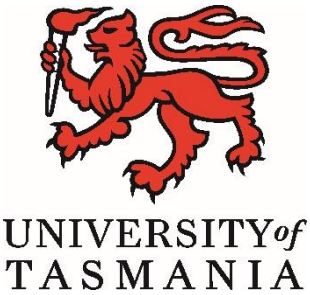

The associations between nutrition, physical activity, brain structure and function in people with and without type 2 diabetes

by

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Submitted in fulfilment of the requirements for the Doctor of Philosophy (Medical Science)

University of Tasmania, July 2020

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Fateme Zabetiantarghi

26 July 2020

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Zabetian-Targhi F, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML (2019) "Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes": The Journal of Nutrition, Volume 149, Issue 10, October 2019, Pages 1805–1811, DOI: https://doi.org/10.1093/jn/nxz139

Author contributions:

Study conception and design: Srikanth VK, Callisaya ML

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PAPER 2: Located in Chapter 4
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PAPER 3: Located in Chapter 5
Zabetian-Targhi F, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML "Adherence to the Australian Dietary Guidelines Is Not Associated with Brain Structure or Cognitive Function in Older Adults": The Journal of Nutrition. Volume 150, Issue 6, June 2020, Pages 1529–1534 DOI: doi: https://doi.org/10.1093/jn/nxaa052.

Author contributions:
Study conception and design: Callisaya ML, Srikanth VK
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PAPER 4: Located in Chapter 6
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Abstract

Background: Dementia is a major public health concern which has a devastating impact on people's quality of life. People with type 2 diabetes (T2D) are at a higher risk of developing dementia as they have a faster rate of cognitive decline and brain atrophy compared to people without T2D. Diet and physical activity are major modifiable risk factors for both dementia and T2D and may be important targets for prevention. However, it is unclear if specific diets and objectively measured physical activity are associated with markers of dementia in people with T2D.

Aims: To examine associations between dietary intake (data-driven dietary patterns, an inflammatory diet, adherence to the Australian Dietary Guidelines), and objectively measured physical activity (steps per day and intensity) with brain structure and cognition. To examine whether any associations with dietary intake differ based on T2D status and if apolipoprotein E-ε4 (APOE-ε4) and insulin-therapy modify any associations with physical activity.

Methods: Data was from the Cognition and Diabetes in Older Tasmanians study, a cross-sectional study of 689 people with (n = 343) and without T2D (n = 346) aged 55–90 years; and the Cognition and Diabetes in Older Tasmanians-Blood Pressure study, which recruited only participants with T2D (n = 220) aged 55-86 years. In all studies, the 80-item Cancer Council of Victoria food frequency questionnaire was used to assess dietary intake. Dietary intake was examined using three methods: dietary patterns calculated using principal component analysis, an inflammatory diet using the dietary inflammatory index (DII), and the Australian Dietary Guidelines Index (maximum score 90, with a higher score indicating greater compliance with the dietary guidelines). An accelerometer was used to obtain measures of step count and time spent on moderate-to-vigorous physical activity averaged over 7 days. In all studies, neuropsychological tests were used to assess six cognitive domains (verbal memory, visual memory, visuospatial function, executive function, verbal fluency, and attention-processing speed) and global cognition. Brain magnetic resonance imaging (MRI) was performed to obtain grey matter, white matter, and hippocampal volumes and markers of small vessel disease (microbleeds, infarcts, and white matter hyperintensities).

Results: There were two dietary patterns (prudent and traditional) for people with T2D and three dietary patterns (prudent, traditional, and Western) for those without T2D. For those without T2D, higher adherence to the Western dietary pattern was associated with lower grey matter volume. However, the association was no longer significant after adjusting for a cardiovascular risk score, mood, and physical activity. The other dietary patterns were not associated with brain structure or

cognitive function. There were no associations between the DII and any of the brain structure variables in fully adjusted models. T2D modified the association between DII and grey matter volume, which was stronger in people without T2D. No associations were observed between the Australian dietary guideline index and brain MRI or cognitive variables. T2D did not modify any associations between diet and brain MRI or cognitive function. Higher daily step count was associated with better attention-processing-speed and greater total hippocampal volume. APOE-ε4 modified the association between moderate-vigorous physical activity and verbal fluency. However, this association was only significant among APOE-ε4 carriers. Insulin-therapy modified the association between moderate-vigorous physical activity and attention-processing speed which was stronger in those taking insulin-therapy.

Conclusion: In this cross-sectional study, there was little evidence of associations between diet and brain health in people with or without T2D. Future prospective studies need to apply a lifespan approach to clarify the effects of early- and mid-life diet on later life brain heath. Higher step count and greater time spent in moderate-vigorous physical activity may be beneficial for different aspects of brain health in people with T2D. Moderate-vigorous physical activity appeared to particularly benefit cognition for APOE-ε4 carriers and those receiving insulin therapy. These factors should be considered in future clinical trials to improve cognitive function in people with T2D.

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Acknowledgements

First and foremost, I wish to express my greatest appreciation to my primary supervisor Associate Professor Michele Callisaya. You have provided me with guidance, courage, and endless support. Your passion for your work has been inspirational. You have constantly motivated me to work harder, think more critically, change my perspective and look deeper.

To my co-supervisor, Dr Kylie Smith, thank you for your contribution, and kind support along the way. To my co-supervisor, Dr Monique Breslin, thank you for all your support, and encouragement. Your knowledge and passion for statistics opened my eyes to a new world. To my advisors, Dr Chris Moran, and Dr Wei Wang thank you for your contribution and guidance.

To my co-author and initial designer of Cognition and Diabetes in Older Tasmanians study, Professor Velandai Srikanth, thank you, this project would not have been possible without you. To my co-author, Professor Wendy Oddy, thank you for generously sharing your knowledge and experience in the field. Thank you also Associate professor Richard Beare for all the technical advice along the way.

To my co-authors from the University of South Carolina, Dr Nitin Shivappa, and professor James Hébert, thank you for your contribution which made the project on the dietary inflammatory index possible. To my co-author, Dr Feitong Wu, thank you for your contribution, and guidance for the physical activity project. To my co-author, Dr Joel M. van Weel, thank you for your help. Thank you to Tim Albion for your technical support. Thanks to the rest of the project team, who provided administrative support- Kate Pubert, Kate Butorac, Nada Alnasser, Chantal Woodhams and Christine Farmer. Thank you to my fellow PhD candidates Dr Saliu Balogun, Johanna Wilson, Dr Dawn Sipmson, Choa Zhu, Dr Larissa Bartlett, Ishanka Munugoda, Oshadi Jayakody, Dr William Cuellar, Anitra Wilson, and Olivia Swann for being so approachable and for helping out whenever needed.

On a personal note, thank you to my family for encouraging me to take this PhD journey far away from home. Thank you to my parents for listening to me and being super supportive. Special thanks to my partner Mitch and his family in Australia who made my PhD journey much easier by providing massive emotional support. Finally, thank you Mitch, for love, encouragement, support, patience, and standing by my side throughout this journey.

Publications directly arising from the work described in this thesis

Published papers

Zabetian-Targhi F, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML (2019) "Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes": The Journal of Nutrition. Volume 149, Issue 10, October 2019, Pages 1805–1811, DOI: https://doi.org/10.1093/jn/nxz139

Zabetian-Targhi F, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML (2019) "Adherence to the Australian Dietary Guidelines Is Not Associated with Brain Structure or Cognitive Function in Older Adults": The Journal of Nutrition. Volume 150, Issue 6, June 2020, Pages 1529–1534 DOI: doi: https://doi.org/10.1093/jn/nxaa052.

Papers submitted

Zabetian-Targhi F, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Shivappa N, Hébert JR, Breslin M, van Weel JM, Callisaya ML "Associations between the dietary inflammatory index, brain volume and small vessel disease" Submitted to the Journal of Academy of Nutrition and dietetics (under review)

Zabetian-Targhi F, Srikanth VK, Beare R, Breslin M, Moran C, Wang W, Wu F, Smith KJ, Callisaya1 ML "The association between physical activity intensity, cognition and brain structure in people with type 2 diabetes " Submitted to the Journal of Gerontology: Series A (under review)

Conference presentations arising from this thesis

2019 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Shivappa N, Hébert JR, Breslin M, van Weel JM, Callisaya ML "The associations between dietary inflammatory index, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. The 43rd Nutrition Society of Australia Annual Scientific Meeting, Newcastle, Australia

2019 **Zabetian-Targhi F**, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML "The associations between adherence to the Australian Dietary Guidelines, cognitive function and brain structure in older adults with and without type 2 diabetes" Poster presentation. The 43rd Nutrition Society of Australia Annual Scientific Meeting, Newcastle, Australia

2019 **Zabetian-Targhi F**, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML "The associations between adherence to the Australian Dietary Guidelines, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation by Callisaya ML. The Alzheimer's Association International Conference, Los Angeles, USA

2019 **Zabetian-Targhi F**, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML "The associations between adherence to the Australian Dietary Guidelines, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. The Australian Dementia Forum, Hobart, Australia

2018 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Poster presentation. The Tasmanian Dementia Symposium, Hobart, Australia

2018 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Webinar presentation. The Nutrition Society of Australia, Hobart, Australia

2018 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Poster presentation by Callisaya ML. The Alzheimer's Association International Conference, Chicago, USA

2018 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Shivappa N, Hébert JR, Breslin M, van Weel JM, Callisaya ML "The associations between adherence to the dietary inflammatory index, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. The Australian Dementia Forum, Sydney, Australia

2017 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. The 10th Asia Pacific Conference on Clinical Nutrition, Adelaide, Australia

2017 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. The School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

2017 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Poster presentation. The Graduate Certificate for Research Conference, University of Tasmania, Hobart, Tasmania, Australia

2017 **Zabetian-Targhi F**, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML "The associations between dietary patterns, cognitive function and brain structure in older adults with and without type 2 diabetes" Oral presentation. Peninsula Clinical School, Central Clinical School, Monash University, Melbourne, Victoria, Australia

Collaborations and memberships during candidature

Collaborating with the scientists from the University of South Carolina who originally developed the dietary inflammatory index for second PhD project entitled: “The association between dietary inflammatory index, cognition, and brain structure.”

Memberships during candidature

Committee member of the Nutrition Society of Australia, Tasmania Branch (2019-2020)
Member of the American Society of Nutrition (2017-present)
Member of the Nutrition Society of Australia (2016-present)

Awards and grants resulting from this thesis material

University of Tasmania International Postgraduate scholarship, Oct 2016- Apr 2020 ($27082)
Travel Award Winner, Nutrition Society of Australia, Oct 2019 ($400)
Travel Award Winner, University of Tasmania, Aug 2017 ($2500)

List of abbreviations

DQES v2	Dietary Questionnaire for Epidemiological Studies Version 2
Aβ42	β-Amyloid42
APOE-ε4	Apolipoprotein E-ε4
CDOT	Cognition and Diabetes in Older Tasmanians
CDOT-BP	Cognition and Diabetes in Older Tasmanians-Blood pressure
COWAT	Controlled Oral Word Associations Tests
CSF	Cerebrospinal fluid
CT	Computer Tomography
DII	Dietary Inflammatory Index
DSM-5	The Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition 2013 update
E-DII	Energy-adjusted Dietary Inflammatory Index
FFQ	Food frequency questionnaire
HEI	Healthy Eating Index
HVLT-R	Hopkins Verbal Learning Test-Revised
MET	Metabolic equivalent of task
MIND	Mediterranean-dietary approaches to stop hypertension Intervention for Neurodegenerative Delay
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NDSS	National Diabetes Services Scheme
NIA-AA	The National Institute on Ageing and Alzheimer's Association
NUTTAB	NUTrient TABles
PCA	Principal component analysis
PET	Positron emission tomography
P-Tau	Phosphorylated Tau
T2D	Type 2 diabetes

1. Introduction

1.1. Preface

Dementia is a major public health concern which has no established pharmacological treatment. The management of modifiable risk factors is thought to be the most effectual way of preventing dementia at this point in time. The focus of this thesis is to examine specific modifiable risk factors (dietary patterns and physical activity) for dementia in relation to dementia markers (brain structure and function). This chapter will start by presenting to the reader the problem of dementia, common markers and known risk factors of dementia. The second part of the introduction will introduce what is known regarding diet and physical activity in relation to brain structure and function and set the scene for the questions examined in this thesis.

1.2. Problem of Dementia

The impact of dementia

Dementia is a syndrome that includes cognitive dysfunction, behavioural and psychological symptoms (1), as well as a decline in physical function which together result in impairment in activities of daily living (2). The greatest impact of dementia is on the quality of life of individuals living with dementia (2). Family members who support people with dementia may often experience stress, anxiety, exhaustion, and problems with their own health (3). There are several forms of dementia. Alzheimer's disease (~ 60% of cases) and vascular dementia (~30% of cases) are the most common forms (4). Other common forms of dementia are dementia with Lewy bodies and frontotemporal dementia (4).

Prevalence and incidence of dementia

According to the World Health Organisation, around 50 million people had dementia worldwide in 2015. This is estimated to rise to 75 million by 2030 and 131.5 million by 2050 (5). Globally there are approximately 10 million new cases of dementia every year which translates into one new case every three seconds (5). The growing numbers could be due to increased life expectancy leading to a growth in the ageing population (5). This increase in life expectancy is mostly attributed to major recent developments in medical treatments and technologies that have decreased the once high infant mortality rate (6). Innovations such as vaccines and clean drinking water have significantly reduced the mortality rate due to "smallpox, scarlet fever, malaria, and cholera" (6). In addition, advances in medical treatment for controlling blood pressure decreased the rate of mortality from conditions such

as stroke, which has also contributed to increased life expectancy (7)."

In Australia, dementia is the second cause of mortality (8) with 459,000 Australians living with the condition (9). Without a medical cure, the number of people living with dementia is projected to increase to 589,807 by 2028 and 1,076,000 by 2058 (9). According to 2017 estimations, there were 250 new cases of dementia per day (10). It is expected the number of new cases of dementia will rise to 318 people per day by 2025 and to more than 650 people by 2056 (10).

The cost of dementia

The total estimated worldwide costs of dementia care were $818 billion in 2015 (5). In 2017, dementia had an estimated cost to Australia of more than $15 billion (10) including at least $4.9 billion per year for the health and aged care sectors (10, 11). This is projected to rise to more than $18.7 billion by 2025, and to more than $36.8 billion by 2056 (10).

1.3. Definitions

The following sections describe the clinical and research definitions of dementia.

Clinical diagnosis of dementia

The Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition 2013 update (DSM-5) is a diagnostic tool that has been released by the American Psychiatric Association (12). Based on the DSM-5, neurocognitive disorders are categorised into major (dementia) or mild disorders. The DSM-5 is based on clinical assessment of cognitive function and a patient history, rather than biological biomarkers. A major or mild neurocognitive disorder is "when there is evidence of substantial cognitive decline from a previous level of performance in one or more cognitive domains". Cognition is measured based on standardised quantified clinical assessment or neuropsychological testing. The cognitive domains include executive function, complex attention, language, learning and memory, social cognition and perceptual-motor skills (12). Cognitive decline in major neurocognitive disorders interferes with a person's ability to independently perform at work or do their usual activities. Whereas cognitive decline in mild neurocognitive disorders does not intervene with a person's independence to perform activities of daily living. The neurocognitive disorders are further classified based on underlying disorders, for example, a major or mild neurocognitive disorder due to Alzheimer's disease, a major or mild Vascular neurocognitive disorder, a major or mild Frontotemporal neurocognitive disorder, a major or mild neurocognitive disorder with Lewy bodies.

Biological processes of Alzheimer's disease

Presence of neuritic plaques and neurofibrillary tangles are the biological hallmark of Alzheimer's disease (13).

Neuritic plaques are extracellular lesions which mainly consist of the amyloid-β42 peptide (Aβ42). Neuritic plaques occur between neurons and disrupt neural networks. The amyloid precursor protein is a natural transmembrane protein in the nervous system which could be metabolised to different Aβ species. Aβ42 is the most toxic form of Aβ that is more prone to aggregate, deposit, and form amyloid plaques (13). The amyloid hypothesis states that the accumulation and deposition of amyloid β (Aβ) peptide is the primary cause of Alzheimer's disease. Meanwhile, evidence has indicated that neurofibrillary tangles have also an important role in the development of Alzheimer's disease (14).

Neurofibrillary tangles are intracellular lesions and mostly include hyperphosphorylated and misfolded Tau proteins. The Tau protein is a microtubule-associated protein which has an important role in the maintenance of the neuronal structure, synaptic plasticity, and axonal transport (13). In healthy neurons, tau normally binds to microtubules. In Alzheimer's disease, however, abnormal chemical changes cause tau to detach from microtubules and stick to other tau molecules to form tangles inside neurons. These tangles block the neuron's transport system and the synaptic connections between neurons (14).

Growing evidence has indicated that inflammation and oxidative stress may contribute to Alzheimer's disease exacerbation. Increased pro-inflammatory cytokines in the brains of people with AD lead to an accumulation of Aβ plaques and tau hyperphosphorylation resulting in the neuronal loss (15).

Definition of Alzheimer's dementia used in research

In 2018, the National Institute on Ageing and the Alzheimer's Association (NIA-AA) provided the 2018 updated research definition for Alzheimer's disease. Alzheimer's disease was defined based on underlying biological processes and biomarkers rather than the clinical symptoms (4).

Since the predominant cerebral pathology in Alzheimer's disease is the accumulation of misfolded proteins (β-Amyloid$_{42}$ (Aβ_{42}), and phosphorylated Tau (P-Tau)) (16), the presence of these two biomarkers is the basis of the biological definition for Alzheimer's disease. Based on the updated definitions, "Alzheimer's pathologic change" would be applied if there was biomarker evidence of Aβ_{42}, but with normal P-Tau. "Alzheimer's disease" would be assigned to individuals who presented evidence of both Aβ_{42} and pathologic P-Tau. "Alzheimer's pathologic change" is regarded as the earlier phase and "Alzheimer's disease" is considered the later phase of the Alzheimer's continuum

(4).

"Neurodegeneration and neural injury" biomarkers are nonspecific indicators of cerebrovascular injury and neuronal damage (4). In the NIA-AA framework, biomarkers of neurodegeneration and neural injury are not applied to define Alzheimer's disease. However, they are used along with Amyloid, and Tau to stage the severity of Alzheimer's disease (4). Biomarkers of neurodegeneration or neural injury include 1) total-Tau as a marker of neural injury at a specific point (17); 2) brain tissue hypometabolism (a marker of neuronal loss and functional impairment) 3) and brain atrophy (a marker of neuronal loss) (18, 19).

Based on the NIA-AA research framework (4), the severity of cognitive impairment is described by the syndromal cognitive staging scheme. This classified the cognitive continuum into the following categories: cognitively unimpaired, mild cognitive impairment and Alzheimer's dementia. Alzheimer's dementia is then further categorised into mild, moderate, and severe dementia. The diagnosis of mild cognitive impairment is based on functional abilities and cognitive assessment (4). Although people with mild cognitive impairment are at a high risk of progressing to dementia, most remain stable or return to normality, and only a small proportion (15.8%) develop dementia (20).

Although the research framework provides a biological definition for Alzheimer's disease, many cases are mixed, and many people do not fit in a distinctive category (4). The clinical definition covers all types of dementia whereas the research definition focuses on Alzheimer's disease pathology, which is the reason it is not recommended for clinical settings (4).

Definition of vascular cognitive impairment

Cognitive impairment of vascular aetiology is categorised as either mild or severe vascular cognitive impairment (vascular dementia) (21). Vascular dementia is the second main form of dementia which occurs as a result of cerebrovascular diseases (21). Cerebrovascular diseases occur in consequence of abnormalities of large vessels, or small arteries of deep brain structures (22).

Small vessel disease indicates a range of pathological, and clinical syndromes resulting from different causes influencing the blood vessels in the brain (23). Small vessel disease leads to the formation of lesions located in the subcortical structures including white matter hyperintensities, lacunar infarcts, microbleeds, or large haemorrhages which are standard biomarkers of vascular dementia in older adults (24-26). Vascular damage may also lead to amyloid burden and brain atrophy (27). The following section will focus on how different biomarkers of dementia are measured.

1.4. Biomarker measurement tools

The pathology of dementia occurs many years before the clinical symptoms (4). Therefore, biomarkers such as brain imaging and fluid biomarkers are commonly used in research to determine those at risk of dementia before clinical symptoms appear.

Cerebrospinal fluid and blood biomarkers

This section describes the cerebrospinal fluid (CSF) and blood biomarkers of dementia. The CSF is a body fluid found in the brain and spinal cord. CSF biomarkers are widely applied in both clinical practice and research. Despite being well-tolerated, CSF sampling is still an invasive procedure; therefore, in recent years researchers have focused on developing biomarkers from more obtainable biological matrices such as blood (28). As blood circulates in the brain, it contains biomarkers that may be effective for the diagnosis of dementia. Although blood draw is easier than CSF sampling, the concentration of biomarkers in the blood is lower than CSF. Therefore, recent research has focused on ultrasensitive immunoassays to examine if the detected blood biomarkers are comparable with the CSF biomarkers in terms of their association with Alzheimer's disease (28).

The most common blood and CSF biomarkers that show changes during Alzheimer's disease include markers of Aβ pathology (low Aβ42 or Aβ42/40), and Tau pathology (high total Tau and P-Tau). There are a large number of other fluid biomarkers which are related to multiple concomitant pathological events related to dementia including neurodegeneration, inflammation, vascular dysregulation or synaptic dysfunction (28).

Imaging

Imaging biomarkers are used to study changes in the structure, function and metabolism of the brain (29). There are different neuroimaging techniques available including Computer Tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI).

Positron emission tomography

PET is a quantitative molecular imaging technique that is complementary to structural imaging for diagnosis and characterisation of disease (30). PET can measure amyloid deposition (31), P-Tau (32) and neuronal activity (lower glucose uptake, and blood flow) (33).

Computer Tomography

A CT scan is a type of X-ray which uses radiation. The main role of CT scans is detecting secondary

causes of cognitive dysfunction, such as tumours and intracranial masses (34). Compared to MRI, CT scans have advantages including lower costs, shorter acquisition time and more widespread availability. CT scans can also be performed in patients with contraindications for MRI (e.g. pacemaker or metal prosthesis) (35). However, CT scans have lower spatial resolution and therefore they are less sensitive compared to MRI in detecting lesions and measuring their progressions.

Magnetic Resonance Imaging

MRI is an imaging technique that applies strong radio waves, and magnetic field gradients to produce images of the body's anatomy (36). MRI does not include X-rays or ionising radiation, which is its advantage over CT and PET scans (36). MRI biomarkers are considered the gold-standard measure for clinical diagnosis of vascular dementia as they provide a detailed picture of any vascular damage, and small vessel disease in the brain (21). MRI biomarkers of brain atrophy (grey matter, white matter, and hippocampal volumes) may reflect cumulative neurodegeneration (37) and are associated with cognitive decline (38). MRI is the most sensitive measure to examine the distribution of cortical atrophy in the research setting (39). MRI measures of regional brain atrophy are valid surrogate biomarkers of dementia and they are well accepted as outcomes to explore pathways underlying dementia (40). In this thesis, MRI is used to obtain measures of brain atrophy (grey matter, white matter, and hippocampal volumes) (40), and small vessel disease (microbleeds, infarcts, and white matter hyperintensities) (24-26). Further details about the MRI methods used in this study will be described in chapter 2.

Diagnosis of dementia is preceded by a preclinical stage and mild cognitive impairment (4). As dementia prevention is important at the early stages (41), this thesis focuses mainly on preclinical and early stages when clinical symptoms of dementia are not manifested. The next section introduces the modifiable and non-modifiable risk factors of dementia.

1.5. Non-modifiable risk factors of dementia

Non-modifiable risk factors of dementia include age, male gender for vascular dementia (42) and female gender for Alzheimer's disease (43). The main genetic risk factor for the development of dementia is the ε-4 polymorphism of the Apolipoprotein E (44). APOE-ε4 carriers are 3 (heterozygous) - to 15 (homozygous) times more at risk of developing late-onset Alzheimer's disease compared to non-carriers (45, 46).

1.6. Modifiable risk factors of dementia

Modifiable risk factors are factors that can be changed in order to prevent dementia. Low education is strongly associated with a higher risk of dementia, and could be addressed particularly in the early stages of life, but also in mid and later life (42, 47). There is a strong association between depression and dementia, which can be modified by medication or psychological therapy (48). Poor sleep (49), and hearing loss (50) are also other modifiable risk factors for dementia. Several factors related to cardiovascular disease such as hypertension (51), stroke (51), type 2 diabetes (T2D) (52, 53), mid-life obesity (54), and smoking (55) are also attributable to the development of dementia (42). Modest alcohol consumption may have a protective role, similar to its known cardiovascular benefits (56). The Mediterranean diet (57, 58) and regular physical activity are also associated with a lower risk of dementia (59). Factors related to cardiovascular disease work through oxidative stress and inflammation to affect brain health (60). This would be discussed further in each section under each risk factor.

The management of modifiable risk factors for dementia is currently considered to be the most effectual method for preventing dementia. Diet and physical activity are important lifestyle factors recommended in the control of T2D and other related conditions such as obesity and hypertension. This thesis was intended to further current understanding of lifestyle risk factors of dementia and expand the existing work on a cohort of people with and without T2D (61). Therefore, the focus of this thesis will be research on two lifestyle factors (diet and physical activity) in a sample of people with and without T2D.

1.7. Type 2 diabetes

Definition

T2D is a chronic disease, defined by hyperglycaemia occurring as a result of a progressive insulin secretory defect and insulin resistance (62). Based on the American Diabetes Association 2011 guidelines, the criteria for the diagnosis of T2D are as follows: “fasting plasma glucose level ⩾7.0 mmol/l (126 mg/dL) or haemoglobin A1c ⩾6.5% (7.7mmol/L) or 2-hour plasma glucose ⩾ 11.1 mmol/l (200mg/dL) after a 75g oral glucose tolerance test or classic symptoms of hyperglycaemia (polyuria, polydipsia, weight loss) or hyperglycaemic crisis with a random plasma glucose ⩾11.1 mmol/l (200mg/dL)” (62).

Prevalence and health implications

Worldwide, in 2019, the prevalence of T2D was 9.3% (463 million people) and is projected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 in people between the ages of 20-79 years (63). According to the Australian National Diabetes Services Scheme (NDSS), in 2020 there are more than 1.3 million Australians with known diabetes aged between 20 and 79 years (64). For every five diagnosed cases of T2D in Australia, there are four undiagnosed cases (65). T2D related long-term complications include macrovascular (stroke and coronary artery disease) (66, 67) and microvascular disease (retinopathy, nephropathy and neuropathy) (66, 68), depression (69), and cognitive decline (70). The following section will focus mainly on T2D related cognitive complications and brain structure abnormalities captured by MRI.

Type 2 diabetes and cognitive decline

T2D nearly doubles the risk of incident dementia (71). Approximately 10% of dementia cases in the world are related to T2D (72). T2D is associated with a faster cognitive decline (73). According to a recent meta-analysis, the cognitive effects of T2D are strongest for episodic memory (effect sizes Cohen's d = -0.51), and cognitive flexibility (d = 0.52), but weaker for logical memory (d = -0.24), verbal fluency (d = -0.35) and processing speed (d = -0.22) (74). In a cross-sectional study, T2D was associated with lower executive function, visuospatial function, visual memory, and processing speed (75). In a longitudinal study, the trajectory of domain-specific cognitive decline was compared between 348 patients with T2D and 397 people without T2D aged 55-90 years, T2D was associated with a decline in verbal fluency and verbal memory over 5 years (70).

Type 2 diabetes and brain structure

T2D is also associated with greater brain atrophy (75, 76) and cerebrovascular disease, including cerebral infarcts (75, 76), altered white matter integrity (77), microbleeds, and white matter hyperintensities (76, 78). Cerebral vessel disease is associated with poorer processing speed (76, 77, 79), executive function (76), memory performance (77) and attention (80) in older adults with T2D (81). In a cross-sectional analysis, T2D was shown to be associated with lower grey matter, and hippocampal volumes (75) which mediated T2D-related cognitive dysfunction (75).

Mechanisms by which type 2 diabetes may affect brain health

T2D could increase the risk of dementia through insulin resistance, central haemodynamic, neuroinflammation, advanced glycation, and aortic stiffness **(See Figure 1.1)** (82). Insulin resistance induces impairments in glucose metabolism and interrupts brain energy balance, subsequently

elevating oxidative stress, and advances glycation which drive pro-inflammatory, pro-amyloid-beta cascades, and neuroinflammation (83). Hyperglycaemia related aortic stiffness (84) is associated with lower brain volume in people with T2D (85).

T2D risk factors (i.e. hyperglycaemia, hypertension, and abdominal obesity) are also associated with an increased risk of dementia. Hyperglycaemia induces the production of advanced glycation end-products, which increase vascular inflammation (86), and contribute to hypertension, lower brain volume (87, 88), and poorer cognition (88). T2D-related obesity is linked to the increased risk of dementia through inducing inflammatory pathways (89) and reduced insulin sensitivity (90). Greater abdominal obesity is associated with lower brain volume (91). The presence of multiple interacting mechanisms calls for preventive approaches to reduce the risk of T2D-related cognitive decline.

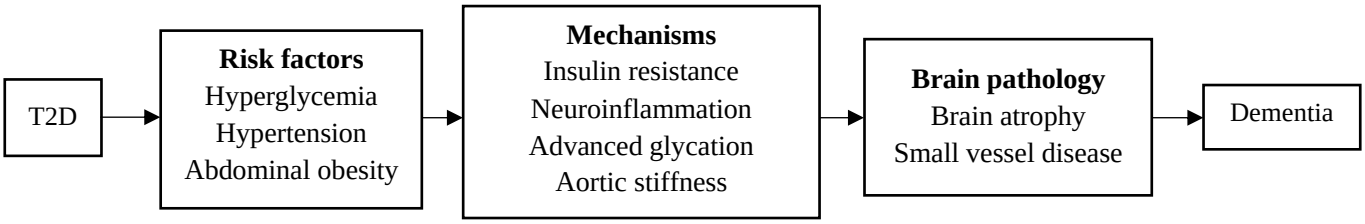


Figure 1.1 Type 2 diabetes pathways to dementia

Risk factors for type 2 diabetes

Dietary intake is a key modifiable factor for prevention of T2D (92). Healthy diets play a significant role in glycaemic control (93) and limiting the incidence of cardiovascular diseases in people with T2D (94). Physical activity is also one of the main factors for the prevention and management of T2D (67, 95). Since having a healthy diet and being physically active are also important factors for the prevention of dementia (96), the next sections will review the literature on the association between nutrition, physical activity, cognitive function, brain volume and small vessel disease in older adults with and without T2D.

1.8. Nutrition

Definition

Diet is the combination of foods that a person eats or drinks. Nutrition is the process of ingestion, and digestion of foods as well as the absorption, and metabolism of nutrients (97). There are two major nutritional approaches for examining the effect of foods and nutrients on health outcomes. The

traditional approach examines the role of single nutrients or foods, whereas the whole diet approach investigates the role of dietary patterns or food combinations.

Traditional research approach in nutritional science

Intake of single foods and nutrients have been shown to be associated with cognitive function and brain structure in older adults (98). Emerging yet contrasting evidence, mainly from observational studies, has pointed to the beneficial effect of some nutrients for brain health, including polyunsaturated fatty acids (99) and vitamins such as the B complex (vitamins B6, B12 and folate) (100), antioxidants (beta-carotene, vitamin A (101, 102), C and E (102)) and vitamin D (100, 103). However, randomised controlled trials have not replicated the beneficial results of observational studies (104, 105).

The traditional approach has certain limitations. Foods and nutrients are usually consumed as part of a diet not in isolation, and many different foods have similar nutrient contents. Moreover, nutrients and minerals could have synergic effects on one another. Therefore, the combined synergic effects and interactions of these nutrients on brain health may not be detected using a single nutrient/food approach (106). Furthermore, the effect of each individual nutrient or food may be too small to show associations with disease, whereas the accumulative effects of various nutrients in a dietary pattern may be large enough to be evident (106). Collectively, this may explain why randomised controlled trials have not replicated results from observational studies regarding the effects of single nutrients or food items on brain health.

Dietary patterns approach

The dietary patterns approach is a whole diet approach that provides methods which are complementary to the traditional approach for examining the relationships between diet and disease. This approach typically considers overall dietary intakes and food combinations as well as the accumulative effects of nutrients and foods consumed together. The dietary patterns approach is a better reflection of typical real-life diet and thus it might be more predictive of disease processes compared to the Traditional approach (107). The following sections will explain the different types of dietary patterns, their methods of measurements, and their association with brain health outcomes.

Types of dietary patterns

Dietary patterns can be defined using two different approaches:

a) A ‘Data-driven’ (posteriori) approach is based on the diet of the study sample (e.g. healthy or unhealthy dietary patterns (107)).

b) A 'Hypothesis-driven' (priori) approach is based on available scientific evidence for specific health outcomes (e.g. certain dietary recommendations or dietary habits identified to be beneficial or harmful for brain health (107)).

Data-driven (posteriori) dietary patterns

Data-driven dietary patterns are usually identified by dimension reduction techniques to summarise dietary data into a few dietary patterns. Dimension reduction techniques are statistical methods used to determine combinations of foods normally consumed together. There are different methods of data reduction to identify data-driven dietary patterns including a) cluster analysis, b) reduced rank regression, c) principal component analysis (PCA) or factor analysis. Although reduced rank regression applies to both a priori and posteriori information, this method is often categorised under the data-driven approach as it is mathematically similar to PCA (107). The following sections introduce each of these methods along with their advantages and disadvantages.

Cluster analysis

Cluster analysis is a data-driven approach that determines clusters of individuals with a similar diet. The clusters are mutually exclusive and are identified based on the frequency of dietary intake, contribution to total energy intake, average intakes of foods and food combinations (106). Once the dietary patterns are identified, further analyses are required to examine the stability and dietary profile of the patterns (106). This technique has some disadvantages. Cluster analysis only groups the individuals who have better adherence to a certain type of diet excluding people who have low adherence to that diet. Therefore, it does not provide a score indicating the degree to which everyone adheres to all dietary patterns. Furthermore, the number of clusters needs to be subjectively selected before performing the analysis which is not the case for other methods such as PCA. Dietary clusters have not been consistent across populations or dietary assessment methods (108).

Only a few studies have used cluster analysis to examine the association between diet and brain health (109, 110). Samieri et al. showed that a cluster analysis derived fish diet in men (n=157) and a fruit and vegetable diet in women (n=267) aged ≥65 were associated with better general cognition (109). Granic et al found that a cluster analysis derived high red meat diet and a high butter diet at baseline were associated with worse global cognition, concentration and focused attention after 3 years of follow-up in 791 British community-dwelling older adults aged 85 and above (110).

Reduced rank regression

Reduced rank regression combines both existing knowledge and exploratory methods to identify

dietary patterns. Reduced rank regression uses predictors (food intakes) and response variables (nutrients, or biomarkers (such as antioxidants, and inflammatory biomarkers)) that are implicated on the pathway between predictors and health outcomes. Reduced rank regression focuses on identifying combinations of food groups, which describe as much variation as plausible in the response variable (111). Dietary patterns identified using reduced rank regression have a correlation with the outcome related response variable that is chosen based on a prior evidence. Therefore, reduced rank regression-derived dietary patterns may be more predictive of health outcomes compared to PCA or cluster analysis. This is a major advantage of reduced rank regression compared to PCA or cluster analysis that do not use prior evidence (112). Despite advantages over others, this method is extremely sensitive to outliers that could mask the low-rank dependence structure between predictors and response variables. Only one study examined the association between reduced rank regression derived dietary patterns and brain health (113). That study used interleukin-6 (an inflammatory marker) as a response variable and found higher adherence to an reduced rank regression derived inflammatory diet (higher intake of fried food, processed meat, red meat, peas and legumes, and a lower intake of whole grains) was associated with cognitive decline in reasoning in a sample of 5083 participants aged 35-55 years (113).

Principal component analysis

PCA is a technique to identify underlying patterns as linear combinations of foods usually eaten together (107). The patterns identified by PCA are linear combinations of food variables which are representative of the variations of food intakes in the original data (112). PCA usually aggregates food items or food groups based on the degree to which they correlate with one another (108). However, the technique has disadvantages. The investigator needs to make a few subjective decisions including selecting the number of food groups, the number of patterns to be extracted by considering factor's eigenvalues and scree plots and setting a cut-off for factor loadings to identify the number of components that have the most contribution in a pattern (108). Therefore, PCA-derived dietary patterns may not be the same across different studies (108). Despite these limitations, PCA has a number of advantages such as giving each participant a continuous score for their adherence to certain dietary patterns (combinations of foods eaten together) (114). PCA has been widely used to examine the association between diet and brain health in older adults (113, 115-120). Thus, for the purpose of this thesis, PCA was used to obtain combinations of foods eaten together. The next section summarises the literature that has previously examined associations between PCA derived dietary patterns, cognitive function and brain structure.

PCA-derived dietary patterns and cognitive function

Studies of older community-dwelling adults (110, 117, 120) found associations between higher adherence to PCA-derived healthy dietary patterns (consisting mainly of vegetables, fruits, and fish) and better general cognition. Those studies also found that higher adherence to PCA-derived unhealthy dietary patterns (including mainly heated processed foods) were associated with poorer general cognition (110, 117, 120). The studies used the Mini-Mental State Examination (MMSE) to measure general cognition (110, 117, 120). MMSE does not examine different cognitive domains as it is a screening tool for cognitive impairment (121).

A cross-sectional study investigated the associations between PCA-derived dietary patterns and cognitive domains in 878 community-dwelling older adults aged 70 years (116). Corley et al. showed that higher adherence to a PCA-derived Scottish Traditional pattern (low in fruit and salad vegetables and high in tinned vegetables, meat pies, beans, pastries, mashed potatoes, sausage rolls, and sauces) was associated with lower verbal ability but not processing speed, memory, or general cognition (116).

A few prospective studies have analysed the association between PCA-derived dietary patterns and cognitive domains in community-dwelling older adults (115, 119, 122). In a sample of 527 Australian older adults, with an average age of 69.3 years, higher adherence to a Western dietary pattern (including, red meats, heated processed foods, sweets, saturated spreads, and beer) was associated with a decline in visuospatial function after 36 months of follow-up (115). Results from the Australian Diabetes, Obesity and Lifestyle study showed that a Western dietary pattern (mainly comprising processed meats, refined grains, and fast food) at baseline was a predictor of poorer memory and processing speed after 12 years in 577 older adults aged >60 years old (119). Results from another longitudinal study indicated that a healthy dietary pattern (mainly included whole grains, fruit, vegetables, fresh dairy products, breakfast cereal, vegetable fat, tea, fish, and nuts) at baseline was associated with better global cognitive function, and verbal memory after 13 years of follow-up (122).

Studies examining the associations between dietary patterns and cognition have been mainly conducted in community-dwelling older adults with less focus on high-risk groups like people with T2D. Although there is not enough biological evidence to show that diet affects the brain differently based on T2D status, people with T2D may have a different dietary intake (123). There was only one study in people with T2D that has examined the association between data-driven dietary patterns and cognitive function (124). The study showed that higher adherence to a PCA-derived healthy dietary pattern (highly included vegetables and fish) was associated with better global cognition in 73

cognitively unimpaired Japanese older adults with T2D aged >65 years (124). However, this study had limitations as the study sample was quite small, and it did not include tests of different cognitive domains.

PCA-derived dietary patterns and brain structure

Besides cognition, it is also important to clarify how dietary patterns are associated with brain structure in order to clarify the potential pathways by which diet may be associated with poorer cognitive function. Only one study examined the association between data-driven dietary patterns and brain structural measures in older adults (118). The Personality and Total Health Through Life study that included 255 Australian adults aged 60-64 years old (118), found higher adherence to a Western dietary pattern (mainly included sausages, roast meat, chips, steak, crisps, hamburgers, and soft drinks) was associated with lower left hippocampal volume, and that higher adherence to a healthy dietary pattern (mainly included salad, fresh vegetables, grilled fish and fruit) was associated with higher left hippocampal volume (118).

Collectively, previous studies mainly focused on the general population and did not focus specifically on people with T2D. Therefore, it is important to explore if the associations between diet and brain health are different based on T2D status. Moreover, no studies examined the association between data-driven dietary patterns, grey and white matter volume measures or small vessel disease in older adults. Future studies need to include brain MRI measurements along with comprehensive neuropsychological tests, to clarify the brain pathways involved in the association between diet and cognitive dysfunction.

Hypothesis-driven (priori) dietary patterns

Hypothesis-driven dietary patterns aim to measure people's adherence to specific evidence-based dietary recommendations (national dietary guidelines) or specific dietary habits (e.g. Mediterranean diet) (106). Hypothesis-driven (priori) dietary patterns include dietary indices and scores. To calculate dietary scores, points are awarded for different recommended components and summed to an accumulated total. Higher scores indicate greater compliance with dietary guidelines or habits. Dietary guidelines are evidence-based national dietary recommendations, which are provided by governments to improve people's health and wellbeing. Dietary indices are developed based on the adherence to these recommendations. For example, the Healthy Eating Index (HEI) is an index that assesses adherence to the U.S. Dietary Guidelines for Americans (125), and the Australian Dietary Guidelines index assesses adherence to dietary recommendations from the Australian National Health

and Medical Research Council (126). There are also other dietary indices that are reflective of people's adherence to evidence-based dietary habits such as Mediterranean diet (127), Mediterranean-dietary approaches to stop hypertension Intervention for Neurodegenerative Delay (MIND) diet (128), or the Dietary Inflammatory Index (DII) which is an indicator of the inflammatory potential of diet (129).

The dietary indices are restricted to the current evidence of diet's relationship with health outcomes (106). Moreover, dietary indices tend to consider favourable features of a diet, for example, specific foods or nutrients, and some indices such as DII, MIND and Mediterranean diet ignore the total effect of dietary intake (112). However, dietary indices are representative of existing knowledge of optimal dietary patterns and enable comparability between different studies. Dietary indices may be easier to interpret and be more understandable by the public compared to data-driven dietary patterns (108). The dietary indices could be applied to assess people's compliance with national dietary guidelines and to examine trends in the population over time. The following section will explain the associations between dietary indices, cognitive function and brain structure in older adults.

Hypothesis-driven dietary patterns and cognitive function and brain structure

Mediterranean diet

The Mediterranean diet is a typical dietary pattern from Mediterranean countries which is composed of a high intake of vegetables, legumes, fruit, nuts, monounsaturated fat (olive oil) and cereals; low intake of saturated fat; moderate intake of meat, dairy foods, fish, poultry, and alcohol (127). Observational studies in older adults indicate that higher adherence to the Mediterranean diet is associated with better cognitive performance (130), less cognitive decline (131), lower risk of developing mild cognitive impairment (132), and Alzheimer's disease (133, 134). Higher adherence to the Mediterranean diet is associated with less brain atrophy (total grey or white matter volume) (135), lower white matter hyperintensities (136), and better structural connectivity (137) in older adults. Furthermore, randomised controlled trials showed that the Mediterranean diet plus olive oil or nuts increased global cognition in comparison with a low-fat diet in older adults after 3 (138) and 6.5 (139) years of dietary interventions.

Mediterranean-dietary approaches to stop hypertension Intervention for Neurodegenerative Delay diet

The MIND diet is a hybrid of dietary components of the Mediterranean diet and Dietary Approaches to Stop Hypertension with modifications that focus on the foods and nutrients associated with brain

health (128). The MIND diet suggests higher intakes of 10 food groups that are good for brain (leafy green vegetables, other vegetables, berries, nuts, beans, seafood, whole grains, poultry, wine, and olive oil) and lower intakes of five food groups that are not good for brain (red meats, cheese, butter and margarine, fried/fast food, pastries and sweets) (128). The MIND diet has been shown to be associated with better cognitive function (140, 141) reduced risk of cognitive decline (142, 143) and Alzheimer's disease (144) in older adults. The next sections will focus on other dietary indices that their associations with brain health are less studied.

Dietary Inflammatory Index (DII)

The DII is a measure of adherence to an inflammatory diet. DII is based on a literature review of 1943 articles that examined the effect of whole foods and dietary constituents on six inflammatory markers (interleukin-1 β, interleukin-4, interleukin-6, interleukin-10, tumour necrosis factor-α and C-reactive protein) (129). The DII is constructed based on 45 food parameters including macro and micronutrients, bioactive components/phytochemicals, foods, herbs and spices. A higher DII suggests higher adherence to an inflammatory diet (129).

The DII approach has some limitations. The inflammatory effects of the food parameters were weighted according to the quality and number of articles published. Despite its limitations, the DII approach includes dietary intakes of food parameters from a variety of cultures that represents dietary inflammatory potential. The approach removes the problem of non-comparability of food parameters' units by using z-scores and percentiles.

The association between DII and cognition and brain structure

Kesse-Guyot et al. found that higher adherence to an inflammatory diet (higher DII) at midlife was associated with lower overall cognitive performance after 13 years of follow-up in 3080 middle-aged adults in a French cohort (145). The same study also showed a negative cross-sectional association between baseline DII and baseline verbal memory, but not executive function (145). A prospective study showed that a higher DII score was associated with greater cognitive impairment after 9.7 years of follow-up in 7085 women aged 65-79 years (146). Moreover, a cross-sectional study indicated that higher DII scores were associated with lower semantic-based memory, episodic memory, working memory, and executive function in a sample of the 1723 older adult aged 60–85 years (147).

Although previous studies investigated the association between DII and cognitive function, the underlying brain pathways by which diet affects cognition is still unknown. Therefore, future research needs to examine the underlying brain pathways using structural brain measures.

Dietary guidelines from other countries

Dietary guidelines are evidence-based national dietary recommendations which are provided by governments to improve people's health and wellbeing. In particular, Dietary Guidelines for Americans and Dutch dietary guidelines are studied in relation to brain health. Healthy Eating Index (HEI) is a measure of adherence to the dietary guidelines for Americans which was released by the United States Department of Agriculture.

A higher HEI score was associated with better global cognition (148, 149), verbal learning (150) and memory (150) in older adults. A higher HEI score was also associated with higher general cognition (MMSE scores) and lower odds of cognitive dysfunction in a sample of 1,269 American adults, aged 45–75 years (148). A lower HEI score was associated with poorer memory and verbal learning in 2090 Americans aged 30-64 years (150).

Moreover, Wengreen et al. examined adherence to the Dietary Guidelines for Americans by creating two food scores that measured individual's intake of recommended and non-recommended foods (149). Authors scored the dietary intakes of individuals based on their adherence to the guidelines' recommended and non-recommended foods. Wengreen et al. found a cross-sectional association between higher adherence to recommended foods and better general cognitive performance (MMSE score) (149). In a prospective analysis, those with the highest adherence to recommended foods at baseline showed a lower cognitive decline over 11 years compared to those with lower adherence. Adherence to non-recommended foods was not associated with MMSE scores (149).

There is only one study that examined the association between adherence to the Dutch dietary guidelines' recommendations and brain health (151). The authors showed that higher adherence to the Dutch dietary guidelines was associated with larger grey, white and hippocampal volume, but not white matter hyperintensities volume, or microbleeds in 4,213 participants aged 45.5–97.5 years old (151). The study did not measure cognitive function.

The dietary guidelines from America and the Netherlands differ from Australia. The Dietary Guidelines for Americans provide recommendations based on the kcal of diet (125), whereas the Australian Dietary Guidelines provide age- and sex-specific recommendations (152). The Dutch dietary guidelines provide general advice for a more plant-based and less animal-based diet with specific recommendations for 15 food groups, however, the recommendations are not specific to kcal of diet, sex and age (153). The guidelines also differ in the way they recommend food components related to brain health. For example, leafy green vegetables, seafood and nuts have specific

recommendations in the Dietary Guidelines for Americans (125) but are combined with other food components in the Australian Dietary Guidelines (152). Moreover, Dutch dietary guidelines differ from the Australian Dietary Guidelines as legumes, nuts, and fish are separately recommended from red meats (151). Given the positive associations between the dietary guidelines from other nations and brain health, as well as the noted differences with the Australian Dietary Guidelines, it would be interesting to examine the association between the Australian Dietary Guidelines and cognitive function and brain structure.

The Australian Dietary Guidelines

The Australian Dietary Guidelines are evidence-based dietary recommendations for improving the Australians' health and wellbeing. The Australian Dietary Guidelines Index is a score to indicate the diet quality of people based on their adherence to the Australian Dietary Guidelines (154). The Australian Dietary Guidelines Index has been validated as a measure of diet quality in an Australian population (126). The majority of the previous studies used the index to evaluate the association between the Australian Dietary Guidelines with cardio-metabolic and cardiovascular risk factors (155-157). However, one recent study examined associations between adherence to the 2013 Australian Dietary Guidelines and general cognition in older adults (158). The Wellbeing, Eating and Exercise for a Long Life study found no association between adherence to the Australian Dietary Guidelines and global cognition in 617 adults aged 55–65 years (158). The cognitive assessment in the study was performed using a screening tool (Telephone Interview of Cognitive Status) and the study did not include brain MRI or examine cognitive domains (158). Therefore, future studies are needed to better understand associations between adherence to the Australian Dietary Guidelines, cognitive domains and brain structural measures in older adults.

Mechanisms by which diet may influence brain health

Diet affects the brain through influencing gut microbiome, obesity, inflammation, oxidative stress, blood-brain barrier integrity, cardiovascular health, angiogenesis, synaptogenesis and neurogenesis **(Figure 1.2).** Angiogenesis is the production of new blood vessels from primary vessels. Synaptogenesis is the process of forming synapses between neurons in the nervous system (159). Neurogenesis is the process of the formation of neurons from neural stem cells.

Diet may modulate levels of gut-derived circulating hormones, nutrients, and immune signals (160) affecting anti-inflammatory activities, which in turn provide neuroprotection (161). Diets can directly influence cognitive function and brain volume by its high or low antioxidant contents (87, 88) through

preventing or promoting advanced glycation end products (162), and vascular inflammation (86).

A high-fat diet can induce obesity. Obesity results in inflammation, oxidative stress and insulin resistance (163), which are also associated with poorer brain health (164). Animal studies indicated that the reduced cognitive function resulting from high-fat diet induced obesity could occur through increased blood-brain barrier permeability (165) and reduced hippocampal glucose transport (166, 167). A high-fat diet induces atherosclerosis and hyperglycaemia (168), which are both associated with poorer cognitive function and brain structure (169). Reduced blood flow due to atherosclerosis may lead to neural loss (170).

Diet can influence hippocampal neurogenesis. A high-calorie diet can increase adults' hippocampal neurogenesis in rodents (171). Healthy dietary components such as cocoa and blueberries and long-chain n-3 fatty acids stimulate neurogenesis in animal studies (172-174)

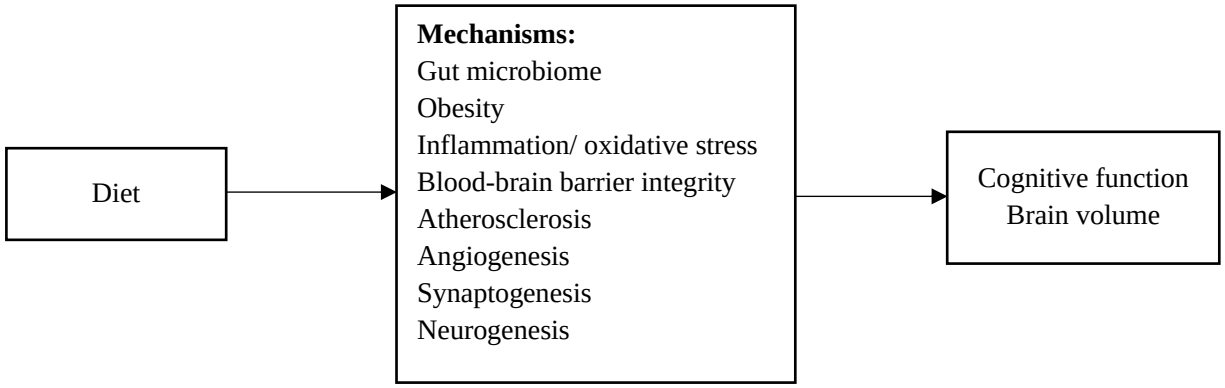


Figure 1.1 The mechanisms by which diet affects brain health

The above section has provided a summary of different types of dietary patterns, and their association with brain health. Physical activity is considered as another important risk factor for dementia. The next section will provide information regarding the definition and methods of measurements for physical activity. The literature will also be reviewed in terms of the current knowledge and gaps for the association between physical activity and brain health in older adults.

1.9. Physical activity

Definition

According to the World Health Organization, "physical activity is defined as any bodily movement produced by skeletal muscles that requires energy expenditure, including activities undertaken while

working, playing, doing household chores, travelling, and engaging in recreational activities" (175). "Exercise is a subcategory of physical activity that is planned, structured, repetitive, and aims to improve or maintain physical fitness" (175). This thesis focuses on physical activity.

Types of physical activity

Physical activity is commonly classified into four main types including aerobic, resistance, stretching and balance. Aerobic activity is defined as "an activity in which the body's large muscles move in a rhythmic manner for a continuous period of time, for example, brisk walking, running, swimming, and bicycling" (176). Aerobic means 'with oxygen' and refers to the use of oxygen during the activity. Resistance activity is defined as "a physical activity that increases skeletal muscle strength, power, endurance, and mass" (176).

Stretching exercises increase flexibility and the ability of a joint to move within a different range of motion (176). Balance activities include walking backwards and standing on one leg which may be improved by strengthening the muscles of the abdomen, back, and legs (176).

The intensity of physical activity

Intensity refers to the extent of the effort needed to perform an activity. The absolute intensity of an activity is defined by the rate of work being conducted. The absolute intensity of aerobic activity is described by the rate of energy expenditure (for example metabolic equivalent of task (MET)) (176). One MET equates to "an oxygen uptake of 3.5 millilitres per kilogram of body weight per minute" (176). The intensity of physical activity is on a continuum including light, moderate and vigorous activities. The intensity of physical activity can be defined as "non-sedentary waking behaviour that requires <3 MET for light-intensity activity (examples include walking at a slow pace (2 kilometres per hour or less), cooking activities, or light household work), 3-5.9 MET for moderate-intensity activity (examples include walking briskly or with a purpose (2.5 to 4 kilometres per hour), mopping or vacuuming, or raking the yard), 6⩽MET for vigorous-intensity activity (walking very fast (4.5 to 5 kilometres per hour), running, carrying heavy loads upstairs, shovelling snow, or participating in strenuous fitness activities" (176).

Methods of measuring physical activity

There are many techniques for the assessment of physical activity, which can be categorised into five groups: calorimetry, behavioural observation, physiological markers (heart rate), questionnaires, and motion sensors (devices). Calorimetry, particularly the doubly labelled water method, is considered the gold standard for assessing physical activity-related energy expenditure. This technique measures

the quantity of water consumed by labelling the hydrogen and oxygen isotopes. Despite being accurate and non-invasive, it is not practical in large cohorts due to the high cost (177). Although direct observation provides an accurate measure of physical activity and is not limited by reporting bias, it is time-consuming, expensive and may lead to changes in participant's behaviour in response to being observed (177). Although heart rate does increase with physical activity, it should be considered with caution as it is also affected by emotional stress (177). Questionnaires provide a subjective (self-reported) assessment of physical activity. Questionnaires are inexpensive, time-efficient, and easy to administer in a large cohort. However, questionnaires can lack sensitivity, miss incidental activities, and are subject to recall bias (over and under-reporting) (177). Objectively measured physical activity does not suffer from recall bias as devices (pedometer or accelerometer) record the activity in real-time. Devices such as accelerometers or pedometer enable more precise measurement of physical activity. Despite providing information on ambulatory activity (step count), and being non-invasive and inexpensive, pedometers do not provide any information on temporal, locomotion, and isometric activities or upper body exercise, and are also not accurate at a very slow speed (177).

Advantages and Disadvantages of using Accelerometry

Objective physical activity measures, such as accelerometry, have been used to overcome limitations of self-report measures (178). Accelerometers are small electronic devices that obtain data on acceleration and deceleration of body mass either uniaxially on the vertical plane or triaxially on the vertical, horizontal, and mediolateral planes (179). They record real-time data on intensity along with frequency, and duration of physical activity and include ambulatory activity (step count) (179). Although accelerometers approximate energy expenditure, they are not able to capture the full energy cost of walking uphill or carrying a load (180). Other limitations are the cost of monitors and time to analyse data. There are also difficulties with remembering to wear the device and monitor placement, especially when the participant needs to wear the device over several days (181).

Using objectively measured physical activity (step count and physical activity intensity) has attracted attention for identifying the association between physical activity, cognition and brain volume. The following section reviews the observational (cross-sectional and longitudinal) studies that used devices such as pedometers or accelerometers to measure physical activity to examine its association with cognitive function and brain MRI structural measures.

Daily step count, and cognition

Previous cross-sectional studies examining the association between step count and cognition found that a higher number of daily steps was associated with better executive function (task switching (182), task switching-processing speed (183-185), inhibitory control (185, 186)), attention-processing speed (184-186), verbal fluency (183) and memory (183, 187). Results from longitudinal studies found lower baseline step count was associated with worse attention/executive function (task switching, processing speed, and inhibitory control) (188). However, most of these studies focused on the general population. There are no studies that have focused on T2D, as a specific high-risk group, to examine the association between daily step count and cognition.

Daily step count and brain structure

Cross-sectional studies examining the association between step count and structural brain volume found that higher daily step count was associated with a greater total (189), and subcortical thalamus and ventral diencephalon volumes (183), as well as thicker medial temporal lobe regions (186, 190). There are no studies examining the association between daily step count and brain structure in people with T2D. Since brain atrophy (decreased white matter and grey matter volumes), and small vessel disease (white matter hyperintensities) are important biomarkers of dementia, it would be interesting to find out whether step count is associated with these biomarkers.

The following section will focus on studies that examined the association between physical activity intensity (light and moderate to vigorous) and brain health.

Physical activity intensity and cognition

Previous studies found cross-sectional associations between objectively measured light physical activity and better word fluency (191), attention processing speed (192), and executive function (task switching) (192, 193) in the general public. However, it is unknown if light physical activity is associated with cognitive domains in people with T2D.

Previous studies in community-dwelling older adults showed that higher time spent in moderate to vigorous physical activity was associated with better executive function (task-switching, (182, 194, 195), verbal fluency, (191, 196), focused attention/inhibitory control) (185), attention processing speed (185) and memory (182, 196, 197). Most of these studies were in the general population, except for two; one that was in people with heart failure (185), and another one in patients with symptomatic peripheral artery disease (197). Prospective studies in a general population of older adults indicated that more time spent in moderate to vigorous physical activity was associated with better verbal

fluency and verbal memory over 3 years (198), maintained semantic memory over 5 years (199) and increased task switching, and processing speed over 12 months of follow-up (200).

However, two studies did not find any associations between moderate to vigorous physical activity, attention processing speed (201), verbal fluency (n=7478; aged 45-74 years) (201), and memory (n=310 aged ≥65 years) (202). The reason could be due to lower average time spent in moderate physical activity (22.6 min/d) (202) and moderate to vigorous physical activity (10.9 min/d) (201) in those studies compared to the ones that observed a significant association between intense physical activity (31.2 min moderate to vigorous physical activity) (191), (37 min moderate to vigorous physical activity) (196), and better cognition.

Physical activity intensity and brain structure

Lower time spent in light physical activity has been found to be associated with severe white matter hyperintensities (203). Greater time spent in light physical activity was associated with higher total brain volume in older adults (189). Prior cross-sectional studies have found associations between greater time spent in moderate to vigorous physical activity and lower grey matter volume (204), hippocampal volume (202, 204) and greater white matter hyperintensities (203) in older adults. However, it is unknown if light or moderate to vigorous physical activity is associated with brain volume and small vessel disease in people with T2D.

The majority of previous studies that examined the association between both light and moderate to vigorous physical activity and brain health found associations for moderate to vigorous physical activity (188, 191, 194-198, 200, 203, 205) but fewer studies found associations for light physical activity (189, 191-193, 203, 205). It is possible that low-intensity physical activity may not provide enough stimulus to improve cognitive function in older adults. Therefore, moderate to vigorous physical activity will be the main measure of physical activity intensity in this thesis.

Knowledge in terms of associations between physical activity intensity and cognition in people with T2D is limited to two studies (206, 207). A study of 1,550 women >70 years with T2D suggested that higher self-reported leisure activity, converted into metabolic equivalent hours per week, was not associated with baseline cognition or decline over an average of 4.2 years (206). A study in people with (n=74) and without T2D (n=71) showed that higher self-reported light exercise on weekdays and moderate exercise on weekends were both associated with better general cognitive function (207). However, these studies used self-reported physical activity. A study in people with (n=258) and without T2D (n=302) found that higher objectively measured daily step count was associated with

better grey matter volume and hippocampal volume (91). There are no studies that have examined whether objectively measured physical activity intensity is associated with measures of brain health (cognition and brain volume) in people with T2D.

Mechanisms by which physical activity may affect brain health

The effect of physical activity on cognition could be through direct pathways. Direct pathways include angiogenesis, synaptogenesis and neurogenesis **(See figure 1.3)**. Vascular endothelial growth factor is one of the main growth factors necessary for angiogenesis. Physical exercise facilitates angiogenesis by increasing the release of Nitric Oxide, and vascular endothelial growth factor (159). Physical exercise enhances neurogenesis, and synaptogenesis in the hippocampus (208) by increasing the production of brain-derived neurotrophic factor (209). Through these processes, physical exercise may then enhance cerebral perfusion, neuronal proliferation, and differentiation (209), which lead to exercise related direct cognitive benefits by improving the brain structure and function including increasing brain volume and improving cerebral connectivity (209).

The effect of physical activity on cognition could be through indirect pathways. Indirect pathways include improving cardiovascular health (decreasing hypertension, hyperglycaemia, insulin resistance, inflammation, and abdominal obesity). Exercise improves blood pressure (210), lipid profiles (210), glycaemic control (211), insulin sensitivity (212), inflammatory biomarkers (213) and reduces fat mass (214), all potential mechanisms associated with cognitive dysfunction in T2D.

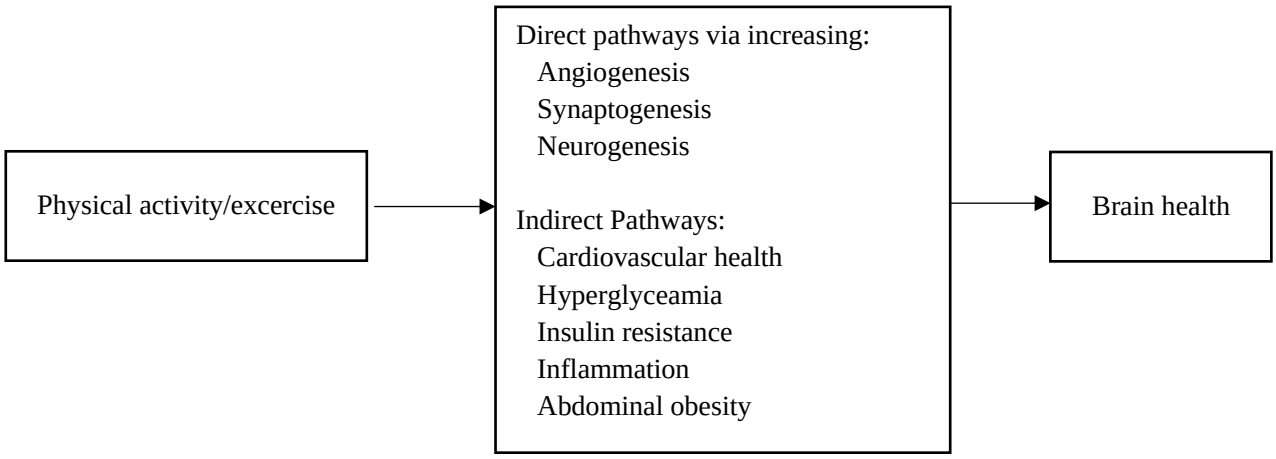


Figure 1.2 The mechanisms by which physical activity/exercise may affect brain health

1.10. Summary

Collectively, diet and physical activity are major risk factors of dementia. However, the associations

between dietary patterns, physical activity and surrogate markers of dementia (cognitive function, brain volume, and small vessel disease) are less studied in older adults with and without T2D. The overall aim of this thesis is to examine the association between dietary patterns, physical activity and brain structure and function in older adults with and without T2D. The following section will outline the research questions of this thesis that will address these gaps in the literature.

Study research questions

In participants with and without T2D:

Study 1 (Chapter 3):

1) Are data-driven dietary patterns associated with cognitive domains, brain volume, and markers of small vessel disease in older adults with and without T2D? Are the associations different based on T2D status?

Study 2 (chapter 4):

2) Is the dietary inflammatory index associated with cognition, brain volume, and small vessel disease? Does T2D modify any associations?

Study 3 (Chapter 5):

1) Is adherence to the Australian Dietary Guidelines associated with cognitive domains, brain volume, and small vessel disease? Does T2D modify any associations?

In participants with T2D:

Study 4 (Chapter 6):

2) Is objectively measured physical activity associated with cognitive domains, brain volume, and small vessel disease? Is the association different based on genetic risk for dementia or severity of T2D?

Thesis structure

This thesis includes both published papers (Chapters 3 and 5) and manuscripts under review (Chapters 4 and 6). The manuscripts in chapters 3, and 5 are presented as published, and the manuscripts in chapters 2 and 6 are presented as submitted.

The structure of this thesis is as follows:

Chapter 2	**Materials and methods**. This chapter will describe the population and participants, study design, outcome measures and other measures used in each study of this thesis.
Chapter 3	**Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes.** This chapter will present findings addressing research question 1 of this thesis. The findings are published: Zabetian-Targhi F, Srikanth VK, Beare R, Moran C, Wang W, Breslin M, Smith KJ, Callisaya ML (2019) *"Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes."* The Journal of Nutrition. Volume 149, Issue 10, October 2019, Pages 1805–1811, DOI: https://doi.org/10.1093/jn/nxz139
Chapter 4	**Associations between the dietary inflammatory index, brain volume and small vessel disease.** This chapter will report findings addressing research question 2 of this thesis. The paper is currently under review with the Journal of Academy of Nutrition and Dietetics.
Chapter 5	**Adherence to the Australian Dietary Guidelines Is Not Associated with Brain Structure or Cognitive Function in Older Adults with and without Type 2 Diabetes.** This chapter will report findings addressing research question 3 of this thesis. The findings have been published: Zabetian-Targhi F, Srikanth VK, Smith KJ, Oddy WH, Beare R, Moran C, Wang W, Callisaya ML. (2020) *"Adherence to the Australian Dietary Guidelines Is Not Associated with Brain Structure or Cognitive Function in Older Adults with and without Type 2 Diabetes".* The Journal of Nutrition. Volume 150, Issue 6, June 2020, Pages 1529–1534 DOI: doi: https://doi.org/10.1093/jn/nxaa052.
Chapter 6	**The association between physical activity intensity, cognition and brain structure in people with type 2 diabetes.** This chapter will report the findings addressing research question 4 of this thesis. The paper is currently under review by the Journal of Gerontology Series A: Medical Sciences.

Chapter 7	**Thesis discussion and conclusions**. This chapter will summarise the findings of the thesis, present conclusions and provide recommendations and future directions.

1.11. Postscript

This chapter provided information on dementia and its risk factors. It provided a summary of the evidence for two modifiable risk factors (diet and physical activity) and their associations with dementia markers (cognition and MRI brain structure). Gaps in the literature were highlighted, and the research questions described will address some of the identified gaps. The following chapter will provide a description of the methods used in the related studies undertaken in this thesis.

2. Methods

2.1 Preface

The broad aim of this thesis is to investigate the relationships between nutrition and physical activity with markers of brain structure (brain volume and small vessel disease) and function (cognition), in older adults with and without T2D. The studies reported in chapters 3-5 of this thesis were conducted using data from the Cognition and Diabetes in Older Tasmanians (CDOT) study. The study in Chapter 6 was conducted using data from the CDOT-Blood Pressure (CDOT-BP) study as it included additional measures of physical activity intensity obtained with an accelerometer.

This chapter provides details regarding the study sample, and measurement of independent (diet and physical activity) and dependent variables (MRI of the brain and cognitive tests) used in the research described in this thesis.

2.2 Study population and recruitment

Cognition and Diabetes in Older Tasmanians study (CDOT)

CDOT is an observational study, designed to investigate the associations of T2D with brain health (cognition and brain structure). This thesis uses data from phase one which was obtained between 2005 and 2011.

Study population and participants of CDOT

A diagram of participants recruitment is provided in **Figure 2.1**. Participants with T2D were enrolled from the National Diabetes Service Scheme (NDSS) which is a commonwealth non-profit governmental program, administered by Diabetes Australia Ltd. People with T2D register voluntarily to the NDSS program and are provided with equipment for monitoring and treatment of diabetes. Those who register through the NDSS can indicate if they would like to be approached to participate in research. The NDSS has a database of people with their postcodes and contact details. Eligible people aged ≥55 years were contacted if they resided in Southern Tasmania (postcodes 7000-7199). People without T2D were randomly recruited from the electoral roll using age (60-85 years) and sex-stratified sampling from the same postcodes. Tasmania is an island state located in the southern part of Australia. The population of southern Tasmania was 239,444 people in 2006 including 46,159 individuals (19%) aged 60 years and above (215).

As participants recruited from NDSS have already been diagnosed for T2D, specific criteria were applied for diagnosis of T2D for people recruited from the electoral roll. Standard criteria applied for the diagnosis of T2D were random plasma glucose ≥11.1 mmol/L, fasting plasma glucose ≥7.0 mmol/L, or 2-h glucose ≥11.1 mmol/L after oral-glucose tolerance test (62). Participants with and without T2D were included if they were community-dwelling and could speak adequate English for cognitive examination. Exclusion criteria were any contradictions to MRI, living in a residential aged care facility or a diagnosis of dementia.

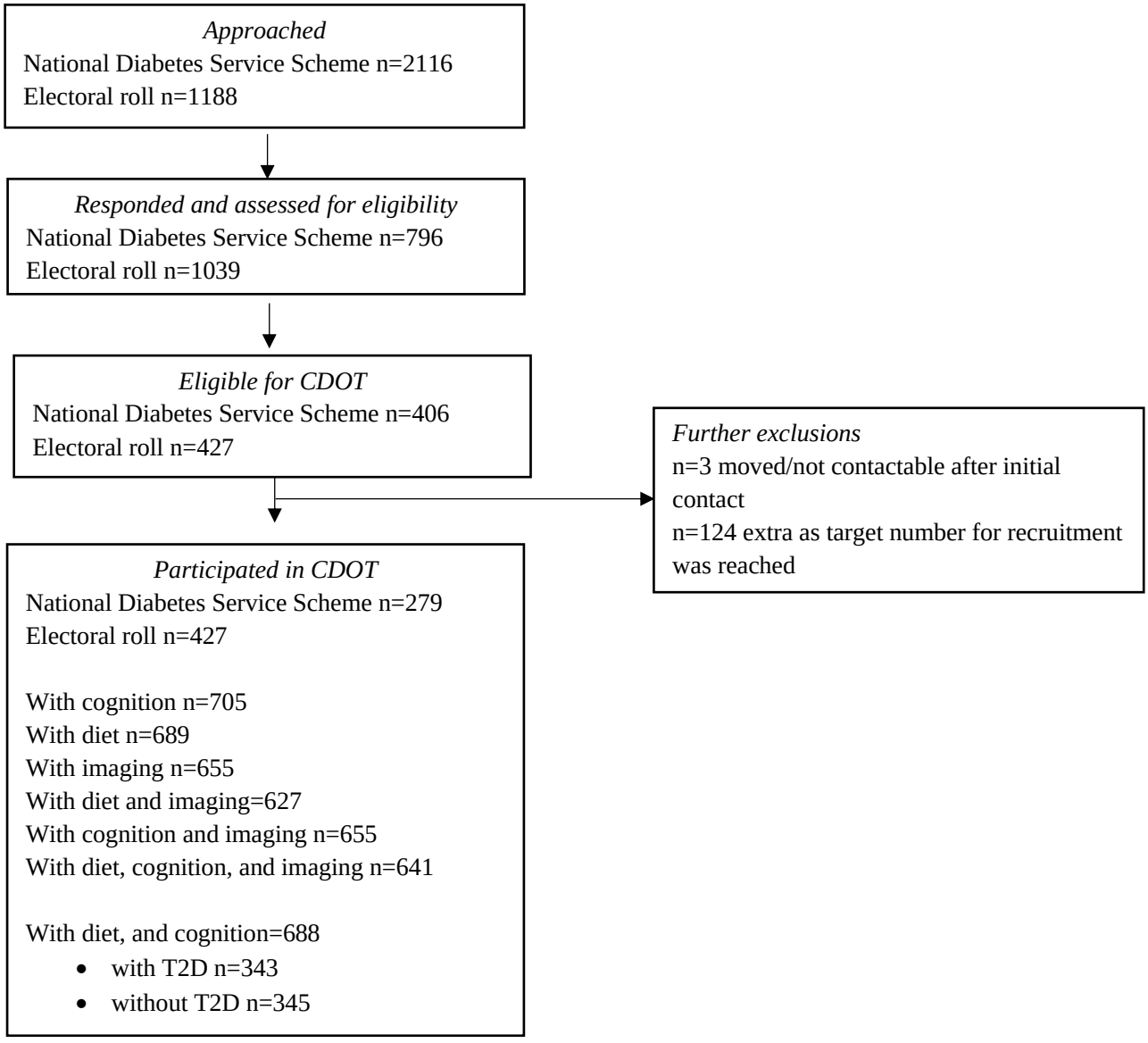


Figure 2.1 Diagram of participants recruitment

Cognition and Diabetes in Older Tasmanians Study – Blood Pressure (CDOT-BP)

CDOT-BP is an observational study (2014-2016) designed to examine the associations between

peripheral and central blood pressure and brain health (cognition and brain structure) in people with T2D.

Study population and participants of CDOT-BP

A total of 83 participants from the original CDOT study agreed to participate in CDOT-BP. In order to obtain additional participants, another mail sent out to 225 NDSS registrants living in Southern Tasmanian postcodes 7000-7199. This produced 103 people acceptances, while 81 did not respond and 41 declined. An additional 10 participants were enrolled through letters sent using Facebook, and 16 people were recruited from the local endocrinology medical clinics. From 212 eligible people, seven had contraindications to MRI and three withdrew, leaving a sample of 202 participants. An additional 50 participants were recruited from the same population source to produce a final total of 252 participants included in this study. Inclusion and exclusion criteria were identical to that of CDOT.

2.3 Funding

The studies were funded by the National Health and Medical Research Council of Australia (project grants 403000,436797 for CDOT, and 1063608 for CDOT-BP).

2.4 Ethical clearance

All participants gave written consent. The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the studies (Ethics approval reference numbers: H0009400 and H7947 for CDOT) and (H0013664 and H0013965 for CDOT-BP).

2.5 Independent Measures

Dietary assessment

In this thesis, dietary data were collected using the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) food frequency questionnaire (FFQ). A copy of this questionnaire is provided in **Appendix 2.1** of this chapter. The DQES v2 is an adjusted version of the FFQ of the Cancer Council of Victoria that was developed in the late 1980s to obtain dietary intake of participants recruited in the Melbourne Collaborative Cohort Study (216). The DQES v2 has been found to be reliable, valid and reproducible in the Australian population (217, 218), including in older adults with T2D (219).

The DQES v2 consists of 80 items including 74 foods and six alcoholic beverages. The 74 food items are classified into four food groups: 1) "cereal foods; sweets and snacks"; 2) "dairy products, meat and fish"; 3) "fruit" and 4) "vegetables (including fresh, frozen, and tinned)". Participants were asked to report their usual dietary intake over the past 12 months using one of 10 response options, ranging from 'never' to 'three or more times per day'. The DQES v2 also covers six types of alcoholic beverages with 10 frequency response options ranging from 'never' to 'every day'. Participants reported their usual portion sizes (small, medium and large) from a series of photographs. Respondents also indicated the types of milk, bread, spread and cheese consumed, and the usual number of fresh vegetables and fruits consumed daily.

Analysis of DQES v2 was completed by the Nutritional Assessment Office of the Cancer Epidemiology division of the Cancer Council of Victoria. Reported frequencies were converted to daily equivalents. Portion sizes were calculated based on participant responses to the serving size pictures in the DQES v2 and information on portion sizes provided by Food Standards Australia and New Zealand (220). The amount of food consumed (grams/day) was calculated by multiplying each portion size (in grams) by the daily equivalent frequencies. Total energy and nutrient intakes were calculated by summing the content for each food across all food items. The information on the energy and nutrient content of each food item was obtained from NUTrient TABles for use in Australia (NUTTAB) 2006 which includes analytical data based on Food Standards Australia New Zealand (220). The nutrient content for beta-carotene (221), fatty acids (222), vitamin E and folate (223) were supplemented by other data where necessary. The nutrients available are listed in **Table 2.1.**

Table 2.1 List of nutrients driven from the DQES v2

Carbohydrate (g/d)	Total fibre (g/d)	Vitamin A (ug/day)
Protein (g/d)	Alcohol (g/d)	Beta-carotene (ug/day)
Total fat (g/d)	Thiamin (mg/d)	Vitamin C (mg/day)
Saturated fatty acids (g/d)	Riboflavin (mg/d)	Vitamin D (ug/day)
Polyunsaturated fatty acids (g/d)	Niacin (mg/d)	Vitamin E (mg/day)
n-3 fatty acids (mg/d)	Pyridoxine (mg/d)	Zinc (mg/day)
n-6 fatty acids (mg/d)	Cobalamin (ug/day)	Iron (mg/day)
Monounsaturated fatty acids (g/d)	Folate (ug/day)	
Total cholesterol (g/d)	Magnesium (mg/day)	

Calculation of dietary patterns

In this thesis, each of the first three studies examined a different dietary pattern approach. The first study focused on the data-driven dietary patterns, the second study examined the Energy-adjusted DII (E-DII), and the third study investigated the Australian Dietary Guidelines index. The following sections explain the methods used to calculate each of the dietary patterns including the FFQ components, statistical analysis, and their strengths and limitations.

Data-driven (Posteriori) Dietary Patterns

To calculate the dietary patterns in Chapter 3, all 80 food items from the FFQ were categorised into 35 food groups based on the similarity of their nutrient profiles, as described in the Composition of Foods Australia guide (224), and an Australian study (225). **Appendix 3.1 Supplementary Table 1 in Chapter 3** describes the food groups and the foods allocated to each group. The food groups were then adjusted for energy intake and presented per 1000 kcal of energy (energy density method) prior to dietary patterns analysis.

PCA was used to obtain data-driven dietary patterns (foods that are usually eaten together) (226). PCA has previously been applied to identify dietary patterns related to cognitive function in people with T2D (124). In order to identify a small number of linear combinations of food components with maximum variance, the initial patterns of PCA were rotated by an orthogonal transformation (107). Varimax rotation was used in this thesis to facilitate the factors' interpretability by improving the separation of food components. A set of uncorrelated factors, or dietary patterns categorised by different foods, were derived. The number of dietary patterns retained was decided based on scree plots (227), and interpretability with factor solutions limited to an eigenvalue of >2.0. Factor loadings equal to and above 0.2 were considered as major contributors to each pattern. A score for each dietary pattern was then calculated as a z-score. Any differences in the results of the PCA between people with T2D compared to those without T2D were also explored. The process of calculating data-driven dietary patterns is summarised in the **Figure-2.2**.

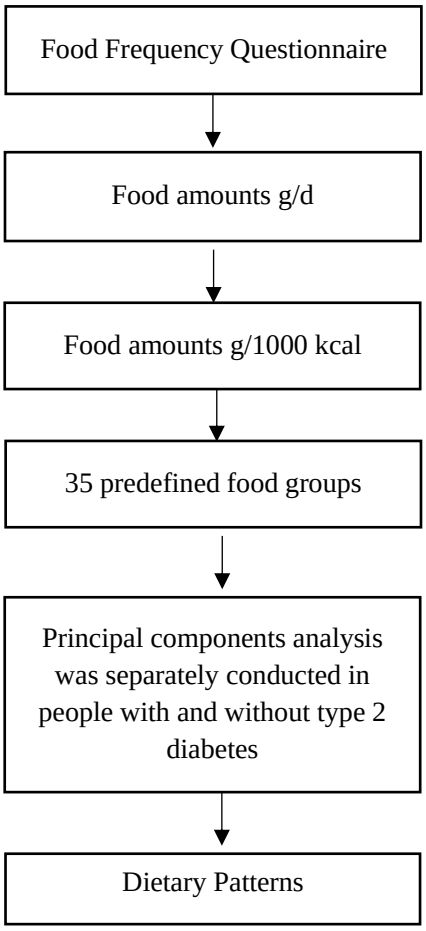


Figure 2.2 The process of calculation of data-driven dietary patterns

Calculation of the Energy-adjusted Dietary Inflammatory Index (E-DII)

The E-DII was calculated based on food parameters demonstrated as having pro- and anti-inflammatory effects (129). A summary of the development of the E-DII is provided in **Figure 2.3**. The validity of the E-DII against inflammatory biomarkers has been shown in previous studies (228, 229). The DQES v2 included data on 27 of the 45 food parameters needed to calculate the E-DII: alcohol, protein, carbohydrate, saturated fat, monounsaturated fat, polyunsaturated fat, total fat, omega 6, omega 3, cholesterol, garlic, onion, vitamin A (retinol), vitamin E, vitamin D, vitamin C, beta-carotene, riboflavin, thiamine, niacin, folate, B6, B12, iron, magnesium, fibre, and zinc.

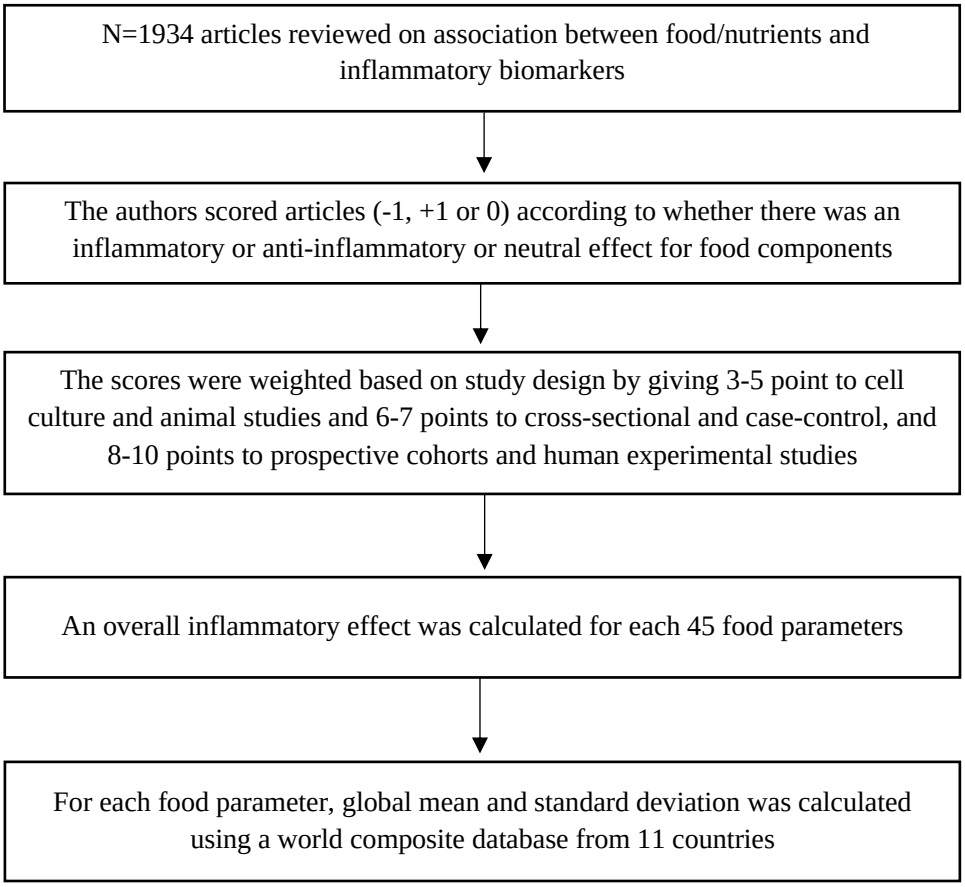


Figure 2.3 The process of DII development by Shivappa et al (129)

Intakes of each parameter were first standardised by subtracting a standard global mean for each food parameter (129) from the participants' reported usual dietary intake and subsequently dividing this by the global standard deviation. These standardised z-scores were transformed to percentiles to decrease the influence of outliers. This value was then doubled, and one was subtracted to achieve an approximately symmetrical distribution centred on 0, with values from -1 to +1. The centred percentile scores for each food parameter were multiplied by a number which was intended to reflect the strength of epidemiological evidence for the pro- or anti-inflammatory effect of that parameter (129). Then the scores were summed across all food parameters with equal weight for each food, to obtain the overall DII score. The E-DII was calculated per 4,180 kJ (1000 kcal) energy content of food parameters consumed. For the study reported in this thesis, the score was calculated by the original authors of the E-DII (129). Higher E-DII values reflect a more pro-inflammatory diet.

Calculation of the Australian Dietary Guidelines index

A Dietary Guidelines Index (DGI) was calculated, which measured adherence to the 2013 Australian Dietary Guidelines. The current Australian Dietary Guidelines (2013 version) were used, as opposed to the guidelines at the time of data collection, as they reflect current nutritional knowledge regarding healthy foods. The DGI was a modified version of a DGI that has been validated in youth and young adults using the 2013 Australian Dietary Guidelines (230). Since the Australian Dietary Guidelines' recommendations are based on daily servings of food (serving/day), in this thesis the amounts of food intakes (g/d) were converted to servings/day. This was done by dividing the absolute amount consumed (g/d) by the recommended serving size (g) in the 2013 Australian Dietary Guidelines (154).

An individual's dietary intake was scored according to the eight recommended dietary components of the 2013 Australian Dietary Guidelines (154). The components of the DGI and the sex- and age-specific criteria to receive the maximum score are reported in **Table 2.2**. The dietary guidelines have recommendations for liquids and added salt but these were not included in the DGI, as the FFQ used in this study did not collect information on these components. For each participant, the total daily servings were calculated by summing up the servings of intakes for all food constituents of each component of the Australian Dietary Guidelines. The Australian Dietary Guidelines index has a maximum score of 90 points. Participants with higher score have greater adherence to the Australian Dietary Guidelines (better diet quality).

Table 2.2 The Australian Dietary Guidelines components and the criteria for maximum scoring.

Guidelines	**Age (year)**	**Serving/day**		**Maximum Score**
		Male	**Female**	
Vegetables, legumes/beans	51 to <70	≥5.5	≥5	10
	≥70	≥5	≥5	
Fruit	>51	≥2	≥2	10
Total cereals	51 to <70	≥6	≥4	5
	≥70	≥4.5	≥3	5
Wholegrains bread/total bread				5 if 100%
Lean meat and alternatives	>51	≥2.5	≥2	5
Lean meat/total meat and alternatives				5 if 100%
Total dairy products	51 to <70	≥2.5	≥4	5
	≥70	≥3.5	≥4	

Low-fat dairy	skim milk, soy milk, reduced-fat milk or no milk.			5
Diet variety – proportion from each of the 5 food groups	2 points awarded for each of the five core food groups when at least one serving is consumed per week.			10
Limiting intakes of saturated fat, alcohol, added salt and sugars	51 to <70	<2.5	<2.5	20
	≥70	<2.5	<2	
Unsaturated fats, oils, and spreads	51 to <70	≤4	≤2	10
	≥70	≤2	≤2	

In summary, each component was scored out of 10, with the exception of the limiting of saturated fat, alcohol, added salt and sugars which had a maximum score of 20 as alcohol was combined to this section. For each component except for low-fat dairy, food and beverages that needed to be limited, unsaturated fats and diet variety, the maximum point was awarded if the amount consumed (serving/day) of that component was equal to, or more than, the recommendation in the 2013 Australian Dietary Guidelines (154). Proportionate scores were calculated if the intake was between the maximum and minimum scoring criteria for all components, except for low-fat dairy, unsaturated fats, and diet variety (230).

For foods and beverages that need to be limited, a proportionate score was given if intake was more than the recommended values but less than twice the recommended amounts. A score of zero was awarded if consumption was twice the recommendations or more. Unsaturated fat was awarded 10 points if intake was equal to or less than the recommendation. A score of zero was given if the intake was more than the recommended values. For low-fat dairy, no points were awarded if the person usually consumed full-fat milk and flavoured milk. No proportionate scores were used for low-fat dairy consumption.

Diet variety was based on the Australian Dietary Guidelines recommendation for people "to enjoy a wide variety of nutritious foods from the five core food groups (vegetables, fruit, whole grains, lean meat, and dairy) every day". Two points were awarded for each of the five core food groups when at least one serving was consumed per week.

2.6 Physical activity assessment

Physical activity was an independent variable for the last study that examined the association between objectively measured physical activity, cognition, and brain structure in people with T2D. Physical activity was assessed with an accelerometer. Accelerometers are lightweight, portable, and non-invasive devices that measure the acceleration of a moving body part in sagittal, coronal and transverse planes. Different types of accelerometers can be worn on the wrist, waist, arm or thigh (231). Accelerometers convert raw acceleration data into counts of activity for a time period . Counts of activity are then converted into intensity classifications by software using established age-based cut points in the general population (232). Accelerometers can also collect data on step count (233). Accelerometers collect data on the activities' date and time which helps with data cleaning prior to analysis. For the purpose of this thesis, an accelerometer was used to collect data on step count and intensity of the activity (ActiGraph GTlM, Pensacola, FL, USA) **(See Figure 2.4)** (234). The ActiGraph GT1M has been demonstrated to be a valid tool for assessing walking across various speeds and gradients (235).

Figure 2.4 ActiGraph GT1M

Participants were asked to wear the accelerometer over their right hip for 7 consecutive days and to fill out a daily monitoring log in which they recorded start and finish times each day. They also recorded any reason and duration for times where the accelerometer was not worn (e.g. sleeping and taking shower).

Data cleaning and processing

When participants returned the Actigraph, raw data was downloaded using software provided by ActiGraph, LLC (ActiLife). The ActiLife software provides the daily step count for each day the accelerometer is worn. The ActiLife software converts the counts of activity to activity intensity (light,

moderate and vigorous) based on established cut-offs. The cut points for physical activity intensity were based on Freedson's study for adults (236): 1951-5724 counts/min (moderate) and >5725 counts/min (vigorous). These cut-points have previously been used in a study of older adults (237).

The accelerometer data were examined and periods of removal were noted. The diaries were checked to calculate the hours per day the accelerometer was worn. Participants' data were included only if they wore the accelerometer for 8 hours per day for at least 5 days (234). The average time spent per day at each intensity of physical activity was measured by dividing physical activity time by the number of days the accelerometer was worn.

2.7 Dependent outcome variables

Cognitive Assessment

For the purpose of this thesis, cognitive domains were created from a comprehensive battery of neuropsychological tests.

Verbal Memory

The Hopkins Verbal Learning Test-Revised (HVLT-R) was used to measure verbal memory. The HVLT-R includes three categories which measure immediate recall, delayed recall, and recognition memory (238). A list of twelve words was read to each participant. Immediate recall was tested by asking participants to repeat the words immediately. The delayed recall was examined by asking participants to remember the words after 20 minutes. Immediate recall for each participant was tested three times, followed by a delayed recall after 20 minutes. To test recognition memory, participants were read a list of twenty-four words and asked to recognise if each word was in the initial list of twelve (238). Participants were scored based on the number of words they repeated correctly. The scores for each task were converted to z-scores. A verbal memory domain was calculated as the average of z-scores on the three categories of HVLT-R. Higher scores on the verbal memory domain indicate better verbal memory function. The test-retest reliability of the HVLT-R three components within 14-134 day intervals is moderate with an intraclass correlation coefficient (ICC)=0.41-0.74 (239). HVLT-R is a valid instrument to use for cognitive assessment in older adults (240).

Visuospatial Function

Visuospatial ability was measured using the Rey-Osterrieth Complex Figure (238). The Rey-Osterrieth Complex Figure Test examines perceptual or constructional organisation (241). It also includes planning and problem-solving strategies. Hence, it is also an indirect measure of executive

function (238). The Rey-Osterrieth Complex figure is provided in **Figure 2.5.** The participants were initially asked to copy the Rey-Osterrieth Complex Figure. The participants were not permitted to turn the piece of paper while doing the test. For scoring, the figure was broken into 18 elements. The participants were given a total score of 36. The task was scored based on the accuracy of reproduction and the placement of the 18 elements within the whole figure. Two points were given if a correct element was placed properly. One point was awarded to an accurate element positioned incorrectly or an incomplete element placed properly. A half-point was given if an element was incomplete but still recognisable and placed improperly (238). Higher points on this test showed better visuospatial function.

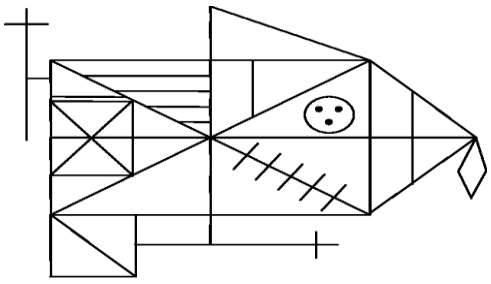


Figure 2.5 Rey Complex Figure Test

Visual Memory

Visual memory was measured using a reproduction of the Rey Complex Figure Test, 20 minutes after an immediate copy of the figure (242). Test-retest reliabilities of both immediate copy and delayed categories of the Rey Complex Figure Test are moderate (ICC=0.76-0.89) in 6 month intervals (242).

Executive Function

The Victoria Stroop Test measures the capability to switch perception and inhibit habitual response (238). The test involves three categories: Dot, Word, and Colour. Each category contains four rows of six items. The Stroop Dot test contains coloured dots, the Stroop Word Test contains coloured words (for example, car, hat, dog), and the Stroop Colour Test comprises names in a different coloured font (for example, the word yellow typed in blue ink). The participants were required to report the colour of the font. The scores were calculated as the time spent to complete each of the three categories (238). Higher scores on all tests show poorer cognitive function. Stroop Colour and Word Tests are shown in **Figure 2.6**. To assess executive function, the variable Stroop Interference

was generated using the Stroop Colour Test z-score minus the Stroop Word Test z-score. The Stroop Dot Test was used to assess attention and processing speed. Test-retest reliabilities of the three components of the Stroop test are excellent within a month interval (ICC=0.83-0.91) (243, 244).

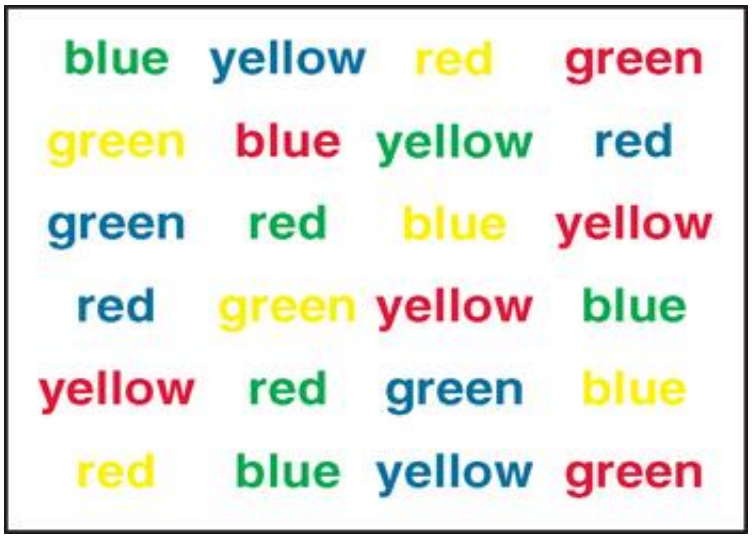


Figure 2.6 Stroop colour and word Test

The Trail Making Test requires the individual to link a set of 25 consecutive targets quickly and accurately. The test consists of two parts: In part A, the targets are all numbers and the individual needs to link them in sequential order. In part B, the target alternates between numbers and letters **(Figure 2.7).** Part A assesses visual attention/processing speed, whereas part B assesses executive function (task switching). If the participant makes an error, the investigator corrects them before the participant moves on to the next target. The task is scored based on the completion time (238). For the physical activity study (chapter 6), the executive function domain was calculated as an average of inverse Stroop Interference z-score (z-score of Stroop Colour minus Stroop Word) and Trails Making Test z-score (z-score of Trail B minus Trail A). Therefore, executive function in the last study includes both components of task switching and inhibitory control (245).

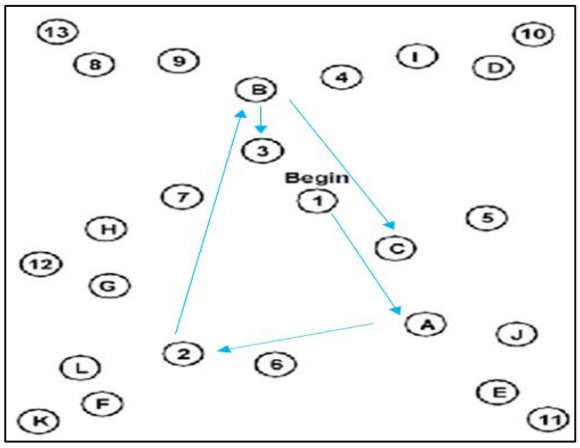


Figure 2.7 Trail making test part B

Verbal Fluency

Verbal fluency was measured by the Controlled Oral Word Associations Tests (COWAT) (238). The COWAT incorporates two categories which examine verbal fluency. The first category involves listing as many words as possible beginning with F without repetition. The second category includes listing as many animals as possible without repetition. Higher scores on the COWAT tests show better verbal fluency (238, 242). The average scores on both categories of COWAT were calculated as the verbal fluency score. The components of COWAT tests have excellent test-retest reliability (within 14-92 days interval) in older people (ICC=0.88) (243).

Attention and Processing Speed

The tests included in this domain were Digit Span, Digit Symbol Coding, the Symbol Search subsets of the Wechsler Adult Intelligence Scale-Third Edition (246), and the Victoria Stroop Dot Test (238). The Digit Span Test contains two tasks. Participants are read lists of two to nine numbers and asked to repeat the same sequence. The difficulty of the test increases as the list of numbers is extended. Then the participants are required to repeat lists of two to eight numbers backwards (238). This test determines the ability to retain auditory attention, and working memory (247). Test-retest reliability for the Digit Span Test is moderate (ICC=0.83) in 3 months interval (248). Higher scores on this test demonstrate better cognitive function. The Total Digit Span Test score is calculated as the sum of Digit Span Forwards and Backwards.

In the Digit Symbol Coding Test, participants were shown nine symbols each with an associated number from 1-9. Then, they were provided with a random list of numbers and asked to match which

symbol was associated with each number. This task is scored based on the number of accurate responses provided in a two minute period (238). A copy of the Digit Symbol Coding Test is provided in **Figure 2.8**.

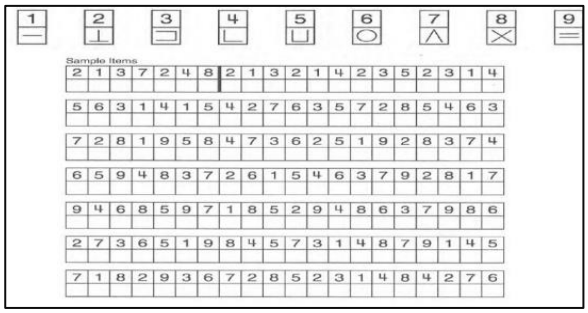


Figure 2.8 Digit Symbol Coding Test

In the Symbol Search Test, participants are given two symbols and asked if they recognise them from a group of five symbols. The test duration is two minutes. The test score is based on the number of accurate responses. Higher scores on these tests indicate better function. The Symbol Search Test is shown in **Figure 2.9**. Both the Symbol Search and Digit Symbol Coding tests showed high test-retest reliability (ICC=0.76) with an average 29 days interval (242).

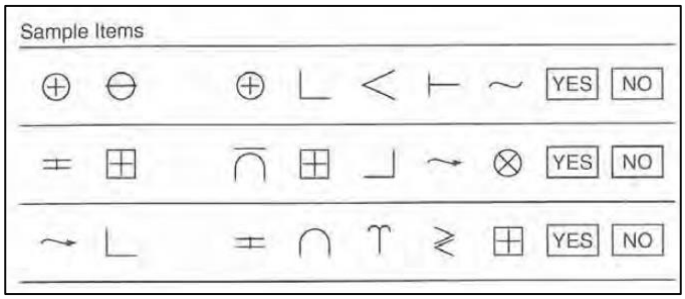


Figure 2.9 Symbol Search Test

Calculation of cognitive scores

To create a score for each domain, a z-score for each cognitive test was calculated by the individual's raw score minus the sample mean score, divided by the sample standard deviation. Stroop scores were reversed so that higher scores translated to better performance. Subsequently, z-scores for each test were averaged to calculate a single composite score for each cognitive domain. Domain scores that included more than one cognitive measure were re-standardised to a standard deviation of one. A global cognitive domain score was calculated as the mean of all domains. These scores were used in

the regression analysis to enable comparison of associations across different cognitive domains. **Table 2.3** lists the raw cognitive tests used for each domain in each chapter of this thesis.

Table 2.3 Individual neuropsychological tests included in each domain

Cognitive domain	Neuropsychological test
Verbal Memory (Chapter 3-6)	Hopkins verbal learning test- revised (immediate, recall, recognition)
Visuospatial function (Chapter 3-6)	Reproduction of the Rey Complex Figure
Visual Memory (Chapter 3-6)	Delayed reproduction of the Rey Complex Figure
Executive function (Chapter 3-5)	Stroop Colour, and Word
Executive function (Chapter 6)	Trails A and B, Stroop Colour, and Word
Verbal Fluency (Chapter 3-6)	COWAT- category and animal
Attention and Processing Speed (Chapter 3-6)	Digit Span, Digit Symbol Coding Symbol Search, and the Victoria Stroop Dot Test

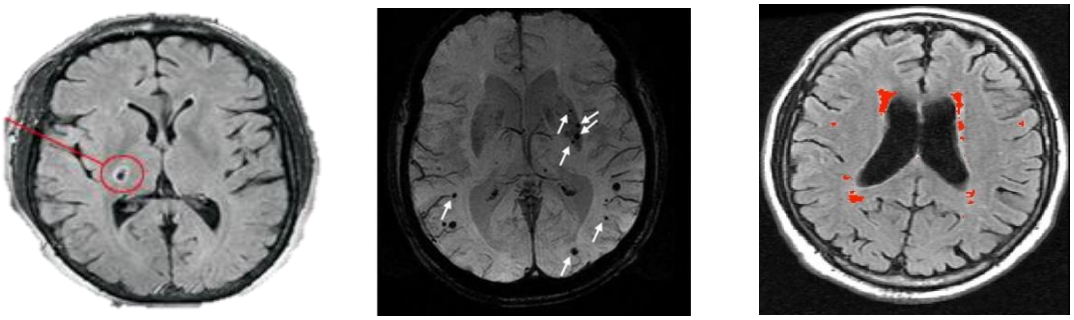
2.8 Magnetic Resonance Imaging-CDOT

For CDOT, markers of small vessel disease were white matter hyperintensity volume, microbleeds and infarcts **(See Figure 2.10)**. The brain volume measures were grey matter, white matter and hippocampal volumes **(See Figure 2.11)**. MRI scans were performed with a single 1.5-T General Electric scanner with the following sequences: high-resolution T1-weighted spoiled gradient echo (repetition time [TR] 35 ms, echo time [TE] 7 ms, flip angle 35°, field of view 24 cm, 120 contiguous slices, isotropic voxel size 1 mm^3), T2-weighted fast spin echo (TR 4,300 ms, TE 120 ms, number of excitations 1, turbo factor 48, voxel size 0.90 x 0.90 x 3 mm); fluid attenuated inversion recovery (TR 8,802 ms. TE 130 ms, inversion Lime 2,200 ms. voxel size 0.50 x 0.50 x 3 mm); gradient echo (TR 0.8 ms, TE 0.015, flip angle 30°, voxel size 0.9 x 0.9 x 7 mm).

Small Vessel Disease

Fully automated white matter hyperintensity segmentation on fluid attenuated inversion recovery sequences was performed using a validated method (249). A voxel counting algorithm calculated the white matter hyperintensity volume. A single trained rater identified the presence of MRI infarcts and microbleeds following with confirmation by consensus between two stroke experts. Infarct was

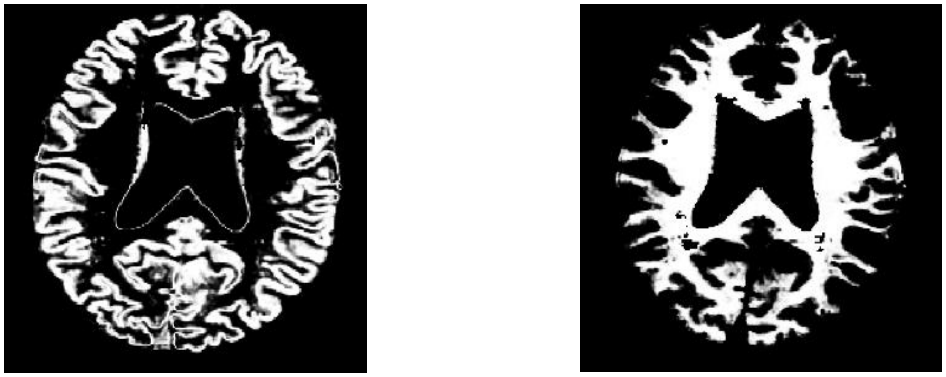
defined as “a hypointensity >3 mm in diameter on three-dimensional T1-weighted and fluid attenuated inversion recovery images with a surrounding hyperintense rim” (250). Microbleeds were defined as “small, rounded, hypointense lesions with clear margins, ranging from 2 to 10 mm on gradient echo sequences”. All measurements were performed with the assessor blinded to the group, age, sex, and other outcome measures.



From left to right- subcortical infarcts, microbleeds, white matter hyperintensities
Figure 2.10 Biomarkers of Small vessel disease

Brain Volumes

The functional MRI of the Brain Linear Image Registration Tool was used to register three-dimensional T1 and GRE sequences in the standard Montreal Neurological Institute space (251). This process has the effect of normalising the brain according to a standard template to take account of variation in brain size. A multispectral segmentation process was applied with the use of three-dimensional T1 and gradient echo sequences. Statistical Parametric Mapping version 5 software (252) was used to produce tissue probability maps of grey and white matter. The images were corrected for volume change that occurred in the result of the normalisation process. Maps of white matter were generated unaffected by white matter hyperintensities by marking locations related to white matter hyperintensities as empty in the tissue probability maps. The tissue probability maps of grey and white matter were produced using Statistical Parametric Mapping version 5 software. Prior voxel-based morphometric analysis, isotropic Gaussian kernel with a full width at half maximum 8 mm was applied to smooth tissue maps. Tissue volumes of the segmented areas (total grey and normal-appearing white matter) were calculated with voxel counting algorithms. A single expert manually segmented both hippocampi by established methods (253). Tissue volumes of the segmented areas were calculated with voxel counting algorithms.



From left to right, grey matter volume, and white matter volume are shown in white.

Figure 2.11 Grey matter volume and white matter volume of the brain.

2.9 Magnetic Resonance Imaging -CDOT-BP

For CDOT-BP, the brain volume measures were grey matter, white matter and hippocampal volumes. The only small vessel disease marker was white matter hyperintensity volume. MRI scans were obtained using a high resolution single 1.5T General Electric Optima scanner. The following structural imaging sequences and parameters were used. For T1, TR = 7.6ms, TE = 2.34ms, flip angle = 12º, FOV = 250mm, 240x240 matrices, and slice thickness = 1mm. For fluid attenuated inversion recovery, TR=9000, TE = 97.1, Flip 160, FOV = 220, acquisition matrix 288x288, and slice thickness 3mm.

Image Processing

Tissue classification for grey matter and white matter volumes was performed using FreeSurfer 5.3. FreeSurfer used a hybrid deformation procedure to remove non-brain tissues (254). FreeSurfer performed for the segmentation of the subcortical white matter and deep grey matter volumetric structures via automated Talairach transformation (255). FreeSurfer performed an automated procedure to normalise intensity (256), and tessellate the grey matter white matter boundary. The process was followed by automated topology correction (257, 258), and surface deformation to make intensity gradients. The grey/cerebrospinal fluid and grey/white borders were placed at the location of the greatest shift in signal intensity (259-261). Volumes of hippocampi were determined based on manual segmentation performed by a single expert.

Small Vessel disease

Intra-subject co-registration between fluid attenuated inversion recovery and T1-weighted images

was performed using Statistical Parametric Mapping (SPM12) software, Wellcome Trust Centre for Neuroimaging. Hypointensities on T1-weighted scans, corresponding to white matter hyperintensities on fluid attenuated inversion recovery scans can cause segmentation errors in FreeSurfer. To account for this, white matter hyperintensities were detected on fluid attenuated inversion recovery using automated thresholding and used to correct the initial tissue classification produced by FreeSurfer. White matter hyperintensities segmentation was carried out using the automated delineation method previously described (249) using white matter masks created by FreeSurfer.

2.10 Covariates

Medical history

Standardised questionnaires were used to obtain information regarding demographics, medical history (including cardiovascular risk factors and disease - past and current smoking, hypertension, angina, stroke, and hyperlipidaemia) and medications use (blood pressure-lowering medications, cholesterol lowering medications, diabetes related medications, and anti-inflammatory medications). The activity of daily living was assessed by the Australian Modified Lawton's Activities of Daily Living scale (262).

Anthropometry measures

Weight (kg) was measured wearing light clothing and without shoes using a Heine portable scale. Height (m) measurements were performed with Leicester stadiometer. Height and weight were measured only once. Body mass index (kg/m^2) was calculated by dividing weight by height squared. Waist circumference was measured as the midpoint between the top of the hip and the lower costal border. Hip circumference was measured as the widest point around buttocks. Waist to hip ratio was calculated via waist circumferences (cm) divided by hip circumferences (cm). Waist and hip circumferences were measured three times and the average was used.

Mood and blood pressure

Mood was assessed with the 15-item Geriatric Depression Scale (263). Systolic and diastolic blood pressure was measured by an Omron M4 sphygmomanometer as the mean of three consecutive seated brachial blood pressure measures from the right arm.

Biochemical measures

Venous blood samples were taken from the antecubital fossa after overnight fasting. Biochemistry analysis of fasting plasma glucose, serum insulin, lipid profile (total cholesterol levels, triglycerides

levels), glycated haemoglobin A1c and inflammatory markers (interleukin-6, tumour necrosis factor-α, C-reactive protein) were performed at the Royal Hobart Hospital, Tasmania, Australia. Insulin resistance was calculated using the homeostatic model assessment for insulin resistance as fasting plasma glucose multiplied by insulin levels divided by 22.5 (264).

2.11 Statistical analysis

Statistical methods will be described in each individual chapter.

2.12 Postscript

This chapter described detailed information on the methods of sampling, data collection and calculation of each dietary pattern, physical activity variables, outcome variables and covariates. The next chapter will provide data analysis and the related results, and discussion of the first study: "Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes.

Appendix 2.1 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 3	○ 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 usually use?

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

4. How much milk do you usually use 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 than 1 slice per day
- ○ 1 slice per day
- ○ 2 slices per day
- ○ 3 slices per day
- ○ 4 slices per day
- ○ 5-7 slices per day
- ○ 8 or more slices per day

7. Which spread 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

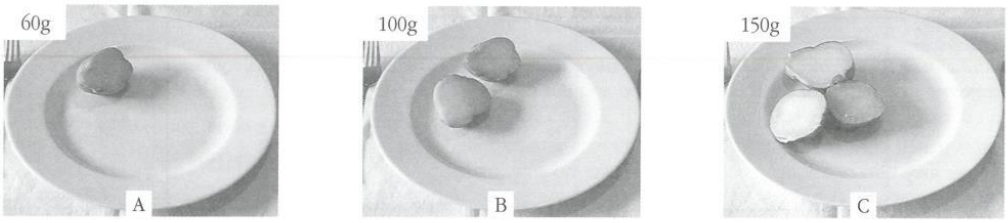
DO NOT WRITE IN THIS AREA.

35045

Pearson 13879

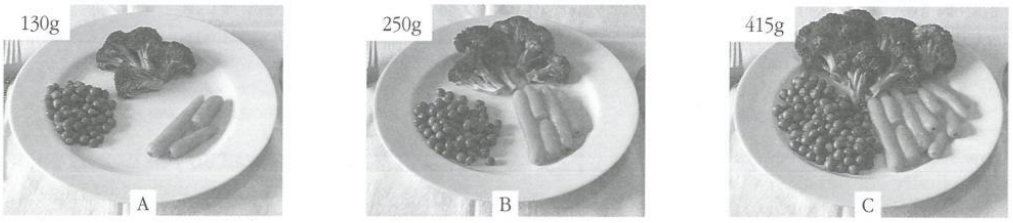
For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually 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



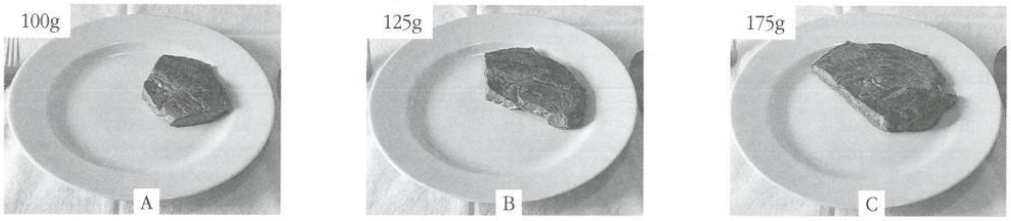
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

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



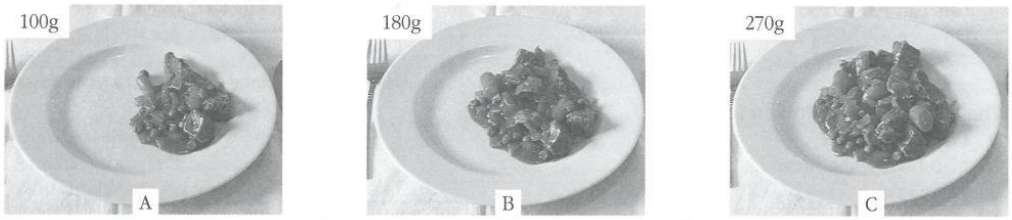
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

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

2

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											
Cheese	B1	○	○	○	○	○	○	○	○	○	○
Ice-cream	B2	○	○	○	○	○	○	○	○	○	○
Yoghurt	B3	○	○	○	○	○	○	○	○	○	○
Beef	B4	○	○	○	○	○	○	○	○	○	○
Veal	B5	○	○	○	○	○	○	○	○	○	○
Chicken	B6	○	○	○	○	○	○	○	○	○	○
Lamb	B7	○	○	○	○	○	○	○	○	○	○
Pork	B8	○	○	○	○	○	○	○	○	○	○
Bacon	B9	○	○	○	○	○	○	○	○	○	○
Ham	B10	○	○	○	○	○	○	○	○	○	○
Corned beef, luncheon meats or salami	B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	B14	○	○	○	○	○	○	○	○	○	○
Fish, 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	○	○	○	○	○	○	○	○	○	○

3

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	D18	○	○	○	○	○	○	○	○	○	○
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 tablets)	D23	○	○	○	○	○	○	○	○	○	○
Mushrooms	D24	○	○	○	○	○	○	○	○	○	○
Zucchini	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	○	○	○	○	○	○	○	○	○	○
White wine (include sparkling wines)	4	○	○	○	○	○	○	○	○	○	○
Fortified wines, port, sherry, etc.	5	○	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc.	6	○	○	○	○	○	○	○	○	○	○

When answering 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 can or stubby of beer = 2 glasses
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.

35045

3. Dietary Patterns Are Not Associated with Brain Atrophy or Cerebral Small Vessel Disease in Older Adults with and without Type 2 Diabetes.

3.1 Preface

Chapter one described dementia as a major public health concern and identified diet as an important modifiable risk factor. There are few prior studies that have examined whether data-driven dietary patterns are associated with brain structure, or whether associations differ based on T2D status. The study reported in this chapter will examine the association between data-driven dietary patterns and brain structure in people with and without T2D. The text of this chapter and the supplementary material in the appendix have been published in *the Journal of Nutrition* (265).

3.2 Introduction

Dementia is a major public health concern which affects approximately 47 million people worldwide (266). There is no effective treatment. Therefore prevention is an urgent priority both to decrease the incidence and to decelerate progression of cognitive decline. Diet and type 2 diabetes (T2D) are among risk factors that could be valuable modifiable targets.

Intake of certain foods, vitamins and antioxidants are related to better cognitive function in the general population (267). Over the past decade, attention has shifted from the impact of single nutrients or foods to a whole diet approach, such as dietary patterns (DPs), which may better reflect the interactive effects of foods in daily eating behaviour (268). A prudent (healthy) diet has been shown to maintain cognition as it contains foods and nutrients with higher antioxidants and anti-inflammatory content that protect the brain against disruptive oxidative stress and inflammatory agents (122, 269). Whereas, it has been observed that a western (unhealthy) diet contains heated processed food that produces advanced glycation end products (162) that lead to cognitive dysfunction (88) through stimulating oxidative stress and inflammatory pathways (270). MRI structural abnormalities are early markers of dementia and may appear before cognitive impairment (26, 271), but few studies have investigated associations between DPs and brain structure (118, 136, 272, 273).

Poor diet is also a risk factor for T2D (274), and people with T2D are at two times higher risk of developing dementia (275). Reductions in cerebral grey and white matter, as well as poorer cognitive

function, are found in people with T2D compared to controls (72, 75). MRI markers of cerebral small vessel disease, such as white matter hyperintensities (WMH) of presumed vascular origin and infarcts in people with T2D have also been associated with lower attention, executive functioning and slower processing speed (271, 276). People with T2D comply less with dietary recommendations particularly on the intake of fruit, vegetables, dairy and grains (123). Therefore, T2D may be an essential factor to consider when examining the relationship between diet and poorer cognitive function or brain structure. However, it is not known if associations between diet and brain health differ among individuals with T2D compare to those without T2D. A higher consumption of western diet increase the risk of developing CVD (277).A higher risk of cardiovascular disease (CVD) such as hypertension, hyperlipidemia, BMI, and stroke at midlife is associated with a higher cognitive decline, WMH, and thiner brain cortex at later life (278-280). Then, CVD risk factors could be potential confounders on the association between diet and brain health. This study aimed to examine the association between DPs and MRI brain structure (volumes of gray matter, white matter, hippocampal and WMH, infarcts and microbleeds) in people with and without T2D. We also examined associations with cognitive variables to compare our results to prior studies.

3.3 Material and Methods

Participants

Participants were recruited into the Cognition and Diabetes in Older Tasmanians study, the details of which have previously been described (75). Briefly, people >55 years with T2D were recruited from the Australian National Diabetes Service Scheme. Participants without T2D were aged ≥60 years and were randomly selected from the Southern Tasmanian electoral roll (75). Any participants with fasting plasma glucose ≥ 7.0 mmol/L, random plasma glucose ≥ 11.1 mmol/L, or 2-h glucose ≥11.1 mmol/L after oral glucose tolerance test were classified as having T2D. The exclusion criteria were living in a nursing home, any contraindication to MRI or a diagnosis of dementia. All participants provided written consent. The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study (Ethics reference numbers: H0009400 and H7947).

Dietary Assessment

Diet was assessed using the Dietary Questionnaire for Epidemiological Studies version 2 (DQES v2), a FFQ developed to measure diet in an ethnically diverse Australian population (281). Previous Australian studies have reported the reliability, validity and reproducibility of the DQESv2 in older

adults with (219, 282) and without T2D (283). The DQES v2 includes 80 items (74 foods and 6 alcoholic beverages). Participants reported their usual intake over the previous year using one of 10 response options, ranging from "never" to "3 or more times per day". Respondents were asked to indicate their usual portion sizes from a series of photographs in the FFQ. Information was also obtained on types of bread, milk, cheese and spread consumed, the usual number of fresh fruits, and vegetables consumed daily. Nutrient intakes and the amounts of each item consumed (grams per day) were calculated by the Cancer Epidemiology Centre of the Cancer Council in Victoria using an Australian food composition NUTTAB 2006 database (220).

Dietary patterns analysis

All 80 food items were classified into thirty-five food groups **(See Appendix 3.1 Supplementary Table 1)** by F.Z, W.H.O, and K.J.S. The food groups were defined based on the similarity of their nutrient profiles as described in the Composition of Foods Australia guide (224), and previous Australian studies (225, 284). We used principal component analysis (PCA) to reduce the size of the food group items into a smaller number of underlying components. Food groups amounts (g) were adjusted for total energy intake by the density method (g/1000 Kcal) prior to PCA (285). The g/1000kcal conversion is not an adequate way to adjust for energy intake. Therefore, regression models were adjusted for total energy intake (286). Varimax rotation was applied to improve the separation of components and facilitate the factor's interpretability. The number of components retained was based on scree plots (227), and interpretability with factor solution limited to an eigenvalue of >2 for participants both with and without T2D. Factor loadings equal to and above 0.2 were considered as major contributors to each pattern. DPs were presented as z-scores (118).

Brain magnetic resonance imaging

We obtained MRI scans with a 1.5-T General Electric scanner using high-resolution T1-weighted spoiled gradient echo (GRE), T2-weighted fast spin echo, and fluid-attenuated inversion recovery (FLAIR), the details of which have been described previously (75). The Functional MRI of the Brain Linear Image Registration Tool was used to register Three-dimensional T1 and GRE sequences in the standard Montreal Neurological Institute space. We applied a multispectral segmentation process and used three-dimensional T1 and GRE sequences. The tissue probability maps of grey and white matter were produced using Statistical Parametric Mapping version 5 software. The images were corrected for the volume change that occurred in the result of the normalization process. Prior voxel-based morphometric analysis, isotropic Gaussian kernel with a full width at half maximum 8 mm was

used to smooth tissue maps. Tissue volumes of the segmented areas (total grey and normal-appearing white matter) were calculated with voxel counting algorithms. A single expert manually segmented both hippocampi. Fully automated WMH segmentation on FLAIR sequences was performed using a validated method (13), and a voxel counting algorithm calculated WMH volume. A single trained rater determined the presence of MRI infarcts and microbleeds which were confirmed by a neurologist and a geriatrician.

Cognitive Assessment

Cognitive function was assessed by a comprehensive battery of validated neuropsychological tests. We generated six cognitive domains using z-scores similar to previous studies (225) and calculated the mean of all domains to give a global cognitive domain score. The units for all the cognitive measures are z-scores, as is common in studies of cognitive function using multiple neuropsychological tests (287). Details regarding the calculation of cognitive domains are provided in the online **Appendix 3.2 supplementary Methods**.

Other Measures

Standardised questionnaires were used to obtain information regarding demographics, medical history (including cardiovascular disease risk factors, self-reports of smoking history, hypertension, angina, stroke, and hyperlipidaemia) and medication use. Mood was assessed with the 15-item Geriatric Depression Scale (263). We measured weight (kg) and height (m) and calculated BMI dividing weight by height squared. Ambulatory activity was measured using a Yamax pedometer over 7 days. Systolic blood pressure was measured by an Omron M4 sphygmomanometer as the mean of three consecutive seated brachial blood pressure measures from the right arm. Hypertension was defined as self-reported history of hypertension, systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or current use of anti-hypertension medication. Venous blood samples were taken from the antecubital fossa after overnight fasting. Biochemistry analysis of fasting plasma glucose, serum insulin, serum lipid profile and serum glycated haemoglobin A1c was performed at the Royal Hobart Hospital, Tasmania, Australia. Insulin resistance was calculated using the homeostatic model assessment for insulin resistance as fasting plasma glucose multiplied by insulin levels divided by 22.5 (288). Patients who had a self-reported history of hyperlipidaemia, cholesterol levels > 6.2 mmol/L or were on cholesterol-lowering medications were defined as having hypercholesterolemia. A continuous cardiovascular disease (CVD) risk score was generated by allocating one point for the presence of each of the CVD risk factors including BMI, hypertension,

hypercholesterolemia, history of past and current smoking, angina, and stroke, and summing the points to give an overall score (289).

Statistical Analysis

Non-normally distributed variables were transformed as appropriate. Square root transformations were applied to the Hopkins Recognition, Stroop Colour and Stroop Dot Time tests and a logarithm transformation for WMH. Student t-tests (for continuous variables) and chi-square tests (for categorical variables) were used to compare means, and proportions of demographic, dietary intake, medical, cognitive and MRI variables between the T2D and non T2D groups. In this study, 76 (11%) of the 689 participants had missing values for ambulatory activity, which were imputed 50 times by multiple imputation procedures using age, sex, education, DPs and CVD risk score. The imputed values for ambulatory activity were then used as a covariate to adjust the regression models.

Multivariable linear (continuous) or binomial logistic regression (categorical variables) were used to examine the associations between each DP z-score and each brain and cognitive variable, initially adjusted for age, sex, education, and energy (continuous MRI variables were additionally adjusted for total intracranial volume) separately in people with and without T2D (model 1). Model 2 was further adjusted for the other confounders (CVD risk score, mood and ambulatory activity) defined based on their biological associations with the predictor (diet) and brain outcomes in our study and covariates applied in the literature (118, 272). To determine the effect of covariates we then examined the change in the beta-coefficient after adding them to the model. The interaction for sex was examined by adding a sex x DP interaction term to the models of the association between DPs and brain health variables. Probability levels less than 0.05 were considered as significant. Statistical analyses were performed in STATA 14.2 (Stata Corp, College Station, TX) software.

3.4 Results

Comparison of general characteristics between groups

Of the 713 participants eligible for the study, 694 participants completed the DQES-v2 dietary questionnaire and had cognitive measurements. Five participants were excluded with dementia (self-reported dementia (n=2) or activity of daily living impairment in combination with cognitive impairment (determined by two neuropsychologists as 1.5 SD below age, sex and education norms) in two or more cognitive domains (n=3). Therefore, 689 participants, 343 with T2D (206 men; 137 women; mean age 67.7± SD 6.8 years) and 346 without T2D (187 men; 159 women; mean age 72.1±

SD 7.1 years) were included in the analyses. Of these, 688 had both cognition and diet data and 627 had both diet and brain MRI measures. Demographic, clinical, and major dietary characteristics of participants before adjustment for age and sex are shown in **Table 3.1.**

The mean duration of T2D was 4.54 ± SD 8.20 years, and 23% were receiving insulin therapy. The sample was mostly Caucasian (99.2% of people without type 2 diabetes and 98.6% of people with type 2 diabetes). **Appendix 3.3 Supplementary Table 2** shows the differences in Mean±SD of cognitive and MRI brain variables between people with and without T2D. People with T2D were more likely to have higher scores on the Hopkins Immediate, Symbol Search, and controlled oral word association tests, and lower scores on Rey complex figure copy, and delay tests ($p<0.05$).

Dietary patterns

DPs were similar among males and females (data not shown), and therefore analyses were not stratified by sex. Two major DPs for people with T2D (prudent and traditional) and three major DPs for people without T2D (prudent, traditional and western) were derived from PCA **(Appendix 3.4 Supplementary Table 3).** For people with T2D, the prudent DP (8% of total variance) was mainly composed of leafy green vegetables, tomato, other vegetables, fresh fruit, yellow and red vegetables, and non-fried fish, and loaded negatively on cake, biscuits and added sugar. The traditional DP (6% of total variance) included high intake of legumes, processed meats, potatoes, yellow and red vegetables, cruciferous, and flavoured milk, and negatively loaded on poultry, nuts, red meat, refined grains, and non-fried fish.

For people without T2D, the prudent DP (8% of total variance) was mainly composed of other vegetables, leafy green vegetables, non-fried fish, cruciferous and poultry, and negatively loaded on chocolate, cake and biscuits. The traditional DP (8% of total variance) included high consumption of yellow and red vegetables, legumes, fried potatoes, processed meats, tomato, and potatoes. The western DP (6% of total variance) mainly included refined grains, red meat, takeaways, wine, beer, saturated spread and negatively loaded on whole grains, low-fat dairy, flavoured milk, and unsaturated spreads. The overall percentages of total variance explained for DPs were 14% for people with T2D and 22% for people without T2D. Total variance of less than 30% is common for the studies on dietary patterns which is comparable to other studies (14.5% (118), 23%(290)).

Table 3.1 Demographic and clinical characteristics of participants with and without type 2 diabetes

Variables	All (*n*=689)	T2D (*n*=343)	Non-T2D (*n*=346)
Male, *n* (%)	393 (57.0)	206 (60.1)	187 (54.0)
Age, y	69.9±7.40	67.7±6.80	72.1±7.10
Education[2], *n* (%)	332 (48.2)	169 (49.2)	163 (47.1)
BMI, (Kg/m^2)	29.1±7.01	31.1±8.60	27.2±4.30
History of Stroke, *n* (%)	58.0 (8.40)	37.0 (10.8)	21.0 (6.00)
History of Hyperlipidaemia, *n* (%)	385 (55.9)	251 (73.2)	134 (38.7)
History of Hypertension, *n* (%)	406 (58.9)	248 (72.3)	158 (45.6)
Past and current smoker, *n* (%)	357 (51.8)	186 (54.2)	171 (49.4)
Angina, *n* (%)	99.0 (14.4)	59.0 (17.2)	40.0 (11.5)
Steps, (*n*/day)	6060±3340	6030±3620	6090±3080
Serum triglycerides, (mmol/L)	1.40±0.80	1.70±0.90	1.20±0.50
Serum total cholesterol, (mmol/L)	4.80±1.10	4.40±0.90	5.20±1.10
Serum $HbA1_c$, (mmol/mol)	6.40±1.20	7.20±1.20	5.60±0.30
Fasting plasma glucose, (mmol/L)	6.50±2.00	7.70±2.30	5.30±0.50
HoMA IR	4.30±9.50	6.40±12.9	2.20±1.50
SBP, (mmHg)	139±20.6	136±18.9	142±21.7
DBP, (mmHg)	78.3±11.4	76.1±10.4	80.3±11.8
BP lowering medication, *n* (%)	368 (88.0)	226 (89.0)	142 (87.0)
Cholesterol medication, *n* (%)	303 (750)	215 (82.0)	88.0 (62.0)
Carbohydrate (g/d)	199±75.3	194±75.8	204±75.6
Protein (g/d)	89.3±40.0	93.5±43.0	85.1±36.0
Total fat (g/d)	72.5±33.0	73.3±34.0	71.8±31.0
Total energy (KJ/d)	7530±2890	7560±2990	7510±2800
Total energy (Kcal/d)	1810±694	1813±717	1800±672

Values are Mean ± SD for continuous unless indicated as frequency (percent) for categorical variables. BP: Blood Pressure, DBP: Diastolic Blood Pressure, HbA_{1c}: Haemoglobin A1c, HoMA IR: Homeostatic Model Assessment of Insulin Resistance, SBP: Systolic Blood Pressure, T2D: Type 2 Diabetes. [1] Had post-high school education.

Regression analysis of dietary patterns and brain MRI and cognitive variables

There were no significant associations between DPs, cognition, brain structure **(Table 3.2)** or cognitive domains variables (**Appendix 3.5 supplementary table 4)** among people with T2D *(P<0.05)***.** For people without T2D, a one SD increase in intake of western DP was associated with 3.09 mL lower grey matter volume (β=-3.03 95%CI: -5.67, -0.38; *P*=0.03). (**Table 3.3**, model 1). However, after the addition of other covariates, the associations were significantly attenuated (β=-1.97 (95%CI: -4.68, 0.74) *P*=0.15). The CVD risk scores changed the coefficient by 18%, and physical activity and mood changed the coefficients by 12% and 10% respectively **(Table 3.3** Model 2**)**. There were no other significant associations between DPs with cognition, brain structure (**Table 3.3)** or cognitive domains **(Appendix 3.6 supplementary table 5)** among people without T2D *(P<0.05)***.** There was no interaction for sex on the association between DPs and cognition or brain structure.

Table 3.2 The association between dietary patterns, and brain structural measures in participants with type 2 diabetes.

DP 1 Prudent (n=343*)*	Model 1[1]	Model 2[3]
	β (95% CI)	β (95% CI)
Gray matter volume [2], mL	0.24 (-2.40, 2.88)	-0.67 (-3.29, 1.94)
White matter volume[2], mL	-0.86 (-3.44, 1.72)	-0.80 (-3.44, 1.84)
Left hippocampal volume[2], mL	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)
Right hippocampal volume[2], mL	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)
WMH volume[2], mL	-0.02 (-0.08, 0.03)	-0.01 (-0.07, 0.05)
	OR (95% CI)	OR (95% CI)
Micro bleeds	1.11 (0.77, 1.60)	1.18 (0.80, 1.73)
Infarcts	0.96 (0.81, 1.14)	0.99 (0.83, 1.19)
DP 2 Traditional (n=343)	β (95% CI)	β (95% CI)
Gray matter volume[2], mL	2.43 (-0.72, 5.58)	1.65 (-1.49, 4.79)
White matter volume[2], mL	0.73 (-2.37, 3.82)	0.76 (-2.42, 3.93)
Left hippocampal volume[2], mL	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)
Right hippocampal volume[2], mL	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.06)
WMH volume[2], mL	-0.07 (-0.14, 0.00)	-0.04 (-0.11, 0.03)
	OR (95% CI)	OR (95% CI)
Microbleeds	1.00 (0.64, 1.58)	0.94 (0.58, 1.54)
Infarcts	0.96 (0.78, 1.19)	0.99 (0.79, 1.23)

[*]*P*<0.05 is significant. Beta coefficients show a one SD increase in the intake of a healthy DP is associated with higher brain volume (positive beta) or lower WMH volume (negative beta); A one SD increase in intake of an unhealthy DP would be associated with lower brain volume (negative beta) or higher WMH volume (positive beta).[1]Model 1: Adjusted for age, sex, education, and energy. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume. [3]Model 2: Model 1 plus additional adjustments for mood, CVD risk score (includes BMI, hypertension, hyperlipidaemia, smoking, angina, and stroke), and ambulatory activity. DP: dietary pattern, WMH: White Matter Hyperintensities.

Table 3.3 The association between dietary patterns, and brain structural measures in participants without type 2 diabetes.

DP 1 Prudent (n=346)	Model 1, [1]	Model 2, [3]
	β (95% CI)	β (95% CI)
Gray matter volume[2], mL	-0.31 (-2.75, 2.13)	-0.57 (-3.08, 1.94)
White matter volume[2], mL	-0.21 (-2.66, 2.24)	-0.90 (-3.42, 1.61)
Left hippocampal volume[2], mL	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.00)
Right hippocampal volume[2], mL	-0.02 (-0.05, 0.02)	-0.03 (-0.06, 0.01)
WMH volume[2], mL	-0.05 (-0.11, 0.01)	-0.05 (-0.11, 0.02)
	OR (95% CI)	OR (95% CI)
Micro bleeds	0.87 (0.66, 1.15)	0.79 (0.59, 1.08)
Infarcts	1.00 (0.83, 1.21)	1.02 (0.84, 1.25)
DP 2 Traditional (n=346)	β (95% CI)	β (95% CI)
Gray matter volume[2], mL	0.45 (-2.09, 2.99)	0.21 (-2.32, 2.73)
White matter volume[2], mL	-2.55 (-2.84, -1.77)	-2.86 (-5.36, 0.35)
Left hippocampal volume[2], mL	-0.02 (-0.05, 0.02)	-0.02 (-0.05, 0.02)
Right hippocampal volume[2], mL	-0.01 (-0.05, 0.02)	-0.01 (-0.05, 0.02)
WMH volume[2], mL	-0.02 (-0.08, 0.04)	-0.01 (-0.08, 0.05)
	OR (95% CI)	OR (95% CI)
Micro bleeds	0.92 (0.67, 1.26)	0.89 (0.63, 1.26)
Infarcts	0.76 (0.59, 0.99)	0.78 (0.59, 1.01)
DP 3 Western (n=346)	β (95% CI)	β (95% CI)
Gray matter volume[2], mL	-3.03 (-5.67, -0.38)*	-1.97 (-4.69, 0.74)
White matter volume[2], mL	-1.11 (-3.79, 1.57)	-0.74 (-3.47, 1.99)
Left hippocampal volume[2], mL	0.01 (-0.02, 0.05)	0.01 (-0.02, 0.05)
Right hippocampal volume[2], mL	-0.01 (-0.04, 0.03)	-0.00 (-0.04, 0.04)
WMH volume[2], mL	-0.01 (-0.07, 0.06)	-0.04 (-0.10, 0.03)
	OR (95% CI)	OR (95% CI)
Microbleeds	0.91 (0.67, 1.23)	0.92 (0.67, 1.27)
Infarcts	0.88 (0.71, 1.08)	0.84 (0.67, 1.05)

[*]*P*<0.05 is significant. Beta coefficients show a one SD increase in the intake of a healthy DP is associated with higher brain volume (positive beta) or lower WMH volume (negative beta); A one SD increase in intake of an unhealthy DP would be associated with lower brain volume (negative beta) or higher WMH volume (positive beta). [1]Model 1: Adjusted for age, sex, education, and energy. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume. [3]Model 2: Model 1 plus additional adjustments for mood, CVD risk score (includes BMI, hypertension, hyperlipidaemia, smoking, angina, and stroke), and ambulatory activity. DP: dietary pattern, WMH: White Matter Hyperintensities.

3.5 Discussion

To our knowledge, this is one of the few studies that has explored the relationship between DPs and brain structure. A higher consumption of a western DP was associated with lower grey matter volume in those with a mean age of 72.1 without T2D; however, this association did not remain significant after adjusting for cardiovascular and other risk factors. There were no other associations between DPs and cognition in people without T2D. We also found no associations between traditional and prudent DPs, cognitive function, and brain structure in people with T2D.

The PCA produced two healthy (traditional and prudent) DPs for people with T2D and one healthy DP (prudent) for people without T2D. While healthy DPs have been investigated in relation to cognitive function (291), studies investigating the relationship with brain structure are less frequent (118). Furthermore, the majority of previously published studies have examined adherence to dietary indices (Priori approach) such as the Mediterranean diet or National dietary guidelines, rather than DPs calculated using a data-driven (Posteriori) approach. Studies using a Priori approach have been inconsistent in their findings. For example, a higher intake of the Mediterranean diet was associated with greater grey and white matter volumes and a lower burden of WMH (135, 136, 273, 292, 293) in some studies, but there were no associations with brain volume (292) or WMH (273) in others. A further study found that higher adherence to the Dutch dietary guidelines was related to higher grey, white and hippocampal volume, but not WMH volume (272). Our non-significant findings may be due to our vegetable based traditional DP, not including fish, nuts, and fruits that are emphasised in the Mediterranean and Dutch dietary guidelines (272, 293). Furthermore, our traditional DP was not solely healthy like the Mediterranean diet, as it also contained processed meat (bacon, ham, and sausages) which is usually eaten with legumes and vegetables as part of the traditional Australian diet. To our knowledge, there is only one study that used the data-driven (Posteriori) approach. This Australian study found that a 1 SD increase in consumption of a prudent DP was associated with a 45.7 mm^3 larger left hippocampal volume in a sample of 255 generally healthy people aged 60-64 years, independent of diabetes, physical activity, hypertension, and smoking (118). However, it is

difficult to compare our study with their results (118) as there was limited description regarding their prudent DP other than it comprised fresh vegetables, salad, fruit and grilled fish. Since the core purpose of a whole food approach is to show the synergistic and cumulative effect of nutrients within DPs (294), it would be interesting in future studies to unravel if the effects on brain health are different if a person eats a healthy diet alone compared to a diet that consists of a mixture of healthy and unhealthy components.

A western DP (higher intake of refined grains, red meat, takeaways, wine, beer, saturated spread as well as lower intake of whole grains, low-fat dairy, flavoured milk, and unsaturated spreads) was associated with a lower grey matter volume in participants without T2D, however this association was not significant after adjusting for CVD and other risk factors. There have been few prior studies investigating data-driven DPs and brain structure. The Australian Personality and Total Health Through Life (PTHTL) study showed that a higher intake of a western (unhealthy) DP (characterised by the consumption of roast meat, sausages, hamburgers, steak, chips, crisps and soft drinks) was associated with a 52.6 mm^3 smaller left hippocampal volume independent of diabetes, physical activity, hypertension, and smoking (118). A nutrient pattern with a high content of trans fatty acids (a characteristic of a western DP) was also related to lower total brain volume independent of hypertension and depression (295). Peripheral inflammatory cytokines can cross the blood-brain barrier to stimulate central inflammation that results in neurodegeneration and cognitive deficits (296). In addition, heat-processed foods may contain high levels of advanced glycation end products (162) that have been associated with poorer cognitive function and cerebral atrophy (88).

The significant associations in our study weakened considerably after adding a CVD risk score to the models. The greater number of factors in our CVD risk score may account for differences in our findings compared to previous studies (118, 295). A higher intake of western diet leads to develop a high risk of CVD (277). CVD risk factors (stroke, hypertension, hyperlipidemia, and obesity) have been related to a higher risk of cognitive decline, WMH, and thinner cortex (278-280). Higher cholesterol can increase the risk of Alzheimer's disease by increasing amyloid deposition in the brain and it also increases the risk of cerebrovascular disease through inducing atherosclerosis (297). CVD risk is likely to be a potential confounder in this study due to the observed associations between CVD risk factors, western diet and grey matter volume; however, prospective study designs are required to clarify if CVD risk is on the pathway between DPs and brain structure.

This study has several strengths. We used a whole diet approach to consider overall eating patterns,

in contrast to a traditional view that examines nutrients and foods in isolation. To explore food patterns, we used a data-driven approach that was independent of priori assumptions. The dietary data were collected using a FFQ that has been previously validated in community-dwelling older Australian populations with (219, 282) and without T2D (283). To our knowledge, this is the first study to explore MRI markers of brain volume and small vessel disease stratified by T2D status. However, there are also limitations. The cross-sectional nature of our study made it impossible to determine causality and capture dietary changes over time and whether CVD risk factors are potential mediators. The FFQ relies on food estimation and may be subject to recall bias; however, it is a well-accepted method for collecting nutritional data in epidemiological studies (298). The DP approach limits the ability to compare our results with other studies. Like other data reduction methods, the number of retained factors is often arbitrarily determined.

In conclusion, although we found that a western diet was associated with lower grey matter volume in people without T2D, these associations were not independent of cardiovascular and other risk factors. Future studies especially longitudinal studies are needed to unravel the direct or indirect effects of DPs on brain health.

3.6 Postscript

The findings from this chapter showed prudent (healthy) and traditional dietary patterns were found for people with T2D, and prudent, traditional and Western dietary patterns (unhealthy) were found for people without T2D. The findings in this chapter suggest that there is an association between a Western dietary pattern and lower grey matter volume in people without T2D. However, this was not independent of cardiovascular risk factors. There were no other significant associations between dietary patterns and brain health.

Systemic inflammation is associated with poorer brain volume and cognition (299). The next chapter of this thesis will examine whether the energy-adjusted dietary inflammatory index (the inflammatory potential of diet) is associated with brain structure in people with and without T2D.

Appendix 3.1 Supplementary Table-1 Food groups and their constituents

Food groups	Variables
Whole grains	High fibre white bread, wholemeal bread, rye bread, multigrain bread, All Bran, bran flakes, Sultanas Bran, FibrePlus muesli, Weetabix, Vita Brits, Weeties, porridge
Refined grains	White bread, crackers, crispbreads, dry biscuits, cornflakes, Nutrigrain, Special K, rice, pasta and noodles
Red meats	Beef, veal, lamb, pork
Processed meat	Processed meat (corned beef, luncheon meats, salami), bacon, ham, sausage, and frankfurter
Poultry	Chicken
Takeaway	Meat pies, quiche, other savoury pastries, hamburger with a bun, pizza
Fried fish	Fried fish, takeaway fish
Other fish	Grilled fish, steamed fish, baked fish, tinned fish (salmon, tuna, sardines)
Fried Potatoes	Fried or roasted potatoes (include hot chips)
Potatoes	Potato cooked without fat
Yellow or red vegetables	Carrots, pumpkin, capsicum
Other vegetables	Beetroot, zucchini, mushrooms, celery, onion, leek, garlic, cucumber
Legumes	Green bean, peas, baked beans, other beans (chickpeas and lentils), bean sprouts, alfalfa sprouts
Cruciferous vegetables	Cabbage, brussels sprouts, broccoli, cauliflower
Leafy green vegetables	Lettuce, endive, salad greens, silverbeet, spinach
Tomato	Fresh and tinned tomato
Fresh fruit	Oranges, citrus fruit, apples, bananas, strawberries, melons (watermelon, rockmelon, honeydew), peaches, nectarines, apricots, pineapple, avocado, mango, pawpaw, pears
Canned fruit	Tinned fruit, frozen fruit
Cakes, biscuits,	Sweet biscuits, cakes (sweet pies, tarts, sweet pastries)

sweets	
Low-fat dairy products	Reduced-fat milk, skimmed milk, low-fat cheese, cottage cheese
Full-fat dairy products	Whole milk, cream cheese, ice cream, full-fat cheese (parmesan, Romano, cheddar, edam camembert, and brie), yoghurt
Soy products	Soya milk, soybean, soybean curd, tofu
Chocolate	Chocolate
Added sugar	Honey, jam, syrup, marmalade, spooned sugar
Crisps	Corn chips and potato crisps, twisties
Nuts	Peanut butter/paste, nuts
Tomato products	Tomato sauce, paste or dried tomato
Eggs	Eggs
Juice	Fruit juices
Saturated spreads	Butter and margarine blends, butter
Unsaturated spreads	Margarine any kind, polyunsaturated margarine, monounsaturated margarine
Beer	Beer low alcohol, beer full alcohol, spirits
Wine	Red wine, white wine, fortified wine
Flavoured milk	Flavoured milk, cocoa, milo
Vegemite	Vegemite, marmite, promite

Appendix 3.2 Methods of analysis for the association between dietary patterns and cognitive domains

The domains created were *verbal memory* (the three components of the Hopkins Verbal Learning test (238)), *visual memory* (Rey-Osterrieth complex figure Delayed reproduction after 20 minutes (238)), *visuospatial function* (Rey-Osterrieth complex figure copy (238)), *executive function* (Stroop interference: Victoria Stroop Colour Test score – Victoria Stroop Word Test score) (238), *Verbal fluency* (the Controlled Oral Word Associations Tests (COWAT) letter and animal categories (238)), as well as *attention-processing speed* (the Digit Span, Digit Symbol Coding and Symbol Search subsets of the Wechsler Adult Intelligence Scale-Third Edition (246), and the Victoria Stroop Dot Test (238)). To create each domain, a z-score for each cognitive test was calculated by the individual's raw score minus the sample mean score, divided by the sample standard deviation. Stroop scores were reversed so that higher scores translated to better performance. Subsequently, z-scores for each test were averaged to compute a single composite score for each domain. Domain scores that included more than one cognitive measure were re-standardised to a standard deviation of one. These scores were used in the regression analysis to allow the comparison of associations between dietary patterns and cognitive domains.

Appendix 3.3 Supplementary Table 2 Cognitive and brain MRI variables

MRI variables and cognitive tests[1]	All (*n*=689) Mean ± SD	T2D (*n*=343) Mean ± SD	Non-T2D (*n*=346) Mean ± SD	P-value
Grey Matter volume, mL	582.7±64.7	581.2±66.3	584.1±63.2	0.57
White Matter volume, mL	456.1±59.0	455.4±62.4	456.8±55.5	0.76
WMH volume, mL	6.5±7.5	5.9±7.0	7.0±7.9	0.08
Brain MRI Infract, *n* (%)	126 (19.6)	72 (22.1)	54 (17.1)	0.13
Brain MRI Microbleeds, *n* (%)	36 (5.6)	14 (4.3)	22 (6.9)	0.11
Digit symbol coding, (*n*)	51.2±14.9	52.2±14.3	50.3±15.6	0.09
Hopkins immediate, (*n*)	22.1±6.4	23.6±5.5	22.1±6.4	<0.01*
Hopkins delay, (*n*)	7.8±2.9	8.0±2.9	7.6±3.0	0.06
Hopkins recognition, (*n*)	10.0±1.8	10.1±1.7	9.9±1.9	0.15
Rey complex copy, (*n*)	30.1±6.1	28.3±6.3	32.0±5.2	<0.01*
Rey complex delay, (*n*)	13.8±6.8	12.8±6.5	14.8±7.0	<0.01*
Digit span, (*n*)	16.0±3.8	16.2±3.7	15.8±3.9	0.15
Symbol search, (*n*)	23.6±7.7	24.6±7.6	22.7±7.8	0.01*
COWAT word, (*n*)	36.3±12.7	35.8±12.8	36.7±12.6	0.32
COWAT category, (*n*)	17.8±4.9	18.4±4.9	17.2±4.9	0.01*
Stroop dot time, (s)	15.7±4.9	16.0±5.1	15.3±4.8	0.06

Stroop word time, (s)	20.6±7.4	20.2±6.2	20.9±8.5	0.16
Stroop colour time, (s)	36.9±17.8	36.6±15.7	37.2±19.5	0.62

[1] Values are Mean ± SD for continuous and frequency (percent) for categorical variables. [*] *P-values<0.05* are considered as significant. *P values* are for comparisons between two groups (T2D and non-T2D) based on t-test and chi square COWAT: Controlled Oral Word Association Test; MRI: magnetic resonance imaging; (n): Number of items answered correctly; T2D: type 2 diabetes; s: seconds; WMH: white matter hyperintensities

Appendix 3.4 Supplementary Table 3 Factor loadings of dietary patterns in people with and without type 2 diabetes

	Type 2 diabetes		No type 2 diabetes		
Dietary patterns	Prudent	Traditional	Prudent	Traditional	Western
Variance %	8%	6%	8%	8%	7%
Yellow red vegetables	0.29	0.24		0.45	
Cruciferous		0.22	0.20		
Potatoes		0.29		0.24	
Legumes		0.36		0.42	
Leafy green vegetables	0.36		0.37		
Other vegetables	0.33		0.42		
Whole grains					-0.34
Takeaways					0.24
Wine					0.22
Beer					0.22
Refined grains		-0.22			0.38
Fried potatoes				0.40	
Processed meats		0.30		0.36	
Tomato	0.34			0.31	
Red meat		-0.22			0.32
Non-fried fish	0.23	-0.20	0.27		
Poultry		-0.32	0.20		
Fresh fruit	0.31		0.32		
Saturated spread					0.21
Unsaturated spread					-0.24
Chocolate			-0.21		
Cake and biscuits	-0.28		-0.30		
Added sugar	-0.22				
Full fat dairy					
Low fat dairy					-0.31
Flavoured milk		0.21			-0.28
Nuts		-0.23			

Appendix 3.5 Supplementary Table 4 The associations between dietary patterns, and cognitive domains in participants with type 2 diabetes.

DP 1 Prudent (n=343)	Model 1[1] β (95% CI)	Model 2[2] β (95% CI)
Global cognition z-score	0.03 (-0.04, 0.09)	0.00 (-0.06, 0.07)
Verbal memory z-score	0.04 (-0.01, 0.10)	0.04 (-0.02, 0.09)
Visual memory z-score	0.02 (-0.04, 0.08)	0.00 (-0.06, 0.06)
Executive function z-score	-0.00 (-0.07, 0.06)	-0.01 (-0.08, 0.05)
Verbal fluency z-score	0.05 (-0.01, 0.11)	0.04 (-0.02, 0.11)
Attention processing speed z-score	0.02 (-0.04, 0.08)	-0.01 (-0.07, 0.05)
Visuospatial function z-score	0.00 (-0.07, 0.07)	-0.01 (-0.08, 0.06)
DP 2 Traditional (n=343)	β (95% CI)	β (95% CI)
Global cognition z-score	0.01 (-0.07, 0.08)	0.00 (-0.07, 0.08)
Verbal memory z-score	-0.01 (-0.07, 0.06)	-0.00 (-0.06, 0.06)
Visual memory z-score	0.03 (-0.04, 0.10)	0.03 (-0.04, 0.10)
Executive function z-score	-0.01 (-0.06, 0.08)	-0.00 (-0.08, 0.07)
Verbal fluency z-score	0.00 (-0.07, 0.08)	0.01 (-0.07, 0.09)
Attention processing speed z-score	-0.04 (-0.11, 0.03)	-0.04 (-0.11, 0.04)
Visuospatial function z-score	0.02 (-0.07, 0.10)	0.01 (-0.08, 0.09)

[1]Model 1: Adjusted for age, sex, education, and energy. Beta coefficients show that a one SD increase in intake of a healthy DP is associated with higher cognition (positive beta) or a one SD increase in intake of an unhealthy DP would be associated with lower cognition (negative beta). [2]Model 2: Model 1 plus additional adjustments for mood, CVD risk score (includes BMI, hypertension, hyperlipidaemia, smoking, angina, and stroke), and ambulatory activity. DP: dietary pattern

Appendix 3.6 Supplementary Table 5 The associations between dietary patterns, and cognitive domains in participants without type 2 diabetes.

DP 1 Prudent (n=346)	Model 1[1]	Model 2[2]
	β (95% CI)	β (95% CI)
Global cognition z-score	0.01 (-0.05, 0.07)	-0.01 (-0.07, 0.04)
Verbal memory z-score	0.01 (-0.05, 0.06)	-0.01 (-0.07, 0.05)
Visual memory z-score	0.04 (-0.03, 0.10)	0.02 (-0.05, 0.09)
Executive function z-score	-0.03 (-0.10, 0.05)	-0.04 (-0.11, 0.03)
Verbal fluency z-score	0.05 (-0.01, 0.11)	0.03 (-0.04, 0.09)
Attention processing speed z-score	0.00 (-0.06, 0.07)	-0.02 (-0.08, 0.05)
Visuospatial function z-score	-0.02 (-0.07, 0.04)	-0.03 (-0.08, 0.02)
DP 2 Traditional (n=346)	β (95% CI)	β (95% CI)
Global cognition z-score	0.02 (-0.04, 0.08)	0.01 (-0.05, 0.07)
Verbal memory z-score	-0.00 (-0.06, 0.06)	-0.02 (-0.07, 0.04)
Visual memory z-score	0.03 (-0.03, 0.10)	0.03 (-0.04, 0.10)
Executive function z-score	0.02 (-0.05, 0.10)	0.02 (-0.06, 0.09)
Verbal fluency z-score	-0.01 (-0.08, 0.06)	-0.02 (-0.09, 0.05)
Attention processing speed z-score	0.05 (-0.02, 0.11)	0.04 (-0.03, 0.10)
Visuospatial function z-score	0.01 (-0.05, 0.06)	0.00 (-0.05, 0.05)
DP 3 Western (n=346)	β (95% CI)	β (95% CI)
Global cognition z-score	0.02 (-0.04, 0.08)	0.04 (-0.02, 0.11)
Verbal memory z-score	0.05 (-0.01, 0.11)	0.08 (0.02, 0.14)
Visual memory z-score	0.03 (-0.05, 0.09)	0.03 (-0.05, 0.10)
Executive function z-score	0.01 (-0.06, 0.09)	0.02 (-0.06, 0.10)
Verbal fluency z-score	-0.01 (-0.08, 0.06)	0.01 (-0.06, 0.08)
Attention processing speed z-score	0.00 (-0.06, 0.07)	0.03 (-0.04, 0.10)
Visuospatial function z-score	-0.00 (-0.06, 0.06)	0.02 (-0.03, 0.08)

[1]Model 1: Adjusted for age, sex, education, and energy. Beta coefficients show that a one SD increase in intake of a healthy DP is associated with higher cognition (positive beta) or a one SD increase in intake of an unhealthy DP would be associated with lower cognition (negative beta). [2]Model 2: Model 1 plus additional adjustments for mood, CVD risk score (includes BMI, hypertension, hyperlipidaemia, smoking, angina, and stroke), and ambulatory activity. DP: dietary pattern

Appendix 3.7 Additional analysis

APOE-ε4 is a genetic risk factor for developing dementia. In accordance with chapter 6, this section aims to investigate the interaction for APOE-ε4 for the associations between the dietary patterns, cognition and brain structure in people with and without T2D. The following tables (supplementary table 6-10) lists the interaction terms beta coefficients, 95% confidence interval and p-values of interaction terms. The effect sizes for APOE-ε4 interactions were quite small and there was no consistent pattern across all the cognitive domains and brain variables.

Supplementary Table 6 The interaction terms for APOE-ε4 for the association between adherence to the Prudent dietary pattern, cognition and brain structure in people with T2D.

	Prudent dietary pattern [1]		
Cognitive function (n=340)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.21	-0.45, 0.03	0.09
Verbal memory, z-score	0.07	-0.16, 0.30	0.55
Visual memory, z-score	0.03	-0.20, 0.26	0.80
Executive function, z-score	-0.24	-0.48, -0.00	0.049*
Verbal fluency, z-score	0.01	-0.24, 0.26	0.91
Attention processing speed, z-score	-0.21	-0.44, 0.02	0.07
Visuospatial function, z-score	-0.12	-0.39, 0.15	0.38
Brain structural measures[2], mL (n=307)			
Grey matter volume, mL	1.63	-8.26, 11.52	0.75
White matter volume, mL	0.50	-9.61, 10.61	0.92
Left hippocampal volume, mL	-0.02	-0.13, 0.09	0.75
Right hippocampal volume, mL	-0.01	-0.13, 0.11	0.81
White Matter Hyperintensities volume, mL	0.12	-0.11, 0.34	0.32
Presence of small vessel disease (n=315)			
Micro-bleeds, (yes/no)	0.75	0.19, 2.98	0.69
Infarcts, (yes/no)	1.11	0.58, 2.15	0.75

[1]adjusted for age, sex, education, energy, mood, CVD risk score, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

Supplementary Table 7 The interaction terms for APOE-ε4 for the association between adherence to the Traditional dietay pattern, cognition and brain structure in people with T2D.

	Traditional dietary pattern [1]		
Cognitive function (n=331)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.04	-0.26, 0.18	0.71
Verbal memory, z-score	-0.10	-0.31, 0.09	0.31
Visual memory, z-score	-0.02	-0.23, 0.18	0.81
Executive function, z-score	0.05	-0.16, 0.26	0.66
Verbal fluency, z-score	0.06	-0.16, 0.29	0.57
Attention processing speed, z-score	-0.02	-0.23, 0.18	0.79
Visuospatial function, z-score	-0.02	-0.26, 0.21	0.84
Brain structural measures[2], mL (n=306)			
Grey matter volume, mL	0.94	-8.00, 9.87	0.84
White matter volume, mL	1.77	-7.38, 10.92	0.70
Left hippocampal volume, mL	-0.04	-0.14, 0.06	0.44
Right hippocampal volume, mL	-0.03	-0.14, 0.08	0.56
White Matter Hyperintensities volume, mL	0.04	-0.16, 0.25	0.69
Presence of small vessel disease (n=315)			
Micro-bleeds, (yes/no)	1.70	0.41, 6.98	0.46
Infarcts, (yes/no)	0.94	0.49, 1.81	0.86

[1]adjusted for age, sex, education, energy, mood, CVD risk score, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

Supplementary Table 8 The interaction terms for APOE-ε4 for the association between adherence to the Prudent dietary pattern, cognition and brain structure in people without T2D.

	Prudent dietary pattern [1]		
Cognitive function (n=324)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.20	-0.40, 0.00	0.05
Verbal memory, z-score	-0.20	-0.42, 0.01	0.07
Visual memory, z-score	0.04	-0.19, 0.26	0.76
Executive function, z-score	-0.33	-0.58, -0.09	0.008*
Verbal fluency, z-score	-0.11	-0.33, 0.12	0.35
Attention processing speed, z-score	-0.12	-0.34, 0.09	0.25
Visuospatial function, z-score	-0.11	-0.29, 0.08	0.26
Brain structural measures[2], mL (n=301)			
Grey matter volume, mL	7.87	-0.81, 16.55	0.08
White matter volume, mL	-6.18	-15.04, 2.69	0.17
Left hippocampal volume, mL	-0.06	-0.18, 0.05	0.27
Right hippocampal volume, mL	-0.10	-0.22, 0.02	0.09
White Matter Hyperintensities volume, mL	-0.21	-0.43, 0.01	0.06
Presence of small vessel disease (n=298)			
Micro-bleeds, (yes/no)	1.94	0.75, 5.05	0.17
Infarcts, (yes/no)	1.37	0.66, 2.82	0.40

[1]adjusted for age, sex, education, energy, mood, CVD risk score, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

Supplementary Table 9 The interaction terms for APOE-ε4 for the association between adherence to the Traditional dietary pattern, cognition and brain structure in people without T2D.

	Traditional dietary pattern [1]		
Cognitive function (n=331)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.10	-0.36, 0.16	0.47
Verbal memory, z-score	-0.11	-0.40, 0.17	0.44
Visual memory, z-score	0.20	-0.11, 0.50	0.20
Executive function, z-score	-0.32	-0.65, 0.01	0.06
Verbal fluency, z-score	-0.03	-0.32, 0.27	0.87
Attention processing speed, z-score	0.08	-0.20, 0.36	0.56
Visuospatial function, z-score	-0.18	-0.43, 0.06	0.14
Brain structural measures[2], mL (n=306)			
Grey matter volume, mL	13.39	2.19, 24.58	0.02*
White matter volume, mL	1.49	-9.93, 12.92	0.80
Left hippocampal volume, mL	0.01	-0.14, 0.16	0.87
Right hippocampal volume, mL	0.10	-0.06, 0.25	0.22
White Matter Hyperintensities volume, mL	-0.20	-0.49, 0.09	0.17
Presence of small vessel disease (n=315)			
Micro-bleeds, (yes/no)	1.09	0.29, 4.16	0.90
Infarcts, (yes/no)	1.89	0.68, 5.20	0.22

[1]adjusted for age, sex, education, energy, mood, CVD risk score, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

Supplementary Table 10 The interaction terms for APOE-ε4 for the association between adherence to the Western dietary pattern, cognition and brain structure in people without T2D.

	Western dietary pattern [1]		
Cognitive function (n=331)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.08	-0.27, 0.11	0.39
Verbal memory, z-score	0.05	-0.15, 0.26	0.60
Visual memory, z-score	-0.08	-0.30, 0.14	0.45
Executive function, z-score	0.05	-0.19, 0.29	0.70
Verbal fluency, z-score	-0.14	-0.35, 0.08	0.20
Attention processing speed, z-score	-0.03	-0.24, 0.17	0.75
Visuospatial function, z-score	-0.19	-0.37, -0.02	0.03*
Brain structural measures[2], mL (n=306)			
Grey matter volume, mL	-4.41	-12.84, 4.02	0.30
White matter volume, mL	1.94	-6.69, 10.56	0.66
Left hippocampal volume, mL	0.02	-0.09, 0.13	0.78
Right hippocampal volume, mL	-0.06	-0.17, 0.06	0.33
White Matter Hyperintensities volume, mL	0.14	-0.08, 0.35	0.22
Presence of small vessel disease (n=315)			
Micro-bleeds, (yes/no)	1.09	0.40, 2.98	0.86
Infarcts, (yes/no)	1.66	0.80, 3.45	0.18

[1]adjusted for age, sex, education, energy, mood, CVD risk score, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

4. Associations between the dietary inflammatory index, brain volume and small vessel disease

4.1 Preface

The results of chapter three revealed very few associations between data-driven dietary patterns cognition and brain. There was only one association between a Western dietary pattern and lower grey matter volume in people without T2D. However, it was not independent of cardiovascular risk factors. For data-driven dietary patterns, the analyses were performed separately in people with and without T2D as PCA results were different for people with T2D compared to those without T2D (Chapter 3). For the dietary indices (chapter 4 and 5), the analyses will be performed in the combined sample as the methods of calculation of indices were the same for each group.

Inflammation is a potential mechanism leading to lower brain volumes and poorer cognition (299). Prior studies have found that an inflammatory diet was negatively associated with cognitive function (145, 147, 300). To develop dietary interventions to improve brain health it is important to understand the brain pathways by which an unhealthy diet may affect cognition. This chapter will investigate if the energy-adjusted dietary inflammatory index (the inflammatory potential of diet) is associated with brain structure and cognitive function. The secondary aim of the study is to examine if T2D modifies any association.

Prior studies have investigated associations between an inflammatory diet and individual cognitive domains (145, 147, 300). Therefore, this chapter will only include a global cognitive composite score to compare results to the prior studies. Since there are no prior studies investigating the association between data-driven dietary patterns, the Australian dietary guidelines index and individual cognitive domains in people with T2D, the individual cognitive domains will only be considered in chapter 3 and 5. The text of this chapter and the supplementary material in the appendix is currently under review for publication in the *Journal of the Academy of Nutrition and Dietetics*.

4.2 Introduction

Approximately 47 million people worldwide live with dementia, which has a devastating effect on quality of life with no effective treatment (266). Diet is a modifiable risk factor that has attracted attention as a potential intervention to prevent or delay the onset of dementia (301). Dietary patterns that consist of high quantities of processed and refined food, processed meat and sweets are thought

to promote inflammation (302) and are associated with greater cognitive decline (303). Previous work from this group examined the associations between dietary patterns (data-driven dietary patterns and adherence to the Australian Dietary Guidelines) and measures of brain atrophy and cerebral small vessel disease (265, 304). However, it is unknown if there are any associations between an inflammatory diet and brain structure.

Brain atrophy and small vessel disease are considered biomarkers of dementia (40). Small vessel disease occurs as a result of abnormalities of small perforating arteries and arterioles supplying deep brain structures (22), and includes infarcts, white matter hyperintensities (WMH), and microbleeds (24-26). Neuro and systemic inflammation are implicated in the pathogenesis of small vessel disease and subsequent cognitive decline (305, 306). Brain atrophy, particularly in the hippocampus is also associated with cognitive decline (307). According to animal studies, obesity-induced inflammation mediates hippocampal synaptic dysfunction (308). It is therefore plausible that a pro-inflammatory diet may contribute to poorer brain health.

The energy-adjusted dietary inflammatory index (E-DII®) is an evidence-based, literature-derived score calculated using data from standard dietary assessments, and aims to reflect the inflammatory potential of an individual's diet (309). Construct validity of the E-DII score has been previously conducted against markers of systemic inflammation (228). Previous studies found that higher E-DII scores (more inflammatory potential) are associated with cognitive decline (145, 300), poorer memory (145, 147), and reduced executive function (147). Brain atrophy and cerebral small vessel disease are known to be on the pathway to cognitive dysfunction (26). However, it is unclear if the E-DII is associated with the brain structural measures. Knowledge regarding the association between E-DII scores and brain structure contributes to the understanding of the mechanisms by which an inflammatory diet affects cognitive function.

Therefore, the aim of this study was to examine the associations between E-DII scores and brain volume, markers of cerebral small vessel disease and global cognitive function using data from the Cognition and Diabetes in Older Tasmanians (CDOT) Study. The hypothesis was that there would be an association between higher E-DII scores and poorer brain health. The CDOT study was designed to address the aim of whether type 2 diabetes (T2D) was associated with lower cognition and to understand the underlying brain mechanisms involved (61). However, there is limited knowledge as to whether the associations between an inflammatory diet and brain health could be different based on T2D status. People with T2D have lower brain volumes than those without T2D (61). It is therefore

plausible that the brain may be more susceptible to a poor diet in those with T2D. Approximately half the participants in the CDOT study had T2D, which allowed this study to explore if T2D was an effect modifier of any observed associations.

4.3 Material and Methods

Participants

Participants with (n=326) and without T2D (n=315) were recruited between 2005 and 2011 as previously described (61). Participants over 55 years of age with T2D were enrolled from the Australian National Diabetes Service Scheme (response rate 38%), and people without T2D aged ≥60 years were randomly selected from the Southern Tasmanian electoral roll (61). The diagnostic criteria for T2D were random plasma glucose ≥ 199.8 mg/dL (11.1 mmol/L), fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L), or 2-h glucose ⩾ 199.8 mg/dL (11.1 mmol/L) after oral glucose tolerance test.

Appendix 4.1 Supplementary Figure 1 shows the diagram for the recruitment of the participants. Participants with and without T2D were not matched for age or sex. Participants were excluded if they lived in a nursing home, had any contraindication to Magnetic Resonance Imaging (MRI) or were diagnosed with dementia. Ethics approval was obtained from the Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee (Ethics reference numbers: H0009400 and H7947). Written informed consent was obtained from all participants.

Dietary Assessment

Dietary assessment was performed using a paper version of the Dietary Questionnaire for Epidemiological Studies version 2 (DQESv2), a semi-quantitative food frequency questionnaire (FFQ) developed by the Cancer Council of Victoria to measure diet in an ethnically diverse Australian population (216). Dietary assessment and brain MRI measurements were completed within two weeks of each other. A previous Australian study has reported the reliability, validity and reproducibility of the DQESv2 in adults with T2D (219). The inflammatory potential of diet was previously captured using this questionnaire (310). Participants estimated their intake of 80 items (74 foods and 6 alcoholic beverages) over the previous year, using 10 response options ranging from “never” to “3 or more times per day”. A series of photographs in the FFQ were used to illustrate options for usual portion sizes. The FFQ also included short questions on the types of bread, milk, cheese and spread usually consumed. The FFQ was self-administered but the responses were checked by a research

assistant to make sure there were no missing responses. Nutrient and total energy intakes and the amounts of each item consumed (grams per day) were calculated by the Cancer Epidemiology Centre (Cancer Council Victoria) using a food composition database known as NUTTAB that contains data on the nutrient content of Australian foods (311).

Calculation of dietary inflammatory index

A detailed description of the E-DII method of calculation and its validation has been published elsewhere (228, 309). The E-DII consists of 45 food parameters based on findings from 1,943 articles that were reviewed and scored for their associations with six inflammatory biomarkers (C-reactive protein (CRP), interleukin-1β, interleukin-4, interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and interleukin-10) (309).

As in many studies, the E-DII was based on fewer (27) than the full list of 45 food parameters, as the FFQ did not have questions to obtain 18 of the food parameters. The included food parameters were as follows: alcohol, carbohydrate, protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, omega 3, omega 6, cholesterol, onion, garlic, vitamin D, vitamin E, vitamin A (retinol), beta-carotene, vitamin C, thiamine, riboflavin, niacin, B6, B12, folate, fibre, iron, magnesium, and zinc. In prior studies, the E-DII was correlated with systematic inflammatory markers even when calculated based on seventeen (312), twenty-five (313), and twenty-six food parameters (147).

As in prior studies (145, 147, 300), dietary intakes were first standardised. This was done by subtracting a standard global mean for each parameter (from a representative world database) (309) from the individual's reported dietary intake and then dividing this by the global standard deviation. These standardised z-scores were converted to percentiles to minimise the effect of outliers. This value was then doubled, and one was subtracted to achieve an approximately symmetrical distribution centred on 0, with values from -1 to +1. The centred percentile scores for each nutritional parameter were weighted to reflect the strength of epidemiological evidence for the pro- or anti-inflammatory effect of that parameter. The weights were based on the number of studies that informed the diet-inflammatory marker relationships in the systematic review (309), with higher weight given to human experimental and prospective cohort studies. Then the scores were summed across all food parameters, to obtain the overall E-DII score. The E-DII was calculated per 1000 kcal (4,180 kJ) energy content of food parameters consumed. In this study, the score was calculated by the original authors of the E-DII (309). Higher E-DII values reflect a more pro-inflammatory diet. In this study, the E-DII score

ranged from –4.7 to 4.5 points, which is comparable to those found in other studies, such as a subsample of the Whitehall II study (−3.35 to 4.23, aged 35-55y) (314), and a nested case-control study within the Northern Sweden Health and Disease Study (−4.16 to 5.04, aged 25-74y) (315).

MRI acquisition

A single 1.5-T General Electric® scanner was used with the following sequences: high-resolution T1-weighted spoiled gradient echo (repetition time [TR] 35 ms, echo time [TE] 7 ms, flip angle 35°, field of view 24 cm, 120 contiguous slices, isotropic voxel size 1 mm3), T2-weighted fast spin echo (TR 4,300 ms, TE 120 ms, number of excitations 1, turbo factor 48, voxel size 0.90 x 0.90 x 3 mm); fluid attenuated inversion recovery (TR 8,802 ms. TE 130 ms, inversion Lime 2,200 ms. voxel size 0.50 x 0.50 x 3 mm); gradient echo (TR 0.8 ms, TE 0.015, flip angle 30°, voxel size 0.9 x 0.9 x 7 mm).

Brain volume measures

Registered 3-dimensional T1 and gradient echo sequences were applied in a multispectral segmentation process (61). The tissue probability maps of grey and white matter were created using Statistical Parametric Mapping® version 5 software. The images were corrected for the volume change that occurred in the result of the normalisation process. Maps of white matter were created by removing WMH from the tissue probability maps. Tissue maps were smoothed using voxel-based morphometric analysis, and isotropic Gaussian kernel with a full width at half maximum 8 mm. Subsequently, voxel counting algorithms were used to calculate tissue volumes of the segmented areas for total grey and normal-appearing white matter. Volumes of hippocampi were determined based on manual tracings performed by a single expert using an established method (253).

Small vessel disease

Fully automated WMH segmentation on T2 fluid-attenuated inversion recovery sequences (316) was performed using a validated method (249). A voxel counting algorithm calculated WMH volume. Presence of microbleeds and infarcts was determined by a single trained rater and confirmed by a neurologist and geriatrician. Infarct and microbleeds were defined based on previously described criteria (61).

Cognitive Assessment

Cognitive function was assessed using a battery of validated neuropsychological tests as follows: the Rey-Osterrieth Complex Figure and a delayed reproduction after 20 minutes (238); the Hopkins Verbal Learning Test (immediate, delay, recognition) (238); the Controlled Oral Word Associations

Tests (letter and animal categories) (238); the Victoria Stroop tests (dot, word, colour) (238); the Digit Span, Digit Symbol Coding and the Symbol Search subsets of the Wechsler Adult Intelligence Scale-Third Edition (246). A z-score for each cognitive test was calculated as the individual's raw score minus the sample's mean score and then divided by the sample standard deviation for each test. The global cognitive domain was calculated as the standardised mean of all tests.

Other Measures

Information on demographics, medical history (including smoking history, hypertension, angina, stroke, and hyperlipidaemia) and medications used were obtained using standardised questionnaires. Mood was assessed with the 15-item Geriatric Depression Scale (263). A research assistant measured height and weight once. Weight was measured wearing light clothing and without shoes using a Heine portable scale. Height measurements were performed with a Leicester stadiometer without shoes. Body mass index was calculated by dividing weight (kilograms) by height (metres) squared. Blood pressure was measured using an Omron M4 sphygmomanometer as the mean of three consecutive seated brachial blood pressure measures from the right arm. Ambulatory activity was measured using a Yamax^{TM} pedometer over 7 days. Venous blood samples were taken from the antecubital fossa after overnight fasting. Biochemistry analysis of fasting plasma glucose, insulin, lipid profile, glycated haemoglobin A_{1c}, CRP, IL-6, and TNF-α were performed. A continuous cardiovascular disease (CVD) risk score was generated by allocating 1 point for the presence of each of the CVD risk factors including obesity (body mass index≥30), hypertension, hypercholesterolemia, history of past and current smoking, angina, and stroke, and summing the points to give an overall score. A continuous anti-inflammatory medication score was derived by summing current use of anti-inflammatory medications (aspirin, oral steroid, non-steroidal anti-inflammatory drugs, fish oil, disease-modifying anti-rheumatic drugs, and metformin).

Statistical Analysis

Non-normally distributed variables were transformed as appropriate. Means (for continuous variables), and proportions (for categorical variables) were used to describe demographic, MRI and cognitive variables across quintiles of E-DII. To confirm the validity of E-DII, age- and sex-adjusted regression analyses were performed to determine associations between E-DII and inflammatory blood markers.

Multivariable linear regression analysis was used to examine the associations between E-DII score and each brain MRI variable and global cognition initially adjusted for age, sex, education and total

energy (model 1). The MRI variables (except for microbleeds and infarcts) were additionally adjusted for height to reflect brain size. Binomial logistic regression was performed to determine the association between the E-DII score and binary MRI variables (presence or absence of infarcts and microbleeds). Model 2 was adjusted for the covariates in model 1 with the addition of mood, ambulatory activity, T2D, the CVD risk and anti-inflammatory medication scores. To examine if T2D was an effect modifier of any association, an E-DII × T2D product term was entered to each model. For ambulatory activity, 44 of 641 participants had missing data. These were imputed using Multiple Imputation by Chained Equations under the missing-at-random assumption (317). Fifty imputed datasets were generated using age, sex, T2D status, education, depression, and a CVD risk score. Statistical analyses were performed using STATA® Version 14.2 (318).

4.4 Results

Out of the 706 people that participated, 705 had cognitive assessment, 689 had completed the DQES-v2 dietary questionnaire, 655 people had brain MRI scans, and 641 participants had cognitive testing, MRI scans, and dietary data **(See Appendix 4.1 Supplementary Figure 1).** The mean (SD) age of participants was 67.7 (6.9) years for people with T2D, and 72.1 (7.2) years for those without T2D. The mean (SD) duration of T2D was 9.2 (9.7) years, and 23% were receiving insulin therapy. Mean (SD) of E-DII was lower in people with T2D -0.2 (1.4) compared to those without T2D -0.5 (1.3) *(P<0.05)*. Demographic and clinical characteristics of participants presented across quintiles of E-DII scores are shown in **Table 4.1** for the 641 people included in the primary analyses. Participants with a more inflammatory diet (Q5) were more likely to be male, have lower education, have a history of stroke or hypertension, use blood pressure medications, and have higher triglycerides levels, energy intake and inflammatory biomarker (CRP) levels compared to those with a lower inflammatory diet (Q1). Participants with a more inflammatory diet (Q5) also had fewer daily steps, lower fasting blood glucose, were less likely to have T2D, a history of hyperlipidaemia or angina or use cholesterol-lowering medications compared to those with a lower inflammatory diet (Q1). The mean brain MRI and global cognitive score of participants across quintiles of E-DII scores are shown in **Table 4.2**. E-DII was positively associated with CRP: (β=0.06 95%CI 0.01, 0.1 *P*=0.03), and IL-6: (β=0.06 95%CI 0.00, 0.1 *P*=0.04), but not TNF-α: (β=0.03 95%CI -0.03, 0.08 *P*=0.32).

Associations between E-DII, brain structure and global cognitive function

The E-DII score was not associated with any of the brain variables or global cognition in model 1 or the fully adjusted model 2 (**Table 4.3)**. There also was no significant modification effect for T2D in

any of the models, except for the association between E-DII and grey matter volume (T2D: β= 1.38 95%CI -3.03, 5.79; without T2D: β= -4.34 95%CI -8.52, -0.16; *P* for interaction term =0.03). The interaction terms for T2D x E-DII are shown in **Appendix 4.2 Supplementary Table 1**. Trends across quintiles of E-DII were tested as a sensitivity analysis. There was no trend across E-DII quintiles and our outcome variables **(see Appendix 4.3 supplementary Table).**

Table 4.1 Characteristics of participants across energy-adjusted dietary inflammatory index (E-DII) quintiles

Dietary Inflammatory Index Quintiles (n=641)	Total n=641	Q1 <-1.5 n=129	Q2 ≥-1.5 to <-0.70 n=128	Q3 ≥-0.70 to <-0.01 n=128	Q4 ≥-0.01 to <0.82 n=128	Q5 ≥0.82 n=128
Characteristics	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Male	363 (57)	64 (50)	72 (56)	64 (50)	74 (58)	89 (70)
Type 2 diabetes	326 (51)	71 (55)	73 (57)	57 (45)	71 (56)	54 (42)
History of Stroke	50 (8)	7 (5)	12 (9)	8 (6)	7 (6)	16 (13)
History of Hyperlipidaemia	359 (56)	73 (57)	78 (61)	71 (56)	73 (57)	64 (50)
History of Hypertension	382 (60)	66 (51)	79 (62)	83 (65)	80 (63)	74 (58)
Past and current smoker	330 (52)	64 (50)	64 (50)	67 (52)	66 (52)	69 (54)
History of Angina	87 (14)	21 (16)	14 (11)	19 (15)	18 (14)	15 (12)
Blood pressure medication	397 (67)	71 (59)	77 (64)	89 (73)	77 (65)	83 (73)
Cholesterol lowering medication	301 (53)	66 (57)	67 (56)	53 (48)	62 (54)	53 (50)
Education (above high school)	306 (48)	63 (49)	63 (49)	61 (48)	58 (45)	61 (48)
	Mean±SD	Mean±SD	Mean±SD	Mean± SD	Mean± SD	Mean±SD
Age, years	69.8 ± 7.4	69.4±6.7	70.1±7.9	70.8±7.6	69.2±7.3	69.7±7.3
Body mass index (kg/m^2)	29.1±7.1	29.3±12.1	28.9±4.9	28.8±4.8	28.9±5.5	29.6±5.2
Mean steps per day [1]	6,151±3373	6,464±3,295	5,943±3,197	5,907±2,883	6,748±4,128	5,729±3,187

Triglycerides (mg/dL) [2]	130.6±67.8	128.8±64.1	133.8±72.0	119.2±49.8	125.9±63.8	146.1±83.4
Total cholesterol (mg/dL) [3]	188.4±44.5	187.9±50.5	187.4±43.2	189.2±44.6	188.9±42.3	188.6±41.9
Energy intake (kcal/day) [4]	7498±2896	1,520±504	1,655±514	1,739±604	1,980±751	2,106±869
C-reactive protein (mg/L)	3.5±7.3	2.6±3.6	3.7±7.7	2.9±2.6	4.4±12.5	4.0±5.7
Tumour necrosis factor α (mg/ml)	1.9±2.2	1.7±2.0	1.8±2.5	2.1±2.5	2.1±2.5	1.8±1.8
Interluekin-6 (mg/L)	1.8±2.7	1.6±2.4	1.8±2.2	1.7±2.1	2.1±4.2	1.7±1.7
Fasting blood glucose (mg/dL) [5]	117.2±36.2	119.2±40.3	123.3±48.1	112.6±28.1	116.6±28.4	113.7±30.1
Systolic blood pressure (mmHg)	139.1±20.5	138.5±21.3	138.6±20.9	140.6±19.0	138.2±20.9	139.4±20.6
Diastolic blood pressure (mmHg)	78.4±11.3	77.4±12.6	77.6±11.1	78.5±8.9	77.7±10.2	80.5±13.2

[1]570/641 people had physical activity data. [2] To convert mg/dL triglycerides to mmol/L, multiply mg/dL by 0.0113. To convert mmol/L triglycerides to mg/dL, multiply mmol/L by 88.6. Triglyceride of 128.8 mg/dL=1.46 mmol/L. [3] To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.0259. To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.7. Cholesterol of 187.9 mg/dL=4.87 mmol/L. [4] To convert kcal energy to kJ, multiply kcal by 4.184. Energy of 1,520 kcal=6360kJ [5] To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.0555. To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0. Glucose of 119.2 mg/dL=6.62 mmol/L.

Table 4.2 Structural brain magnetic resonance imaging variables and general cognition across quintiles of the energy-adjusted dietary inflammatory index (E-DII) scores

Energy-adjusted dietary inflammatory index quintiles (n=641) [1]	Q1 <-1.5 n=129	Q2 ≥-1.5 to <-0.70 n=128	Q3 ≥-0.70 to <-0.01 n=128	Q4 ≥-0.01 to <0.82 n=128	Q5 ≥0.82 n=128
Characteristics	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Cognitive composite z-score	-0.05±0.9	0.2±0.9	0.07±0.9	0.09±0.9	-0.1±0.9
Grey matter volume, mL	580.5±66.5	579.8±55.6	579.9±67.7	587.4±58.6	588.1±69.4
White matter volume, mL	454.2±58.0	453.1±52.2	457.5±52.0	458.8±60.8	459.3±67.2
Left hippocampal volume, mL	2.4±0.5	2.4±0.5	2.4±0.5	2.4±0.5	2.5±0.6
Right hippocampal volume, mL	2.5±0.5	2.5±0.5	2.5±0.5	2.6±0.5	2.6±0.6
WMH volume [2], mL	5.8±6	5.7±5.9	7.5±9.6	5.9±6.1	7.4±8.8
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Microbleeds, n (%)	6 (5)	6 (5)	10 (8)	5 (4)	9 (7)
Infarcts, n (%)	27 (21)	25 (20)	30 (23)	21 (17)	22 (17)

[1] Means and SD have not been adjusted for covariates [2] WMH: white matter hyperintensities

Table 4.3 Associations between the energy-adjusted dietary inflammatory index (E-DII), brain magnetic resonance imaging and cognition

	Energy-adjusted dietary inflammatory Index [1]					
	Model 1			Model 2		
Brain health variables (n=641)	β	95%CI	*P*	β	95%CI	*P*
Cognitive composite z-score	0.01	-0.04, 0.06	0.70	0.01	-0.04, 0.06	0.70
Grey matter volume, mL	-1.15	-4.30, 2.00	0.47	-1.70	-4.83, 1.43	0.29
White matter volume, mL	-1.16	-4.18, 1.87	0.45	-2.14	-5.18, 0.90	0.17
Left hippocampal volume, mL	0.02	-0.01, 0.05	0.18	0.005	-0.02, 0.03	0.69
Right hippocampal volume, mL	0.02	-0.01, 0.05	0.28	0.0005	-0.03, 0.03	0.97
White matter hyperintensities, mL	0.02	-0.03, 0.07	0.52	0.008	-0.04, 0.06	0.77
	OR	95%CI	*P*	OR	95%CI	*P*
Microbleeds, yes/no	1.05	0.82, 1.34	0.73	1.03	0.80, 1.32	0.82
Infarcts, yes/no	0.93	0.80, 1.08	0.37	0.92	0.79, 1.07	0.28

[1] Model 1 adjusted for age, sex, education, and energy. All the models were also adjusted for height (measure of brain size) except for cognitive score, microbleeds, and infarcts. Model 2 adjusted for Model 1 plus an additional adjustment for mood, type 2 diabetes , cardiovascular score, ambulatory physical activity, and anti-inflammatory medication score.

4.5 Discussion

To our knowledge, this is the first study to investigate associations between E-DII score and brain structure. There was no evidence of any association between E-DII score and measures of brain volume, cerebral small vessel disease or global cognition. However, in one of the seven brain MRI outcomes there was a statistically significant modification effect for T2D status on the associations between E-DII score and grey matter volume such that the effect was stronger in people without T2D.

It was hypothesised that a higher E-DII score would be associated with lower brain volume and a greater burden of small vessel disease based on prior studies that found associations between Western diets and lower hippocampal volume (118) or poorer cognition (115, 319). For example, higher adherence to a Western diet (processed meat, chips, crisps and sweets) was associated with lower hippocampal volume even after adjusting for CVD risk factors (118). Contrary to the hypothesis, there were no associations between E-DII score and grey and white matter volume or cerebral small vessel disease. Interestingly, in line with findings from this study, a prior study did not find any association between E-DII score and cerebrovascular disease (incidence of endarterectomy of neck arteries post stroke or transient ischaemic attack) or stroke over 11 years (320). Null results may have been due to the relatively low burden of WMH (median volume 3.81mL), microbleeds (19.5%) or infarcts (5.5%) in the present study or the low incidence of cerebrovascular disease (0.9%) or stroke (0.6%) in the prior study (320).

Additionally, a secondary hypothesis was that T2D would modify associations between the E-DII and brain structure such that associations would be stronger in those with T2D. This hypothesis was based on the knowledge that T2D is associated with lower grey matter volume and a higher burden of small vessel disease (61). There was only one significant modification effect for T2D on the association between E-DII and grey matter volume. Surprisingly, the association was stronger among people without T2D, which was in the opposite direction than expected. However, this should be viewed with caution as it was one of seven outcomes and the effect appeared small. This was consistent with previous results from the same dataset, where little evidence was found for association between data-driven dietary patterns, and adherence to the Australian Dietary Guidelines and brain MRI (265, 304).

Although there were weak positive associations between E-DII scores and CRP and IL-6, the E-DII score was not significantly associated with global cognitive function in this study. This is in contrast to prior studies in which higher E-DII scores were associated with lower global cognition (145),

memory (145, 147), and executive function (147). The Supplementation with Antioxidant Vitamins and Minerals study included 3080 participants aged 45-60 years with 13 years follow-up (145). The National Health and Nutrition Examination Survey was cross-sectional and included 1,723 older adults who were 60–85 years of age (147). Differences in findings may be because these studies both used multiple 24-hour dietary recalls, which are more accurate methods of dietary assessment compared to a single FFQ (145, 147). Moreover, the effect may be stronger if the cumulative effect of diet is examined over time (145). Furthermore, previous studies used cognitive composite scores with different cognitive tests to the present study (145, 147). Therefore, the different tests included in these studies to assess cognition may be another reason for the varied findings.

Strengths of this study include the use of brain MRI, to allow the investigation of potential pathways of an inflammatory diet on brain health for the first time. Having two cohorts of people; i.e., with and without T2D, allowed us to investigate if any observed associations differed by T2D status. The E-DII has been validated previously and correlated with a variety of inflammatory markers in multiple studies (312, 321, 322). Furthermore, diet was assessed using a validated questionnaire.

Despite its strengths, this study had some limitations. As noted, the data were cross-sectional, and therefore, it was not possible to infer causality based on temporality. This is an important consideration given the fact that diet can change over time and it would be expected that long-term habitual exposures would be the important determinants of brain volume. The E-DII may be subject to some limitations, such as the score only partly reflecting the knowledge of inflammatory effects of individual foods, and the scoring relies on weightings based on study design. FFQs have known measurement error, including dietary misreporting due to social desirability (323-325), which may lead to underestimation of the associations between E-DII and cognitive and brain structural outcomes. Additionally, the FFQ used in this study did not include all parameters included in the original E-DII score (27 of the 45 possible parameters were available). The items not included were selenium, eugenol, ginger, saffron, trans fatty acids, turmeric, tea, flavan-3-ol, flavones, flavanols, flavanones, anthocyanidins, isoflavones, pepper, thyme, oregano, rosemary, and caffeine. These items are mostly consumed in low amounts and all of them are anti-inflammatory except for trans fatty acids (309). Therefore, E-DII scores may have underestimated the anti-inflammatory properties of participants' diets in this study (229).

In conclusion, there was little cross-sectional evidence of an association between E-DII score and brain volume, cerebral small vessel disease or cognitive function in a cohort of participants with and

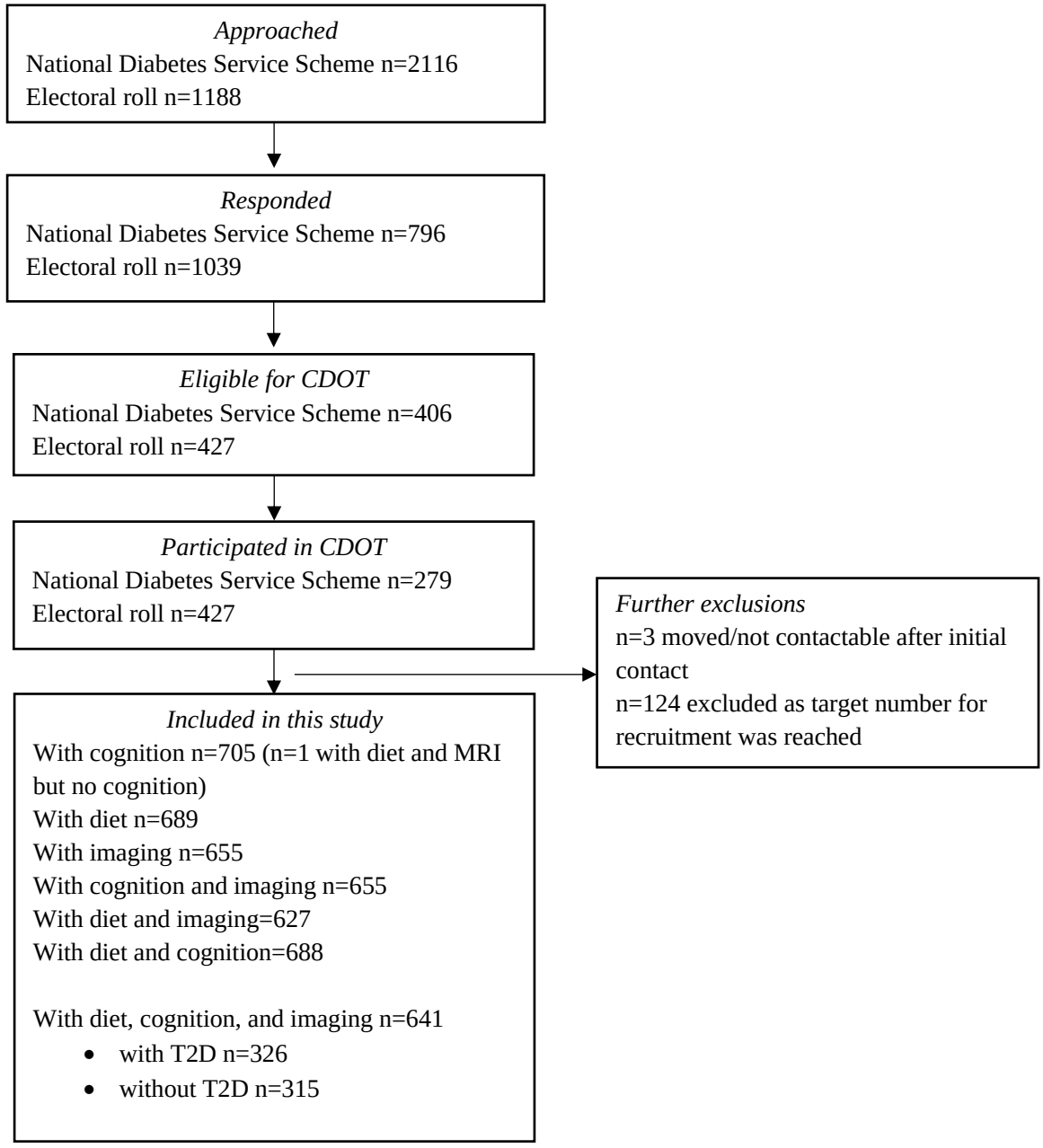
without T2D. There was a modification effect for T2D on the association between the E-DII and grey matter volume, but the effect size was small. The E-DII score was based on fewer (27) than the possible 45 food parameters, a number which is average when dietary data are collected by FFQs. Future studies should use 24-hour recall or a more comprehensive dietary assessment method. Future studies, especially of prospective design, are needed to examine the relationship between E-DII score and brain structure and function.

4.6 Postscript

Apart from one significant effect modification observed among multiple models, there were no significant associations between the E-DII and brain structure. The significant modification effect suggested that the association between a higher E-DII score and lower grey matter volume was stronger in people without T2D. This association was independent of vascular health factors and anti-inflammatory medication use.

It is important to promote healthy dietary behaviours to protect the brain against cognitive decline, and dementia. The Australian Dietary Guidelines provide dietary recommendations for health and wellbeing. The next chapter of this thesis will examine whether adherence to the Australian Dietary Guidelines is associated with cognitive domains and brain structure in people with and without T2D. The secondary aim of the study is to examine if T2D modifies any associations

Appendix 4.1 Supplementary Figure 1 Participant recruitment diagram



Appendix 4.2 Supplementary Table 1 Interactions between type 2 diabetes and energy-adjusted dietary inflammatory index

Brain variables (n=641)	β for T2D[1] x E-DII[2] Interaction terms[3]	95% CI	*P*
General cognition z-score	-0.01	-0.09, 0.08	0.09
Grey matter volume, mL	6.33	0.51, 12.15	0.03
White matter volume, mL	5.02	-0.66, 10.70	0.08
Left hippocampal volume, mL	-0.03	-0.08, 0.02	0.24
Right hippocampal volume, mL	0.002	-0.05, 0.06	0.93
White matter hyperintensities, mL	0.02	-0.08, 0.12	0.64
	OR for T2D[1] x E-DII[2] Interaction terms[3]	95% CI	*P*
Microbleeds	1.09	0.75, 1.36	0.95
Infarcts	0.89	0.54, 1.49	0.66

[1] T2D: type 2 diabetes, [2] E-DII: energy-adjusted dietary inflammatory index, [3] Models were adjusted for age, sex, education, energy, mood, type 2 diabetes , cardiovascular score, ambulatory physical activity, and anti-inflammatory medication score. All the models were also adjusted for height (measure of brain size) except for cognitive score, microbleeds, and infarcts.

Appendix 4.3 Supplementary Table 2 Association between E-DII cognition and brain volumes across E-DII quintiles

	Energy-adjusted dietary inflammatory index (E-DII) quintiles[1] (n=641)					
	Model 1			Model 2		
	β	95%CI	*P*	β	95%CI	*P*
Cognitive composite z-score						
E-DII Quintile 2	**0.25**	**0.05, 0.45**	**0.02**	**0.27**	**0.08, 0.46**	**0.01**
E-DII Quintile 3	0.16	-0.05, 0.36	0.13	0.16	-0.04, 0.35	0.12
E-DII Quintile 4	0.14	-0.06, 0.35	0.18	0.18	-0.02, 0.04	0.08
E-DII Quintile 5	0.05	-0.16, 0.27	0.61	0.05	-0.16, 0.26	0.63
E-DII continuous (trend)	0.00	-0.05, 0.05	0.96	0.00	-0.04, 0.05	0.93
Grey matter volume, mL						
E-DII Quintile 2	-2.21	-14.98, 10.57	0.74	-1.31	-13.85, 11.24	0.84
E-DII Quintile 3	1.52	-11.45, 14.49	0.82	1.13	-11.62, 13.89	0.86
E-DII Quintile 4	4.57	-8.68, 17.83	0.50	4.47	-8.57, 17.50	0.50
E-DII Quintile 5	-2.08	-15.51, 11.34	0.76	-3.40	-16.71, 9.92	0.62
E-DII continuous (trend)	0.27	-2.76, 3.30	0.86	-0.08	-3.08, 2.93	0.96
White matter volume, mL						
E-DII Quintile 2	-2.86	-15.17, 9.45	0.65	-4.09	-16.33, 8.14	0.51
E-DII Quintile 3	3.71	-8.79, 16.21	0.56	1.67	-10.77, 14.11	0.79
E-DII Quintile 4	1.20	-11.57, 13.97	0.85	-0.11	-12.83, 12.60	0.99
E-DII Quintile 5	-3.66	-16.60, 9.27	0.58	-7.12	-20.10, 5.87	0.28
E-DII continuous (trend)	-0.29	-3.21, 2.63	0.85	-0.99	-3.92, 1.95	0.51
Left hippocampal volume, mL						
E-DII Quintile 2	-0.01	-0.13, 0.11	0.90	-0.02	-0.13, 0.09	0.75
E-DII Quintile 3	-0.03	-0.15. 0.09	0.63	-0.07	-0.18, 0.04	0.23
E-DII Quintile 4	0.03	-0.09, 0.15	0.67	0.00	-0.11, 0.12	0.94
E-DII Quintile 5	0.05	-0.07, 0.18	0.39	-0.02	-0.13, 0.09	0.79
E-DII continuous (trend)	0.01	-0.01, 0.04	0.34	-0.00	-0.03, 0.02	0.91
Right hippocampal volume, mL						

E-DII Quintile 2	-0.02	-0.15, 0.11	0.78	-0.02	-0.14, 0.09	0.68
E-DII Quintile 3	-0.03	-0.16, 0.10	0.64	-0.07	-0.19, 0.05	0.23
E-DII Quintile 4	0.05	-0.09, 0.18	0.51	0.02	-0.10, 0.14	0.74
E-DII Quintile 5	0.04	-0.10, 0.17	0.60	-0.04	-0.17, 0.08	0.53
E-DII continuous (trend)	0.01	-0.02, 0.04	0.40	-0.00	-0.03, 0.02	0.78
White matter hyperintensities volume, mL						
E-DII Quintile 2	0.03	-0.19, 0.24	0.80	-0.01	-0.22, 0.20	0.92
E-DII Quintile 3	0.02	-0.19, 0.24	0.83	-0.01	-0.23, 0.20	0.91
E-DII Quintile 4	0.00	-0.22, 0.23	0.98	-0.02	-0.24, 0.20	0.88
E-DII Quintile 5	0.05	-0.17, 0.28	0.63	0.01	-0.22, 0.23	0.96
E-DII continuous (trend)	0.00	-0.04. 0.06	0.74	0.00	-0.05, 0.05	0.99
	OR	95%CI	*P*	OR	95%CI	*P*
Microbleeds, yes/no						
E-DII Quintile 2	1.07	0.34, 3.34	0.91	1.07	0.34, 3.33	0.91
E-DII Quintile 3	1.48	0.50, 4.39	0.48	1.43	0.48, 4.26	0.52
E-DII Quintile 4	1.06	0.32, 3.48	0.93	1.04	0.31, 3.46	0.95
E-DII Quintile 5	1.34	0.43, 4.16	0.62	1.26	0.40, 3.98	0.69
E-DII continuous (trend)	1.06	0.82, 1.36	0.65	1.05	0.81, 1.35	0.73
Infarcts, yes/no						
E-DII Quintile 2	1.01	0.55, 1.86	0.96	0.94	0.51, 1.75	0.85
E-DII Quintile 3	1.00	0.54, 1.86	0.99	0.96	0.51, 1.80	0.89
E-DII Quintile 4	0.82	0.43, 1.57	0.55	0.74	0.38, 1.44	0.38
E-DII Quintile 5	0.71	0.36, 1.38	0.31	0.66	0.33, 1.32	0.24
E-DII continuous (trend)	0.92	0.79, 1.06	0.25	0.90	0.77, 1.05	0.18

[1]Model 1 adjusted for age sex, education, height, and energy. Model 2 adjusted for Model 1 plus an additional adjustment for mood, type 2 diabetes , cardiovascular score, ambulatory physical activity, and anti-inflammatory medication score.

Appendix 4.4 Additional analysis

APOE-ε4 is a genetic risk factor for developing dementia. In accordance with chapter 6, this section aims to investigate the interaction for APOE-ε4 for the associations between the E-DII, cognition and brain structure. The following table lists the interaction terms beta coefficients, 95% confidence interval and p-values of interaction terms. The effect sizes for APOE-ε4 interactions were quite small and there was no consistent pattern across all the cognition and brain variables.

Supplementary Table 3 The interaction terms for APOE-ε4 for the association between E-DII, cognition and brain structure in people with and without T2D.

	The energy adjusted dietary inflammatory index (E-DII)		
	β for interaction terms[1]	95% CI	*P* value
Global cognition, z-score (n=614)	0.05	-0.05, 0.15	0.31
Brain structural measures[2], mL (n=601)			
Grey matter volume, mL	-1.78	-8.24, 4.68	0.59
White matter volume, mL	1.61	-4.71, 7.94	0.62
Left hippocampal volume, mL	0.02	-0.03, 0.08	0.47
Right hippocampal volume, mL	0.01	-0.05, 0.07	0.63
White Matter Hyperintensities volume, mL	0.06	-0.05, 0.17	0.28
Presence of small vessel disease (n=614)			
Micro-bleeds, (yes/no)	0.81	0.49, 1.34	0.41
Infarcts, (yes/no)	0.98	0.70, 1.36	0.88

[1]adjusted for age, sex, education, energy, height, mood, type 2 diabetes, cardiovascular score, ambulatory physical activity, and anti-inflammatory medication score. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease.

5. Adherence to the Australian Dietary Guidelines is not associated with brain structure or cognitive function in older adults

5.1 Preface

The results of chapter four revealed very few associations between the dietary inflammatory index cognitive function and brain structure. There was only one significant modification effect which suggested that the association between the higher energy-adjusted dietary inflammatory index and lower grey matter volume was stronger in people without T2D.

It is essential to evaluate the Australian Dietary Guidelines against brain health outcomes. This chapter will examine whether adherence to the Australian Dietary Guidelines is associated with cognitive domains and brain structure. The text of this chapter and the supplementary material in the appendix have been published in *the Journal of Nutrition* (304).

5.2 Introduction

Dementia is a major public health concern that affects approximately 47 million people around the globe (326). Dementia is characterised by a wide range of neuropsychological and behavioural problems that interfere with an individual's quality of life. Since there is no established cure for dementia, prevention is an urgent priority (41). Diet is an important modifiable risk factor for dementia (327). To date much of the research regarding diet and brain health has been conducted on single foods or nutrients, whereas the dietary pattern approach has been less researched in the field (291). There are two main approaches for dietary patterns: the data-driven approach (265) and diet quality indices.

Diet quality indices are particularly important as they often reflect adherence to national dietary guideline recommendations, and the best diet for health and wellbeing (126). Few studies have examined the associations between adherence to these guidelines and measures of brain health such as cognitive function or brain structure on Magnetic Resonance Imaging (MRI). Higher adherence to the United States Department of Agriculture Dietary Guidelines (2005 and 2010) was associated with better global cognition (148, 149), verbal learning (150) and memory (150) in older adults. Moreover, greater adherence to the Dutch dietary guidelines was associated with larger brain volume in generally healthy older adults (151). In Australia, there has only been one study that examined associations

between adherence to the Australian Dietary Guidelines and cognition, and this was in children (328); this study indicated that higher adherence to the Australian Dietary Guidelines in early life is associated with better cognitive ability later in childhood. Knowledge regarding associations between adherence to the Australian Dietary Guidelines and both cognition and brain structure may help to develop recommendations regarding the optimal diet to maintain brain health.

Type 2 diabetes (T2D) doubles the risk of developing dementia (329) and is associated with lower cerebral grey and white matter, as well as poorer cognitive performance (61, 330). However, it is not known if associations between diet quality indices and brain health differ among participants with and without T2D. In a sample of people with and without T2D, this study aimed to: 1) examine the associations between compliance with the Australian Dietary Guidelines measured using a dietary guideline index (DGI) and cognition and brain structure and 2) determine if T2D is an effect modifier of any associations.

5.3 Methods

Participants

This study is a secondary analysis of the Cognition and Diabetes in Older Tasmanians (CDOT) study. CDOT was designed to examine the associations between T2D and brain structure and function, the details of which have been described elsewhere (61). Briefly, people >55 years with T2D were recruited through the Australian National Diabetes Service Scheme and participants without T2D aged ≥60 years were randomly selected from the Southern Tasmanian electoral roll (61). The enrolment of participants and measurement were performed between 2005 and 2011. The criteria for diagnosing people with T2D were fasting plasma glucose ≥ 7.0 mmol/L, random plasma glucose ≥ 11.1 mmol/L, or 2-h glucose ≥11.1 mmol/L after an oral glucose tolerance test. People living in a nursing home or who had a diagnosis of dementia were excluded. Assessment of all measures were the same for people with and without T2D. All participants provided informed written consent. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee (Ethics reference numbers: H0009400 and H7947).

Dietary Assessment

Dietary intake was assessed using the Dietary Questionnaire for Epidemiological Studies version 2 (DQESv2) food frequency questionnaire (FFQ), which was developed to measure diet in an ethnically

diverse Australian population (281). The reliability, validity and reproducibility of the DQESv2 has been reported in previous Australian studies in older adults both with (219) and without (331) T2D. Participants determined their intake of 80 items (74 foods and 6 alcoholic beverages) over the past 12 months, using 10 response options ranging from "never" to "3 or more times per day". A series of photographs in the FFQ were used to elucidate usual portion sizes. The FFQ also included short questions on the types of bread, milk, cheese and spread usually consumed. The Cancer Epidemiology Centre of the Cancer Council Victoria calculated the energy intake and amounts of each item consumed in grams per day.

The Australian Dietary Guidelines index (DGI)

The Australian DGI is a food-based score designed to reflect the diet quality of individuals according to their compliance with the current 2013 Australian Dietary Guidelines (152). Current guidelines were applied, as opposed to the guidelines at the time of data collection, as they reflect contemporary nutritional knowledge regarding healthy foods. We used a validated Australian DGI 2013 method that has been described previously (332). Dietary intakes of individuals were scored according to eight recommended dietary components: diet variety; daily servings of fruit, vegetables, cereals [total cereal and proportion of whole grain bread to total bread], meat and alternatives [lean meat and alternatives and proportion of lean meat to total meat and alternatives], dairy and alternatives [total dairy products and low-fat dairy]; unsaturated fats, and limiting intake of foods containing saturated fat, alcohol, added salt and sugars (152). The dietary components for liquids were not included, as the FFQ used in this study did not collect information on these components.

The scoring criteria and types of food included in each category are listed in **(Appendix 5.1 Supplementary Table 1)**. **(Appendix 5.2 Supplementary Table 2)** defines the serving sizes of the food components based on Australian Dietary Guidelines. Each component was scored out of 10, with the exception of 'limiting intake of foods containing saturated fat, alcohol, added salt and sugars' which had a maximum score of 20. Maximum scores were given when participants met the age- and sex-specific recommendations (152). Proportionate scores were calculated for all items except for low fat dairy, unsaturated fats, and diet variety. The proportionate scores were awarded if the intakes were between the maximum and minimum scoring criteria (332). The DGI has a maximum score of 90 points. A higher score indicates greater compliance to the dietary guidelines (better diet quality).

Cognitive Assessment

Cognitive function was assessed using a comprehensive battery of validated neuropsychological tests.

We generated six cognitive domains using z-scores, similar to the Gardener et. al study (303). The domains created were *verbal memory* (the three components of the Hopkins Verbal Learning test (238)), *visual memory* (Rey-Osterrieth Complex Figure delayed reproduction after 20 minutes (238)), *visuospatial function* (Rey-Osterrieth Complex Figure copy (238)), *executive function* (Stroop interference: Victoria Stroop colour test score minus the Victoria Stroop word test score (238)), *Verbal fluency* (the letter and animal categories of Controlled Oral Word Associations Tests (238)), as well as *attention-processing speed* (the Digit Span, Digit Symbol Coding and Symbol Search subsets of the Wechsler Adult Intelligence Scale-Third Edition (246), and the Victoria Stroop dot test (238)). To create each domain, a z-score for every cognitive test was calculated by the individual's raw score minus the sample mean score, divided by the sample standard deviation. Stroop scores were reversed so that higher scores translated to better performance. Subsequently, z-scores for each test were averaged to compute a single composite score for each domain. Domain scores that included more than one cognitive measure were re-standardised to a standard deviation of one. Global cognitive function was calculated as the average of all the cognitive domain z-scores. These z-scores were used in the regression analysis to allow comparison of associations across the cognitive domains.

Brain magnetic resonance imaging

A 1.5T General Electric MRI scanner was used to obtain T1-weighted spoiled gradient echo, gradient echo, and fluid-attenuated inversion recovery images. They were processed using previously described methods (61, 249), to measure volumes of grey matter, white matter and white matter hyperintensities (WMH). Volumes of hippocampi were determined based on manual tracings performed by a single expert. Presence of microbleeds was determined by a single trained rater and confirmed by a neurologist and geriatrician.

Other Measures

Standardised questionnaires were used to obtain information regarding demographics, medical history (including vascular health factors - smoking history, hypertension, angina, stroke, and hyperlipidemia) and medication use. Mood was assessed with the 15-item Geriatric Depression Scale (263). Weight (kg) was measured wearing light clothing and without shoes using a Heine portable scale. Height (m) measurements were performed with Leicester stadiometer. Body mass index was calculated by dividing weight by height squared. Ambulatory activity was measured using a Yamax pedometer over 7 days. Systolic blood pressure was measured by an Omron M4 sphygmomanometer as the mean of three consecutive seated brachial blood pressure measures from the right arm.

Hypertension was defined as self-reported history of hypertension, systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg and/or current use of anti-hypertension medication. Biochemistry analysis of fasting plasma glucose was measured using a Roche Cobas 6000 analyser with hexokinase. Patients who had a self-reported history of hyperlipidemia, cholesterol levels >6.2 mmol/L or were on cholesterol-lowering medications were defined as having hypercholesterolemia. Since cognitive dysfunction could be partly due to vascular dysfunction (333), we generated a score based on known vascular health factors related to cognition (smoking, obesity, stroke, angina, hypertension and hyperlipidaemia) to consider their confounding effects. A continuous vascular health score was generated by allocating one point for the presence of each of the following vascular health factors: obesity (body mass index ≥30kg/m^2), hypertension, hypercholesterolemia, history of past and current smoking, angina, and stroke, and summing the points to give an overall score. Activity of daily living was assessed by Australian Modified Lawton's Activities of Daily Living scale (334).

Statistical Analysis

Non-normally distributed variables were transformed as appropriate. Square root transformations were applied to ambulatory activity, Hopkins recognition, Stroop colour and Stroop dot time tests and a logarithm transformation was applied to WMH. In this study, 76 (11%) of the 688 participants had missing values for physical activity, which were imputed by multiple imputation procedures using age, sex, education, energy, DGI and all other covariates in the model, with values imputed 50 times. The imputed values for ambulatory activity were then used as a covariate in the regression models.

The associations between DGI and each cognitive domain were examined using multivariable linear regression, initially adjusted for age, sex, education and total energy intake (model 1). Energy intake was included so that the results could be interpreted as being independent of the total amount of food consumed. Similarly, multivariable linear regression analyses were used to examine associations between DGI and continuous MRI brain variables (model 1 as above with additional adjustment for total intracranial volume) and binomial logistic regression for categorical variables including infarcts and microbleeds (model 1 as above). Model 2 included additional adjustments for mood, vascular health score, T2D, and ambulatory activity. Confounders were defined based on the current literature (118, 151) and their biological associations with the predictor (diet) and the cognitive and brain outcomes. In sensitivity analyses, we identified participants who potentially under- or over-reported their energy intake using the Goldberg cut-off (335) and Schofield equations (336) using a physical

activity level (PAL) equal to 1.55 (the WHO value for `light' activity (337), and repeated the analyses. To examine effect modification for T2D, we entered a DGI ×T2D product term into each model. Effect modification for sex was tested using the same method. Probability levels less than 0.05 were considered significant. Statistical analyses were performed in STATA 14.2 (Stata Corp, College Station, TX) software.

5.4 Results

Of the 713 participants recruited into the study, 693 participants completed the dietary questionnaire and cognitive tests. We excluded 18 people who did not answer any of the FFQ questions. At the clinic, the questionnaires were carefully checked by research staff to ensure there were no missing responses. We excluded five participants with dementia. Three people had self-reported dementia and two people had impairment in activity of daily living in combination with cognitive impairment, which was determined by two neuropsychologists as 1.5 SD below age, sex and education norms in two or more cognitive domains. The participants flow diagram is shown in **(Appendix 5.3 Supplementary Figure 1)**. Hence, we analyzed the data from 688 participants, 343 with T2D (mean age 67.7± SD 6.9 years) and 345 without T2D (mean age 72.1± SD 7.2 years). Of these, 641 (T2D n=326; without T2D n=315) had brain MRI measures. The mean duration of T2D was 4.6±8.2 years, and 23% were receiving insulin therapy. Demographic, clinical characteristics and major dietary characteristics of participants are shown in **Table 5.1. Table 5.2** shows the DGI scores for the full sample and stratified by T2D status. The mean DGI score was 56.5±10.6 for people with T2D and 53.1 ±10.5 for people without T2D.

Associations between DGI, cognitive function and brain structure

Associations between DGI, cognitive domains and brain structural variables are shown in **Table 5.3**. The DGI was not associated with any of the cognitive domains or structural brain measures in model 1 or the fully adjusted model 2. In sensitivity analyses, we excluded participants classified as under-reporting (n=150) or over-reporting (n=12) their energy intake. There were no meaningful changes in the beta-coefficients for the association between diet quality and brain health measures. Non-linearity was examined for all associations by adding a squared and cubic term of the dietary guidelines index to the regression models. None of the non-linear associations were significant.

Effect modification by type 2 diabetes and sex

We observed no effect modification by T2D or sex on the association between DGI and cognition or

Table 5.1 Demographic and clinical characteristics of participants with and without type 2 diabetes

Variables	All (*n*=688)	T2D (*n*=343)	Non-T2D (*n*=345)
Characteristics[1]			
Male, *n* (%)	393 (57.1)	206 (60.1)	187 (54.2)
Age, y	69.9 ± 7.4	67.7 ± 6.9	72.1 ± 7.2
Education[2], *n* (%)	331 (48.1)	169 (49.3)	162 (47.0)
Body mass index, Kg/m^2	29.2 ± 7.1	31.1 ± 8.6	27.3 ± 4.3
Medical History			
History of Stroke, *n* (%)	58 (8.4)	37 (10.8)	21 (6.1)
History of Hyperlipidaemia, *n* (%)	385 (55.9)	251 (73.2)	134 (38.8)
History of Hypertension, *n* (%)	406 (59.0)	248 (72.3)	158 (45.8)
Past or current smoker, *n* (%)	357 (51.9)	186 (54.2)	171 (49.6)
Angina, *n* (%)	99 (14.4)	59 (17.2)	40 (11.6)
Clinical Characteristics			
Steps/day, (*n*/day)	6070 ± 3340	6030 ± 3620	6100 ± 3070
Serum total cholesterol, (mmol/L)	4.8 ± 1.2	4.4 ± 1.0	5.3 ± 1.2
Fasting plasma glucose, (mmol/L)	6.5 ± 2.1	7.7 ± 2.3	5.3 ± 0.6
Systolic BP, (mmHg)	138.8 ± 20.5	136.2 ± 19.0	141.5 ± 21.7
Diastolic BP, (mmHg)	78.3 ± 11.4	76.2 ± 10.4	80.4 ± 11.9
BP lowering medication, *n* (%)	368 (88.0)	226 (89.0)	142 (87.0)
Cholesterol medication, *n* (%)	303 (750)	215 (82.0)	88.0 (62.0)
Dietary Characteristics			
Total energy (KJ/d)	7530 ± 2890	7560 ± 2990	7510 ± 2800
Total energy (Kcal/d)	1810 ± 694	1810 ± 717	1800 ± 672

brain structure *(P<0.05).*

[1] Values are means ± SDs for continuous variables unless indicated as frequency (%) for categorical variables. [2] Post-high school education BP: Blood Pressure, T2D: type 2 diabetes

Table 5.2 Mean ± SD values for each component of the Australian Dietary Guidelines index for all participants and those with and without type 2 diabetes

Dietary guidelines index score	All (n=688)	T2D (n=343)	Non-T2D (n=345)
Vegetables score, 10 points	5.2 ± 2.2	5.2 ± 2.2	5.2 ± 2.1
Fruit, 10 points	8.2 ± 2.6	8.1 ± 2.7	8.2 ± 2.5
Total cereal and bread, 5 points	4.3 ± 1.1	4.3 ± 1.1	4.3 ± 1.0
Whole grains bread as a proportion of total bread, 5 points	3.8 ± 2.1	4.0 ± 1.9	3.6 ± 2.2
Total lean meat and alternatives, 5 points	3.9 ± 1.2	4.0 ± 1.2	3.7 ± 1.2
Proportion of lean meats to total meat and alternatives, 5 points	4.1 ± 0.6	4.2 ± 0.6	4.1 ± 0.6
Total dairy, 5 points	2.6 ± 1.2	2.6 ± 1.2	2.6 ± 1.2
Low-fat dairy, 5 points	3.0± 2.4	3.3 ± 2.4	2.7 ± 2.5
Limit intake of saturated fat, alcohol, added salt and sugars, 20 points	7.0 ± 8.2	8.0 ± 8.5	6.0 ± 7.8
Unsaturated fats, 10 points	7.7 ± 4.2	7.7 ± 4.2	7.6 ± 4.3
Diet variety, 10 points	5.1± 1.3	5.1 ± 1.3	5.1 ± 1.3
Total dietary guidelines index score, 90 points	54.8± 10.7	56.5± 10.6	53.1 ± 10.5

The values are Mean ±SD. Criteria for awarding maximum points: For vegetables: 51-70 years: Male (M): ≥5.5 serving/day (s/d) Female (F): ≥5 (s/d); ≥70 years: M: ≥5 (s/d) F: ≥5 (s/d); For fruit: ≥2 (s/d); For cereal: 51-70 years: M: ≥6 (s/d) F: ≥4 (s/d); ≥70 years: M: ≥4.5 (s/d) F: ≥3 (s/d); For whole grain proportion: 100%; For Lean meat and alternatives: M: ≥2.5 (s/d); F: ≥2 (s/d); For lean meat proportion: 100%; For total dairy 51-70 years: M: ≥2.5 (s/d); F: ≥4 (s/d); ≥70 years: M: ≥3.5 (s/d); F: ≥4 (s/d); For low fat diary: Intake of Skim, soy or reduced fat milk or no milk. For limited foods: 51-70 years: M: <=2.5 (s/d) F: <=2.5(s/d); ≥70 years: M:<=2.5 (s/d) F: <=2 (s/d); For healthy fats: 51-70 years: M: <=4 (s/d), F: <=2 (s/d); >70 years: M: <=2 (s/d), F: <=2 (s/d); For diet variety: the maximum score awarded when at least one serving of the components of five core food categories were consumed per week. T2D: type 2 diabetes

Table 5.3 The association between the Australian Dietary Guidelines index, cognitive function, and brain structural measures in Tasmanian older adults with and without type 2 diabetes.

	The Australian Dietary Guidelines index[1]	
	Model 1,[2]	Model 2,[4]
	β (95% CI)	β (95% CI)
Global cognition, z-score (n=688)	-0.04 (-0.09, 0.02)	-0.04 (-0.09, 0.02)
Verbal memory, z-score	-0.01 (-0.06, 0.04)	-0.04 (-0.10, 0.02)
Visual memory, z-score	-0.02 (-0.09, 0.04)	-0.01 (-0.06, 0.06)
Executive function, z-score	0.02 (-0.05, 0.08)	0.02 (-0.05, 0.08)
Verbal fluency, z-score	-0.02 (-0.08, 0.05)	-0.02 (-0.08, 0.04)
Attention processing speed, z-score	-0.03 (-0.09, 0.03)	-0.05 (-0.11, 0.01)
Visuospatial function, z-score	-0.05 (-0.11, 0.02)	-0.04 (-0.09, 0.02)
Brain structural measures[3], mL (n=641)	Model 1,[2] β (95% CI)	Model 2,[4] β (95% CI)
Gray matter volume, mL	-0.97 (-3.47, 1.53)	-1.37 (-3.83, 1.08)
White matter volume, mL	-0.50 (-2.92, 1.95)	-0.14 (-2.60, 2.33)
Left hippocampal volume, mL	-0.01 (-0.05, 0.02)	0.01 (-0.02, 0.04)
Right hippocampal volume, mL	-0.01 (-0.05, 0.02)	-0.00 (-0.03, 0.03)
White Matter Hyperintensities volume, mL	-0.02 (-0.08, 0.04)	0.01 (-0.05, 0.06)
Presence of small vessel disease (n=641)	Model 1,[2] OR (95% CI)	Model 2,[4] OR (95% CI)
Micro-bleeds, (yes/no)	1.05 (0.78, 1.42)	1.09 (0.81, 1.48)
Infarcts, (yes/no)	1.01 (0.85, 1.20)	1.02 (0.85, 1.22)

[1]The β co-efficient represents the increase (positive β) or decrease (negative β) in cognitive Z-scores and brain volumes associated with a 10-point increase in dietary guidelines index score. [2]Model 1: adjusted for age, sex, education, and energy. [3]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume. [4]Model 2: Model 1 plus additional adjustments for mood, vascular health score, type 2 diabetes, and ambulatory activity.

5.5 Discussion

To our knowledge, this is the first study to investigate associations between adherence to the Australian Dietary Guidelines (using a DGI), cognition and brain MRI among older adults. In this cross-sectional study, the Australian DGI was not associated with cognition or structural brain measures. No effect modification was observed for T2D.

Prior studies that examined associations between adherence to the other national dietary guidelines

and brain health have been inconsistent. Our results are in line with studies that showed higher adherence to the Dietary Guidelines for Americans or Canadians (Healthy Eating Index (HEI)) was not associated with global cognition (2 studies: n=1499; age >50 years; race=99.4% White (338) and n=373; age 68-82 years; study population=Quebec residents (339)), verbal fluency (n=1499; age >50 years; race=99.4% White (338)), executive function (2 studies: n=1499; age>50 years; race=99.4% White (338) and n=1,269; age 45-75 years; study population= Puerto Ricans (148)), memory or attention (n=1,269; age 45-75 years; study population= Puerto Ricans (148)). However, our results are inconsistent with studies that showed that a higher adherence to the Dietary Guidelines for Americans (measured using the HEI and recommended food score) was associated with better global cognition (2 studies: n=3634; age 65 years (149) and n=1,269; age 45-75 years; study population= Puerto Ricans (148)), verbal learning and memory (n=2090; age 30-64 years; race=51.2% African American, 48.8% White (150)). Very few studies have examined associations between adherence to national guidelines and brain structure. Similar to our study, higher adherence to the Dutch dietary guidelines was not associated with WMH volume in n=4,213 older adults with a mean aged 65.7 years (151), but unlike our study higher adherence was associated with greater brain volume.

Differing results may emerge from variations in calculating the DGI, including the foods included in each component of the score and the recommended serving sizes, as well as difference in studies' populations, age ranges, and cognitive outcome measures (148-151). For example, the American HEI-2010, which is based on the Dietary Guidelines for Americans, scores intake of foods per 1000 Kcal of total energy intake. Whereas the Australian DGI is based on age-sex specific food recommendations. Foods that are considered healthy for the brain such as dark green vegetables, beans, seafood, legumes, and nuts are recommended individually and scored separately in the American HEI-2010 (150), and Dutch diet quality index (151). In contrast, the Australian DGI-2013 groups these items into vegetables and lean meats and alternatives (152).

Australian Dietary Guidelines are based on broad selections of foods that are associated with wider aspects of health (152), whereas the Mediterranean diet, and the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet focus on specific dietary components that originally were associated with lower risk of cardiovascular disease (340) and have been also shown to be related to better brain health (141, 341). The reason for seeing no associations in our study might be that the Australian DGI does not individually score components related to a healthy brain such as green leafy vegetables, berries, beans, fish, poultry, nuts, olive oil, and red wine (141, 342, 343). For example, fish, nuts, poultry and beans are scored along with red meats. There are also no separate

recommendations for leafy green vegetables, berries, olive oil, or red wine. The Australian Dietary Guidelines could potentially be improved by separately recommending fish, nuts, beans, leafy green vegetables, berries, olive oil, and red wine as per the Mediterranean diet and MIND diet which have been associated with both cardiovascular (340) and brain health outcomes (141, 341).

There are several limitations to our study. The study was cross-sectional, and it is possible that dietary intake earlier in the life course, for example in midlife and even childhood, may influence cognition in older adulthood to a greater extent than diet in later life. The Australian diet has changed over the last few decades by embracing foods from Asia and Europe, compared to traditional British foods (344). Therefore, the participants' current diet may be quite different to their diet from earlier life stages. For example, Australians adults aged >19 years ate more whole fruit, a greater diversity of vegetables, more brown and wholegrain cereals, beans, peas, and pulses, as well as less refined sugar in 2011 compared to 1995 (345).

Furthermore, people with T2D had a slightly higher DGI score than those without T2D. This may be because those with T2D consumed a healthier diet to help manage their diabetes, or they may be more likely to report socially desirable answers. Further research is needed to determine if there are critical or sensitive periods where diet can influence cognition in later life. FFQs have known measurement errors, including biased self-reporting due to social desirability (323) and this would reduce the variability of the DGI score, potentially leading to underestimated associations between DGI and cognitive and brain outcomes. Furthermore, our FFQ did not include some of the Australian Dietary Guidelines' items such as fluids (coffee, tea, soft drinks, and water). An additional limitation is that older adults may have a limited ability to recall intake of dietary items over the previous 12-month period. Given the association between hydration and better cognition (346), lack of fluids measures in this study may underestimate the association between DGI and brain health. This study also had specific strengths. This includes using a validated questionnaire to measure diet, a comprehensive battery of neuropsychological tests and measures of brain structure using MRI, enabling us to investigate the association of an Australian DGI with a number of cognitive domains and brain structure for the first time. Having participants well characterised for T2D allowed us to investigate the effect modification by T2D status.

In conclusion, we found no association between the Australian DGI and multiple cognitive domains or brain structure. Prospective studies are needed to examine whether long-term adherence to the Australian Dietary Guidelines is associated with better brain health.

5.6 Postscript

The findings from this chapter revealed no association between adherence to the Australian Dietary Guidelines and brain health. This could be because the Australian Dietary Guidelines do not incorporate separate recommendations for brain-healthy foods. Similar associations were observed for people with T2D compared to those without T2D.

Physical activity is another important risk factor for dementia and cognitive decline. The next chapter of this thesis will focus on the association between physical activity, cognitive domains and brain structure in people with T2D.

Appendix 5.1 Supplementary Table 1 Components and scoring of the 2013 Australian dietary guideline index.

Dietary Guidelines	Description	Criteria for maximum score	Minimum score	Maximum score
Enjoy a wide variety of nutritious foods from the five groups every day (vegetables, fruit, whole grains, lean meat, and dairy)	Food variety: 2 points awarded for each of the five core food categories when at least one serving is consumed per week.	100%	0	10
Eat plenty of vegetables of different types and colours, and legumes/beans	Total servings of vegetables consumed per day: potato, tomato, tomato paste, capsicum, lettuce, cucumber, celery, beetroot, carrot, cabbage, cauliflower, broccoli, spinach, green bean, sprouts, legumes (peas, baked beans, soybeans/tofu, other beans (lentil, chickpea)), pumpkin, onion, garlic, mushroom, and zucchini.	51-70 years: M: ≥5.5 F: ≥5 ≥70 years: M: ≥5 F: ≥5	0	10

Enjoy a wide variety of fruit	Total servings of fruit consumed per day: tinned fruit, orange, apple, pear, banana, melon, pineapple, strawberries, apricot, peach, mango, and fruit juice.	M: ≥2 F: ≥2	0	10
Grain (cereal) foods, mostly wholegrain and/or high cereal fibre varieties, such as bread, cereals, rice, pasta, noodles, polenta, couscous, oats, quinoa and barley	Total servings of grains per day: All-Bran, bran flakes, Weetabix, cornflakes, porridge, muesli, rice, pasta, and bread.	51-70 years: M: ≥6 F: ≥4 ≥70 years: M: ≥4.5 F: ≥3	0	5
	The proportion of whole grains bread to total bread: sum of wholemeal bread, rye bread, multi-grain bread to sum of high fibre white bread, white bread, wholemeal bread, rye bread, multi-grain bread.	100%	0	5

Lean meats and poultry, fish, eggs, tofu, nuts and seeds, and legumes/beans	Total servings of lean meats and alternatives per day: Beef, veal, chicken, lamb, pork, grilled fish, tinned fish, eggs, baked beans, soybean/tofu, other beans, peas, nuts.	M: ≥2.5 F: ≥2	0	5
	Proportion of lean meat and alternatives to total meat and alternatives.	100%	0	5
Milk, yoghurt, cheese and/or their alternatives, mostly reduced fat	Total servings of dairy products per day: cheese (hard cheese, firm cheese, soft cheese, ricotta/cottage cheese, cream cheese, low-fat cheese), yoghurt, milk (Full cream, reduced fat cream, skim milk, flavoured milk, and soya milk).	51-70 years: M: ≥2.5 F: ≥4 ≥70 years: M: ≥3.5 F: ≥4	0	5
	Low fat/reduced fat dairy: Type of milk usually consumed.	Skim or soy milk =5 No milk =5 Reduced fat=5	Full fat milk=0 Flavoured milk=0	5
Limit intake of foods containing saturated fat, alcohol, added salt, and added	Servings per day of margarine, margarine blends, butter, sweet biscuits, cakes, meat pies, corn chips (crisps), ice cream, fried potatoes, pizza, hamburger, jam, sugar, chocolate, crackers, vegemite, and alcohol.	51-70 years: M: ≤2.5 F: ≤2.5 ≥70 years: M: ≤2.5 F: ≤2	51-70 years M: >2.5 F:>2.5 ≥70 years M: >2.5 F: >2	20

sugars				
A small allowance of unsaturated oils, fats or spreads	Servings per day of unsaturated spreads and oils: monounsaturated fat margarine, polyunsaturated fat margarine, avocado, peanut butter.	51-70 years: M: ≤4 F: ≤2; >70 years: M: ≤2 F: ≤2;	M>4, F>2	10

Notes: F: female M: male

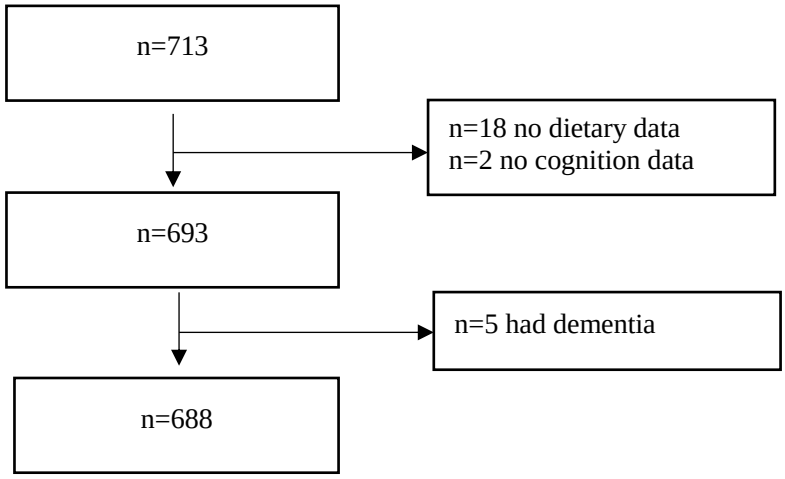
Appendix 5.2 Supplementary Table 2 The serving sizes of the food components based on Australian Dietary Guidelines.

Food items	Amount of a serving [1]
Vegetables	75 g
Fruit	150 g
Fruit juice	125 mL
Bread slice	40 g
Pasta, rice [2]	75-120 g
Cornflakes, muesli, wheat cereal flakes, All Bran, bran flakes, Weetbix	30 g
Porridge	120 g
Nuts	30 g
Beef, veal, lamb, pork	65 g
Chicken	80 g
Grilled fish, tinned fish, fried fish	100 g
Eggs	120 g
Sausage [2]	50-70 g
Processed meat, ham, bacon, hamburger [2]	50-60 g
Baked beans, other beans, peas	150 g
Soybean and Tofu	170 g
Hard cheese, firm cheese, soft cheese, low-fat cheese	40 g
Ricotta, cream cheese	120 g
Yoghurt	200 g
Flavoured milk, soy milk, full cream milk, reduced-fat milk, skim milk,	250 mL
Red wine, white wine (1 standard drink)	100 mL
Fortified wine (1 standard drink)	60 mL
Spirits (1 standard drink)	30 mL
Full beer (1 standard drink)	267 mL
Low beer (1 standard drink)	400 mL
Vegemite (347)	6 g

Butter (Saturated spreads)	20 g
Margarine blend [3]	15 g
Sweet biscuits	35 g
Cakes	40 g
Meat pie, fried potato	60 g
Crackers and Crisps	30 g
Ice cream	75 g
Pizza [4]	58 g
Jam	60 g
Sugar [5]	37.5 g
Chocolate	25 g
Peanut butter, unsaturated margarine	10 g
Avocado (348)	30 g

[1] The serving sizes are based on the Australian Dietary Guidelines 2013 (152) except where external references are provided. [2] Mean value was used when a range for serving sizes was provided by Australian Dietary Guidelines 2013 (152). [3] For margarine blend, the serving size was calculated as the mean values for saturated (20g) and unsaturated spreads (10g). [4]The Australian Dietary Guidelines 2013 state that a serving of discretionary foods is 600kJ (152). Since pizza has 1044 KJ per 100 g (220), 58g of pizza is a serving of discretionary food (600KJ). [5] There are 1600kJ per 100g of sugar (220), therefore 37.5 g of sugar is a serving of discretionary food (600kJ)

Appendix 5.3 Supplementary Figure 1 participants flow diagram



Appendix 5.4 Additional analysis-A

The main results showed no association between the total score of adherence to the Australian Dietary Guidelines and brain health. It was hypothesised that the individual scores on guidelines might have cancelled each other and driven the association to null. Therefore, the association between each individual guideline and two main brain health markers (grey matter volume, and global cognition) was investigated using the general linear regression model adjusted for the covariates. There was an association between higher adherence to the guidelines on lean protein (both total and proportion) and better global cognition. There were associations between higher adherence to fruit, alcohol, and discretionary foods, and lower global cognition. There was a significant association between higher adherence to vegetable guidelines and better grey matter volume. There was an association between higher adherence to healthy oils and lower grey matter volume. T2D was not an effect modifier for any of the associations. In conclusion, the associations were small and not conclusive.

Supplementary Table 3 The association between adherence to the individual Australian Dietary Guidelines, global cognition and grey matter volume in people with (n=343) and without T2D (n=346).

	Global cognition		Grey matter volume	
Diet quality score	β	95% CI	β	95% CI
Vegetables	0.02	-0.02, 0.05	**2.11**	**0.01, 4.2**
Fruit	**-0.03**	**-0.06, -0.01**	-1.30	-2.9, 0.3
Total cereal	0.05	-0.02, 0.12	0.20	-4.3, 4.7
Whole grains proportion	0.01	-0.02, 0.04	-0.06	-2.1, 1.9
Total lean protein	**0.07**	**0.00, 0.13**	0.04	-4.2, 4.3
Lean protein proportion	**0.12**	**0.02, 0.22**	-2.50	-9.4, 4.5
Total dairy	0.03	-0.02, 0.09	0.19	-3.5, 3.9
Low fat dairy	0.02	-0.01, 0.05	-0.43	-2.4, 1.5
Alcohol	**-0.02**	**-0.03, -0.00**	-0.40	-1.4, 0.6
Extra/Discretionary foods	**-0.01**	**-0.02, -0.00**	-0.14	-0.8, 0.5
Healthy oils	0.01	-0.01, 0.02	**-1.11**	**-2.1, -0.1**
Diet variety	-0.01	-0.06, 0.04	-0.11	-3.4, 3.2

Models are adjusted for age, sex education, and energy (Continuous brain MRI structure measures are additionally adjusted for total intracranial volume), mood, vascular risk score and ambulatory activity, and type 2 diabetes.

Appendix 5.5 Additional analysis-B

APOE-ε4 is a genetic risk factor for developing dementia. In accordance with chapter 6, this section aims to investigate the interaction for APOE-ε4 for the associations between the adherence to the Australian dietary guidelines and cognitive domains and brain structure. The following table lists the interaction terms beta coefficients, 95% confidence interval and p-values of interaction terms. The effect sizes for APOE-ε4 interactions were quite small and there was no consistent pattern across all the cognitive domains and brain variables.

Supplementary Table 4 The interaction terms for APOE-ε4 for the association between adherence to the Australian Dietary Guidelines, cognition and brain structure in people with and without T2D.

	The Australian Dietary Guidelines Index[1]		
Cognitive function (n=655)	β for interaction terms	95% CI	*P* value
Global cognition, z-score	-0.02	-0.03, -0.00	0.01*
Verbal memory, z-score	-0.00	-0.02, 0.01	0.09
Visual memory, z-score	0.00	-0.01, 0.02	0.96
Executive function, z-score	-0.02	-0.04, -0.01	0.008*
Verbal fluency, z-score	-0.01	-0.03, 0.00	0.16
Attention processing speed, z-score	-0.01	-0.03, 0.00	0.06
Visuospatial function, z-score	-0.00	-0.02, -0.00	0.21
Brain structural measures[2], mL (n=608)			
Grey matter volume, mL	0.23	-0.38, 0.84	0.46
White matter volume, mL	0.17	-0.45, 0.79	0.59
Left hippocampal volume, mL	-0.00	-0.01, 0.02	0.18
Right hippocampal volume, mL	-0.00	-0.01, 0.00	0.67
White Matter Hyperintensities volume, mL			
Presence of small vessel disease (n=613)			
Micro-bleeds, (yes/no)	1.00	0.94, 1.08	0.90
Infarcts, (yes/no)	1.04	0.99, 1.09	0.11

[1]adjusted for age, sex, education, energy, mood, vascular health score, type 2 diabetes, and ambulatory activity. [2]MRI variables (except for infarcts and microbleeds) adjusted additionally for total intracranial volume except for White Matter Hyperintensities volume and small vessel disease. * *P* value<0.05

6. The association between physical activity intensity, cognition and brain structure in people with type 2 diabetes

6.1 Preface

The results of chapter 5 revealed no associations between the adherence to the Australian Dietary Guidelines, cognition and brain volume measures. Physical activity is another essential risk factor for dementia. It is unknown whether the objectively measured high-intensity physical activity is associated with better brain health in people with T2D. There are only a few studies examining the association between daily step count, and brain structure in people with T2D. This chapter will examine whether objectively measured physical activity (daily step count and moderate to vigorous physical activity) are associated with cognitive domains and brain structure. As a secondary aim, this chapter will examine whether the associations are modified based on the genetic risk of dementia (being APOE-ε4 carrier) or T2D severity (receiving insulin-therapy). The text of this chapter and the supplementary material in the appendix is submitted to *by the Journal of Gerontology: Series A.*

6.2 Introduction

Dementia is a major public health concern and has adverse effects on an individual's quality of life (326). Low levels of physical activity (PA) may be one of the most important risk factors for dementia (349). A higher daily step count and greater time spent in moderate to vigorous physical activity (MVPA) are associated with better brain health (cognition and brain volume (182, 183, 202)). However, little is known as to whether MVPA is associated with better brain health in people at greater risk of dementia, such as those with type 2 diabetes (T2D) (330).

The effect sizes for T2D related cognitive dysfunction appear stronger for the domains of executive function (task switching, and inhibitory control) and processing speed (350). In addition, T2D has shown to be associated with lower grey and hippocampal volume, as well as greater levels of small vessel disease (61). A study of older women with T2D found that higher self-reported leisure activity, converted into metabolic equivalent hours per week, was not associated with baseline cognition or decline over an average of 4.2 years (206). Whereas, a study of a combined sample of people with and without T2D (51% with T2D) showed that more time spent in self-reported light exercise during weekdays, or moderate exercise during weekend days, was associated with better general cognitive function (207). Inconsistent results from prior studies could be partly explained by recall bias due to

using self-reported PA. A study of a combined sample of people with and without T2D found that pedometer measured step count was associated with higher grey matter and hippocampal volume (91). However, that study did not include measures of PA intensity (91). There are no studies to our knowledge that have examined whether objectively measured MVPA is associated with measures of brain health in people with T2D.

APOE-ε4 and insulin-therapy are two factors that may modify the association between PA and brain health. APOE-ε4 is a risk factor for dementia (351), but the literature in the general population is inconsistent as to whether APOE-ε4 modifies associations between PA and cognition, with some studies showing a benefit for APOE-ε4 carriers (352, 353), some for APOE-ε4 non-carriers (354-356) and others no difference (357-359). People who are taking insulin usually have greater severity of T2D and dysglycemia (360). It is possible that PA may have less benefit for cognition in this group due to a greater accumulation of changes in the brain (361) or due to difficulty controlling blood glucose levels (362). However, it is currently uncertain in people with T2D whether the association between PA and brain health is different for APOE-ε4 carriers or those taking insulin.

Therefore, the aim of this study was to examine the association between objectively measured PA (daily step count, and MVPA) with different cognitive domains and brain structure in people with T2D. A secondary aim was to explore if APOE-ε4 or insulin-therapy status modified any associations between PA and brain health. As exercise may have the strongest effects on attention-processing speed (245), executive function (245) and hippocampal volume (363) in the general population, we hypothesised that greater levels of objectively measured PA (MVPA and step count) would be associated with better outcomes for these brain functions and structure.

6.3 Material and Methods

Participants

This is a secondary analysis from the Cognition and Diabetes in Older Tasmanians Blood Pressure study (CDOT-BP). CDOT-BP aimed to examine the associations between peripheral and central blood pressure on cognition and brain structure in people with T2D. Participants (n=252) over 55 years of age with T2D were enrolled in the study from the Australian National Diabetes Service Scheme, the Royal Hobart Hospital, general advertising, and social media. The diagnostic criteria for T2D were random plasma glucose ≥11.1 mmol/L, fasting plasma glucose ≥7.0 mmol/L, or 2-h glucose ≥11.1 mmol/L after oral glucose tolerance test. Participants were excluded if they lived in a

nursing home, had any contraindication to Magnetic Resonance Imaging (MRI) or were diagnosed by a doctor with dementia. All participants provided informed written consent. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee (H0013664 and H0013965).

Measurement of PA

Participants were asked to wear an accelerometer (ActiGraph GTlM, Pensacola, FL, USA) over the right hip for seven days and to fill out a daily monitoring log in which they recorded start and finish wear times. They also recorded any duration and reason for periods where the accelerometer was removed (e.g. sleeping and taking a shower). Participants were included in the analysis if they wore the accelerometer for at least 8 hours per day for 5 days (234).

To obtain the average total step count and time spent in moderate and vigorous PA per day, each measure was divided by the number of valid days of accelerometer wear. Freedson's cut points for adults were used to convert daily counts of PA to different levels of PA intensity (236). Since the average time of engagement in vigorous PA was low (0.13±0.6 minutes per day (min/d)), we combined the time spent in moderate and vigorous PA to facilitate the analysis of data.

Cognitive measurements

A comprehensive battery of validated neuropsychological tests was used to measure cognitive function. We generated seven cognitive domains using z-scores similar to a prior study (265). The domains created were *verbal memory* (the three components of the Hopkins Verbal Learning test (238)), *visual memory* (Rey-Osterrieth complex figure Delayed reproduction after 20 minutes (238)), *visuospatial function* (Rey-Osterrieth complex figure copy (238)), *executive function* (Inhibitory control: Stroop interference (Victoria Stroop Colour Test score – Victoria Stroop Word Test score), and Task switching: Trails Making Tests (B-A) (238)), *verbal fluency* (the Controlled Oral Word Associations Tests letter and animal categories (238)), as well as *attention-processing speed* (the Digit Span, Digit Symbol Coding and Symbol Search subsets of the Wechsler Adult Intelligence Scale-Third Edition (246), and the Victoria Stroop Dot Test (238)). To create each domain, a z-score for each cognitive test was calculated as the individual's raw score minus the sample mean score, divided by the sample standard deviation. Stroop scores were reversed so that higher scores translated to better performance. Subsequently, z-scores for each test were averaged to compute a single composite score for each domain. We also calculated a *global cognitive domain* score that was the mean of all domains. These scores were used in the regression analysis to allow the comparison of

associations across cognitive domains.

Imaging

High resolution MRI scans were obtained using a single 1.5T GE Optima scanner (GE Healthcare, Chicago, USA). The following structural imaging sequences and parameters were used. For T1, TR = 7.6ms, TE = 2.34ms, flip angle = 12º, FOV = 250mm, 240x240 matrices, and slice thickness = 1mm. For Fluid Attenuated Inversion Recovery (FLAIR), TR=9000, TE = 95.1, Flip 160, FOV = 220, acquisition matrix 288x288, and slice thickness 3mm.

Image Processing

Structural measures (grey matter and white matter volumes) were obtained with FreeSurfer software 5.3. White matter hyperintensities were segmented using a semi-automated procedure based on our previous approaches (61), and the resulting masks used to correct the FreeSurfer white matter segmentation. Volumes of hippocampi were determined based on manual segmentation performed by a single expert.

Other Measures

Information on age, sex, education and medical history (including history of smoking, hypertension, stroke, T2D duration, and insulin-therapy) were obtained using standardised questionnaires. Waist circumference was measured as the midpoint between the top of the hip and the lower costal border. Hip circumference was measured as the widest point around buttocks. Waist to hip ratio was calculated via waist circumferences (cm) divided by hip circumferences (cm). Mood was assessed with the 15-item Geriatric Depression Scale (263). Blood pressure was measured using an Omron M4 sphygmomanometer as the mean of three consecutive seated brachial blood pressure measures from the right arm. Venous blood samples were taken from the antecubital fossa after overnight fasting. APOE-ε4 genotype was derived from whole blood DNA. Participants were considered APOE-ε4–positive if they had at least one copy of APOE-ε4 allele. Dietary data were collected using an 80-item food frequency questionnaire that had been validated in people with T2D (219). A dietary guidelines index was used to estimate diet quality, calculated as adherence to the 2013 Australian Dietary Guidelines based on method explained previously (304). A higher score indicates a better diet quality.

Statistical Analysis

Participant characteristics were presented descriptively. Multivariable linear regression analysis was

used to examine the associations between PA measures (daily step count per 500 steps/d, and MVPA per 10 min/d) with each cognitive domain and brain MRI variable initially adjusted for age, sex, and education (Model 1). Model 2 was further adjusted for smoking history, prior stroke, and diet quality. The covariates were chosen based on their known residual confounding effect. Five (2.3%) of the 220 participants had missing values for diet quality, which were imputed 50 times by multiple imputation procedures using age, sex, education, smoking and stroke status. The models with MRI outcome variables were additionally adjusted for total intracranial volume to reflect brain size.

Since abdominal obesity, systolic blood pressure, and mood could plausibly be on the pathway between PA and brain health, we tested mediation by adding each factor separately to the fully adjusted models to determine any change in the beta coefficients. To examine if APOE-ε4 or insulin-therapy modified any associations between PA and brain health (cognition and brain structure), we entered a PA × APOE-ε4 product (or PA × insulin-therapy) term into all models. Statistical analyses were performed using STATA® 14.2 (Stata Corp, College Station, TX, USA) software. Diagnostic tests were performed to check for non-linearity, residuals and influential points for all models.

6.4 Results

Out of 252 participants recruited to the study, 232 wore accelerometers and had cognitive data. Five participants were excluded as they wore the accelerometer for less than 5 days of at least 8 hours. Nineteen participants had no accelerometer diary, from which seven were excluded due to a pattern of steps that were not consistent with wear over 7 days (e.g fluctuating between implausibly high and low step counts). That left 220 participants who had both PA and cognitive data, and 165 participants who had both PA and brain MRI data measured with the same scanner and protocol.

The mean age of participants was 67.9 (SD 6.4) years and 60% (n=132) were male. Twenty-six percent (n=57) of participants were APOE-ε4 positive and 20% (n=43) were on insulin therapy. The median duration of T2D was 11 years. **Table 6.1** shows the demographic and clinical characteristics of the participants. **Appendix 6.1 Supplementary Table 1** shows the mean ± SD of all cognitive tests and brain MRI variables.

PA and cognition

Table 6.2 shows the results for the association between PA (the two alternate measures of step count, and MVPA) and each of the seven cognitive domains. Out of 14 models, there was one statistically significant association between step count and cognition. A higher step count was associated with

better attention-processing speed in the fully adjusted model (β=0.022 95%CI 0.003, 0.041 *P*=0.023). This association remained significant after adding mood and systolic blood pressure to the model, where each changed the coefficients by less than 10%. Adding waist to hip ratio attenuated the association by 23% (β=0.017 95%CI 0.001, 0.035 *P*=0.07). There were no other significant associations between step count or MVPA and the other cognitive domains in overall sample (*P>0.05*).

Table 6.1 Characteristics of participants

Characteristics	Participants with cognitive data n=220	Participants with MRI data n=165
Age (years), mean±SD	67.9±6.4	68.3±6.6
Sex (male), n (%)	133 (60.0)	100 (60.6)
Education, (at least high school), n (%)	181 (81.9)	137 (83.0)
History of stroke, n (%)	10 (4.5)	10 (6.1)
History of hypertension, n (%)	162 (73.3)	118 (71.5)
Past and current smoking, n (%)	115 (52.0)	75 (45.5)
Waist to hip ratio, mean±SD	1.0±0.08	1.0±0.08
Systolic blood pressure, (mmHg), mean±SD	127.8±11.8	127.9±10.5
Diastolic blood pressure, (mmHg), mean±SD	76.4±8.3	75.9±7.2
Geriatric Depression Scale score, mean±SD	2.3±2.5	2.4±2.6
Apolipoprotein E-ε4 allele carriers, n (%)	57 (26.4)	43 (26.4)
Receiving insulin-therapy, n (%)	43 (19.6)	33 (20.0)
Steps per day, mean±SD	5777±2485	5755±2548
MVPA, (min/d), mean±SD	21.4±20.4	21.8±21.7

Table 6.2 The association between daily step count, moderate to vigorous physical activity and cognitive function in people with type 2 diabetes.

Steps/day (per 500 steps/day)[1]				
n=220	Model 1		Model 2	
Cognitive domain (z-score)	β	95%CI	β	95%CI
Executive function	-0.002	-0.020, 0.016	-0.001	-0.018, 0.018
Attention-processing speed	**0.022**	**0.004, 0.041***	**0.022**	**0.003, 0.041***
Verbal fluency	-0.009	-0.031, 0.013	-0.009	0.031, 0.014
Verbal memory	-0.004	-0.026, 0.018	-0.002	-0.025, 0.020
Visual memory	-0.002	-0.029, 0.025	-0.001	-0.027, 0.028
Visuospatial function	0.017	-0.010, 0.043	0.017	-0.009, 0.044
Global cognition	0.004	-0.010, 0.017	0.005	-0.009, 0.018
MVPA time (per 10 min/d)				
Cognitive domain (z-score)	β	95%CI	β	95%CI
Executive function	-0.019	-0.064, 0.026	-0.015	-0.061, 0.029
Attention-processing speed	0.040	-0.006, 0.087	0.042	-0.005, 0.089
Verbal fluency	-0.020	-0.075. 0.035	-0.023	-0.078, 0.032
Verbal memory	0.001	-0.056, 0.056	-0.001	-0.055, 0.057
Visual memory	-0.001	-0.070, 0.067	0.004	-0.064, 0.072
Visuospatial function	0.027	-0.040, 0.095	0.032	-0.034, 0.099
Global cognition	0.005	-0.029, 0.038	0.007	-0.027, 0.041

[1] Model 1: adjusted for sex, age, and education; Model 2: adjusted for model 1 + smoking, stroke, and diet quality. MVPA: Moderate-to-vigorous physical activity. * $p<0.0$

Table 6.3 The association between daily step count, moderate to vigorous physical activity and brain volume measures in people with type 2 diabetes.

Steps/day (per 500 steps/day)				
n=165	Model 1		Model 2	
Brain volume (mL)	β	95%CI	β	95%CI
Hippocampal volume	**0.027**	**0.004, 0.049***	**0.028**	**0.005, 0.051***
Gray matter volume	1.068	-0.049, 2.185	1.076	-0.064, 2.216
White matter volume	0.698	-0.786, 2.181	0.767	-0.721, 2.256
WMH volume	-0.110	-0.293, 0.073	-0.101	-0.287, 0.086
MVPA time (per 10 min/d)				
Brain volume (mL)	β	95%CI	β	95%CI
Hippocampal volume	0.046	-0.009, 0.101	0.048	-0.008, 0.105
Gray matter volume	2.562	-0.155, 5.280	2.636	-0.114, 5.387
White matter volume	0.365	-3.252, 3.983	0.744	-2.858, 4.346
WMH volume	-0.279	-0.724, 0.167	-0.282	-0.731, 0.167

Model 1: adjusted for sex, age, education and total intracranial volume; Model 2: adjusted for model 1 + smoking, stroke, and diet quality. MVPA: Moderate-to-vigorous physical activity; WMH: White matter hyperintensity * $p<0.05$

PA and brain volumes

Table 6.3 shows the results for the association between PA (the two alternate measures of step count, and MVPA) and each of four brain structural measures. Out of eight models, there was one statistically significant association for step count. A higher step count was associated with greater hippocampal volume in the fully adjusted model (β=0.028 95%CI 0.005, 0.051 *P*=0.019). The association remained unchanged after adding waist to hip ratio, mood and systolic blood pressure to the models (all less than 10% change in the beta-coefficient). There were no other significant associations between PA and brain MRI measures (*P>0.05*).

Effect modification: APOE-ε4 and insulin-therapy

Out of the 44 models that were tested for effect modification, there were two statistically significant terms **(See Appendix 6.2 and 6.3 Supplementary Table 2 and 3)**. APOE-ε4 modified the association between MVPA and verbal fluency in the fully adjusted model (*P* for interaction = 0.002; APOE-ε4 carriers β = 0.137 95%CI 0.027, 0.249; APOE-ε4 non-carriers β

= -0.070 95%CI -0.131, -0.009) (Figure 6.1) and insulin-therapy modified the association between MVPA and attention-processing speed in the fully adjusted model (*P* for interaction=0.019; Insulin-therapy = β 0.161 95%CI 0.044, 0.278; No insulin-therapy = β 0.017 95%CI -0.033, 0.068) (Figure 6.2).

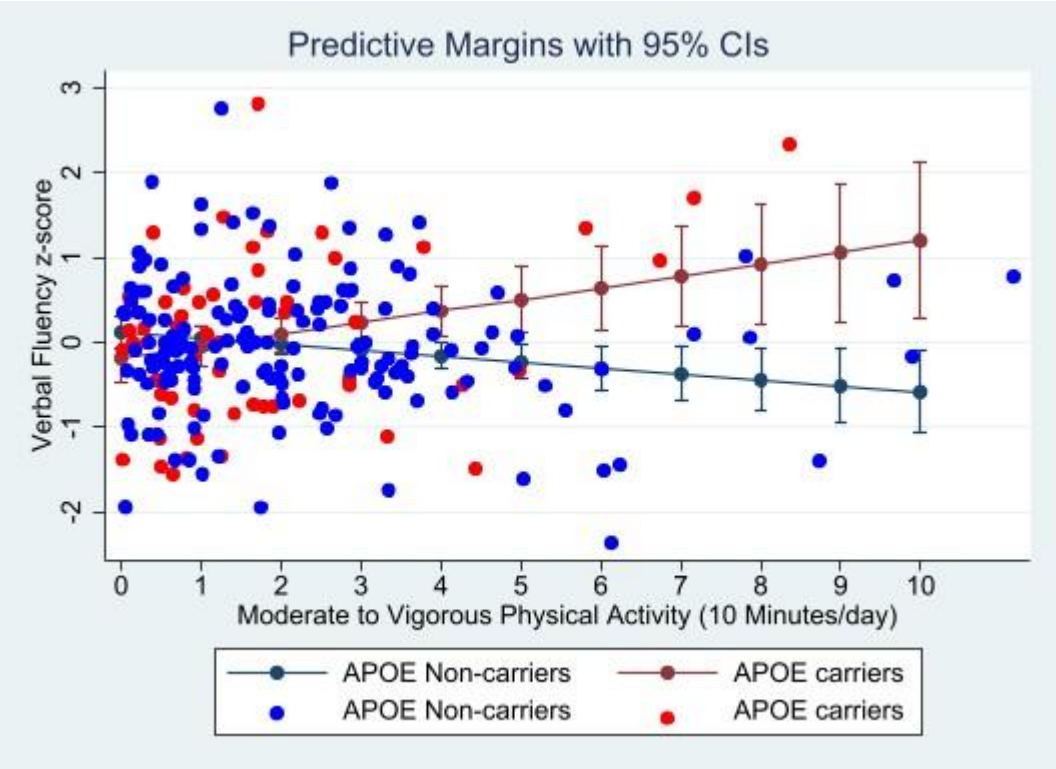


Figure 6.1 - The modification effect of APOE-ε4 for the associations between moderate to vigorous physical activity and verbal fluency

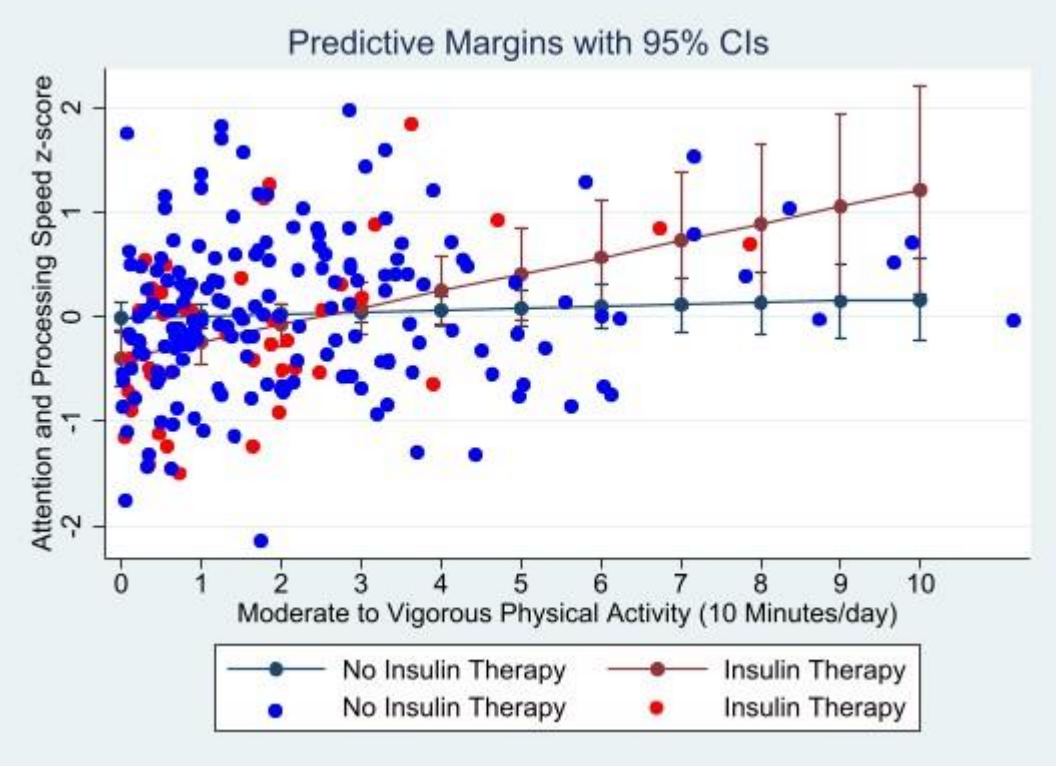


Figure 6.2- The modification effect of insulin therapy for the association between moderate to vigorous physical activity and attention and processing speed

6.5 Discussion

This is the first study to our knowledge to examine both the associations between objectively measured daily step count and MVPA with brain function and structure in people with T2D. We observed that a higher daily step count was associated with better attention-processing speed and greater hippocampal volume. APOE-ε4 and insulin therapy status modified associations between MVPA and cognition, such that the association between MVPA and verbal fluency was stronger in APOE-ε4 carriers and the association between MVPA and attention-processing speed was significant in those taking insulin-therapy. There were no other significant associations between PA and brain health.

Associations between step count, cognition and brain structure

The findings were consistent with our hypothesis regarding the association between a higher daily step count and better attention-processing speed and hippocampal volume. An association between greater daily steps and better attention-processing speed was in line with prior studies

in the general population of older adults (184) and those with heart failure (183). However, there were no associations with verbal fluency (183, 184) or executive function (task switching (182-184)) which was not consistent with previous observational studies or our hypothesis. Furthermore, a 2 year randomised controlled trial compared exercise (walking, resistance and flexibility training) with health education, and found a benefit of exercise on global cognition and memory, but not attention or executive function in people with T2D (364). Differences in findings may be due to differences in sample characteristics, cognitive tests measured, study design, timeframes or duration and intensity of physical activity. Interestingly, we found that waist to hip ratio attenuated the association between daily step count and attention-processing speed by 23%, suggesting a partial mediation role for abdominal obesity. This could be due to the effect of PA on decreasing visceral fat accumulation (365) and obesity related systemic inflammation (366) which affects the brain.

Our findings suggest that interventions aimed at increasing ambulatory activity could be tested in future randomised controlled trials to improve attention-processing speed in people with T2D. Additionally, the association between each extra 500 daily steps and 0.028 mL of hippocampal volume was consistent with the current knowledge that exercise may increase hippocampal volume via neurogenesis (367). However, based on a prior study, people with T2D (average age 67.8) had a hippocampal volume of on average 0.84 mL less than those without T2D (average age 72.1) (61). This would mean our effect size translates to people with T2D requiring at least an extra 15,000 steps per day to return to a hippocampal volume comparable to those without T2D. An increase of 15,000 extra steps would take participants mean step count to approximately 21,000, which may be hard to achieve in terms of motivation or in the face of T2D related comorbidities.

Associations between MVPA, cognition and brain structure

In line with our hypothesis, greater time spent in MVPA was associated with better performance in verbal fluency and attention processing speed, but this was stronger in APOE-ε4 carriers and those taking insulin-therapy respectively. In contrast, we found no associations between MVPA and hippocampal volume or other measures of executive function. Our findings were partially in line with a previous study that found APOE-ε4 carriers showed stronger associations between higher self-reported total PA, converted into metabolic equivalent min per week, and better verbal fluency in the general population (353). Greater levels of MVPA in people with the APOE-ε4 genotype could help with reducing other

metabolic risk factors related to APOE-ε4 such as atherosclerosis (368) which is linked to cognitive impairment (369). However, our results differed from the studies that found APOE-ε4 carriers get less benefit from PA (354-356), possibly because these previous studies mostly considered self-reported activities (354-356). Moreover, the significant association between higher MVPA and better attention processing speed for those on insulin therapy were inconsistent with our hypothesis that PA would have less benefit on cognition in those taking insulin-therapy. The cognitive benefits of MVPA for those on insulin-therapy warrant further research.

Surprisingly, our study did not replicate previous studies that found significant associations between moderate PA (mean 26.4 min/day) and better executive function (task switching) (194) in older adults or associations between moderate PA (mean 22.6 min/d) and greater hippocampal volume (202) in older adults with mild cognitive impairment. The reason might be due to the slightly lower level of MVPA in our study (average: 21.4 min/d) compared to the previous study (26.4 min/d) (194) in older adults, equivalent to an extra 35 min/week. It is possible that people with T2D might need more time in MVPA to alter executive function.

This study was novel as it was the first to investigate associations between objectively measured PA intensity and brain health in a sample of well-categorised people with T2D. The use of an accelerometer to measure PA, rather than by self-report, reduced the chance of recall or social desirability bias (370). A comprehensive battery of neuropsychological tests enabled examination of different cognitive domains. Furthermore, the addition of MRI structural measures allowed exploration of potential brain pathways. This study also has some limitations. Due to the cross-sectional design of the study, we could not determine causality or whether early or midlife PA is associated with cognition in later life. Due to the cross-sectional design, our effect size represents between-person differences. Prospective studies are needed to confirm these results based on within-person differences. Accelerometers cannot capture all types of activity such as swimming or cycling. We did not have other brain measures such as β-amyloid, which has previously shown to be associated with moderate intensity PA (371). Finally, the results need to be considered with caution due to multiple testing of significance.

In conclusion, among people with T2D, a higher number of steps per day may be beneficial for attention-processing speed and hippocampal volume. Greater time spent in MVPA seemed to specifically benefit attention-processing speed for APOE-ε4 carriers and verbal fluency for

those receiving insulin therapy. Future randomised controlled trials of exercise in people with T2D need to consider these factors.

6.6 Postscript

The findings of this chapter showed a higher daily step count was associated with better attention-processing-speed and greater total hippocampal volume. Moderate to vigorous physical activity was associated with better verbal fluency in APOE-ε4 carriers and better attention-processing speed in those receiving insulin therapy. There were no other significant associations.

The next chapter will provide a summary of the results, clinical and research implications and the future directions of the studies of this thesis.

Appendix 6.1 Supplementary Table 1 Cognitive test results and brain MRI measurements in participants with type 2 diabetes

MRI variables (n=165)	Mean±SD
Total hippocampal volume, mL	565.5±57.1
Grey Matter volume, mL	490.6±70.8
White Matter volume, mL	6.9±0.8
WMH volume, mL	4.2±5.9
Cognitive tests (n=220)	
Digit symbol coding, (n)	55.6±14.1
Hopkins immediate, (n)	24.9±5.2
Hopkins delay, (n)	8.6±2.7
Hopkins recognition, (n)	10.4±1.5
Rey complex copy, (n)	26.4±4.6
Rey complex delay, (n)	14.1±5.3
Digit span, (n)	18.7±3.7
Symbol search, (n)	26.9±6.6
COWAT word, (n)	37.8±12.0
COWAT category, (n)	19.3±4.8
Stroop dot time, (s)	13.7±2.9
Stroop word time, (s)	17.9±4.1
Stroop colour time, (s)	32.6±13.1
Trails A, (s)	32.5±10.7
Trails B, (s)	76.9±39.8

COWAT: Controlled Oral Word Association Test; MRI: Magnetic resonance imaging; (n): Number of items answered correctly; s: seconds; WMH: White matter hyperintensities

Appendix 6.2 Supplementary Table 2 Interactions between insulin-therapy and physical activity on cognitive domains, and brain volumes in participants with type 2 diabetes

Cognitive domains, z-score (n=220)	**β for insulin-therapy x steps (per 500/day) interaction term**	**95% CI**	***P***
Executive function	0.012	-0.030, 0.053	0.570
Attention-processing speed	0.040	-0.003, 0.083	0.070
Verbal fluency	-0.009	-0.060, 0.043	0.740
Verbal memory	0.003	-0.049, 0.055	0.919
Visual memory	-0.008	-0.072, 0.056	0.809
Visuospatial function	0.019	-0.043, 0.082	0.538
Global cognition	0.001	-0.022, 0.041	0.547
Brain volumes mL, (n=165)			
Grey matter volume	-1.935	-4.930, 1.060	0.204
White matter volume	2.066	-1.772, 5.904	0.289
Hippocampal volume	-0.009	-0.070, 0.052	0.781
White matter hyperintensities	-0.427	-0.960, 0.016	0.058
Cognitive domains, z-score (n=220)	**β for insulin-therapy x MVPA per (10 min/d) interaction term**	**95% CI**	***P***
Executive function	-0.023	-0.143, 0.097	0.711
Attention-processing speed	**0.149**	**0.025, 0.274**	**0.019**
Verbal fluency	-0.014	-0.162, 0.133	0.847
Verbal memory	0.028	-0.122, 0.179	0.713
Visual memory	0.069	-0.114, 0.252	0.459
Visuospatial function	0.122	-0.056, 0.301	0.177
Global cognition	0.055	-0.035, 0.145	0.228
Brain volumes mL, (n=165)			
Grey matter volume	-2.509	-10.457, 5.439	0.534
White matter volume	4.564	-5.603, 14.731	0.377

Hippocampal volume	-0.027	-0.190, 0.135	0.741
White matter hyperintensities	-0.595	-1.896, 0.705	0.367

Note: Models were adjusted for age, sex, education, mood, stroke, smoking and diet quality. Brain volume measures were additionally adjusted for total intracranial volume as a measure of brain size. MVPA: Moderate to vigorous physical activity.

Appendix 6.3 Supplementary Table 3 Interactions between apolipoprotein E-ε4 (APOE-ε4) and physical activity on cognitive domains, and brain volumes in participants with type 2 diabetes

Cognitive domains, z-score (n=216)	**β for APOE-ε4 x steps (per 500/day) interaction term**	**95% CI**	***P***
Executive function	0.025	-0.016, 0.066	0.232
Attention-processing speed	0.014	-0.029, 0.056	0.523
Verbal fluency	0.057	0.008, 0.107	0.023
Verbal memory	0.037	-0.013, 0.088	0.149
Visual memory	-0.012	-0.074, 0.051	0.717
Visuospatial function	-0.006	-0.068, 0.056	0.853
Global cognition	0.019	-0.012, 0.050	0.219
Brain volumes mL, (n=165)			
Grey matter volume	1.254	-1.476, 3.983	0.366
White matter volume	-3.637	-7.199, -0.076	0.050
Hippocampal volume	0.017	-0.039, 0.073	0.553
White matter hyperintensities	0.069	-0.381, 0.519	0.763
Cognitive domains, z-score (n=216)	**β for APOE-ε4 x MVPA per (10 min/d) interaction term**	**95% CI**	***P***
Executive function	0.035	-0.06, 0.137	0.498
Attention-processing speed	0.055	-0.051, 0.160	0.310
Verbal fluency	**0.197**	**0.076, 0.317**	**0.002**
Verbal memory	0.059	-0.067, 0.186	0.356
Visual memory	-0.071	-0.226, 0.084	0.365
Visuospatial function	0.006	-0.148, 0.160	0.938
Global cognition	0.047	-0.030, 0.124	0.232
Brain volumes mL, (n=163)			
Grey matter volume	2.922	-3.851, 9.694	0.395
White matter volume	-4.707	-13.648, 4.235	0.300

Hippocampal volume	0.017	-0.039, 0.073	0.553
White matter hyperintensities	-0.035	-1.150, 1.080	0.951

Note: Models were adjusted for age, sex, education, mood, stroke, smoking and diet quality. Brain volume measures were additionally adjusted for total intracranial volume as a measure of brain size. MVPA: Moderate to vigorous physical activity

Appendix 6.4 Additional analysis

Investigating the association between light physical activity and brain health was not part of the aims of this chapter. There is evidence of the association between greater levels of engagement in light physical activity and better brain health. Therefore, the results of the association between light physical activity, cognition, and brain volumes are provided in this appendix. There was no association between light physical activity and measures of brain health in this thesis.

Supplementary Table 4 The association between daily light physical activity, cognitive function and brain volumes in people with type 2 diabetes

	Light physical activity time per 10 min/d	
Cognitive domains, z-score (n=220)	β	95%CI
Executive function	0.0002	-0.013, 0.013
Attention	0.012	-0.0016, 0.026
Verbal memory	-0.002	-0.018, 0.014
Visual memory	-0.001	-0.022, 0.019
Verbal fluency	-0.004	-0.021, 0.012
Visuospatial function	0.007	-0.013, 0.027
Global cognition	0.002	-0.079, 0.012
Brain volumes mL, (n=165)	β	95%CI
Grey matter volume, mL	0.490	-0.36, 1.35
Total hippocampal volume, mL	0.011	-0.0062, 0.028
White matter volume, mL	0.190	-0.91, 1.30
WMH volume, mL	-0.045	-0.18, 0.095

The model adjusted for sex, age, education, smoking, stroke, diet quality. Brain volume measures were additionally adjusted for total intracranial volume.

7. Summary

7.1 Background and aim of this thesis

Dementia is a significant public health concern (326). Symptoms of dementia include progressive cognitive decline, psychological, motor and behavioural changes, which lead to impairments in activities of daily living (1, 2). It is imperative to prevent or delay the onset of dementia as there is no established cure. Modifiable risk factors for dementia include nutrition, physical activity and type 2 diabetes (T2D) (372).

Dietary intake is associated with cognitive decline and increased risk of dementia (57, 58, 373, 374). Previous observational studies suggest that certain foods and nutrients are associated with better brain health (98). However, findings have not been replicated in randomised controlled trials (104, 105). This may be because nutrients and foods are consumed as part of a diet, not in isolation (104, 105). In contrast, dietary pattern approaches (data-driven dietary patterns, and dietary indices) focus on the nutrient and food combinations. They, therefore, could be more predictive of disease processes (107). Dietary indices such as the Mediterranean diet (130, 375), the Mediterranean-dietary approaches to stop hypertension Intervention for Neurodegenerative Delay (MIND) diet (140, 141), and international dietary guidelines from other countries (148, 149, 151) have been investigated in relation to brain health. However, few studies have investigated whether certain types of dietary patterns (derived from principal component analysis (110, 116-118, 120)) or dietary indices (the dietary inflammatory index (145, 147), and the Australian Dietary Guidelines (158)) are associated with brain health in older people. The majority of these studies used cognitive screening tools (110, 117, 120, 158). Only a few studies examined cognitive domains (116, 145, 147) and even fewer included brain imaging (118). Measures of structural brain imaging may provide information about the pathways by which diet may affect cognitive function.

People with T2D are at higher risk of developing dementia (329) and may have different dietary habits compared to those without T2D (123). There is only one study of people with T2D that examined the association between data-driven dietary patterns and general cognition (124). However, there are no studies to date that have examined the association between the dietary inflammatory index or the Australian Dietary Guidelines and brain structure. Examining associations between different dietary patterns/indices and brain health would assist in tailoring preventative interventions for people with T2D.

Physical inactivity is associated with both T2D and a greater risk of dementia (59). Previous studies found that greater intensity of physical activity is associated with better cognition and greater brain volume in older people (376). However, it is unknown if there are similar benefits for people with T2D. There are only two studies that have specifically examined people with T2D to examine the associations between self-reported physical activity intensity and general cognition (206, 207). Objectively measured physical activity would provide more accurate information on the intensity of physical activity measures (178).

Therefore, the main aims of this thesis were to investigate the associations between:

1) data-driven dietary patterns, cognition, and brain structure and to examine whether the associations are different in people with T2D compared to those without T2D.

2) a dietary inflammatory index, cognition, and brain structure and to examine whether T2D modifies any associations.

3) adherence to the Australian Dietary Guidelines, cognition, and brain structure and to examine whether T2D modifies any associations.

4) objectively measured physical activity, cognition, and brain structure and to examine if the genetic risk for dementia (APOE-ε4), or T2D severity (insulin therapy) modifies any associations.

7.2 Methods

For the first three aims, participants were recruited from the Cognition and Diabetes in Older Tasmanians (CDOT) study which includes a sample of people (aged 55-85 years) with and without T2D. For the last aim, participants were recruited from the CDOT-Blood Pressure study which was a sample of people with T2D. In both studies, participants were >55 years old. The exclusion criteria were having dementia, living in a nursing home or having a contraindication for MRI. In all studies, the 80-item Cancer Council of Victoria food frequency questionnaire (FFQ) was used to assess dietary intake. Dietary intake was assessed using three methods: data-driven dietary patterns calculated using principal component analysis, an inflammatory diet using the energy-adjusted dietary inflammatory index and the Australian Dietary Guidelines Index. Physical activity (steps per day and intensity) was measured using an accelerometer over 7 days. A battery of neuropsychological tests was used to obtain information on cognitive domains (verbal memory, visual memory, visuospatial function, executive function, verbal fluency, attention-processing speed, and global cognition). MRI was performed to obtain grey, white matter, and hippocampal volumes and markers

of small vessel disease (microbleeds, infarcts, and white matter hyperintensity volume).

7.3 Major findings and implications of the dietary studies

To follow are summaries of the major findings of each chapter, along with summaries of implications.

Chapter 3: Dietary patterns are not associated with brain atrophy or cerebral small vessel disease in older people with and without type 2 diabetes

The aim of this study was to examine the associations between data-driven dietary patterns, cognitive domains, brain volumes and small vessel disease in people with and without T2D. This is one of the first studies to date to examine dietary patterns and their association with brain imaging (brain volumes and small vessel disease).

There were two dietary patterns (prudent and traditional) for people with T2D and three dietary patterns (prudent, traditional, and Western) for those without T2D. For people without T2D, higher adherence to the Western dietary pattern was associated with lower grey matter volume in the initial model, adjusted for age, sex, education and energy. The addition of a cardiovascular risk score, mood and physical activity weakened the association such that it was no longer significant for the Western dietary pattern. This was mainly driven by the cardiovascular risk score (model attenuated by 20%). Vascular disease might plausibly be on the pathway between diet and brain health. There were no other significant associations between dietary patterns and brain health measures.

Chapter 4: Associations between the dietary inflammatory index, brain volume and small vessel disease

The main aim of the study was to investigate the association between the energy-adjusted dietary inflammatory index, brain volumes, small vessel disease and general cognition in people with and without T2D. The secondary aim was to explore whether T2D modifies any of the associations. This is the first study to date to examine the associations between the energy-adjusted dietary inflammatory index and measures of brain imaging.

Apart from one significant interaction term observed among multiple models, the main findings were not significant. The only significant finding was that the association between the energy-adjusted dietary inflammatory index and lower grey matter volume was stronger in people without T2D. There was a weak positive correlation between the energy-adjusted dietary inflammatory index and blood levels of inflammatory biomarkers.

Chapter 5: Adherence to the Australian Dietary Guidelines is not associated with brain structure or cognitive function in older people

The main aim of the study was to investigate the association between the Australian dietary guidelines, cognitive domains, brain volumes, and small vessel disease in people with and without T2D. The secondary aim was to explore whether having T2D modified any associations. To date, this is the first study in older people to evaluate associations between adherence to the Australian dietary guideline recommendations and brain imaging and different cognitive domains.

No associations were found between adherence to the Australian Dietary Guidelines and cognitive function, brain volume or small vessel disease. T2D did not modify any associations.

Implications for practice - the first three studies on diet

Data-driven dietary patterns provide information about the sample's usual dietary patterns particularly the combination of foods consumed together. That could be a combination of healthy, unhealthy or a mix of healthy and unhealthy food components which define different data-driven dietary patterns. This type of analysis is particularly useful to gain an insight into the diet consumed by a sample or population. However, the hypothesis-driven dietary patterns are pre-defined based on current research knowledge in the literature. The dietary inflammatory index measures the adherence to an inflammatory diet based on understanding of the association between inflammatory biomarkers, nutrients, and food components. The Australian dietary guidelines index measures people's adherence to the evidence-based national dietary recommendation. Although they seem to be different methods with different purposes, dietary patterns derived based on both approaches could overlap in specific food components. For example, a data-driven healthy dietary pattern could include some of the healthy food components of the national dietary guidelines and a data-driven unhealthy dietary pattern could contain some of the components with similar nutrient content of the dietary inflammatory index. Despite applying different dietary methods, overall, there was very little evidence found to show that the dietary patterns examined in this thesis were associated with brain health. The studies in this thesis did not find sufficient evidence in terms of which diet is the most effective for dementia prevention. Taken together with the prior literature, the recommendations for dementia prevention would be based on the Mediterranean diet.

Despite applying different dietary methods, overall, there was very little evidence found to show that the dietary patterns examined in this thesis were associated with brain health. This was surprising as prior studies found Western dietary patterns (110, 116-118, 120), or the energy-adjusted dietary inflammatory index (145, 147), were associated with brain health. Based on prior evidence (118, 151),

it was hypothesised that data-driven healthy dietary patterns and adherence to the Australian Dietary Guidelines would be associated with better brain structure, while data-driven unhealthy dietary patterns and the energy-adjusted dietary inflammatory index would be associated with poorer brain structure.

The Australian Dietary Guidelines recommended avoiding unhealthy foods such as heated processed foods to improve health and wellbeing and prevent T2D (154). Similar unhealthy foods were featured in the Western dietary pattern and the inflammatory diet in this thesis. The weak evidence of the association between unhealthy diets and lower grey matter volume in people without T2D should be considered with caution due to multiple comparisons. Despite our findings, there are still many benefits of adopting healthy dietary behaviours, as a healthy diet (Mediterranean diet and Dietary Approaches to Stop Hypertension DASH diet) has been shown to benefit a number of risk factors for dementia, such as hypertension, and cardiovascular disease (377, 378). Adherence to the Australian Dietary Guidelines has been shown to reduce risk factors for cardiovascular disease (155-157).

The Australian Dietary Guidelines have been developed to improve overall health and wellbeing (154). Based on the findings from this thesis, adherence to the Australian Dietary Guidelines was not associated with brain health. That could be because the Australian Dietary Guidelines include broad recommendations for general health and do not have separate recommendations for the food components that are important for brain health. For example, the Mediterranean-DASH Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) diet includes components for dark green vegetables, nuts, seafood, berries and olive oil and has been effective in improving brain health, which may be due to high contents of nutrients and antioxidants that are protective against cognitive decline, and brain atrophy (143, 375, 379). The beneficial nutrients include mono-unsaturated and polyunsaturated fatty acids, vitamin B, D, C, and minerals (143, 379). Specific recommendations for foods that have been shown to benefit brain health could be considered for inclusion in future versions of the Australian Dietary Guidelines.

Even adding separate recommendations for brain health may not change people's dietary behaviours. High adherence to the Australian Dietary Guidelines in older adults is associated with level of education and socioeconomic status (380). The mean adherence of people to the Australian Dietary Guidelines was poor based on the results of this thesis which is consistent with results from a prior study in older adults (380). An increase in people's knowledge and understanding of the dietary guidelines' recommendations may be necessary for improving healthy dietary habits (381). The media, clinicians, schools, and public health campaigns have important roles in improving people's

knowledge of the Australian Dietary Guidelines and building healthy dietary behaviours. Policy changes that may influence people's dietary habits and reduce the number of unhealthy foods that people eat, include increasing taxes (382), repricing, or reformulating products to make them healthier (383).

In this thesis people with T2D had a healthier diet compared to people without T2D. This was evident as the Western dietary pattern was only detected in people without T2D. In addition, people with T2D had lower adherence to an inflammatory diet and better adherence to the Australian dietary recommendations. This might imply that people with T2D are more conscious about their diet than people without T2D, because people with T2D monitor glycaemic levels and may also be trying to lose weight. A previous qualitative study reported that while participants with T2D might improve their diet quality immediately after diagnosis, they found it challenging to maintain healthy dietary behaviours (384). This could be due to participants being uncertain of ideal dietary habits or insufficient time with health professionals to discuss ways to change and maintain behaviours (384). However, observations in this thesis could also be due to reporting bias. Our study provided only a snapshot of dietary habits. It would be interesting for future studies to understand if people with T2D who adopt a healthier diet after diagnosis and maintain it over time also reduce their risk of dementia.

Dieticians and general practitioners are essential to promote healthier dietary habits for those with and without T2D. In Australia, the accessibility of dietetics care through Medicare for a general older person is only possible if the person has a chronic condition, and only five visits are subsidised per year. This may limit accessibility and may not be sufficient to change long term behaviours. A healthy diet should be taught and promoted from an early age, where schools have an important role to play.

Chapter 6: The association between physical activity intensity, cognition and brain structure in people with type 2 diabetes

This study aimed to examine the relationship between objectively measured physical activity (moderate to vigorous physical activity and daily steps count) and cognition and brain structure among participants with T2D. The secondary aim of this study was to explore whether genetic risk for dementia (APOE-ε4) or T2D severity (insulin-therapy) modified any associations. This is the first study in people with T2D to examine associations between objectively measured physical activity intensity with cognitive domains, and brain structure.

A higher step count was associated with better attention processing speed (which was partially mediated by abdominal obesity) and larger hippocampal volume. APOE-ε4 modified associations

between moderate to vigorous physical activity and verbal fluency, such that associations were stronger in APOE-ε4 carriers. Insulin therapy modified associations between moderate to vigorous physical activity and attention processing speed, such that the association was stronger in people receiving insulin therapy. There were no other significant associations.

Implications for practice - physical activity

Based on the physical activity guidelines for Australians, "adults older than 65 years should complete at least 30 minutes per day (min/d) of moderate-intensity physical activity on most, preferably all, days of the week plus at least 2 days per week of progressive resistance exercises, and balance training" (385). The recommended physical activity specifically for people with T2D is to complete 30 min/d of physical activity for good health or 45-60 min/d to lose weight (386). In our sample, of participants with T2D, the average time of engagement in moderate to vigorous physical activity was 21 min/d. Therefore, on average people with T2D in this thesis did not meet recommendations.

In this thesis, participants did not have dementia, but potentially some may have had mild cognitive impairment. The Australian physical activity recommendations for older people with mild cognitive impairment or subjective cognitive decline include "engaging in at least 150 minutes per week (21.4 min/d) of moderate aerobic activity or 90 minutes (12.9 min/d) of vigorous intensity aerobic activity per week" (387). People with mild cognitive impairment also need to engage in activities that help to improve or maintain balance. It also recommended for this group to engage in at least 2 days per week of progressive resistance exercises (387). Resistance training may benefit brain health (388) but unfortunately we are unable to determine if participants met the guidelines for these other types of activities. Resistance training also has beneficial effects for glycemic control in people with T2D (389). Future studies need to examine whether the recommendations should be different if a person has both mild cognitive impairment and T2D.

In this thesis, associations between moderate to vigorous physical activity and brain health were observed in APOE-ε4 carriers and people receiving insulin therapy. The average time spent in moderate to vigorous physical activity was 18.2 min/d for APOE-ε4 carriers and 16.5 min/d for those receiving insulin therapy which is even less than the average in the overall sample. It needs to be further studied as to whether physical activity recommendations should be more intense or more frequent for APOE-ε4 non-carriers and those with diabetes not on insulin.

There are no physical activity guidelines including recommendations for daily step count. Based on evidence from the literature, 7,000 to 10,000 daily steps is recommended for healthy older adults with

at least 3,000 of those steps being at a brisk pace. Those with a disability or chronic illness (including diabetes) are recommended to complete 6,500 to 8,500 steps per day (390). The average daily step count in our study was 5,777 steps per day. The mean daily step count was 5,245 steps for APOE-ε4 carriers and 4,996 steps for people receiving insulin therapy. Overall, in this thesis, the average daily steps were less than recommendations for people with chronic diseases such as T2D.

Based on the results of this thesis, each extra 500 daily steps was associated with 0.028 mL of additional hippocampal volume. However, based on a prior study, people with T2D (average age 67.8) had a hippocampal volume of on average 0.84 mL less than those without T2D (average age 72.1) (8). This would mean our effect size translates to people with T2D requiring at least an extra 15,000 steps per day to return to a hippocampal volume comparable to those without T2D. An increase of 15,000 extra steps would take the mean step count of participants to approximately 21,000. The feasibility of this increase may need further research. People with T2D may need to engage in more steps per day than current recommendations to gain brain health benefits. In future versions of physical activity guidelines, an increase may be needed in the amount of recommended daily steps for people with T2D to improve brain health.

7.4 Implications, limitations and future directions for research

The work in this thesis has contributed to the body of evidence on associations between diet (dietary patterns, dietary indices), objectively measured physical activity (daily step and physical activity intensity) and measures of brain structure and function in people with and without T2D. There are however gaps which warrant further research. Ideas for future studies are listed below.

Longitudinal studies

The data for this study could not prove causation due to the cross-sectional design. Therefore, the study findings should be tested in future prospective studies. In addition, current diet and physical activity are not representative of lifetime habits. Therefore, the longitudinal relationship between diet, physical activity, cognition and markers of brain structure should be examined through a lifespan approach to determine the best time to intervene (early- or mid-life) and the most appropriate strategies to reduce the prevalence of dementia.

According to the studies from this thesis, moderate to vigorous physical activity and daily steps may target different aspects of brain health. Future longitudinal studies need to examine moderate to vigorous physical activity and step count over time to investigate which forms (including resistance

training) of physical activity are more beneficial to brain health for people with T2D, and if they affect brain health through different mechanisms.

In all three dietary studies, people with T2D showed healthier dietary habits than those without T2D. Studies in this thesis did not collect information on the dietary habits of participants before being diagnosed with T2D. There may have been changes in people's dietary habits in the period before diagnosis to a few years after diagnosis. Change in diet may have an effect on brain health. It would be interesting to track the changes in dietary habits before and after T2D diagnosis to investigate if any changes in diet affect brain health.

The reasons why adherence to the Australian Dietary Guidelines was not associated with brain health should be further investigated. These findings are in line with another recent Australian study (158). Future studies could compare the Australian Dietary Guidelines with dietary guidelines from other countries or other dietary indices in the same sample to determine which diets are most protective against dementia and cognitive decline.

Randomised controlled trials

Prior randomised controlled trials compared exercise (walking, resistance and flexibility training) with health education and found a benefit of exercise on global cognition and memory, in people with T2D but not in those without T2D (364). Future randomised controlled trials need to examine whether the effects of physical activity/exercise on brain health are different based on insulin therapy and genetic risk status in people with T2D. This may help clinicians tailor physical activity routines. Future studies need to examine whether there is a threshold for daily steps where brain health improves. Future randomised controlled trials could also examine whether increasing the number of steps (>20, 000/d) or engagement in high-intensity exercise at mid-life is feasible in people with T2D and if it is effective for reducing the rate of cognitive decline.

This thesis found little evidence for associations between specific dietary patterns and brain health. However, physical activity seemed to give slightly more benefit to the T2D group compared to non-T2D group. This thesis considered nutrition and physical activity separately. However, nutrition and physical activity likely interact as common lifestyle behaviours in dementia prevention (391). Prior studies showed that individuals with increased activity levels over a 10-year period are more likely to report a better diet quality (392). Very few studies have investigated the effect of a combined diet and physical activity intervention for cognitive improvement (393). The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) trial (n=2654)

implemented a multidomain intervention (n=1260), including exercise, nutrition, health education, and cognitive training for 2 years compared to n=629 control group in at-risk older adults without substantial cognitive impairment (393). The multidomain intervention group showed a greater improvement in global cognition, processing speed, and executive functioning compared to the control group (393). Future research needs to investigate the effect of multidomain interventions of lifestyle behaviours (nutrition and physical activity) for dementia prevention.

More sensitive tools

Accelerometers are typically considered as the gold standard in physical activity assessment, as they are more reliable compared to other methods, and instead of just measuring steps per day (pedometers) they can measure physical activity intensity (394). The cognitive tests used in this thesis have been shown to be sensitive to mild cognitive decline due to ageing (243). The magnetic resonance imaging measures used in this thesis may have a lack of sensitivity to detect small differences. Future studies could consider other methods including blood biomarkers or other types of brain imaging such as PET-amyloid, functional MRI or diffusion tensor imaging that could be more sensitive to small pathological changes in the brain.

The FFQ used in this thesis is a valid, reliable and widely-used dietary assessment tool (219) which facilitates comparison of the results with other datasets. An advantage of using an FFQ is to get a measure of usual intake in large-scale epidemiological studies in a relatively simple, cost-effective and time-efficient manner (286). However, FFQs may also lead to information or recall bias. FFQs are often criticised for their less accurate estimations of dietary intake compared to other dietary assessment methods such as a 3-day dietary record and a 24-h dietary recall (395). Participants' current diet may influence their answer to their average dietary intake over the past 12 months. It is also difficult for participants to calculate or recall their diet in the past 12 months. Ideally, an objective measure such as a nutrient biomarker panel that reflects dietary pattern adherence could be employed in future studies as an additional approach to circumvent the limitations of participant self-reported dietary intake. However, the FFQ used in this study was previously evaluated as correctly capturing most nutrient intakes in comparison to a 3-day food record (219). Moreover, the FFQ used in this thesis did not include all 45 food components needed for the dietary inflammatory index calculation. Therefore, future studies need to consider using more comprehensive and dietary assessment tools (e.g. multiple 3-day dietary records, or 24-h dietary recalls). In addition, the FFQ used in this thesis was not specific for brain health and future studies might consider using an FFQ that include items related to brain health such as berries, olive oil, olives, and seafoods in addition to fish.

The Mediterranean diet was not considered in this thesis. Some of the components of the Mediterranean diet (such as olive oil, seeds, herbs or spices) were not captured by the FFQ used in this thesis. In addition, there have been a number of studies that have already examined the association between the Mediterranean diet, cognitive function (130-134) and brain structure (135-137). However, there is only one study that investigated whether association between the Mediterranean diet and incident cognitive impairment is different based on T2D status (396). Future studies are needed to investigate associations between the Mediterranean diet, individual cognitive domains and brain structure in people with T2D.

To remove the confounding effect of energy intake, people with implausibly high and low values of energy intakes were removed in a sensitivity analysis in chapter 5. However, the main results remained the same after removal of those people. People with implausibly high and low energy intake were identified in the samples for chapter 3-4. However, those people were not removed from the analyses because they included 40% of the sample. Meanwhile, the regression models were adjusted for total energy intake to address the confounding effect of energy for all of the regression models across chapter 3-5. Future studies need to consider the removal of extreme energy values as a method for considering the confounding effect of energy intake.

In this thesis, the characteristics of participants were similar for the full sample (n=705) compared to those included in the analyses (n=688) (Appendix 7.1). Our sample appears representative of the general population of Australians. For the first three studies, out of 343 people with T2D, 72% had hypertension, while 71% of older adults (aged ≥65 years) are reported to have hypertension in general population (27). For the first three studies, out of 345 people without T2D, 46% had hypertension. Similarly, according to the Australian Bureau of Statistics, 44.5% of older adults aged ≥65 years, have hypertension (28). In this thesis, the effect of diet or physical activity on brain health might be small and difficult to capture. Future studies need to use a larger sample size to provide greater statistical power to detect small but important differences in diet or physical activity.

We used APOE-ε4 as a genetic marker of dementia. However, dementia is likely to occur as a result of complex interactions between other genetic factors, the environment and lifestyle behaviours. The inclusion of APOE-ε4 in personalised medicine at this time is controversial, but in the future may be considered as part of a broader panel of information including other genetic markers and risk factors (398).

7.5 Concluding remarks

The studies presented in this thesis were designed to address the gaps in the literature in relation to association between modifiable risk factors (diet and physical activity), cognitive function, brain volume and small vessel disease. There was limited and inconsistent evidence that diet (assessed using three different methods: data-driven dietary patterns, a dietary inflammatory index, and the Australian Dietary Guidelines) or physical activity were associated with brain health in older people. With reference to the research questions, the conclusions are as follows:

1) There was evidence of an association between higher intake of a Western dietary pattern and lower grey matter volume in people without T2D, although this disappeared when including other cardiovascular risk factors in the model. There were no other significant associations.

2) There was evidence of effect modification for T2D on the association between a higher intake of an inflammatory diet and lower grey matter volume, such that the association was stronger in people without T2D. This association was independent of cardiovascular risk factors and use of anti-inflammatory medication. There were no other significant associations.

3) There was no association between adherence to the Australian Dietary Guidelines and brain health. The associations were not different based on T2D status.

4) In people with T2D, higher daily step count was associated with better brain health (attention and hippocampal volume). The association between higher moderate to vigorous physical activity, and verbal fluency was stronger in APOE-ε4 carriers. The association between higher moderate to vigorous physical activity, and attention-processing speed was significant in people receiving insulin therapy. There were no other significant associations.

5) Overall, despite applying different dietary patterns methods, there was very little evidence found to show that the dietary patterns examined in this thesis were associated with brain health. Physical activity instead appeared to be more beneficial to those with T2D. Future studies need to include multidomain interventions of both diet and physical activity for the prevention of dementia in people with T2D.

Appendix 7.1 Supplementary Table 1-The comparison of the characteristics of full sample with people who had data on diet and cognition

Characteristics	n=705 Full sample	n=688 with diet and cognition
Age, (Mean±SD)	69.9±7.4	69.9±7.4
Type 2 Diabetes, n (%)	394 (49.3)	343 (49.9)
Male, n (%)	400 (56.7)	393 (57.1)
Education[1], n (%)	337 (47.8)	331 (48.1)
History of Hypertension, n (%)	414 (58.7)	406 (59.0)
History of Smoking, n (%)	367 (52.1)	357 (51.9)
History of Stroke, n (%)	60 (8.5)	58 (8.4)
History of angina, n (%)	102 (14.5)	99 (14.4)

[1]High school and above

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