



School of Medicine,

Department of Medical Gerontology

**Folate and Vitamin B₁₂ in The Irish Longitudinal Study on
Ageing cohort: associations with cognitive function**

A thesis submitted to the University of Dublin, Trinity
College, for the Degree of Doctor of Philosophy in the
School of Medicine.

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Declaration

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A handwritten signature in black ink, appearing to read 'Deirdre O'Connor', written in a cursive style.

Date: 31st May 2023

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LIST OF ABBREVIATIONS

AD - Alzheimer's disease	HA – Health Assessment
AF - Animal Fluency	HCY - homocysteine
ATC - Anatomical Therapeutic Chemical	HHCY – hyperhomocysteinemia
BBB - blood-brain-barrier	IADL - Instrumental Activity of Daily Living
BMI - body mass index	ICC – Intraclass correlation coefficients
BP – blood pressure	IPAQ - International Physical Activity Questionnaire
CAPi - computer-aided personal interview	IQR – interquartile range
CES-D - Center for Epidemiologic Studies Depression Scale	IRR - incidence rate ratio
CHD – coronary heart disease	ISBP – impaired stabilisation of blood pressure
CI – confidence interval	LBM - lean body mass
CNS – central nervous system	MCI - mild cognitive impairment
CR - caloric restriction	MedDiet - Mediterranean diet
CSF – cerebrospinal fluid	MFAF - Mandatory folic acid fortification
CVD - Cardiovascular disease	MIND - Mediterranean–DASH Intervention for Neurodegenerative Delay
DHF - Dihydrofolate	MMSE - Mini Mental State Examination
DHFR - Dihydrofolate reductase	MoCA - Montreal Cognitive Assessment
DNA - deoxyribonucleic acid	MRI - magnetic resonance imaging
DASH - Dietary Approaches to Stop Hypertension	MS - Metabolic syndrome
DSST - Digit Symbol Substitution Test	MSyn - Methionine synthase
EPIC - European Prospective Investigation of Cancer and Nutrition	MTHFR - Methylene tetrahydrofolate reductase
EU - European Union	NADPH - Nicotinamide adenine dinucleotide phosphate
FAD - Flavin adenine dinucleotide	NANS – National Adult Nutrition Survey
FA - folic acid	NHANES - National Health and Nutrition Examination Survey
FBDGs - food based dietary guidelines	
FDR – false-discovery rate	
FFQ - food frequency questionnaire	
FP - food pyramid	
GMB - gut microbiota	

NO – nitric oxide
NTD - neural tube defect
OH - orthostatic hypotension
OR - odds ratio
PA - pernicious anaemia
PGA - Pteroylglutamic acid
PPI - Proton pump inhibitors
RCT - randomised controlled trial
RNA - ribonucleic acid
RDA - recommended daily allowance
RTEBCs - Ready-to-eat breakfast cereals
SALSA - Sacramento Area Latino Study
on Ageing
SCFA - short-chain fatty acids
SCQ - Self-Completion Questionnaire
SD - Standard deviation
SLÁN - Survey of Lifestyle, Attitudes and
Nutrition
T2DM - type-2 diabetes mellitus
TILDA - The Irish Longitudinal Study on
Ageing
THF - Tetrahydrofolate
TIA - transient ischemic attack
TUL - Tolerable Upper Intake Level
UK - United Kingdom
US - United States
UVB - ultraviolet B
WHO - World Health Organization

SUMMARY OF THESIS

The aim of this Ph.D. thesis is to examine nutrition, cognitive and autonomic dysfunction in the older population in Ireland. One review and five separate quantitative studies are presented in this thesis, which use data from The Irish Longitudinal Study on Ageing (TILDA), a large population representative cohort study of adults over the age of 50 residing in Ireland. The thesis focuses on the relationship between folate and vitamin B₁₂ with cognitive dysfunction and commonly observed manifestations of autonomic dysfunction - orthostatic hypotension (OH). Nutritional behaviour among the population in the form of compliance with national food-based dietary guideline (FBDGs) are also examined.

Chapter summaries:

Background and Literature Review

Chapter One provides an overview of the background literature related to public health nutrition changes, technological advancements, and the impact on the ageing population. It introduces the role of the B-vitamins folate and vitamin B₁₂ in maintaining metabolic function and discusses the "*methylfolate trap hypothesis*" that suggests vitamin B₁₂ deficiency impairs overall folate metabolism. The chapter also examines the nutritional behaviours of older adults in Ireland. **Chapter Two** reviews the existing evidence on the relationships between folate, vitamin B₁₂, and cognitive function, and blood pressure control (as an indicator of autonomic function) in older adults. It reviews studies examining the role of folate and vitamin B₁₂ in cognitive dysfunction and blood pressure control among older adults.

Methodology

Chapter Three gives an overview of the methodologies employed in TILDA, provides a detailed description of the data acquisition across the longitudinal

elements of the study, and describes the measures used in each of the individual studies that make up this thesis.

Five Quantitative Studies

Chapter Four presents the first empirical study of the thesis, which aims to quantify plasma folate and vitamin B₁₂ status in a nationally representative population of older adults in Ireland. It determines the prevalence of low status and deficiency rates of folate and vitamin B₁₂ and investigates the factors influencing these vitamin concentrations. **Chapter Five** presents the second empirical study, which investigates the cross-sectional associations between folate, vitamin B₁₂, and two tests of global cognitive function in older adults. It tests the hypothesis that lower concentrations of folate and vitamin B₁₂ are associated with poorer cognitive health. **Chapter Six** focuses on the third empirical study, which examines the longitudinal associations between folate concentrations and changes in several domain of cognitive performance over eight years. It investigates the hypothesis that low folate status is associated with accelerated cognitive decline over time. **Chapter Seven** presents the fourth empirical study of my thesis, which investigates the cross-sectional and longitudinal associations between plasma folate, vitamin B₁₂ concentrations, and orthostatic hypotension (OH) in older adults. It examines the hypothesis that low levels of folate and vitamin B₁₂ may increase the risk of OH.

Chapter Eight focuses on the fifth and final empirical study, which aims to describe and summarise the consumption frequencies and compliance levels of food groups as defined by national dietary recommendations. It examines the dietary patterns and adherence to food guidelines among older adults in Ireland.

Discussion and Conclusions

Chapter Nine provides a discussion of the main findings of the thesis and their implications. It summarises the key findings from each empirical study and their contribution to the overall understanding of the relationships between folate, vitamin B₁₂, cognitive function, orthostatic hypotension, and adherence to dietary

guidelines in older adults. The chapter concludes by highlighting the need for age-specific and sustainable nutritional guidance for older adults.

Overall, the thesis investigates the associations between folate, vitamin B₁₂, cognitive function, orthostatic hypotension, and dietary patterns in older adults, providing valuable insights into the role of nutrition in ageing and important health outcomes.

LAY ABSTRACT

This is a comprehensive thesis exploring the role of folate and vitamin B₁₂ in cognitive function, orthostatic hypotension, and adherence to food-based dietary guidelines among older adults. The thesis comprises nine main chapters, encompassing literature review, empirical studies, and analyses of a nationally representative dataset.

The introductory chapters establish the backdrop for the thesis by discussing the demographic shift towards an ageing society and the rising prevalence of age-related conditions such as neurodegeneration and dementia. The importance of optimising nutrition, particularly folate and vitamin B₁₂, in reducing the risk of these pathologies is highlighted. The "methylfolate trap hypothesis" is introduced, suggesting that a deficiency in vitamin B₁₂ impairs overall folate metabolism. Safety concerns regarding the masking of vitamin B₁₂ deficiency by folic acid (FA) supplementation are also addressed.

The thesis proceeds with five empirical studies utilising the dataset. The first study investigates the prevalence of folate and vitamin B₁₂ deficiencies among older adults in Ireland and identifies demographic and lifestyle determinants associated with low vitamin levels. Findings indicate high rates of deficiency, particularly among the oldest age group and individuals with lower asset wealth. Gender disparities and the impact of economic status and lifestyle factors on vitamin levels are also discussed. The subsequent study examines plasma folate and vitamin B₁₂ concentrations as predictors of cognitive function. Results demonstrate that elevated folate levels do not pose increased cognitive risk, but higher concentrations of folate are associated with better global cognitive function. The third study focuses on longitudinal associations between baseline folate status and cognitive decline, revealing that low folate levels are linked to declines in global cognitive function and episodic memory over an eight-year period. The fourth study investigates the relationship between plasma folate and vitamin B₁₂ concentrations and orthostatic hypotension (OH) in older adults. While univariate longitudinal associations are observed, the addition of antidepressant medication attenuates these associations. Finally, the fifth study

examines adherence to food-based dietary guidelines among older adults in Ireland. The findings highlight inadequate consumption of essential nutrient-rich foods and overconsumption of energy-dense, unhealthy foods.

In conclusion, this thesis provides valuable insights into the relationships between folate, vitamin B₁₂, cognitive function, orthostatic hypotension, and dietary patterns in the older adult population. The findings emphasise the need for tailored nutritional guidance and strategies to address the challenges faced by older adults in maintaining optimal health and preventing chronic diseases. This research contributes to the growing body of knowledge regarding the importance of folate and vitamin B₁₂ in promoting healthy ageing and underscores the significance of public health policies aimed at improving the nutritional well-being of older adults.

AIMS AND HYPOTHESIS OF THE THESIS

Overall Aim of Thesis

The aim of this Ph.D. thesis is to investigate whether inadequate plasma folate and vitamin B₁₂ status are associated with cognitive decline and to examine whether these associations may be by means of autonomic dysfunction or inappropriate nutrition behaviours in the population.

Aim 1: To review the role of folate and vitamin B₁₂ in cognitive dysfunction and blood pressure control among older adults.

Objective 1.1: To conduct a review of the literature to determine the role lower concentrations of folate and vitamin B₁₂ play in cognitive impairment and blood pressure control.

Objective 1.2: To establish the potential benefit of maintaining micronutrient sufficiency for sustaining the health of the ageing population using several vitamins known to be of high prevalence of insufficiency in older adults.

Hypothesis 1: I hypothesised that lower concentrations of specific micronutrients would be implicated in poorer cognitive health and that the co-occurrence of multiple nutrient deficiencies confers greatest risk to health in older adults.

This is disseminated in Chapter Two; Paper 1 - *Sustaining an ageing population: The role of micronutrients in frailty and cognitive impairment.*

Aim 2: To quantify plasma folate and vitamin B₁₂ status in a nationally representative population of older adults.

Objective 2.1: To measure deficiency and low status rates of folate and vitamin B₁₂ of The Irish Longitudinal Study on Ageing (TILDA) at baseline.

Objective 2.2: To investigate the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in the older adult population of Ireland.

Hypothesis 2: I sought to test the hypothesis that among the population of older adults in Ireland there would be high rates of deficiency and low levels of folate and vitamin B₁₂, due, in part, to a liberal but voluntary practice of fortification of some foods in Ireland.

This is disseminated in Chapter Four: Paper 2 - *Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: Evidence from the Irish Longitudinal Study on Ageing (TILDA)*.

Aim 3: To describe the prevalence of distinct folate and vitamin B₁₂ status groups among older adults in Ireland.

Objective 3.1: To characterise older community-dwelling adults in Ireland by distinct vitamin B₁₂ and folate groups

Objective 3.2: To examine the associations of combinations of vitamin B₁₂ and folate status and global cognitive function.

Hypothesis 3: I hypothesised that a low vitamin B₁₂ status coupled with high blood concentrations of folate does not confer greater risk to global cognitive performance in this older population group.

This is disseminated in Chapter Five: Paper 3 - *Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA)*.

Aim 4: To investigate longitudinal associations of folate concentrations with changes in cognitive performance.

Objective 4.1: To examine sequential assessments of cognitive performance longitudinally at two-, four-, six- and eight-years follow-up.

Hypothesis 4: I hypothesised that low folate was associated with accelerated cognitive decline in older people living in Ireland.

This is disseminated in Chapter Six: Paper 4 – *Low folate predicts accelerated cognitive decline: 8-year follow-up of 3,140 older adults in Ireland.*

Aim 5: To ascertain the cross-sectional and longitudinal associations of plasma folate with total vitamin B₁₂ concentrations and orthostatic hypotension in a large, nationally representative cohort of community-dwelling adults aged 50 years and older.

Objective 5.1: To use a reproducible standard of measurement and an accurate assessment of beat-to-beat blood pressure (BP) to determine the cross-sectional and longitudinal associations with folate and vitamin B₁₂.

Hypothesis 5: I hypothesised that low levels of folate and vitamin B₁₂ would increase the risk of orthostatic hypotension.

This is disseminated in Chapter 7: Paper 5 - *Orthostatic Hypotension: Associations with Folate and Vitamin B₁₂.*

Aim 6: To describe and summarise consumption frequencies and determine compliance levels of food groups as defined by national dietary recommendations among older adults in Ireland.

Objective 6.1: To describe and summarise consumption frequencies and percentage compliance with the food-based dietary guidelines in place in older adults in Ireland.

Objective 6.2: To establish the socio-demographic, health and lifestyle determinants for compliance with certain food group-level recommendations in this population.

Hypothesis 6: I hypothesised that compliance with food-based dietary guidelines would be low among adults aged 50 and older in Ireland.

This is disseminated in Chapter Eight: Paper 6 - Nutrition *behaviour and compliance with Irish food-based dietary guidelines: a cross-sectional study.*

DISSEMINATION OF THESIS

Peer Reviewed Publications

1. Laird EJ, O'Halloran AM, Carey D, **O'Connor DMA**, Kenny RA, Molloy AM. Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: evidence from the Irish Longitudinal Study on Ageing (TILDA). *Br J Nutr*. 2018 Jul;120(1):111-120
2. **O'Connor DMA**, Laird EJ, Carey D, O'Halloran AM, Clarke R, Kenny RA, Molloy AM. Plasma concentrations of vitamin B 12 and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA). *British Journal of Nutrition*. 2020 Sep;124(6):602-10.
3. **O'Connor DMA**, Scarlett S, De Looze C, O'Halloran AM, Laird E, Molloy AM, Clarke R, McGarrigle CA, Kenny RA. Low folate predicts accelerated cognitive decline: 8-year follow-up of 3140 older adults in Ireland. *European Journal of Clinical Nutrition*. 2022 Jan 13:1-8.
4. **O'Connor DMA**, Molloy AM, Kenny RA, O'Halloran AM. Sustaining an ageing population: The role of micronutrients in frailty and cognitive impairment. *Proceedings of the Nutrition Society*. 2023 Mar 20:1-4.

Papers Under Review

1. **O'Connor DMA**, Leahy S, Kenny RA, McGarrigle CA. Nutrition behaviour and compliance with Irish food-based dietary guidelines: a cross-sectional study.

Papers in Preparation

1. **O'Connor DMA**,..., Kenny RA, McGarrigle CA. Orthostatic Hypotension: Associations with Folate and Vitamin B₁₂.

Presentations

- '*Variations in vitamin B₁₂ and folate balance: implications for cognitive function? Findings from The Irish Longitudinal Study on Ageing (TILDA).*' 11th International Conference on Homocysteine and One-Carbon Metabolism (ICHOCM) Aarhus University, Aarhus, Denmark. May 2017. **[Poster Presentation].**
- '*Variations in vitamin B₁₂ and folate balance: implications for cognitive function? Findings from The Irish Longitudinal Study on Ageing (TILDA).*' The Nutrition Society: Irish Section Meeting. Dublin, Ireland – June 2017. **[Oral Presentation].**
- '*Low Vitamin B₁₂ and High Folate Status-Cause for Concern? Findings from The Irish Longitudinal Study on Ageing (TILDA).*' IGS 66th Annual & Scientific Meeting, Sept 2018: Transforming Ageing Across Borders. Cavan, Ireland – September 2018. **[Poster Presentation].**
- '*Low B-12 and High Folate Status – Cause for Concern? Evidence from The Irish Longitudinal Study on Ageing (TILDA).*' Federation of American Societies for Experimental Biology (FASEB) Folic Acid, Vitamin B₁₂ and One-Carbon Metabolism Scientific Research Conference. Nova Scotia, Canada – August 2018. **[Oral Presentation].**
- '*Low Vitamin B₁₂ and High Folate Status-Cause for Concern? Findings from The Irish Longitudinal Study on Ageing (TILDA).*' IGS 66th Annual & Scientific Meeting, Sept 2018: Transforming Ageing Across Borders. Cavan, Ireland – September 2018. **[Poster Presentation].**
- '*High folate status is positively associated with cognitive function, irrespective of B₁₂ status. Findings from The Irish Longitudinal Study on Ageing (TILDA).*' Longitudinal Studies Conference, 30 May–01 June 2018, Wellcome Genome Campus, Hinxton, UK. **[Poster Presentation].**
- '*High folate status is positively associated with cognitive function, irrespective of B₁₂ status. Findings from The Irish Longitudinal Study on Ageing (TILDA).*' The Nutrition Society: Irish Section Meeting. Coleraine, N. Ireland – June 2018. **[Oral Presentation].**

- *'Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA).'* *Irish Section of the Nutrition Society 2019 [Oral Presentation].*
- *"Dietary Patterns and Associations with Ageing: Evidence from The Irish Longitudinal Study on Ageing (TILDA)." 13th European Nutrition Conference, FENS 2019 Dublin, Ireland – October 2019. [Oral Presentation].*
- *'Adherence to healthy eating guidelines in the Irish population: Evidence from the Irish Longitudinal Study on Ageing.'* *British and Irish Longitudinal Studies of Ageing (BILS) meeting, 3-4th February 2020, University of Manchester, UK. [Oral Presentation].*
- *'Low folate predicts accelerated cognitive decline: 8-year follow-up of 3140 older adults in Ireland.'* *FASEB: Folic Acid, Vitamin B₁₂ and One-Carbon Metabolism Conference 2020 [Online Conference Oral Presentation]. Nominated & Won – Best Short Presentation.*

News and Media Coverage

Paper 2 – Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: Evidence from the Irish Longitudinal Study on Ageing (TILDA).

- “New study finds one in eight older adults in Ireland are deficient in vitamin B₁₂.” https://www.tcd.ie/news_events/articles/new-study-finds-one-in-eight-older-adults-in-ireland-are-deficient-in-vitamin-b12/ (June, 2018).
- “Low B-12 and folate levels in mature adults ‘of concern’.” <https://www.medicalnewstoday.com/articles/322287> (June, 2018).
- “One in eight older Irish adults vitamin B₁₂ deficient, study finds.” <https://www.nutraingredients.com/Article/2018/06/26/One-in-eight-older-Irish-adults-vitamin-B12-deficient-study-finds> (June, 2018).
- “Vitamin B₁₂ and folate deficiencies in older adults partly attributed to voluntary fortification policy, researchers say.” <https://www.nutraingredients-usa.com/Article/2018/07/10/Vitamin-B12-and-folate-deficiencies-in-older-adults-partly-attributed-to-voluntary-fortification-policy-researchers-say> (June, 2018).

Paper 3 - Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA).

- “High folate levels in the older population: implications for fortification in Ireland.” <https://tilda.tcd.ie/news-events/2020/2018-olderadults-folate/> (June, 2020)
- “High levels of folic acid in blood do not impact on brain function of older people – Irish researchers.” <https://www.independent.ie/irish-news/health/high-levels-of-folic-acid-in-blood-do-not-impact-on-brain-function-of-older-people-irish-researchers-39286596.html> (June, 2020)

- “Study shows high folate levels associated with improved cognition in older adults.” <https://www.news-medical.net/news/20200616/Study-shows-high-folate-levels-associated-with-improved-cognition-in-older-adults.aspx> (June, 2020).

Paper 4 – Low folate predicts accelerated cognitive decline: 8-year follow-up of 3,140 older adults in Ireland.

- “Trinity scientists link low folate to risk of cognitive decline in later life.” <https://tilda.tcd.ie/news-events/2022/2203-low-folate-linked-cognitivedecline/> (January, 2022).
- “Low Folate Linked To Risk Of Cognitive Decline In Later Life.” <https://scienceblog.com/527773/low-folate-linked-to-risk-of-cognitive-decline-in-later-life/> (January, 2022).

ETHICAL APPROVAL AND PEER REVIEW

The Irish Longitudinal Study on Ageing was approved by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin, and all participants provided written informed consent prior to participation in the study.

As outlined at the beginning of this thesis, six papers (partially or as a whole) included in the thesis have been published or are under peer review with international journals (one in Chapter One: Introduction and Background, Chapter Two: Literature Review, and Chapter Four, Chapter Five, Chapter Six, Chapter Seven (in preparation) and Chapter Eight (submitted)). The quantitative studies included as the papers in Chapters Four to Seven are the main focus of the thesis, with those in Chapter One and Two informing the Background and Literature Review. Content relating to Chapters Four, Five, Six and Eight was presented as platform presentations and/or has featured in news and media outlets (as outlined in the section '**Dissemination of Thesis**'; *page 8*).

All data capture, storage, handling, and retrieval procedures have been audited by the internal audit office of Trinity College Dublin (TCD) and complied with TCD policy for handling patient-identifiable information. TILDA is registered as a data controller with the Office of the Data Protection Commissioners and complies with all national data protection legislation.

STATEMENT OF AUTHORSHIP

The studies that form this thesis are the product of a combined effort of several individuals and institutions, in which I, the author, played an integral part. The thesis was based on analysis of data from Waves 1 to 5 of The Irish Longitudinal Study on Ageing (TILDA). Professor Rose Anne Kenny is the Principal Investigator of TILDA, one of a family of longitudinal studies which have used the Health and Retirement Study (HRS) in the United States as a template. Professor Rose Anne Kenny conceived and designed the TILDA study, with input from an initial core group consisting of Professor Kenny, Dr Hilary Cronin, Dr Claire O'Regan, Professor Patricia Kearney, Dr Yumiko Kamiya, Dr George Savva and Professor Brendan Whelan. The design of study protocols and procedures and adherence to both, in addition to collection and management of the TILDA dataset, is co-ordinated and delivered by the TILDA research nurses and health care professionals and the management team with input from the oversight board, the steering committee and the scientific advisory board (<https://tilda.tcd.ie/people/>).

Central to this thesis was data from the health assessment component of TILDA. The concentrations of plasma folate and total vitamin B₁₂ from Wave 1 blood samples were analysed in Professor Anne Molloy's lab in TCD. The active stand protocol and pre-processing of active stand data prior to incorporation into the Wave 1 dataset has been published previously by Dr Ciarán Finucane and Dr Matthew O'Connell.

While others collected and processed the datasets used in the thesis, I conceived and developed the aims and objectives and designed, analysed, interpreted, and wrote the components of this thesis myself. I was responsible for checking the data for accuracy and coding errors. I recoded and generated new variables for this analysis. I instigated, planned, conducted, analysed, and interpreted the studies in Chapters Four, Five, Six, Seven and Eight. I ensured that methodology was consistent and accurate. I was supervised throughout the work programme of this thesis by Professor Rose Anne Kenny (primary supervisor), and later with additional supervision from Dr Christine McGarrigle, who in particular provided statistical advice and supervision regarding individual studies.

I co-authored Paper 2 (Chapter Four) and wrote all components of the other papers published, submitted for publication and in preparation from this thesis (Paper 1, Paper 3, Paper 4, Paper 5 and Paper 6 (as outlined in the section **‘Dissemination of Thesis’**; *page 8*). As co-authors on those papers, the following reviewed the papers for content and approved final versions prior to journal submission: Professor Rose Anne Kenny and Dr Christine McGarrigle, where applicable.

The core TILDA data collection and preparation were funded by the Irish Government, via the Health Research Board (HRB), The Atlantic Philanthropies and Irish Life plc. The vitamin biomarker analysis was supported by the Irish Department of Agriculture, Food, and the Marine through the grant 13F492: The Nutritional Biomarker Database Enhancement Initiative of (‘BIO-TILDA’ 2013–2015).

The financial sponsors played no role in the design, execution, analysis, and interpretation of data or in the writing of this thesis.

CHAPTER ONE: INTRODUCTION AND BACKGROUND

Introduction to Chapter One

This chapter introduces the concepts of ageing and nutrition and investigates how ageing can alter dietary requirements and nutritional status. It discusses dietary behaviours and compliance with public health guidelines in older people in Ireland and the role of nutrition in cognition as we age. The chapter also introduces the specific micronutrients folate and vitamin B₁₂, and their role in metabolism and cognitive function. Finally, the concept of the *methylfolate trap hypothesis* is discussed.

1.1. Ageing and nutrition in the 21st century

Unprecedented technological advancements and improvements in medicine and public health over the past two centuries have contributed to the exponential growth of the global population from one billion in 1800 to an estimated eight billion in 2023 (1, 2, 3). This population boom can be attributed to the decline in infant mortality and corresponding increase in life expectancy from 40 to 80 years in developed nations (4, 5, 6). The increase in life expectancy, coupled with reductions in fertility has led to an ageing population, which is in turn compounded by recent declining birth rates, particularly in developed nations (7). The combination of these demographic processes has resulted in an ageing population that is growing in the developed world, with comparable tendencies appearing in the developing world (7). It is estimated that by 2030, one in six Europeans will be over the age of 60, and by 2040, one in four will be over the age of 85 (8). The population of Ireland is undergoing a similarly rapid increase in its ageing demographic. The proportion of over 65s in Ireland rose by 19.1% between 2011 and 2017 (9). The most recent estimates suggest Ireland's population is ageing faster than any other country in Europe today (**Figure 1.1.**) (10).

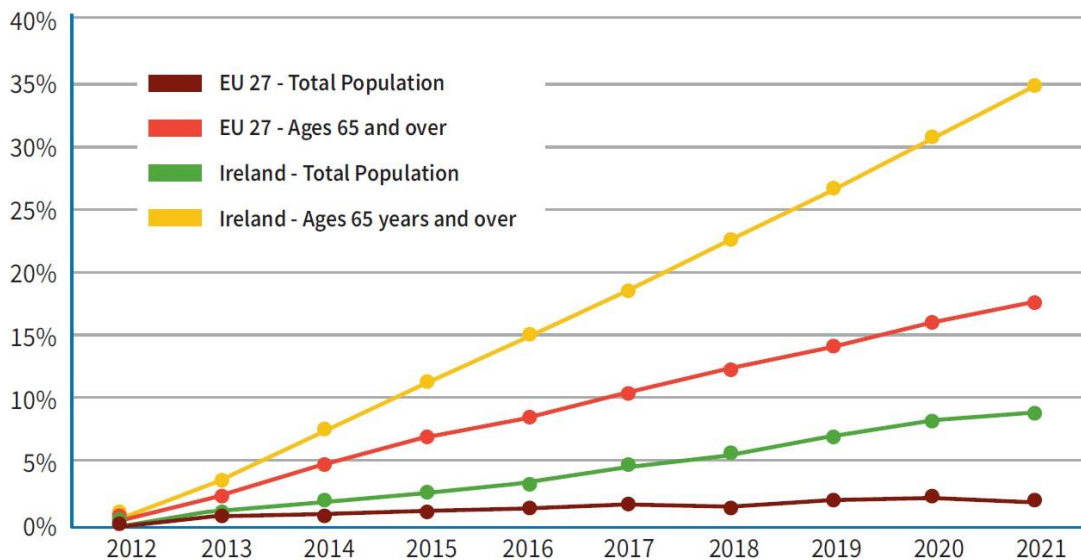


Figure 1.1. Cumulative Percentage Increases in Population, All Ages and Age 65+ for Ireland and EU-27, 2012 to 2021 (10)

This dramatic increase has been partially driven by improved delivery of healthcare, treatments, and prevention of disease (11, 12). However, there remains some disparity in the proportion of healthy life years, i.e., time spent in a healthy state, during this extended life expectancy. Of those aged 65 years and older in the European Union (EU) the life expectancy was 20 years in 2018 (13), with the expected healthy life years from this age between 8.4-9.8 years, while it was higher still in Ireland (10.9-12.0 years) (14). There is a greater incidence of illness burden as we age, resulting in increased strain on healthcare systems and economic cost (15, 16). For the individual, disease burden can result in increased healthcare utilisation and influence independent living, physical and cognitive functioning, and quality of life (17, 18, 19, 20, 21, 22, 23).

The aforementioned increases in life expectancy and ageing populations have led to increases in prevalence of neurodegenerative diseases and dementia associated with ageing. These conditions have a substantial societal and emotional cost. It has been suggested that there may be as many as 50 million dementia cases worldwide (24), with prevalence estimates of two to four percent by age 65 and 15% by age 80 (25). Up to 10 million people in Europe suffer from dementia, with costs expected to exceed €250 billion by 2030 (26). In the absence of effective pharmacological treatments to halt or reverse the mechanisms

underlying age-related cognitive decline, it is of huge public health importance to focus attention to methods of prevention, with lifestyle approaches representing the most promising avenue for future research. There is a growing need to address and understand the factors that lead to cognitive decline and impairment in old age, to extend healthy life years and reduce economic and healthcare burdens. Diet and nutritional status are factors that can readily be modified.

The brain undergoes neural development until approximately 30 years of age (27), after which a slow process of atrophy occurs, with clinical signs of neurodegeneration not typically appearing until later in life. It is hypothesised that this trajectory is determined by a complex interaction between genetic, endogenous, and environmental factors (28). This process is typically slow in nature, providing a significant window of opportunity for preventive intervention strategies. Nutrition has been identified as a potentially effective intervention for reducing the risk of age-related pathologies such as neurodegeneration and dementia (29).

Studies of nutritional epidemiology suggest that healthy diets and several candidate nutrients may play a protective role in brain ageing outcomes (30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40). Existing evidence suggests that certain nutrients or food constituents, including specific vitamins, flavonoids, and long-chain fatty acids, may have a positive effect on cognitive function (30, 31). Other than a focus on target nutrients, the most optimal preventative avenues are often suspected to be based on multi-nutrient approaches, as suggested by findings from the Three City observational study (32) and other cohort studies (33, 34, 35, 36, 37, 38), whole diet approaches, as well as multi-domain approaches incorporating changes to nutrition amongst other lifestyle factors such as exercise (31, 34, 41). Several recent observational and intervention studies have incorporated approaches of specific nutrients, such as the VITACOG trial (39), which focused on the efficacy of specific B-vitamins to lower biomarkers related to cognitive decline, and whole diet approaches, such as with the HELIAD study (40) which investigated adherence to a Mediterranean diet (MedDiet) pattern and brain health. These multi-nutrient approaches highlight specific nutrients that impact brain ageing.

1.2. Age-related changes in diet and nutritional status

1.2.1. Caloric Restriction

Energy consumption and body weight have an impact on longevity and quality of life in older age. It has been shown that caloric restriction (CR), defined as a reduction in ad libitum energy intake, increases life span in numerous species (42), however, implications for humans remain unclear. Long-term randomised studies applying CR to human subjects are impractical, however, some studies spanning several years have yielded promising outcomes (43, 44). For example, >2 year of CR in young- and middle-aged non-obese adults led to relative reductions in cardiometabolic risk factors (43). In contrast, among older individuals (≥75 years), research indicates that overweight and obesity are associated with the lowest all-cause mortality rate (44). Additional research is required to clarify the roles of both energy intake patterns and weight status in optimal ageing.

1.2.2. Challenges to changing nutritional needs

Contemporaneous changes to the environment and lifestyle may make it difficult for older adults to adhere to dietary recommendations. Changing family dynamics mean that older adults may have less support and face significant obstacles in obtaining recommended nutrient-dense diets. Older age is associated with changes in taste and smell (45), loss of appetite (46), dental and chewing problems (47), and limitations in mobility and access to affordable and high-quality fresh food (48). This is especially concerning because older people require more nutrient-dense foods to meet their changing nutritional needs. Despite lower energy requirements, age-related inefficiencies in absorption and utilisation increase the requirement for certain essential nutrients (49, 50, 51). Moreover, older adults frequently fall below recommended intake levels; several nutrients, including protein, omega-3 (n-3) fatty acids, dietary fibre, carotenoids, calcium, magnesium, potassium, and vitamins B₆, B₁₂, D, and E, have been highlighted as typically under-consumed and a health risk among older adults, according to national surveys and observational cohort studies (52).

It is important to consider that recommended daily allowance (RDA) intake levels for micronutrients may not always provide optimal intake for the older population, because in general, they are determined based on studies conducted in younger, healthy populations. Nutrients specifically identified by the 2015 Dietary Guidelines report as particular “shortfall nutrients” for US adults aged 70 years and older include calcium, vitamin D, dietary fibre, and potassium, with protein noted as a macronutrient of concern (53). In addition, medications used for common chronic conditions associated with age can alter nutrient requirements by interfering with absorption or metabolism (54). For example, long-term use of acid-blocking medications, such as proton pump inhibitors (PPI) may contribute to the development of vitamin B₁₂ deficiency (55). With disease and medication use, there may be a need for adapted nutritional requirements. To make nutrient recommendations in these metabolically altered states, evidence from randomised controlled trials involving older adults with such diseases is required.

1.2.3. Food Supplementation

While food supplements may be helpful in the case of specific nutrient deficiencies, in circumstances when health conditions or medications interfere with absorption or metabolism of specific nutrients, trials of specific nutrient supplements have been underwhelming, highlighting that whole foods are important (56). Some exceptions to this include vitamin B₁₂ supplements which may correct deficiency associated with atrophic gastritis and when using acid-blocking medications, metformin, or other interfering medications like methotrexate (57); and vitamin D supplements for individuals who receive inadequate ultraviolet B (UVB) light exposure from sunlight and during the winter months in northern latitudes (58). Higher amounts of vitamin E might also be needed to maintain an optimal immune response and to enhance resistance to respiratory infections (59). It is possible that some nutrient supplementation trials may have failed to elucidate effects due to the absence of a rigorous assessment of individual nutritional status to identify those in need of specific supplements. Still, even though food supplements are useful in the face of inadequate intakes of certain nutrients, food-based approaches to meet

requirements must be promoted as a priority. Inadequate intakes of fruit, vegetables, legumes, whole grains, nuts or seeds, fish, lean meat, poultry, and low-fat fluid dairy products (e.g., milk and yoghurt) are common among older adults, while excessive intakes of refined grain products, processed and fatty meats, fried foods, solid fats, and added sugars are prevalent (60).

Excess accumulation of certain nutrients may also contribute to disease. The body is less able to excrete pre-formed vitamin A as retinol, so this vitamin should not be consumed in excess (61). Iron, a commonplace nutrient deficiency in earlier life-cycle phases, can accumulate with age, and high intakes of heme-iron (from meat or supplements) at older ages have been associated with increased risk of cardiovascular disease (CVD); therefore, excessive intakes should also be avoided (62). In addition, some micronutrients, particularly minerals, have a narrow window between requirement and Tolerable Upper Intake Level (TUL) (63), meaning that global supplementation is not considered a panacea for this problem due to the risk of toxicity. Judicious use of supplements based on their identified or likely deficiency is generally recommended.

1.2.4. *Nutritional Frailty*

The term “*nutritional frailty*” has been used to describe a condition characterised by sudden significant weight loss, loss of muscle mass and strength, or an essential loss of physiologic reserves, making the individual susceptible to disability (64, 65). It has been recognised that increasing numbers of older adults may be frail, but simultaneously obese (66). Malnutrition is defined as deficient energy and macro- and/or micronutrient status (due to inadequate food intake, malabsorption, and/or increased metabolism frequently associated with inflammation), which can result in measurable biological changes and tissue and/or functional ability loss (67). Diagnosis of undernutrition is based on the presence of chronic inadequate intake, loss of weight, body fat, and muscle mass, or impairment of physical function (68). Consequently, undernutrition occurs in older adults when they do not consume enough protein to preserve skeletal muscle, lack vitamin B₁₂, resulting in neurological deterioration, or have low vitamin D intakes, resulting in decreased calcium storage in the bone. Nutritional risk is typically defined as the presence of factors that reduce food intake and, if

not mitigated, inevitably result in malnutrition (69). Low appetite and insufficient consumption contribute to nutritional fragility, sarcopenia, and physical fragility. Regardless of the terminology used (nutrition or malnutrition risk, mal- or undernutrition, or nutritional frailty), it is important to note that undernutrition is preventable (48); however, if left untreated, additional negative functional, health, and quality-of-life outcomes occur (70).

Insufficient consumption of nutrient-dense foods is the primary cause of undernutrition among older adults living in the community (71). Prevalence estimates of undernutrition and nutrition risk depend on the measurements employed and whether upstream approaches are considered when selecting risk factors to include in screening instruments (72). Over 35 percent of older adults living in the community have been found to be malnourished (71, 72). Despite the considerable interest recently shown in nutrition risk and/or undernutrition, these conditions remain under-diagnosed and - treated in older adults living in the community (69), potentially promoting the high prevalence of malnutrition identified at admission to hospital (71, 72).

1.2.5. Overnutrition in older people

Although many nutrients are lacking in the diets of older adults (52), overconsumption of other nutrients contributes to overnutrition and the risk of obesity and chronic diseases. These include saturated fats from fatty meats, processed meat, and whole-fat dairy products, trans fats from margarine, shortening, and processed baked goods, refined carbohydrates from soft drinks, white bread, and white rice, and sodium from canned and processed foods (52). Restricting energy-dense foods while maximising nutrient density is an important nutrition strategy for healthy ageing. However, it is important to note that the current applicability of body mass index (BMI) guidelines have recently been called into question, as measures of body composition and adiposity in older adults have raised doubts about the wisdom of restricting energy intake to reduce body weight in otherwise healthy older adults. Some research (44) indicates that overweight and mild obesity are associated with the lowest all-cause mortality in older people (>75 years). Moreover, older individuals with overweight or mild obesity who exhibit a survival advantage did not exhibit

sarcopenia (73). Recent findings suggest that this observation may be partially explained by the inclusion of metabolically healthy overweight and obese older people, without an elevated mortality risk in population studies of BMI and mortality (74).

Nutritional issues and an increased risk of malnutrition contribute to frailty through a combination of sociological, biological, and cognitive factors. Frailty, cognitive function, and nutrition are all important aspects of healthy ageing. However, despite the growing recognition of the importance of these factors, there is still much to learn about their interrelationships. Further research is needed to better understand the complex links between frailty, cognitive function, and nutrition, which could ultimately inform the development of more effective interventions and treatments to support healthy ageing.

1.3. Nutrition behaviour and status among older adults in Ireland

A balanced diet is crucial for healthy ageing, as it can impact chronic diseases (75) and cognitive function (76, 77). Nutrition plays a critical role in promoting healthy later life years, while malnutrition remains a widespread problem among older adults, accounting for 11% of Ireland's healthcare expenditure (78). As described in **Section 1.2, “Age- related changes in diet and nutritional status”**, the ageing process is typically accompanied by physiological change, comorbidities, digestive issues, early satiety, taste alterations, social factors, and reduced food access, contributing to a decreased food intake with age (79, 80, 81), thereby increasing nutritional risk and potentially precipitating accelerated loss of muscle mass, the development of frailty and malnutrition (82). As appetite and overall energy needs can decrease (83), the demand for protein and micronutrients remains the same or even increases, necessitating the consumption of nutrient-dense foods (84). Physical challenges, such as food availability, accessibility, and preparation, in addition to social determinants, such as marital status, living alone, and social exclusion, are among the complex factors influencing the dietary patterns and habits of older people (85, 86, 87). In order to meet macronutrient and micronutrient requirements and maximise the

likelihood of good health, it is vital for this varied and vulnerable group to achieve optimal nutritional intake.

1.3.1. Dietary Guidelines and intakes

Dietary recommendations for the older adults typically include the consumption of high-fibre foods, fruits and vegetables, adequate protein, low-fat dairy sources, and the restriction of foods high in saturated fat, salt, and sugar. Food-based dietary guidelines (FBDGs) compliance is associated with a reduction in the risk of frailty (88), all-cause mortality, CVD mortality and coronary heart disease (89). FBDGs in Ireland are based on the food pyramid (FP) model, which specifies a recommended number of daily (average) servings from the major food groups, represented by shelves of the FP (90). FBDGs are created to encourage adults of all ages to consume a broad and varied diet. Few studies of dietary intake and adherence in the Irish general population have included healthy subsamples of older adults (91, 92).

A recent study analysed the nutritional status and dietary intakes of a sample of community-dwelling adults in the south of Ireland aged 65 years and over (93) and revealed the prevalence of malnutrition was low, 1.2%, which appears to be consistent with previous research in the same region (92). However, the small-scale study (n 162) by O'Connell et al, concluded that up to 21.0 were 'at risk' of malnutrition. Age had no significant effect on male nutrient intakes, whereas among females, vitamin C and D intakes decreased with age. There were clear gender differences in dietary intake, with a higher prevalence of inadequate diet in male subjects. Sugars, snacks, and fats were consumed excessively, particularly by men, while dairy intake fell short of recommendations (93). Further, they reported that energy (54.9%), fibre (82.7%), calcium (58.6%), magnesium (62.3%), iron (54.9%), folate (66.0%), vitamin D (93.2%), and vitamin E (61.1%) were the most frequently reported deficiencies. The prevalence of dietary folate deficiency was higher in the oldest females.

Another small study of older adults (n 63) in Ireland in receipt of meals on wheels showed that over one-third (38.5%) were malnourished or at-risk of malnutrition and over half (52.3%) were overweight or obese (94). The energy content of the meals contributed 35-40% of the recommended dietary

allowance (RDA) for older men and 42-45% of the RDA for older women. The levels of vitamin C (25.3%), vitamin D (11.6%), folate (24.8%), and calcium (20.9%) in the evaluated meals fell below one- third of the Irish RDA for these nutrients (94).

A recent study revealed that the incidence of malnutrition among community-dwelling adults aged 50 and older in Ireland was 10.7% over a two-year period. This study revealed a variety of risk factors for the development of malnutrition, including marital status, recent hospitalisation, difficulty walking and stair climbing (95). While evidence suggests that dietary inadequacy is prevalent among older adults (92, 96, 97) individual nutrient deficiency rates reported in Ireland vary (92, 98).

1.4. The role of nutrition in cognitive decline and impairment

Cognitive decline and impairment are prevalent in older adults (99, 100, 101, 102, 103). Normative values of cognitive function in healthy older adults in Ireland showed that cognitive function when measured using tests of global cognition declined with age (104). Cognitive impairment can range from mild impairment to more severe forms of impairment, such as dementia and decline is experienced by individuals at different rates. Whereas the prevalence of mild cognitive impairment (MCI) was 10.1% in a nationally representative study of community-dwelling older adults in Ireland (105), other research reports ranges between 7.4% to as high as 24.3% in a study of low- and middle-income countries, suggesting heterogeneity in the risk of development of MCI (105, 106).

Dementia is characterised by functional impairment and while MCI may not immediately result in functional difficulties, the incidence of dementia is much higher among those with MCI compared to those without (100, 107). MCI is viewed as a precursor to dementia. Amnesic MCI is most often linked to risk of Alzheimer's disease (AD) (107, 108). A prospective study of individuals with MCI reported a conversion rate to AD of 12% per year over a four-year follow-up period (109). As the ageing demographic shifts, the prevalence of cognitive impairment will increase creating a global public health challenge (110, 111, 112). MCI and dementia can have serious implications for the lives of older

adults, their carers and society. Cognitive impairment, even when mild, can lead to consequences such as driving cessation with negative implications for the lifestyle of older adults and increased risk of depressive symptoms (99, 113, 114, 115). Dementia severely inhibits independent living and function, resulting in high rates of institutionalisation and often a need for constant care (110, 112). There is a growing need to better understand risk factors for MCI in order to prevent or delay the onset of dementia and its economic and personal consequences (111).

Nutrition is a modifiable risk factor that has been associated with many non-communicable diseases linked to dementia, such as diabetes and CVD (116, 117). There is mounting evidence that a healthy diet plays a crucial role in maintaining cognitive health and delaying cognitive decline (118, 119, 120). Certain nutrients (e.g., folate, vitamin B₁₂, flavonoids, vitamin D, and certain lipids) or food groups (e.g., seafood, vegetables, and fruits), and possibly moderate alcohol and caffeine consumption are associated with cognitive outcomes in older adults, but the evidence is mostly from observational studies and infrequently from clinical trials.

Several longitudinal studies have reported associations between certain dietary patterns or nutrients and brain-volume loss (121, 122) and integrity (123), with some clinical trials confirming these results (124, 125). Individuals with nutrient deficiencies or a genetic predisposition to cognitive impairment may be more susceptible to the protective effects of some nutrients and food groups. Identification of potentially distinct associations between these subgroups should be a research priority for the future. Oxidative stress is thought to be a major contributor to neurodegeneration and depression (126), thus, antioxidants such as vitamin C (127, 128, 129, 130), vitamin E (131) and β -carotene may be important (132), but no clear conclusions can be made. Overall, a substantial body of research, largely from observational studies, indicates a direct impact of nutrition on clinical indicators of cognitive status in older persons.

It is important to acknowledge the complex and synergistic relationships between nutrients. Vitamins and micronutrients often act as collections of co-factors, therefore interventions using singular or closely related compounds may have a

focus that is too narrow. As a result of the complex biological interactions between the various components of the diet, it has been suggested that using a whole-diet approach, through the study of dietary patterns rather than individual nutrients or food groups, might help to understand the role that diet can play in chronic disease (133).

The MedDiet diet is typified by higher intakes of fruits, vegetables, fish, beans, legumes and healthy fats and a low intake of processed foods, red meat, sweets, and non-essential and ultra-processed foods and has been shown to benefit cardiovascular health and longevity (134, 135). Adherence to MedDiet-type dietary patterns has also been linked to a decreased risk of cognitive impairment (136, 137, 138, 139), dementia, and progression from MCI to dementia (140, 141, 142, 143, 144, 145, 146). Patterns similar to the MedDiet, such as DASH (Dietary Approaches to Stop Hypertension) (147), and MIND (Mediterranean–DASH Intervention for Neurodegenerative Delay) (138, 148) have also been shown to be protective of cognitive function as people age. While the diets share many components, there are some differences; for example, only the MIND and MedDiet specify olive oil as the primary source of fat, and only the MIND diet includes berries in the fruit component (133). Numerous food components (such as fruits, vegetables, and nuts (149) and fatty fish (150, 151) and their nutrient components (such as polyphenols (152) and n-3 polyunsaturated fatty acids) (153) are believed to protect neurocognitive function in older adults. Ballarini et al. (2021) recently observed that higher MedDiet adherence led to larger grey matter volume, better memory, lower amyloid beta, and lower phosphorylated tau (154). Thus, there is mounting evidence that this type of diet pattern may protect against AD. These findings were confirmed by two clinical trials that compared the MedDiet pattern with nuts or olive oil against recommendations to restrict dietary fat (142, 155).

Such dietary patterns are no longer typical among Western populations in countries such as Australia, the United States, the United Kingdom and Ireland. On average, older adults in Ireland consume less than the recommended intake of fruits, vegetables, and legumes (156), while eating more than the suggested servings of discretionary foods (98). This is consistent with the Western-style

diet, which is characterised by a high intake of processed foods, refined sugars, and low intakes of fruits and vegetables (157). Various studies have demonstrated that the typical Western dietary pattern is related to poorer cognitive outcomes (158, 159, 160).

1.4.1. The connections between diet, gut microbiota, and cognitive decline

The connection between diet and cognitive decline and dementia is well established. Epidemiologic evidence indicates diets high in saturated fats such as the Western diet (WD) (161) and obesity (162, 163). Are associated with increased risk for developing AD and dementia. Furthermore, the incidence of AD is higher in countries that typically consume high-fat diets as opposed to low-fat diets (161). However, human data suggests, in general, that high-fat diets and obesity are risk factors for AD (164). It is widely acknowledged that a poor diet, characterised by a high fat and sugar content, leading to obesity and metabolic syndrome (MS), also has extensive negative effects on cognition (165). This relationship is mediated by mechanisms including inflammation, insulin resistance, and brain-blood-barrier (BBB) impairment. A poor quality diet is responsible for various changes in the composition and condition of gut microbiota (GMB), which is also associated with certain symptoms of MS symptoms and a significant increase in systemic and cerebral inflammation (166). Multiple mouse studies have demonstrated that AD model mice that consume high-fat diets have accelerated neuropathology (167, 168, 169, 170), increased neuroinflammation (167, 168, 169, 170) and decreased performance on AD-related behaviour tests (168, 169, 170, 171, 172, 173). However, there have also been some studies that have not observed an effect of high fat diet on AD-related neuropathology (172, 173, 174).

Diet is one of the most crucial factors that influences GMB composition. For instance, Mediterranean, ketogenic, vegan, and gluten-free diets beneficially alter GMB composition in humans (175, 176, 177, 178, 179, 180, 181) (182) suggesting healthier and more varied diets are more likely to precipitate healthy and varied microbiome. Furthermore, the GMB plays a large role in the development and progression of obesity (183) and MS (184). The GMB may

regulate peripheral inflammation, which modulates the development and progression of obesity (185, 186).

There is growing evidence that the composition of the microbiota can affect cognitive function. Changes in the population of GMB have recently been hypothesised to be one of the major factors in the pathogenesis of schizophrenia, depression, anxiety, autism, and multiple sclerosis. Several mechanisms have been demonstrated to link the central nervous system (CNS) and the GMB, including disturbances to vagal nerve signalling, BBB impairment, inflammation, and abnormal neurotransmitter production, possibly via the microbiota's production of specific short-chain fatty acids (SCFAs), urease, and D- lactate. All of these findings suggest that a combination of factors, rather than a single factor or mechanism, is responsible for the association between nutrition, GMB, and cognitive function (166).

There is evidence of the connection between diet, GMB, and AD (187, 188). While the links between diet and AD, diet and GMB, and GMB and AD have been investigated, there are no published experiments that examine all three factors simultaneously. Nagpal et al. (2019) conducted a study with 17 patients (11 with MCI and 6 cognitively normal) who followed a modified Mediterranean-ketogenic diet for 6 weeks, followed by 6 weeks of following an American Heart Association-recommended diet. They observed that a modified Mediterranean-ketogenic diet could alter the GMB and SCFA production in human MCI which correlated with amyloid measured in cerebrospinal fluid (CSF) (189). The possible mechanism that the GMB plays the mediating role between consumption of a diet with high in fat and sugar and the cognitive deficits is summarised in **Figure 1.2**.

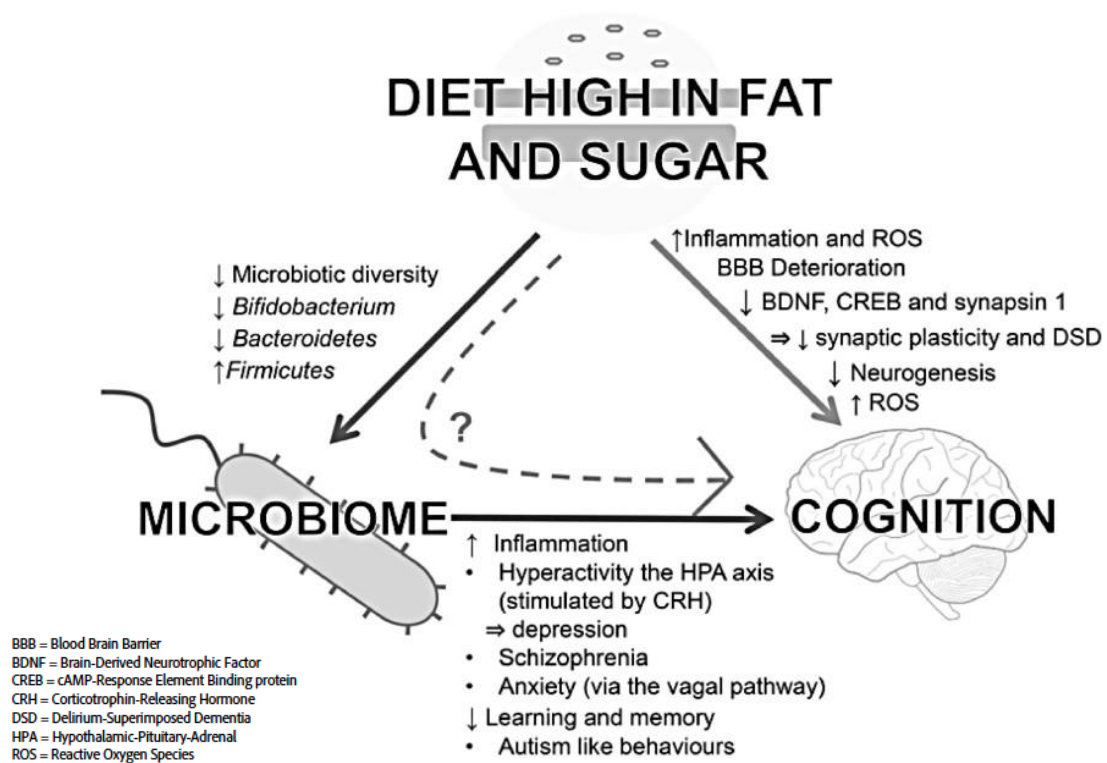


Figure 1.2. The proposed mechanisms behind the detrimental relationships between a diet high in fat and sugar, cognition, and the microbiome (166).

Future longitudinal studies are required to determine if dietary interventions can modify AD phenotypes via the GMB. In addition, further animal model experiments are necessary to determine whether GMB modifications are required for dietary interventions to modify AD phenotypes. Dietary modification of the GMB, which regulates peripheral and neuroinflammation, and which can affect the progression of AD pathology, suggests a connection between these factors.

1.5. An overview of folate and vitamin B₁₂

Folate and folic acid (FA) are forms of the water-soluble vitamin, B9. Folate is found naturally in food, while FA is the synthetic form of this vitamin found in supplements and fortified foods. Folate is essential for the life-sustaining processes of DNA synthesis, replication, and repair. It is also essential for protein biosynthesis, an essential process for cellular viability. Sources of folate include

beans, peas, lentils, oranges, whole-wheat products, liver, asparagus, beets, broccoli, Brussels sprouts, and spinach, and FA-containing supplements or fortified foods (190). Meanwhile, vitamin B₁₂ is also a water-soluble compound that is synthesised by certain bacteria in the gastrointestinal tract of animals and is then absorbed by the host animal. Vitamin B₁₂ is concentrated in animal tissues, hence it is found only in foods of animal origin (191). Foods that are high in vitamin B₁₂ include liver, meat, eggs, and dairy foods, in addition to synthetic supplements and fortified foods, where applicable.

A deficiency of folate (<7nmol/L) has been linked to ageing processes in the brain (192). Deficiency and low folate are frequently observed in countries without mandatory folic acid fortification (MFAF), such as the United Kingdom (193) and Ireland (194), where mandatory fortification is not in place to prevent neural tube defects (195). Since the implementation of MFAF of grains in the United States to reduce the incidence of neural tube defects, the prevalence of deficient and low folate status among the population has decreased significantly (195). Estimates of vitamin B₁₂ deficiency in older populations range from 5 to 40 percent, based on the chosen measurement marker and deficiency threshold (196). Folate and vitamin B₁₂ are required for DNA synthesis and repair, the metabolism of amino acids, and methylation reactions. In addition, these B-vitamins are necessary for the efficient metabolism of homocysteine (HCY), a cytotoxic amino acid that is a by-product of biological methylation reactions (197). In addition to causing neuropathy and anaemia, even a moderate deficiency in vitamin B₁₂ will lead to an increase in plasma HCY because one of the primary routes of HCY metabolism involves recycling HCY to methionine via MSyn.

1.5.1. Folate and vitamin B₁₂ status among the older population in Ireland

The status of folate and vitamin B₁₂ among older adults is of significant concern. Currently, the EU does not enforce MFAF in food. However, a noteworthy exception within the EU is observed in Ireland and the United Kingdom, where a custom of voluntary food fortification policy exists, which often includes the addition of FA. Ready-to-eat breakfast cereals (RTEBCs) are the most commonly fortified foods contain FA, although not all RTEBCs

undergo fortification (198). A recent investigation, as part of the National Adult Nutrition Survey (NANS) conducted by Hopkins et al. (2015) pertaining to the folate and vitamin B₁₂ status in Ireland was constrained by its focus on a limited sample size of adults aged over 50 years (n 363), thereby restricting the applicability of the findings. is (194). The average serum folate levels in those aged >50 were 28.1 nmol/L, which is considered to be in the “low- normal range”. Meanwhile the average vitamin B₁₂ concentration among the subsample age >50 was 281 pmol/L, which is considered to be within the normal range. The authors did not disclose however the proportion of the sample that could be categorised as having deficient or low status for either B-vitamin. Furthermore, the study lacked an expansive collection of demographic and clinical information, consequently limiting the scope of its analysis.

It is important to note that older adults in Ireland may not necessarily consume foods that are fortified or rich in vitamin B₁₂, such as dairy products, which are a recommended source of this nutrient. Given this, and the paucity of nationally representative data, detailed characterisation of the folate and vitamin B₁₂ status of older adults in Ireland is crucial in order to understand the prevalence of deficiencies and the potential implications for their health and well-being.

1.5.2. The role of folate and vitamin B₁₂ in one carbon metabolism

Folate needs to be in its fully reduced form, tetrahydrofolate (THF; **Figure 1.3.**), to be biochemically active. Therefore, the synthetic form of the vitamin found in supplements and fortified foods, FA requires modification by the action of the enzyme dihydrofolate reductase (DHFR) to form THF (199). In two consecutive nicotinamide adenine dinucleotide phosphate (NADPH) -dependent reactions, dihydrofolate (DHF) and then THF are produced. However, the reduction of FA is a slow process influenced by individual differences in DHFR activity (199). Therefore, exposure to high oral doses of FA may result in the presence of unmetabolized FA in the bloodstream (200), which some claim may be associated with negative health effects (201). Once THF enters the folate cycle, it gains a methyl group from the conversion of serine to glycine in a vitamin B₆-dependent reaction to form 5,10-methyleneTHF. Riboflavin, also known as

vitamin B2, also participates in one-carbon metabolism in its active co-factor forms flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Pyridoxine- phosphate oxidase requires FMN for the formation of pyridoxal 5'-phosphate from pyridoxine phosphate, the active form of vitamin B6. MTHFR, which requires FAD as a cofactor, converts 5,10-methyleneTHF to 5MTHF. MSyn then converts 5MTHF to THF, thereby completing the cycle. The latter conversion requires vitamin B12 (i.e., methylcobalamin) as a cofactor and simultaneously enables the re-methylation of HCY to methionine and then SAM, the universal methyl donor, which is essential for a variety of methylation processes, including DNA methylation. DNA methylation is the addition of a methyl group to the DNA base cytosine, which can alter gene transcription and reduce enzyme production (202). Thus, apart from folate, three other B-vitamins play essential roles in one-carbon metabolism, as they are required for the activity of the various enzymes within the folate cycle.

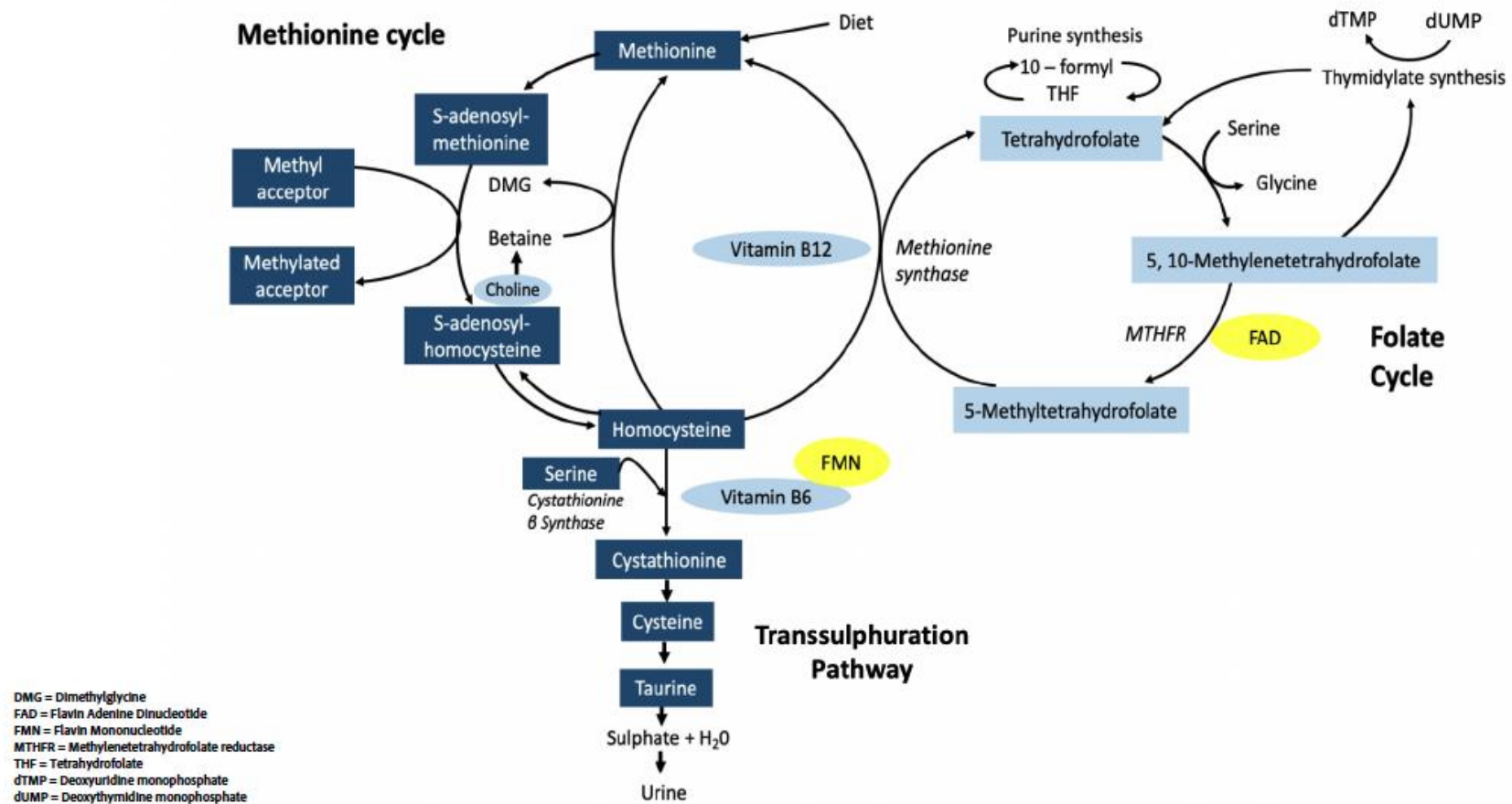


Figure 1.3. One Carbon Metabolism Pathway (203)

1.5.3. Interactions between folate and vitamin B₁₂ – the methylfolate trap hypothesis

Undoubtedly, the debate surrounding the MFAF of staple grains and flour is not without controversy. The contention that excessive FA can mask vitamin B₁₂ deficiency in older people is divisive. Folate and vitamin B₁₂ have a metabolic interrelationship that must be comprehended in order to comprehend several central aspects of this controversy.

The form of folate used in both supplements and fortified foods containing FA is called pteroylglutamic acid (PGA), which is not typically found naturally occurring in foods. The use of the term ‘folic acid’ or ‘FA’ throughout the thesis refers specifically to PGA. As described in the previous section, the metabolic functions of folate and vitamin B₁₂ intersect in the methylation cycle. MSyn, one of the vitamins B₁₂-dependent enzymes, functions in the methylation cycle, one of the folate cycles (203) (see **Figure 1.3.**). The Methylation cycle is required to maintain the supply of the methyl donor, SAM, and if it is disrupted, a wide variety of methylated products are diminished. A reduction in myelin basic protein methylation, as observed in pernicious anaemia (PA) and other causes of vitamin B₁₂ deficiency, results in subacute combined degeneration of the spinal cord. Myelin damage and abnormal neurotransmitter metabolism are hypothesised to be caused by methylation abnormalities (204). While rare, subacute combined spinal cord degeneration is one of the presenting conditions in PA. The major presenting symptom of PA is a megaloblastic anaemia with identical morphology to that of folate deficiency. The ‘*methyl trap hypothesis*’ explains the relationship between methylation cycle impairment, insufficient DNA biosynthesis, and anaemia (205). This hypothesis suggests that when the folate cofactor 5-methyltetrahydrofolate (5MTHF) is formed, the enzyme that forms the cofactor, 5,10- methyleneTHF reductase, cannot utilise it in the back reaction in vivo. Therefore, the only way for this folate cofactor to be recycled to THF (and thus participate in DNA biosynthesis and permit cell division) is via the vitamin B₁₂-dependent enzyme MSyn (see **Figure 1.3.**). When the enzyme's activity is compromised, as it would be in PA, the cellular folates become increasingly “trapped” as 5MTHF. Consequently, the cell will experience a

form of folate deficiency, as it will have sufficient usable folate, but it will be trapped in a form that cannot be used for DNA biosynthesis. The resulting anaemia will be identical to that of true folate deficiency. Therefore, there will be neuropathy and/or anaemia in PA (206). Treatment with intramuscular administration of vitamin B₁₂ reactivates the enzyme MSyn, allowing myelination to resume. Folate, trapped as (enter the correct form) will also be released, allowing DNA synthesis and erythrocyte production to resume, thereby correcting anaemia. When the synthetic FA enters the cell, it is first converted via dihydrofolate to THF (**Figure 1.3.**). This process begins outside of the methyl trap and can therefore initiate DNA biosynthesis, thereby treating anaemia. While such FA is also ultimately trapped as 5MTHF, if it is given continuously and in quantities sufficient to exceed the intestinal mucosal cells' capacity to convert it to 5MTHF, the FA will maintain DNA biosynthesis and appear to have treated the anaemia. Treatment with FA administration, however, cannot restart the methylation cycle, which is blocked at the vitamin B₁₂-dependent enzyme MSyn. Thus, neuropathy will persist and only be diagnosed later, when vitamin B₁₂ treatment may only partially reverse nerve damage.

1.6. Summary

Technological advancements and improvements in public health have contributed to a population boom and an ageing population. Age-related pathologies such as neurodegeneration and dementia are becoming more prevalent. Nutrition plays a role in almost all bodily functions, including in the brain and as such it has been identified as a potentially effective method for reducing the risk of these pathologies.

Age-related changes in diet and nutritional status can impact longevity and quality of life in older adults. They may require more nutrient-dense foods to meet their changing nutritional needs, but can often experience physical and societal obstacles to obtaining recommended diets and limitations in mobility and access to affordable and high-quality fresh food.

Malnutrition is a widespread problem among older adults in Ireland and is often compounded by the physiological changes associated with increasing age. Nutrition is a modifiable risk factor that can contribute to cognitive decline. Folate and vitamin B₁₂ are essential co-factors in one-carbon metabolism and are necessary for DNA synthesis, replication, and repair. Deficiency in folate and vitamin B₁₂ can lead to various health problems, such as megaloblastic anaemia, neuropathy, cognitive impairment, and higher rates of mortality from CVD and cancer. The prevalence of folate and vitamin B₁₂ deficiency in older populations varies depending on the country, with Ireland having a high rate of low status among the older population. The voluntary fortification policy of Ireland is ineffective in maintaining their status of those age 50 and older. The contention that excessive FA can mask vitamin B₁₂ deficiency in older, raising concern over the safety of MFAF is controversial. The metabolic functions of these B-vitamins intersect in the methylation cycle, which is required to maintain the supply of the methyl donor SAM. The '*methylfolate trap hypothesis*' states that B₁₂ deficiency impairs overall folate metabolism because 5MTHF becomes metabolically trapped. Synthetic FA, whether obtained by supplementation or MFAF, can appear to treat anaemia but may conceal the underlying and potentially irreversible neuropathy caused by vitamin B₁₂ deficiency.

In this thesis, I explore how variations in concentrations of vitamin B₁₂ and folate may impact cognition function in ageing in a population of community dwelling older adults and the possible relationship that a common manifestation of cardiovascular ageing, orthostatic hypotension, may have with folate and vitamin B₁₂ status – due to its known associations with cognitive function and evidence linking folate and vitamin B₁₂ to blood pressure control. Finally, I examine the nutritional behaviours of older adults in Ireland.

Figure 1.4. presents a schematic summary of this PhD thesis. The analyses conducted in this thesis will help to improve the understanding of the nutritional status and cognitive function of older adults, while also shedding light on the relationship between orthostatic hypotension and folate and

vitamin B₁₂ status. The results of this study will provide valuable objective data that can be used to raise public health awareness and inform healthcare professionals about the importance of proper nutrition in older adults. By highlighting the nutritional behaviours of older adults in Ireland, this study will also help reduce negative outcomes associated with poor nutrition and cognitive decline in this population.

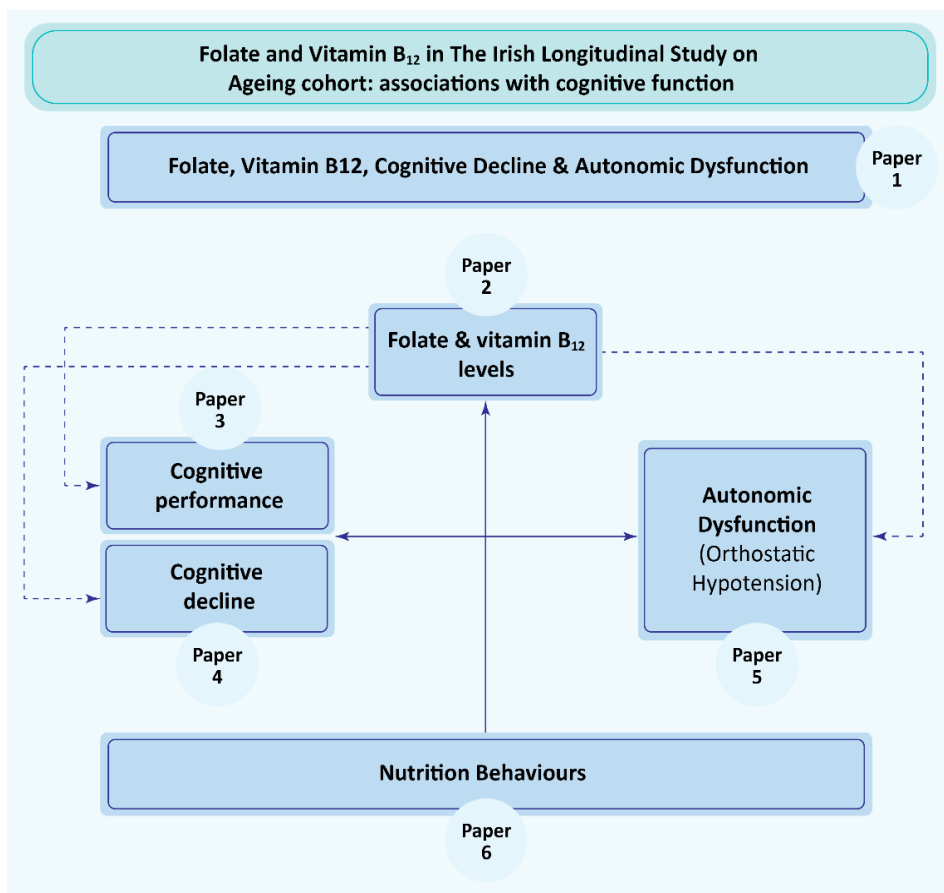


Figure 1.4. Schematic overview of thesis

CHAPTER TWO: FOLATE, VITAMIN B₁₂, COGNITIVE DECLINE AND AUTONOMIC DYSFUNCTION – LITERATURE REVIEW

Introduction to Chapter Two

It is well known that vascular risk factors play a role in the pathogenesis of cognitive impairment and dementia. The link between the specific vitamins, folate and vitamin B₁₂ and dementia is complex, with low folate and low vitamin B₁₂ levels, and in specific circumstances, high folate concentrations being linked to cognitive impairment or decline pressure influencing the risk of dementia. In this chapter, the literature examining folate and vitamin B₁₂, and cognitive outcomes is reviewed. Their impact on blood pressure control has also been proposed as a potential mechanism for their influence of cognitive function, given its own established relationship with cognitive dysfunction.

In this chapter, **Aim 1** is to review the role of folate and vitamin B₁₂ in cognitive dysfunction and blood pressure control among older adults.

Objectives 1.1 is to conduct a review of the literature to determine the role lower concentrations of folate and vitamin B₁₂ play in cognitive impairment and blood pressure control. **Objective 1.2** is to establish the potential benefit of maintaining micronutrient sufficiency in sustaining the health of the ageing population using several vitamins known to have a high prevalence of low status in older adults.

List of Published Work from Chapter Two

Published Tables

Table 2.1. Summary of the existing relationship between selected micronutrients and cognitive impairment

Published Sections

2.1. The role of folate and vitamin B₁₂ in cognitive decline

2.1. The role of folate and vitamin B₁₂ in cognitive decline

B-vitamins that are involved in one-carbon metabolic pathways have been studied extensively for their potential effect on cognitive impairment and dementia (197). Both vitamin B₁₂ and folate, in addition to vitamin B₆ and riboflavin, are required for DNA synthesis and repair, amino acid metabolism and methylation reactions. Further, these B-vitamins are required for efficient metabolism of HCY, the cytotoxic intermediary amino acid that is a downstream product of biological methylation reactions (197).

Observational evidence suggests that the inhibition of methylation reactions may influence cognitive impairment in ageing (197) and there is growing interest in the possibility that a loss of cognitive function, may partly be explained by inadequate status of these vitamins (207, 208, 209, 210). Severe vitamin B₁₂ deficiency, such as that seen in pernicious anaemia, causes severe neurological consequences including sensory and motor neuropathy. Low or deficient vitamin B₁₂ status is associated with depression (211) and altered mental status and cognitive decline (212). It also reduces the availability of folate for DNA synthesis (212). Age-related deficiencies in folate transport and metabolism, use of anti-folate drugs, genetic factors, and excessive alcohol consumption are among the factors that contribute to vitamin B₁₂ and folate insufficiency (213), frequently seen in older persons (193). In TILDA, the prevalence of older people with deficient or low vitamin B₁₂ (<185 pmol/L) and folate (<10 nmol/L) status were 12% and 15%, respectively (214).

Several epidemiological studies have shown cross-sectional and prospective associations between low vitamin B₁₂ (212, 215) and folate (216, 217, 218, 219, 220, 221, 222, 223, 224, 225), and the risk of cognitive impairment and dementia, while others have not (226). As demonstrated in the longitudinal study described in **Chapter Six (Figure 6.2.)** of this thesis (227) low baseline folate levels can predict a reduction in overall cognitive function and episodic memory in older persons who were cognitively healthy, making them a potential key marker for the risk of early decline (227). This was consistent

with other studies showing low folate status was associated with higher risks of cognitive impairment or dementia (216, 217, 218, 219, 220, 221, 222, 223, 224, 225). Data pertaining to the relationship between folate and vitamin B₁₂ and cognitive impairment are summarised in **Table 2.1**

Table 2.1. Summary of the existing relationship between folate and vitamin B₁₂ and cognitive impairment

Micronutrient	Major sources	Main findings associated with cognitive impairment and related measures (references)
Vitamin B ₁₂	Animal products, fortified foods, supplements	• Low or deficient B₁₂ concentrations associated with depression (211) and cognitive decline (212) (215)
Folate	Animal products; milk; leafy vegetables; legumes fortified foods, supplements	• Low folate associated with the risk of cognitive impairment and dementia (216, 217, 218, 219, 220, 221, 222, 223, 224, 225)

In addition, selected observational data have suggested that older adults with simultaneous low vitamin B₁₂ and high folate status had higher risks of anaemia and cognitive impairment or decline (228, 229, 230), given that high-dose FA treatment was shown to temporarily mask clinical symptoms in persons with pernicious anaemia (231). However, the causal relevance of these associations remains uncertain with conflicting results (232, 233, 234). TILDA reported that those with low B₁₂ combined with high folate status did not have any adverse associations with cognitive performance compared. In contrast, the study demonstrated that higher concentrations of folate were associated with small, but statistically significant higher scores for global cognitive performance in this setting (235).

Evidence from randomised controlled trials (RCTs) of B-vitamins have shown no consistent benefit of supplementation on cognitive outcomes. FA supplementation was associated with improved domain-specific cognitive

performance in RCTs with relatively large samples and ≥ 2 years follow-up (236, 237, 238). One trial that examined individuals with high HCY to exclude causes other than low folate concentrations and FA supplementation (0.8mg oral FA per day) was associated with improved memory, processing speed and sensorimotor speed after three years (237). Supplementation was more effective in improving processing speed in those with high baseline HCY levels ($>12.9 \mu\text{mol/L}$) and in improving information processing and sensorimotor speed in those with low baseline vitamin B₁₂ concentrations ($<250 \text{ pmol/L}$) (237). Another trial, testing combinations of folate, vitamins B₆ and B₁₂, and n-3 fatty acids for 4 years were effective in preserving semantic memory or temporal orientation in a subgroup of participants with previous coronary artery disease or ischaemic stroke, but not in the total trial population of 1,748 men and women aged 45-80 years (239). These observations suggest that individuals with high baseline HCY, low baseline vitamin B concentrations, or established cardiovascular and cerebrovascular disease might benefit most from vitamin B supplementation.

HCY has been touted as an indicator of the progression of cognitive dysfunction and dementia over time (240). Several lines of evidence support this HCY hypothesis, also known as the association between elevated HCY (plasma total HCY $> 15 \text{ mol/L}$) and cognitive dysfunction (240). The evidence shows that hyperhomocysteinemia (HHCY) has been related to cerebral macroangiopathy and microangiopathy, endothelial dysfunction, impaired nitric oxide (NO) activity, and increased oxidative stress (241). Likewise, increased HCY can trigger inflammatory processes, which play important roles in developing neurocognitive disorders (242). Cumulative incidence of HHCY was reported at 26.6% in 112 cases of dementia in a cohort study on 816 individuals aged > 65 years from an Italian population-based survey over an average follow-up of 4 years (218). While most of the literature and trials that have been carried out have been underpinned by the hypothesis that lowering HCY, which is associated with cognitive impairment, by B-vitamin supplementation, it is plausible that biological mechanisms other than HHCY may underlie the associations between B-vitamins and cognitive impairment.

Vitamins and nutrients often function as a collection of cofactors, therefore interventions using singular or closely related compounds may have too narrow a focus and do not account for the complexity of the synergistic interactions between nutrients. This is illustrated by another trial using FA, B₆, and B₁₂, showing treatment was effective only in those with high baseline n-3 fatty acid concentrations. In fact, n-3 fatty acid status was protective against brain atrophy only in the presence of B-vitamin supplementation, suggesting that both are needed for effectiveness (243). Other proposed mechanisms include impaired methylation, misincorporation of uracil into DNA, attenuation of inflammation, vascular care modification of cerebral FA deficiency and antioxidant activity.

To further examine the association between these B-vitamins, homocysteine, and cognitive decline, the VITACOG trial recruited 270 community-dwelling participants over 70 years of age with MCI and randomly assigned them to receive either a daily placebo or tablet containing 0.8 mg FA, 0.5 mg vitamin B₁₂, and 20 mg vitamin B₆ for two years (125). It was discovered that only participants with an adequate level of n-3 fatty acids benefited from B-vitamin supplementation. Brain atrophy, as measured by structural magnetic resonance imaging (MRI), was reduced by 30% in the B-vitamin- treated group following treatment. In key grey matter regions, including the medial temporal lobes, there was a further reduction in atrophy in the active treatment group, with regional grey matter loss of 0.6% compared to 5.2% in the placebo group (244). Regarding the association with homocysteine, total brain atrophy in the placebo group doubled across the levels of baseline homocysteine, whereas in the active treatment group there was no relationship between HCY at baseline and atrophy, such that subjects in the top quartile of HCY levels demonstrated a 53% slowing of the rate of brain atrophy (125). In addition, subjects receiving B-vitamins demonstrated a slower rate of decline in episodic memory, semantic memory, and global cognition, as measured by the Mini-Mental State Examination (MMSE), but only in subjects with baseline HCY levels above the median (> 11 mol/L), and improvements in clinical and functional outcomes occurred in subjects with higher baseline HCY levels (> 13mol/L) These results suggested that the disease mechanisms

underlying MCI and subsequent cognitive and clinical aspects may be modifiable by B-vitamins, particularly in individuals with elevated HCY levels (197).

Other trials in which B-vitamins were administered and beneficial effects on cognition were observed have been discussed, as have the possible reasons for these discrepancies (197, 245, 246). In particular, a meta-analysis (247) claimed that there was no beneficial effect of B-vitamins on ‘cognitive ageing’; however, this analysis is difficult to interpret because cognition was only assessed once in 74% of the participants, and cognitive decline over time could not be determined in these subjects.

A review by Zhang and colleagues (248) found that a high folate level was associated with a 32% lower risk of cognitive decline in cross-sectional studies compared to a low folate level. In prospective studies that measured folate blood concentrations and folate intake, no significant association was found. Due to moderate heterogeneity among studies assessing folate blood concentrations, sensitivity analysis revealed that the association was not significant when four studies were excluded (224, 249, 250, 251). The same review found that individuals with high vitamin B₁₂ blood concentrations had a lower risk of dementia compared to those with low vitamin B₁₂ concentrations in cross-sectional studies. No significant association was found in prospective studies that assessed either vitamin B₁₂ blood concentrations or intakes (248).

2.1.1. The special case of high folate and low vitamin B₁₂

It has been hypothesised that the combination of high folate intake (in the form of FA) and low vitamin B₁₂ status may impair cognitive performance in older adults. Initial case reports and observational studies, as summarised by Field and Stover (252) and Berry (253) supported the hypothesis that excessive folate exposure exacerbates the cognitive impairment resulting from low vitamin B₁₂ status. However, as it stands, the relationship between variations of folate and vitamin B₁₂ concentrations and potential impact on cognitive function is poorly understood and epidemiological evidence is

conflicting. In addition, selected observational data have suggested that older adults with high folate and low vitamin B₁₂ status had higher risks of anaemia and cognitive impairment or decline (228, 229, 230), given that high-dose FA treatment temporarily masks clinical symptoms in people with pernicious anaemia (231). In a later cross-sectional study using the US National Health and Nutrition Examination Survey (NHANES) dataset (215) of >2000 subject aged 60+ years. They found low serum vitamin B₁₂ (< 150 pmol/L) as associated with cognitive impairment both independently and in an interactive manner with high folate levels of folate (>74.1 nmol/L) (*n* 54 in interaction group), the Digit Symbol Substitution Test (DSST) and the Animal Fluency test (AF). However, the causal relevance of these associations remains uncertain with conflicting results (232, 233, 234).

In other studies, the consumption of FA with or without vitamin B₁₂, may help prevent cognitive impairment among older people, particularly in populations when low folate status is commonplace, possibly through the lowering plasma HCY levels (254, 255) or other mechanisms. In The Netherlands, a 3-year randomised double-blind placebo-controlled trial of >800 older adults (aged 50–70 years, all vitamin B₁₂-sufficient) found that FA supplements of 800 mg per day improved cognitive function (237). In this trial, domains of cognitive function associated with ageing were found to improve, and these changes were associated with reductions in HCY (237). In a previous similar trial (2-year randomised double-blind placebo-controlled trial in New Zealand involving >276 older adults (≥65 years) with elevated HCY levels (≥13 mmol/l) using high-dose supplements of FA (1000 mg), vitamin B₁₂ (500 mg) and vitamin B₆ (10 mg) as the homocysteine-lowering intervention) no beneficial effects of homocysteine-lowering on cognition were found (256). The lack of effect observed in this earlier study has been attributed in part to the arguably less sensitive test used to evaluate cognitive function, the MMSE (256). In the Dutch study, five distinct cognitive domain tests were utilised. The protective effects of folate on cognitive function are dependent on adequate vitamin B₁₂ status, according to a large-scale analysis of the relationship between blood levels of folate and vitamin B₁₂ in older adult

participants (>60 years, n = 1459) in the 1999–2002 NHANES and measures of cognitive function (228). If vitamin B₁₂ status is adequate, then FA (even at high intake levels) appears to be protective of cognitive function, according to this study (228, 257). In cases where vitamin B₁₂ status was inadequate, however, it was reported that high FA consumption exacerbated cognitive impairment (228, 257). In the paper described in **Chapter Five (Paper 3)** of this thesis, we reported that no adverse associations were observed among those with the combination of high folate and low vitamin B₁₂ status with respect to cognitive performance. Instead, the study found that higher folate concentrations were linked to marginally better global cognitive performance (235).

These divergent effects of FA intake depending on vitamin B₁₂ status need to be confirmed. The two randomised controlled trials described earlier (237, 258) cannot contribute to this evidence because either a high dose of vitamin B₁₂ was administered with FA (256), or the trial only included individuals who were vitamin B₁₂ replete (237). Collectively, these recent reports indicate a beneficial effect of FA food fortification on cognitive function of older adults, but this effect may be dependent on adequate vitamin B₁₂ status. In a review by the US National Toxicology Program (259) concluded that while there is some evidence from observational studies, the available data were limited and there are no randomised controlled trials designed to answer this question. The available evidence indicates that cognitively healthy individuals who consume large quantities of FA do not have an increased risk of cognitive impairment (259). Thus, the main implication for FA food fortification is to emphasise the importance of ensuring older adults with low vitamin B₁₂ status are identified and treated.

2.2. Folate, vitamin B₁₂ and evidence in blood pressure control

The importance of folate insufficiency in BP control has been studied primarily due to its key role in HCY regulation. In the absence of adequate folate, an accumulation of HCY can be induced (260). HHCY, as a result of folate insufficiency, can lead to homocysteine-induced arteriolar constriction,

increased sodium reabsorption, renal dysfunction, and increased arterial stiffness (261), resulting in increased BP. High concentrations of HCY have been associated with elevated BP and linked to chronic heart failure (262). Folate and vitamin B₁₂ deficiencies are the common causes of HHCY. Treatment of HHCY with FA has been shown to reduce plasma homocysteine, which itself can cause endothelial cell injury (263). Data pertaining to the relationship between folate and vitamin B₁₂ and cognitive impairment are summarised in **Table 2.2**. Interestingly, among those with HHCY and coronary artery disease (CAD), a folate-rich diet, including a variety of fruit, vegetables and fortified RTEBCs was as effective as FA capsules in decreasing total plasma HCY concentrations and adds further support to the recommendation of those diets to prevent CVD (264). It is well established that intervention with FA, along with vitamin B₁₂ and sometimes vitamin B₆, can reduce plasma HCY concentrations as reported in a large meta-analysis of randomised controlled trials (265). Of the B-vitamins investigated, folate has been shown to be most effective, even at doses as low as 0.2 mg/day when maintained for a long enough period of intervention (i.e., 24 weeks) (265, 266). Since the effect of FA on vasodilation has already been well established (267), it is unsurprising that several epidemiologic studies have investigated the relationship between folate and related B-vitamins, BP control and various aspects of vascular health. Some epidemiological studies have investigated the relationship between higher folate and vitamin B₁₂ dietary intakes and lower BP levels (268) or decreased risk of hypertension (269, 270, 271). There is limited scientific evidence supporting a direct correlation between vitamin B₁₂ and blood pressure control. In the evaluation of patients with symptoms of autonomic dysfunction, such as orthostatic hypotension, vitamin B₁₂ deficiency should be considered. One study suggested that patients presenting with OH (a type of low BP) should be screened for vitamin B₁₂ deficiency even if they do not exhibit clinical neurologic impairment or the typical haematological features associated with vitamin B₁₂ deficiency (272). However, it is plausible that low levels of vitamin B₁₂ affect blood pressure control via the trapping of folate to create a functional folate deficiency and potentially elevated HCY levels. Vitamin B₁₂ is involved in the production of

red blood cells and the formation of DNA, and it may also play a role in maintaining healthy blood vessels. Clinical vitamin B₁₂ deficiency (<148pmol/L) has been implicated as a direct cause of OH according to one case study (273). Together these data may suggest that a higher level of folate, when facilitated by adequate levels of B₁₂, may reduce the risk of hypertension and wider vascular dysfunction. It is important to note that more research is needed to fully understand the relationship between vitamin B₁₂ and blood pressure regulation.

Table 2.2. Summary of the existing relationship between folate and vitamin B₁₂ and blood pressure control.

Micronutrient	Major sources	Main findings associated with blood pressure control and related measures (references)
Vitamin B ₁₂	Animal products, fortified foods, supplements	• Vitamin B₁₂ deficiency is associated with orthostatic hypotension (273). • Higher vitamin B₁₂ dietary intakes are associated with lower BP levels / decreased risk of hypertension (268).
Folate	Animal products; milk; leafy vegetables; legumes fortified foods, supplements	• Serum folate levels were not associated increased odds of hypertension (274), but higher dietary intakes are associated with lower BP (268) or decreased risk of hypertension (269, 270, 271). • Low folate intakes are a risk factor for CVD (275, 276, 277) • FA trials (278) (279). and fortification (280, 281, 282) associated with a reduced risk of stroke and lower systolic and diastolic BP (283, 284), recent stroke or transient ischemic attack (285).

Studies focusing on FA supplementation have shown interesting results. However, there are few published studies examining blood vitamin levels in this context. A recent study reported that serum folate levels were not associated with systolic BP, diastolic BP, or odds of hypertension in Korean adults (274). FA fortification is associated with a reduced risk of stroke (280, 281, 282), and while observational data indicate that low folate intake from the diet is a risk factor for CVD, evidence from clinical trials is inconsistent.

Trials of FA supplementation have shown that high-dose supplementation may lower systolic and diastolic BP (283, 284). Many trials investigating CVD have been underpowered, necessitating meta-analyses to provide conclusive evidence regarding the possible protective effects against coronary heart disease (CHD) (275, 276, 277). A recent large clinical trial involving 8000 patients with recent (less than 7 months) stroke or transient ischemic attack (TIA) demonstrated the safety and efficacy of 2 mg of FA supplementation in combination with 25 mg of vitamin B₆ and 500 g of vitamin B₁₂ in reducing the risk of recurrent serious vascular events (285). A more recent meta-analysis of 30 randomised controlled trials (n 82,334) found decreased risks of CVD and stroke among those receiving FA supplementation, provides support for a modest benefit of FA supplementation for the prevention of stroke. FA supplementation was associated with a 10% reduction in the risk of stroke and a 4% reduction in the overall risk of CVD. A greater benefit for CVD was observed in participants without a history of CVD or with lower plasma folate levels at baseline, as well as in studies with a greater reduction in HCY levels. There was no significant benefit or harm with FA supplementation for the risk of CHD observed (286). Furthermore, another meta- analysis of 12 RCTs involving 16,958 patients with pre-existing vascular disease showed that the overall relative risks (RR) were 0.86 (95% CI, 0.71–1.04) for stroke after administration of FA for 6 months (279). FA could improve vascular function and decrease the risk of lacunar brain infarcts, and vascular dementia, by decreasing serum HCY levels and vascular care.

Although the majority of previous CVD studies were designed to examine HCY reduction in relation to CVD risk, some also examined the relationship between B- vitamins and blood pressure. One of the largest studies, the VISP (Vitamin Intervention for Stroke Prevention) trial, which involved supplementing 3,649 cerebral infarct patients with high or low doses of FA for two years, successfully decreased HCY, but showed no effect on BP (287). Similarly, McMahon and colleagues reported a significant reduction in HCY in response to FA, vitamin B₆ and B₁₂ supplementation for two years, but no effect on BP (258). Of note, a primary prevention trial in 20,702 hypertensive Chinese adults reported that FA supplementation (0.8 mg/day), for a median

duration of 4.5 years, reduced the risk of stroke in those with elevated cholesterol by as much as 31% (278). One small study involving 130 participants supplemented with high doses of FA (5 mg/day) and vitamin B₆ (250 mg/day) for two years reported a HCY reduction and a corresponding reduction in SBP (3.7 mmHg) (283). A further study of 24 male smokers reported a small but significant reduction in HCY (2.6 mol/L) and a decrease of SBP of 8 mmHg. However, this particular study was conducted on adults that engaged in smoking, which raises HCY and BP (284, 288). If HCY was causally associated with elevated BP, intervention studies to lower it would invariably demonstrate a corresponding BP response; however the literature is controversial (289), suggesting there may be no mechanistic association between HCY and BP.

Separate meta-analyses estimated that a reduction in HCY by 25% could reduce the risk of stroke by up to 24% and heart disease by 16% (290, 291). To subsequently establish a cause-and-effect relationship between elevated HCY and CVD outcomes, a number of randomised controlled trials with B-vitamins were conducted; however, despite significant HCY lowering, the majority failed to show a reduction in CVD risk in response to intervention with B-vitamins (300). Of note however, a secondary analysis of the Heart Outcomes Prevention Evaluation 2 trial (HOPE 2) and two subsequent meta-analyses demonstrated that interventions with FA and related B-vitamins could reduce the risk of stroke (282, 292, 293). This effect was confined to trials that were three years in length or greater (suggesting that trials of shorter duration may have underestimated or missed this benefit) and in trials involving participants with no previous history of stroke. However, the vast majority of the previous trials were secondary prevention trials in patients with overt CVD, and as such, participants would be less likely to respond to B-vitamin intervention. The balance of evidence therefore suggests that any beneficial effects of B-vitamins would be most relevant in the prevention rather than the treatment of CVD.

The associations between folate and BP may also be due to biological factors other than HHCY. Folate may have beneficial effects on BP by direct action

on endothelial cells (294). The active form of folate, 5MTHF, increases endothelial function by enhancing the activity of NO within the endothelial cells of blood vessels. Thus, inadequate levels of serum folate can increase BP through endothelial dysfunction (295). This is supported by two studies that demonstrate oral FA supplementation improves endothelial function in vivo (296, 297).

Further, none of the aforementioned studies considered the influence of MTHFR genotypes on BP response. The MTHFR 677TT genotype independently increases CVD risk (290, 298, 299, 300, 301). It is assumed that HCY is responsible for this association, which is found to be highest in TT genotypes and lowest in CC genotypes, with CT genotypes having intermediate concentrations (289). Recently, evidence suggests that this polymorphism may independently increase CVD risk. Meta- analyses of the TT genotype and CVD risk show a stronger association for stroke (298, 301) than for heart disease (290, 299) (300, 302). The relationship is stronger in hypertension risk studies than CVD outcomes (303, 304, 305, 306). Hypertension is a leading stroke risk factor, so modulating the blood pressure phenotype in MTHFR 677TT genotype individuals may prevent or delay stroke.

2.3. Conclusions

This chapter discusses the relationships between folate and vitamin B₁₂, with cognitive function and blood pressure regulation. The potential impact of these B-vitamins on cognitive function and decline was explored. Observational evidence suggests that a deficiency in folate and/or vitamin B₁₂ may partly explain age-related decline in cognitive function. Folate and vitamin B₁₂ are required for DNA synthesis and repair, amino acid metabolism, and methylation reactions. There is a reasonable number of published studies that have shown associations between low levels of folate and vitamin B₁₂ and the risk of cognitive impairment and dementia. RCTs of B-vitamins have shown inconsistent benefits of supplementation on cognitive outcomes, but FA supplementation was associated with improved domain-

specific cognitive performance in RCTs with relatively large samples and ≥ 2 years follow-up. While most of the published literature and trials have been underpinned by the hypothesis that lowering HCY, which is associated with cognitive impairment, will improve cognitive outcomes by action of B-vitamin supplementation, it is plausible that biological mechanisms other than HHCY may explain the associations between B-vitamins and cognitive impairment.

It has been hypothesised that the combination of high folate intakes (in the form of FA) may exacerbate the cognitive dysfunction resulting from low vitamin B₁₂ status, by masking the vitamin B₁₂ inadequacy, thereby avoiding detection and appropriate treatment. However, as it stands, the potential impact on cognitive function is poorly understood and epidemiological evidence is conflicting with the causal relevance of these associations remaining uncertain.

Folate deficiency can lead to an accumulation of HCY, which can cause arterial stiffness, renal dysfunction, and high blood pressure. Folate and vitamin B₁₂ deficiency are frequent causes of HCY accumulation, which is treatable with supplemental folate. Vitamin B₁₂ deficiency can itself, lead to a functional folate deficiency.

Epidemiological studies have demonstrated a correlation between higher folate and vitamin B₁₂ consumption and reduced blood pressure. FA supplementation has been linked to a decreased risk of stroke and CVD, and it may improve vascular function and reduce the risk of brain infarcts and vascular dementia by lowering serum HCY levels. However, additional research is required to fully understand the relationship between blood concentrations of folate and vitamin B₁₂, their interrelationship, and blood pressure regulation.

This thesis explores the status of plasma folate and total vitamin B₁₂ and the socio- economic determinants of these (**Chapter Four**). It then investigates the relationship between plasma folate and total vitamin B₁₂ and longitudinal cognitive decline in a population representative sample and their interaction

with cognitive function (**Chapter Five** and **Chapter Six**). This thesis also investigates whether these B-vitamins are associated with orthostatic hypotension, as a measure of autonomic dysfunction, over time (**Chapter Seven**). Finally, it sets the scene for dietary intakes and adherence to food-based recommendations among the population of those aged 50 and older in Ireland (**Chapter Eight**).

CHAPTER THREE: METHODOLOGY

Introduction to Chapter Three

In this Ph.D. thesis, I used quantitative analysis of TILDA to examine the aims and hypotheses laid out at the beginning of the thesis (see **Aims and Hypotheses of the Thesis**; page 5). All data were derived from TILDA. This chapter describes in detail the methodology of the thesis, the study population and design, and the measures and analysis strategies used. This thesis includes five quantitative studies that use data from TILDA.

3.1. Study Design

3.1.1. Overview of TILDA

TILDA is a nationally-representative, longitudinal study of adults aged 50 years and older, and their spouse of any age, in the Republic of Ireland. TILDA collects extensive information on the social, economic and health circumstances of participants.

Interviews are repeated every two years and referred to as waves of data collection. The study is comprised of three main components: 1) A structured computer-assisted personal interview (CAPI); 2) an optional self-completion questionnaire (SCQ) collecting more sensitive information; and 3) an optional comprehensive health assessment (HA) carried out by trained research nurses in a health centre. TILDA was established to provide baseline information on the older population in Ireland and contribute to our understanding of the causal processes involved in ageing by tracking changes over time among the ageing population (307). TILDA was originally modelled on the Health and Retirement Study (HRS) questionnaire to allow for maximum comparability with a family of longitudinal studies worldwide following a similar design (308).

3.1.2. Sampling Design

TILDA used a random sampling procedure (RANSAM) (309) to recruit participants for the first wave of data collection. The original target population was any adult aged 50 years or older, in addition to their spouse of any age, living in a residential address in the Republic of Ireland. The Irish Geodirectory, a comprehensive listing of all residential addresses in the Republic of Ireland, was used as the sampling frame for TILDA. The sampling process was comprised of three-stages. Firstly 3,155 clusters were identified which comprised of between 50 and 1,180 addresses. Of these, 640 clusters, and 50 addresses within each cluster were randomly selected, identifying 32,000 unique residential addresses. It was estimated that 25,600 of these households would achieve the required sample size threshold of 8,000 participants. **Figure 3.1.** shows the distribution of the randomly selected clusters used to form the TILDA sample. Trained interviewers were required to identify eligibility of each selected address by determining whether a member of the household was aged 50 years or older. Further detail on the sampling and design of TILDA has been published elsewhere (307, 310, 311). The response rate for TILDA was 62%, with over 8,000 people taking part in the study.

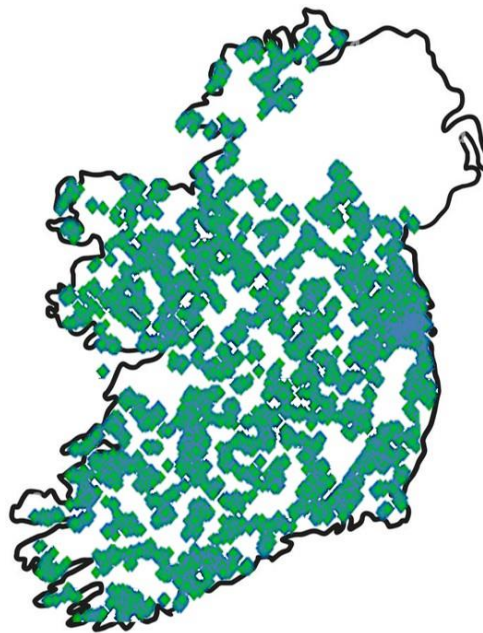


Figure 3.1. Location of clusters selected at random for inclusion in TILDA

3.1.3. Data Collection

As mentioned above, there are three distinct components to TILDA by which data on the study participants is acquired. The first, a computer-assisted personal interview (CAPI) was administered by trained interviewers at each wave of data collection in the participants' own home. The CAPI collects detailed information on demographics, lifestyle, social, behavioural, mental, and physical health. The second component, a self-completion questionnaire (SCQ) was provided for participants which they were invited to complete in their own time and send by return post to TILDA's research premises. The

Demographic data Education Childhood health Migration history Marital status and marriage history Social circumstances Transfer to (and from) children Transfer to (and from) parents (Instrumental) activities of daily living Helpers Social connectedness Participation in social/recreation activities Relationship quality (SCQ) Employment and lifelong learning Employment situation Job history Lifelong learning Retirement and expectations Planning for retirement Expectations Income and assets Source of income Assets Transport Transportation Driving Medication Health-care utilization	Physical health Self - rated health Limited long- standing illness/disability Sensory function Cardiovascular disease Non - cardiovascular chronic illness Falls/fear of falling/steadiness Chronic pain Incontinence Medical screening Mental health Self- reported mental health Depression Life satisfaction Anxiety (SCQ) Worry (SCQ) Loneliness (SCQ) Perceived stress (SCQ) Stressful life events (SCQ) Quality of life (SCQ) Cognitive health Self-rated memory Orientation Word-list learning (immediate and delayed recall) Verbal fluency Prospective memory Behavioral health Smoking Physical activity Sleep Alcohol (SCQ) Diet (SCQ) Ageing perceptions (SCQ)

data captured in the CAPI and SCQ are summarised in **Figure 3.2.**

Figure 3.2. Summary of data collected in TILDA; CAPI and SCQ

The third component of TILDA involves a comprehensive health assessment (HA), carried out in a dedicated health centre. The HA is carried out every four

to six years, with full health centre data available for Waves 1 and 3 of TILDA to date. Objective measures of health in participants who attended the health centre was collected. Participants that were unable or unwilling to attend the health centre were alternatively offered an in-home, abbreviated HA where a subset of features was measured. Data collected in the in-centre assessments are summarised in **Table 3.1**. Health centre data included in this thesis will be described in detail later in this chapter.

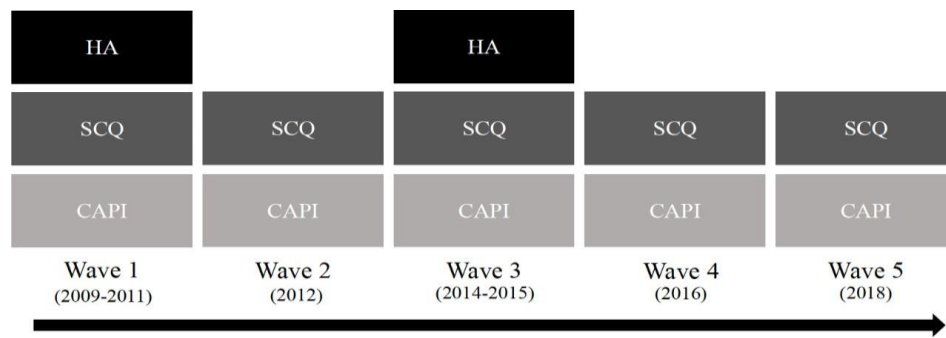
Table 3.1. TILDA data collected during health centre assessment

Neuropsychological	Cardiovascular	Mobility	Sensory	Anthropometric/Other
MMSE	Pulse wave velocity	TUG	Visual Acuity	Height
MOCA	Seated and standing blood pressure	Repeated Chair stands	Contrast sensitivity	Weight
SART	Phasic blood pressure responses	GAITRite assessment	Macular pigment density	Waist and hip circumference
Picture memory	Heart rate variability		Retinal photographs	Grip strength
NART	Cerebral perfusion		Multisensory integration (SHAMS)	Heel ultrasound
Visual reasoning	ECG			Blood samples
Choice reaction time				Hair samples
Colour trail tests				Accelerometry (subset)
Depressive symptoms				MRI Brain (subset)
Anxiety symptoms				Dental assessment offered

MMSE – Mini-Mental State Examination; MOCA – Montreal Cognitive Assessment; SART – Sustained Attention to Response task; NART – National Adult Reading test; ECG – Electrocardiogram; TUG – Timed up and go; SHAMS – Sound induced flash illusion; MRI – Magnetic Resonance Imaging.

3.1.4. Longitudinal Data Acquisition

TILDA consists of a series of “waves” of data collection, carried out every two years, with HA data collected every four to six waves. The data utilised in this thesis comprises data from the first to fifth waves of TILDA. The first wave of data collection was carried out from 2009-2011 and included a CAPI, SCQ and HA. The second wave of data was collected in 2012 and included a CAPI and SCQ, and the third wave of data was collected between 2014 – 2015, including a CAPI, SCQ and health assessment. Data collection waves are summarised in **Figure 3.3**.



CAPI – Computer-Assisted Personal Interview; SCQ – Self Completion Questionnaire; HA – Health Assessment

Figure 3.3. Data collection waves 1-5 of TILDA

3.1.4.1. Wave 1

The first wave of data collection took place between October 2009 and February 2011 (312). Interviews were conducted in 6,279 households with 8,175 individuals aged 50 years and older, and a further 329 younger spouses, participating in the study (n 8,504). This represented a response rate of 62.0% (313). A comprehensive HA was also offered to participants who completed an interview. Cognitive, cardiovascular, gait and vision testing were conducted, and anthropometric measurements and blood samples were taken. The HA for Wave 1 was carried out in a centre based in Dublin or Cork. Where the participant was unable to travel, a modified home-based assessment was offered (314).

3.1.4.2. Wave 2

Wave 2 interviews were carried out between April 2012 and January 2013 (315). Participants were invited to take part in the structured interview and complete an optional self-completion questionnaire. Of the original core sample (n 8,504), 7,207 participants completed the self-interview at Wave 2. Further, Wave 2 introduced new types of interviews. A proxy interview was carried out where a participant was unable to complete an interview due to a physical or cognitive impairment (n 78). Where a participant of the previous wave had subsequently passed away, an end-of life (EOL) interview was

sought with a close family member or friend of the participant (*n* 160). In addition to the core sample, 170 new participants completed a CAPI at Wave 2. These participants were individuals who shared a household with a TILDA participants from Wave 1 but did not take part in the first set of interviews.

3.1.4.3. Wave 3

Interviews for Wave 3 took place between March 2014 and October 2015 (316). A total of 6,403 participants completed the wave (self-interview), with a further 119 proxy interviews and 206 end-of-life interviews carried out. The optional HA was offered again at Wave 3, with 5,270 participants completing this in a health assessment centre base in Dublin. Interviews were also collected for 29 new participants outside of the core sample.

3.1.4.4. Wave 4

Between January 2016 and December 2016 (317), interviews for Wave 4 were completed. Self-interviews were completed by 5,445 participants, proxy interviews by 117 participants and 206 end-of-life interviews were completed. Interviews were collected for 13 new participants outside of the core sample.

3.1.4.5. Wave 5

Interviews for Wave 5 were carried out between January 2018 and December 2018. Self-interviews were completed by 4,980 participants, proxy interviews by 123 participants and 171 end-of-life interviews were completed. A further 3 new participants also completed Wave 5 interviews.

Figure 3.4. displays an overview of the modules included at each wave of data collection (Waves 1-5), and the attrition rates of the core Wave 1 sample.

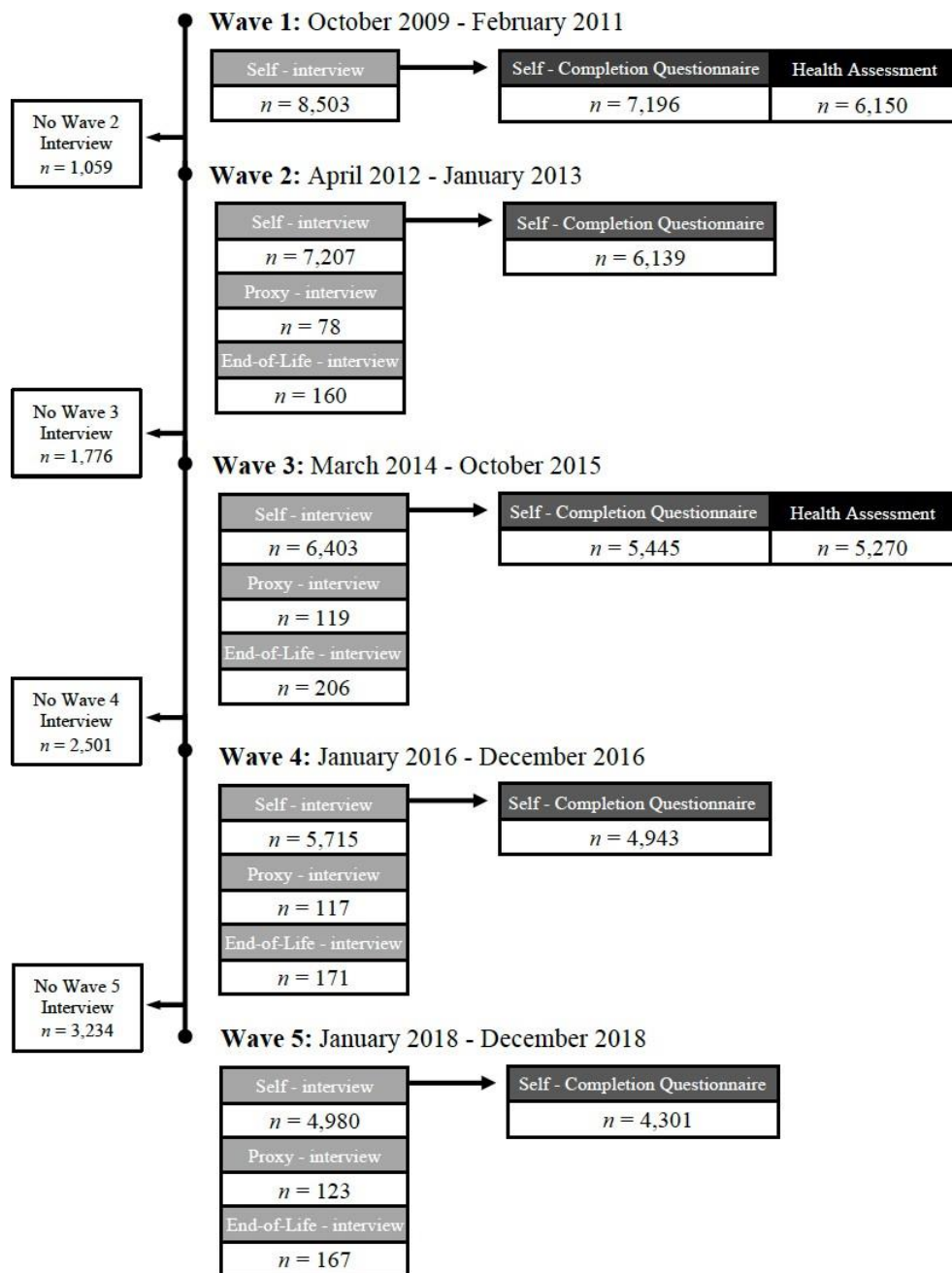


Figure 3.4. TILDA sample (Wave 1 – Wave 5)

3.2. Measures

This Ph.D. thesis examined the relationship between plasma folate and vitamin B₁₂ concentrations and cognitive function and blood pressure control, in addition to dietary behaviour that may influence these factors. To do this, specific measurements within the study were employed which are described in detail in this section.

Details of measures covering outcome, predictor and confounding variables are provided in the respective methodology of each study. Measures of folate and vitamin B₁₂, cognitive and blood pressure control were the primary focus of the thesis. The B- vitamins were measure by gold-standard microbiological assay. Cognitive function was assessed by the Mini-Mental State Examination (MMSE) (318), the Montreal Cognitive Assessment (MoCA) (319), a verbal fluency task, immediate and delayed verbal memory tests. Blood pressure control was measured by OH. Dietary intake was measured by FFQ. **Figure 3.5.** illustrates how the availability of measurements varied across wave of data collection.

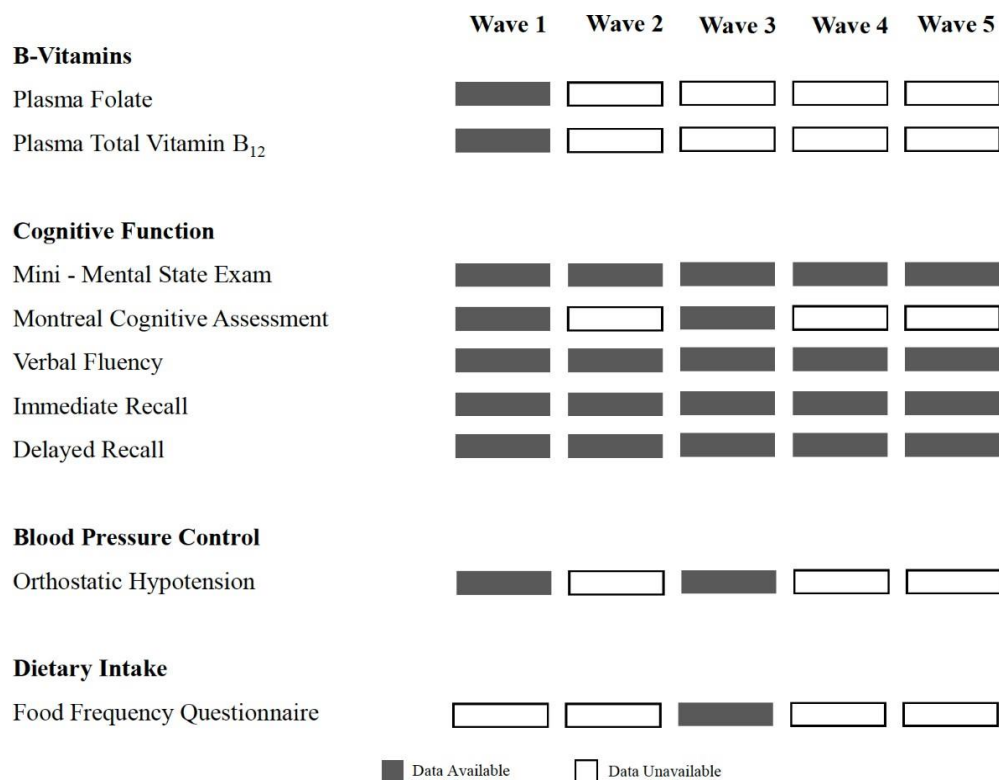


Figure 3.5. Measurements in each wave of data collection (Wave 1 to Wave 5).

3.2.1. Folate and Vitamin B₁₂ Measurement

Non-fasting whole blood samples were collected between 09:30 and 16:30 by venepuncture into 10 mL K₂EDTA (*potassium ethylenediaminetetra-acetic acid*) tubes (BD, Becton, Dickinson Limited, Oxford, UK) during the HA by a trained phlebotomist and were transported at 2°C –8°C rpm for 15 min) and aliquots were labelled and stored at –80°C within 48 hours of collection, until required for analysis. Unless stated otherwise, biomarker analyses were automated and carried out on archived plasma samples between 2014 and 2016 by an International Organization for Standardization accredited clinical pathology laboratory at a teaching hospital in Dublin, Ireland. The date of blood collection was documented and seasonality was determined: Winter (December–February), spring (March–May), summer (June– August), and autumn (September–November). Plasma folate and total plasma vitamin B₁₂ concentrations were determined by microbiological assays, *Lactobacillus casei* (*L. rhamnosis* (NCIB 10463; ATCC 27773)), *limit of detection 0.6 nmol/L* (320), respectively *Lactobacillus leichmannii* (*L. leichmanii* (*delbrueckii*) (NCIB 12519, ATCC 43787)), *limit of detection 3.7 pmol/L* (321). Detection ranges of the analysed samples for folate and vitamin B₁₂ were 0.9–642.2 nmol/L and 45.4–1941.4 pmol/L, respectively. **Figure 3.6.** depicts the distribution of plasma folate concentrations in among those aged 50 and older in Wave 1 of TILDA. The inter-assay coefficients of variability (CV) for plasma folate and total vitamin B₁₂ were <10.9%.

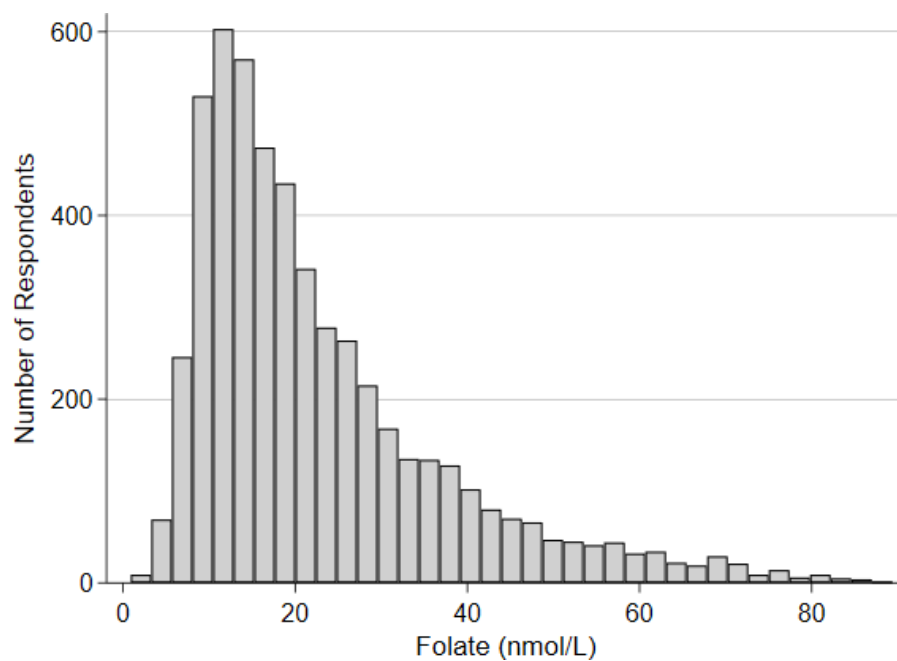


Figure 3.6. Histogram of plasma folate (nmol/L) at Wave 1.

The distribution of plasma total vitamin B₁₂ concentrations are shown in **Figure 3.7**.

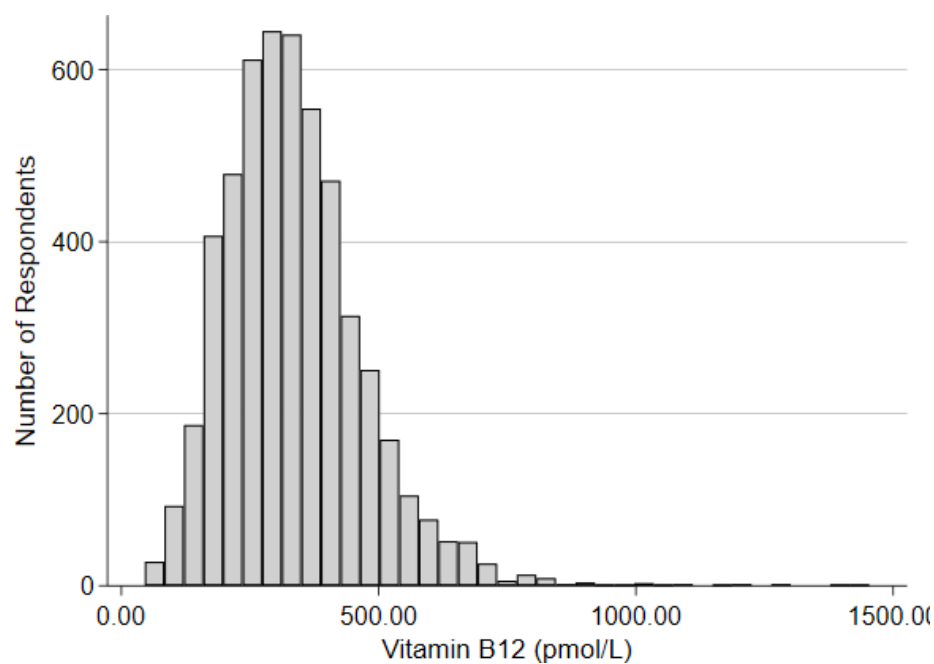


Figure 3.7. Histogram total cobalamin (vitamin B₁₂) pmol/L at Wave 1.

3.2.2. *Cognitive Function*

3.2.2.1. Mini-Mental State Examination (MMSE)

Participants completed the MMSE at each wave of TILDA. The MMSE measures global cognitive function and is used to screen for cognitive impairment (318).

Participants were asked a series of questions which assess attention, orientation, memory, language, and visuospatial ability. Scores range from 0-30.

3.2.2.2. Montreal Cognitive Assessment (MoCA)

The MoCA was administered by research nurses during the HA in Wave 1 and Wave 3 of TILDA. The MoCA also measures global cognitive function, covering attention, orientation, executive function, memory, language, and visuospatial ability (319).

Participants were asked a series of questions which assess attention, orientation, memory, language, and visuospatial ability. Scores range from 0-30. The MoCA is understood to be more sensitive at detecting mild cognitive change in a wide variety of settings (322).

3.2.2.3. Verbal Fluency

Verbal fluency was used to assess executive function at each wave of TILDA. Participants were asked to name as many different animals as possible in 60 seconds. This assessment requires participants to apply mental flexibility, self-initiated activity, abstraction, language processing and orientation (317, 323, 324, 325).

3.2.2.4. Immediate and Delayed Recall

An immediate and delayed word list recall were used to assess memory measuring verbal learning and recall (325). Participants were read a list of ten words and asked to recall as many of the words as possible immediately after (immediate recall). This was repeated twice, and the scores were added together to give a total immediate recall score. At a later stage in the interview, approximately 15 minutes after the list has been read, the

participant was asked to again recall as many words as possible (delayed recall).

3.2.3. *Autonomic function: Orthostatic Blood Pressure Measurement*

Participants who came to the health centre for assessment at Wave 1 and Wave 3 underwent an active stand (AS) test, in which a research nurse measured the beat-to-beat blood pressure response to standing up. Non-invasive measurement of the beat-to-beat blood pressure was conducted using a Finometer Midi device (Finapres Medical Systems). The assessment of orthostatic blood pressure used a standardised protocol. In a quiet room with a temperature between 21 and 23 degrees Celsius, participants lay prone for 10 minutes prior to standing. The systolic blood pressure was measured as the mean value between 60 and 30 seconds before standing. The participants were instructed to stand promptly, aided by the nurse when necessary. The monitoring of systolic BP, diastolic BP, and heart rate continued for an additional 120 seconds. Participants were asked whether they had experienced symptoms such as light-headedness, dizziness, or unsteadiness following the AS.

At the first wave, blood pressure was computed at 10-second intervals using 5-second moving averages around each point. Given the use of moving averages, the 120 second time point for most participants would include edge effects; therefore, BP was estimated at 10 second intervals up to 110 seconds post-stand. At Wave 3, BP data were smoothed using a moving average of 10 seconds around each time point. During the first wave, the Finometer's graphical user interface data was used to determine the stand location.

At wave 3, the point of stand was determined using the data from the height correction unit. Previous analysis of the TILDA data has shown that most participants stabilise their BP within 40 seconds of stand (326). OH at 40 seconds has been found to be associated with outcomes in TILDA, including falls and cognitive decline (327, 328). In addition, a study conducted by Juraschek et al. using data from the Atherosclerosis Risk in Communities (ARIC) study found that the first two measurements of blood pressure after standing were most predictive of outcomes (329). The first measurement was

taken at a mean of 28 seconds ($\pm$ 5.4 seconds standard deviation) with a standard deviation of 5.4 seconds. The second measurement was taken at a mean of 53 seconds ($\pm$ 7.5 seconds standard deviation) with a standard deviation of 7.5 seconds. As a result, in this study, the 40-second time point to evaluate OH as an indicator of impaired blood pressure stabilisation is used. OH was also assessed at 110 seconds, which represents a subset of participants with OH with more severe symptoms. These participants had OH throughout the duration of the stand. Continuous blood pressure monitoring is depicted in **Figure 3.9** during AS.

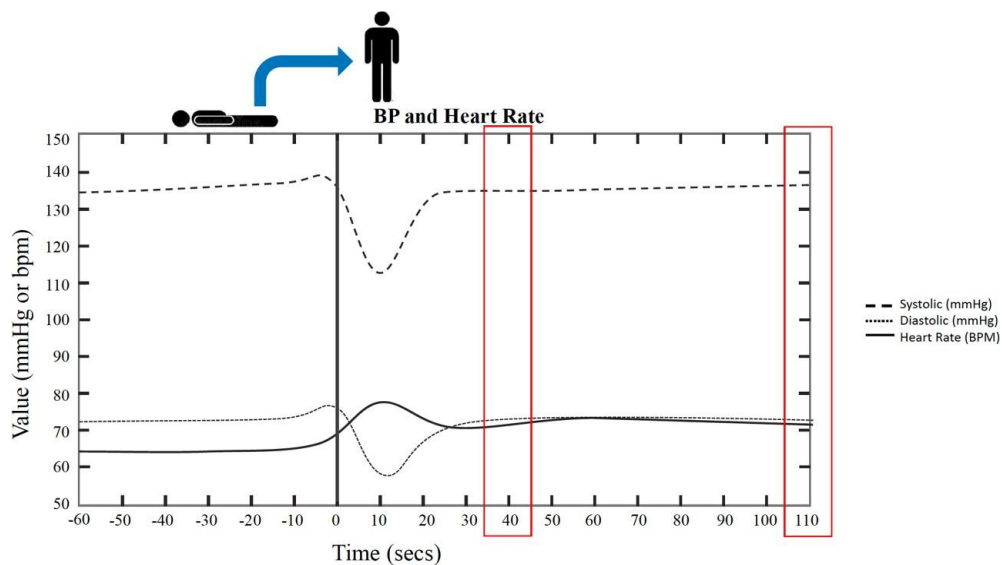


Figure 3.9. Orthostatic blood pressure assessment.

3.2.4. Dietary Intake

In the third wave of TILDA (2014) a Food Frequency Questionnaire (FFQ) was completed by 5,206 participants, as part of the SCQ. The semi-quantitative FFQ with 52 items was adapted from the Survey of Lifestyle, Attitudes, and Nutrition (SLÁN) 2007 (91), and the European Prospective Investigation of Cancer and Nutrition (EPIC) study (330). The FFQ was created to evaluate the diet quality and habitual frequency of the 52 food items or aggregate groups within the main food groups eaten in the average Irish diet. Food groups were defined in accordance with the Irish FP guidelines in order to compare dietary patterns with national dietary recommendations (331).

Participants noted the frequency with which they consumed each item over the previous year. Typical weights and portion sizes were estimated using guidelines McCance and Widdowson’s food composition tables (332) and the Irish Food Portion Size database (333). Responses for each food item/aggregate group came from nine distinct frequency categories: never or less than once a month, 1-3 times per month, once a week, 2-4 times per week, 5-6 times per week, once a day, 2-3 times per day, 4-5 times per day, and 6+ times per day. **Figure 3.10** displays an example of a food item from the FFQ (for full TILDA FFQ, see **Appendix 11.3**). Mean daily consumption and compliance with the 2012 FP recommendations for each food category were used to assess dietary quality. The sum of the items was then used to compute an overall measure of compliance to the entire FP, with a theoretical range of 0 (no compliance) to 6 (full compliance). Furthermore, compliance with suggestions for the Fruit and Vegetables, Milk, Yogurt, and Cheese, and Meat, Poultry, Fish, Eggs, Beans, and Nuts shelves was investigated separately.

Potatoes, Rice, Pasta (medium serving)	Never/ less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Potatoes, including boiled, mashed, baked potatoes, but excluding roast potatoes, chips or potato products (e.g. waffles)	☐	☐	☐	☒	☐	☐	☐	☐	☐

Figure 3.10. Example of a food item from the FFQ

3.3. Statistical Analysis

The statistical analyses in this Ph.D. thesis were conducted using Stata 14.2 and Stata 15.1 (334, 335). Suitable analysis strategies were developed for each paper to investigate the study’s primary objectives. In the methodology section of each paper, analysis strategies are described in full detail. Here, a brief summary of the respective analyses is provided.

This first empirical study in this Ph.D. thesis reported the folate and vitamin B₁₂ status of a large, nationally representative sample of the older population

of Ireland – using Wave 1 of the TILDA dataset. It investigated the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in this population. (**Chapter Four, Paper 2**). The second study analysed cross-sectional associations of plasma folate and total vitamin B₁₂ groups with two measures of global cognitive function from Wave 1 of TILDA (**Chapter Five, Paper 3**). The third study was a longitudinal assessment of cognitive function and plasma folate concentration, examining trajectories of cognitive function with respect to baseline folate status (Wave 1) at 2-, 4-, 6- and 8-year follow-up (**Chapter Six, Paper 4**). The fourth study comprised cross-sectional and longitudinal analyses of OH over four years with respect of baseline folate and vitamin B₁₂ status at baseline (**Chapter Seven, Paper 5**). Finally, the fifth quantitative study in this thesis includes a cross-sectional exploration of habitual dietary intakes, using an FFQ at Wave 3 of TILDA. Multivariable logistic regression was used to investigate whether different patterns of compliance dietary recommendations were associated with socio-demographic, health and lifestyle behaviours, social, and cognitive characteristics of the population (**Chapter Eight, Paper 6**).

CHAPTER FOUR: PAPER 2 – VOLUNTARY FORTIFICATION IS INEFFECTIVE TO MAINTAIN THE VITAMIN B₁₂ AND FOLATE STATUS OF OLDER IRISH ADULTS: EVIDENCE FROM THE IRISH LONGITUDINAL STUDY ON AGEING (TILDA)

Introduction to Chapter Four

This section of the chapter addresses **Aim 2** and **Objectives 2.1** and **2.2** of the thesis. Given the lack of reliable and scalable nationally representative data pertaining to folate and vitamin B₁₂ status among older adults in Ireland, this chapter aimed to quantify plasma folate and vitamin B₁₂ status in a nationally representative population of older adults. This was done by measuring deficiency and low status rates of plasma folate and vitamin B₁₂ sample obtained during Wave 1 of TILDA. Further, this chapter investigates the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in the older adult population of Ireland.

In the following paper, I test the hypothesis that among the population of older adults in Ireland have high rates of deficiency and low levels of folate and vitamin B₁₂, due, in part to a liberal but voluntary practice of fortification of some foods in Ireland. The following data is from the second published work from my thesis, “*Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: evidence from the Irish Longitudinal Study on Ageing (TILDA)*” (**Appendix 11.1.2**).

List of Published Work from Chapter Four

Published Figures

Figure 4.1. Frequency distribution of plasma folate (a) and total vitamin B₁₂ (b) of older Irish adults from The Irish Longitudinal Study on Ageing study by age group.

Published Tables

Table 4.1. Mean plasma folate with weighted prevalence of status by demographic and lifestyle characteristics

Table 4.2 Mean plasma total vitamin B₁₂ with weighted prevalence of status by demographic and lifestyle characteristics

Table 4.3 Determinants of plasma folate concentrations (nmol/L l) in older

Irish adults Table 4.4 Determinants of plasma total vitamin B₁₂

concentrations (pmol/l) in older

Published Sections

4.1 Abstract

4.2. Introduction

4.3. Methods

4.4. Results

4.5. Discussion

4.1. Abstract

Mandatory fortification of staple grains with FA and/or vitamin B₁₂ is under debate in many countries including Ireland, which has a liberal, but voluntary, fortification policy. Older adults can be at risk of both deficiency and high folate status, although little is known on the actual prevalence and the major predictors. Population prevalence estimates from older adults ($n = 5290 \geq 50$ years) TILDA (Wave 1) are presented here. Measures included plasma folate and total vitamin B₁₂, whereas predictors included detailed demographic, socio-economic, geographic, seasonal and health/lifestyle data. The prevalence of deficient or low folate status was 15% whereas the prevalence of vitamin B₁₂ status (<185 pmol/L) was 12%. High folate status (>45 nmol/L) was observed in 8.9 %, whereas high vitamin B₁₂ status was observed in 3.1 % (>601 pmol/L). The largest positive predictor of folate concentration was FA supplement use (6.0; 95 % CI 3.0, 9.0 nmol/L; $P < 0.001$) followed by being female and statin medications. The largest negative predictor was geographic location (-5.7 ; 95 % CI -6.7 , -4.6 ; $P < 0.0001$) followed by seasonality and smoking. The largest positive predictor of vitamin B₁₂ concentration was self-reported vitamin B₁₂ injection and/or supplement use (coefficient 51.5 pmol/; 95 % CI 9.4, 93.6; $P = 0.016$) followed by sex and geographic location. The largest negative predictor was metformin use (-33.6 ; 95% CI -51.9 , -15.4 ; $P < 0.0001$). B-vitamin status in older adults is affected by health and lifestyle, medication, sampling period and geographic location. We observed a high prevalence of low folate and vitamin B₁₂ status, indicating that the current policy of voluntary fortification is ineffective for older adults.

4.2. Introduction

National surveys have shown that the proportion of older adults within most populations (defined as those >60 years) is increasing, and by 2050 global projections indicate that the number of older adults worldwide will be more than 2 billion (21% of the population) (8). With such profound shifts in demographics also comes the corresponding increase in the prevalence of the chronic diseases of ageing including CVD, cognitive impairment and

associated co-morbidities (336). Maintenance of optimal nutritional status has been cited as an achievable goal in preventing or delaying the development of chronic disease (337), and recent emphasis has been placed on the protective roles of micronutrients, including the B-vitamins.

Both folate and vitamin B₁₂ are essential co-factors in intracellular one-carbon metabolism, which is involved in DNA synthesis, cellular regulation processes and amino acid metabolism (338). Both vitamins are thought to be associated with cardiovascular health and cognitive function. A recent meta-analysis (*n* 82 334) indicated a 10% lower risk of stroke and a four percent lower risk of overall CVD with FA supplementation (286), whereas in a longitudinal study of older adults (>60 years, *n* 501) higher concentrations of vitamin B₁₂ were associated with a decreased rate of brain volume loss over 6 years (121). Estimates of vitamin B₁₂ deficiency in older populations range from 5 to 40%, depending on the marker of measurement and the deficiency cut-off selected (196). The consequences of a B₁₂-deficient state can include the development of megaloblastic anaemia, irreversible demyelinating neurological disease/paresthesias and the potential for impaired immune and cognitive function (339). Hence, the early detection of deficiency and awareness of the factors that can affect B₁₂ status is important. In terms of folate, since the introduction of mandatory FA fortification of grains in the USA to reduce the incidence of neural tube defects, the prevalence of deficient and low folate status in the US population has substantially decreased (195). Conversely, there is an accompanying increased incidence of high blood folate concentrations (>45 nmol/L), which has led to concerns of potential adverse effects, especially when combined with low vitamin B₁₂ status, as frequently observed in older adults (201).

To date, MFAF does not occur in the EU. Uniquely within the EU, however, both Ireland and the UK have a voluntary but liberal food fortification policy that includes FA. The foods most commonly fortified with FA are RTEBCs, but not all are fortified (198, 340). In addition, there are reports of varying degrees of FA enrichment of foods, and enrichment also appears to be inconsistent across time and across products (341). Moreover, foods that are

fortified or rich in vitamin B₁₂ are not necessarily consumed by the older population. For instance, dairy foods, which are a particularly good source of vitamin B₁₂, are only consumed in the recommended amounts by only 4 percent of older adults in Ireland (342). A recent Irish national survey that previously examined vitamin B₁₂ and folate status was limited to a small number of older adults and had a narrow range of demographic and clinical data (194). Therefore, the aims of this study were (i) to report the folate and vitamin B₁₂ status of a large, nationally representative sample of the older population of Ireland ($n > 5000$) and (ii) to investigate the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in this population, which is subject to a voluntary, but liberal, food fortification policy.

4.3. Methods

4.3.1. Study population

Participants were recruited as part of TILDA, a nationally representative cohort of community-dwelling adults aged ≥ 50 years, designed to investigate how the health, social and economic circumstances of the older Irish population interact in the determination of ‘healthy’ ageing. Full details of the study design, sampling and methodology have been published previously (313). The first wave of data collection (Wave 1) was conducted between October 2009 and July 2011, using a stratified clustered procedure to randomly sample postal addresses from the Irish Geo-Directory (a listing of all residential addresses in the Republic of Ireland). All postal addresses in Ireland were assigned to one of 3155 geographic clusters using RANSAM (a random sampling design for Ireland) where all household residents aged ≥ 50 years were eligible to participate; a sample of 640 of these clusters was selected, stratified by socio- economic group and geography to maintain a population representative sample. Clusters were selected with a probability proportional to the number of individuals aged ≥ 50 years in each cluster. At Wave 1, 8175 adults completed a computer-aided personal interview (CAPI) (response rate of 62 %). Approximately 72.1 % ($n = 5895$) consented to, and

participated in, a health assessment that included donating a blood sample. In total, folate measurements were available for 90.7 % (n 5350) and B₁₂ measurements were available for 88.5 % (n 5219) of these participants. Missing data were randomly dispersed across clusters and were generally because of sample assay failures or insufficient sample volume. The study was conducted according to the guidelines set out in the Declaration of Helsinki, and ethical approval was granted by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin.

4.3.2. Lifestyle and supplement use

During the CAPI, data were collected regarding sex, age, province of residence (Leinster – East/South East; Munster – South/South West; Ulster and Connacht – North and West), habitation status (living alone yes/no), current smoker status (yes/no), current alcohol status (yes/no) and household housing wealth (measure of economic resource ‘asset wealth’ coded above or below the average for the sample). A list of up to twenty medications including both prescription and non-prescription medications and dietary/vitamin supplements were recorded by interviewers. Once entered into the data set, they were assigned World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification codes and dichotomised to yes/no for FA supplement use (single tablet or multi-vitamin) and for use of vitamin B₁₂ (injection or single tablet or B₁₂ in multi-vitamin). Classification codes were also dichotomised to yes/no for the commonly prescribed medications of older adults, which have been associated with B-vitamin concentrations including metformin, PPI, statins and thyroxin medications (55, 343). Self-reported physical activity was assessed by the International Physical Activity Questionnaire (IPAQ). Physical activity levels were also dichotomised and defined according to the IPAQ categories: physically active = minimally or health enhancing physically active; not physically active = inactive or insufficiently active. During the health assessment, anthropometric measurements including height (to the nearest 0.01 m) and weight (to the nearest 0.01 kg) were measured with Seca™ (Seca

Ltd) using a standardised protocol. BMI was then calculated and obesity was classified as a BMI $>30 \text{ kg/m}^2$. Seasonality was examined and defined as winter (December through February), spring (March through May), summer (June through August) and autumn (September through November).

4.3.3. *Blood samples*

A non-fasting blood sample was collected by venepuncture into one 10-fml K₂EDTA tube (BD, Becton, Dickinson Limited) by a trained phlebotomist. Samples were kept chilled and centrifuged (3000 rpm for 15 min) and aliquots were labelled and stored at -80°C until required for analysis. Plasma folate and total vitamin B₁₂ concentrations were determined by microbiological assays, as previously described (320) (321). The inter-assay CV for plasma folate and vitamin B₁₂ was 0.9 %. Folate status profiles (nmol/L) were defined as follows: <6.8 , deficient; $6.8\text{--}10.0$, low; $>10\text{--}23.0$, low-normal; $>23.0\text{--}45.0$, normal; >45.0 , high (344). Vitamin B₁₂ status profiles (pmol/L) were defined as follows: <148 , deficient; $148\text{--}<185$, low; $185\text{--}<258$, low normal; $>258\text{--}601$, normal; and >601 , high (345, 346). These cut-offs were selected to allow for comparison with previous studies and to give an indication of the population range.

4.3.4. *Statistical analyses*

Base population weights were calculated by comparing TILDA with the 2011 Irish Census with respect to age, sex, educational attainment, marital status and urban vs. rural residence (weights reflected the reciprocal of the selection probability). The staged stratified clustering sample design of TILDA was accounted for when computing CI and standard errors. Prevalence estimates and descriptive statistics were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether respondents provided a blood sample. TILDA participants with plasma folate or vitamin B₁₂ concentrations more than three standard deviations (SD) above the mean or who were missing data on other covariates were excluded from the analysis, resulting in 5,290 results

for plasma folate and 5,158 results for total vitamin B₁₂ (the +3SD cut-off was applied to remove outlying high values, likely attributable to measurement error). Weighted prevalence estimates are reported as percentages with 95% CI. Weighted geometric means are presented along with folate and vitamin B₁₂ concentrations categorised by the stated biomarker cut-offs, stratified by the population characteristics. Pairwise comparisons of proportions by folate and vitamin B₁₂ status cut-offs were computed across variables of interest, and false-discovery rate correction for multiple comparisons was applied ($P < 0.041$, achieving $q < 0.05$). Multivariable linear regression estimated the effects of health, lifestyle, geographic and socio-economic factors on folate and vitamin B₁₂ concentrations. Multiple imputation (twelve cycles) was used to impute missing information on asset wealth in the final statistical models (information was missing for <5% of participants). All analyses were carried out using STATA 14 (StataCorp).

4.4. Results

The population characteristics of the TILDA cohort have been published comprehensively elsewhere (313). In short, of the participants included in this combined analysis, the mean age was 63.6 years (age range 50–98) and 53% were female. The proportion who reported currently consuming alcohol was 76.8%, whereas 15.6% were current smokers. The prevalence of FA supplementation was 2.8%, with significantly more women than men reporting supplementation use (3.6 v. 2.0%; $P < 0.001$, respectively). The prevalence of reported combined vitamin B₁₂ supplementation (injection and/or supplements) was 2.6%, with women reporting significantly higher usage than men (3.5 v. 1.7%; $P < 0.001$, respectively).

4.4.1. Prevalence of biomarker status

The estimated distribution of both folate and vitamin B₁₂ status by age group is displayed in **Figure 4.1**. The prevalence of deficient or low folate status was 15%, whereas the prevalence of deficient or low vitamin B₁₂ status was 12%. High folate status (>45 nmol/L) was observed in 8.9 %, while high vitamin

B₁₂ status (>601 pmol/L) was observed in just 3.1 % of the population. The prevalence estimates by demographic and lifestyle factors are displayed in **Table 4.1** for folate and **Table 4.2** for vitamin B₁₂. Participants reporting supplement use had significantly higher concentrations of both plasma folate and total vitamin B₁₂ and had lower rates of deficiency compared with non-supplement users. For both biomarkers, men had significantly lower concentrations as did the oldest old (>80 years) ($P<0.001$). For both folate and vitamin B₁₂, those who were obese, smoked or reported lower mean asset wealth had significantly lower biomarker concentrations and higher rates of deficiency/low status. Seasonality was also significantly associated with both biomarker concentrations ($P<0.001$). Concentrations of folate were highest in spring but lowest in autumn (19.5 v. 16.4 nmol/L), while total vitamin B₁₂ levels were the highest in spring and the lowest in summer (318.9 v. 304.5 pmol/L) (but we note that in the regression analyses, the effects of seasonality were only robust for folate concentrations in autumn v. winter). When examined by geographic area, participants residing in the North and West of the Island (Connaught and Ulster) had lower plasma folate compared with those living in the East and South East (Leinster region) (16.2 v. 21.1 nmol/L). For medications, only metformin use was significantly associated with lower total vitamin B₁₂ concentrations (272.9 v. 315.7 pmol/L; $P<0.0001$) and a higher prevalence of deficiency (11.0 v. 4.8 %; $P<0.0001$). There were no statistically significant differences in the concentration of folate and vitamin B₁₂ by statin, PPI or thyroid medication use (data not shown).

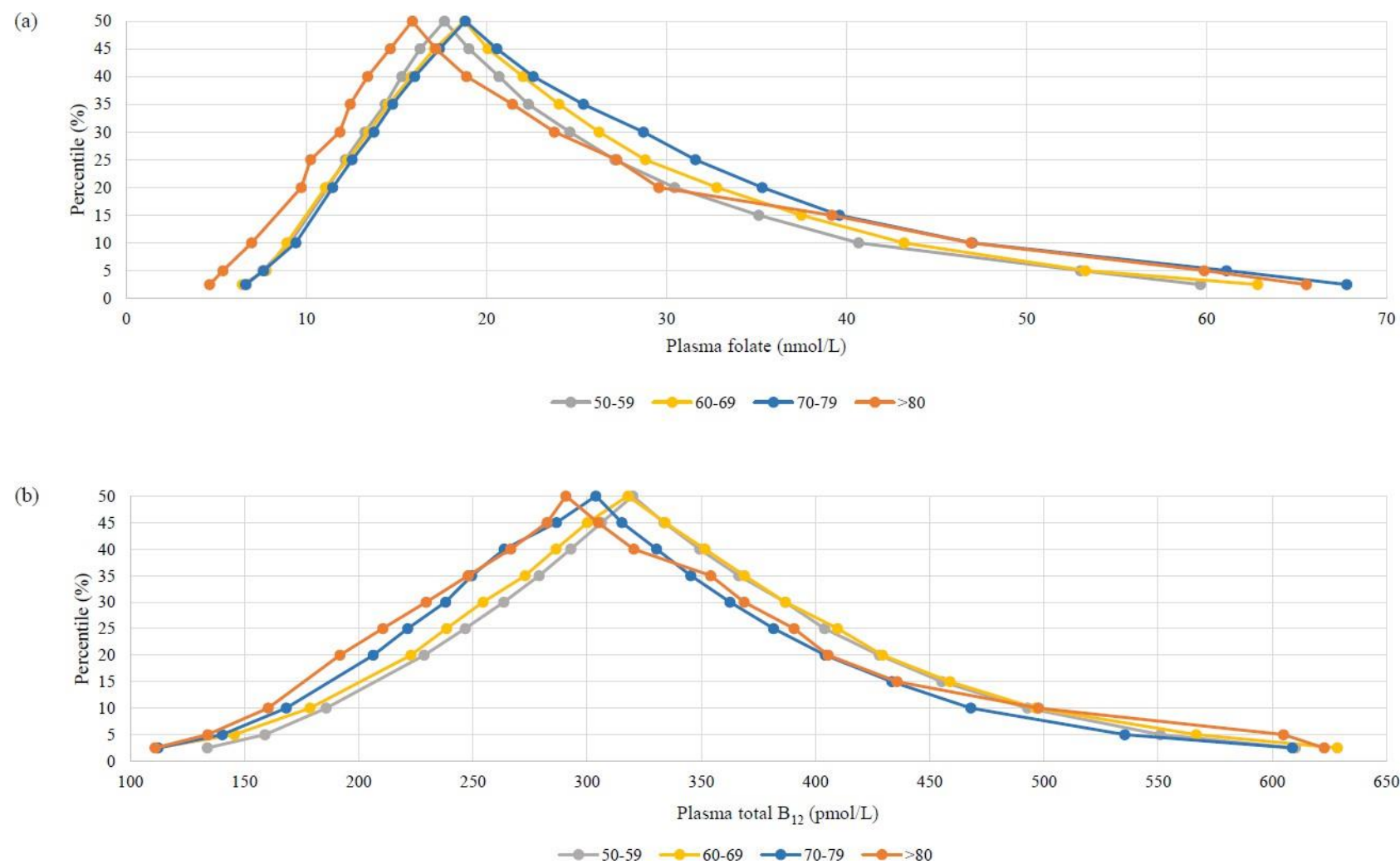


Figure 4.1. Frequency distribution of plasma total folate (a) and vitamin B₁₂ (b) of older adults, by age group

Table 4.1. Mean plasma folate with weighted prevalence of status by demographic and lifestyle characteristics† (Weighted means and prevalence estimates and 95% confidence intervals)

Characteristics	Subjects (n)	Plasma folate									
		Average	<10 nmol/L (n 731)		10–23 nmol/L (n 2610)		> 23–45 nmol/L (n 1530)		> 45 nmol/L (n 479)		
		(nmol/L)	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Supplements	162	24.5	20.7, 29.1	8.0	3.9, 15.3	35.5	28.0, 43.7	29.3	21.7, 38.1	27.2	19.9, 35.9
No supplements	5128	18.5	18.1, 19.0	15.1	13.8, 16.4	49.8	48.2, 51.4	26.7	25.2, 28.1	8.4	7.4, 9.4
<i>P</i>		0.0013*		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Sex											
Male	2476	17.9	17.4, 18.4	15.9	14.2, 17.6	51.9	49.7, 54.0	25.0	23.1, 26.9	7.2	6.0, 8.5
Female	2814	19.5	18.9, 20.1	14.0	12.4, 15.6	47.0	44.9, 49.1	28.4	26.5, 30.2	10.6	9.8, 12.1
<i>P</i>		<0.00001*		0.3284		0.2314		0.0009*		<0.00001*	
Age (years)											
50–59	2256	18.3	17.8, 18.9	14.3	12.6, 16.1	52.2	49.9, 54.4	26.0	23.9, 28.0	7.5	6.3, 8.9
60–69	1784	19.1	18.4, 19.7	14.5	12.7, 16.5	47.8	45.2, 50.3	28.8	26.5, 31.0	8.9	7.3, 10.7
70–79	968	19.8	18.9, 20.9	12.7	10.5, 15.3	48.0	44.2, 51.6	28.0	24.8, 31.4	11.3	9.0, 14.0
>80	282	16.7	15.2, 18.4	23.1	17.7, 29.6	45.1	38.4, 51.8	21.0	16.1, 26.6	10.8	7.6, 15.2
<i>P</i>		<0.00001*		0.0004*		0.041		0.0309*		0.0235*	
Obesity											
BMI >30 kg/m²	1782	17.6	17.0, 18.2	16.8	14.7, 19.0	51.8	49.0, 54.4	24.4	22.1, 26.7	7.1	5.8, 8.6
BMI <30 kg/m²	3493	19.2	18.7, 19.8	14.0	12.5, 15.4	48.3	46.4, 50.2	27.8	26.1, 29.5	9.9	8.7, 11.3
<i>P</i>		<0.00001*		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Smoking Status											
Smoker	828	16.7	15.9, 17.6	19.2	16.5, 22.2	51.5	47.7, 55.2	22.6	19.4, 26.1	6.7	4.9, 8.8
Non-smoker	4462	19.2	18.7, 19.7	13.9	12.5, 15.2	48.9	47.2, 50.6	27.7	26.2, 29.2	9.5	8.3, 10.7
<i>P</i>		<0.00001*		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Alcohol Consumption											
Alcohol consumer	3738	19.0	18.5, 19.5	14.0	12.6, 15.4	49.1	47.3, 50.9	27.7	26.0, 29.4	9.2	8.0, 10.4
Non-consumer	1123	18.1	17.4, 19.0	16.8	14.2, 19.6	48.9	45.7, 52.1	25.3	22.6, 28.0	9.0	7.2, 11.1
<i>P</i>		0.056		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Physical Activity											

Physically active	3728	18.7	18.2, 19.3	14.5	13.1, 16.0	49.8	47.9, 51.5	26.6	25.0, 28.3	9.1	7.9, 10.3
Not physically active	1516	18.5	17.8, 19.2	15.7	13.5, 18.1	48.8	45.9, 51.6	27.0	24.5, 29.6	8.5	6.8, 10.4
<i>P</i>		0.571		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Habitation											
Lives alone	1011	18.2	17.3, 19.1	17.9	15.2, 20.9	46.8	43.2, 50.3	25.2	22.2, 28.3	10.1	8.1, 12.4
Does not live alone	4279	18.8	18.4, 19.3	13.9	12.6, 15.2	50.3	48.5, 51.9	27.2	25.7, 28.7	8.6	7.5, 9.7
<i>P</i>		0.2069		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Asset wealth											
< Mean Asset wealth	2683	17.6	17.1, 18.2	16.3	14.6, 18.2	51.9	49.7, 54.00	24.7	22.8, 26.6	7.1	5.9, 8.3
> Mean Asset wealth	2335	20.3	19.6, 21.1	12.3	10.6, 14.2	46.3	43.9, 48.6	30.0	27.8, 32.2	11.4	9.6, 11.3
<i>P</i>		<0.00001*		<0.00001*		<0.00001*		0.0633		0.2547	
Season											
Summer	1335	19.1	18.3, 19.8	13.1	11.0, 15.4	49.6	46.6, 52.5	29.7	26.9, 32.6	7.6	6.0, 9.5
Autumn	1069	16.4	15.6, 17.2	19.8	16.7, 23.3	51.5	47.7, 55.1	25.7	22.6, 29.0	3.0	1.9, 4.3
Winter	1145	19.3	18.4, 20.3	13.6	11.4, 16.0	49.4	45.9, 52.8	24.7	21.9, 27.6	12.3	10.2, 14.7
Spring	1738	19.5	18.8, 20.3	14.0	12.0, 16.0	48.1	45.3, 50.8	26.5	24.2, 28.9	11.4	9.5, 13.6
<i>P</i>		<0.00001*		0.0004*		0.5235		0.0852		<0.00001*	
Location											
East/South East	2604	21.1	20.4, 21.9	10.5	8.9, 12.0	46.4	44.2, 48.5	30.1	28.2, 32.1	13.0	11.3, 14.8
South/South West	1398	16.2	15.7, 16.8	20.3	17.7, 22.9	52.2	49.4, 54.9	23.2	20.9, 25.7	4.3	3.3, 5.4
North and West	1088	17.0	16.3, 17.7	18.0	15.4, 20.6	52.7	49.1, 56.2	23.6	20.7, 26.8	5.7	4.3, 7.5
<i>P</i>		<0.0001*		<0.0001*		0.0008*		<0.0001*		<0.0001*	

*Statistically significant after adjustment for multiple comparisons ($P < 0.041$).

† P values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

This table has been published the paper *Laird et al., 2018*, please see **Appendix 11.1.2**

**Table 4.2. Mean plasma total vitamin B₁₂ with weighted prevalence of status by demographic and lifestyle characteristics†
(Weighted means and prevalence estimates and 95% confidence intervals)**

Characteristics	Subjects (n)	Vitamin B ₁₂									
		Average (pmol/l)	95% CI	<148 pmol/L (n 237) %	95% CI	148– <185 pmol/L (n 320) %	95% CI	185– <258 pmol/L (n 994) %	95% CI	>258 pmol/L (n 3627) %	95% CI
Supplements and/or B₁₂ injection	147	352.3	326.8, 378.3	2.9	1.0, 7.8	3.9	1.7, 8.5	14.7	9.7, 21.5	78.5	70.7, 84.5
No supplements or B₁₂ injection	5011	312.5	308.3, 316.4	5.1	4.4, 5.9	7.0	6.1, 7.8	19.6	18.4, 20.7	68.3	66.8, 69.7
<i>P</i>		0.0022*		<0.0001*		<0.0001*		<0.0001*		<0.0001*	
Sex											
Male	2428	303.4	298.5, 308.7	5.1	4.1, 6.2	7.5	6.3, 8.8	20.2	18.5, 22.0	67.2	65.0, 69.1
Female	2730	323.2	317.5, 329.0	5.0	4.1, 6.1	6.3	5.2, 7.5	18.7	17.1, 20.3	70.0	67.8, 71.8
<i>P</i>		<0.0001*		0.8516		0.3239		0.5619		0.0147*	
Age (years)											
50–59	2196	320.7	314.7, 326.5	4.0	3.0, 5.1	5.7	4.6, 6.9	17.9	16.2, 19.6	72.4	70.3, 74.4
60–69	1757	317.1	310.4, 324.0	5.4	4.2, 6.7	6.1	4.8, 7.6	19.7	17.8, 21.8	68.8	66.3, 71.1
70–79	929	299.2	290.0, 308.3	6.4	4.8, 8.5	8.9	6.9, 11.3	22.6	19.4, 25.9	62.1	58.2, 65.7
>80	276	297.2	281.2, 313.2	6.6	4.2, 10.1	11.1	7.7, 15.6	19.8	15.2, 25.2	62.5	56.3, 68.2
<i>P</i>		<0.0001*		0.0431		0.0013*		0.1011		<0.0001*	
Obesity											
BMI >30 kg/m²	1734	298.9	292.7, 305.5	5.9	4.6, 7.3	8.2	6.6, 9.9	21.6	19.6, 23.7	64.3	61.7, 66.7
BMI <30 kg/m²	3410	321.1	315.7, 326.1	4.7	3.8, 5.6	6.2	5.3, 7.2	18.3	16.8, 19.7	70.8	68.9, 72.5
<i>P</i>		<0.0001*		0.0053*		0.0032*		<0.0001*		<0.0001*	
Smoking status											
Smoker	803	296.8	287.3, 306.2	6.9	5.0, 9.4	7.3	5.5, 9.6	21.3	18.4, 24.4	64.5	60.7, 68.0
Non-smoker	4355	317.5	313.2, 322.2	4.7	3.9, 5.4	6.8	5.9, 7.7	19.0	17.7, 20.3	69.5	67.9, 71.0
<i>P</i>		0.0002*		<0.0001*		<0.0001*		<0.0001*		<0.0001*	
Alcohol consumption											
Alcohol consumer	3638	314.3	309.7, 319.3	4.8	4.0, 5.8	6.3	5.4, 7.2	19.0	17.5, 20.3	69.0	68.1, 71.6
Non-consumer	1103	315.7	306.6, 324.7	5.4	4.0, 7.1	8.1	6.3, 10.2	19.5	17.0, 22.2	67.0	63.7, 70.1
<i>P</i>		0.8346		<0.0001*		<0.0001*		<0.0001*		<0.0001*	

Physical activity											
Physically active	3637	312.5	305.2, 320.0	5.5	4.6, 6.5	6.1	5.2, 7.0	18.9	17.5, 20.2	69.5	67.8, 71.1
Not physically active	1479	313.9	309.0, 318.6	4.2	3.1, 5.5	8.6	7.0, 10.5	20.9	18.5, 23.3	66.3	63.4, 69.0
P	0.8000			<0.0001*		0.0008*		<0.0001*		<0.0001*	
Habitation											
Lives alone	988	304.8	295.4, 314.3	6.5	4.8, 8.7	7.8	6.1, 10.0	20.2	17.5, 23.0	65.5	62.0, 68.7
Does not live alone	4170	316.4	311.8, 320.7	4.6	3.9, 5.3	6.6	5.7, 7.5	19.2	17.9, 20.5	69.6	62.0, 68.7
P	0.0319*			<0.0001*		<0.0001*		<0.0001*		<0.0001*	
Asset wealth											
<Mean asset wealth	2622	309.0	303.4, 314.3	5.9	4.8, 6.9	7.2	6.1, 8.4	19.1	17.6, 20.5	67.8	65.7, 69.7
> Mean asset wealth	2270	320.0	314.7, 326.1	4.0	3.0, 4.9	6.4	5.1, 7.5	19.9	18.1, 21.7	69.7	67.6, 71.8
P	0.0080*			<0.0001*		0.0004*		0.0004*		<0.0001*	
Season											
Summer	1290	304.5	296.8, 312.2	5.2	4.0, 6.6	7.8	6.2, 9.7	22.4	19.8, 25.1	64.6	61.5, 67.6
Autumn	1057	311.5	302.4, 320.7	6.3	4.7, 8.2	8.4	6.6, 10.5	17.7	15.1, 20.5	67.6	64.4, 70.6
Winter	1113	317.9	309.4, 326.5	4.6	3.3, 6.1	5.7	4.3, 7.5	20.6	18.0, 23.3	69.1	65.8, 72.2
Spring	1695	318.9	312.5, 325.8	4.6	3.4, 6.0	6.0	4.7, 7.4	17.6	15.8, 19.6	71.8	69.4, 74.0
P	<0.0001*			0.3126		0.0520		0.0173*		0.0031*	
Location											
East/South East	2553	310.4	305.2, 316.1	5.5	4.4, 6.6	7.1	5.9, 8.4	19.5	17.9, 21.1	67.9	65.9, 69.9
South/South West	1562	311.5	304.5, 318.9	4.5	3.3, 5.8	7.0	5.6, 8.7	20.5	18.3, 22.9	68.0	65.2, 70.6
North and West	1043	324.0	314.7, 333.7	5.1	3.8, 6.8	6.1	4.7, 7.8	17.7	15.5, 20.1	71.1	67.8, 74.0
P	<0.0001*			0.4841		0.6132		0.2476		0.2387	

* Statistically significant after adjustment for multiple comparisons ($P < 0.041$).

† P values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

This table has been published the paper *Laird et al., 2018*, please see **Appendix 11.1.2**.

4.4.2. Regression analyses

The largest positive predictor of plasma folate concentration was FA supplement use (6.0 nmol/L; 95% CI 3.0, 9.0); $P<0.001$) followed by being female and statin medication use (**Table 4.3**). The largest negative predictor was geographic location (North, West and South v. East and South East; $P<0.001$) followed by data being sampled in the autumn, smoking and obesity.

In a multivariable linear regression analysis (**Table 4.4**) the largest positive predictor of total vitamin B₁₂ concentration was self-reported injection and/or supplement use (coefficient 51.5 pmol; 95% CI 9.4, 93.6; $P=0.016$). This was closely followed by being female and geographic location (North and West v. East and South East). The largest negative predictor was metformin medication use (−33.6; 95 % CI −51.9, −15.4; $P<0.001$). Other smaller negative predictors included smoking and obesity. FA supplement use was not a significant predictor of total vitamin B₁₂ concentration.

Table 4.3. Determinants of plasma folate concentrations (nmol/L) in older adults (Regression coefficients and 95% confidence intervals)

Characteristics	Coefficient	Linearized		95% CI
		SE	P	
Age (years)	0.35	0.29	0.237	−0.23, 0.93
Sex				
Female	2.04	0.43	<0.0001	1.19, 2.90
BMI				
Obese*	−2.16	0.47	<0.0001	−3.10, −1.23
Smoking status				
Current	−2.27	0.61	<0.0001	−3.49, −1.05
Physical activity				
Inactive†	−0.28	0.54	0.607	−1.35, 0.79
Folate supplement use				
User‡	6.02	1.53	<0.0001	3.01, 9.03
Season sampled				
Spring	−0.09	0.74	0.900	−1.54, 1.36
Summer	−1.37	0.72	0.059	−2.79, 0.05
Autumn	−4.81	0.69	<0.0001	−6.18, −3.44
Alcohol use				
Consumer	0.72	0.55	0.192	−0.36, 1.81
Living arrangement				
Alone	−0.33	0.66	0.616	−1.64, 0.97
Province				
Ulster and Connacht	−4.86	0.67	<0.0001	−6.17, −3.54
Munster	−5.70	0.55	<0.0001	−6.79, −4.61
Asset wealth				
> Mean asset wealth	1.59	0.52	0.003	0.56, 2.63

Metformin use				
User	0.61	1.17	0.598	−1.68, 2.91
Statin use				
User	1.36	0.57	0.019	0.22, 2.49
PPI use				
User	−1.11	0.70	0.114	−2.50, 0.26
Thyroxine use				
User	0.90	0.92	0.332	−0.92, 2.72

PPI, proton pump inhibitors.

* Obesity = BMI >30 kg/m².

† Physical activity levels were defined by IPAQ categories.

‡ Folate-containing supplements (including multi-vitamins) were defined using the World Health Organization's Anatomical Therapeutic Chemical codes.

This table has been published the paper *Laird et al., 2018*, please see **Appendix 11.1.2**.

Table 4.4. Determinants of plasma total vitamin B₁₂ concentrations (pmol/L) in older adults (Regression coefficients and 95% confidence intervals)

Characteristics	Coefficient	Linearized		95% CI
		SE	P	
Age (years)	-0.94	0.25	<0.0001	-1.44, -0.45
Sex				
Female	17.91	4.13	<0.0001	9.80, 26.03
BMI				
Obese*	-21.70	4.33	<0.0001	-30.21, -13.20
Smoking status				
Current	-22.39	5.62	<0.0001	-33.43, -11.34
Physical activity				
Inactive†	0.69	5.72	0.584	-8.11, 14.39
B₁₂ injection/supplement use				
User‡	51.53	21.42	0.016	9.45, 93.61
Folate supplement use				
User‡	-20.59	16.92	0.224	-53.82, 12.63
Season sampled				
Spring	3.13	5.72	0.584	-8.11, 14.39
Summer	-9.10	6.31	0.150	-21.49, 3.29
Autumn	-1.46	6.51	0.822	-14.25, 11.31
Alcohol use				
Consumer	-7.04	5.18	0.175	-17.23, 3.13
Living arrangement				
Alone	7.17	5.65	0.205	-3.93, 18.28
Province				

Ulster and Connacht	15.60	5.85	0.008	4.10, 27.11
Munster	3.15	4.86	0.517	−6.93, 12.69
Asset wealth				
> Mean asset wealth	8.89	4.35	0.042	0.33, 17.45
Metformin use				
User	−33.69	9.29	<0.0001	−51.94, −15.43
Statin use				
User	3.46	4.52	0.444	−5.41, 12.34
PPI use				
User	6.48	6.05	0.285	−5.41, 18.37
Thyroxine use				
User	6.05	8.64	0.484	−10.93, 23.03

PPI, proton pump inhibitors.

* Obesity = BMI >30 kg/m².

† Physical activity levels were defined by IPAQ categories.

‡ Vitamin B₁₂ injections or vitamin B₁₂-containing supplements (including multi-vitamins) were defined using the World Health Organization's Anatomical Therapeutic Chemical codes.

This table has been published the paper *Laird et al., 2018*, please see **Appendix 11.1.2**

4.5. Discussion

To my knowledge, this study is the first to present prevalence data for folate and vitamin B₁₂ status and their determinants in a large representative sample of the older Irish population. In a country with a voluntary fortification policy, the estimated prevalence of folate deficiency/low folate status is 15% or one in seven people aged >50 years. These data are in stark contrast to countries with mandatory fortification (of enriched cereal grains or flour) such as the US where rates of low folate status (<10 nmol/L) have been reported in only 1.2% of those aged ≥60 years (post-fortification) (347). Such a high rate of low folate status is important given the implications of sub-optimal concentrations, particularly for older adults. Deficiencies in folate can result in impaired biosynthesis of DNA with clinical symptoms including the development of megaloblastic anaemia, alopecia, achromotrichia and neuropathy (348, 349). Longer-term, low folate status has been associated with higher rates of cognitive impairment, CVD and cancer mortality (210, 350). For instance, in the US National Health and Nutritional Examination Survey (NHANES) (1999–2010), women with folate concentrations of approximately 20 nmol/L had a 1.5-fold increased all-cause mortality risk in comparison with those >20 nmol/L folate concentrations (350). In terms of vitamin B₁₂, the estimated prevalence of deficiency and low status (<185 pmol/L) in older adults in Ireland is 12% or one in eight. This data are similar to observations across other older adult surveys (aged >60 years). In NHANES (1999–2002), the prevalence of vitamin B₁₂ deficiency (<148 pmol/L) was about three percent, whereas seven percent had a low status (<185 pmol/L) (195). In three combined UK surveys (*n* 3,511), one in twenty people (aged 65–74 years) and one in ten people (aged >75 years) had vitamin B₁₂ deficiency (defined as <150 pmol/L) (193, 351). These observed high rates of vitamin B₁₂ deficiency in older adults are of concern given that early diagnosis is important to avoid irreversible neurologic damage. It is noteworthy that metformin therapy has been shown to negatively affect vitamin B₁₂ status, and it was identified as the strongest negative predictor of B₁₂ status in this study. Additionally, metformin may influence folate status by inhibiting its absorption in the gut. However, the current study did not find significant associations between metformin use and folate levels.

Both folate and vitamin B₁₂ status have been examined previously in a much smaller, representative study of older Irish adults (*n* 358) (194). TILDA data for folate are somewhat lower than those observed in the National Adult Nutrition Study (NANS), which may be explained by using the matrix of plasma instead of serum (352). Interestingly, the plasma folate data from TILDA were closely aligned with a study from Northern Ireland (*n* 662) where the folate concentrations of low-medium consumers of FA fortified foods were similar (16.2–22.6 nmol/L) (353). In NANS, the average vitamin B₁₂ concentration for adults >50 years was 281 pmol/L, which is close to the value of 308 pmol/L observed in the current study. However, unlike the NANS or Hoey *et al.* study, which were both limited to a small sample number and a narrow range of potential determinants, use of the TILDA data allowed for the examination of a wide range of possible health and lifestyle determinants. We observed that the oldest old (>80 years) had the highest rates of both low folate status and B₁₂ deficiency (23.1 and 6.6%, respectively). Older adults can be at a particular risk of deficiency owing to increased malabsorption from atrophic gastritis, infection or medication interactions (339). Interestingly, we also observed that men and those with a lower asset wealth also had lower folate and vitamin B₁₂ concentrations. It has been reported that men often display a lower B-vitamin status than women (194, 354), which in some studies has been attributed to a higher intake of supplements (which also is a factor in the current study). In addition, it has been observed that older men tend to have a poorer awareness of healthy diet recommendations compared with women who were also more likely to have healthier dietary patterns (92, 355). Few studies have examined measures of economic wealth and B-vitamins. Pfeiffer *et al.* (354) in NHANES (2003–2006) reported that those with a lower poverty income ratio had a significantly lower serum folate concentration. Similar dietary mechanisms may explain the decrease in B-vitamin status with age and lower asset wealth including compromised food quantity, quality and variety and lower fruit and vegetables intakes in those with lower incomes (92, 356).

Obesity and smoking were also observed as risk factors for lower B-vitamin status in the current study. Obesity has been associated previously with higher erythrocyte folate and lower serum folate in 3,767 adults from NHANES. The suggested mechanisms included volumetric dilution, lower folate intakes, lower folate intestinal

absorption and higher folate uptake by red blood cells (357). This is of concern for Ireland, as over half of the over 50s population are classified as centrally obese, with only 16 % of men and 26% of women having a normal BMI (358). Smoking has also been associated with lower B-vitamin concentrations in both the British National Diet and Nutrition Survey and in the US NHANES (2003–2006) (354, 359). It has been hypothesised that NO or hydrogen sulphide could oxidise or compete for the vitamin B₁₂ cobalt atom, whereas tetrahydrofolates could react with cyanates to form biologically inactive derivatives (360).

Uniquely, we were also able to examine the associations with geographic location and with seasonality. We observed that plasma concentrations of folate were lowest in autumn and concentrations of total vitamin B₁₂ were lowest in summer compared with winter. Some (361), but not all studies (362, 363) examining folate concentrations after exposure to UV light from sun-bed use or outdoor exposure have observed a lower status. For example, in a small study of forty-five Australian women (aged 18–47 years), UV exposure appeared to reduce the effectiveness of FA supplementation (364). Other studies have also observed that UV exposure may affect vitamin B₁₂ status. In a cohort of 1,013 older Chileans, solar radiation was associated with vitamin B₁₂ deficiency (365). Similar mechanisms for both folate and vitamin B₁₂ have been proposed including direct and indirect photo-degradation by UV radiation and by the formation of free radicals (366, 367). These observations may not only be important for B-vitamin status but also for other micronutrients as UV exposure is the main determinant of vitamin D status. However, geographic variations in vitamin levels may simply reflect underlying socioeconomic factors rather than purely environmental influences. Regions with lower socioeconomic status, such as the North and West of Ireland, often face challenges like reduced food security and limited access to diverse, nutrient-rich foods, which can contribute to lower folate and vitamin B₁₂ levels. These areas might have higher rates of economic deprivation, which can limit individuals' ability to access adequate nutrition and healthcare, thereby impacting vitamin status. Additionally, disparities in dietary patterns and supplement use across different socioeconomic groups may also play a significant role. Therefore, while seasonality and UV exposure could be important factors, socioeconomic disparities likely contribute substantially to the observed geographic differences in vitamin levels.

It is important to note that FA supplement use was not a contributor to vitamin B₁₂ concentrations or status in the TILDA population, as there have been some concerns that high folate status could exacerbate the clinical effects of vitamin B₁₂ deficiency (368). Within TILDA, 8.9 % had a folate concentration >45 nmol/L. This is lower than the frequency observed either in NANS (19 %) (194) or in post-fortification NHANES in the USA (38 %) (369). The same methodology was used in all three studies and the folate measurements for this study and for NANS were carried out in the same laboratory. It is unclear what the clinical implications are of high folate status. Previously, high folate concentrations have been correlated with unmetabolized FA in plasma (370), which has been suggested to be a driver of some adverse health effects (370). In a cross-sectional study of 219 older adults (>65 years), higher folate concentrations were associated with lower brain volumes (371), whereas a higher cancer incidence was reported in the folate and B₁₂ intervention group of the B-PROOF study of older adults, with the effect on cancer incidence more pronounced in the oldest old (>80 years) (372). FA has been suggested to be a preventive agent for cancer initiation but a promoter of established neoplastic cells (276). However, this effect is still not clear as higher levels of folate have been associated with a lower risk of lung cancer diagnosis in a case-control study of 366 adults (373) whereas lower folate status in comparison with high status has been associated with a higher risk of all-cause mortality (350).

The major strength of this study is that it is the largest nationally representative older population sample reported with detailed data on a wide range of potential determinants. A limitation is that no dietary intake data were available including the consumption of fortified food products or the dosage of B-vitamin supplements taken. However, previous studies have shown a weak relationship between intake and blood biomarker data for these micronutrients (194, 353). One recent study has indicated that dietary intake data and biomarkers reflect the same underlying folate status with a systematic underestimation bias in intake data (374). Furthermore, this study was limited to the use of plasma folate and total vitamin B₁₂ as indicators of status with the corresponding benefits and drawbacks associated with each of these biomarkers (i.e. plasma folate is subject to variations from recent dietary intake, while total vitamin B₁₂ measurement in blood is known to have relatively low sensitivity for estimating vitamin B₁₂ deficiency (375)). It is also important to note

that because of the recruitment sampling criteria these findings may not be applicable to non-Caucasians, older adults in institutional care or those with severe cognitive impairment.

In summary, we have observed high rates of low folate and vitamin B₁₂ status coupled with a much smaller rate of high folate status in the older population of Ireland. Ideally, encouraging older persons to improve their normal eating practices and to follow the FP has the potential to enhance the folate and vitamin B₁₂ status of this population group. However, compliance to healthy eating recommendations, as well as practical issues such as cooking practices, bioavailability, medication intakes and age-related changes to the gastrointestinal tract, means that the holistic unfortified approach can be ineffectual. TILDA data (and others previously) indicate that the current policy of voluntary fortification is ineffective at preventing the occurrence of deficiency and low blood status. Serious consideration must now be given to the implementation of mandatory FA and vitamin B₁₂ fortification. Such a policy would ensure that the requirements of the ‘at-risk’ older adult population are met while also helping to reduce the prevalence of folate-responsive neural tube defects in the unborn child. The continued delay in consideration of this implementation could put >180,000 and 145, 000 older adults in Ireland at risk of the health consequences of low folate and vitamin B₁₂ status, respectively.

CHAPTER FIVE: PAPER 3 - PLASMA CONCENTRATIONS OF FOLATE AND VITAMIN B₁₂ AND GLOBAL COGNITIVE FUNCTION IN AN OLDER POPULATION: CROSS-SECTIONAL FINDINGS FROM THE IRISH LONGITUDINAL STUDY ON AGEING

Introduction to Chapter Five

In this chapter, I address **Aim 3** and **Objectives 3.2** and **3.3** of the thesis. The hypothesis that a low vitamin B₁₂ status, coupled with high blood concentrations of folate does not confer greater risk to global cognitive performance in this older population group, is explored.

Using cross-sectional analyses from Wave 1 of TILDA, this chapter aimed to characterise the population of community dwelling adults in Ireland ≥50 years by variations of folate and vitamin B₁₂ concentrations, and to estimate the prevalence of those groups. The associations of these combinations of folate and vitamin B₁₂ status and global cognitive function could then be established. The following data is from the third publish work from my thesis, “*Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA).*” (**Appendix 11.1.3**).

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Figure 5.3. Associations of vitamin B₁₂ and folate status and global cognition (MoCA) – aged ≥ 50 years and ≥ 60 years.

Figure 5.4. Associations between concentrations of folate (continuous) and quintiles of vitamin B₁₂ and global cognition - aged ≥ 60 years.

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Supplementary Table 5.1. Additional sample characteristics, by vitamin B₁₂ and folate groups

Supplementary Table 5.2. Selected sample characteristics, by vitamin B₁₂ and folate groups – stratified by age

Supplementary Table 5.3. Associations of concentrations of vitamin B₁₂ and folate status with global cognition (MMSE & MoCA).

Supplementary Table 5.4. Multivariable associations of concentrations of vitamin B₁₂ and folate status with measures of global cognition (MMSE and MoCA), aged ≥ 50 years.

Supplementary Table 5.5. Multivariable associations vitamin B₁₂ and folate groups with measures of global cognition (MMSE and MoCA), aged ≥ 60 years.

Supplementary Table 5.6. Sensitivity Analysis: Associations of vitamin B₁₂ and folate groups with global cognition (MMSE & MoCA).

Supplementary Table 5.7. Associations between concentrations of folate (continuous) and quintiles of vitamin B₁₂ with global cognition in those aged ≥ 60 years.

Supplementary Table 5.8. Associations between concentrations of vitamin B₁₂ (continuous) and quintiles of folate with global cognition in those aged ≥ 60 years.

Published Sections

5.1 Abstract

5.2. Introduction

5.3. Methods

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5.5. Results

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5.1. Abstract

Introduction

The uncertainty surrounding high intakes of FA and associations with cognitive decline in older adults with low vitamin B₁₂ status has been an obstacle to MFAF for many years.

Methods

We estimated the prevalence of combinations of low/normal/high vitamin B₁₂ and folate status and compared associations with global cognitive function using two approaches of individuals in a population-based study of those aged ≥ 50 years in the Republic of Ireland. Cross-sectional data from 3,781 men and women from Wave 1 of TILDA were analysed. Global cognitive function was assessed by Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

Prevalence estimates for combinations of vitamin B₁₂ (plasma vitamin B₁₂ $<$ or ≥ 258 pmol/L) and folate (plasma folate $\leq$ or > 45.3 nmol/L) concentrations were generated. Negative binomial regression models were used to investigate the associations of vitamin B₁₂ and folate status with global cognitive function.

Results

1.5% (n 51) had low vitamin B₁₂ (< 258 pmol/L) and high folate (> 45.3 nmol/L) status. Global cognitive performance was not significantly reduced in these individuals when compared to those with normal status for both B-vitamins (n 2,433). Those with *normal B₁₂/high folate* status (7.6%) had better cognitive performance (MMSE: incidence rate ratio (IRR) 0.82, 95% CI [0.68-0.99]; $P=0.043$, MoCA: IRR 0.89 [0.80-0.99]; $P=0.025$).

Conclusions

We demonstrated that high folate status was not associated with lower cognitive scores in older adults with low vitamin B₁₂ status. These findings provide important safety information that could guide fortification policy recommendations in Europe.

5.2. Introduction

Cognitive impairment has been associated with low vitamin B₁₂ (212) and low folate status (217). In addition, some previous observational data have suggested that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia and cognitive impairment or decline (228, 229, 230), however, the causal relevance of these associations remains uncertain.

Vitamin B₁₂ and folate participate in one carbon metabolism, critical for the formation of DNA and ribonucleic acid (RNA), neurotransmitters, membrane lipids and proteins. Because of these functions, there has been growing interest in the possibility that age- related conditions, in particular loss of cognitive function, may partly be explained by inadequate status of these vitamins (207, 208, 209, 210). Vitamin B₁₂ deficiency (plasma vitamin B₁₂ < 48 pmol/L) is associated with severe neurological consequences including sensory and motor neuropathy, altered mental status and cognitive decline (212). Vitamin B₁₂ deficiency also reduces the availability of folate for DNA synthesis (212).

Since the introduction of mandatory fortification of staple foods with FA to reduce neural tube effects (NTD), high folate status (>45.3 nmol/L) is frequently observed in countries such as the United States (US) and Canada. Recent research has raised concerns regarding the adverse implications of simultaneous low vitamin B₁₂, and high folate status given that high-dose FA treatment was shown to temporarily mask clinical symptoms in persons with pernicious anaemia (231).

While some studies have reported that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia, cognitive impairment and decline (228, 229, 230), other large studies conducted in diverse populations have not replicated such adverse associations (232, 233, 234). There is considerable heterogeneity among the published studies, including differences in vitamin status cut-offs, biochemical methodologies and the instruments used to assess cognitive function (259). Likewise, large-scale randomised controlled trials have failed to demonstrate any beneficial effects of supplementation with vitamin B₁₂ or FA on global or domain specific cognitive function (376).

Ireland has one of the highest rates of NTD in Europe (377), and supplementation initiatives have been shown to be ineffective (378), yet one major obstacle impeding population-based food fortification is the concern that high folate status in the presence of low vitamin B₁₂ status could have a detrimental effect on cognitive function in older people. In this study adults aged 50 years and older from TILDA, the prevalence of older people with deficient or low vitamin B₁₂ status (<185 pmol/L) was 12%, while high folate status (>45.3 nmol/L) was observed in nine percent of the population (214). TILDA affords a unique opportunity to investigate whether there is an association between cognitive performance and variations of vitamin B₁₂ and folate in a large, nationally representative population sample of older adults. I hypothesise that a low vitamin B₁₂ status, coupled with high blood concentrations of folate does not confer greater risk to global cognitive performance in this older population group.

5.3. Methods

5.3.1. Study population

Cross-sectional data from Wave 1 of TILDA were analysed. TILDA is a nationally representative prospective cohort study on ageing of community-dwelling adults aged 50 and older, living in the Republic of Ireland (ROI). Details of the study design have been reported previously (310, 311, 379). Briefly, Wave 1 took place between October

2009 and February 2011. 8,174 adults aged ≥ 50 years from 6,279 households completed a computer-assisted personal interview (CAPI), representing a response rate of 62%. Of the total sample, 84.6% completed the self-completion questionnaire (SCQ) (n 7,196) and 72.1% (n 5,895) participated in a health assessment which included blood measurements. Plasma vitamin B₁₂ measurements were available for 88.5% (n 5,219) and plasma folate for 90.7% (n 5,350) of participants. Missing data were due to insufficient sample and / or assay failures. Individuals who did not complete both standardised assessments of cognitive function, the Mini Mental State Examination (MMSE) (318) and Montreal Cognitive Assessment (MoCA) (319), were not included in this analysis, giving a final analytical sample of 3,871 (**Figure 5.1.**). Ethical approval was obtained from the Faculty of Health Sciences Research

Ethics Committee of Trinity College Dublin. All participants were fully informed of the study and gave written consent.

5.3.2. *Assessment of cognitive function.*

Global cognitive function was assessed using the MMSE and MoCA. The MMSE is a brief 30-point test that is used to screen for cognitive impairment, commonly used in clinical practice to screen for dementia and assesses orientation, recall, attention, calculation, language abilities and visuospatial ability. The MoCA, also used to screen for dementia, is particularly sensitive to mild cognitive deficits (319, 380), with additional assessments of executive function, abstraction, and visuospatial ability. Both tools have scores ranging from 0 to 30.

5.3.3. *Analysis of B-vitamins*

A non-fasting blood sample was collected by venepuncture into one 10ml K2EDTA tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10ml ethylene diamine tetra-acetic acid (EDTA; BD, Becton, Dickinson Limited) tubes for long term storage (Sarstedt; Numbrecht, Germany). Samples were kept chilled and were centrifuged (3000 rpm for 15 minutes). Aliquots of plasma were labelled and stored at -80°C within 48 hours of blood collection and stored until required for analysis. The TILDA protocol for blood sample collection, processing, and storage is detailed elsewhere (307). Plasma vitamin B₁₂ and folate concentrations were determined by microbiological assay; *Lactobacillus leichmannii* (*L. leichmannii* (*delbrueckii*) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/L (321) and *Lactobacillus casei* (*L.rhamnosis* (NCIB 10463; ATCC 27773)), limit of detection 0.6 nmol/L (320), respectively. Inter-assay coefficients of variability (CV) for both methods were < 10.9%.

5.3.4. *Sample Size and Power Calculation*

Due to the relatively low number of participants in the "at-risk" group (*low B₁₂/high folate*) in this study (n = 51), a post hoc power calculation was performed to assess the sample size required to detect a moderate effect size ($\Delta = 0.5$). Using a significance level of 5% ($\alpha = 0.05$) and 80% power ($1-\beta = 0.80$), the estimated sample size per group was determined using the formula:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \cdot 2\sigma^2}{\Delta^2}$$

where n is the required sample size per group, $Z_{\alpha/2}$ is the critical value corresponding to a 5% significance level (1.96), Z_{β} is the critical value for 80% power (0.84), σ represents the standard deviation of the outcome (assumed to be 1 in the absence of exact data), and Δ is the minimum detectable effect size (assumed to be 0.5, a typical moderate effect size in cognitive studies).

$$n = \frac{(1.96 + 0.84)^2 \cdot 2 \cdot 1^2}{0.5^2} = \frac{15.68}{0.25} = 62.72$$

Calculations indicate that approximately 63 participants per group would be required to achieve 80% power. Given the 51 participants in the *low B₁₂/high folate* group, the present study may be underpowered to detect moderate effect sizes, which suggests that findings should be interpreted with caution.

5.3.5. Other Covariates

Socio-demographic characteristics examined included age, gender, educational attainment, mean asset wealth and habitation status. Lifestyle characteristics comprised self-reported physical activity (International Physical Activity Questionnaire (IPAQ)), smoking status, alcohol consumption, depressive symptoms (Center for Epidemiologic Studies Depression Scale (CES-D)) (381) and history of stroke. Use of medications (up to 20 prescription and non-prescription medications, including food supplements) were classified by WHO ATC classification codes.

Anthropometric measurements included height, weight, BMI and handgrip strength (average two recordings from dominant hand using a dynamometer). Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg and/or antihypertensive medication use. Diabetes was defined as diabetes or prediabetes by measurement of glycated haemoglobin (HbA_{1c}) (382), self-reported diabetes diagnosis and/or use of anti-glycaemic medications.

5.3.6. Exclusion Criteria

Individuals without a health assessment and blood sample were excluded (n 3,248). Those for whom vitamin B₁₂ and / or folate concentrations could not be measured, or whose measurement values exceeded the 99th percentiles and participants in receipt of vitamin B₁₂ injections, were omitted from the analysis (n 450). To rule out participants with possible MCI, additional exclusionary criteria were applied to both MMSE and MoCA results. Those with MMSE scores of less than 24 (383) and MoCA below the age and education normative cut-offs of Rosetti *et al.*, (2011) (384) were also omitted (n 650).

5.4. Statistical Analysis

5.4.1. Descriptive Statistics

While there is no international consensus regarding the cut-offs for both vitamin B₁₂ and folate status, we adopted the widely used cut-offs identified in the literature. For plasma vitamin B₁₂ (pmol/L) these included: < 148 (deficient); 148 - < 185 (low status); 185 - < 258 (low/normal status); > 258 - > 601 (normal) and > 601 (high) (345, 346). For plasma folate (nmol/L), the cut-offs included: 10 – 23.0 (low/normal); > 23.0 - 45.3 (normal) > 45.3 (high) (233, 344). To compare with previous studies, a four-level categorical variable was created on the basis of vitamin B₁₂ (< or $\geq$ 258 pmol/L) (345, 346) and folate concentrations ($\leq$ or > 45.3 nmol/L) (195) and was defined as: (i) *normal B₁₂/normal folate* ($\geq$ 258 pmol/L, $\leq$ 45.3 nmol/L), (ii) *normal B₁₂/high folate* ($\geq$ 258 pmol/L, > 45.3 nmol/L), (iii) *low B₁₂/high folate* (< 258 pmol/L, > 45.3 nmol/L), and (iv) *low B₁₂/normal folate* (< 258 pmol/L, $\leq$ 45.3 nmol/L).

Non-normally distributed continuous variables were described as medians and interquartile ranges (IQR) for each group, stratified by sample characteristics, biomarkers, and cognitive tests. These were compared using Kruskal-Wallis and Mann–Whitney U tests for pairwise comparisons. Categorical variables were compared using Chi-squared tests or Fisher's exact tests, as appropriate. A false-discovery rate (FDR) correction for multiple comparisons was applied ($P < 0.013$, achieving FDR $q < 0.05$) to adjust for the number of individual tests carried out (i.e.,

q indicates that the average alpha level across the family of tests does not exceed 0.05, following correction).

5.4.2. Regression Analyses with Global Cognitive Function as Outcome Variable

Associations of vitamin B₁₂ and folate with cognitive function and other covariates were explored in a series of regression analyses. The relationship between cognitive function and groups of vitamins B₁₂ and folate status was analysed with models appropriate for the distribution of the outcome variables, MMSE (continuous) and MoCA (continuous). Visualisation of the data and detailed inspection of model residuals demonstrated that neither outcome was normally distributed after appropriate removal of outliers.

Therefore, MMSE and MoCA were analysed using negative binomial regression models (due to overdispersion) with the outcome representing the count of errors made during the test (30 minus the raw score) due to their left skewed distributions, consistent with previous reports using TILDA data (385).

The results of these analyses are presented as the incidence rate ratio (IRR) for each model, relative to the reference category, *normal B₁₂/normal folate*, as previously performed in this cohort (386). For both cognitive tests, three models were investigated: unadjusted (Model 0); adjusted for socio-demographic covariates - age, sex, educational attainment, mean asset wealth and habitation status (Model 1) and adjusted for socio-demographic, lifestyle, health indicators and medications - physical activity, smoking, alcohol consumption, BMI, mean grip strength, hypertension, diabetes, depressive symptoms, FA supplement use, PPI, statin and thyroid medication use and history of previous stroke (Model 2).

These analyses were then repeated in the sample aged ≥ 60 years ($n = 2,070$), for more direct comparison with other studies in this field. Further, a sensitivity analysis was performed including the participants with lower cognitive scores, aged ≥ 50 years and ≥ 60 years, omitted from the main analysis.

Additionally, quintiles of vitamin B₁₂ concentrations were created based on plasma vitamin B₁₂ concentrations in those aged ≥ 60 years. An interaction term was

included to examine whether the association between plasma folate (as a continuous variable) differed across quintiles of vitamin B₁₂, using the highest quintile of vitamin B₁₂/folate (Q5) as the reference category (the reverse instantiation was also analysed, i.e., continuous vitamin B₁₂ with quintiles of folate).

Population weights were calculated for the regression analyses by comparing the TILDA population with the 2011 Irish Census with respect to age, sex, educational attainment, marital status, and urban versus rural residence (weights reflected the reciprocal of the selection probability). Prevalence estimates, descriptive statistics and model coefficients were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether a blood sample was provided. The staged, stratified, and clustered sample design of TILDA was considered when estimating confidence intervals and standard errors. Analyses were carried out using Stata 15 (StataCorp, College Station, TX, USA).

5.5. Results

5.5.1. Baseline Characteristics of the Study Population

The study design and exclusion criteria are detailed in **Figure 5.1**. The mean (SD) age was 61.8 years (8.8) (range 50 - 98 years); 50.7% were female (*n* 3,871). Median (IQR) MMSE and MoCA scores were 29 (28 - 30) and 26 (24 - 28), respectively. Vitamin B₁₂ concentrations ranged from 45.4 – 719 pmol/L, with 4.8% [95% CI: 4.1 – 5.7] classified as deficient (< 148 pmol/L) and 25.5% [24.1 – 27.0] with low vitamin B₁₂ status (148 – 258 pmol/L). Folate concentrations were 0.9 – 82 nmol/L, 5.7% [4.9 – 6.7] had high folate status [45.3 – 60 nmol/L], with an additional 3.3% [2.8 – 4.0] classified as very high folate status (> 60 nmol/L), similar to rates observed in the TILDA population (214). The prevalence of *normal B₁₂/normal folate* status was 62% [60.2 - 63.8], *normal B₁₂/high folate* was 7.6% [6.6 - 8.8], *low B₁₂/high folate* was 1.5% [1.1 - 2.0] and *low B₁₂/normal folate* was 28.9% [27.3 – 30.6].

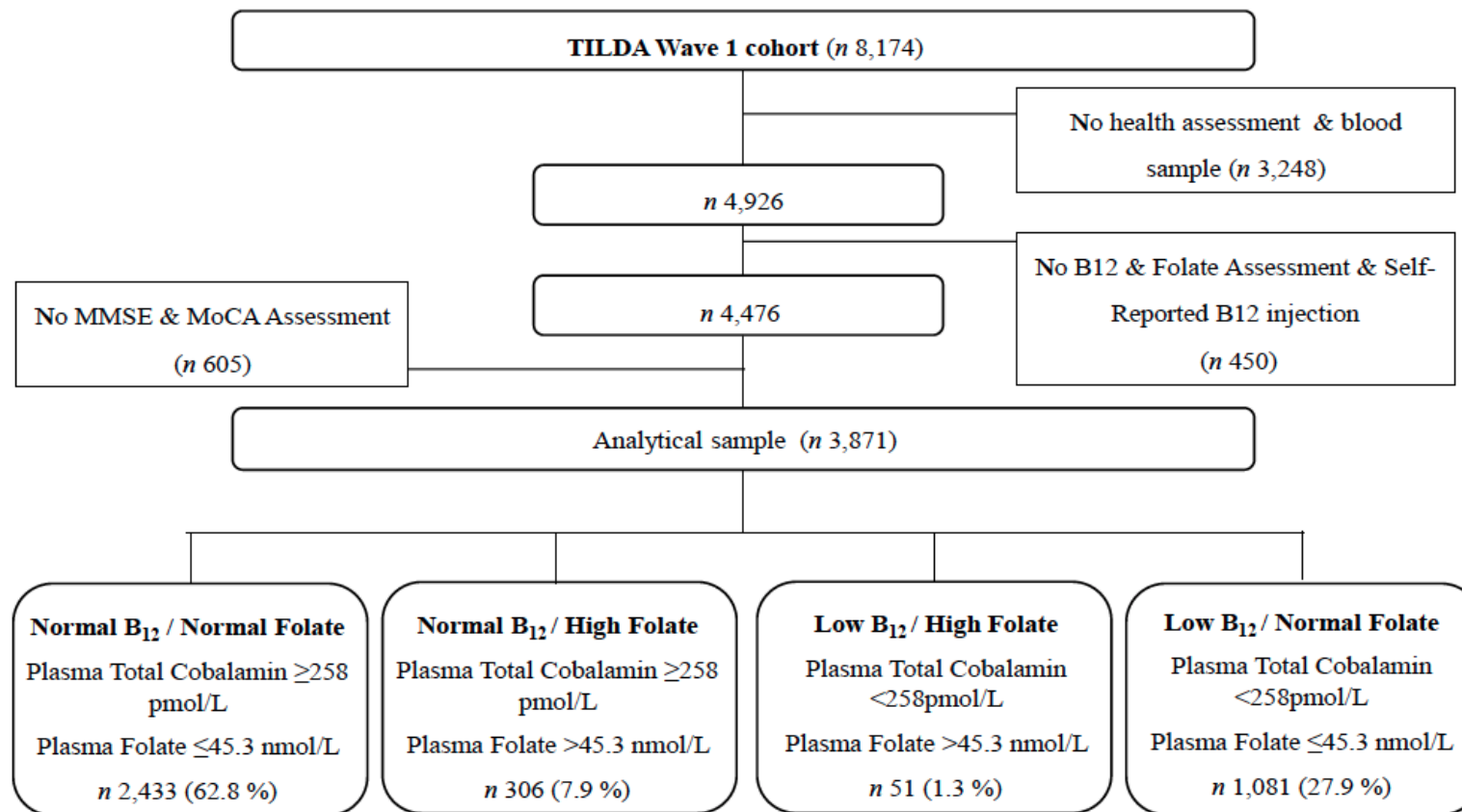


Figure 5.1. Flow Diagram – Analytical sample design and selection.

This figure has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

5.5.2. Characteristics of the Study Population, by vitamin B₁₂ and folate groups

Sample characteristics, stratified by vitamin B₁₂ and folate groups are detailed in **Table 5.1**, with additional sample characteristics displayed in **Supplementary Table 5.1**. All groups were older than the reference category, *normal B₁₂/normal folate*, and were more likely to be hypertensive. When compared to the reference category, subjects with *normal B₁₂/high folate* were more likely to be female, have above average household wealth, lower mean grip strength and BMI and were less likely to be categorised as obese. This group was also more likely to report taking a FA supplement and had better global cognitive scores than the reference group. Those with *low B₁₂/high folate* status were more likely to be obese, have above average household wealth and be hypertensive and diabetic relative to the reference category. Subjects with *low B₁₂/normal folate* status had lower levels of educational attainment, higher overall BMI and were more likely to smoke and to be obese, hypertensive, diabetic and thus to use metformin (**Supplementary Table 5.1**). These groups were further examined by age- group (**Supplementary Table 5.2**), though no additional relevant significant findings were observed.

Table 5.1. Basic sample characteristics, grouped by concentrations of vitamin B₁₂ and folate

		Vitamin B ₁₂ ≥ 258 pmol/L		Vitamin B ₁₂ < 258pmol/L		P for comparison across all groups
		Folate ≤ 45.3 nmol/L 'Normal B ₁₂ / Normal Folate' (n 2,433)	Folate > 45.3 nmol/L 'Normal B ₁₂ / High Folate' (n 306)	Folate > 45.3 nmol/L 'Low B ₁₂ / High Folate' (n 51)	Folate ≤ 45.3 nmol/L 'Low B ₁₂ / Normal Folate' (n 1,081)	
n (%)	3,871					
Age (years)	3,871	60 (55, 67) ¹	62 (56, 68)	66 (57, 73)	61 (55, 68)	0.0001
P		Reference	0.0050*	0.0015*	0.0105*	
Over 80 years [n (%)]	95/3,871	52 (2.1)	5 (1.6)	4 (7.8)	34 (3.2)	0.017
P		Reference	0.5204	0.1347	0.0967	
Females	2,045/3,871	1,292 (53.1)	198 (64.7)	23 (45.1)	531 (49.1)	0.0000
P		Reference	0.0001*	0.2602	0.0294	
Obese [n (%)]	1,274/3,866	777 (31.9)	72 (23.5)	24 (47.1)	401(37.1)	0.0000
P		Reference	0.0013*	0.0338*	0.0032*	
Vitamin B12 (pmol/L)	3,871	361.3 (308.7, 435.2)	421.0 (344.7, 514.0)	214.2 (160.1, 241.3)	205.9 (170.3, 235.4)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Folate (nmol/L)	3,871	18.8 (13.1, 26.9)	56.4 (50.0, 65.5)	55.4 (49.1, 61.5)	14.7 (10.7, 22.0)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Folic Acid Supplement Use [n (%)]	123/3,871	68 (2.8)	22 (7.2)	7 (13.7)	26 (2.4)	0.0000
P		Reference	0.0038*	0.0251	0.4969	
MMSE (Global Cognition)	3,871	29 (28.0, 30.0)	29 (29.0, 30.0)	29 (28.0, 30.0)	29 (28.0, 30.0)	0.0170
P		Reference	0.0003*	0.9448	0.7175	
MoCA (Global Cognition)	3,871	26 (24.0, 28.0)	26 (25.0, 28.0)	26 (24.0, 27.0)	26 (24.0, 28.0)	0.0054
P		Reference	0.0243*	0.5625	0.0338	

¹ Non-normally distributed continuous variables were described as medians and interquartile ranges (IQR) (all such values), and compared using Kruskal-Wallis test as appropriate, and Mann–Whitney U test for pairwise comparisons with the reference category. Categorical variables were compared using the chi-square tests of Fisher's exact test, as appropriate

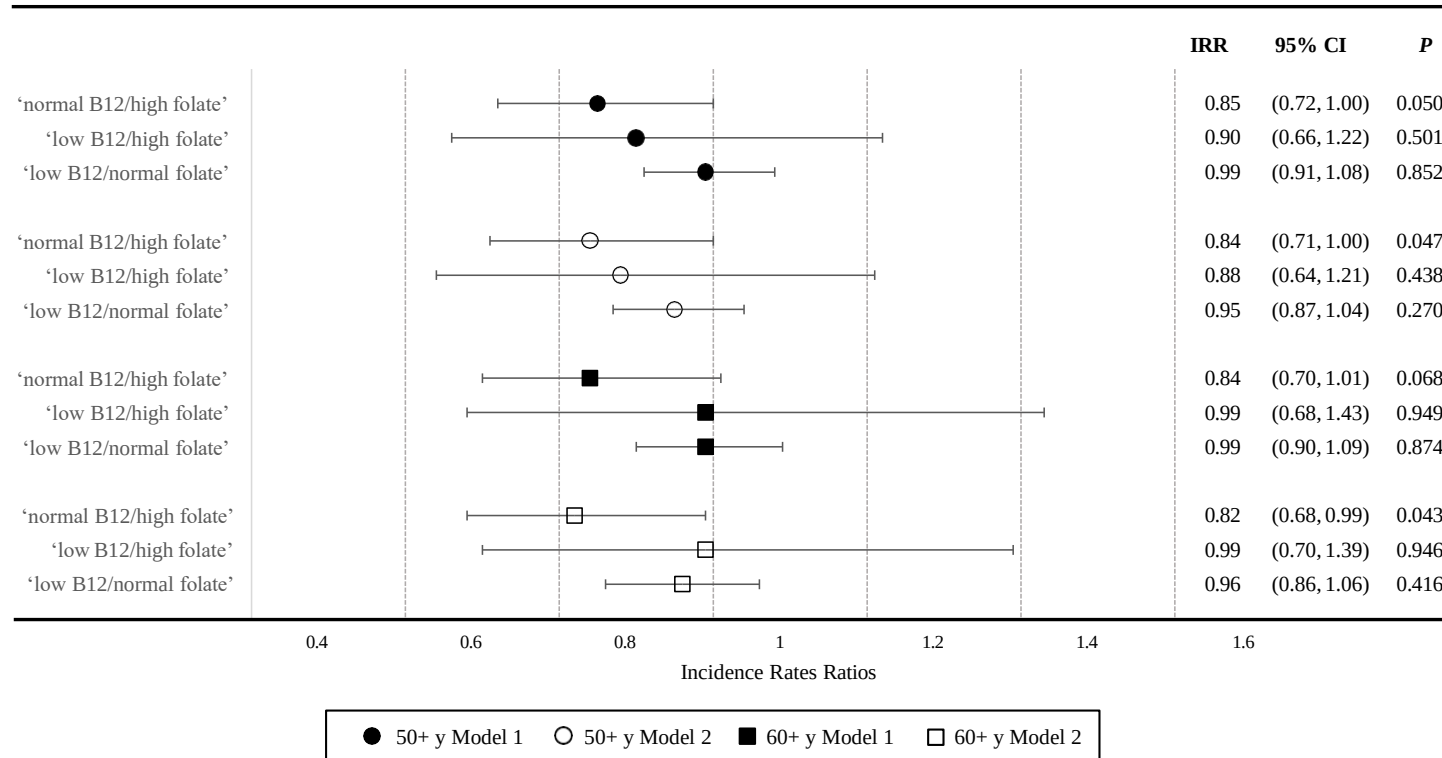
*Statistically significant after adjustment for multiple comparisons (value < 0.013)

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

5.5.3. Associations with Combinations of Vitamin B₁₂ and Folate Status

Figure 5.2 and **Supplementary Table 5.3** display the associations between the groups of vitamin B₁₂ and folate status in those aged ≥ 50 and ≥ 60 years for MMSE. Relative to *normal B₁₂/normal folate*, having *low B₁₂/high folate* was not associated with a significant increase in MMSE error rates for Models 0, 1 or 2. Further, for all three models there were statistically significant decreases in MMSE error rates for those in the *normal B₁₂/high folate* group in both the ≥ 50 and ≥ 60 years groups (Model 2: 0.84 [0.71 - 1.00]; $P = 0.047$ and 0.82 [0.68 - 0.99]; $P = 0.043$, respectively). **Figure 5.3.** and **Supplementary Table 5.3** show the associations for MoCA. Similarly, *low B₁₂/high folate* status was not associated with increases in error rates in the three models tested. However, having *normal B₁₂/high folate* was associated with significant decreases in MOCA error rates for Models 0, 1 and 2, respectively, in those aged ≥ 50 years (0.91 [0.83 - 0.99]; $P = 0.031$, 0.92 [0.85 - 1.00]; $P = 0.040$ and 0.91 [0.85 - 1.00]; $P = 0.042$) and ≥ 60 years (0.87 [0.78 - 0.97]; $P = 0.014$, 0.91 [0.83 - 1.00]; $P = 0.044$ and 0.89 [0.80 - 1.00]; $P = 0.025$). Results of the full multivariable analyses for Models 1 and 2 are presented in **Supplementary Tables 5.4 and 5.5**. Sensitivity analysis indicated that those in the *low B₁₂/high folate* groups for each model and in the subgroup aged ≥ 60 years, were not more likely to make errors with the MMSE or MoCA compared to those within the reference category (**Supplementary Table 5.6**).

MMSE

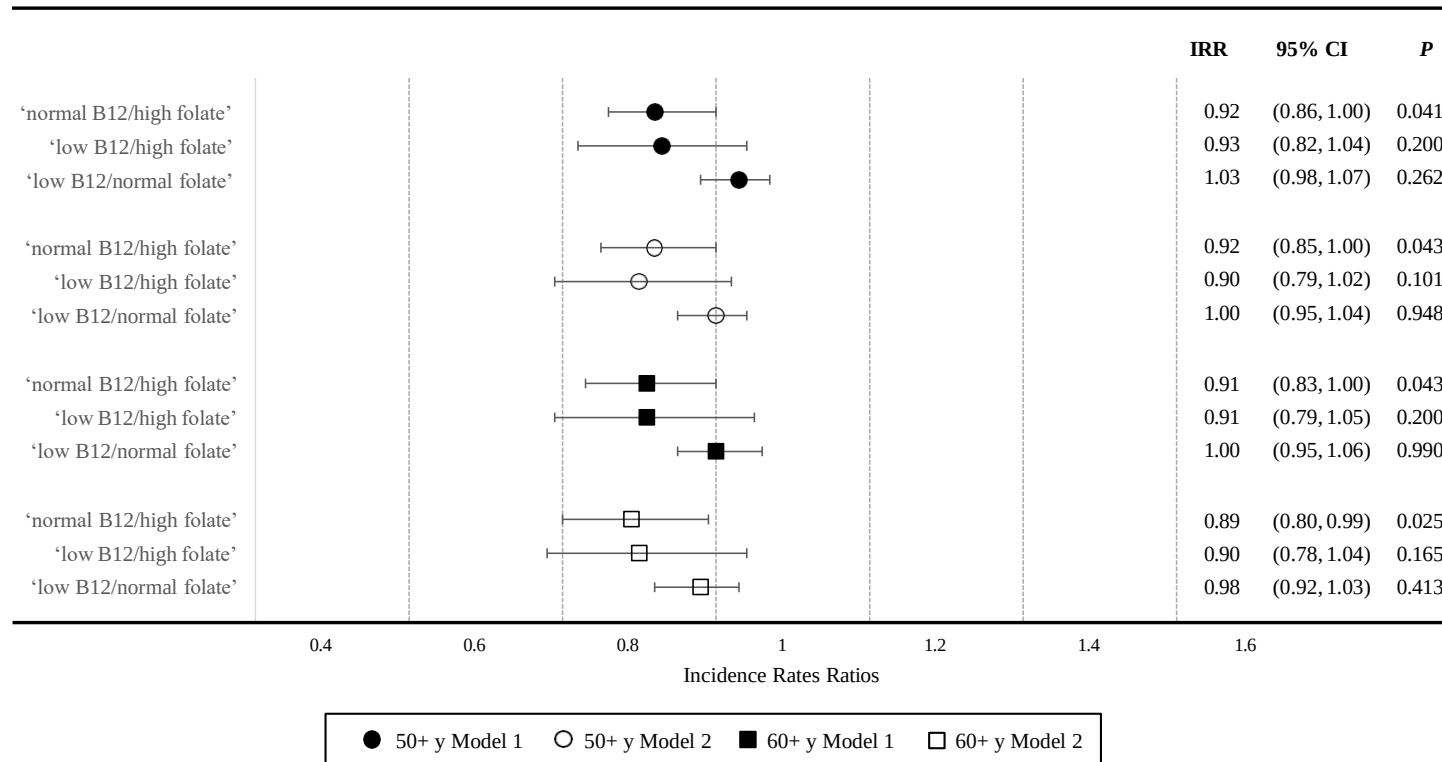


● Aged ≥ 50 years Model 1 (Socio-demographic) (MMSE error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status);
 ○ Aged ≥ 50 years Model 2 (Socio-demographic, Lifestyle, Health Indicators & Medications) (Model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status & depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke)
 ■ Aged ≥ 60 years Model 1 (Socio-demographic) (MMSE error rate and B₁₂ and folate groups controls for age, sex, educational attainment, mean asset wealth and habitation status);
 □ Aged ≥ 60 years Model 2 (Socio-demographic, Lifestyle, Health Indicators and Medications) (Model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

Figure 5.2. Associations of vitamin B₁₂ and folate status and global cognition (MMSE) – aged ≥ 50 years and ≥ 60 years (incidence rate ratios; IRR and 95% CIs).

This figure has been published in the paper O'Connor et al., 2020, please see Appendix 11.1.3.

MoCA



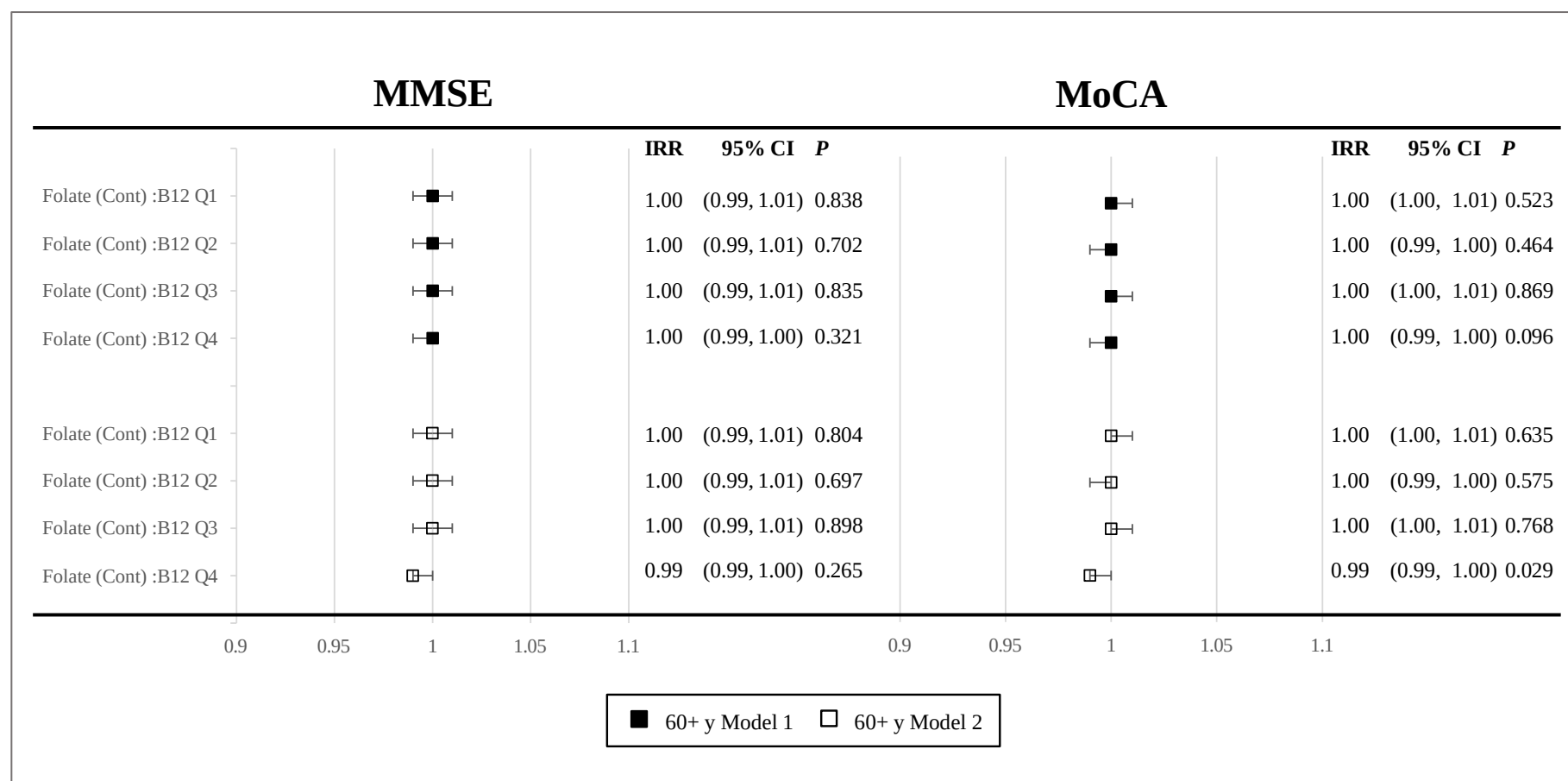
● Aged ≥ 50 years Model 1 (Socio-demographic) (MoCA error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status);
○ Aged ≥ 50 years Model 2 (Socio-demographic, Lifestyle, Health Indicators & Medications) (Model 1 + controls for physical activity, smoking status & alcohol consumption, BMI, grip strength, hypertension status, diabetes status & depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke)
■ Aged ≥ 60 years Model 1 (Socio-demographic) (MoCA error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status);
□ Aged ≥ 60 years Model 2 (Socio-demographic, Lifestyle, Health Indicators & Medications) (Model 1 + controls for physical activity, smoking status & alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

Figure 5.3. Associations of vitamin B₁₂ and folate status and global cognition (MoCA) – aged ≥50 years and ≥60 years (incidence rate ratios; IRR and 95% CIs).

This figure has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

5.5.4. Associations with Quintiles of Vitamin B₁₂ and Continuous Folate Concentrations

Additional models, in subjects aged ≥ 60 years, examined folate concentrations on a continuum, interacted with quintiles (Q1 - Q5) of vitamin B₁₂ (Q1 vitamin B₁₂, 45.4 - 225 pmol/L (n 409); Q2 225 – 289 pmol/L (n 427); Q3 289 – 350 pmol/L (n 408); Q4 350 - 428 pmol/L (n 415); Q5 428 – 719 pmol/L (n 412)). These demonstrated there was no evidence of interactions with respect to MMSE and MoCA error rates (**Figure 5.4**). For MoCA, Model 2 revealed a small yet statistically significant decreases in the rate of errors for those with the highest vitamin B₁₂ concentrations (**Supplementary Table 5.7**). The reverse instantiation was also tested (i.e., vitamin B₁₂ as the continuous variable, interacted with quintiles of folate). This analysis showed no association of error rates with decreasing concentrations of vitamin B₁₂ (**Supplementary Table 5.8**)



■ Aged ≥ 60 years Model 1 (Socio-demographic) (Global cognition measure and vitamin B₁₂ and folate concentrations with controls for age, sex, educational attainment, mean asset wealth and habitation status);
□ Aged ≥ 60 years Model 2 (Socio-demographic, Lifestyle, Health Indicators and Medications) (Model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

Figure 5.4. Associations between concentrations of folate (continuous) and quintiles of vitamin B₁₂ and global cognition - aged ≥60 years (incidence rate ratios; IRR and 95% CIs).

This figure has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

5.6. Discussion

The present study estimated the prevalence of groups of vitamin B₁₂ and folate status in a nationally representative sample of older adults in Ireland and investigated the hypothesis that low vitamin B₁₂ status in conjunction with high folate status, is associated with increased risk of cognitive decline in older people (228, 229, 230). This study demonstrated that the 1.5% of the population with *low B₁₂/high folate* status did not have any adverse associations with cognitive performance compared to those with normal status for both B-vitamins. In contrast, higher concentrations of folate were associated with small, but statistically significant higher scores for MMSE and MoCA performance. In contrast to previous research using single measures of global cognition in older cohorts, this study employed two clinically validated methods of global cognitive function measurement simultaneously.

There are a number of possible explanations as to why these findings diverge with those of the NHANES, Framingham Heart Study and three combined cohorts of Moore and colleagues (2014) (228, 229, 230). In NHANES (*n* 1,457), concentrations of high plasma folate (> 59 nmol/L) with low vitamin B₁₂ (plasma B₁₂ < 148 pmol/L or methylmalonic acid (MMA) > 0.21 mmol/L) (*n* 42) were associated with poorer cognitive performance cross-sectionally. The latter study was conducted in the US, a region implementing MFAF and where the use of supplements containing FA was common, leading to extremely high concentrations of plasma folate and the presence of circulating unmetabolized FA (228, 387, 388). Thus, very high concentrations (plasma folate >59 nmol/L) were observed in 20.7% of the NHANES cohort, compared to just 3.3% (plasma folate ≥ 60 nmol/L) in the present study. The use of FA supplements was uncommon amongst older adults in Ireland, ranging between 7.2 – 13.7% amongst the two groups with the highest status. In addition, the Digit Symbol-Substitution subtest (DSST) of the Wechsler Adult Intelligence Scale III was used in NHANES to examine cognitive performance in attention and processing speed, while the present study assessed global cognitive performance using both MMSE and MoCA. From the Framingham Heart Study (229), the data of which pertained to pre- mandatory fortification in the US (1986–1990), Morris *et al.*, (2012) reported faster rates of decline in MMSE were associated with vitamin B₁₂ concentrations of < 257 pmol/L and plasma folate of > 20.2 nmol/L - after 8-year

follow up (n 24). While the vitamin B₁₂ cut-off used was similar to the current study, we applied a higher threshold to define high folate status and used a cognitively intact sample. Furthermore, there were twice as many participants included in the current analysis.

Moore *et al.*, (2014) also suggested that high folate or FA supplements may be detrimental to cognition in older people with low vitamin B₁₂ concentrations (n 1,354) (230). Compared to the present study, a similar vitamin B₁₂ cut-off (< 250 pmol/L) was used, and red cell folate of $>1,594$ nmol/L, which approximate to those employed in the Framingham Heart Study investigation and had 39 participants in the low vitamin B₁₂/high folate group. However, the sample was recruited from three separate clinical cohorts in Australia, a region also implementing MFAF. In addition, 480 subjects had established AD and 187 had confirmed MCI. This is in contrast with our study, all of whom were free living and cognitively robust (n 3,871). In addition, Moore *et al.*, (2014) (230) used the binary outcome of MMSE of < 24 to define cognitive impairment, whereas our study used MMSE and MoCA as a continuous outcome to investigate the range of risk to cognitive performance.

The findings of the present study are consistent with other studies using European population data; those of Clarke *et al.*, (2008) (232) and Doets *et al.*, (2014) (234) showing no association of low vitamin B₁₂/high folate status with an increased risk to cognitive function. In addition, the Sacramento Area Latino Study on Aging (SALSA) in America (233) did not demonstrate a negative association with cognitive function (n 1,535) using a vitamin B₁₂ deficiency cut-off of < 148 pmol/L and high folate of >45.3 nmol/L, although biochemical anomalies were observed.

To our knowledge, this is the largest such study to use a nationally representative sample of community-dwelling older adults that were cognitively unimpaired, employing rigorous exclusion criteria for cognitive performance to ensure accurate investigation of early cognitive changes and to mitigate potential confounding. Another strength of the current study is the use of two tests of global cognitive performance in parallel. Most other studies in this field have investigated the MMSE or variations of it to determine cognitive performance or impairment. This study also measured global cognitive performance using the MoCA, an arguably better

screening tool for subtle cognitive decline and mild cognitive deficits (319, 380). In addition, we are the first study to explore interactions of vitamin B₁₂ and folate concentrations with respect to MoCA and we controlled for a large extent of covariates to account for factors that may affect B-vitamin status, particularly medications and food supplement use.

A limitation of the present study, consistent with previous research, was the low number of participants in the "at-risk" group (*low B₁₂/high folate*), with only 51 participants. While a power calculation was conducted, it did not meet the required 63 participants per group, indicating that the study is likely underpowered. Although not ideal, this suggests that many studies in this field may also face similar power limitations. A sufficiently powered study would require a larger sample size in the *low B₁₂/high folate* group or could use a case-control design, comparing cases with cognitive impairment to matched controls by B-vitamin status, to test this hypothesis more effectively. Further, future research could consider more advanced methods like Mendelian randomization to address sample size challenges associated with examining extreme ends of the vitamin concentration distribution.

Another limitation was the cross-sectional nature of the present study and therefore we cannot predict decline within the at-risk group. Moreover, the present study was limited to the use of plasma vitamin B₁₂ and folate as indicators of status, acknowledging the advantages and disadvantages associated with these biochemical measures (i.e., total vitamin B₁₂ in blood has low sensitivity for estimating vitamin B₁₂ deficiency (375) when compared to other biomarkers, whereas plasma folate can be subject to variations from recent dietary intakes). In addition, dietary intake data at Wave 1 was not ascertained, therefore we could not estimate dietary exposure of both vitamin B₁₂ and folate. It is also important to note that because of the recruitment sampling criteria and rigorous exclusion criteria in TILDA that these findings may not be generalisable to non-Caucasians, older adults in institutional care or those with cognitive impairment.

Finally, we acknowledge the potential reverse causality and bi-directional relationship between global cognitive performance and vitamin B₁₂ and folate status and that the possible association between B-vitamin status and cognitive impairment in older adults

might reflect effects of such vitamins on AD or cerebrovascular disease, which are the primary pathogenic processes underlying cognitive impairment in older adults.

In summary, the present study was undertaken to assess the possible hazards for cognitive function in older people associated with voluntary FA fortification in Ireland and found no evidence that high blood folate concentrations affected the associations of low vitamin B₁₂ status with cognitive impairment. While reassuring, it is essential to exercise caution in interpreting these findings, as the study's cross-sectional design and limited at-risk sample size could mask subtle associations. These findings suggest that higher folate status may be protective of global cognitive function irrespective of vitamin B₁₂ status, in a representative sample of community dwelling population of older people but requires prospective investigation.

Despite cautious interpretation, the findings still carry significant implications for public health policy on folic acid fortification in Ireland and Europe. They provide some reassurance to healthcare providers and policymakers that higher folate status may not increase the risk of cognitive impairment in older adults. Furthermore, the results support the possibility of the introduction of mandatory FA fortification, targeted vitamin B₁₂ supplementation recommendations, and public awareness campaigns to improve the overall nutritional status of older populations.

In light of these findings, the following recommendations are made to the Irish Government and Department of Health. First, it is recommended that Ireland implement mandatory folic acid fortification of staple foods such as bread and flour. The current study highlights that voluntary fortification is insufficient, particularly for older adults, with up to 15% of older adults in the ROI having deficient or low folate status. Mandatory fortification has been successful in countries like the US in reducing neural tube defects and improving overall folate status, and similar benefits could be expected in Ireland. Second, public health campaigns should be launched to promote vitamin B₁₂ supplementation, especially among those aged 50 and above. Given that folic acid fortification does not address the issue of low vitamin B₁₂ status, which remains prevalent in older adults, it is crucial to encourage B₁₂ supplementation to maintain balanced nutrient intake and avoid potential risks arising from folate- B₁₂ interactions.

Third, the government of Ireland should also establish a monitoring system to track folate and vitamin B₁₂ levels across various population groups. This would allow for informed adjustments in the event of MFAF to the levels as needed to maintain optimal nutrient balance and prevent potential adverse outcomes, such as unmetabolized folic acid accumulation. Finally, public awareness campaigns should emphasize the importance of a balanced diet and the role of supplements in maintaining adequate folate and vitamin B₁₂ levels, particularly for older adults who may have difficulty achieving adequate intake through diet alone.

5.7. Supplemental Information

Supplementary Table 5.1. Additional sample characteristics, by vitamin B₁₂ and folate groups

		Vitamin B ₁₂ ≥258 pmol/L		Vitamin B ₁₂ <258pmol/L		P for comparison across all groups
		Folate ≤45.3 nmol/L	Folate >45.3 nmol/L	Folate ≤45.3 nmol/L	Folate >45.3 nmol/L	
		'Normal B ₁₂ / Normal Folate' (n 2,433)	'Normal B ₁₂ / High Folate' (n 306)	'Low B ₁₂ / High Folate' (n 51)	'Low B ₁₂ / Normal Folate' (n 1,081)	
n (%)	3,871					
Educational Attainment	3,871					
None/Primary	846	502 (20.6)	54 (17.7)	14 (27.5)	276 (25.5)	0.002
P		Reference	0.2005	0.2841	0.0017*	
Secondary	1,727	1108 (45.5)	131 (42.8)	22 (43.1)	466 (43.1)	0.515
P		Reference	0.3641	0.7342	0.1801	
Tertiary/Higher	1,298	823 (33.8)	121 (39.5)	15 (29.4)	339 (31.4)	0.052
P		Reference	0.0535	0.4980	0.1485	
Household Wealth – above average [n (%)]	1,897 / 3,712	1,184 (50.6)	179 (61.9)	32 (65.3)	502 (48.6)	0.000
P		Reference	0.0010*	0.0417*	0.2227	
Living Alone [n (%)]	629 / 3,871	381 (15.7)	56 (18.3)	7 (13.7)	185 (17.1)	0.4940
P		Reference	0.2578	0.6944	0.2859	
Physical Activity Level (IPAQ) [n (%)]	3,871					
Low	1,051	647 (26.6)	83 (27.1)	12 (23.5)	309 (28.6)	0.6050
P		Reference	0.8439	0.6136	0.2249	
Moderate	1,376	870 (35.8)	123 (40.2)	19 (37.3)	363 (33.6)	0.1850
P		Reference	0.1353	0.8285	0.2093	
High	1,445	916 (37.7)	100 (32.7)	20 (39.2)	409 (37.8)	0.3720
P		Reference	0.0824	0.8223	0.9163	

Smokers [n (%)]	568 / 3,871	344 (14.1)	33 (10.8)	9 (17.7)	182 (16.8)	0.0340
P		<i>Reference</i>	<i>0.0793</i>	<i>0.5188</i>	<i>0.0442*</i>	
Alcohol Consumers [n (%)]	2,889 /3,601	1,825 (80.1)	233 (80.9)	36 (73.5)	795 (80.7)	0.6400
P		<i>Reference</i>	<i>0.7384</i>	<i>0.3038</i>	<i>0.6759</i>	
BMI (kg/m²)	3,866/3,871	27.9 (25.2, 31.0)	27.3 (24.2, 29.8)	29.7 (26.8, 31.7)	28.5 (25.6, 31.8)	0.0001
P		<i>Reference</i>	<i>0.0006*</i>	<i>0.0759</i>	<i>0.0006*</i>	
Grip Strength	3,813	24.8 (19.3, 33.5)	22.3 (17.5, 30. 8)	26.0 (18.0, 34.0)	25.8 (19.3, 33.3)	0.0008
P		<i>Reference</i>	<i>0.0003*</i>	<i>0.9916</i>	<i>0.4080</i>	
Hypertension [n (%)]	1,573/3,854	927 (38.2)	135 (44.3)	29 (56.9)	482 (44.9)	0.0000
P		<i>Reference</i>	<i>0.0454*</i>	<i>0.0085*</i>	<i>0.0002*</i>	
Diabetes [n (%)]	250/3,832	131 (5.5)	20 (6.6)	9 (17.7)	90 (8.4)	0.000
P		<i>Reference</i>	<i>0.4498</i>	<i>0.0242*</i>	<i>0.0023*</i>	
CES-D (Depressive Symptoms)	3,821/3,871	3.0 (1.0, 7.0)	3.0 (1.0, 7.0)	4.0 (0.0, 7.0)	3.0 (1.0, 7.0)	0.9961
P		<i>Reference</i>	<i>0.6359</i>	<i>0.9264</i>	<i>0.7284</i>	
Creatinine (µmol/L)	3,871	77.0 (66.0, 89.0)	75.5(64.0, 85.0)	76 (66.0, 91.0)	77 (67.0, 88.0)	0.0217
P		<i>Reference</i>	<i>0.0093*</i>	<i>0.4537</i>	<i>0.2719</i>	
Statin Use [n (%)]	1,125/3,871	703 (28.9)	89 (29.1)	21 (41.2)	312 (28.8)	0.2970
P		<i>Reference</i>	<i>0.8786</i>	<i>0.0803</i>	<i>0.9845</i>	
Thyroid Medication Use [n (%)]	253/3,871	159 (6.5)	26 (8.5)	4 (7.8)	64 (5.9)	0.396
P		<i>Reference</i>	<i>0.2465</i>	<i>0.7331</i>	<i>0.4828</i>	
Proton Pump Inhibitors [n (%)]	440/3,871	280 (11.5)	32 (10.4)	11 (21.8)	117 (10.8)	0.117
P		<i>Reference</i>	<i>0.5737</i>	<i>0.0857</i>	<i>0.5499</i>	
Metformin [n (%)]	139/3,871	66 (2.7)	10 (3.3)	5 (9.8)	58 (5.4)	0.000
P		<i>Reference</i>	<i>0.6098</i>	<i>0.0928</i>	<i>0.0005*</i>	
History of Stroke	43/3,871	27 (1.1)	3 (1.0)	0 (0)	13 (1.2)	0.983
P		<i>Reference</i>	<i>0.8301</i>	<i>0.0000</i>	<i>0.8137</i>	

1 Non-normally distributed continuous variables were described as medians and interquartile ranges (IQR) (all such values), and compared using Kruskal-Wallis test as appropriate, and Mann–Whitney U test for pairwise comparisons with the reference category. Categorical variables were compared using the chi-square tests of Fisher’s exact test, as appropriate.

*Statistically significant after adjustment for multiple comparisons (q value <0.013)

This table has been published in the paper *O’Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.2. Selected sample characteristics, by vitamin B₁₂ and folate groups – stratified by age

n (%)	3,871	'Normal B₁₂/Normal Folate' (n 2,433)	'Normal B₁₂/High Folate' (n 306)	'Low B₁₂/High Folate' (n 51)	'Low B₁₂/Normal Folate' (n 1,081)	P for comparison across groups¹
Age < 50 years (n 3,871)						
Age, mean (SD)	3,871	60 (55, 67)	62 (56, 68)	66 (57, 73)	61 (55, 68)	0.0001
P		Reference	0.0016*	0.0011*	0.0033*	
Female [n (%)]	2,045/3,871	1,292 (53.1)	199 (64.8)	23 (45.1)	531 (49.1)	0.0000
P		Reference	0.0001*	0.2602	0.0294	
Vitamin B₁₂ (pmol/L)	3,871	361.3 (308.7, 435.2)	420.9 (344.6, 514.0)	214.2 (160.1, 241.3)	205.9 (170.3, 235.4)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Folate (nmol/L)	3,871	18.8 (13.1, 26.9)	56.5 (50.0, 65.7)	55.4 (49.1, 61.5)	14.7 (10.7, 22.0)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Creatinine (μmol/L)	3,871	77.0 (66.0, 89.0)	76.0 (64.0, 85.0)	76 (66.0, 91.0)	77 (67.0, 88.0)	0.0217
P		Reference	0.0130	0.4399	0.2356	
Folic Acid Supplement Use [n (%)]	123/3,871	68 (2.8)	22 (7.2)	7 (13.7)	26 (2.4)	0.0000
P		Reference	0.0039*	0.0251	0.4969	
MMSE	3,871	29 (28.0, 30.0)	29 (29.0, 30.0)	29 (28.0, 30.0)	29 (28.0, 30.0)	0.0219
P		Reference	0.0128*	0.6987	0.1751	
MoCA	3,871	26 (24.0, 28.0)	26 (25.0, 28.0)	26 (24.0, 27.0)	26 (24.0, 28.0)	0.0054
P		Reference	0.0503	0.4997	0.0131	
Age < 60 years (n 1,801)						
n (%)	1801	(n 1,187)	(n 125)	(n 15)	(n 474)	
Female [n (%)]	975/1,801	633 (53.3)	82 (65.6)	8 (53.3)	252 (53.2)	0.068
P		Reference	0.0065*	0.9997	0.9521	
Vitamin B₁₂ (pmol/L)	1,801	361.4 (308.4, 438.4)	413.9 (336.5, 501.3)	198.6 (148.2, 230.5)	205.9 (171.3, 234.7)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Folate (nmol/L)	1,801	18.1 (12.7, 25.2)	55.4 (49.7, 62.4)	55.9 (52.7, 63.2)	14.9 (11.2, 22.3)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Creatinine (μmol/L)	1,801	75 (65, 86)	70 (61, 81)	69 (62, 84)	74 (65, 84)	0.0221
P		Reference	0.0041*	0.2433	0.7001	
Folic Acid Supplement Use [n (%)]	58/1,801	38 (3.2)	8 (6.4)	1 (6.7)	11 (2.3)	0.086
P		Reference	0.1565	0.6043	0.3063	
MMSE	1,801	29 (29, 30)	30 (29, 30)	30 (29, 30)	29 (29, 30)	0.1640
P		Reference	0.0643	0.1827	0.8986	
MoCA	1,801	27 (25, 28)	27 (25, 28)	27 (26, 28)	26 (25, 28)	0.2391
P		Reference	0.3307	0.6915	0.1195	

Age 60 - 69 years (n 1,359)						
n (%)		(n 845)	(n 115)	(n 17)	(n 382)	
Female [n (%)]	718/1,359	446 (52.8)	78 (67.8)	7 (41.2)	187 (49.0)	0.003
P		Reference	0.0014*	0.3504	0.2147	
Vitamin B ₁₂ (pmol/L)	1,359	367.4 (413.4, 439.2)	431.2 (357.1, 521.5)	216.0 (187.3, 251.5)	210.7 (173.9, 237.6)	0.0001
P		Reference	0.0001*	0.0000*	0.0000*	
Folate (nmol/L)	1,359	19.6 9 (13.5, 27.9)	56.8 (50.7, 65.7)	51.1 (48.1, 56.4)	14.6 (10.3, 22.2)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Creatinine (μmol/L)	1,359	78 (66.5, 89.5)	76 (65, 85)	78 (71, 91)	79 (68, 88)	0.3360
P		Reference	0.1464	0.5650	0.5129	
Folic Acid Supplement Use [n (%)]	43/1,359	22 (2.6)	9 (7.8)	3 (17.7)	9 (2.4)	0.001
P		Reference	0.0427	0.1153	0.7947	
MMSE	1,359	29 (28, 30)	29 (29, 30)	29 (28, 30)	29 (28, 30)	0.0755
P		Reference	0.6832	0.6832	0.8383	
MoCA	1,359	26 (24, 28)	27 (25, 28)	27 (26, 28)	26 (25, 28)	0.2391
P		Reference	0.0074*	0.4045	0.3567	
Age 70 - 79 years (n 616)						
n (%)	616	(n 349)	(n 61)	(n 15)	(n 191)	
Female [n (%)]	300/616	182 (52.2)	34 (55.7)	7 (46.7)	77 (40.3)	0.041
P		Reference	0.6057	0.6870	0.0081*	
Vitamin B ₁₂ (pmol/L)	616	352.4 (305.9, 422.6)	401.5 (341.9, 522.8)	216.4 (156.5, 241.3)	205.0 (169.6, 236.0)	0.0001
P		Reference	0.0001*	0.0000*	0.000*	
Folate (nmol/L)	616	20.1 (13.6, 29.6)	59.9 (50.2, 69.0)	55.6 (47.0, 64.6)	14.8 (11.3, 22.3)	0.0001
P		Reference	0.0000*	0.0000*	0.0000*	
Creatinine (μmol/L)	616	80 (69, 93)	81 (68, 95)	83 (72, 99)	82 (72, 97)	0.6782
P		Reference	0.8518	0.5813	0.2852	
Folic Acid Supplement Use [n (%)]	19/616	8 (2.3)	3 (4.90)	3 (20.0)	5 (2.6)	0.013
P		Reference	0.3664	0.0991	0.8174	
MMSE	616	29 (28, 30)	29 (28, 30)	29 (28, 29)	29 (27, 30)	0.1310
P		Reference	0.3820	0.8018	0.0540	
MoCA	616	25 (24, 27)	26 (24, 27)	24 (24, 26)	25 (23, 27)	0.3896
P		Reference	0.4702	0.4152	0.2614	

Age ≥ 80 years (n 95)						
n (%)	95	(n 52)	(n 5)	(n 4)	(n 34)	
Female [n (%)]	52/95	31 (59.6)	5 (100)	1 (25.0)	15 (44.1)	0.153
P		Reference	0.1915	0.1850	0.1637	
Vitamin B₁₂ (pmol/L)	95	329.9 (292.6, 399.7)	386.2 (304.8, 608.4)	216.0 (162.5, 244.0)	176.2 (149.6, 222.9)	0.0001
P		Reference	0.2952	0.0009*	0.0000*	
Folate (nmol/L)	95	18.5 (12.4, 28.4)	52.2 (47.7, 57.3)	61.4 (53.4, 63.8)	11.7 (9.2, 16.4)	0.0001
P		Reference	0.0001*	0.0009*	0.0001*	
Creatinine (μmol/L)	95	87.5 (71.5, 104.5)	80.5 (70, 87)	92 (77, 104)	89.5 (75, 109)	0.7626
P		Reference	0.4588	0.8113	0.6333	
Folic Acid Supplement Use [n (%)]	3/95	0 (0)	2 (40.0)	0 (0)	1 (2.9)	0.008
P		Reference	0.1172	.	0.3199	
MMSE	95	28 (27, 29)	27 (27, 29)	27 (25.5, 28)	28 (27, 30)	0.4271
P		Reference	0.3375	0.1907	0.6680	
MoCA	95	23.5 (21, 26)	22.5 (20, 27)	24 (21.5, 26)	25 (21, 27)	0.4840
P		Reference	0.4840	0.7607	0.1240	

¹ Non-normally distributed continuous variables were described as medians and interquartile ranges (IQR) (all such values), and compared using Kruskal-Wallis test as appropriate, and Mann-Whitney U test for pairwise comparisons with the reference category. Categorical variables were compared using the chi-square tests of Fisher's exact test, as appropriate

*Statistically significant after adjustment for multiple comparisons (q value <0.013).

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.3. Associations of concentrations of vitamin B₁₂ and folate status with global cognition (MMSE & MoCA): (incidence rate ratios; IRR, standard errors; SE and 95% Confidence Intervals; 95% CIs)

	Model 0					Model 1				Model 2					
	IRR	SE	95% CIs		P	IRR	SE	95% CIs		P	IRR	SE	95% CIs		P
≥ 50 years	n 3,871					n 3,712					n 3,299				
MMSE															
‘normal B ₁₂ /high folate’	0.83	0.07	0.70	0.98	0.030	0.85	0.07	0.72	1.00	0.050	0.84	0.07	0.71	1.00	0.047
‘low B ₁₂ /high folate’	1.24	0.25	0.84	1.83	0.274	0.90	0.14	0.66	1.22	0.501	0.88	0.14	0.64	1.21	0.438
‘low B ₁₂ /normal folate’	1.07	0.05	0.98	1.17	0.131	0.99	0.04	0.91	1.08	0.852	0.95	0.04	0.87	1.04	0.270
MoCA															
‘normal B ₁₂ /high folate’	0.91	0.04	0.83	0.99	0.031	0.92	0.04	0.86	1.00	0.041	0.92	0.03	0.85	1.00	0.043
‘low B ₁₂ /high folate’	1.08	0.08	0.93	1.25	0.303	0.93	0.06	0.82	1.04	0.200	0.90	0.06	0.79	1.02	0.101
‘low B ₁₂ /normal folate’	1.06	0.03	1.01	1.12	0.011	1.03	0.02	0.98	1.07	0.262	1.00	0.02	0.95	1.04	0.948
≥ 60 years	n 2,070					n 1,972					n 1,761				
MMSE															
‘normal B ₁₂ /high folate’	0.82	0.08	0.67	0.99	0.042	0.84	0.08	0.70	1.01	0.068	0.82	0.08	0.68	0.99	0.043
‘low B ₁₂ /high folate’	1.23	0.27	0.81	1.89	0.319	0.99	0.18	0.68	1.43	0.949	0.99	0.17	0.70	1.39	0.946
‘low B ₁₂ /normal folate’	1.08	0.05	0.98	1.20	0.119	0.99	0.05	0.90	1.09	0.874	0.96	0.05	0.86	1.06	0.416
MoCA															
‘normal B ₁₂ /high folate’	0.87	0.05	0.78	0.97	0.014	0.91	0.04	0.83	1.00	0.043	0.89	0.05	0.80	0.99	0.025
‘low B ₁₂ /high folate’	1.01	0.09	0.86	1.20	0.877	0.91	0.07	0.79	1.05	0.20	0.90	0.07	0.78	1.04	0.165
‘low B ₁₂ /normal folate’	1.04	0.03	0.98	1.11	0.150	1.00	0.03	0.95	1.06	0.99	0.98	0.03	0.92	1.03	0.413

Reference group = ‘normal vitamin B₁₂ /normal folate’, IRR =1.00

This table has been published in the paper *O’Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.4. Multivariable associations of concentrations of vitamin B₁₂ and folate status with measures of global cognition (MMSE and MoCA), aged ≥ 50 years: (incidence rate ratios; IRR and 95% Confidence Intervals; 95% CIs)

Multivariable models:	MMSE		MoCA	
	Model 1	Model 2	Model 1	Model 2
n	3,712	3,299	3,712	3,299
<i>Concentrations of vitamin B₁₂ and folate status</i>				
'normal B₁₂/high folate'	0.85*	0.84*	0.92*	0.92*
	[0.72,1.00]	[0.71,1.00]	[0.86,1.00]	[0.85,1.00]
'low B₁₂/high folate'	0.90	0.88	0.92	0.9
	[0.66,1.22]	[0.64,1.21]	[0.82,1.04]	[0.79,1.02]
'low B₁₂/normal folate'	0.99	0.95	1.03	1.00
	[0.91,1.08]	[0.87,1.04]	[0.98,1.07]	[0.95,1.04]
Age (y)				
50-59	1.00	1.00	1.00	1.00
	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]
60-69	1.11*	1.16**	1.12***	1.11***
	[1.02,1.21]	[1.05,1.27]	[1.07,1.16]	[1.06,1.16]
70-79	1.41***	1.44***	1.27***	1.23***
	[1.28,1.56]	[1.28,1.62]	[1.21,1.35]	[1.15,1.31]
80+	1.86***	1.88***	1.62***	1.54***
	[1.58,2.18]	[1.60,2.21]	[1.47,1.78]	[1.38,1.72]
Sex				
Male	1.00	1.00	1.00	1.00
	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]
Female	0.89**	0.82***	0.99	0.90**
	[0.83,0.96]	[0.73,0.92]	[0.95,1.02]	[0.85,0.96]
Educational Attainment				
None/Primary	1.00	1.00	1.00	1.00
	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]	[1.00,1.00]

Secondary	0.60*** [0.56,0.65]	0.60*** [0.55,0.66]	0.80*** [0.76,0.83]	0.81*** [0.77,0.85]
Tertiary/Higher	0.34*** [0.30,0.38]	0.36*** [0.32,0.40]	0.48*** [0.46,0.51]	0.50*** [0.47,0.53]
Above Average Asset Wealth	0.91* [0.85,0.99]	0.92 [0.85,1.00]	0.94** [0.91,0.98]	0.94** [0.90,0.98]
Living Alone		0.99 [0.89,1.11]		1.00 [0.95,1.06]
<i>Physical Activity Level (IPAQ)</i>				
Low		1.00 [1.00,1.00]		1.00 [1.00,1.00]
Moderate		0.95 [0.86,1.04]		1.00 [0.94,1.05]
High		0.97		1.00
			[0.88,1.06]	[0.95,1.05]
Smokers			1.10 [0.98,1.23]	1.04 [0.98,1.11]
Alcohol Consumers			1.02 [0.93,1.12]	0.98 [0.93,1.02]
<i>BMI</i>				
Normal			1.00 [1.00,1.00]	1.00 [1.00,1.00]
Overweight			1.08 [0.97,1.19]	1.04 [0.99,1.10]
Obese			1.10 [0.99,1.23]	1.10** [1.03,1.16]
Mean grip strength			0.99 [0.99,1.00]	0.99*** [0.99,1.00]
Hypertension			1.02 [0.95,1.10]	1.02 [0.98,1.06]
Diabetes			1.07	1.08*

	[0.92,1.23]	[1.00,1.16]
CES-D (Depressive Symptoms)	1.00	1.00
	[1.00,1.01]	[1.00,1.01]
Folic Acid Supplement Use	1.21	1.08
	[0.75,1.96]	[0.81,1.43]
Proton Pump Inhibitors	1.00	0.98
	[0.89,1.12]	[0.92,1.04]
Statin Use	0.96	1.01
	[0.87,1.05]	[0.96,1.06]
Thyroid Medication Use	1.06	1.01
	[0.92,1.23]	[0.93,1.09]
Previous Stroke	1.19	1.07
	[0.84,1.67]	[0.89,1.29]

Reference group = 'normal B₁₂/normal folate', IRR =1.00, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.5. Multivariable associations vitamin B₁₂ and folate groups with measures of global cognition (MMSE and MoCA), aged ≥ 60 years: (incidence rate ratios; IRR and 95% Confidence Intervals; 95% CIs).

Multivariable models: n	MMSE	MoCA			
		Model 1 1,972	Model 2 1,761	Model 1 1,972	Model 2 1,761
<i>Concentrations of vitamin B₁₂ and folate status</i>					
'normal B ₁₂ /high folate'		0.84 [0.70,1.01]	0.82* [0.68,0.99]	0.91* [0.83,1.00]	0.89* [0.80,0.99]
'low B ₁₂ /high folate'		0.99 [0.68,1.43]	0.99 [0.70,1.39]	0.91 [0.79,1.04]	0.9 [0.78,1.04]
'low B ₁₂ /normal folate'		0.99 [0.90,1.09]	0.96 [0.86,1.06]	1.00 [0.95,1.06]	0.98 [0.92,1.03]
<i>Age (y)</i>					
60-69		1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]
70-79		1.29*** [1.17,1.42]	1.25*** [1.13,1.39]	1.15*** [1.08,1.21]	1.11*** [1.05,1.18]
80+		1.68*** [1.44,1.96]	1.63*** [1.39,1.91]	1.45*** [1.32,1.61]	1.40*** [1.25,1.57]
<i>Sex</i>					
Male		1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]
Female		0.89* [0.81,0.98]	0.82** [0.71,0.94]	0.96 [0.91,1.01]	0.88** [0.81,0.96]
<i>Educational Attainment</i>					
None/Primary		1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]	1.00 [1.00,1.00]

Secondary	0.66*** [0.60,0.73]	0.63*** [0.57,0.70]	0.80*** [0.76,0.85]	0.80*** [0.75,0.85]
Tertiary/Higher	0.41*** [0.36,0.46]	0.40*** [0.35,0.46]	0.50*** [0.47,0.54]	0.52*** [0.48,0.55]
Above Average Asset Wealth	0.87** [0.79,0.96]	0.88* [0.80,0.98]	0.93** [0.88,0.98]	0.92** [0.87,0.97]
Living Alone		1.08 [0.95,1.23]		1.01 [0.95,1.09]
<i>Physical Activity Level (IPAQ)</i>				
Low		1.00 [1.00,1.00]		1.00 [1.00,1.00]
Moderate		0.95 [0.85,1.07]		1.00 [0.93,1.07]
High		0.98 [0.87,1.10]		0.99 [0.92,1.06]
Smokers		1.07 [0.92,1.25]		1.03 [0.94,1.13]
Alcohol Consumers		1.04 [0.94,1.16]		0.97 [0.91,1.02]
<i>BMI</i>				
Normal		1.00 [1.00,1.00]		1.00 [1.00,1.00]
Overweight		0.97 [0.86,1.10]		1.02 [0.95,1.09]
Obese		0.99 [0.87,1.13]		1.07 [0.99,1.15]
Mean grip strength		0.99 [0.99,1.00]		0.99** [0.99,1.00]

Hypertension	0.99 [0.90,1.10]	1.00 [0.95,1.05]
Diabetes	1.11 [0.95,1.30]	1.04 [0.94,1.14]
CES-D (Depressive Symptoms)	1.01 [1.00,1.01]	1.00 [1.00,1.01]
Folic Acid Supplement Use	1.14 [0.72,1.80]	1.08 [0.80,1.46]
Proton Pump Inhibitors	0.98 [0.86,1.12]	0.97 [0.90,1.05]
Statin Use	0.97 [0.87,1.08]	1.01 [0.96,1.08]
Thyroid Medication Use	1.06 [0.89,1.26]	1.01 [0.91,1.12]
Previous Stroke	0.94 [0.65,1.35]	1.03 [0.83,1.27]

Reference group = 'normal B₁₂/normal folate', IRR =1.00, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**

Supplementary Table 5.6. Sensitivity Analysis: Associations of vitamin B₁₂ and folate groups with global cognition (MMSE & MoCA); (incidence rate ratios; IRR, standard errors; SE and 95% Confidence Intervals; 95% CIs).

	Model 0					Model 1				Model 2				P	
	IRR	SE	95% CIs		P	IRR	SE	95% CIs		P	IRR	SE	95% CIs		
≥ 50 years	n 4,463					n 4,263				n 3,765					
MMSE															
‘normal B ₁₂ /high folate’	0.85	0.08	0.70	1.03	0.088	0.88	0.08	0.74	1.05	0.157	0.88	0.08	0.74	1.05	0.145
‘low B ₁₂ /high folate’	1.17	0.22	0.82	1.68	0.379	0.85	0.14	0.63	1.17	0.319	0.83	0.14	0.61	1.15	0.267
‘low B ₁₂ /normal folate’	1.15	0.06	1.04	1.26	0.004	1.06	0.46	0.98	1.16	0.162	1.02	0.05	0.93	1.12	0.656
MoCA															
‘normal B ₁₂ /high folate’	0.89	0.04	0.81	0.98	0.021	0.90	0.04	0.83	0.98	0.020	0.92	0.40	0.84	1.00	0.042
‘low B ₁₂ /high folate’	1.01	0.08	0.86	1.19	0.873	0.86	0.06	0.74	1.00	0.043	0.86	0.07	0.73	1.01	0.069
‘low B ₁₂ /normal folate’	1.07	0.03	1.03	1.13	0.004	1.03	0.02	0.99	1.08	0.152	1.01	0.03	0.97	1.07	0.517
≥ 60 years	n 2,426					n 2,298				n 2,040					
MMSE															
‘normal B ₁₂ /high folate’	0.81	0.84	0.66	0.99	0.044	0.85	0.08	0.71	1.03	0.101	0.84	0.08	0.69	1.02	0.084
‘low B ₁₂ /high folate’	1.16	0.23	0.78	1.17	0.457	0.97	1.18	0.67	1.40	0.870	1.00	0.19	0.69	1.45	0.991
‘low B ₁₂ /normal folate’	1.18	0.07	1.05	1.33	0.005	1.10	0.06	0.99	1.24	0.075	1.07	0.07	0.95	1.20	0.279
MoCA															
‘normal B ₁₂ /high folate’	0.87	0.05	0.78	0.98	0.026	0.90	0.04	0.82	1.00	0.061	0.90	0.05	0.81	1.00	0.070
‘low B ₁₂ /high folate’	0.97	0.09	0.80	1.17	0.737	0.86	0.08	0.72	1.03	0.113	0.89	0.09	0.73	1.09	0.250
‘low B ₁₂ /normal folate’	1.08	0.03	1.01	1.15	0.016	1.04	0.03	0.98	1.10	0.169	1.02	0.03	0.96	1.09	0.506

Reference group = ‘normal B₁₂/normal folate’, IRR =1.00, **P*<0•05, ***P*<0•01, ****P*<0•001

This table has been published in the paper *O’Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.7. Associations between concentrations of folate (continuous) and quintiles of vitamin B₁₂ with global cognition in those aged ≥ 60 years (incidence rate ratios; IRR, standard errors; SE and 95% Confidence Intervals; 95% CIs).

<i>n</i>	Model 1					Model 2				
	IRR	SE	95% CIs		P	IRR	SE	95% CIs		P
	1,972					1,761				
MMSE										
Folate (Cont.): B ₁₂ Q1	1.00	0.01	0.99	1.01	0.838	1.00	0.01	0.99	1.01	0.804
Folate (Cont.): B ₁₂ Q2	1.00	0.01	0.99	1.01	0.702	1.00	0.01	0.99	1.01	0.697
Folate (Cont.): B ₁₂ Q3	1.00	0.01	0.99	1.01	0.835	1.00	0.01	0.99	1.01	0.898
Folate (Cont.): B ₁₂ Q4	1.00	0.01	0.99	1.00	0.321	0.99	0.00	0.99	1.00	0.265
MoCA										
Folate (Cont.): B ₁₂ Q1	1.00	0.00	1.00	1.01	0.523	1.00	0.00	1.00	1.01	0.635
Folate (Cont.): B ₁₂ Q2	1.00	0.00	0.99	1.00	0.464	1.00	0.00	0.99	1.00	0.575
Folate (Cont.): B ₁₂ Q3	1.00	0.00	1.00	1.01	0.869	1.00	0.00	1.00	1.01	0.768
Folate (Cont.): B ₁₂ Q4	1.00	0.00	0.99	1.00	0.096	0.99	0.00	0.99	1.00	0.029

Reference group = 'Folate (Continuous): B₁₂ Q5, IRR =1.00; Q1 vitamin B₁₂, 45.4 - 225 pmol/L (*n* 409), Q2 225 – 289 pmol/L (*n* 427), Q3 289 – 350 pmol/L (*n* 408); Q4 350 - 428 pmol/L (*n* 415); Q5 428 – 719 pmol/L (*n* 412).

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**.

Supplementary Table 5.8. Associations between concentrations of vitamin B₁₂ (continuous) and quintiles of folate with global cognition in those aged ≥ 60 years (incidence rate ratios; IRR, standard errors; SE and 95% Confidence Intervals; CIs).

<i>n</i>	Model 1					Model 2				
	IRR	SE	95% CIs		P	IRR	SE	95% CIs		P
			1,972					1,761		
MMSE										
B ₁₂ (Cont.): Folate Q2	1.00	0.00	1.00	1.00	0.588	1.00	0.00	1.00	1.00	0.867
B ₁₂ (Cont.): Folate Q3	1.00	0.00	1.00	1.00	0.254	1.00	0.00	1.00	1.00	0.559
B ₁₂ (Cont.): Folate Q4	1.00	0.00	1.00	1.00	0.637	1.00	0.00	1.00	1.00	0.820
B ₁₂ (Cont.): Folate Q5	1.00	0.00	1.00	1.00	0.438	1.00	0.00	1.00	1.00	0.560
MoCA										
B ₁₂ (Cont.): Folate Q2	1.00	0.00	1.00	1.00	0.342	1.00	0.00	1.00	1.00	0.558
B ₁₂ (Cont.): Folate Q3	1.00	0.00	1.00	1.00	0.534	1.00	0.00	1.00	1.00	0.882
B ₁₂ (Cont.): Folate Q4	1.00	0.00	1.00	1.00	0.687	1.00	0.00	1.00	1.00	0.764
B ₁₂ (Cont.): Folate Q5	1.00	0.00	1.00	1.00	0.400	1.00	0.00	1.00	1.00	0.389

Reference group = 'B₁₂ (Continuous): Folate Q1, IRR =1.00; Q1 folate, 1 - 11 nmol/L (n 376), Q2 11 – 16 nmol/L (n 401), Q3 16 – 22 nmol/L (n 379); Q4 22 – 33 nmol/L (n 439); Q5 33 – 81 nmol/L (n 475)

This table has been published in the paper *O'Connor et al., 2020*, please see **Appendix 11.1.3**

CHAPTER SIX: PAPER 4 - LOW FOLATE PREDICTS ACCELERATED COGNITIVE DECLINE: 8-YEAR FOLLOW-UP OF 3,140 OLDER ADULTS IN IRELAND.

Introduction to Chapter Six

In this chapter, I address **Aim 4** and **Objective 4.1** of the thesis; I investigate the longitudinal associations of folate concentrations with changes in cognitive performance in community-dwelling people over time using sequential assessments of cognitive performance longitudinally at two, four, six- and eight-years follow-up. I hypothesise that low folate was associated with accelerated cognitive decline in older people living in Ireland. The following data is from the fourth published work included in this thesis; *Low folate predicts accelerated cognitive decline: 8-year follow-up of 3,140 older adults in Ireland. (Appendix 11.1.4).*

List of Published Work from Chapter Six

Published Figures

Figure 6.1. Flow Diagram – Analytical sample design and selection

Figure 6.2. Univariate and multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall)

Published Tables

Table 6.1. Details of Cognitive Tests.

Table 6.2. Unadjusted Cognitive Function Scores for All Eligible and Available Participants at Each Wave.

Table 6.3 Characteristics of sample at baseline (2009-2011), according to plasma folate concentrations in quintiles.

Supplementary Table 6.1. Variables missing at each wave.

Supplementary Table 6.2. Characteristics of sample at baseline (2009-2011), according to B₁₂ status.

Supplementary Table 6.3a. Multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) - Model 1.

Supplementary Table 6.4a. Multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) – Model 2.

Supplementary Table 6.5. Sensitivity Analysis: Multivariable longitudinal associations between baseline folate quintiles and cognitive assessments

*(MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall),
sample aged 65 and over: Model 2.*

Published Sections

6.1 Abstract

6.2 Introduction

6.3 Methods

6.4 Statistical Analysis

6.5 Results

6.6 Discussion

6.1. Abstract

Objective

To examine associations of plasma folate concentrations and risk of global and domain- specific cognitive decline in older people.

Methods

Data of 3,140 participants from TILDA, a nationally representative cohort of adults aged ≥ 50 years were used over 8-year follow-up. Biannual cognitive assessments included the Mini-Mental State Examination (MMSE), verbal fluency and immediate and delayed word recall tests (Waves 1-5) and the Montreal Cognitive Assessment, (MoCA) (Waves 1 and 3). Plasma folate concentrations were measured in stored blood collected at baseline. Mixed-effects Poisson and linear regression determined associations between baseline folate concentrations and cognition.

Results

In multivariable-adjusted models of those aged ≥ 50 years at baseline, low folate at baseline (<11.2 nmol/L) was associated with higher proportions of MMSE errors (incidence rate ratio [IRR]=1.10; 95% confidence interval [CI] (1.00,1.21),

lowest vs. highest quintile) over eight years. Plasma folate <21.8 nmol/L predicted declines in episodic memory for immediate (beta [β]=-0.26; 95% CI(-0.48,-0.03), $\beta=-0.29$; 95% CI(-0.50, 0.08) and $\beta=-0.29$; (-0.50,-0.08), for lowest three vs. highest quintile) and delayed recall ($\beta=-0.20$; 95% CI(-0.38,-0.01), $\beta=-0.18$; 95% CI(-0.37,-0.01) and $\beta=-0.19$; (-0.36,-0.01) lowest three vs. highest quintile). There were no significant associations in a subsample aged ≥ 65 years.

Conclusion

In those aged ≥ 50 years, lower concentrations of folate may have differential relationships with cognitive domains. Folate <11.2 nmol/L predicted a decline in global cognitive function, while <21.8 nmol/L predicted poorer episodic

memory. Low folate was associated with accelerated decline in cognitive function and is an important marker for cognitive decline among older people.

6.2. Introduction

Approximately 7% of the world's population aged ≥ 65 years have dementia, a major cause of disability and mortality, that increases exponentially with age (389). Cognitive impairment, a precursor to dementia, can range from mild to severe and is characterised by a deterioration in attention, executive function, learning and memory (390).

Maintenance of cognition is essential for functional independence in ageing. The optimisation of nutritional status has been suggested to be a possible modifiable factor for prevention of cognitive impairment (76) and may be involved in the delay or prevention of chronic diseases including dementia (31, 77), CVD (117), and structural changes in the brain (121, 122, 125).

Folate and vitamin B₁₂ are essential co-factors in one-carbon metabolism, DNA- methylation and nucleotide synthesis, processes that occur in all tissues in the body including the brain and have been linked to neurodegeneration (391). Both vitamins are required for efficient metabolism of HCY, a cytotoxic intermediary amino acid produced by the methionine cycle (391) that has been associated with CVD and stroke, as well as cognitive decline (246). The importance of folate insufficiency in cognitive impairment and dementia has been extensively studied in recent years due to its key role in HCY regulation (197). Several epidemiological studies have shown cross-sectional and prospective associations between low folate and the risk of cognitive impairment and dementia (216, 217, 218, 219, 220, 221, 222, 223, 224).

Overt folate deficiency (<7 nmol/L) has been associated with accelerated ageing processes in the brain (192). While rarely observed in countries where MFAF of staple grains is in place to reduce the incidence of NTD (195), deficiency and low folate are frequently observed in countries without MFAF such as the UK (193) and Ireland (194). TILDA demonstrated a 15% prevalence of low-to-

deficient folate status (<10 nmol/L) among older adults living in a setting of voluntary fortification, highlighting its lack of effectiveness for maintenance of folate status in older people (214).

Using TILDA data, we investigated the longitudinal associations of folate concentrations with changes in cognitive performance in community-dwelling people, including sequential assessments of cognitive function at two, four, six- and eight-years follow-up. We hypothesised that low folate was associated with accelerated cognitive decline in older people living in Ireland.

6.3. Methods

TILDA is a nationally representative prospective cohort study of community-dwelling adults aged ≥ 50 years living in the Republic of Ireland. The study design and sampling procedure have been described previously (313). Briefly, participants were asked to complete a structured interview at each wave, capturing information on their social, financial and health circumstances. At baseline (2009-2011), 8,504 participants were recruited, and data were collected every two years thereafter, with objective health assessments conducted at alternate waves. Data for this study were obtained from Waves 1 to 5 of TILDA, collected between 2009 and 2018. The median interval between interviews for participants between Wave 1 to Wave 2 was 2.0 years, Wave 2 to Wave 3 was 2.3 years, Wave 3 to Wave 4 was 1.7 years and Wave 4 to Wave 5 was 2.0 years. Ethical approval was obtained at each data collection from the Faculty of Health Sciences Research Committee, Trinity College Dublin. Written consent was obtained from all participants.

6.3.1. Assessment of Cognitive Function

Participants underwent a battery of cognitive assessments at each wave which included the Mini-Mental State Examination (318) (MMSE; Global Cognition), a verbal fluency test (Semantic Memory) and immediate and delayed word recall tests (Episodic Memory). The Montreal Cognitive Assessment, (MoCA; Global Cognition) was measured at Waves 1 and 3 (319). **Table 6.1.** describes

the details of the cognitive tests. Data are presented by the cognitive domains for each test. Both the MMSE and MoCA scores are subject to ceiling effects, therefore the numbers of errors were calculated with the outcome representing the count of errors made during the test (30 minus the raw score) and used in analyses rather than total score.

Table 6.1. Details of Cognitive Tests.

Domain	Test	Assessed in Waves	Scoring	Administration	Effect Measure	Interpretation of Effects
Global Cognition	MMSE	1,2,3,4,5	Total number of errors (0-30)	Participants were asked questions comprising attention, memory, orientation, language, and spatial ability	Rate Ratio	>1 = worse cognition
	MoCA	1,3		Participants were asked questions comprising attention, memory, orientation, language, executive function, and spatial ability	Rate Ratio	>1 = worse cognition
Semantic Memory	Verbal fluency	1,2,3,4,5	Total number of words	Participants were asked to name as many animals as possible in 60 seconds	β coefficient	<0 = worse cognition
Episodic Memory	Immediate recall	1,2,3,4,5	Total number of words (0-20)	Participants were read a list of ten words on two occasions and asked to recall as many words as possible from the list immediately after	β coefficient	<0 = worse cognition
	Delayed recall	1,2,3,4,5	Total number of words (0-10)	Participants were asked to recall as many words as possible from the list read previously (approximately 15 minutes delay)	β coefficient	<0 = worse cognition

Notes: Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall. CAPI: computer-assisted personal interview; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

6.3.2. Measurement of B-vitamins

In Wave 1 during the health assessment, a non-fasting blood sample was collected by venepuncture into one 10ml K2EDTA tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10ml ethylene diamine tetra-acetic acid (EDTA; BD, Becton, Dickinson Limited) tubes for long term storage (Sarstedt; Numbrecht, Germany). Samples were kept chilled and were centrifuged (3000 rpm for 15 minutes). Aliquots of plasma were labelled and stored at -80°C within 48 hours of blood collection and stored until required for analysis. The protocol for blood sample collection, processing, and storage used is detailed elsewhere (307). Blood samples were analysed for plasma folate and B₁₂ determined by microbiological assay; *Lactobacillus casei* (*L. rhamnosis* (NCIB 10463; ATCC 27773)), limit of detection 0.6 nmol/L (320) and *Lactobacillus leichmannii* (*L. leichmannii* (*delbrueckii*) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/L (321), respectively. Inter-assay coefficients of variability (CV) for both methods were <10.9%.

6.3.3. Covariates

Socio-economic covariates included were age, sex and educational attainment (primary, secondary or tertiary / higher); lifestyle characteristics including physical activity (Short-Form International Physical Activity Questionnaire) (392), smoking status, alcohol use and use of FA supplements; and health characteristics included weight and height (BMI derived), (kg/m²), depressive symptoms (Center for Epidemiologic Studies Depression Scale (CES-D) scale) (393), hypertension (doctor diagnosis or prescribed medications) and diabetes (doctor diagnosis or prescribed medications). Plasma B₁₂, (categorised as < or ≥258pmol/L, vitamin D (plasma 25-hydroxyvitamin D) and lutein concentrations were also included. Medications (up to 20 prescription and non- prescription medications, including food supplements) were classified by WHO ATC classification codes. These covariates were chosen as they have previously been or were expected to be associated with cognitive performance or folate metabolism.

6.3.4. *Analytical Sample*

Of the 8,504 participants interviewed in Wave 1, 3,871 were eligible at baseline after exclusion criteria (**Figure 6.1**). Individuals aged <50 years (n 330), without health assessment data (n 3,140) and blood samples (n 108), in receipt of B₁₂ injections (n 5), and for whom folate and/or B₁₂ measurements exceeded the 99th percentiles (n 74 and n 371, respectively) were excluded from the analysis. Individuals with MMSE scores of <24 (383) and MoCA below established age and education normative cut-offs (384) at baseline were also excluded (n 605) to remove participants with possible MCI. Prospective analyses used eligible baseline participants with two or more follow-up cognitive measurements and those who did not receive B₁₂ injections in subsequent waves, yielding a final analytical sample of 3,140 participants. Data on missing samples are presented in **Supplementary Table 6.1**.

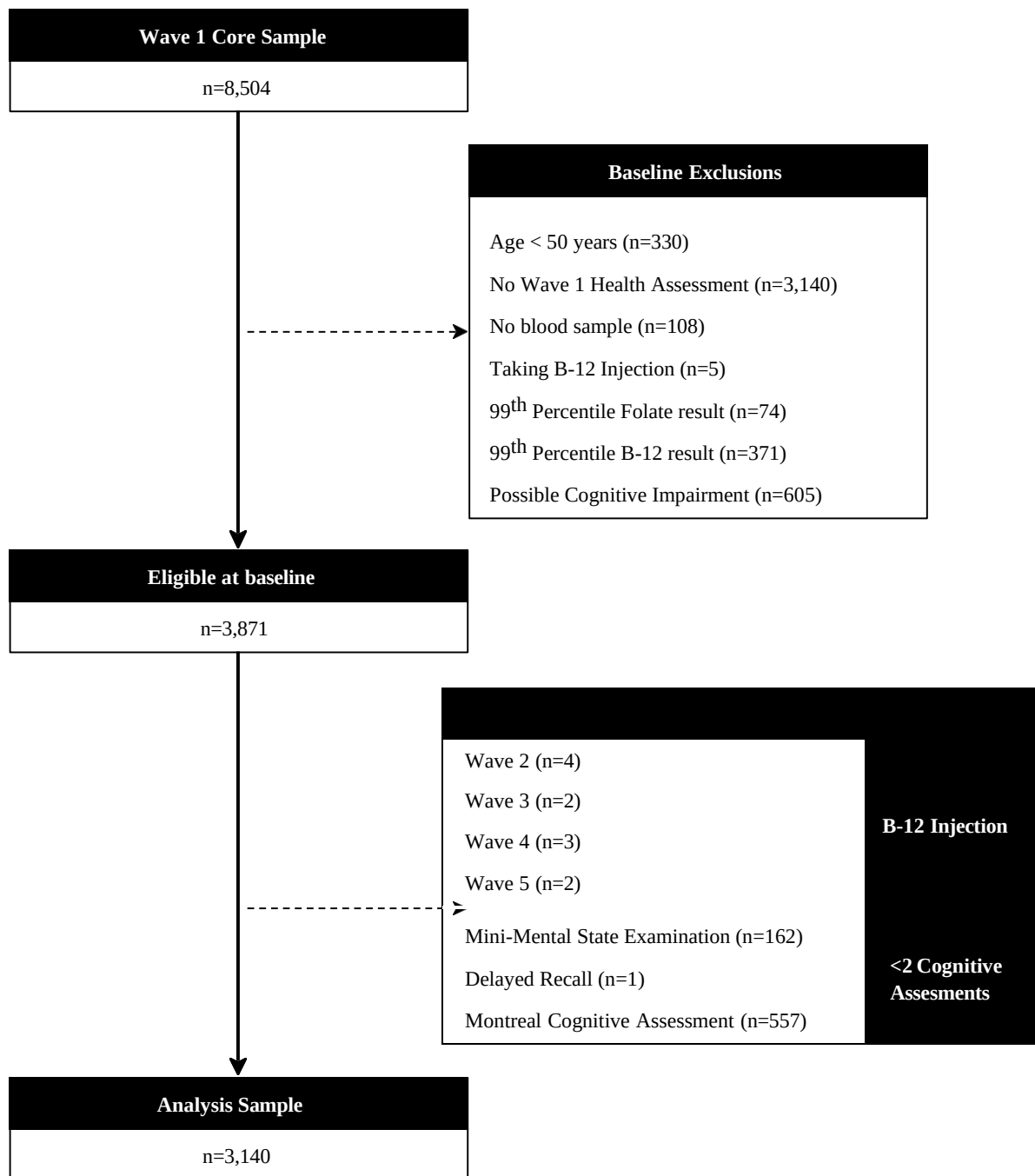


Figure 6.1. Flow Diagram – Analytical sample design and selection

This figure has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

6.4. Statistical Analysis

Baseline characteristics of the sample were summarised by quintiles of plasma folate. Categorical variables were examined using percentages and continuous variables were explored using means and standard deviations, or medians and interquartile ranges, where appropriate. Performance scores for all cognitive tests were obtained for each wave. Histograms and Q-Q plots were used to assess marginal distributions of cognitive variables. Mixed-effects models were used to examine the longitudinal associations between baseline folate status and cognitive function and take account for clustering of repeated measures within the same individual (394).

Cognitive function scores for MMSE and MoCA errors were right-skewed and Poisson models were used. Verbal fluency, immediate and delayed recall were modelled using linear regression models. Models were adjusted for covariates as defined previously and treated as time-varying fixed effects, where appropriate. Age squared (age^2) was added to each model to account for the curvilinear relationship of age and cognition.

Creatinine was included as a continuous covariate in the initial test models for Model 2, however this yielded no additional effects so in the interest of maintaining the most parsimonious model possible, creatinine was removed from the final version of Model 2. Interaction terms for time and predictor variables were included to model the associations between folate quintile group membership at baseline and change in cognitive function at subsequent waves within the respective groups.

Intraclass correlation coefficients (ICC) for the linear models were calculated to measure the proportion of variance attributable to variance between individuals. We initially ran random intercept only models for cognitive function measures without predictor variables, revealing intraclass correlation coefficients of 0.52 (95% CI 0.50,0.54), 0.52 (95% CI 0.51,0.54) and 0.57 (95% CI 0.55,0.58), suggesting that 52%, 52% and 57% of the variation in verbal fluency, immediate and delayed recall was within individuals, respectively. To examine whether differences in overall folate quintile group

membership (between-person) were associated with cognitive function over time, interactions between folate status and wave were included as covariates in the models. This allowed us to address in the same model whether participants with low folate on average had worse cognitive function and whether these effects changed over time. Model type was dependent on the distribution of the cognitive outcome variable.

Separate models were run for each cognitive outcome with the theoretically most favourable folate quintile (highest) used as the reference category. The analysis allowed us to examine if the effect of the association across quintiles of folate was apparent over the full exposure distribution (or was limited to the extremes). Model 1 are unadjusted regression models. Model 2 adjusts for age, age squared, sex, socio-demographic, behavioural, lifestyle and medical covariates. Statistical significance was defined as $P < 0.05$ and were performed using Stata 15.1 (StataCorp, College Station, TX). [OBJ]

6.5. Results

6.5.1. Baseline Characteristics

The mean (standard deviation) age of the baseline sample was 62.1 years (7.2), 52.7% were female. Cognitive scores for participants at each wave show small differences between waves, with the most notable decline evident with verbal fluency (**Table 6.2**). Sample characteristics were compared by quintiles of folate concentrations at Wave 1 and are summarised in **Table 6.3**. Relative to those in the highest quintile, participants in the lowest quintile of folate at baseline ($<11.2\text{nmol/L}$) were more likely to be male, smoke, classified as obese ($\text{BMI} \geq 30$), have higher levels of creatinine, report depressive symptoms, and have lower mean B_{12} and vitamin D. Furthermore, they were less likely to use a FA supplement. Cognitive function scores did not differ across quintile groups at Wave 1. **Supplemental Table 6.2.** summarises the sample characteristics that were compared by B_{12} groups (Deficient $<148\text{pmol/L}$, Low $148 - <258\text{pmol/L}$ and Normal $\geq 258\text{pmol/L}$).

Table 6.2. Unadjusted Cognitive Function Scores for All Eligible and Available Participants at Each Wave.

	Wave 1	Wave 2	Wave 3	Wave 4	Wave 5
Global Cognition					
MMSE Errors^a	1 (0-2)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)
MoCA Errors^a	4 (2-5)	-	3 (2-5)	-	-
Semantic Memory					
Verbal Fluency^b	22.4 ± 6.9	20.5 ± 5.9	19.9 ± 5.7	19.6 ± 5.7	19.5 ± 5.7
Memory					
Immediate Recall^b	14.1 ± 2.8	14.5 ± 2.8	14.3 ± 2.9	14.2 ± 3.0	14.1 ± 3.0
Delayed Recall^b	6.6 ± 2.1	6.6 ± 2.3	6.5 ± 2.3	6.4 ± 2.4	6.4 ± 2.4

Notes: Available cognitive data varies for each test depending on whether it was included in the interview or health assessment; data from Wave 1 (collected October 2009 to July 2011; N = 3,123–3,140), Wave 2 (collected February to December 2012; N = 3,077–3,095), Wave 3 (collected March 2014 to December 2015; N = 2,962–3,136), Wave 4 (collected January to December 2016; N = 2,919–2,939) and Wave 5 (collected January 2018 to December 2018; N = 2,664–2,691). MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall.

^amedian (IQR) (IQR= interquartile range)

^b mean ± SD (SD = Standard Deviation)

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

Table 6.3. Characteristics of sample at baseline (2009-2011), according to plasma folate concentrations in quintiles.

Characteristic n	All Participants 3,140	Plasma Folate Level, nmol/L					p-value ¹
		Quintile 1 0.9-11.2 nmol/L 573	Quintile 2 11.2-15.6 nmol/L 638	Quintile 3 15.7-21.8 nmol/L 629	Quintile 4 21.8-32.5 nmol/L 646	Quintile 5 32.6-81.3 nmol/L 654	
Age, mean ± SD	61.4 ± 8.0	61.6 ± 8.4	60.7 ± 8.0	60.6 ± 7.7	61.7 ± 8.0	62.3 ± 7.9	<0.001
Female, N (%)	1,661 (52.9)	290 (50.6)	303 (48.4)	330 (52.5)	345 (53.4)	387 (59.2)	<0.01
Educational Attainment, N (%)							
Primary	639 (20.4)	121 (21.1)	139 (21.8)	132 (21.0)	131 (20.3)	116 (17.7)	0.418
Secondary	1,377 (43.9)	264 (46.1)	272 (42.6)	277 (44.0)	284 (44.0)	280 (42.8)	
Tertiary	1,124 (35.8)	188 (32.8)	227 (35.6)	220 (35.0)	231 (35.8)	258 (39.5)	
Lives with someone, N (%)	2,639 (83.7)	94 (16.4)	106 (16.6)	106 (16.9)	91 (14.1)	114 (17.4)	0.537
Smoke, N (%)	426 (13.6)	91 (15.9)	113 (17.7)	75 (11.9)	75 (11.6)	72 (11.0)	<0.01
Alcohol Use, N (%)	2,379 (80.4)	415 (78.6)	474 (79.8)	486 (81.5)	495 (80.1)	509 (81.7)	0.658
BMI, mean ± SD	27.5 ± 4.7	27.8 ± 4.8	27.9 ± 5.2	27.6 ± 4.4	27.6 ± 4.4	27.0 ± 4.5	<0.05
Obese, N (%)	1013 (32.4)	191 (33.5)	218 (34.3)	219 (35.0)	215 (33.5)	170 (26.1)	<0.001
Exercise Group, N (%)							
Low	836 (26.6)	158 (27.6)	170 (26.7)	164 (26.1)	174 (26.9)	170 (26.0)	0.855
Moderate	1,116 (35.5)	207 (36.1)	209 (32.8)	227 (36.1)	232 (35.9)	241 (36.9)	
High	1,188 (37.8)	208 (36.3)	259 (40.6)	238 (37.8)	240 (37.2)	243 (37.2)	
Creatinine, mean (95% CI)*	78.9 [78.4,79.6]	81.4 [79.9,82.9]	79.1 [77.7 80.4]	79.1 [78.1,81.1]	78.2 [76.9,79.6]	76.9 [75.6,78.1]	<0.01
Women, mean (95% CI)*	70.4 [69.8,71.1]	71.8 [70.3,73.3]	70.4 [68.8,71.9]	70.5 [69.1,72.0]	69.8 [68.4,71.2]	69.9 [68.5,71.3]	0.883
Men, mean (95% CI) *	88.8 [87.9,89.7]	91.6 [89.4,93.8]	87.4 [85.6,89.1]	89.5 [87.2,91.8]	87.9 [86.0,89.9]	87.5 [85.8,89.3]	<0.01
Folic Acid Supplement Use, N (%)	107 (3.4)	13 (2.3)	8 (1.3)	20 (3.2)	22 (3.4)	44 (6.7)	<0.001
Number of Medications, mean ± SD	2.3 ± 2.4	2.3 ± 2.6	2.2 ± 2.6	2.0 ± 2.1	2.4 ± 2.4	2.4 ± 2.4	0.061
High blood pressure, N (%)	1,259 (40.3)	245 (43.0)	259 (40.7)	224 (35.8)	257 (40.1)	274 (42.0)	0.102
Diabetes, N (%)	192 (6.2)	32 (5.6)	36 (5.7)	36 (5.8)	48 (7.6)	40 (6.2)	0.588
History of Stroke, N (%)	30 (1.0)	6 (1.1)	5 (0.8)	9 (1.4)	2 (0.3)	8 (1.2)	0.278
CESD, mean ± SD	5.2 ± 6.5	5.3 ± 7.0	5.2 ± 6.7	5.4 ± 6.5	5.3 ± 6.5	4.8 ± 6.0	0.561

Folate nmol/L, mean (95% CI)*	23.1 [22.6,23.6]	8.8 [8.7,9.0]	13.4 [13.2,13.5]	18.6 [18.5,18.7]	26.4 [26.2,26.6]	46.5 [45.6,47.3]	<0.001
B₁₂ pmol/L, mean (95% CI)*	333.5 [330.6,337.5]	290.8 [282.2,299.5]	300.2 [292.3,308.2]	335.6 [327.2,344.0]	349.2 [340.8,357.6]	386.5 [377.2,395.0]	<0.001
Vitamin D nmol/L, mean (95% CI)*	53.4 [52.5,54.2]	46.4 [44.6,48.3]	49.6 [47.9,51.5]	51.7 [50.0,53.5]	56.8 [55.0,58.7]	62.8 [61.0,64.6]	<0.001
Lutein nmol/L, mean (95% CI)*	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	<0.001
MMSE errors, median (IQR)	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-1)	0.094
MoCA errors, median (IQR)	4 (2-5)	4 (2-6)	4 (2-5)	4 (2-5)	4 (2-5)	3.5 (2-5)	<0.05
Verbal Fluency, mean ± SD	22.4 ± 6.9	22.3 ± 6.8	22.4 ± 7.2	22.5 ± 6.7	22.3 ± 6.8	22.5 ± 6.8	0.979
Immediate Recall, mean ± SD	14.1 ± 2.8	14.0 ± 2.9	14.0 ± 2.9	14.1 ± 0.8	14.3 ± 2.8	14.3 ± 2.8	0.102
Delayed Recall, mean ± SD	6.6 ± 2.1	6.4 ± 2.2	6.5 ± 2.1	6.5 ± 2.1	6.5 ± 2.2	6.7 ± 2.1	0.289

SD, standard deviation; CI, confidence interval.

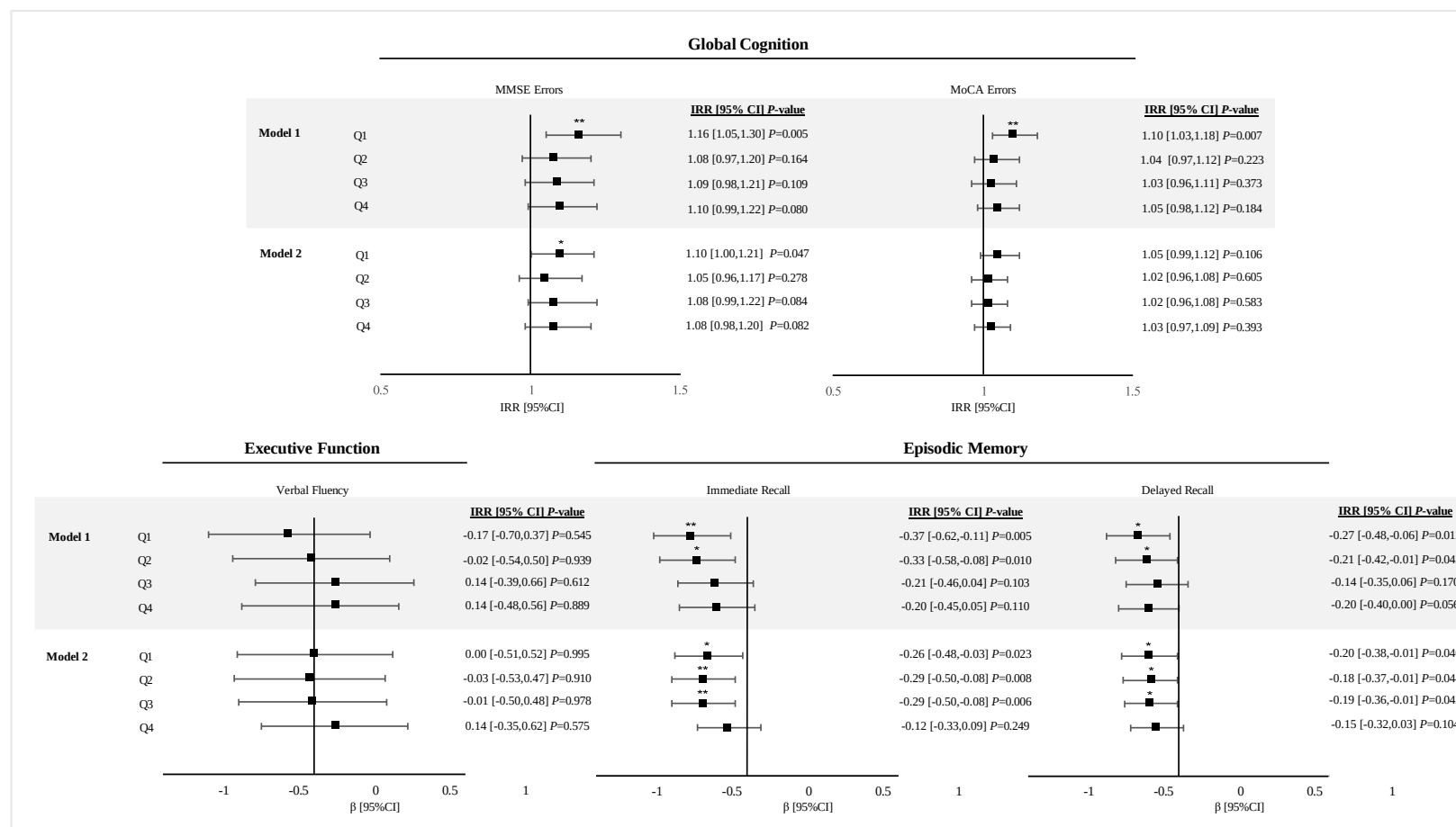
*geometric mean

¹Significance testing used one-way ANOVAs for normally distributed continuous characteristics, Kruskal-Wallis for non-normally distributed continuous characteristics and chi-square tests for categorical characteristics.

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

6.5.2. Regression Analyses

Regression analyses examining associations between baseline folate and cognitive function are presented in **Figure 6.2**. Multivariable-adjusted models demonstrated that folate levels of <11.2 nmol/L (lowest quintile) at baseline were associated with increases of MMSE errors (Incidence Rate Ratio [IRR]=1.10; 95% confidence interval [95% CI]1.00,1.21), over eight years when compared to the reference category, the highest quintile (32.6-81.3 nmol/L). Baseline folate levels <21.8nmol/L (quintiles 1-3) predicted an overall decline in episodic memory for both immediate (beta [β]=-0.26; (95% CI-0.48,-0.03), β =-0.29; (-0.50,-0.08) and β =-0.29; (-0.50,-0.08), for quintiles 1-3 vs. highest quintile) and delayed recall (β =-0.20; 95% CI= -0.38 to -0.01, β =-0.18; 95% CI -0.37,0.01 and β =-0.19; (-0.36,-0.01) for quintiles 1-3 vs. highest quintile) over eight years. In Model 1, significant increases in the number of MoCA errors amongst those in the lowest quintile of folate (<11.2 nmol/L) were observed (IRR=1.10; 95% CI 1.03 to 1.18) over four years, however, this effect was attenuated with adjustment for additional covariates (Model 2). Results for Models 1 and 2 are presented in **Supplemental Tables 6.3** and **6.4**, respectively.



Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L); Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L). ^aIRR = Incident Rate Ratio; ^b β =Beta Coefficient; ** $P<0.01$ * $P<0.05$

Model 1 = Basic model. Longitudinal cognitive variable and quintile of baseline folate controlling for time (wave).

Model 2 = Socio-demographic, Health Indicators, Lifestyle, & Medical model. Model 1+ additional adjustment for age, age2, sex, educational attainment, baseline vitamin B₁₂ status (<or $\geq$ 258pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, anti-hypertensive medications.

Figure 6.2. Univariate and multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall).

This figure has been published in the paper O'Connor et al., 2022, please see **Appendix 11.1.4**

6.5.3. *Sensitivity Analysis*

Regression estimates of the sensitivity analysis conducted on sub-samples of participants are presented in **Supplemental Tables 6.5**. Among the sub-sample aged ≥ 65 years, there were no significant associations between baseline folate and changes in cognitive function.

6.6. Discussion

This study investigated the hypothesis that low folate status was associated with accelerated cognitive decline by examining the longitudinal associations between baseline folate status and repeated measures of five tests of cognitive function. To our knowledge, this is the largest longitudinal study to examine this issue using repeated measurements of tests exploring multiple cognitive domains. The results indicate that over an eight-year follow-up period, low folate (<11.2 nmol/L) was associated with decline in global cognitive function and a modest insufficiency (<21.8 nmol/L) predicted declines in both immediate and delayed recall.

Folate insufficiency is often observed among older Europeans and increases in prevalence with age (193). Causes of deficiency include decreased dietary intake and malabsorption, age-related impairments in folate transport and metabolism, use of anti-folate medications, genetic factors, and excessive alcohol intake (213). These findings are consistent with other studies showing low folate status was associated with higher risks of cognitive impairment or dementia (216, 217, 218, 219, 220, 221, 222, 223, 224). In one study of older adults (≥ 60 years), those with MCI or dementia had significantly lower serum folate levels than healthy controls. Subjects in the lowest tertile (<13.5 nmol/L) had an adjusted Odds Ratio (OR) of 3.8 (95% CI: 1.3, 11.2; $P=0.018$) for dementia (216). Further, a meta-analysis examining thirteen studies (≥ 60 years) demonstrated low folate was associated with general and specific impairments in cognition such as attention, episodic and visuospatial memory, or abstract reasoning (OR=1.66, 95% CI: 1.40, 1.96) (222).

In the current study, the results for episodic memory add to emerging evidence that folate may have differential relationships with specific domains of cognition. This may be due to its role in *hippocampal* neurogenesis, as shown in animal models (395, 396). The hippocampus is among the most important structures in the brain involved in memory (397) and is critical in the pathogenesis of diseases such as AD and dementia. High concentrations of HCY have been associated with hippocampal atrophy (398). Adequate folate is essential for balanced HCY levels and normal protein methylation processes in the brain, needed for cell signalling pathways, therefore it is conceivable that B-vitamin-related memory deficits could be due to

changes of hippocampal neuronal processes linked to encoding information in episodic memory.

One of the interesting findings of this study was the association between low folate levels and higher numbers of MMSE errors over time, but not in the subsample aged ≥ 65 years from baseline. This may seem unexpected at first; however, it can be explained by the limitations of the MMSE as a tool for detecting subtle cognitive changes. While the MMSE is widely used, it is often considered a relatively crude measure of cognitive function, particularly in older adults, as it primarily assesses orientation, memory, and attention but lacks sensitivity in capturing more nuanced cognitive deficits. Other more sensitive measures of cognitive function, such as the Montreal Cognitive Assessment (MoCA), are better suited to detect early signs of cognitive impairment in areas such as executive function and memory, which the MMSE does not assess as thoroughly. Therefore, it is possible that the more sensitive cognitive tests used in this study—such as immediate and delayed recall—better captured the impact of low folate levels on cognitive decline.

The lack of significant findings in the subsample aged ≥ 65 years at baseline could also be due to the more pronounced cognitive decline already present in this group, making it more challenging to detect the subtle, longitudinal effects of low folate status that might be more evident in younger age groups. Cognitive reserve and age-related physiological changes may also obscure folate's impact on cognitive decline in this older cohort.

Our results also suggest that there may be a continuum of folate levels related to performance even within the 'low' and 'low-normal' ranges established in literature (214). Over eight years, low folate was associated with declining MMSE, a routine screening test often regarded as a crude indicator of cognitive performance.

While MMSE was a key test in many studies assessing B-vitamins and cognitive function, additional tests were included to add granularity to our study and to explore subtle changes across separate domains of cognition. Using more sensitive tests, such as MoCA and episodic memory assessments, allowed us to capture the effects of low folate on cognitive decline more precisely. Using folate quintiles, a modest

insufficiency at baseline predicted a decline in episodic memory, suggesting it may be subtle enough to predict deleterious effects among more sensitive cognitive tests.

Evidence from randomised controlled trials (RCTs) using FA and other B-vitamins to lower HCY have shown no consistent benefit of supplementation over approximately five years on cognitive outcomes. FA supplementation was associated with improved domain-specific cognitive performance in RCTs with large samples and ≥ 2 years follow-up (236, 237, 238). Another trial examined individuals with high HCY to exclude causes other than low folate concentrations and FA supplementation (0.8mg oral FA per day) was associated with improved memory, processing speed and sensorimotor speed after three years (237).

Furthermore, supplementation of daily oral 0.4mg FA and 100 μ g B₁₂ promoted improvement in cognitive function after two years, particularly in immediate and delayed recall (238), while FA supplementation (0.4mg per day for 2 years) for individuals with MCI (*n* 180) was associated with better performance in tests of both memory and attention (223). However, a meta-analysis of 11 RCTs involving 37,485 individuals by Clarke et al., concluded that HCY-lowering by supplementation with B-vitamins for five years had no significant effect on individual domains or global cognitive function (247). Further, a recent systematic review and meta-analysis of 31 RCTs of B-vitamin supplementation in individuals with and without cognitive impairment also concluded that B-vitamins did not improve cognitive performance or slow cognitive decline compared to placebo (399). The interpretation of such findings should however consider the limitations of available data and lack of an obvious benefit of lowering HCY using B-vitamins, rather than evidence of no effect. Inconsistencies among RCT results may be explained by chance, bias, power issues or possible methodological differences between the studies, including FA fortification and trial duration, thereby making it difficult to draw firm conclusions.

It is plausible that biological mechanisms other than HHCY may underlie the associations between low folate and cognitive impairment, with proposed mechanisms including impaired methylation and mis-incorporation of uracil into DNA. The complex synergistic interactions between nutrients are also important to consider. Vitamins and nutrients often function as a collection of cofactors,

therefore interventions using singular or closely related compounds may have too narrow a focus. This is illustrated by another trial using FA, vitamin B₆ and B₁₂, showing treatment was effective only in those with high baseline n-3 fatty acid concentrations. In fact, n-3 fatty acid status was protective against brain atrophy only in the presence of B-vitamin supplementation, suggesting that both are needed for effectiveness (243).

The major dietary source of folate is green leafy vegetables and lower incidence of AD or dementia has been associated with higher dietary folate intake (400). Thus, even if low folate status is a surrogate for a less healthy diet and consequently is associated with poorer cognitive function, it is important nonetheless to consider what it tells us about individuals with lower folate levels. The present study shows that those with lower baseline folate were more likely to be male, smokers and obese, to have lower mean B₁₂ and vitamin D levels, but also had higher creatinine levels. They were less likely to use a FA supplement, which may be an indicator of lower socio-economic status. While it is possible that those taking dietary supplements engage in healthier behaviours which may positively impact cognitive performance, FA-containing supplement use was low, ranging from 3-6% in the whole sample (Table 6.3).

Limitations were present. The analysis was limited to the use of non-fasting plasma folate and total vitamin B₁₂ and additional B₁₂-biomarkers such as methyl-malonic acid and holotranscobalamin were not measured. The B-vitamin analysis was conducted at one time point, thus there is a possibility of regression dilution. Longitudinal studies are subject to attrition; therefore, selection bias cannot be ruled out leading to under- or over-estimation of observed associations. This, and the likelihood that cognitive decline commenced earlier than age 65, may explain the lack of associations observed in the subsample aged ≥ 65 years. While our analysis controlled for a wide range of known covariates it is impossible to eliminate the risk of residual confounding. The factors controlled for in the statistical analyses were chosen *a priori* to be consistent with previous publications using folate and cognitive function in this population (235). Finally, the sample consisted of high-functioning, healthy and younger community-dwelling participants and may not represent the older population living in residential care.

This study has several strengths. The five cognitive tests examined encompassed three domains, global cognition, semantic and episodic memory. The longitudinal design included multiple assessments over eight years, objective assessments of vitamin biomarkers using gold standard methods and detailed phenotypic data pertaining to socio-demographic and lifestyle factors, diseases and medication use in a large, well- characterised sample from one population. The use of mixed-effects models in addition to the consideration of relevant covariates and interactions in the analyses, adds to the rigor of the current study. This study was performed in Ireland, a country that does not have MFAF, and this should be considered a strength. With a high prevalence of folate insufficiency observed in the population, it allows reliable assessment of the associations with cognitive function over time.

In summary, this study has shown that a decline in global cognitive performance and episodic memory among older adults that were cognitively normal can be predicted by low baseline folate levels, and therefore it may be an important indicator for risk of early decline. Additionally, the limitations of the MMSE as a crude measure of cognitive function are evident, highlighting the need to use more sensitive tests like the MoCA and episodic memory recall to capture subtle cognitive changes. This could have implications for clinical and public health policy recommendations for prevention of cognitive decline in older people.

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Supplemental Table 6.1. Variables missing at each wave.

	Wave 1 (n 3,140)	Wave 2 (n 3,095)	Wave 3 (n 3,140)	Wave 4 (n 2,949)	Wave 5 (n 2,707)
MMSE	0	18	25	30	43
MoCA	0	-	178	-	-
Verbal Fluency	16	0	4	10	16
Immediate Recall	1	0	4	15	17
Delayed Recall	17	0	6	15	17
<i>Covariates</i>					
Folate	0	-	-	-	-
Vitamin B₁₂	0	-	-	-	-
Vitamin D	0	-	-	-	-
Lutein	68	-	-	-	-
Age	0	0	0	0	0
Sex	0	0	0	0	0
Educational Attainment	0	0	0	0	0
Smoking	0	0	1	0	1
Alcohol Use	181	244	295	281	314
BMI	14	41	70	89	116
Exercise Group	0	27	150	0	0
Folate Supplement	0	0	0	0	0
Anti-Hypertensive Medication	0	0	0	16	3
Diabetes	20	0	0	0	0
CES-D	38	29	13	19	27

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

Supplemental Table 6.2. Characteristics of sample at baseline (2009-2011), according to vitamin B₁₂ status.

Characteristic n	All Participants	B ₁₂ Level, pmol/L			p-value ¹
		Deficient <148 pmol/L	Low ≥148 – <258 pmol/L	Normal ≥258 pmol/L	
	3,140	124 (4.0)	773 (24.6)	2,243 (71.4)	
Age, mean ± SD	61.4 ± 8.0	63.2 ± 8.2	61.8 ± 8.3	61.1 ± 7.9	<0.001
Female, N (%)	1661	65 (52.4)	384 (49.7)	1,212 (54.0)	0.111
Educational Attainment, N (%)					
Primary	639 (20.4)	34 (27.4)	175 (22.6)	430 (19.2)	<0.05
Secondary	1,377 (43.9)	58 (46.8)	328 (42.2)	991 (44.2)	
Tertiary	822 (36.7)	32 (25.8)	270 (34.9)	822 (36.7)	
Lives with someone, N (%)	2,629 (83.7)	98 (79.0)	640 (82.8)	1,891 (83.7)	0.217
Smoke, N (%)	426 (13.6)	21 (16.9)	122 (15.8)	283 (12.6)	<0.05
Alcohol Use, N (%)	2,379 (80.4)	94 (78.3)	580 (81.0)	1,705 (80.3)	0.778
BMI, mean ± SD	27.5 ± 4.7	28.0 ± 4.8	28.0 ± 5.1	27.4 ± 4.5	<0.05
Obese, N (%)	1,013 (32.4)	44 (35.5)	277 (36.0)	692 (31.0)	0.061
Exercise Group, N (%)					
Low	836 (26.6)	35 (28.2)	226 (29.2)	575 (25.6)	0.332
Moderate	1,116 (35.5)	44 (35.5)	25 (33.3)	815 (36.3)	
High	1,188 (37.8)	45 (36.3)	290 (37.5)	853 (38.0)	
Creatinine, mean (95% CI) *	76.9 [76.3,77.5]	78.5 [75.4,81.7]	77.5 [76.3,78.7]	76.7 [76.0,77.4]	0.310
Women, mean (95% CI) *	69.0 [68.4,69.6]	69.0 [68.4,69.6]	69.3 [68.0,70.6]	68.8 [68.0,69.5]	0.338
Men, mean (95% CI) *	87.0 [86.2,87.8]	86.9 [81.9,92.3]	86.5 [84.9,88.1]	87.1 [86.2,88.1]	0.725
Folic acid supplement Use, N (%)	108 (3.4)	2 (1.6)	26 (3.4)	79 (3.5)	0.520

Number of Medications, mean ± SD	2.3 ± 2.4	2.0 ± 2.2	2.5 ± 2.6	2.2 ± 2.3	0.440
High blood pressure, N (%)	1,259 (40.3)	59 (48.8)	332 (43.2)	868 (38.8)	<0.05
Diabetes, N (%)	192 (6.2)	11 (8.9)	63 (8.2)	118 (5.3)	<0.01
History of Stroke, N (%)	30 (1.0)	21 (1.1)	1 (0.8)	2 (0.7)	0.650
CESD, mean ± SD	5.2 ± 6.5	5.6 ± 8.1	5.4 ± 6.8	5.1 ± 6.3	0.452
Folate nmol/L, mean (95% CI)*	19.5 [19.1,19.9]	14.0 [12.6,15.5]	16.4 [15.8,17.0]	21.1 [20.6,21.6]	<0.001
B₁₂ pmol/L, mean (95% CI)*	311.3 [307.0,315.5]	113.3 [108.4,118.5]	210.1 [207.9,212.3]	376.9 [373.3,380.6]	<0.001
Vitamin D nmol/L, mean (95% CI)*	53.4 [52.5,54.2]	50.0 [46.4,53.9]	50.0 [48.4,51.6]	54.8 [53.8,55.8]	<0.001
Lutein nmol/L, mean (95% CI)*	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.2 [0.2,0.2]	0.121
MMSE errors, median (IQR)	1.0 ± 1.2	1.1 ± 1.3	1.1 ± 1.3	1.0 ± 1.2	0.648
MoCA errors, median (IQR)	3.9 ± 2.5	4.3 ± 2.6	4.0 ± 2.5	3.9 ± 2.5	0.052
Verbal Fluency, mean ± SD	22.4 ± 6.9	23.0 ± 7.2	22.0 ± 6.8	22.5 ± 6.9	0.182
Immediate Recall, mean ± SD	14.1 ± 2.8	13.5 ± 3.1	14.1 ± 2.8	14.2 ± 2.8	<0.05
Delayed Recall, mean ± SD	6.6 ± 2.1	6.3 ± 2.2	6.6 ± 2.1	6.5 ± 2.1	0.258

SD, standard deviation; CI, confidence interval

*Geometric mean

¹Significance testing used one-way ANOVAs for normally distributed continuous characteristics, Kruskal-Wallis for non-normally distributed continuous characteristics and chi-square tests for categorical characteristics.

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

Supplemental Table 6.3a. Multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) - Model 1

n	MMSE Errors			MoCA Errors			Verbal Fluency			Immediate Recall			Delayed Recall		
	IRR	[95% CI]	P	IRR	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P
	2,393			2,393			2,393			2,393			2,393		
Fixed Effects															
Q1	1.16	[1.05,1.30]	0.005	1.10	[1.03,1.18]	0.007	-0.17	[-0.70,0.37]	0.545	-0.37	[-0.62, -0.11]	0.005	-0.27	[-0.48, -0.06]	0.012
Q2	1.08	[0.97,1.20]	0.164	1.04	[0.97,1.12]	0.223	-0.02	[-0.54,0.50]	0.939	-0.33	[-0.58, -0.08]	0.010	-0.21	[-0.42, -0.01]	0.043
Q3	1.09	[0.98,1.21]	0.109	1.03	[0.96,1.11]	0.373	0.14	[-0.39,0.66]	0.612	-0.21	[-0.46,0.04]	0.103	-0.14	[-0.35,0.06]	0.170
Q4	1.10	[0.99,1.22]	0.080	1.05	[0.98,1.12]	0.184	0.14	[-0.48,0.56]	0.889	-0.20	[-0.45,0.05]	0.110	-0.20	[-0.40,0.00]	0.056
Wave 2	0.84	[0.80,0.88]	0.000	-	-	-	-1.86	[-2.06, -1.65]	0.000	0.35	[0.25,0.45]	0.000	0.07	[-0.01,0.16]	0.213
Wave 3	0.93	[0.88,0.97]	0.003	0.90	[0.88,0.92]	0.000	-2.46	[-2.67, -1.26]	0.000	0.15	[0.06,0.25]	0.002	-0.01	[-0.09,0.07]	0.243
Wave 4	0.95	[0.90,0.99]	0.046	-	-	-	-2.89	[-3.10,2.26]	0.000	0.03	[-0.07,0.13]	0.468	-0.17	[-0.25, -0.08]	0.000
Wave 5	0.93	[0.88,0.98]	0.006	-	-	-	-3.08	[-3.30, -2.86]	0.000	-0.14	[-0.25, -0.04]	0.001	-0.27	[-0.36, -0.18]	0.000
Random Effects															
σ²Individual	0.62	[0.57,0.67]		0.24	[0.22,0.27]		18.48	[17.28,19.77]		4.36	[4.11,4.63]		3.03	[2.87,3.22]	
σ²Residual							17.00	[16.52,17.49]		3.98	[3.88,4.08]		2.31	[2.24,2.37]	
ICC							0.52	[0.50,0.54]		0.52	[0.51,0.54]		0.57	[0.55,0.58]	

Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L).

Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L).

^aIRR = Incident Rate Ratio;

^b β =Beta Coefficient

Model 1 = Basic model. Longitudinal cognitive variable and quintile of baseline folate controlling for time (wave).

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

Supplemental Table 6.3b. Multivariable longitudinal associations between baseline vitamin B₁₂ and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) - Model 1

	MMSE Errors			MoCA Errors			Verbal Fluency			Immediate Recall			Delayed Recall		
	IRR	[95% CI]	P	IRR	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P
n	2,393			2,393			2,393			2,393			2,393		
Fixed Effects															
Vitamin B ₁₂ ≥ 258pmol/L	1.08	[0.98,1.319]	0.190	1.02	[0.94,1.09]	0.290	0.02	[-0.40,0.44]	0.912	-0.12	[-0.38, 0.13]	0.320	-0.11	[-0.30, 0.08]	0.254
Wave 2	0.85	[0.81,0.89]	0.000	-	-	-	-1.80	[-2.04, -1.56]	0.000	0.30	[0.20,0.40]	0.000	0.05	[-0.04,0.14]	0.271
Wave 3	0.91	[0.86,0.95]	0.003	0.89	[0.85,0.92]	0.001	-2.50	[-2.70, -2.30]	0.000	0.20	[0.08,0.32]	0.012	-0.01	[-0.09,0.07]	0.345
Wave 4	0.93	[0.89,0.97]	0.012	-	-	-	-2.87	[-3.12,-2.62]	0.000	0.06	[-0.04,0.16]	0.223	-0.16	[-0.25, -0.06]	0.018
Wave 5	0.91	[0.87,0.96]	0.006	-	-	-	-3.02	[-3.27, -2.77]	0.000	-0.13	[-0.23, -0.03]	0.003	-0.23	[-0.32, -0.14]	0.004
Random Effects															
σ ² Individual	0.60	[0.56,0.65]		0.22	[0.20,0.26]		16.80	[15.70,17.90]		4.22	[4.02,4.42]		2.89	[2.71,3.08]	
σ ² Residual							15.00	[14.40,15.60]		3.80	[3.72,3.88]		2.31	[2.17,2.31]	
ICC							0.56	[0.54,0.58]		0.54	[0.52,0.56]		0.55	[0.53,0.57]	

Reference category: Plasma vitamin B₁₂ <258pmol/L

^aIRR = Incident Rate Ratio;

^bβ=Beta Coefficient

Model 1 = Basic model. Longitudinal cognitive variable and quintile of baseline vitamin B₁₂ controlling for time (wave).

Supplemental Table 6.4a. Multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) - Model 2

n	MMSE Errors			MoCA Errors			Verbal Fluency			Immediate Recall			Delayed Recall		
	IRR	[95% CI]	P	IRR	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P
	2,393			2,393			2,393			2,393			2,393		
Fixed Effects															
Q1	1.10	[1.00,1.21]	0.047	1.05	[0.99,1.12]	0.106	0.00	[-0.51,0.52]	0.995	-0.26	[-0.48, -0.03]	0.023	-0.20	[-0.38, -0.01]	0.040
Q2	1.05	[0.96,1.17]	0.278	1.02	[0.96,1.08]	0.605	-0.03	[-0.53,0.47]	0.910	-0.29	[-0.50, -0.08]	0.008	-0.18	[-0.37, -0.01]	0.044
Q3	1.08	[0.99,1.22]	0.084	1.02	[0.96,1.08]	0.583	-0.01	[-0.50,0.48]	0.978	-0.29	[-0.50, -0.08]	0.006	-0.19	[-0.36, -0.01]	0.042
Q4	1.08	[0.98,1.20]	0.082	1.03	[0.97,1.09]	0.393	0.14	[-0.35,0.62]	0.575	-0.12	[-0.33,0.09]	0.249	-0.15	[-0.32,0.03]	0.104
Wave 2	0.76	[0.69,0.84]	0.000	-	-	-	-1.34	[-1.88, -0.81]	0.000	0.58	[0.34,0.82]	0.000	0.07	[-0.12,0.26]	0.478
Wave 3	0.73	[0.66,0.80]	0.000	0.91	[0.86,0.96]	0.001	-1.41	[-1.97, -0.84]	0.000	0.50	[0.25,0.75]	0.000	0.22	[0.02,0.42]	0.035
Wave 4	0.73	[0.66,0.81]	0.000	-	-	-	-2.09	[-2.67, -1.51]	0.000	0.56	[0.31,0.82]	0.000	0.16	[-0.05,0.36]	0.140
Wave 5	0.70	[0.63,0.78]	0.000	-	-	-	-1.80	[-2.43, -1.18]	0.000	0.49	[0.21,0.76]	0.001	0.25	[0.03,0.48]	0.027
Random Effects															
σ²Individual	0.35	[0.31,0.38]		0.11	[0.10,0.13]		14.6	[13.7,15.6]		2.52	[2.35,2.70]		1.93	[1.80,2.06]	
σ²Residual							17.4	[16.9,17.9]		3.91	[3.80,4.02]		2.28	[2.21,2.34]	
ICC							0.46	[0.44,0.48]		0.39	[0.37,0.41]		0.46	[0.44,0.48]	

Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L).

Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L).

^aIRR = Incident Rate Ratio;

^b β =Beta Coefficient

Model 2 = Socio-demographic, Health Indicators, Lifestyle, & Medical model. Longitudinal cognitive variable and quintiles of baseline folate with adjustment for time (wave), age², sex, educational attainment, baseline vitamin B₁₂ status (<or $\geq$ 258pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, anti-hypertensive medications.

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

Supplemental Table 6.4b. Multivariable longitudinal associations between baseline vitamin B₁₂ and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall) - Model 2

	MMSE Errors			MoCA Errors			Verbal Fluency			Immediate Recall			Delayed Recall		
	IRR	[95% CI]	P	IRR	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P
n	2,393			2,393			2,393			2,393			2,393		
Fixed Effects															
Vitamin B ₁₂ ≥ 258pmol/L	1.03	[0.92,1.15]	0.562	1.04	[0.97,1.11]	0.453	0.06	[-0.45,0.58]	0.781	-0.08	[-0.30,0.14]	0.637	-0.07	[-0.29, 0.15]	0.521
Wave 2	0.90	[0.86,0.92]	0.092	-	-	-	-1.50	[-1.90,-1.10]	0.225	0.23	[0.11,0.35]	0.091	0.02	[-0.08,0.12]	0.651
Wave 3	0.95	[0.89,0.95]	0.145	0.93	[0.87,0.98]	0.224	-1.60	[-2.10,-1.10]	0.316	0.12	[0.02,0.22]	0.347	-0.05	[-0.15,0.05]	0.397
Wave 4	0.97	[0.92,1.01]	0.213	-	-	-	-1.80	[-2.30,-1.30]	0.478	0.03	[-0.07,0.13]	0.552	-0.10	[-0.20,0.00]	0.125
Wave 5	0.92	[0.86,0.98]	0.132	-	-	-	-2.00	[-2.50,-1.50]	0.239	-0.05	[-0.15,-0.05]	0.295	-0.15	[-0.25,-0.05]	0.203
Random Effects															
σ ² Individual	0.64	[0.59,0.69]		0.23	[0.20,0.26]		16.90	[15.80,17.90]		4.20	[3.98,4.42]		3.00	[2.80,3.20]	
σ ² Residual							16.00	[15.00,17.00]		4.00	[3.88,4.12]		2.25	[2.18,2.32]	
ICC							0.54	[0.52,0.56]		0.54	[0.52,0.56]		0.56	[0.54,0.58]	

Reference category: Plasma vitamin B₁₂ <258pmol/L

^aIRR = Incident Rate Ratio;

^bβ=Beta Coefficient

Model 2 = Socio-demographic, Health Indicators, Lifestyle, & Medical model. Longitudinal cognitive variable and quintiles of baseline folate with adjustment for time (wave), age, age², sex, educational attainment, baseline vitamin B₁₂ status (<or≥ 258pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, anti-hypertensive medications

Supplemental Table 6.5. Sensitivity Analysis: Multivariable longitudinal associations between baseline folate quintiles and cognitive assessments (MMSE, MoCA, Verbal Fluency, Immediate Recall and Delayed Recall), sample aged 65 and over: Model 2.

n	MMSE Errors			MoCA Errors			Verbal Fluency			Immediate Recall			Delayed Recall		
	IRR	[95% CI]	P	IRR	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P	β	[95% CI]	P
	2,393			2,393			2,393			2,393			2,393		
Fixed Effects															
Q1	1.08	[0.94,1.24]	0.265	1.06	[0.96,1.16]	0.263	0.32	[-0.50,1.15]	0.446	-0.24	[-0.64,0.15]	0.226	-0.11	[-0.45,0.23]	0.526
Q2	0.99	[0.86,1.13]	0.832	0.98	[0.90,1.08]	0.690	0.19	[-0.60,0.99]	0.637	-0.08	[-0.46,0.30]	0.679	0.08	[-0.25,0.41]	0.626
Q3	1.07	[0.93,1.23]	0.337	0.96	[0.88,1.06]	0.407	0.38	[-0.43,1.19]	0.357	-0.03	[-0.41,0.36]	0.894	0.13	[-0.20,0.47]	0.434
Q4	1.09	[0.96,1.25]	0.199	1.04	[0.95,1.14]	0.348	0.02	[-0.76,0.80]	0.957	-0.07	[-0.45,0.30]	0.711	-0.09	[-0.42,0.23]	0.582
Wave 2	0.77	[0.68, 0.87]	0.000	-	-	-	-1.41	[-2.19, -0.62]	0.000	0.79	[0.42,1.15]	0.000	0.09	[-0.21,0.39]	0.552
Wave 3	0.76	[0.66, 0.87]	0.000	0.97	[0.90,1.06]	0.526	-1.97	[-2.81, -1.12]	0.000	0.54	[0.14,0.94]	0.008	0.15	[-0.17,0.48]	0.361
Wave 4	0.75	[0.65,0.86]	0.000	-	-	-	-2.64	[-3.54, -1.75]	0.000	0.27	[-0.14,0.69]	0.203	-0.21	[-0.56,0.14]	0.237
Wave 5	0.76	[0.65,0.90]	0.001	-	-	-	-2.39	[-3.40, -1.38]	0.000	0.28	[-0.19,0.75]	0.248	-0.02	[-0.42,0.37]	0.906
Random Effects															
σ ² Individual	0.26	[0.22,0.31]		0.08	[0.06,0.10]		12.71	[11.29,14.03]		2.76	[2.43,3.13]		2.25	[2.01,2.53]	
σ ² Residual							16.51	[15.71,17.35]		4.49	[4.27,4.72]		2.56	[2.44,2.69]	
ICC							0.43	[0.40,0.47]		0.38	[0.35,0.41]		0.46	[0.44,0.50]	

Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L).

Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L).

^aIRR = Incident Rate Ratio;

^b β =Beta Coefficient

Model 2 = Socio-demographic, Health Indicators, Lifestyle, & Medical model. Longitudinal cognitive variable and quintiles of baseline folate with adjustment for time (wave), age, age², sex, educational attainment, baseline vitamin B₁₂ status (<or≥ 258pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, anti-hypertensive medications

This table has been published in the paper *O'Connor et al., 2022*, please see **Appendix 11.1.4**

CHAPTER SEVEN: PAPER 5 – ORTHOSTATIC HYPOTENSION: ASSOCIATIONS WITH FOLATE AND VITAMIN B₁₂

Introduction to Chapter Seven

In this chapter, I address **Aim 5** and **Objective 5.1** of the thesis; investigating the cross- sectional and longitudinal associations of OH with baseline plasma folate and vitamin B₁₂, in a large nationally representative older adult population (n >4000, aged ≥50 years).

This chapter aimed to examine the longitudinal associations between plasma folate and total vitamin B₁₂ concentrations and orthostatic hypotension in a large, nationally representative cohort of community-dwelling people using a traceable standard of measurement and an accurate assessment of beat-to-beat blood pressure (BP). This tested the underlying hypothesis that low levels of folate and vitamin B₁₂ may increase the risk of vascular dysfunction, such as orthostatic hypotension. The following data is from the fifth quantitative study from my thesis (in preparation), “*Orthostatic Hypotension: Associations with Folate and Vitamin B₁₂.*”-(**Appendix 11.1.2**).

7.1. Abstract

Background: Folate and vitamin B₁₂ deficiency can contribute to endothelial dysfunction and increase the risk of clinical neuropathies due to the effects on the sympathetic nervous system.

Objective: Examine cross-sectional and longitudinal associations between plasma folate and total vitamin B₁₂ concentrations and orthostatic hypotension (OH) over four years in a large, nationally representative cohort of community-dwelling people using a traceable standard of measurement and an accurate assessment of beat-to-beat blood pressure (BP).

Methods: This study used participants aged ≥ 50 years from TILDA (n 4172, aged ≥ 50 years) and investigated the relationship between blood plasma folate and vitamin B₁₂ levels at baseline (Wave 1) and OH (Waves 1 and 3). Impaired stabilisation of BP on standing was defined as a sustained drop of ≥ 20 mm Hg systolic BP or ≥ 10 mm Hg diastolic BP up to 40 seconds post stand (impaired stabilisation of BP on standing [ISBP]). Participants who sustained a drop of ≥ 20 mm Hg systolic BP or ≥ 10 mm Hg diastolic BP throughout the 110 seconds stand (OH110) were also assessed. Data were analysed using multivariable regression models, adjusting for relevant covariates.

Results: There was no evidence of a significant relationship between plasma folate and vitamin B₁₂ concentrations and indicators of impaired stabilisation of blood pressure or OH in older adults. These findings were observed after adjusting for relevant covariates, including age, sex, BMI, smoking status, alcohol consumption, and medication use.

Conclusions: This is the first and largest population study to investigate the relationship between plasma folate and vitamin B₁₂ concentrations and OH in older adults. The results suggest that low levels of folate and vitamin B₁₂ do not increase the risk of OH or impaired stabilisation of blood pressure in this population. However, further research is needed to explore the potential biological factors linking folate and blood pressure control.

7.2. Introduction

Orthostatic hypotension (OH) is typically defined as a sustained decrease of at least 20 mmHg in systolic blood pressure (BP) or 10 mmHg in diastolic BP within three minutes of standing (401). Previous data published by TILDA has shown the prevalence of impaired BP stabilisation upon standing of 15.6%, rising with age in both men and woman (326). OH is an independent risk factor for several unfavourable outcomes such as a higher risk of falls (402), cardiovascular events (403), depression (404), cognitive dysfunction (405) and all-cause mortality (406). Although treatment with medication is common in cases of OH, the identification of simple, modifiable risk factors with low potential for adverse effects could present a positive step for the management of this condition.

As a water-soluble B-vitamin, folate is naturally present in a wide variety of foods, such as vegetables, fruit, beans, nuts, peas, eggs, seafood, dairy products, grains, meat, fortified foods or as FA supplements (190), while vitamin B₁₂ can be obtained from animal products and supplements (191). One-carbon metabolism is driven by the folate and methionine cycles that together regulate DNA synthesis and methylation reactions (as discussed in **Chapter One, Section 1.5.2 “The role of folate and vitamin B₁₂ in one carbon metabolism”**, and **Figure 1.4**). MSyn is the rate-limiting enzyme in one-carbon metabolism, which converts HCY - a cytotoxic intermediary amino acid associated with CVD, stroke, and cognitive decline (246), to methionine (Met) using 5MTHF as a methyl donor and vitamin B₁₂ as an essential cofactor (407). Therefore, the vitamins, folate and vitamin B₁₂ are critical for one-carbon metabolism, DNA-methylation, and nucleotide synthesis, processes that occur in all tissues of the body, including in the cardiovascular system.

Since the effect of FA on vasodilation has been well established (267), it is unsurprising that epidemiologic studies have investigated the relationship between folate and related B-vitamins, BP control and related cardiovascular parameters. Several studies have focused on the relationship between higher levels folate and vitamin B₁₂ intakes and lower BP levels (268) or decreased risk of hypertension (269, 270, 271). Trials have shown that high-dose FA supplementation may lower

systolic and diastolic BP (283, 284) while a meta-analysis of 30 randomised controlled trials found decreased risks of CVD and stroke among those receiving FA supplementation (286). There are few published studies examining blood folate levels however, with those that have been published showing mixed results. One recent study showed that serum folate levels were not significantly associated with systolic BP, diastolic BP, or odds of hypertension in Korean adults (274). To our knowledge, there are no quantitative studies to date exploring the association between folate and OH, while clinical deficiency of vitamin B₁₂ has been implicated as a direct cause of OH with just one case study (273). Together these data may suggest that a higher level of folate, facilitated by adequate levels of vitamin B₁₂, may reduce the risk of hypertension and wider vascular dysfunction. Therefore, I hypothesise that low levels of folate and vitamin B₁₂ may have the opposite effect by increasing the risk of vascular dysfunction, such as OH.

Thus, the aim of this study is to investigate the cross-sectional and longitudinal associations of OH with baseline plasma folate and vitamin B₁₂, in a large nationally representative older adult population (n 4172, aged ≥ 50 years).

7.3. Methods

7.3.1. Study Population

TILDA is a prospective cohort study in a nationally representative sample of community-dwelling adults in the Republic of Ireland aged 50 years. The study design and sampling method have been described elsewhere previously (313). At each wave, participants were asked to complete a structured interview that collected data on their social, financial, and health status. At baseline (2009–2011), 8503 participants were recruited; thereafter, data were collected every two years, and objective health assessments were performed every four years. Data for this study were obtained from Waves 1 and 3 of TILDA, collected between 2009 and 2015 (313). Ethical approval was obtained at each data collection from the Faculty of Health Sciences Research Committee, Trinity College Dublin. Written informed consent was obtained from all participants. All experimental procedures were conducted according to the Declaration of Helsinki, and all evaluations were done by

trained research nurses. The Irish Social Science Data Archive at University College Dublin and the Interuniversity Consortium for Political and Social Research at the University of Michigan have made anonymised data and materials publicly accessible at <http://www.tilda.ie>.

7.3.2. *Assessment of Orthostatic Hypotension*

Participants who attended for health assessment underwent an active stand, a non-invasive assessment of beat-to-beat BP response to standing following 10 minutes of supine rest, using digital photoplethysmography (Finometer MIDI device; Finapres Medical Systems). Under the supervision of a certified health nurse, all measurements were taken in a quiet room with an ambient temperature between 21°C and 23°C. Baseline BP was defined as the mean value between 60-30 seconds prior to standing during rest. Up to 110 seconds post-stand, BP was taken and approximated at 10-second intervals using 5-second moving averages around each time point.

In this study, two independent measures of BP response to standing were examined. Previous analyses from this population have demonstrated that the majority stabilised their BP within 40 seconds of stand (326) and therefore impaired stabilisation of BP was defined as a sustained drop of ≥ 20 mm Hg systolic BP or ≥ 10 mm Hg diastolic BP up to 40 seconds post stand (impaired stabilisation of BP on standing [ISBP]). Further, participants whose BP dropped by ≥ 20 mm Hg systolic BP or ≥ 10 mm Hg diastolic BP throughout the 110 second stand were analysed to indicate those participants with the most severe OH (OH110). The OH110 group exhibits a prolonged reduction in BP, as defined by the American Autonomic Society (401).

7.3.3. *Assessment of Folate and Vitamin B₁₂*

Non-fasting blood samples were collected during the Wave 1 health assessment by venepuncture into one 10ml K2EDTA tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10ml ethylene diamine tetra-acetic acid (EDTA; BD, Becton, Dickinson Limited) tubes for long term storage (Sarstedt; Numbrecht, Germany). The samples were kept chilled and centrifuged (3000 rpm for 15 minutes). Within 48 hours of blood collection, aliquots of plasma were labelled

and stored at - 80°C until analysis. The protocol for collecting, processing, and storing blood samples in TILDA is described elsewhere (313). Blood samples were analysed for plasma folate and vitamin B₁₂ determined by microbiological assay; *Lactobacillus casei* (L. rhamnosis (NCIB 10463; ATCC 27773)), limit of detection 0.6 nmol/L (320) and *Lactobacillus leichmannii* (L. leichmanii (delbrueckii) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/L (321), respectively. Both methods had inter-assay coefficients of variability (CV) of 10.9%.

7.3.4. *Covariates*

The covariate information utilised in the study was collected during the computer-assisted personal interview and health assessment. Socio-economic covariates consisted of age, sex, and educational attainment (primary, secondary, or tertiary/higher).

Lifestyle characteristics encompassed smoking status, as well as the use of FA and vitamin B₁₂ containing supplements. Health characteristics included self-reported cardiovascular conditions (hypertension, angina, heart attack, heart failure, diabetes mellitus, stroke, transient ischemic attack, irregular heart rhythm, and heart murmur), which were consolidated into a categorical variable (0, 1-2, or ≥ 3 conditions), as well as diabetes (doctor diagnosis or prescribed medications). Obesity was measured using a BMI > 30 kg/m². Additionally, plasma vitamin B₁₂ deficiency was included, categorised as $<$ or ≥ 148 pmol/L. Information on medication use (up to 20 prescription and non-prescription medications, including food supplements) was gathered during the computer-assisted personal interview and was classified using WHO ATC classification codes. The following medications were controlled for: antihypertensive medications ((ATC C02), diuretics (ATC C03), β -blockers (ATC C07), calcium channel blockers (ATC C08), and renin-angiotensin system agents (ATC C09)); antidepressant medications (ATC N06A). These covariates were chosen due to their previously established or anticipated associations with OH or folate and vitamin B₁₂ metabolism.

7.3.5. Statistical Analysis

Baseline characteristics of the analytical sample, summarised by quintiles of plasma folate (**Table 7.1.**) and vitamin B₁₂ status (**Table 7.2.**). Quintiles of plasma folate and total vitamin B₁₂ were created based concentrations in those aged ≥ 50 years at baseline. Categorical variables were examined using percentages and continuous variables were explored using means and standard deviations. Group differences were compared using ANOVA for continuous variables and χ^2 for categorical variables.

Using logistic regression, cross-sectional associations between ISBP and quintiles of the respective B-vitamins were evaluated. Univariate and multivariable analyses were conducted while controlling for age, gender, baseline BP, supplement use, antidepressant medication, antihypertensive medication, number of cardiovascular conditions, smoking, alcohol consumption, and BMI. Similarly, univariate logistic regression was used to examine the association between OH110 and B-vitamins, followed by multivariable logistic regression controlling for relevant confounders. In the cross-sectional component of the analysis, Model 1 is an unadjusted logistic regression model (Model 1 = Basic model. Cross-sectional OH (ISBP or OH110) and quintile of the relevant B-vitamin concentration (Quintile 5 = reference category).

Model 2 makes additional adjustment for socio-economic factors (Model 2 = Socio-economic model. Cross-sectional OH (ISBP or OH110) and quintiles of the relevant B- vitamin concentration (Quintile 5 = reference category) with adjustment for age, sex, and educational attainment). Covariates selected for the third fully adjusted model were the most parsimonious variables to represent socio-economic and health indicators of the population (Model 3 = Cross-sectional OH (ISBP or OH110) and quintiles of quintiles of the relevant B-vitamin concentration (Quintile 5 = reference category) with adjustment for age, sex, educational attainment, smoking status, alcohol consumption, vitamin B₁₂ deficiency, antihypertensive medication use, anti-depressive medication use, PPI use, no. of cardiovascular conditions, mean grip strength (dominant hand), diabetes status and BMI conditions.

The longitudinal associations between baseline B-vitamin levels and OH were examined using mixed-effects logistic regression models that accounted for

clustering of repeated measurements within individuals (394). The present study utilised adjusted models for covariates, treating them as time-varying fixed effects when appropriate. To model the associations between folate quintile group membership at baseline and change in OH at subsequent waves within the respective groups, interaction terms for time and predictor variables were incorporated. The first prospective model examined the associations between OH over four year and baseline folate status (Model 1= Longitudinal OH (ISBP or OH110) variable and quintiles of baseline folate with adjustment for time (wave)). Model 2 made additional adjustments for socio-economic factors (Model 2 = Longitudinal OH (ISBP or OH110) variable and quintiles of baseline folate with adjustment for time (wave), age, sex, vitamin B₁₂ deficiency (148 pmol/L), time-interacted with educational attainment. Model 3 accounts for additional time- interacted variables (Model 3 = Fully adjusted model. Longitudinal OH (ISBP or OH110) variable and quintiles of baseline folate with adjustment for time (wave), age, sex, time interacted with educational attainment anti-hypertensive medication use, anti- depressant medication use and BMI). Furthermore, interactions between quintiles of the B- vitamins and wave were included as covariates in the models to investigate whether differences in overall B-vitamin quintile group membership (between-person) were associated with OH over time.

7.4. Results

Of the 8,504 participants interviewed during the first wave, 4,481 were eligible at baseline after the application of exclusion criteria. Individuals aged <50 years (*n* 332) without health assessment data and blood samples (*n* 3,242) and for whom folate or vitamin B₁₂ measurement exceeded the 99th percentiles (*n* 443), were excluded from the analysis. There were a further 309 participants who did not have adequate active stand data for analysis. Therefore, the final sample for analysis was 4,172 participants.

7.4.1. Cross Sectional: Baseline sample characteristics

Population characteristics of the TILDA cohort are detailed elsewhere (313). Of the 4,172 included in this analysis, the baseline mean age (SD) was 61.6 years (8.2) and

52.6% were female. Sample characteristics compared by plasma folate and total vitamin B₁₂ concentrations are summarised in **Tables 7.1. & 7.2.**, respectively. Participants who had deficient or low in folate status, were more likely to be younger and male, to smoke and have higher BMI when compared with those with sufficient status. In addition, they were less likely to consume alcohol or achieve third level educational attainment (**Table 7.1.**). They were also more likely to have higher mean grip strength and BMI. With respect to vitamin B₁₂, those with deficient or low status were more likely to be older, male, to be current smokers, use antihypertensive medications, have diabetes and higher mean systolic blood pressure and BMI than compared to those with sufficient vitamin B₁₂ status. They were also less likely to have achieved third level educational attainment or higher (**Table 7.2.**).

Table 7.1. Baseline Characteristics by plasma total vitamin B₁₂ status (n 4,172)

Participant Characteristics	Deficient <6.8nmol/L (n 106)	Low ≥6.8- <23.0nmol/L (n 2,527)	Normal ≥23.0- <45.3nmol/L (n 1,180)	High ≥45.3- <60.0nmol/L (n 228)	Very High ≥60.0nmol/L (n 131)	P Value
Folate, nmol/L	5.5±1.2	14.5±4.3	31.6±6.1	52.0±4.2	68.4±5.8	0.0000
Age, y	62.5±9.5	61.4±8.4	62.1±8.2	62.3±8.0	64.5±8.8	0.0001
Female sex, % (n)	52.6 (60)	50.4 (1362)	55.9 (708)	62.0 (158)	64.1(91)	0.000
Educational Attainment (base none/primary), % (n)						
Secondary	36.8 (42)	43.4 (1172)	41.1 (519)	40.4 (104)	35.9 (51)	0.017
Tertiary/ higher	32.5 (37)	34.3 (927)	38.7 (489)	39.5 (101)	44.4 (63)	
Smoking status (base never), % (n)						
Past	30.7 (35)	38.6 (1044)	40.8 (517)	39.5 (101)	38.7 (55)	0.002
Current	21.1 (24)	16.5 (446)	11.8 (149)	11.4 (29)	12.0 (17)	
Alcohol Consumption, % (n)	72.4 (76)	78.8 (1952)	80.5 (958)	78.9 (191)	78.5 (106)	0.352
Antihypertensive medications, % (n)	34.2 (39)	32.5 (879)	32.4 (410)	34.12 (87)	35.9 (51)	0.898
Antidepressant medications, % (n)	7.9 (9)	5.6 (151)	6.0 (76)	7.8 (20)	4.9 (7)	0.516
Proton Pump Inhibitors, % (n)	10.5 (12)	11.7 (315)	11.7 (147)	11.4 (29)	12.0 (17)	0.997
Folate-containing supplements, % (n)	3.5 (4)	2.0 (55)	3.6 (45)	9.8 (25)	4.9 (7)	0.000
No. of CVD conditions, % (n)						
1-2	11.4 (13)	12.5 (337)	12.3 (156)	11.4 (30)	12.7 (18)	0.980
≥3	0.9 (1)	0.7 (20)	0.9 (11)	0.4 (1)	0.0 (0)	
Grip Strength, Dominant Hand	26.2±10.0	27.1±10.2	26.3±9.7	25.3±9.4	24.0±8.9	0.0001
Diabetes mellitus, % (n)	7.9 (9)	5.9 (159)	6.6 (83)	7.8 (20)	4.9 (7)	0.584
Mean systolic BP, mmHg	136.7±19.2	134.5±19.4	134.2±19.1	136.4±20.2	137.8±21.5	0.0930
Mean diastolic BP, mmHg	82.7±11.4	82.4±11.2	82.1±10.8	83.0±11.3	82.3±10.8	0.7684
BMI, kg/m²	29.1±6.5	28.8±4.9	28.2±4.8	27.9±4.6	27.4±4.0	0.0000
Sustained OH @40sec, % (n)	11.3 (12)	10.1 (256)	11.6 (136)	7.5 (17)	15.3 (20)	0.129
Sustained OH @110sec, % (n)	5.7 (6)	3.8 (96)	5.9 (69)	4.9 (11)	6.1 (8)	0.060

Table 7.2. Baseline Characteristics by total vitamin B₁₂ status (n 4,172)

Participant Characteristic	Vitamin B ₁₂			P Value
	Deficient <148 pmol/L (n 195)	Insufficient ≥148-<258pmol/L (n 1,041)	Sufficient ≥258nmol/L (n 2,936)	
Vitamin B₁₂, pmol/L	117.1±24.4	211.6±30.3	388.4±98.4	0.0000
Age, y	63.9±9.3	62.4±8.6	61.4±8.12	0.0000
Female sex, % (n)	51.0 (103)	48.6 (541)	54.8 (1735)	0.001
Educational Attainment (base none/primary), % (n)				
Secondary	43.1 (87)	40.2 (447)	42.8 (1354)	0.000
Tertiary/ higher	23.8 (48)	35.5 (395)	37.1 (1174)	
Smoking status (base never), % (n)				
Past	35.6 (72)	37.7 (419)	39.8 (1261)	0.025
Current	20.3 (41)	16.8 (187)	13.8 (437)	
Alcohol Consumption, % (n)	77.3 (143)	79.4 (803)	79.2 (2337)	0.902
Antihypertensive medications, % (n)	41.1 (83)	32.9 (365)	32.1 (1018)	0.032
Antidepressant medications, % (n)	4.5 (9)	5.5 (61)	6.1 (193)	0.509
Proton Pump Inhibitors, % (n)	9.4 (19)	11.8 (131)	11.7 (370)	0.606
Vitamin B₁₂ supplements, % (n)	1.00 (2)	2.3 (25)	3.0 (95)	0.123
No. of CVD conditions, % (n)				
1-2	11.9 (24)	11.8 (131)	12.6 (399)	0.201
≥3	1.9 (4)	0.9 (10)	0.6 (19)	0.207
Grip Strength, Dominant Hand	25.7±9.5	27.0±10.3	26.6±9.9	0.2385
Diabetes mellitus, % (n)	11.9 (24)	7.8 (87)	5.3 (167)	0.000
Mean SBP, mmHg	136.2±19.9	136.5±20.5	134.0±19.0	0.0006
Mean DBP, mmHg	82.5±10.4	82.9±11.4	82.1±11.0	0.1416
BMI, kg/m²	29.3±4.9	29.1±5.1	28.3±4.7	0.0000
Sustained OH @40sec	12.3 (24)	10.2 (106)	10.6 (311)	0.673
Sustained OH @110sec	6.7 (13)	4.0 (42)	4.6 (135)	0.264

Groups were compared using ANOVA for continuous variables and χ^2 for categorical variables.

Body mass index; CVD, cardiovascular diseases; DBP, diastolic blood pressure; HR heart rate; and SBP, systolic blood pressure.

*Data are means±SD or percentage as appropriate.

7.4.2. Cross-sectional: Regression analyses of OH and B-vitamins

Univariate logistic regression (Model 1) was used to assess an association between ISBP and quintiles of plasma folate (Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L) Quintile 5, (32.6-81.3 nmol/L)) found that, compared with the reference group (Quintile 5, highest concentrations), those with lower concentrations of plasma folate (odds ratio [OR], 0.94;95% CI, 0.69-1.28;P=0.689, OR=0.91; 95% CI, 0.67-1.23; P=0.528, OR=0.74; 95% CI, 0.53-1.01; P=0.060, OR=0.98; 95% CI, 0.73-1.33; P=0.910) were no more likely to have evidence of ISBP upon active stand. In multivariable models, controlling for age, sex educational attainment (Model 2), and further adjustment for smoking status and alcohol consumption, antihypertensive and antidepressant medications, PPI use, number of cardiovascular conditions, diabetes mellitus, BMI, and grip strength (Model 3), there was no evidence of a cross-sectional association between plasma folate and ISBP (**Table 7.3.**).

Univariate logistic regression used to assess the association between OH110 and quintiles of plasma folate found that, compared with Quintile 5, Quintile 2 (11.2-15.6 nmol/L) (OR=0.44; 95% CI 0.27-0.72, P=0.001) had significantly lower odds of OH110. On controlling for all potential confounders, the statistically significant effect for Quintile 2 persisted (OR=0.49; 95% CI,0.29-0.83; P=0.008) (**Table 7.3.**).

Table 7.3. Cross-sectional univariate and multivariable logistic regression models to assess for association between quintiles of plasma folate and orthostatic hypotension

Characteristics	ISBP									OH110								
	Model 1 (4,172)			Model 2 (4,171)			Model 3 (3,808)			Model 1 (4,172)			Model 2 (3,820)			Model 3 (3,820)		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Q1	0.94	0.69-1.28	0.689	1.04	0.76,1.44	0.787	1.08	0.77-1.52	0.662	0.65	0.42-1.01	0.055	0.70	0.44-1.09	0.116	0.70	0.43-1.14	0.149
Q2	0.91	0.67-1.23	0.528	1.04	0.76-1.43	0.812	1.06	0.76-1.48	0.735	0.44	0.27-0.72	0.001	0.49	0.30-0.80	0.004	0.49	0.29-0.83	0.008
Q3	0.74	0.53-1.01	0.060	0.86	0.62-1.19	0.361	0.90	0.63-1.27	0.539	0.66	0.43-1.01	0.058	0.75	0.48-1.16	0.194	0.81	0.51-1.29	0.378
Q4	0.98	0.73-1.33	0.910	1.08	0.79-1.09	0.637	1.04	0.74-1.44	0.836	0.71	0.47-1.09	0.119	0.76	0.50-1.17	0.215	0.81	0.52-1.27	0.351
Age, y				1.08	1.06-1.09	0.000	1.07	1.06-1.09	0.000				1.06	1.05-1.08	0.000	1.06	1.03-1.08	0.000
Female sex				1.42	1.15-1.74	0.001	1.24	0.89-1.73	0.195				1.71	1.26-2.32	0.001	1.58	0.97-2.58	0.068
Educational Attainment (ref: none/primary)																		
Secondary				0.82	0.63-1.06	0.137	0.86	0.65-1.14	0.293				0.76	0.53-1.10	0.142	0.82	0.55-1.21	0.316
Tertiary/ higher				0.93	0.71-1.20	0.563	1.02	0.77-1.37	0.877				0.66	0.45-0.97	0.036	0.70	0.46-1.06	0.094
Smoking status (ref: never)																		
Past							1.16	0.91-1.48	0.238							0.90	0.63-1.29	0.580
Current							1.52	1.10-2.10	0.012							1.08	0.67-1.75	0.744
Alcohol Consumption							0.83	0.64-1.07	0.151							0.92	0.64-1.33	0.660
B₁₂ deficiency (<148 pmol/L)							0.97	0.59-1.60	0.918							1.63	0.88-3.03	0.124
Antihypertensive medication use							1.00	1.00-1.01	0.131							1.30	0.91-1.86	0.142
Antidepressant medication use							2.25	1.55-3.26	0.000							2.30	1.40-3.78	0.001
Proton Pump Inhibitor use							0.90	0.64-1.26	0.531							1.12	0.70-1.77	0.645

No. of CVD conditions (ref: none)

1-2	1.07	0.79-1.47	0.648	1.02	0.64-1.63	0.660
≥3	0.79	0.25-2.47	0.683	0.48	0.06-3.85	0.491
Grip Strength, Dominant Hand	0.99	0.98-1.01	0.446	1.00	0.97-1.02	0.764
Diabetes mellitus (ref: no)	1.48	0.99-2.23	0.057	1.48	0.81-2.69	0.204
BMI, kg/m²	0.95	0.92-0.97	0.000	0.92	0.88-0.96	0.000

Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L).

Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.3 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L).

*OR = Odds Ratio.

Model 1 are unadjusted regression models. (Model 1 = Basic model. Cross-sectional OH (ISBP or OH110) and quintile of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category).) Model 1 are unadjusted regression models.

Model 2 = Socio-economic model. Cross-sectional OH (ISBP or OH110) and quintiles of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category) with adjustment for age, sex, and educational attainment. Model 3 = Fully adjusted model- socio-economic and health indicators. Cross-sectional OH (ISBP or OH110) and quintiles of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category) with adjustment for age, sex, educational attainment, smoking status, alcohol consumption, vitamin B₁₂ deficiency, antihypertensive medication use, anti-depressive medication use, proton pump inhibitor use, no. of cardiovascular conditions, mean grip strength (dominant hand), diabetes status and BMI conditions.

Regarding vitamin B₁₂, there was no statistically significant difference in the likelihood of ISBP or OH110 by quintiles of vitamin B₁₂ concentrations (Q1 = Quintile 1 (total vitamin B₁₂ 45.4 – 224.8 pmol/L.); Q2 = Quintile 2 (total vitamin B₁₂ 224.9 – 288.7 pmol/L); Q3 = Quintile 3 (total vitamin B₁₂ 228.8 – 350.0 pmol/L); Q4 = Quintile 4 (total vitamin B₁₂ 350.0 – 428.1 pmol/L using the highest quintile of vitamin B₁₂ (Q5=Quintile 5 (total vitamin B₁₂ (428.5 -727.8 pmol/L) as the reference category (**Table 7.4.**).

Table 7.4. Cross-sectional univariate and multivariable logistic regression models to assess for association between quintiles of plasma vitamin B₁₂ and orthostatic hypotension

Characteristics	ISBP									OH110								
	Model 1 (4,172)			Mode 2 (4,171)			Model 3 (3,820)			Model 1 (4,172)			Mode 2 (4,171)			Model 3 (3,820)		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Q1	0.97	0.72-1.32	0.870	0.88	0.64-1.21	0.429	0.81	0.58-1.14	0.231	0.93	0.60-1.45	0.758	0.87	0.55-1.37	0.549	0.87	0.54-1.41	0.578
Q2	0.81	0.59-1.11	0.192	0.80	0.58-1.11	0.187	0.82	0.59-1.15	0.254	0.76	0.47-1.20	0.192	0.77	0.48-1.24	0.281	0.80	0.48-1.28	0.323
Q3	0.87	0.64-1.19	0.383	0.87	0.63-1.20	0.402	0.84	0.60-1.17	0.301	0.83	0.52-1.30	0.383	0.6	0.54-1.37	0.521	0.87	0.54-1.41	0.570
Q4	1.01	0.75-1.36	0.958	1.00	0.73-1.36	0.977	0.95	0.68-1.32	0.749	0.93	0.60-1.45	0.958	0.93	0.60-1.46	0.768	0.90	0.56-1.43	0.647
Age, y				1.08	1.06-1.09	0.000	1.08	1.06-1.09	0.000				1.06	1.05-1.08	0.000	1.06	1.04-1.08	0.000
Female sex				1.40	1.14-1.72	0.002	1.24	0.89-1.72	0.206				1.75	1.28-2.38	0.000	1.58	0.97-2.57	0.068
Educational Attainment (ref: none/primary)																		
Secondary				0.82	0.63-1.06	0.125	0.85	0.64-1.13	0.270				0.76	0.53-1.09	0.136	0.81	0.64-1.20	0.288
Tertiary/ higher				0.92	0.71-1.20	0.531	1.00	0.75-1.33	0.978				0.68	0.46-0.99	0.046	0.69	0.45-1.05	0.079
Smoking status (ref: never)																		
Past							1.14	0.90-1.46	0.285							0.87	0.61-1.24	0.448
Current							1.58	1.15-2.19	0.005							1.06	0.66-1.70	0.817
Alcohol Consumption																		
Antihypertensive medication use							0.84	0.65-1.08	0.170							0.92	0.64-1.33	0.668
Antidepressant medication use							1.08	0.84-1.38	0.552							1.31	0.92-1.87	0.137
Antidepressant medication use							2.24	1.60-3.25	0.000							2.35	1.44-3.85	0.001
Proton Pump Inhibitor use							0.88	0.63-1.23	0.470							0.07	0.68-1.70	0.766

No. of CVD conditions (ref: none)						
1-2	1.02	0.74-1.40	0.902		0.90	0.56-1.44 0.662
≥3	0.78	0.25-2.44	0.672		0.43	0.05-3.36 0.417
Grip Strength, Dominant Hand	1.00	0.98-1.01	0.624		1.00	0.97-1.03 0.992
Diabetes mellitus (ref: no)	1.42	0.95-2.15	0.091		1.34	0.73-2.44 0.344
BMI, kg/m²	0.95	0.93-0.98	0.000		0.92	0.88-0.96 0.000

Reference category: Q5 = Quintile 5 (total vitamin B₁₂ 428.5 -727.8 pmol/L).

Q1 = Quintile 1 (total vitamin B₁₂ 45.4 – 224.8 pmol/L.); Q2 = Quintile 2 (total vitamin B₁₂ 224.9 –288.7 pmol/L.); Q3 = Quintile 3 (total vitamin B₁₂ 228.8 – 350.0 pmol/L.); Q4 = Quintile 4 (total vitamin B₁₂ 350.0 – 428.1 pmol/L).

^aOR = Odds Ratio.

Model 1 are unadjusted regression models. (Model 1 = Basic model. Cross-sectional OH (ISBP or OH110) and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category).

Model 2 = Socio-economic model. Cross-sectional OH (ISBP or OH110) and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category) with adjustment for age, sex, and educational attainment.

Model 3 = Fully adjusted model- socio-economic and health indicators. Cross-sectional OH (ISBP or OH110) and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category) with adjustment for age, sex, educational attainment, smoking status, alcohol consumption, antihypertensive medication use, anti-depressive medication use, proton pump inhibitor use, no. of cardiovascular conditions, mean grip strength (dominant hand), diabetes status and BMI conditions.

7.4.3. Prospective: Regression analyses of OH and B-vitamins

Regression analyses examining associations between baseline folate and vitamin B₁₂ with OH are presented in **Tables 7.5.** and **7.6.**, respectively. Regarding folate, univariate analysis (Model 1) revealed those in Quintile 3 of folate had reduced risk of ISBP (OR=0.69; 95% CI, 0.48,0.99, P=0.045), however, this association was attenuated with adjustment for relevant confounders (Models 2 and 3).

Univariate analysis revealed that varying concentrations of folate at baseline were associated with reduced odds of OH110 over four years (OR=0.57; 95% CI 0.36,0.90, P=0.016) (OR=0.61; 95% CI 0.39,0.95, P=0.030) for Q2 and Q3 vs. the reference category, Q5 (32.6-81.3 nmol/L), however similarly to ISBP, these associations were attenuated with adjustment for relevant confounders (Models 2 and 3) (**Table 7.5.**). In terms of vitamin B₁₂, there were no associations in univariate or multivariable models adjusted for appropriate confounders, over four years (**Tables 7.6.**).

Table 7.5. Multilevel logistic regression models to assess for association between folate and orthostatic hypotension, over four years

n	ISBP									OH110								
	Model 1			Model 2			Model 3			Model 1			Model 2			Model 3		
	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P
		3,406			3,406			3,404			3,406			3,406			3,404	
Fixed Effects																		
Q1	0.89	[0.63,1.27]	0.526	0.94	[0.67,1.32]	0.734	1.11	[0.78,1.60]	0.557	0.71	[0.46,1.11]	0.135	0.75	[0.48,1.15]	0.184	0.99	[0.62,1.60]	0.973
Q2	0.93	[0.66,1.31]	0.678	1.02	[0.72,1.42]	0.930	1.27	[0.89,1.80]	0.193	0.57	[0.36,0.90]	0.016	0.62	[0.39,0.98]	0.040	0.89	[0.54,1.45]	0.640
Q3	0.69	[0.48,0.99]	0.045	0.80	[0.56,1.13]	0.198	1.32	[0.93,1.86]	0.119	0.61	[0.39,0.95]	0.030	0.69	[0.44,1.07]	0.096	1.07	[0.67,1.70]	0.775
Q4	1.00	[0.71,1.40]	0.995	1.07	[0.77,1.48]	0.689	1.20	[0.85,1.70]	0.301	0.73	[0.48,1.13]	0.157	0.77	[0.51,1.18]	0.236	1.39	[0.89,2.16]	0.150
Wave 3	0.77	[0.62,0.93]	0.008	0.60	[0.48,0.74]	0.000	0.63	[0.16,2.47]	0.511	0.66	[0.49,0.89]	0.006	0.57	[0.42,0.77]	0.000	0.08	[0.01,0.56]	0.011
Random Effects																		
σ² Individual	1.96	[1.25,3.07]	1.50	0.90,2.48]		1.34	[0.58,3.09]	1.34	[0.58,3.09]	0.98	[0.34,2.81]		0.85	[0.25,2.88]				
σ² Residual																		
ICC	0.37	[0.27,0.48]	0.31	0.21,0.43		0.30	[0.20, 0.42]	0.29	[0.15,0.48]	0.23	[0.09,0.46]		0.20	[0.07,0.47]				

Reference category: Q5 = Quintile 5 (Plasma Folate 32.6 - 81.3 nmol/L).

Q1 = Quintile 1 (Plasma Folate 0.9 - 11.2 nmol/L); Q2 = Quintile 2 (Plasma Folate 11.2 - 15.6 nmol/L); Q3 = Quintile 3 (Plasma Folate 15.7 - 21.8 nmol/L); Q4 = Quintile 4 (Plasma Folate 21.8 - 32.5 nmol/L).

*OR = Odds Ratio.

Model 1= Longitudinal OH (ISBP or OH110) variable and quintiles of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category) with adjustment for time (wave).

Model 2 = Longitudinal OH (ISBP or OH110) variable and quintiles of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category) with adjustment for time (wave), age, sex, vitamin B₁₂ deficiency (148pmol/L), time-interacted with educational attainment.

Model 3 = Fully adjusted model. Longitudinal OH (ISBP or OH110) variable and quintiles of plasma folate (Quintile 5 32.6-81.3 nmol/L = reference category) with adjustment for time (wave), age, sex, time interacted with educational attainment anti-hypertensive medication use, anti-depressant medication use, BMI.

Table 7.6. Multilevel logistic regression models to assess for association between vitamin B₁₂ and orthostatic hypotension, over four years

n	ISBP									OH110								
	Model 1 3,406			Model 2 3,406			Model 3 3,404			Model 1 3,406			Model 2 3,406			Model 3 3,404		
	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P	OR	[95% CI]	P
Fixed Effects																		
Q1	1.19	[0.84,1.68]	0.336	1.05	[0.75,1.47]	0.773	1.08	[0.74,1.56]	0.703	1.02	[0.66,1.59]	0.922	0.96	[0.48,1.15]	0.858	0.83	[0.50,1.39]	0.484
Q2	0.89	[0.62,1.26]	0.500	0.83	[0.59,1.17]	0.296	0.92	[0.65,1.30]	0.644	0.71	[0.45,1.13]	0.146	0.71	[0.39,0.98]	0.139	0.74	[0.46,1.19]	0.311
Q3	0.91	[0.64,1.29]	0.594	0.91	[0.64,1.28]	0.575	1.11	[0.79,1.56]	0.552	0.92	[0.59,1.42]	0.696	0.94	[0.44,1.07]	0.792	0.83	[0.52,1.31]	0.414
Q4	1.00	[0.71,1.42]	0.975	0.99	[0.71,1.39]	0.969	1.09	[0.77,1.53]	0.624	0.76	[0.48,1.20]	0.240	0.77	[0.51,1.18]	0.258	1.04	[0.67,1.60]	0.870
Wave 3	0.76	[0.62,0.93]	0.008	0.60	[0.49,1.05]	0.000	0.64	[0.16,2.47]	0.519	1.33	[0.57,0.89]	0.006	0.57	[0.42,0.77]	0.000	0.08	[0.01,0.56]	0.011
Random Effects																		
σ² Individual	1.93	[1.23,3.04]		1.47	[0.89,2.46]		1.37	[0.80,2.33]		1.33	[0.57,3.07]		0.98	[0.35,2.79]		0.86	[0.26,2.87]	
σ² Residual																		
ICC	0.37	[0.27,0.48]		0.31	[0.21,0.43]		0.29	[0.20,0.42]		0.29	[0.15,0.48]		0.23	[0.09,0.46]		0.21	[0.07,0.47]	

Reference category: Q5 = Quintile 5 (total vitamin B₁₂ 428.5 -727.8 pmol/L).

Q1 = Quintile 1 (total vitamin B₁₂ 45.4 – 224.8 pmol/L.); Q2 = Quintile 2 (total vitamin B₁₂ 224.9 –288.7 pmol/L.); Q3 = Quintile 3 (total vitamin B₁₂ 228.8 – 350.0 pmol/L.); Q4 = Quintile 4 (total vitamin B₁₂ 350.0 – 428.1 pmol/L).

*OR = Odds Ratio.

Model 1= Longitudinal OH (ISBP or OH110) variable and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category) with adjustment for time (wave).

Model 2 = Longitudinal OH (ISBP or OH110) variable and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category) with adjustment for time (wave), age, sex and time-interacted with educational attainment.

Model 3 = Fully adjusted model. Longitudinal OH (ISBP or OH110) variable and quintiles of total vitamin B₁₂ (Quintile 5 428.5 -727.8 pmol/L = reference category) with adjustment for time (wave), age, sex, time interacted with educational attainment anti-hypertensive medication use, anti-depressant medication use, BMI.

7.5. Discussion

We investigated the hypothesis that low levels of folate and vitamin B₁₂ may increase the risk of OH. To our knowledge, this is the first and therefore largest and most comprehensive population study to investigate the relationship of plasma folate and vitamin B₁₂ concentrations with OH in older adults.

We found univariate longitudinal associations between folate and OH over 4 years. However, when controlled for anti-depressant medications (Model 3) the associations were attenuated. No cross-sectional or prospective associations between vitamin B₁₂ with indicators of impaired stabilisation of BP or OH after adjustment for relevant covariates were observed.

Due to the well-established associations between endothelial function and folate, as well as the clinical neuropathies caused by folate and vitamin B₁₂ deficiency on the sympathetic nervous system, this study was conducted to investigate the associations with OH. The significance of folate deficiency in blood pressure regulation has been studied primarily due to its central role in HCY regulation. HCY levels can become elevated in the absence of adequate folate. High HCY levels have been associated with hypertension. FA treatment has been shown to reduce plasma HCY, which can induce endothelial cell injury. Low or insufficient levels of serum folate induce an accumulation of HCY (260) which can lead to HCY -induced arteriolar constriction, increased sodium reabsorption, renal dysfunction, and increased arterial stiffness (261), resulting in increased BP. The associations between folate and BP may also be due to biological factors other than HHCY. It has been shown that folate may have favourable effects on BP by acting directly on endothelial cells (294). The active form of folate, 5MTHF, increases endothelial function by enhancing the activity of NO within endothelial cells of blood vessels. Thus, inadequate levels of serum folate can increase BP through endothelial dysfunction (295). This is supported by two studies that demonstrate oral FA supplementation improves endothelial function in vivo (296, 297).

Our results show that OH is unlikely to be associated with these B-vitamins or predicated by baseline vitamin status. The prevalence of OH increases with age and

is high in this population (326). In a previous study from TILDA, the prevalence ISBP on stand in the first wave of data collection (2009-2011) rose from 14.3% of men and 16.9% of women aged 50-59 to 43.4% of men and 39.9% of women over the age of 80 (326). However, this is likely related to causes such as muscle weakness, baroreflex issues and dehydration in separate work published by our research group, we demonstrated that the current practice of voluntary food fortification in Ireland is insufficient to maintain adequate folate and vitamin B₁₂ status of older adults in Ireland (214). In fact, 15% and 12% of the population, respectively, were found to have low or deficient folate (<10 nmol/L) and vitamin B₁₂ status (<185 pmol/L). However, there is little evidence from randomised controlled trials linking these vitamins to blood pressure regulation and other common causes and conditions associated with severe OH. Studies have shown that OH is independently associated with unfavourable outcomes such as a higher risk of depression (404) and cognitive dysfunction (405). Further, prospective data from our study has shown low vitamin B₁₂ is associated with incident depression (211) and low folate predicts accelerated cognitive decline (227). In addition, higher levels of folate, irrespective of vitamin B₁₂ levels were associated with better global cognitive performance (235). Despite these common associations, after adjusting for relevant confounders, we found no association between folate or vitamin B₁₂ and indicators of ISBP or OH110.

While there was no consistent association between folate and vitamin B₁₂ and OH observed, lower concentrations of folate were associated increased risk of OH110 in the longitudinal analysis in the unadjusted (both ISBP and OH110) and the socio-economic (OH110 only) models (Models 1 and 2, respectively). However, these effects were attenuated in the fully adjusted longitudinal models, which included adjustment for antidepressants. The attenuation of the association may be linked to the direct effects of antidepressant medications on the autonomic nervous system, which are well-established. Many antidepressants, particularly tricyclics and serotonin-norepinephrine reuptake inhibitors (SNRIs), are known to affect blood pressure regulation by altering the sympathetic and parasympathetic nervous system balance, potentially leading to OH. Therefore, it is more likely that the observed effect of antidepressants on OH in this study is due to their impact on autonomic function rather

than a mediation pathway through depression or folate status. This suggests that while antidepressants may be a confounding factor, their mechanism of action on OH is independent of folate-related pathways.

Despite the lack of consistent findings in the current study, the potential value of maintaining adequate folate and vitamin B₁₂ in the management and management of dysfunction cannot be discounted, as it is possible that our follow-up time was not long enough to observe an effect. Furthermore, our study showed no increased risk of OH with higher concentrations of folate or vitamin B₁₂. Thus, the achievement of optimal status of these B-vitamins in this cohort could have benefits for overall and cognitive well-being without an adverse consequence for BP.

This study has several strengths that should be acknowledged. It is the first study exploring this relationship between folate vitamin B₁₂ and OH of its kind. Additionally, it has been conducted in a large, nationally representative sample. Recognised methods for folate and vitamin B₁₂ analysis were used in addition to reported assay coefficient of variation for quality control. The repeated beat-to-beat measurement of blood pressure (BP) response to standing using a finometer allowed for a precise assessment of BP behaviour in response to orthostasis and can detect more subtle BP fluctuations. The prospective analysis uses a wide range of covariates. Some of the limitations must also be acknowledged. Causality cannot be examined due to B-vitamins only being available at baseline. TILDA does not use the traditional definition of OH, and the cardiovascular disorders were self-reported. In addition, because of the sampling criteria, our findings may not be applicable to non-whites, older adults in institutional care or those with severe cognitive impairment. Further, dehydration was not measured in this cohort and it is possible that 4-year follow-up was not long enough to see any change.

From our findings in the first and thus largest study yet investigated, we conclude that there is no association between baseline folate and vitamin B₁₂ and OH over four years among community-dwelling older adults. Positive univariate associations between folate and OH over 4 years were attenuated by the addition of antidepressant medication. This attenuation, however, is more likely due to the direct effects of antidepressants on the autonomic nervous system rather than a mediation pathway

involving folate and depression, as originally hypothesized. Antidepressants, particularly tricyclics and SNRIs, can influence autonomic function, affecting blood pressure regulation and leading to OH. Therefore, future research should focus on distinguishing the role of antidepressants from other mechanisms, including vitamin deficiencies, in the context of OH. While B-vitamins and depression (409), as well as depression and OH (408), are relevant in the broader context of cognitive decline (410), it appears that antidepressant use exerts a distinct influence on autonomic function. Thus, future work should carefully examine the distinct pathways between B-vitamin deficiency, autonomic dysfunction, and OH, while considering the independent effects of antidepressant medications.

CHAPTER EIGHT: PAPER 6 - NUTRITION BEHAVIOUR AND COMPLIANCE WITH IRISH FOOD-BASED DIETARY GUIDELINES: A CROSS-SECTIONAL STUDY

Introduction to Chapter Eight

In this chapter, I address **Aim 6** and **Objective 6.1** of the thesis. The chapter describes and summarises the consumption frequencies of food groups as defined by national dietary recommendations among older adults in Ireland and aims to determine the compliance levels with the FP within this population. I establish the socio-demographic, health and lifestyle determinants for compliance with certain food group-level recommendations in this population. The hypothesis underling this quantitative study is that compliance levels with Irish FBDGs were low, with few meeting food-group level recommendations. This may assist policymakers in the development of targeted interventions to improve dietary habits and thus nutritional status of the older population in Ireland.

The following data is from the sixth quantitative work included in this thesis (currently under review), “*Nutrition behaviour and compliance with Irish food-based dietary guidelines: a cross-sectional study*”.

8.1. Abstract

Background: A varied diet in addition to physical activity contributes to healthy ageing. Representative data describing compliance with national guidelines and nutritional recommendations among older adults in Ireland is lacking. This study investigated compliance with recommendations in a representative sample of Irish people aged ≥ 50 years.

Methods: A food frequency questionnaire (FFQ) was completed as part of TILDA, a nationally representative study of ≥ 50 s living in Ireland (n 5,206). Characteristics associated with compliance with national dietary guidelines were investigated using multivariable logistic regression models.

Results: Compliance levels with food-group recommendations from the FP were generally low, with particularly poor adherence among older participants. Only 15% of participants aged 75 and older met the recommendation for fruit and vegetable intake (five or more servings per day). Women were significantly more likely to meet both the fruit and vegetable recommendation (Odds Ratio [OR]=2.13; 95% CI [1.81, 2.50]) and the recommendation for milk, yogurt, and cheese consumption (OR=1.48; 95% CI [1.20, 1.81]). Higher educational attainment was associated with a greater likelihood of meeting the fruit and vegetable recommendation (OR=1.69; 95% CI [1.34, 2.12]), and higher levels of physical activity were also linked to improved compliance with both the fruit and vegetable recommendation (OR=1.51; 95% CI [1.27, 1.80] for moderate activity and OR=1.70; 95% CI [1.40, 2.07] for high activity) and the milk, yogurt, and cheese recommendation (OR=1.25; 95% CI [1.00, 1.56] for moderate activity vs. low).

Conclusion: The determinants of compliance with specific food groups were socially patterned and individuals aged ≥ 50 years in Ireland may need new targeted and age-specific tools to see dietary recommendations met in this population group.

8.2. Introduction

A balanced diet is an important aspect of health and wellbeing and has long been advocated for healthy ageing. Diet can influence age-related chronic diseases such as CVD, type-2 diabetes mellitus (T2DM), cancer and osteoporosis (75) and may be a modifiable risk factor for prevention of cognitive impairment (76) and dementia (77), highlighting the essential role that nutrition plays in contributing to healthy later life years. An increasingly older population, with up to one in five of those living in Ireland reaching ≥65 years by 2041 (9), and the corresponding rise in prevalence of age-related diseases, leads to greater demands on healthcare services, costs and long-term care services (411).

The ageing process is typically accompanied by physiological change, comorbidities, digestive issues and deteriorations of sensory capacity (79). This can alter the nutritional intakes of older people and precipitate accelerated loss of muscle mass, the development of frailty and malnutrition (82). When coupled with regular physical activity, a healthy diet is associated with increased life expectancy, improved quality of life and reduced risk of age-related chronic diseases (412). Factors affecting dietary patterns and habits of older people are complex but include physical challenges such as food availability, accessibility and preparation (85) in addition to social determinants such as marital status, living alone and social exclusion (86, 87).

Dietary recommendations for older people include consumption of high fibre foods, fruit and vegetables, adequate protein, sources of low-fat dairy and limiting foods high in saturated fat, salt, and sugar. While nutritional recommendations are, in principle, the same for all healthy adults, with the physiological alterations that can occur with age (e.g. body composition, gastrointestinal tract, water balance and bone health), nutritional requirements can diverge from those of the general population. As appetite and overall energy requirements can decrease, the demand for protein and micronutrients remains the same or even increases creating a need for more nutrient- dense food options (84). It is particularly important to consume adequate high-quality

protein to maintain muscle and bone health, to improve strength and physical function (413).

Compliance with FBDGs is associated with a reduction in the risk of frailty (88), all-cause mortality, CVD mortality and coronary heart disease (89). In Ireland, FBDGs are based on the FP model, which specifies a recommended number of daily (average) servings from the main food groups, represented as shelves of the FP (90). FBDGs are developed to encourage broad and varied diets in adults of all ages. Few studies of dietary intake and compliance in the general population in Ireland have included healthy subsamples of older adults (91). While one study examined nutritional intake (92), there is little representative evidence specific to the older population that explores dietary characteristics and age-specific determinants of compliance with recommendations in a potentially nutritionally vulnerable population group. To our knowledge, this is the largest study of consumption frequencies and determinants of compliance with FBDGs in a representative sample of the older adults in Ireland.

The objectives of the present study are threefold: (i) to summarise the consumption frequencies of food groups from the FP among older adults in Ireland; (ii) to determine the compliance levels with the FP within this population, and (iii) to establish the socio- demographic, health and lifestyle determinants for compliance with certain food group- level recommendations in this population. This may assist policymakers in the development of targeted interventions to improve dietary habits and thus nutritional status of the older population in Ireland.

8.3. Methods

8.3.1. Participants and Study Methodology

TILDA is a stratified probability sample of 8,172 community-dwelling men and women aged ≥ 50 years, resident in Ireland. The study interviewed participants between October 2009 and February 2011 and assessed social, economic, health, lifestyle and behaviour and attitudes of people resident in Ireland. The response rate

was 62%. Participants were re-interviewed in Wave 2 (2012) and Wave 3 (2014) with corresponding response rates of 82% and 90%, respectively. This study uses data from TILDA Wave 3 (2014) in which participants completed a Food Frequency Questionnaire (FFQ) within a self-completion questionnaire (SCQ), completed by 5,206 participants. The methodological details have been reported elsewhere (310) but briefly, participants were interviewed by trained interviewers in their own home using computer-assisted personal interviewing (CAPI), and an SCQ, including more sensitive questions, was returned by mail. The sampling frame was the Irish GeoDirectory, a mapping of all residential addresses in the Republic of Ireland. A multi-stage probability sample of addresses was chosen using the RANSAM sampling procedure (311). All household residents aged 25 years and their spouse/partner were eligible to participate. This study was approved by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin, and participants provided written informed consent prior to participation in the study.

8.3.2. *Measures*

8.3.2.1. Dietary Intake

The 52-item semi-quantitative questionnaire was adapted from the SLÁN 2007 (91), and the EPIC study (330). The FFQ was designed to assess diet quality and habitual frequency of the 52 food items or aggregate groups within the primary food groups consumed in the typical Irish diet. Food groups were defined according to recommendations from the Irish FP, to enable comparison of dietary patterns with national dietary recommendations (331). Participants indicated habitual frequency of consumption of each item over the last year. Typical weights and portion size estimates were based on recommendations established by food composition tables (332) and the Irish Food Portion Size database (333). For each food item/aggregate group, answers were from nine different frequency categories: never or less than once a month, 1-3 times per month, once a week, 2-4 times per week, 5-6 times per week, once a day, 2-3 times per day, 4-5 times per day, and 6+ times per day, as

shown in **Figure 8.1**. Dietary quality was indicated by mean daily consumption and compliance to the 2012 FP recommendations for each food group (**Figure 8.1.**). To measure compliance within each group participants were scored: complying with recommendations for this shelf (1), or not complying to this shelf (0). An overall measure of compliance to the whole FP was then calculated as the sum of the items, with a theoretical range of 0 (no compliance) to 6 (full compliance). In addition, compliance with recommendations for Fruit and Vegetables, Milk, Yogurt and Cheese and Meat, Poultry, Fish, Eggs, Beans and Nuts shelves were examined separately.

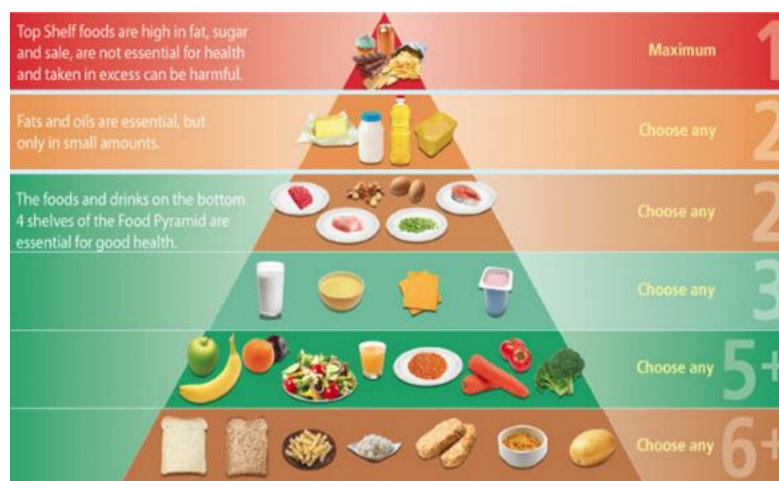


Figure 8.1. Irish Food Pyramid, 2010.

8.3.2.2. Covariates

The covariates included were chosen on theoretical grounds. Socio-demographic characteristics included age, sex, educational attainment (primary or none vs. secondary or higher), marital status (married or cohabiting vs. single, separated or divorced, or widowed), household income (lowest quintile vs. other four quintiles), location (Dublin vs. urban/not Dublin, rural) and employment status (employed vs. retired, or other).

Health behaviours included smoking status (never, past, current), alcohol consumption (no vs. yes), and physical activity using the international physical activity questionnaire (IPAQ) (392). Self-reported physical health measures included

BMI (calculated; (normal ($18.5 \leq \text{BMI} < 25$) vs. underweight (< 18.5), overweight (≤ 25 - < 30), obese (≥ 30)), disability status (not disabled vs. Instrumental Activity of Daily Living (IADL) disability only, or any ADL disability). Self-rated health (excellent or very good vs. good, or fair/poor) and self-rated dental health (excellent or very good vs. good, or fair/poor) were also included. Social participation was measured as taking part in any social groups (yes vs. no). Cognitive impairment was defined as Mini Mental State Examination Score (MMSE) < 24 (318).

8.3.2.3. Statistical Analysis

Descriptive characteristics for categorical variables are presented as total number (N) and percentage (%), continuous variables were described as the mean with standard deviation (SD) or median with range. Multivariable logistic regression was used to investigate whether different patterns of compliance with selected elements of the FP were associated with socio-demographic, health and lifestyle behaviours, social, and cognitive characteristics of the population. Two models were tested, Model 1 adjusted for age, sex, educational attainment, income, location, employment and marital status, while Model 2 made additional adjustment for physical activity levels, smoking status, alcohol consumption, self-reported BMI, disability status, self-rated health and dental health, social participation and cognitive function. Statistical significance was set to $p < 0.05$. TILDA uses survey weights to account for differential selection into the cohort, attrition at each wave and for the complex sample design. These weights were used in estimating prevalence. All analyses were performed using Stata 15.1 (StataCorp, College Station, TX).

8.4. Results

The mean age of the population was 67.0 years (± 8.7) and over half were female (54.9%). Characteristics of the study population are presented in **Table 8.2**.

Table 8.2. Descriptive characteristics

Characteristics	Number of TILDA participants	Weighted prevalence estimates (%) of general population
Sample Size	5,206	
Socio-demographics		
Age, mean $\pm$ SD	67.0 $\pm$ 8.7	66.5 $\pm$ 9.3
Age		
50-64 years	2,318	49.8
65 -74 years	1,797	27.7
75+ years	1,091	22.5
Gender		
Male	2,355	47.5
Female	2,851	52.5
Educational attainment		
Primary/none	1,244	34.8
Secondary	2,097	44.2
Third/higher	1,864	21.0
Income quintiles		
Lowest	965	20.9
2	783	15.7
3	846	16.0
4	866	15.1
Highest	862	15.2
Area		
Dublin	1,283	25.6
Urban/not Dublin	1,474	30.3
Rural	2,449	44.2
Employment Status		
Employed	1,607	33.0
Retired	2,528	43.2
Other	1,065	23.8
Marital Status		
Married	3,640	67.9
Single	448	9.1
Divorced/separated	370	7.8
Widowed/Widowed	748	15.1

Health Behaviours		
Smoking		
Never	2,372	43.6
Past	2,245	42.5
Current	587	13.8
Alcohol Consumer		
No	1,663	36.2
Yes	3,230	63.8
Physical activity level		
Low	1,899	39.4
Moderate	1,780	35.1
High	1,274	25.5
Physical Health		
Self-Reported BMI (calculated)		
Underweight (<18.5)	49	1.0
Normal (18.5≤BMI<25)	1,739	32.7
Overweight (25≤BMI<30)	2,179	43.6
Obese (≥ 30)	1,066	22.8
Disability Status		
Not disabled	4,796	90.7
IADL disability only	15	3.4
Any ADL disability	255	5.8
Self-Rated Health		
Self-Rated Health		
Excellent/very good	2,531	45.1
Good	1,778	35.3
Fair/Poor	896	19.6
Self-rated Dental Health		
Excellent/very good	2,113	38.5
Good	2,169	42.4
Fair/Poor	920	19.1
Social		
Social Participation		
No	2,539	53.3
Yes	2,664	46.6
Cognitive Function		
MMSE, mean ± SD	28.6 ± 1.9	28.4 ± 2.0
MMSE ≥ 24	213	5.4

SD, standard deviation

Detailed consumption frequencies of each food group in the FP as servings/day by sample characteristics (mean (SD)) are shown in **Supplementary Table 8** and described in Supplementary Information. Percentage compliance for characteristics within selected shelves of the FP are detailed in **Table 8.3**. Women (percentage compliance [%] = 23.9; 95% confidence interval [CI] (22.1,25.7)), those with higher levels of educational attainment (%=26.5; 95% CI (24.2,29.0) tertiary or higher vs. none/primary), income (%=24.2%; 95% CI (21.0,27.6) highest vs. lowest quintile) and physical activity (%=22.1; 95% CI (19.7,24.7)) had significantly higher levels of compliance with the recommendation of 5+ servings of fruit and vegetables per day.

Conversely, those that smoked (%=13.8; 95% CI (10.8,17.4)), were overweight (%=18.0; 95% CI (16.3,19.8)) or had any ADL (%=11.5; 95% CI (7.9,16.6)), reported fair/poor self-rated (%=16.0; 95% CI (13.5,18.8)) and dental health (%=15.4; 95% CI (13.1,18.0)) and no social participation had significantly lower percentage compliance (%=17.4; 95% CI (17.8,19.1)). There was also an age gradient evident with Fruit and Vegetable compliance with the percentage of those aged ≥ 75 years reported significantly lower compliance than younger age groups (%=15.5; 95% CI (13.3,18.0) vs. 50-64 years and 65-74 years). With respect to the Milk, Yoghurt and Cheese recommendation, there were few differences among the groups, with those that do not consume alcohol having a higher percentage compliance (%=13.2; 95% CI (11.4,15.1)). Secondary level education (%=26.5; 95% CI (24.5,28.6)), higher income (%=26.4; 95% CI (23.2,30.0)) and higher BMI (%=24.2; 95% CI (22.2,26.2)) showed a higher percentage compliance for the Meat, Poultry, Fish, Eggs, Beans and Nuts recommendation (2 servings per day), while people who were single (%=21.9; 95% CI (17.7,26.9)) or divorced/separated (%=21.0; 95% CI (16.7,26.0)) or underweight (%=6.8; 95% CI (2.5,17.0)) had significantly lower levels.

Table 8.3. Percentage compliance with recommendations for shelves of the Food Pyramid: Fruit and Vegetables, Milk, Yogurt and Cheese and Meat, Poultry, Fish, Eggs, Beans and Nuts recommendations.

Characteristic	Compliance with Fruit and Vegetable recommendation (5+ per day)	Compliance with Milk, Yoghurt and Cheese recommendation (3 servings per day)	Compliance with Meat, Poultry, Fish, Eggs, Beans and Nuts recommendation (2 servings per day)
Percent (95% CI)	21.7 (20.1,22.8)	11.9 (11.0,12.7)	24.3 (23.1,25.5)
Socio-demographics			
Age			
50-64 years	20.9 (19.0,22.9)	11.5 (10.1,13.1)	24.9 (22.9,27.0)
65 -74 years	20.2 (18.3,22.4)	11.8 (10.2,13.6)	22.9 (20.8,25.2)
75+ years	15.5 (13.3,18.0) ^{a,b}	11.0 (9.0,13.3)	23.8 (21.2,26.6)
Gender			
Male	14.8 (13.3,16.3)	10.1 (8.8,11.5)	25.7 (23.7,27.7)
Female	23.9 (22.1,25.7) ^c	12.7 (11.4,14.1)	22.7 (21.0,24.5)
Educational attainment			
Primary/none	14.8 (12.7,17.2)	11.2 (9.4,13.2)	21.4 (19.1,24.0)
Secondary	19.8 (18.1,21.6) ^d	11.2 (9.9,12.7)	26.5 (24.5,28.6) ^d
Third/higher	26.5 (24.2,29.0) ^{d,e}	12.5 (10.8,14.4)	23.6 (21.5,25.9)
Income quintiles			
Lowest	15.3 (12.7,18.2)	10.9 (8.9,13.3)	19.7 (17.1,22.5)
2	18.4 (15.6,21.6)	10.7 (8.4,13.4)	23.1 (19.9,26.7)
3	16.4 (13.9,19.2)	11.5 (9.1,14.4)	24.4 (21.3,27.9)
4	23.3 (20.4,26.5) ^f	10.7 (8.7,13.2)	26.1 (22.8,29.6) ^f
Highest	24.2 (21.0,27.6) ^{f,g,h}	12.5 (10.2,15.4)	26.4 (23.2, 30.0) ^{f,g}
Not recorded	20.1 (17.3,23.3)	12.5 (10.0,15.5)	26.4 (23.2, 29.9)
Area			
Dublin	20.4 (18.0,23.0)	11.0 (9.1,13.1)	24.8 (22.2, 27.6)
Urban/not Dublin	20.9 (18.5,23.4)	11.7 (9.9,13.7)	22.9 (20.4,25.5)
Rural	18.1 (16.5,19.9)	11.6 (10.2,13.1)	24.6 (22.7,26.6)
Employment Status			
Employed	20.5 (18.4,22.9)	10.8 (9.1,12.7)	25.3 (22.9,27.8)
Retired	19.2 (17.6,21.0)	11.9 (10.6,13.4)	23.3 (21.5,25.2)
Other	18.8 (16.4,21.6)	11.5 (10.0,13.8)	24.0 (21.3,27.0)
Marital Status			
Married	20.5 (19.0,22.0)	12.1 (10.9,13.4)	25.7 (24.1,27.4)
Single	17.8 (14.2,22.0)	9.1 (6.4,12.8)	21.9 (17.7,26.9) ⁱ
Divorced/ separated	18.5 (14.2,23.8)	12.1 (8.7,16.6)	21.0 (16.7,26.0) ⁱ
Widowed	16.9 (14.0,20.3)	9.8 (7.9,12.2)	19.9 (17.0,23.1)
Health Behaviours			
Smoking			

Never	21.0 (19.3,22.9)	12.2 (10.7,13.8)	25.0 (23.1,27.1)
Past	19.9 (18.1, 21.8)	9.9 (8.7,11.3)	24.2 (22.1,26.4)
Current	13.8 (10.8,17.4)j,k	14.0 (11.1,18.0)	20.9 (17.7,24.6)
Alcohol Consumer			
Yes	20.8 (19.3,22.5)	10.7 (9.5,12.0)	25.2 (23.6,27.1)
No	18.1 (16.0,20.3)l	13.2 (11.4, 15.1)l	22.6 (20.4,25.0)
Physical Activity Level			
Low	16.0 (14.2,18.0)	10.1 (8.7,11.7)	23.9 (21.7,26.2)
Moderate	22.1 (20.0,24.4)m	11.8 (10.2,13.7)	25.1 (22.9,27.6)
High	22.1 (19.7,24.7)m	12.5 (10.4,14.9)	23.8 (21.2,26.7)
Physical Health			
BMI			
Underweight (<18.5)	18.2 (8.4,35.0)	22.6 (11.4,40.0)	6.8 (2.5,17.0)n
Normal (≤18.5-<25)	21.5 (19.4,23.8)	11.4 (9.8,13.1)	23.5 (21.3,25.9)
Overweight (≤25-<30)	18.0 (16.3,19.8)n	12.0 (10.4,13.7)	24.2 (22.2, 26.2)n
Obese (≥ 30)	20.4 (17.8,23.3)	10.9 (9.1,13.2)	26.3 (23.3,29.5)
Disability Status			
Not disabled	20.1 (18.8,21.4)	11.5 (10.5,12.6)	24.5 (23.1,26.0)
IADL disability only	18.4 (12.3,26.5)	15.2 (9.7,22.9)	17.5 (11.7,25.8)
Any ADL disability	11.5 (7.9,16.6)o	8.8 (5.9, 13.1)	21.8 (16.7,28.0)
Self-Rated Health			
Self-Rated Health			
Excellent/very good	21.6 (19.9,23.5)	12.6 (11.2,14.1)	24.1 (22.3,26.2)
Good	18.8 (16.9,21.0)	10.3 (8.8,12.0)	25.3 (23.2,27.5)
Fair/Poor	16.0 (13.5,18.8)p	11.0 (8.8,13.6)	22.0 (19.2,25.0)
Self-rated Dental Health			
Excellent/very good	21.9 (20.0,24.0)	11.5 (10.1,13.2)	24.4 (22.2,26.7)
Good	19.3 (17.5,21.2)q	11.3 (9.9,12.9)	24.5 (22.4,27.0)
Fair/Poor	15.4 (13.1,18.0)q	11.6 (9.5,14.1)	22.7 (19.7, 25.9)
Social			
Social Participation			
Yes	22.0 (20.3,23.7)	12.3 (10.9,13.9)	25.1 (23.4,27.0)
No	17.4 (17.8,19.1)r	10.7 (9.4,12.2)	23.2 (21.3,25.2)
Cognitive Function			
MMSE > 24	19.8 (18.5,21.1)	11.5 (10.5,12.6)	24.3 (22.9,25.7)
MMSE  24	13.5 (9.6, 18.8)	10.6 (6.8,16.2)	21.5 (16.1,28.0)

Significant difference ($p < 0.05$) between sample groups calculated with Kruskal-Wallis- and Conover-Inman-Tests: a) different to aged 50-64; b) different to aged 65-74; c) Female vs Male; d) different to primary; e) different to secondary; f) different to low income; g) different to 2nd; h) different to 3rd; i) different to married; j) different to never; k) different to past smoker; l) different to alcohol consumer; m) different to low PA; n) different to normal BMI; o) different to not disabled; p) different to excellent self-rated health; q) different to excellent self-rated dental health; r) social participation yes/no.

The results of multivariable logistic regression analysis investigation the associations of characteristics of older population with compliance with recommendations from selected shelves of the FP are shown in **Table 8.4**. Following adjustment for putative confounders, compliance with any 3 or more recommendations from the FP was associated with moderate (Odds Ratio [OR] = 1.49; 95% CI (1.16, 1.92)) and high (OR=1.35;95% CI (1.02,1.80)) levels of physical activity. The determinants independently associated with compliance with recommendations for consumption of Fruit and Vegetables were being female (OR=2.13; 95% CI (1.81,2.50)), having a tertiary or higher education level (OR=1.69 95% CI (1.34,2.12), and reporting higher levels of physical activity (OR=1.51; 95% (1.27,1.80), OR=1.70; 95% CI (1.40,2.07), moderate and high). The determinants associated with non-compliance with this recommendation were being a current smoker (OR =0.67; 95% (0.51, 0.89)), being widowed (OR=0.75; 95% (0.59, 0.97)) and reporting fair/poor self-rated dental health (OR=0.80; 95% (0.65, 1.00)). Determinants independently associated with complying with the recommendation for Milk, Yogurt and Cheese included being female (OR=1.48: 95% (1.20,1.81)), and reporting moderate physical activity (OR=1.25; 95%CI (1.00, 1.56)); while consuming alcohol was associated with non-compliance ((OR=0.81; 95% CI (0.66,0.98)). Compliance with the recommendation for Meat, Poultry, Fish, Eggs, Beans and Nuts was associated with being in the highest income quintile (OR=1.37; 95% CI (1.03, 1.81)) and having a second level education (OR=1.30; 95% CI (1.07,1.58)), while non-compliance with the recommendation was associated with being widowed and underweight.

Table 8.4. Multivariable logistic regression analysis of determinants of compliance with recommendations from selected shelves of the FP.

	Compliance with any 3 or more shelves		Compliance with any 3 or more shelves		Compliance with Fruit and Vegetable recommendation		Compliance with Milk, Yogurt and Cheese recommendation		Compliance with Meat, Poultry, Fish, Eggs, Beans and Nuts recommendation	
	Model 1		Model 2		Model 2		Model 2		Model 2	
n	5,105		4,468		4,496		4,497		4,497	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Age Group (ref: 50-64 years)										
65-74 years	0.89	[0.68,1.15]	0.86	[0.65,1.13]	1.08	[0.89,1.31]	1.04	[0.81,1.33]	0.90	[0.75,1.08]
75 + years	0.83	[0.60,1.15]	0.88	[0.62,1.27]	0.97	[0.75,1.25]	1.03	[0.75,1.41]	1.17	[0.93,1.48]
Sex (ref: Male)										
Female	1.15	[0.94,1.42]	1.22	[0.97,1.53]	2.13***	[1.81,2.50]	1.48***	[1.20,1.81]	0.91	[0.78,1.06]
Education (ref: primary)										
Secondary	1.19	[0.90,1.58]	1.18	[0.87,1.62]	1.15	[0.92,1.44]	1.08	[0.83,1.40]	1.30**	[1.07,1.58]
Tertiary	1.31	[0.98,1.76]	1.18	[0.87,1.62]	1.69***	[1.34,2.12]	1.08	[0.83,1.40]	1.13	[0.91,1.39]
Income Quintile (ref: lowest)										
2	1.39	[0.94,2.08]	1.39	[0.90,2.14]	1.18	[0.88,1.58]	1.04	[0.73,1.47]	1.09	[0.83,1.44]
3	1.33	[0.89,2.00]	1.35	[0.87,2.09]	1.05	[0.78,1.42]	0.95	[0.66,1.36]	1.29	[0.98,1.70]
4	1.32	[0.89,1.97]	1.35	[0.88,2.07]	1.29	[0.97,1.70]	1.00	[0.70,1.42]	1.29	[0.98,1.69]
Highest	1.31	[0.84,2.02]	1.23	[0.77,1.97]	1.34	[0.98,1.83]	1.13	[0.77,1.66]	1.37*	[1.03,1.81]
Location (ref: Dublin)										
Urban/not Dublin	0.81	[0.62,1.06]	0.80	[0.60,1.08]	1.00	[0.82,1.22]	1.11	[0.86,1.44]	0.88	[0.73,1.07]
Rural	0.94	[0.74,1.20]	0.95	[0.73,1.24]	0.95	[0.79,1.14]	1.14	[0.90,1.44]	1.02	[0.85,1.22]
Employment (ref: employed)										
Retired	1.03	[0.78,1.35]	0.97	[0.73,1.30]	1.08	[0.89,1.33]	1.21	[0.94,1.57]	0.97	[0.80,1.18]
Other	1.05	[0.78,1.41]	1.04	[0.76,1.44]	1.01	[0.72,1.30]	0.97	[0.80,1.27]	1.13	[0.91,1.40]
Marital status (ref: married or cohabiting)										
Single	0.97	[0.66,1.42]	1.01	[0.68,1.52]	0.90	[0.67,1.20]	0.87	[0.60,1.25]	0.79	[0.59,1.05]
Divorced/separated	0.71	[0.45,1.12]	0.76	[0.47,1.22]	0.84	[0.62,1.15]	0.89	[0.61,1.32]	0.92	[0.69,1.23]
Widowed	0.76	[0.54,1.09]	0.80	[0.55,1.15]	0.75*	[0.59,0.97]	0.80	[0.58,1.09]	0.77*	[0.61,0.99]

Smoking status (ref: never smoked)								
Past smoker	1.22	[0.97,1.53]	1.08	[0.93,1.26]	0.95	[0.78,1.17]	0.95	[0.82,1.11]
Current smoker	1.15	[0.79,1.68]	0.67**	[0.51,0.89]	1.26	[0.93,1.72]	0.96	[0.75,1.23]
Physical activity (ref: low)								
Moderate	1.49**	[1.16,1.92]	1.51***	[1.27,1.80]	1.25*	[1.00,1.56]	0.95	[0.80,1.12]
High	1.35*	[1.02,1.80]	1.70***	[1.40,2.07]	1.15	[0.89,1.47]	0.86	[0.75,1.23]
Alcohol consumer (ref: no)								
Yes	0.85	[0.68,1.07]	1.03	[0.87,1.21]	0.81*	[0.66,0.98]	1.10	[0.94,1.29]
BMI (ref: Normal)								
Underweight (<18.5)	0.54	[0.13,2.28]	0.98	[0.45,2.13]	1.14	[0.47,2.79]	0.25*	[0.08,0.83]
Overweight (≤25 - <30)	0.94	[0.74,1.20]	0.92	[0.78,1.09]	1.09	[0.88,1.34]	0.97	[0.82,1.13]
Obese (≥ 30)	1.15	[0.87,1.54]	1.08	[0.88,1.33]	1.08	[0.83,1.40]	1.13	[0.93,1.37]
Disability status (ref: not disabled)								
IADL disability only	1.17	[0.59,2.33]	0.90	[0.54,1.49]	1.37	[0.80,2.35]	0.63	[0.37,1.05]
Any ADL disability	0.83	[0.44,1.53]	0.68	[0.44,1.05]	1.13	[0.71,1.78]	0.87	[0.61,1.26]
Self-Rated Health (ref: excellent/very good)								
Good	1.02	[0.80,1.30]	1.04	[0.88,1.23]	0.92	[0.74,1.14]	1.15	[0.98,1.34]
Fair/Poor	0.98	[0.69,1.38]	1.05	[0.83,1.33]	1.01	[0.76,1.35]	1.09	[0.87,1.36]
Self-rated Dental Health (ref: excellent/ very good)								
Good	0.89	[0.71,1.12]	0.88	[0.75,1.04]	1.11	[0.90,1.36]	0.98	[0.84,1.15]
Fair/Poor	0.74	[0.54,1.02]	0.80*	[0.65,1.00]	1.03	[0.79,1.35]	0.97	[0.79,1.19]
Social Participation (ref: no)								
Yes	1.15	[0.92,1.43]	1.10	[0.95,1.29]	1.15	[0.95,1.40]	1.00	[0.86,1.15]
Cognitive Function (ref: MMSE>24)								
MMSE >24	0.48	[0.19,1.21]	1.13	[0.70,1.80]	1.13	[0.67,1.89]	1.16	[0.78,1.72]

Exponentiated coefficients; 95% confidence intervals in brackets

* p<0.05, **p<0.01, *** p<0.001

8.5. Discussion

This study shows the majority of older adults did not comply with the national dietary guidelines outlined by the Irish FP. Older adults under-consumed foods that are high in fibre and essential micronutrients (e.g. whole-grain and starchy carbohydrates and fruit and vegetables), while over-consuming energy-dense foods high in fats, salts and sugar, by a factor of almost three. These types of habitual dietary intakes over long periods are concerning as highly-processed foods from within this group have very little nutritional value and contribute to many adverse health conditions including obesity, T2DM, hypertension and CVD (414). A gradual over-consumption of these energy-dense and highly palatable foods may have led to a positive energy balance within this population, as demonstrated by the high levels of overweight and obesity observed in the study population (up to 75%) (358).

Malnutrition, be it by under- or over-consumption, can affect common health conditions in older adults. For example, inadequate intakes of calcium and vitamin D can cause osteoporosis and bone fractures (415). Data from TILDA has indicated that 16% of women and 2% of men report a doctors' diagnosis of osteoporosis (325), and up to 11% of this population were at risk of malnutrition (95). Furthermore just 11.9% of the population of adults aged ≥50 years meeting recommendations of foods from the Milk, Yoghurt and Cheese group, which constitutes major and affordable sources of, calcium, and vitamin D, in addition to B-vitamins and high-quality protein.

These findings are consistent with previous research demonstrating strong and pervasive socio-economic determinants of fruit and vegetable intake among older adults in the United States (416) and Canada (417) and younger population from Ireland (91). Less advantaged groups tend to have poorer quality diets which can contribute to other health inequalities in the prevalence of obesity, T2DM and CVD (418).

Results suggest that overall compliance with recommendations across all six shelves of the FP as a whole was low, and no specific socio-demographic or health or social associations were evident. There was some evidence that a more active lifestyle was associated with better diet, as increased physical activity levels were independently

associated with complying with three or more shelves. However, this accounted for less than 10% of this population.

Of particular concern was the failure of large proportions of the older population to meet the basic recommendations of the most nutrient-dense food groups and arguably the most nutritionally relevant groups among older people, Fruit and Vegetables, Milk, Yogurt and Cheese, and Meat, Poultry, Fish, Eggs, Beans and Nuts shelf. Previous research among the general adult population of Ireland has indicated that messages associated with the FP are well understood but may not translate to daily food choices, with just one in four in the general consuming ≥ 5 servings/day of fruit and vegetables (419).

We also observed age, gender and socio-economic differences in determinants of compliance many of the FP recommendations. A clear age gradient was also observed in the percentage of Fruit and Vegetable compliance, with those aged 75 years and older reporting significantly lower compliance than younger age groups. Women were more likely to comply with recommendations, particularly with Fruit and Vegetables, which had low compliance overall, while socio-economic differences in compliance were evident with both higher income and higher educational attainment associated with higher compliance within some food shelves.

To improve compliance with the FP recommendations, these findings highlight the need for targeted, practical interventions that account for socio-economic disparities and the distinct barriers faced by older adults. Public health campaigns could be tailored to specific groups, such as men, individuals with lower incomes or education levels, and those aged 75 and over, who demonstrated lower compliance. These campaigns should promote cost-effective, nutrient-dense foods and raise awareness about the nutritional importance of consuming sufficient fruit, vegetables, and high-quality protein. Community-based programs, such as cooking classes or meal-planning workshops, could be designed to teach older adults how to incorporate these foods into their diets despite financial or physical limitations. Furthermore, increasing access to affordable, nutritious foods through subsidies or voucher systems for low-income older adults could directly address socio-economic barriers to compliance.

As protein absorption and intake decreases with age, loss of lean body mass (LBM), sarcopenia and frailty can occur (82). Individuals in older age groups would therefore benefit from increasing their intake across the Milk, Yogurt and Cheese and the Meat, Poultry, Fish, Eggs, Beans and Nuts shelves, which are high in protein and micronutrients. Exercise should be combined with this increased protein intake and other nutritional interventions, to maintain LBM and loss of strength (420).

We also found that lifestyle and health habits may affect nutrition behaviour. Smoking and poorer self-rated dental health were associated with non-compliance with the Fruit and Vegetables recommendation, which may be linked to advancing age as the group age 75 year and older had significantly lower percentage compliance with this food group. Alcohol consumption was associated with non-compliance with Milk, Yogurt, and Cheese, recommendations. Finally, widowhood and being underweight independently predicted non-compliance with the Meat, Poultry, Fish, Eggs, Beans, and Nuts recommendations. Further work is needed to disentangle the causal pathways between these confounders and poor compliance with the FP.

Our study has several strengths. We used detailed phenotypic data pertaining to socio- demographic and lifestyle factors, diseases and medication use in a large, well- characterised sample from one population. In addition, the consideration of relevant covariates highlighted in previous studies adds to the rigor of the current study. One strength is the examination of consumption frequencies by food group-level. Nutrients and food constituents are not consumed in isolation, but rather in meals consisting of foods with complex combinations of nutrients that may interact. Moreover, intake of several nutrients is usually correlated (e.g., meat protein and saturated fat, vitamin A and C). Therefore, it is problematic to examine the independent health effect of individual nutrients with such effects often too small to detect, however, the combined effect of many within broad dietary patterns could be large enough to be detectable.

Further, the reported results are comparable with other data, allowing potential cross-country comparisons on food consumption behaviour. Notwithstanding these strengths, the study limitations must be acknowledged. The cross-sectional design limited our ability to make inferences about causal relationships between FBDG

compliance and lifestyle factors. The use of self-reported questionnaires is subject to potential inaccuracies, recall and reporting biases. If we consider an FFQs propensity to overestimate intakes, our study population is performing even worse than our estimates suggest with respect to average daily servings of foods.

The analysis controlled for a wide range of appropriate covariates, however there may still be residual confounding. Model covariates were chosen *a priori* for consistency with the literature and were theoretically plausible, but it is possible that other unidentified confounding factors could have influenced the results. Finally, the sample consisted of high functioning, healthy and community-dwelling participants and may not represent the older population living in residential care.

FBDGs such as the FP are important instruments for public health nutritionists and stakeholders for planning and deliverance of dietary recommendations to the general public, however, many studies have shown that education-based instruments such as the FP may not be effective in the translation of recommendations to practice, particularly in those who lack interest in health and diet (421). Since the data in the present study present data was collected (2014), the Department of Health has published an updated FP (December 2016) containing age-specific dietary guidelines for the first time (90).

The key differences between these and the guidelines employed in the present study are increased intake of Fruit and Vegetables, a decrease in the suggested maximum levels of items from the Foods and Drinks high in Fat, Salt and Sugar shelf, and a reduction in appropriate daily servings of Breads, Cereals, Potatoes, Pasta and Rice, which is sex- and activity-level specific. When considering how poorly the older population performed with the former FP, it is apparent how inaccessible new recommendations are and that compliance would be even lower than in the present study. This gap between knowledge and practice suggests that current methods of conveying dietary advice may not be sufficiently impactful for the older population, particularly without accompanying interventions that address access, affordability, and personalised education.

While the changing nutritional needs of older adults are considered somewhat in the newer FBDGs, we feel these recommendations are unattainable without proper education and awareness and that a more focused approach to achieving optimal nutrition intake may be more appropriate with this older population.

To improve compliance with these updated guidelines, it would be essential to engage older adults through tailored strategies, including public health education campaigns that emphasize the relevance of proper nutrition as individuals age. Engaging healthcare providers, such as general practitioners and dietitians, to deliver targeted advice during routine health check-ups would increase the accessibility of this information. Nutritional interventions designed for older adults should also involve community support, such as nutritional workshops, peer-led initiatives, and local health promotion activities, to encourage better compliance through social support and practical guidance.

Our results demonstrate the translational paucity of the dietary recommendations for older people and provide an important impetus for additional development of these tools and for the introduction of new strategies to enhance the compliance and knowledge of this population subgroup. This highlights a potential gap between public health recommendations and the real-world needs of older adults, suggesting that these guidelines should be revised to reflect the specific barriers this population faces.

Given the ageing demographic there is an urgent need for a current national nutrition policy that focuses on older people as a varied and potentially vulnerable group for lifestyle-related illness, which should include policies to minimise the risk of both over- and under-nutrition, and to promote inclusive measures that ensure appropriate education and awareness, food availability, accessibility and affordability as people age. Such interventions have been effective in changing dietary intake in older adults in Europe (422). For older adults in Ireland, FBDGs should highlight specific selections within food group to emphasise foods with a high ratio of nutrients to energy (nutrient density) to ensure adequate nutrient intakes, and should be narrowed to reflect lower energy needs with increasing age.

To conclude, the current study investigated the associations of nutritional behaviour and compliance with FBDGs. Findings indicate that recommendations of the FP model are largely unattainable and therefore not suitable in this population of mid-life and older adults. This disconnect between dietary guidelines and actual intake poses serious challenges for public health interventions aimed at older adults. This could have implications for public health policy recommendations for general health and wellbeing, weight management, and prevention of chronic disease in older people in Ireland. Moving forward, developing age-focused, nutritionally sustainable dietary guidance will be critically important to effectively support older adults' health needs, along with strategies that address socio-economic and practical barriers to compliance.

8.6 Supplemental Information

8.6.1 Consumption frequencies

Mean consumption was 4.0 servings/day of foods from the Bread, Cereals, Potatoes, Pasta and Rice shelf. More daily servings of foods from this shelf were consumed by those ≥ 75 years, males, with primary or no formal education, living in rural locations, married and those that did not consume alcohol. Mean consumption of Fruit and Vegetables was 3.6 servings/day. Consumption levels were higher among women, those with higher educational attainment, higher income, former and non-smokers, those without IADL/ADL, with excellent self-reported health and dental health, with higher social participation and those with and higher cognition. The mean consumption of foods from the Milk, Yogurt and Cheese shelf was 2.3 servings/day among older adults. Mean daily consumption levels of Meat, Poultry, Fish, Eggs, Beans and Nuts were 2.0 servings/day. Individuals with lower levels of educational attainment reported higher number of servings from this group, whereas those with an IADL disability consumed less food from this shelf. On average, older adults consumed 2.4 servings/day of foods from the Reduced Fat Spreads and Oils group of the FP. Those aged ≥ 75 years consumed more servings from this shelf, older adults with excellent or very good self-rated health consumed less. Mean daily consumption levels for the Foods and Drinks high in Fat, Salt and Sugar group were 2.9 servings/day.

Supplemental Table 8. Consumption frequencies (weighted estimates) of food groups associated with the Food Pyramid (mean serving per day (95% CI))

Characteristic	Bread, Cereals, Potatoes, Pasta and Rice (N = 5,127)	Fruit and Vegetables (N = 5,146)	Milk, Yogurt and Cheese (N = 5,162)	Meat, Poultry, Fish, Eggs, Fat Beans and Nuts (N = 5,129)	Reduced Spreads and Oils (N = 5,126)	Foods and Drinks high in Fat, Salt and Sugar (N = 5,111)
Mean Consumption (servings), 95% CI	4.0 (3.9,4.1)	3.6 (3.5,3.7)	2.3 (2.2,2.3)	2.0 (1.9,2.0)	2.4 (2.4,2.5)	2.9 (2.8,3.0)
Socio-demographics						
Age						
50-64 years	3.9 (3.7,4.0)	3.7 (3.5,3.8)b	2.3 (2.2,2.4)	1.9 (1.9,2.0)	2.3 (2.2,2.4)	2.8 (2.7,3.0)b
65-74 years	4.1 (3.9,4.3)a	3.6 (3.5,3.8)b	2.2 (2.2,2.3)	1.9 (1.8,2.0)	2.5 (2.4,2.7)a	2.8 (2.7,3.0)b
75+ years	4.2 (4.0,4.4)a	3.4 (3.2,3.6)	2.2 (2.1,2.4)	2.0 (1.9,2.2)	2.7 (2.5,2.9)a	3.2 (3.0,3.5)
Gender						
Male	4.3 (4.2,4.4)	3.2 (3.1,3.3)	2.2 (2.1,2.3)	2.0 (1.9,2.1)	2.5 (2.4,2.6)	3.0 (2.8,3.1)
Female	3.7 (3.6,3.9)c	4.0 (3.8,4.1)c	2.4 (2.3,2.4)c	1.9 (1.8,1.9)c	2.4 (2.3,2.5)c	2.9 (2.7,3.0)c
Educational attainment						
Primary/none	4.2 (4.0,4.4)	3.2 (3.1,3.4)	2.3 (2.2,2.4)	2.1 (2.0,2.2)	2.6 (2.4,2.8)	3.1 (2.9,3.4)
Secondary	3.9 (3.8,4.1)	3.6 (3.5,3.8)d	2.3 (2.2,2.3)	1.9 (1.9,2.0)d	2.3 (2.3,2.4)d	2.9 (2.8,3.0)d
Third/higher	3.8 (3.7,3.9)d	4.1 (4.0,4.3)d,e	2.3 (2.2,2.3)	1.8 (1.7,1.9)d	2.3 (2.2,2.4)d	2.6 (2.5,2.7)d,e
Income quintiles						
Lowest	3.8 (3.6,4.1)	3.3 (3.0,3.5)	2.2 (2.1,2.3)	2.0 (1.8,2.2)	2.5 (2.3,2.7)	2.9 (2.7,3.2)
2	4.0 (3.8,4.2)	3.5 (3.3,3.7)	2.3 (2.2,2.4)	1.9 (1.8,2.0)	2.4 (2.2,2.5)	3.0 (2.7,3.2)
3	4.1 (3.9,4.3)	3.4 (3.2,3.6)	2.3 (2.2,2.4)	1.9 (1.8,2.1)	2.6 (2.4,2.8)	3.1 (2.8,3.4)
4	4.2 (4.0,4.5)	3.8 (3.6,4.1)f	2.3 (2.2,2.5)	2.0 (1.9,2.2)	2.5 (2.3,2.6)	2.8 (2.5,3.0)
Highest	3.8 (3.7,4.0)	4.0 (3.8,4.3)f,g,h	2.2 (2.1,2.3)	1.9 (1.9,2.0)	2.3 (2.2,2.5)	2.8 (2.6,3.0)
Not recorded	4.0 (3.8,4.2)	3.7 (3.4,3.9)	2.3 (2.2,2.4)	1.9 (1.8,2.0)	2.4 (2.2,2.6)	2.9 (2.7,3.1)
Area						

Dublin	3.7 (3.6,3.9)	3.7 (3.5,3.9)	2.2 (2.1,2.3)	1.8 (1.8,1.9)	2.3 (2.2,2.4)	2.8 (2.6,3.0)
Urban/not Dublin	4.0 (3.8,4.2)	3.6 (3.4,3.8)	2.3 (2.2,2.4)	2.0 (1.9,2.1)	2.4 (2.3,2.6)	3.1 (2.9,3.3)
Rural	4.2 (4.0,4.3)i	3.6 (3.4,3.7)	2.3 (2.2,2.3)	2.0 (1.9,2.1)i	2.5 (2.4,2.6)i	2.9 (2.8,3.0)
Employment Status						
Employed	4.0 (3.8,4.1)	3.8 (3.6,3.9)	2.3 (2.2,2.4)	2.0 (1.9,2.1)	2.4 (2.2,2.5)	2.9 (2.7,3.0)
Retired	4.1 (4.0,4.3)	3.6 (3.5,3.7)	2.2 (2.2,2.3)	2.0 (1.9,2.0)	2.5 (2.4,2.6)	2.9 (2.8,3.1)
Other	3.9 (3.7,4.1)	3.4 (3.2,3.6)	2.4 (2.3,2.5)j	1.9 (1.8,2.0)	2.4 (2.2,2.5)	2.9 (2.7,3.2)
Marital Status						
Married	4.1 (4.0,4.2)	3.7 (3.6,3.8)	2.3 (2.2,2.4)	2.0 (1.9,2.0)	2.5 (2.4,2.6)l	2.9 (2.8,3.0)
Single	4.0 (3.7,4.4)	3.3 (3.0,3.6)	2.2 (2.1,2.4)	2.0 (1.8,2.3)	2.3 (2.0,2.6)	3.2 (2.8,3.5)
Divorced/ separated	3.5 (3.2,3.7)k	3.5 (3.1,3.9)	2.1 (1.9,2.2)	1.9 (1.7,2.1)	2.0 (1.8,2.3)	2.7 (2.3,3.1)
Widowed	3.8 (3.6,4.0)	3.4 (3.2,3.7)	2.2 (2.1,2.4)	1.9 (1.7, 2.1)	2.6 (2.4,2.8)l	2.9 (2.7,3.1)
Health Behaviours						
Smoking						
Never	4.1 (4.0,4.3)	3.8 (3.7,4.0)m	2.3 (2.2,2.4)n	1.9 (1.8,2.0)	2.5 (2.4,2.7)	3.0 (2.9,3.2)
Past	3.9 (3.8,4.0)	3.6 (3.5,3.8)m	2.2 (2.1,2.2)	2.0 (1.9,2.1)	2.4 (2.3,2.5)	2.7 (2.6,2.9)o
Current	3.9 (3.6,4.2)	3.0 (2.7,3.2)	2.5 (2.3,2.7)n	2.1 (1.9,2.3)	2.3 (2.1,2.4)	3.0 (2.8,3.3)
Alcohol Consumer						
Yes	3.8 (3.7,3.9)	3.6 (3.6,3.8)	2.2 (2.2,2.3)	2.0 (1.9,2.0)	2.3 (2.2,2.4)	2.7 (2.6,2.9)
No	4.4 (4.2,4.5)p	3.6 (3.4,3.7)p	2.3 (2.2,2.4)	1.9 (1.8,2.0)	2.6 (2.5,2.8)p	3.2 (3.0,3.4)p
Physical Activity Level						
Low	4.0 (3.8,4.1)	3.4 (3.2,3.6)	2.3 (2.2,2.4)	2.0 (1.9,2.1)	2.4 (2.3,2.6)	2.9 (2.8,3.1)
Moderate	4.0 (3.8,4.2)	3.7 (3.5,3.9)q	2.3 (2.2,2.4)	1.9 (1.8,2.0)	2.4 (2.3,2.5)	2.8 (2.7,3.0)
High	4.1 (3.9,4.2)	3.8 (3.6,4.0)q	2.2 (2.2,2.3)	2.0 (1.9,2.1)	2.4 (2.3,2.6)	2.9 (2.7,3.2)
Physical Health						
BMI (calculated)						
Underweight (<18.5)	4.8 (3.3,6.2)	3.5 (2.5,4.5)	2.4 (2.0,2.7)	2.1 (1.3,2.9)	2.5 (1.5,3.4)	3.2 (2.6,3.9)

Normal (18.5≤BMI<25)	3.9 (3.8,4.1)	3.7 (3.6,3.9)	2.3 (2.2,2.4)	1.9 (1.8,2.0)	2.3 (2.2,2.4)	2.8 (2.6,2.9)
Overweight (25≤BMI<30)	4.0 (3.9,4.1)	3.5 (3.4,3.6)r	2.3 (2.2,2.3)	2.0 (1.9,2.0)	2.4 (2.3,2.5)	2.9 (2.8,3.1)
Obese (≥ 30)	4.2 (4.0,4.4)	3.6 (3.4,3.9)	2.2 (2.1,2.4)	2.1 (1.9,2.2)r	2.6 (2.4,2.8)r	3.0 (2.8,3.3)
Disability Status						
Not disabled	4.0 (3.9,4.1)	3.6 (3.5,3.8)	2.3 (2.2,2.3)	2.0 (1.9,2.0)	2.4 (2.3,2.5)	2.9 (2.8,3.0)
IADL disability only	3.8 (3.4,4.1)	3.5 (2.9,4.0)	2.3 (2.0,2.6)	1.7 (1.5,1.8)	2.6 (2.1,3.2)	3.6 (2.6,4.5)s
Any ADL disability	4.0 (3.6,4.4)	3.0 (2.7,3.4)s	2.2 (2.0,2.4)	2.0 (1.7,2.2)	2.5 (2.2,2.8)	3.1 (2.7,3.5)
Self-Rated Health						
Self-Rated Health						
Excellent/very good	3.9 (3.8,4.0)	3.9 (3.7,4.0)	2.3 (2.2,2.3)	1.9 (1.8,1.9)	2.3 (2.2,2.4)	2.8 (2.6,2.9)
Good	4.2 (4.0,4.3)t	3.5 (3.3,3.7)t	2.3 (2.2,2.4)	2.0 (1.9,2.2)	2.6 (2.5,2.7)t	3.0 (2.8,3.1)
Fair/Poor	4.0 (3.8,4.2)	3.2 (3.0,3.5)t	2.2 (2.1,2.3)	2.0 (1.9,2.1)	2.4 (2.2,2.6)	3.2 (2.9,3.5)t
Self-rated Dental Health						
Excellent/very good	4.0 (3.8,4.1)	3.9 (3.7,4.0)	2.3 (2.3,2.4)	2.0 (1.9,2.1)	2.4 (2.3,2.5)	2.8 (2.6,2.9)
Good	4.1 (4.0,4.2)	3.5 (3.4,3.7)u	2.2 (2.2,2.3)u	1.9 (1.9,2.0)	2.4 (2.3,2.6)	3.0 (2.8,3.1)u
Fair/Poor	3.9 (3.7,4.1)	3.2 (3.0,3.5)u	2.3 (2.1,2.4)	2.0 (1.8,2.1)	2.5 (2.3,2.7)	3.0 (2.7,3.2)u
Social						
Social Participation						
Yes	4.1 (4.0,4.2)	3.9 (3.8,4.0)	2.3 (2.3,2.4)	2.0 (1.9,2.1)	2.5 (2.4,2.6)	2.9 (2.8,3.1)
No	3.9 (3.8,4.1)	3.4 (3.2,3.5)v	2.2 (2.2,2.3)v	1.9 (1.9,2.0)	2.4 (2.3,2.5)	2.9 (2.7,3.0)
Cognitive Function						
MMSE > 24	4.0 (3.9,4.1) w	3.6 (3.5,3.7)w	2.3 (2.2,2.3)	2.0 (1.9,2.0)w	2.4 (2.4,2.5)	2.9 (2.8,3.0)w
MMSE ≤ 24	4.2 (3.6,4.7)	3.0 (2.6,3.5)	2.3 (2.0,2.5)	2.2 (1.9,2.4)	2.4 (2.0,2.9)	3.5 (2.9,4.1)

Significant difference (p<0.05) between sample groups calculated with Kruskal-Wallis- and Conover-Inman-Tests:

a) different to aged 50-64 years; b) different to age 75+ years; c) Female vs Male; d) different to primary; e) different to secondary; f) different to low income; g) different to 2nd; h) different to 3rd; i) different to Dublin;

j) different to retired; k) different to married; l) different to separated/divorced; m) different to current smoker; n) different to past smoker; o) different to never smoked; p) different to alcohol consumer; q)

different to low PA ;r) different to normal BMI; s) different to not disabled; t) different to excellent; u) (dental) different to excellent; v) different to social participation (yes); w) different to MMSE <24.

CHAPTER NINE: FINDINGS

Introduction to Chapter Nine

This Ph.D. thesis had two overarching aims; (i) to investigate whether inadequate plasma folate and vitamin B₁₂ status are associated with cognitive decline and (ii) to examine whether these associations may arise from inappropriate nutrition behaviours and blood pressure dysregulation. Specifically, I set out to examine associations between the two B-vitamins, folate and vitamin B₁₂, and cognitive function. I also aimed to examine, for the first time, indices of blood pressure dysregulation, OH, with respect to these B- vitamins, an often-implicated mechanism in the association between CVD and cognitive impairment. In addition, I assessed whether the community-dwelling population over 50 years in Ireland were compliant with dietary recommendations, given the direct impact dietary behaviours have on ensuring adequate nutrition and optimal micronutrient status among older adults.

In this chapter, the key findings of the thesis are summarized and discussed. Further, potential limitations are addressed, and the possible clinical implications of the findings are outlined. Lastly, I offer recommendations for future research as well as public health policy.

9.1. Main Findings of Thesis

9.1.1. *Introduction and Literature Review*

Chapter One and **Chapter Two** evaluated and discussed relevant background literature. It was evident that many of the recent changes that have occurred including technological advancements and improvements in public health have led to a population boom and an ageing society. In turn, age-related conditions like neurodegeneration and dementia are becoming more prevalent, and while optimising nutrition has emerged as a potential avenue for reducing the risk of neurodegenerative pathologies the age-related changes in diet and nutritional status can impact the longevity and quality of life of older adults, by means of malnutrition.

The interaction between folate and vitamin B₁₂ in the methylation cycle is crucial for maintaining proper metabolic function. The "*methylfolate trap hypothesis*", best summarised in **Figure 1.4** and **Section 1.5.3 (Chapter One)** suggests that a deficiency of vitamin B₁₂ hampers folate metabolism, trapping the active form of folate and impairing overall folate metabolism. Synthetic FA, whether obtained through supplementation, voluntary enrichment or MFAF could therefore plausibly neuropathy and myelopathy caused by vitamin B₁₂ deficiency which has raised safety concerns.

Chapter Two explored the background literature relating to the links between folate and vitamin B₁₂ levels on cognitive function among older adults. Additionally, it investigated the potential relationship between OH, a common manifestation of cardiovascular ageing, and folate and vitamin B₁₂ status, given their associations with cognitive function and blood pressure control. Furthermore, the nutritional behaviours of older adults in Ireland were examined. This chapter reviewed the existing evidence for **Hypothesis 1** in this thesis, "*lower concentrations of specific micronutrients may be implicated in poorer cognitive health and that the co-occurrence of multiple nutrient deficiencies confers greatest risk to cognitive health in older adults*". In the chapter, I sought to review the role of folate and vitamin B₁₂ in cognitive dysfunction and blood pressure control among older adults. **Chapter Two** satisfied **Objective 1.1**, determining the role that lower concentrations of folate and vitamin B₁₂ play in cognitive impairment and blood pressure control. The chapter also addressed **Objective 1.2** by establishing the potential benefit of maintaining micronutrient sufficiency for sustaining the health of the ageing population using several vitamins known to have a high prevalence of low status in older adults. Observational evidence suggests that deficiencies in folate and/or vitamin B₁₂ may contribute to age-related cognitive decline. Multiple studies have found links between low folate and vitamin B₁₂ levels and a higher risk of cognitive impairment and dementia. However, RCTs examining the effects of B-vitamin supplementation on cognitive outcomes have produced inconsistent results. Some RCTs have shown specific improvements in specific cognitive abilities after taking FA supplements for two years or longer. It is important to note that while the main focus has been on the hypothesis that reducing levels of HCY, which are associated

with cognitive impairment, can lead to better cognitive outcomes through B- vitamin supplementation, there could be other biological mechanisms involved. This information is discussed in my recently published review paper (**Paper 1**) (423). The possibility of FA exacerbating cognitive dysfunction resulting from low vitamin B₁₂ status by masking the deficiency and preventing appropriate treatment has been suggested but remains poorly understood, with conflicting epidemiological evidence regarding the causal relevance of these associations.

Folate deficiency can lead to elevated HCY levels, which in turn can contribute to arterial stiffness, renal dysfunction, and high blood pressure. Both folate and vitamin B₁₂ deficiencies are known causes of HCY accumulation, and folate supplementation can effectively treat this condition. Additionally, vitamin B₁₂ deficiency can lead to functional folate deficiency.

Epidemiological studies have indicated a correlation between higher folate and vitamin B₁₂ intake and reduced blood pressure. FA supplementation has been associated with a decreased risk of stroke and CVD, as well as potential improvements in vascular function and reduced risk of brain infarcts and vascular dementia by lowering serum HCY levels.

However, **Chapter Two** reveals that further research is needed to fully comprehend the relationship between plasma concentrations of folate and vitamin B₁₂, their interplay, and blood pressure regulation.

9.1.2. Five Empirical Studies investigating folate and vitamin B₁₂, cognitive function, orthostatic hypotension and adherence to food-based dietary guidelines

Following on from the literature review set out in **Chapter Two**, which addressed thesis **Aim 1** and **Objectives 1.1 and 1.2**, were the five distinct empirical studies of the thesis. All studies were derived from the TILDA dataset and included both cross-sectional and longitudinal analyses. Baseline (Wave 1) plasma folate and vitamin B₁₂ concentrations were analysed as cross-sectional predictor variables in the first of the five studies, presented in **Chapter Four (Paper 2)**. Several measures of cognitive function (global and cognitive domain specific) were analysed as predictor variables in the cross-sectional and longitudinal analyses encompassed in the second (**Chapter**

Five, Paper 3) and third (**Chapter Six, Paper 4)** empirical studies of this thesis, respectively, while the outcome variables include plasma concentrations of the B-vitamins at baseline. In the fourth empirical study (**Chapter Seven, Paper 5**), longitudinal beat-to-beat blood pressure data from Waves 1 and 3 of the AS were analysed as a predictor variable, while concentrations of plasma folate and vitamin B₁₂ from Wave 1 were included as outcome variables.

Aim 2 of my thesis was to "quantify plasma folate and vitamin B₁₂ status in a nationally representative population of older adults." This was carried out by satisfying **Objectives 2.1** and **2.2**: "to measure deficiency and low status rates of folate and vitamin B₁₂ in TILDA at baseline, and to investigate the demographic, socio-economic, geographic, seasonal, health, and lifestyle determinants of these vitamin concentrations in the older adult population of Ireland." This was achieved in **Chapter Four (Paper 2)**, which is the first quantitative study included in my thesis. In Ireland, this study provided the first comprehensive prevalence data regarding the status of folate and vitamin B₁₂, as well as the factors influencing them, within a significant and representative sample of adults aged 50 years and older.

Concentrations of plasma folate (nmol/L) were categorized into five status profiles: <6.8, deficient; 6.8–10.0, low; >10–23.0, low normal; >23.0–45.0, normal; >45.0, high. Total vitamin B₁₂ (pmol/L) was also categorized into five status profiles: <148, deficient; 148–<185, low; 185–<258, low normal; >258–601, normal; and >601, high. One in seven adults aged 50 and older could be classified as having deficient/low folate status (<10 nmol/L), and up to one in eight were classified as having deficient/low vitamin B₁₂ status. The distributions of both B-vitamins among the population aged 50 and older are depicted in Figure 4.1. It was observed that the oldest old (>80 years) had the highest rates of both low folate status and vitamin B₁₂ deficiency, while men and those with lower asset wealth were more likely to have lower folate and vitamin B₁₂ concentrations.

Several studies, including those by Hopkins et al. (2015) and Pfeiffer et al. (2013) (194, 354), have reported that men tend to exhibit lower levels of B-vitamins compared to women. One possible explanation for this difference, as observed in **Chapter Four**, is the higher consumption of supplements by women. Furthermore, it has been observed that older men tend to have a limited understanding of

recommended healthy dietary practices compared to women, who also exhibit healthier eating habits (92, 355). There is a scarcity of studies examining the relationship between economic status and B-vitamins. However, Pfeiffer et al. (2013), in their NHANES study conducted from 2003 to 2006, found that individuals with a lower poverty income ratio had significantly lower levels of serum folate. Similar dietary factors may account for the decline in B-vitamin levels with age and lower asset wealth, such as reduced food quantity, quality, and variety, as well as lower intake of fruits and vegetables among individuals with lower incomes (92, 356).

In this thesis, obesity and smoking were identified as risk factors for lower B-vitamin levels. Obesity is associated with higher erythrocyte folate and lower serum folate levels, potentially due to volumetric dilution, reduced folate intake, decreased folate absorption, and increased folate uptake by red blood cells (357). In Ireland, over half of people aged 50 years and older are centrally obese (358). Smoking has also been linked to lower B-vitamin concentrations, possibly due to oxidation or competition for the cobalt atom in vitamin B₁₂ and the formation of biologically inactive folate derivatives (360).

In **Chapter Four**, I sought to test the hypothesis that among the population of older adults in Ireland there would be high rates of deficient/low levels of folate and vitamin B₁₂, due, in part to a liberal but voluntary practice of fortification of some foods in Ireland. Substantial rates of low folate and vitamin B₁₂ status were observed. Encouraging older adults to improve their eating habits could potentially enhance their folate and vitamin B₁₂ levels. However, compliance with healthy eating recommendations, practical issues like cooking practices and medication intake, as well as age-related gastrointestinal changes, can limit the effectiveness of a holistic unfortified approach. The data from **Chapter Four**, suggest that voluntary fortification fails to prevent deficiencies and low blood levels of these B-vitamins.

The third aim of my thesis was, *“to establish the prevalence of distinct folate and vitamin B₁₂ status groups among older adults in Ireland”*. I sought to do this by satisfying **Objective 3.1 and 3.2** *“to characterise older community-dwelling adults in Ireland by distinct vitamin B₁₂ status and global cognitive function”* in **Chapter**

Five (Paper 3). This paper tested my hypothesis that low vitamin B₁₂ status in conjunction with high folate status, is associated with increased risk of cognitive decline in older people (228, 229, 230). The study demonstrated that the 1.5% of the population aged 50 years and older with simultaneous high folate and low vitamin B₁₂ did not have any adverse associations with cognitive performance compared to those with normal status for both B-vitamins (**Figures 5.2 and 5.3**). Interestingly, the study demonstrated that higher concentrations of folate were associated with small, but statistically significant higher scores for global cognitive function among those aged 50 and older in Ireland. The results showed no evidence that high blood folate levels affected the relationship between low vitamin B₁₂ levels and cognitive impairment. The study did not find any differences in cognitive performance, suggesting that among the older population of Ireland, at least, elevated folate levels did not pose an increased risk to the study population. Conversely, in this population, the data indicated that higher folate levels might actually protect global cognitive function regardless of vitamin B₁₂ status in older individuals living in the community.

Building on the work completed in **Chapters Four and Five**, **Aim 4** of the thesis was “*to investigate longitudinal associations of folate concentrations with changes in cognitive performance*”. This is addressed in **Chapter Six**, the findings of which were published in **Paper 4**, which satisfies **Objective 4.1**, “*to examine sequential assessments of cognitive performance longitudinally at two, four, six- and eight-years follow-up.*” in relation for folate and plasma B₁₂ status. Using five tests of cognitive function, this study investigated the hypothesis that low folate status was associated with accelerated cognitive decline. By examining the longitudinal associations between baseline folate status and repeated measures of cognitive function over time, I demonstrated that over an eight years low folate (<11.2nmol/L) was associated with decline in global cognitive function and a modest insufficiency (<21.8nmol/L) predicted declines in both immediate and delayed recall, markers of episodic memory (227).

The fifth aim of my thesis, “*to ascertain the cross-sectional and longitudinal associations between plasma folate and total vitamin B₁₂ concentrations and orthostatic hypotension in a large, nationally representative cohort of community-*

dwelling adults aged 50 years and older”, was addressed in **Chapter 7**. This was done so by satisfying **Objectives 5.1** and **5.2**. It investigated the hypothesis that low plasma levels of folate and vitamin B₁₂ may increase the risk of OH in the older population. This is the first and therefore largest and most comprehensive population study to investigate the relationship of plasma folate and vitamin B₁₂ concentrations with OH in older adults. Univariate longitudinal associations between folate and OH over 4 years were demonstrated. However, any associations were attenuated by the addition of antidepressant medication use within the model. No cross-sectional or prospective associations between plasma vitamin B₁₂ and indicators of impaired stabilisation of BP or OH after adjustment for relevant covariates were detected.

In the final study of the thesis, I aimed to describe and summarise consumption frequencies and determine compliance levels of food groups as defined by national dietary recommendations (**Aim 6**). This provided a “real-world” synopsis of actual food intake and patterns in the older population, through which dietary adequacy must be maintained. This was achieved by describing and summarising consumption frequencies and percentage compliance with the FBDGs in place and establish the sociodemographic, health and lifestyle determinants for compliance with certain food group-level recommendations in this population (**Chapter Eight, Paper 6**).

Unsurprisingly, this study revealed a concerning trend among older adults in their adherence to national dietary guidelines in Ireland, as the majority of participants failed to comply with the recommended guidance. Notably, there was an insufficient consumption of fibre- and essential micronutrient- rich foods, such as whole-grain carbohydrates, fruit and vegetables, and dairy foods. Conversely, there was a notable overconsumption of energy-dense foods high in fats, salts, and sugars, nearly three times beyond the recommended levels. This habitual dietary pattern consisting of highly processed and nutritionally deficient foods contributes to various adverse health conditions, including obesity, T2DM, hypertension, and CVD. These findings underscore the need for the development of improved dietary recommendations and strategies tailored specifically for older adults. In conclusion, this study's findings suggest that dietary recommendations outlined by the FP model are largely unattainable and unsuitable for mid-life and older adults. This has significant implications for public health policy, as it pertains to overall health and well-being,

weight management, and the prevention of chronic diseases among the older population in Ireland. Moving forward, age-specific and sustainable nutritional guidance will be of utmost importance to address these challenges effectively.

9.2. Methodological Considerations

9.2.1. Limitations

Potential limitations of each of the individual papers included in my thesis have been addressed in their respective chapters. There are some limitations common to all of the papers published.

Firstly, with respect to the nutritional markers in my thesis, the analyses of B-vitamins in **Papers 2, 3, 4 and 5** were limited to the use of non-fasting plasma folate and total vitamin B₁₂ as indicator of status, which although recognised methods of measurements (supported by assay coefficient of variation for quality control), are not as rigorous as the use of supporting vitamin B₁₂ biomarkers such as methylmalonic acid and holotranscobalamin. Acknowledging the disadvantages associated with these biochemical measures (i.e. plasma folate is subject to variations from recent dietary intake, while total vitamin B₁₂ measurement in blood is known to have relatively low sensitivity for estimating vitamin B₁₂ deficiency (375). In addition, HCY was not measured in TILDA. Across the relevant studies (**Papers 2,3,4 and 5**), B-vitamin analysis was conducted at one time point, thus there is a possibility of regression dilution, therefore causality cannot be established. A related limitation of the studies in **Papers 2, 3 4 and 5** is that no dietary intake data were available at baseline of TILDA to coincide with the blood samples, nor the consumption of fortified food products or the dosage of B-vitamin supplements taken were recorded. However, previous studies have shown a weak relationship between dietary intake and blood biomarker data for these micronutrients (194, 353). One recent study has indicated that dietary intake data and biomarkers reflect the same underlying folate status with a systematic underestimation bias in intake data (374).

Secondly, many of the covariates that were used to control for potential confounding, such as relevant medical history, relied on participant self-report. The Irish healthcare

system lacks electronic data collection and integration between hospitals or between hospitals and primary care, necessitating the use of self-reporting as the most effective approach to gather such data. Consequently, self-reporting was facilitated through the CAPI in TILDA, which were conducted consistently by trained interviewers. However, it should be noted that individuals with cognitive impairment might have a higher likelihood of providing inaccurate information regarding their medical conditions. With the exception of one study in my thesis (**Chapter Eight, Paper 6**), self-reported variables were employed for adjusting confounding factors and were not the primary focus. Furthermore, all study participants underwent dementia screening prior to receiving an invitation to participate, and those exhibiting significant cognitive impairment were excluded from the study.

Thirdly, data obtained during the AS was utilised in the HA. Due to the substantial number of health assessments conducted on participants residing in various locations across the country, it was not practically feasible to standardise lifestyle factors such as the time elapsed since their last meal, consumption of caffeine or alcohol, smoking habits, medication timing, or the specific time of day when the active stand was performed. Nonetheless, the AS protocol itself was standardised among participants and administered by trained research nurses adhering to standardised protocols. OH is a diverse condition with various underlying causes, both neurogenic and non-neurogenic, which could potentially affect outcomes. However, the specific underlying causes of OH in the participants included in **Paper 5** presented in this thesis were unknown.

Finally, in all studies in my thesis, the study sample consisted of high-functioning, highly motivated, healthy and community-dwelling participants and may not represent the older population living in residential care and may not be generalisable to non-Caucasians, those older adults in institutional care or those with dementia.

9.2.2. *Strengths*

The main strength of the thesis is its use of the TILDA dataset itself. TILDA provides a large deeply phenotyped cohort of adults aged 50 and older in Ireland that is based on a nationally representative sample. The sample represents 1 in 156 people aged over

50 in Ireland. The CAPI was carried out by trained interviewers, and health assessments were carried out by trained research nurses using standardised protocols.

Furthermore, in all studies in this thesis, the large sample size is a key strength. Among studies of B-vitamins, B-vitamins and cognitive function B-vitamins and blood pressure control, and food consumption patterns, those included in this thesis are among the largest reported in the literature. Comprehensive assessment of covariates in the TILDA dataset and the application of rigorous statistical methods allowed a large number of confounders to be considered in this thesis.

9.3. Conclusions and Future Research

The work in this thesis demonstrated that inadequate plasma folate and vitamin B₁₂ status are implicated in cognitive health in the population. The findings of my comprehensive literature review, presented in **Chapter 2**, indicate that lower, albeit varying levels of folate and vitamin B₁₂, are associated with compromised cognitive health. Additionally, the co-occurrence of multiple nutrient deficiencies confers greatest risk to health in older adults which is important when considering the findings presented in **Chapter 8 (Paper 6)**. This thesis also underlines the necessity of implementing targeted public health interventions, such as mandatory folic acid fortification and increased vitamin B₁₂ supplementation, to address these deficiencies.

The thesis demonstrated that among the population of older adults in Ireland there are high rates of deficiency and low levels of folate and vitamin B₁₂. It will be of the utmost importance to measure the blood folate and vitamin B₁₂ levels in later waves of TILDA (e.g., at Wave 6) to examine change in status *within* individuals over time. This would allow future work to build on the cross-sectional relationship observed in **Chapter Five, Paper 3** and indeed the longitudinal association between folate and with cognitive decline in **Chapter Six, Paper 4**. Additionally, a continuous tracking system of folate and vitamin B₁₂ levels in the population, as well as the monitoring of fortification and supplementation effectiveness, is crucial to fine-tune these public health initiatives.

As discussed previously, there are a number of processes that may underlie these associations between B-vitamins and cognitive function. The measurement of HCY in the Wave 1 data to correspond with the existing folate and vitamin B₁₂ would be an important addition to future studies to determine its significance as the key underlying mechanism in keeping with the discussions of my thesis. Further, studies exploring MRI data (Wave 3) in conjunction with B-vitamins and change in cognitive function and dietary patterns (Wave 3 may add to the understanding of the physiological mechanisms behind these associations. Such studies would also help identify potential physiological markers that can be integrated into policy frameworks for improved health screening and nutritional recommendations.

While I did not find a persistent association between OH and the B-vitamins over 4 years (**Chapter Seven, Paper 5**), this may be a result of the low prevalence of OH and the relatively short follow-up period. Follow-up at future waves of TILDA will allow for explorations of whether OH is associated with B-vitamins over longer follow-up periods. Interestingly, I did demonstrate univariate longitudinal associations between folate and OH over 4 years, but these associations were attenuated with the addition of antidepressant medication use. This suggests that the attenuation is more likely due to the direct effects of antidepressants on the autonomic nervous system, rather than a mediation pathway involving folate and depression. Antidepressants, particularly tricyclics and SNRIs, are known to affect blood pressure regulation by influencing autonomic nervous system function. Given what we know of the relationships between OH and cognitive decline (405), depression (408), and folate and vitamin B₁₂ with these outcomes (227, 235, 409), future research should focus on differentiating the independent effects of antidepressant medications from potential pathways involving vitamin deficiencies. Bidirectionality within these pathways should also be considered, especially given the well-known effects of medications on blood pressure regulation and behaviour. It is recommended that future policy discussions account for the role of medications, especially antidepressants, in modulating B-vitamin effects, to ensure a holistic approach to nutritional and pharmacological interventions in older adults.

Finally, this thesis showed that compliance with FBDGs was low among adults aged 50 and older in Ireland (**Chapter Eight, Paper 6**). This is a critical finding in the

overall context of the thesis. It supports the proposition of the literature review (Chapter Two) that co-occurrence of multiple nutrient deficiencies confers greatest risk to health in older adults, including cognitive function. This strongly highlights the need for the Irish Government and the Department of Health to implement and promote age-specific dietary guidelines, particularly aimed at older adults, who may find it challenging to meet basic dietary requirements through diet alone. Such initiatives should include practical steps to improve access to and affordability of nutrient-rich foods. If older adults are continually unable to meet basic dietary guidelines, this needs to be communicated effectively to policymakers to drive action. This policy should include mandatory folic acid fortification, targeted vitamin B₁₂ supplementation, and public awareness campaigns to inform older adults of their nutritional needs, ensuring that both under-nutrition and over-nutrition risks are addressed.

Effective interventions in Europe have demonstrated the potential for changing dietary habits among older adults, emphasising the importance of revising Ireland's FBDGs to emphasise nutrient-dense food choices that align with age-related lower energy requirements. This is particularly important in the context of a system of voluntary fortification that itself is also ineffective in maintaining adequate status of specific micronutrients, such as folate. Future work to hypothetically model differing levels of folate and vitamin B₁₂ fortification based on the consumption frequencies measured in the TILDA sample could help to inform public health nutrition policy in Ireland. These efforts will ensure that the nutritional status of older adults in Ireland is optimized, and may reduce the prevalence of cognitive decline and cardiovascular issues associated with poor nutrition.

In conclusion, my thesis has explored the intricate connections between folate, vitamin B₁₂, cognitive function, blood pressure regulation in the form of orthostatic hypotension, and dietary patterns in older adults. Through a series of empirical studies, it has shed light on the prevalence of low folate and vitamin B₁₂ status in the older population and its potential impact on cognitive performance and orthostatic hypotension. These findings hold significant implications for public health policy, emphasizing the need for Ireland to implement mandatory folic acid fortification and to promote targeted vitamin B₁₂ supplementation, particularly among older adults.

The findings highlight the importance of maintaining adequate levels of folate and vitamin B₁₂ for optimal cognitive function and overall health in older adults. Additionally, the thesis emphasises the significance of adhering to food-based dietary guidelines tailored to the needs of older individuals to ensure sufficient nutrient intake. The insights gained from this research provide a foundation for developing age-specific nutritional interventions and policies to promote healthy ageing and enhance the quality of life for older adults. These measures, if implemented, will lead to sustained improvements in nutritional health and longevity, and could prevent the progression of nutrient-related cognitive and cardiovascular conditions in the older population.

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CHAPTER ELEVEN: APPENDICES

Appendix 11.1. Published Works

*Appendix 11.1.1 – Paper 1- Sustaining an ageing population:
The role of micronutrients in frailty and cognitive impairment.*

*Appendix 11.1.2 - Paper 2 - Voluntary fortification is ineffective
to maintain the vitamin B₁₂ and folate status of older Irish adults:
Evidence from the Irish Longitudinal Study on Ageing (TILDA).*

*Appendix 11.1.3 - Paper 3 - Plasma concentrations of vitamin B₁₂
and folate and global cognitive function in an older population:
cross-sectional findings from The Irish Longitudinal Study on
Ageing (TILDA).*

*Appendix 11.1.4 - Paper 4 – Low folate predicts accelerated
cognitive decline: 8-year follow-up of 3,140 older adults in
Ireland.*

Appendix 11.2. Copyright Permissions

Appendix 11.3. Food Frequency Questionnaire

Appendix 11.1. Published Works

*Appendix 11.1.1 – Paper 1 - Sustaining an ageing population:
The role of micronutrients in frailty and cognitive impairment.*

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Conference on ‘Food and nutrition: Pathways to a sustainable future’ Symposium four: Sustaining an ageing population

Sustaining an ageing population: the role of micronutrients in frailty and cognitive impairment

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Age-related frailty and cognitive decline are complex multidimensional conditions that significantly impact the ability of older adults to sustain functional capacity and independence. While underlying causes remain poorly understood, nutrition continually emerges as one associated risk element. Many studies have addressed the importance of adequate nutrition in delaying the onset of these conditions, but the specific role of micronutrients is not well established. The consideration of pre-frailty as an outcome variable is also limited in the current literature. In this review, we focus on the potential value of maintaining micronutrient sufficiency to sustaining the health of the ageing population. Using data from the Irish longitudinal study on ageing, we consider several vitamins known to have a high prevalence of low status in older adults and their impact on pre-frailty, frailty and cognitive impairment. They include vitamin B₁₂ and folate, both of which are associated with multiple biological mechanisms involved in long-term health, in particular in cognitive function; vitamin D, which has been associated with increased risk of musculoskeletal disorders, depression and other chronic diseases; and the carotenoids, lutein and zeaxanthin, that may help mitigate the risk of frailty and cognitive decline via their antioxidant and anti-inflammatory properties. We show that low concentrations of folate and carotenoids are implicated in poorer cognitive health and that the co-occurrence of multiple nutrient deficiencies confers greatest risk for frailty and pre-frailty in the Irish longitudinal study on ageing cohort. These health associations contribute to evidence needed to optimise micronutrient status for health in the older adult population.

Micronutrients: Frailty: Cognitive decline: Ageing: Healthspan

Abbreviations: COVID-19, coronavirus disease; FA, folic acid; HCA, homocysteine; RCT, randomised controlled trials; TILDA, The Irish longitudinal study on ageing.

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Background

Global population ageing

Unprecedented technological developments coupled with advances in medicine and public health over the past two centuries has contributed to exponential growth in the global population from 1 billion in 1800, to a projected 8 billion in 2023^(1–3). This population growth can be attributed to profound reductions in child mortality and the rapid increase in life expectancy from 40 to 80 years in developed countries^(4–6). The extension in life expectancy has resulted in an increasing population of older adults, a situation exacerbated by lower fertility rates, especially in developed countries⁽⁷⁾. In 2018, for the first time in human history, the number of people aged ≥ 65 years surpassed those aged ≤ 5 years of age globally⁽⁸⁾. The combination of these two demographic phenomena has resulted in a growing, yet increasingly ageing population throughout the developed world; with similar patterns emerging in the developing world⁽⁷⁾. By 2030, one-in-six Europeans will be aged over 60 years and by 2040 one-in-four older adults will be aged over 85 years⁽⁹⁾. This global phenomenon of population ageing presents pressing challenges to the sustainability of our healthcare, social care and welfare systems, particularly if increases in lifespan are decoupled from increases in healthspan.

Lifespan and healthspan

Increases in the total number of years lived (lifespan) are not keeping pace with gains in the number of years lived free of disease and disability (healthspan). The gap between lifespan and healthspan is estimated at 9 years⁽¹⁰⁾. More pessimistic estimates suggest that as much as one-fifth of life may be lived with chronic disease⁽¹¹⁾. Chronic conditions of mid- and later-life account for 4 out of every 5 years lived with disability, while four conditions – CVD, cancer, diabetes and respiratory diseases – account for 80% of chronic disease-related deaths^(12,13).

Current health and social care strategies are largely ineffective in closing the gap between lifespan and healthspan. The increasing demand on healthcare and social support services resulting from living longer, with a growing burden of disease and disability, is becoming ever more apparent to governments, policy makers, service planners and stakeholders.

This is underscored by high profile programmes targeting the extension of healthy lifespan such as the United Nations Sustainable Development Goals and the WHO Decade of Healthy Ageing. To achieve measurable gains in healthspan, thereby sustaining the ageing population, a more coordinated and cohesive approach across medicine, science, government and wider civic society will be necessary⁽¹⁴⁾. Fundamental to this will be targeting modifiable risk and ameliorating factors that are useful in terms of preventing, monitoring and intervening in the onset and progression of age-related conditions.

Biological v. chronological age

Chronological age is associated with declines in physical and cognitive health, the risk of adverse health outcomes and mortality, and the increased use of health and social care services^(13,15). However, older adults of the same age are not at the same risk for these outcomes⁽¹⁶⁾. Ageing is a dynamic and heterogeneous process and there is substantial variability in how we age biologically. Biological ageing occurs due to damage and dysregulation at the cellular (macromolecules and cells), physiological (tissue, organ, system) and functional (organismal) level, ultimately manifesting in the decline of physical and cognitive function^(17,18). Indeed, the heterogeneity in the pace of biological ageing becomes more pronounced, with more divergent trajectories, at older ages^(17,19). During the recent coronavirus disease (COVID-19) pandemic, many countries advised adults aged ≥ 70 years to shield to prevent infection and mortality and protect health services⁽²⁰⁾. However, using chronological age to characterise individual mortality risk as the basis to implement a blanket policy for older adults at the population level had its problems. Such policies did not take account of the heterogeneity in the pace of ageing among older adults and failed to recognise the consequent harms of physical deconditioning, social isolation, loneliness, depression and decreased quality of life^(21–23).

In this narrative review, we will focus on the potential value of micronutrients and maintaining micronutrient sufficiency, to sustaining the health of the ageing population. We will pay particular attention to two common and interlinked conditions of ageing, frailty and cognitive impairment, which significantly impact the ability of older adults to sustain functional capacity and independence. The graphical abstract depicts the role of micronutrient status and the physiological systems that underpin frailty and cognitive impairment.

Frailty and cognitive decline in older adults

Frailty and the disability cascade

As discussed earlier, not everyone of the same age is at the same risk of adverse health outcomes. Frailty captures this differential biological risk that is distinct from, but related to, chronological ageing⁽²⁴⁾. It is a common condition in older adults, although it is not an inevitable part of ageing. While recognised as a clinical syndrome, frailty is not a medical diagnosis because it can have different underlying causes in different individuals. Frailty is characterised by multisystem loss of physiological reserve, systemic decompensation in response to stressors (e.g. infection, medication change or a change in living arrangements) and increased risk of adverse outcomes including falls, disability and mortality, independently of chronological age⁽²⁵⁾. It is also predictive of increased use of health and social care services⁽²⁶⁾. Frailty is a dynamic process that can be viewed on a continuum. An older person can transition in either direction between robustness or non-frailty, pre-frailty (an intermediate sub-clinical state) and frailty^(27–29).



Thus, it can represent a transition between healthy ageing and disability, and is a target condition for the prevention of disability and the extension of healthy life years^(30–32).

The gold standard methodology for the assessment and management of frailty is comprehensive geriatric assessment. Comprehensive geriatric assessment is a holistic and interdisciplinary assessment of the individual, resulting in a personalised care plan, and has been demonstrated to reduce the risk of disability, cognitive decline, long-term residential care and death^(33,34). However, this approach is unfeasible for systematic case finding at the population level. The frailty phenotype^(35,36), the frailty index^(37,38) and the clinical frailty scale^(39,40) are widely accepted screening instruments for diagnosing frailty at the population level. The choice of frailty instrument depends largely on the type of clinical or research setting, the participant or patient group, availability of trained personnel, time constraints and administrative burden⁽⁴¹⁾. Internationally, the prevalence of frailty is 4–59% among adults aged ≥65 years, depending on the frailty instrument applied and population under study⁽⁴²⁾.

Associations between frailty and cognitive impairment

The study of the relationship between frailty and cognitive decline is complex with several different approaches considered including the examination of effects of separate domains on one another, temporal studies and bidirectionality.

Physical function (often as individual components of the frailty phenotype) and its association with cognitive impairment have been examined. For example, slow gait speed⁽⁴³⁾ and a decline in grip strength⁽⁴⁴⁾ have been linked to cognitive impairment and poorer performance on tests of memory, verbal skills, spatial skills and processing speed, respectively. Further, some studies have investigated the temporal relationship between the reduction of muscle strength and cognitive ability, suggesting that cognitive decline may precede physical decline^(45,46), although the evidence suggests that this effect is attenuated when other comorbidities are considered⁽⁴⁷⁾.

While a number of studies have linked frailty to cognitive decline⁽⁴⁸⁾, with some indicating frailty predicts global cognitive decline and incident Alzheimer's disease⁽⁴⁹⁾, many investigations have focused on the associations between frailty and individual domains of cognitive function. In a recent longitudinal study, participants who were frail showed deficits on assessments of verbal fluency and information processing speed over a 12-year period⁽⁵⁰⁾.

Current evidence suggests that worsening frailty among older adults is considered a precursor to cognitive impairment, to a lesser extent, the reverse may also be true^(43,44). Frailty and cognitive impairment have a variety of underlying causes, both conditions can predict incident dementia, and each may influence the other. As they are highly correlated with advancing age, it is expected that the two will interact as people age^(45,46).

Understanding their co-occurrence and interplay could therefore shed light on pathophysiology, management and prevention. The combination of two perspectives that are typically treated individually presents a challenge in the study of frailty and cognitive impairment. Additionally, only a few studies have explicitly investigated frailty and cognitive impairment in this manner. Although frail older persons may perform poorly on cognitive tests, they may not show significant changes in the cognitive tests, according to research that looked at the bidirectionality of frailty and individual domains of cognitive function^(50,51). Conversely, some researchers have examined a bidirectional association between components of frailty indices, namely physical function, and cognition. A significant predictive value of baseline handgrip strength on the onset of further cognitive decline was recently confirmed by a longitudinal study conducted on an American population over a 20-year period⁽⁵²⁾. Interestingly, the authors also highlighted a significant bidirectional relationship in which the absence of cognitive deficit or the presence of increasing baseline cognitive deficit severity related to progressively higher risks of weaker handgrip strength⁽⁵²⁾. The consistent bidirectional association between physical and cognitive functions has also been validated in a large Korean population over 8 years, with results suggesting that these conditions might share common pathways such as oxidative stress or chronic inflammation⁽⁵³⁾. Oxidative stress⁽⁵⁴⁾ and chronic inflammation⁽⁵⁵⁾ are associated with both frailty and with cognitive decline.

No matter the method of measurement, frailty is a broader, more comprehensive concept that incorporates deficits across various domains and incorporates many aspects of physical function. Thus, rather than focusing solely on physical function, using the concept of frailty allows for the assessment of a more comprehensive measure of health and susceptibility. Few longitudinal studies have explored a bidirectional relationship between frailty and cognitive impairment. One such study by Godin *et al.*⁽⁵⁶⁾ identified a significant bi-directional relationship across two waves of SHARE, a study based in Europe.

Cognitive frailty

Another strategy for the exploration of the relationship between frailty and cognitive impairment is the concept of cognitive frailty. Defined by the International Academy of Nutrition and Aging/International Association of Gerontology and Geriatrics as '*the simultaneous presence of physical frailty operationalized with the frailty phenotype model and cognitive impairment diagnosed with a CDR score of 0.5 among older adults without concurrent Alzheimer's disease (AD) or any other form of dementia*', it is used to characterise people who have both features but have not been clinically diagnosed with dementia⁽⁵⁷⁾. The inclusion of cognitive measures in the assessment of frailty can improve the predictive validity of the phenotype regarding unfavourable health and is important for assessing both physical and cognitive function in older adults for the planning of timely interventions.



The role of nutrition in frailty and cognitive impairment

Nutrition and frailty are intrinsically linked. Unintentional weight loss is a key susceptibility factor for frailty, according to Fried *et al.*⁽³⁶⁾. Primary sarcopenia, due in part to macro- and micro-nutrient deficiencies, is common among frail populations^(58,59) with up to 90% of older persons who are malnourished also being more frail^(58,60,61).

While many studies have focused on the impact of macronutrient (e.g. protein) intake on frailty, fewer have reported associations between explicit frailty and the circulating micronutrients captured within the scope of this review^(62–68) (Table 1). Previous investigations have exhibited design limitations including modest or female-only or relatively young samples or have used a single frailty measure^(62–67). However, a study from our research group used a large representative sample of adults aged 50 and over, to demonstrate that lower levels of lutein, zeaxanthin and vitamin D were associated with three different measures of frailty, and also that these relationships were evident in measures of prefrailty (Fig. 1)⁽⁶⁸⁾.

Nutrition is a modifiable risk factor that has been associated with many non-communicable diseases linked to dementia, such as diabetes and CVD^(69,70). Lifelong nutrition may also have a direct effect on brain function, for example, longitudinal studies have reported associations between certain dietary patterns or nutrients and brain-volume loss^(71,72) and integrity⁽⁷³⁾, with some clinical trials confirming these results^(74,75). Oxidative stress is thought to be a major contributor to neurodegeneration and depression⁽⁷⁶⁾; thus, antioxidants such as vitamin C^(77–80), E⁽⁸¹⁾ and β -carotene may be important⁽⁸²⁾, but no clear conclusions can be made. Overall, a substantial body of research, largely from observational studies, points to a direct impact of lifelong nutrition on clinical indicators of cognitive status in older persons.

Vitamin B₁₂ and folate

The role of vitamin B₁₂ and folate in frailty

Vitamin B₁₂ and folate have been linked to various chronic diseases of ageing such as CVD, diabetes and cognitive impairment^(83,84). These water-soluble micronutrients are essential co-factors in one-carbon metabolism, DNA-methylation and nucleotide synthesis, serving a regulatory effect in all tissues in the body, including systemic inflammation⁽⁸⁴⁾. Therefore, it has been proposed that these B vitamins provide a fundamental framework for comprehending the onset and development of frailty, by their modulating effect on cellular processes. Deficiency or low serum levels of vitamin B₁₂ (<400 pg/ml, equivalent to <295.1 pmol/l) seem to have a negative effect on conditions such as sarcopenia⁽⁸⁵⁾ and other musculoskeletal disorders⁽⁸⁶⁾ related to frailty. Conversely, it has been observed that ageing and frailty can lead to vitamin B₁₂ deficiency⁽⁶⁰⁾. Low folate has also been independently associated with frailty^(62,64),

but this relationship has not been observed consistently^(67,68).

The role of vitamin B₁₂ and folate in cognitive impairment

B-vitamins that are involved in one-carbon metabolic pathways have been studied extensively for their potential effect on cognitive impairment and dementia⁽⁸⁷⁾. Both vitamin B₁₂ and folate, in addition to vitamin B₆ and riboflavin, are required for DNA synthesis and repair, amino acid metabolism and methylation reactions. Further, these B-vitamins are required for efficient metabolism of homocysteine (HCY), a cytotoxic intermediary amino acid that is a downstream product of biological methylation reactions⁽⁸⁷⁾. Observational evidence suggests that the inhibition of methylation reactions may influence cognitive impairment in ageing⁽⁸⁷⁾ and there is growing interest in the possibility that a loss of cognitive function may partly be explained by inadequate status of these vitamins^(88–91). Severe vitamin B₁₂ deficiency, such as that seen in pernicious anaemia, causes severe neurological consequences including sensory and motor neuropathy. Low or deficient vitamin B₁₂ status is associated with depression⁽⁹²⁾ and altered mental status and cognitive decline⁽⁹³⁾. It also reduces the availability of folate for DNA synthesis⁽⁹³⁾. Age-related deficiencies in folate transport and metabolism, use of anti-folate drugs, genetic factors and excessive alcohol consumption are among the factors that contribute to vitamin B₁₂ and folate insufficiency⁽⁹⁴⁾, frequently seen in older persons⁽⁹⁵⁾. In Irish longitudinal study on ageing (TILDA), the prevalence of older people with deficient or low vitamin B₁₂ (<185 pmol/l) and folate (<10 nmol/l) status was 12% and 15%, respectively⁽⁹⁶⁾.

Several epidemiological studies have shown cross-sectional and prospective associations between low vitamin B₁₂⁽⁹³⁾ and folate^(97–106), and the risk of cognitive impairment and dementia. As demonstrated previously by our group⁽¹⁰⁷⁾, low baseline folate levels can predict a reduction in overall cognitive function and episodic memory in older persons who were cognitively healthy, making them a potential key marker for the risk of early decline (Fig. 2). This was consistent with other studies showing low folate status was associated with higher risks of cognitive impairment or dementia^(97–106).

In addition, selected observational data have suggested that older adults with simultaneous low vitamin B₁₂ and high folate status had higher risks of anaemia and cognitive impairment or decline^(108–110), given that high-dose folic acid (FA) treatment was shown to temporarily mask clinical symptoms in persons with pernicious anaemia⁽¹¹¹⁾. However, the causal relevance of these associations remains uncertain with conflicting results^(112–114). TILDA reported that those with low B₁₂ combined with high folate status did not have any adverse associations with cognitive performance compared. In contrast, the study demonstrated that higher concentrations of folate were associated with small, but statistically significant higher scores for global cognitive performance in this setting⁽¹¹⁵⁾.

Table 1. Summary of the existing relationship between selected micronutrients, frailty and cognitive impairment

Micronutrient	Major sources	Main findings associated with frailty and related measures (references)	Main findings associated with cognitive impairment and related measures (references)
Vitamin B ₁₂	Animal products	- Low serum B₁₂ concentrations associated with frailty⁽⁶²⁾ - Increasing MMA – 1.66–2.33 times greater odds of being frail compared to non-frail⁽⁶⁹⁾ - Serum B₁₂ 15 % lower in individuals with sarcopenia^(60,69)	Low or deficient B₁₂ concentrations associated with depression⁽⁶³⁾ and cognitive decline⁽⁶⁹⁾
Folate	Animal products; milk; leafy vegetables; legumes	- Low serum folate associated with frailty⁽⁶²⁾ - Higher folate significantly and negatively associated with frailty^(60,64,65) - Supplementation with folate, vitamins B₆, B₁₂, D and calcium improves frailty among community-living older persons⁽¹⁶⁶⁾	Low folate associated with the risk of cognitive impairment and dementia^(67,168)
Vitamin D	Fish oils; red meat; fortified foods (e.g. ready to eat breakfast cereals, milk)	- Odds of being frail were higher for those participants with lowest vitamin D^(62,63) - Higher vitamin D was significantly and negatively associated with frailty⁽⁶⁴⁾ - Three measures of frailty were associated with lower levels vitamin D⁽⁶⁹⁾ - Vitamin D supplementation is linked to improved gait speed and muscle strength in the elderly⁽¹²⁹⁾	Lower vitamin D was linked to faster cognitive ageing and worsening cognitive test scores^(143,144)
Xanthophyll carotenoids (lutein, zeaxanthin)	Leafy vegetables and eggs	- Association between frailty and lutein/zeaxanthin⁽⁶²⁾ - Lowest quartile of serum carotenoids – increased risk of becoming frail⁽⁶³⁾ - Measures of frailty were associated with lower levels of lutein and zeaxanthin⁽⁶⁸⁾ - Zeaxanthin lower in subjects who are physically, cognitively or psychologically frail⁽¹⁶²⁾ - Lower plasma lutein and zeaxanthin concentrations were linked to decreased grip, hip and knee strength in community-dwelling older women, according to a cross-sectional study⁽¹⁶³⁾	Higher lutein and zeaxanthin have been positively associated with cognitive performance⁽¹⁶⁴⁾
Composite of micronutrients		- Frail women were more likely to have at least two or more micronutrient deficiencies (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, retinol, α-tocopherol, 25 (OH) D, vitamin B₆, B₁₂ and folate)⁽⁶⁹⁾ - The number of nutritional deficiencies (retinol, α-tocopherol, 25-hydroxyvitamin D, vitamin B₆, vitamin B₁₂, folate, selenium, zinc and total carotenoids) associated with incident frailty⁽⁶³⁾ - Low intake of more than three (protein, vitamin D, E, C and folate)⁽⁶⁴⁾ - Three separate measures of frailty were associated with deficiencies in 5 – of B₁₂, folate, vitamin D lutein and zeaxanthin⁽⁶⁹⁾	

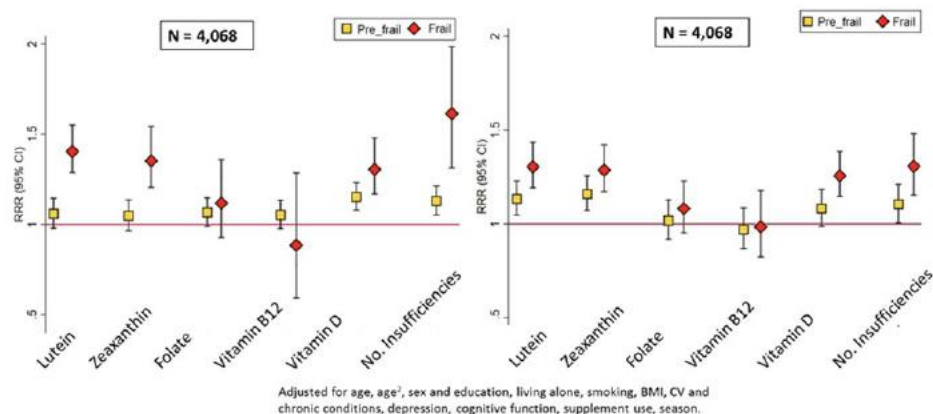


Fig. 1. Micronutrient insufficiency associated with pre-frailty and frailty as indexed by the frailty phenotype and frailty index. Adapted from O'Halloran et al., *J Am Med Dir Assoc.* 2020.

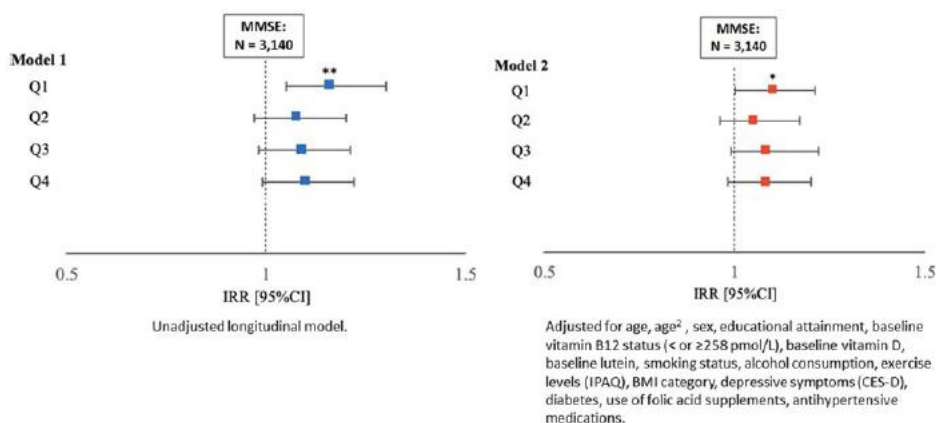


Fig. 2. Low folate predicts cognitive decline over 8 years. Adapted from O'Connor et al., *Eur J Clin Nutr.* 2022.

Evidence from randomised controlled trials (RCT) of B-vitamins has shown no consistent benefit of supplementation on cognitive outcomes. FA supplementation was associated with improved domain-specific cognitive performance in RCT with relatively large samples and ≥ 2 years follow-up^(116–118). One trial that examined individuals with high HCY to exclude causes other than low folate concentrations and FA supplementation (0.8 mg oral FA daily) was associated with improved memory, processing speed and sensorimotor speed after 3 years⁽¹¹⁷⁾. Supplementation was more effective in improving processing speed in those with high baseline HCY levels ($>12.9 \mu\text{M/l}$) and in improving information

processing and sensorimotor speed in those with low baseline vitamin B₁₂ concentrations ($<250 \text{ pmol/l}$)⁽¹¹⁷⁾.

In another trial, testing combinations of folate, vitamins B₆ and B₁₂, and *n*-3 fatty acids for 4 years were effective in preserving semantic memory or temporal orientation in a subgroup of participants with previous coronary artery disease or ischaemic stroke, but not in the total trial population of 1748 men and women aged 45–80 years⁽¹¹⁹⁾. These observations suggest that individuals with high baseline HCY, low baseline vitamin B concentrations or established cardiovascular and cerebrovascular disease might benefit most from vitamin B supplementation.



While most trials that have been carried out have been underpinned by the hypothesis that lowering HCY, which is associated with cognitive impairment, by B-vitamin supplementation, it is plausible that biological mechanisms other than hyperhomocysteinaemia may underlie the associations between B-vitamins and cognitive impairment. Other proposed mechanisms include impaired methylation and misincorporation of uracil into DNA. Vitamins and nutrients often function as a collection of cofactors, therefore interventions using singular or closely related compounds may have too narrow a focus and do not account for the complexity of the synergistic interactions between nutrients. This is illustrated by another trial using FA, B₆ and B₁₂, showing treatment was effective only in those with high baseline *n*-3 fatty acid concentrations. In fact, *n*-3 fatty acid status was protective against brain atrophy only in the presence of B-vitamin supplementation, suggesting that both are needed for effectiveness⁽¹²⁰⁾.

Vitamin D

The role of vitamin D in frailty

Due to its well-established relationship with bone and muscle health, vitamin D intake is vital for the ageing population. Vitamin D is known to regulate calcium homeostasis, bone mineralisation and inflammatory response. Vitamin D deficiency (25(OH)D < 30 nmol/l) has been consistently reported to be highly prevalent in older adults⁽¹²¹⁾ with data from TILDA suggesting a prevalence of 13%⁽¹²²⁾. Low vitamin D also has been consistently associated with frailty^(62–64) and prefrailty in the TILDA cohort (Fig. 1)⁽⁶⁸⁾. Evidence linking low vitamin D levels and incident phenotype frailty has been shown in both meta- and longitudinal analyses^(123–128). After a 3-year follow-up, Vogt *et al.* observed that participants (>65 years) with baseline vitamin D levels < 37.5 nmol/l, compared with ≥ 75 nmol/l, were more likely to become pre-frail or frail⁽¹²⁷⁾. A meta-analysis revealed that vitamin D supplementation is linked to improved gait speed and muscle strength in the older persons⁽¹²⁹⁾. Similar to this, a meta-analysis of intervention trials reported that calcium and vitamin D supplementation may help prevent fractures in older persons⁽⁵⁷⁾. In addition, vitamin D supplementation was associated with increased global DNA methylation levels and reduced epigenetic ageing^(130,131). However, the exact role of vitamin D intake in older adults remains unclear, in part due to limitations in intervention study design and targeting of appropriate populations.

Frailty, COVID-19 and vitamin D

The presence of co-morbidity and frailty in older adults has been associated with a higher risk of undesired outcomes and mortality due to COVID-19 and poorer response to COVID-19 vaccination⁽¹³²⁾. Therefore, the identification of potentially accessible and low-cost health and lifestyle behaviours that could attenuate this risk in those with frailty remains a high priority.

Recent research has highlighted that vitamin D may have an important function within the immune system. Expression of the vitamin D receptor has been identified on a variety of cells of the immune system including macrophages, T lymphocytes, dendritic cells and monocytes and may act as a modulator through its ability to alter cytokine secretion⁽¹³³⁾. For instance, low vitamin D status has been previously associated with markers of inflammation and an enhanced pro-inflammatory profile in older Irish adults⁽¹³⁴⁾. Pro-inflammatory cytokines have been implicated in increased severity of COVID-19 and positive modulation of these interleukins by vitamin D has been hypothesised⁽¹³⁵⁾. Early observational evidence suggested that countries with either a programme of mandatory vitamin D food fortification or higher exposure to UVB vitamin D forming light had lower incidence of COVID-19 and death rates in comparison to countries without fortification or low light exposure^(135,136). Actual vitamin D intervention studies have produced mixed results with little to no effect in healthy populations but positive effects in the at-risk frail populations⁽¹³⁷⁾.

The role of vitamin D in cognitive impairment

The body of evidence for the function of vitamin D in maintaining brain health has been growing since the discovery of the vitamin D receptor in the brain⁽¹³⁸⁾. Several different neurobiological pathways have been linked⁽¹³⁹⁾. A meta-analysis⁽¹⁴⁰⁾ observed an inverse dose-response relationship between the concentrations of vitamin D and risk of dementia or Alzheimer's disease.

Systematic reviews and meta-analyses have demonstrated that Alzheimer's disease patients' serum vitamin D status is lower than that of healthy controls, and that this is related to worse cognitive results^(141,142). Reduced vitamin D status has been linked to faster cognitive ageing and worsening cognitive test scores, according to longitudinal studies^(143,144). In addition, Hooshmand *et al.* used MRI to show that having more vitamin D was linked to larger brain volumes⁽¹⁴⁵⁾.

Large cross-sectional and prospective investigations revealed that a higher risk of depression was associated with decreased serum vitamin D status^(146–149). A thorough systematic analysis that incorporated data from cross-sectional, prospective and RCT studies concluded that having reduced vitamin D status may increase the chance of developing late-life depression⁽¹⁴⁷⁾. More recently, an extensive meta-analysis of 41 RCT (*n* 53 235) found that vitamin D supplementation reduced the occurrence of depressive symptoms⁽¹⁵⁰⁾. However, experimental evidence of the effect of vitamin D supplementation is scarce, with a recent review suggesting that a role for vitamin D supplementation in enhancing cognition (separate from depression) in adults cannot be supported based on evidence to date⁽¹⁵¹⁾. The variability of vitamin D concentrations, cognitive tests used, supplementation doses and the samples' characteristics (i.e. ethnicity or number of participants who are deficient) may explain the ambiguity in the findings.



Lutein and zeaxanthin

The role of lutein and zeaxanthin in frailty

Xanthophyll carotenoids have long been implicated in improving visual outcomes and disease progression in individuals with age-related macular degeneration. More recently, a putative protective role for these compounds in other chronic diseases of ageing has emerged, including cancer⁽¹⁵²⁾, CVD⁽¹⁵³⁾, diabetes⁽¹⁵⁴⁾, neurodegenerative disease⁽¹⁵⁵⁾ and bone health⁽¹⁵⁶⁾. Citrus fruits, spinach, kale, broccoli, maize and other vegetables and fruits are the main sources of lutein and zeaxanthin in the diet⁽¹⁵⁷⁾. The biological mechanisms underpinning these associations may lie in their antioxidant⁽¹⁵⁸⁾ and anti-inflammatory properties^(159,160) and the promotion of cell membrane stabilisation⁽¹⁶¹⁾. These mechanisms likely explain why inverse associations between carotenoid levels and disease risk have been observed for several age-associated conditions with an inflammatory or oxidative stress aetiology. Consequently, they may influence multi-system dysregulation which has been proposed to underlie the frailty syndrome.

Several studies have shown associations between lutein and zeaxanthin and frailty^(62,63,68,162), in addition to physical deficits including decreased grip, hip and knee strength in community-dwelling older women, according to a cross-sectional study⁽¹⁶³⁾. Fig. 1 displays the results of a recent cross-sectional examination from our group that demonstrated that plasma lutein and zeaxanthin concentrations were negatively correlated with prefrailty and frailty across three different frailty instruments⁽⁶⁸⁾.

The role of lutein and zeaxanthin in cognitive impairment

Carotenoids have been proposed to have anti-inflammatory effects in addition to their antioxidant characteristics, by interacting with inflammatory cellular signalling cascades⁽¹⁵⁹⁾. Lutein and zeaxanthin – xanthophyll carotenoids with antioxidant and anti-inflammatory characteristics – are present in the retina and the brain and have neuroprotective properties. High concentrations of these carotenoids have been positively related to cognitive performance⁽¹⁶⁴⁾. Higher plasma lutein and zeaxanthin were independently associated with better composite scores in the areas of executive function, memory and global cognition. Additionally, Feeney *et al.* discovered evidence linking increased plasma zeaxanthin with better processing speed⁽¹⁶⁴⁾. Although the results of large population studies and clinical trials have been somewhat mixed, a recent review demonstrated a direct relationship among cognitive functions, macular pigment and the intake of lutein and zeaxanthin⁽¹⁶⁵⁾.

Dietary patterns, frailty and cognitive impairment

It is important to acknowledge the complex and synergistic relationships between nutrients. Vitamins and micronutrients often act as collections of co-factors, therefore

interventions using singular or closely related compounds may have a focus that is too narrow. Interestingly, several studies have reported an increasing likelihood of frailty^(62–64,68) with increasing accumulation of micronutrient insufficiencies. This is supported by a study that found supplementation with folate, vitamins B₆, B₁₂, D and calcium improved frailty among community-living older persons⁽¹⁶⁶⁾.

Because of the complex biological interactions between the various components of the diet, it has been suggested that using a whole-diet approach, through the study of dietary patterns rather than individual nutrients or food groups, might help to elucidate the role of diet in chronic diseases, such as frailty and cognitive impairment in older people. The Mediterranean diet is a good example of using dietary patterns to characterise dietary intake. Adherence to a Mediterranean-type diet pattern, known for its benefits on cardiovascular health and longevity^(167,168) has also been linked to a decreased risk of frailty^(169–174) and cognitive impairment⁽¹⁷⁵⁾.

With respect to frailty, data from a 6-year longitudinal study revealed a lower risk of frailty in participants with a high Mediterranean diet score⁽¹⁷³⁾. A recent meta-analysis, examining adherence to the Mediterranean diet and risk of frailty, indicated those with strongest adherence had a 56% decreased risk of frailty⁽¹⁷⁶⁾. Further, a study found that older women with type-2 diabetes who were at risk for frailty from the nurses' health study benefited from better adherence to a Mediterranean diet⁽¹⁷⁷⁾. Therefore, it seems in addition to consuming a Mediterranean-type diet to possibly treat frailty, later adoption of a Mediterranean-type diet may act as a limiting factor for the development of frailty^(177,178).

Other dietary patterns have shown comparable results. Higher healthy eating index scores were inversely related to lower risks of physical frailty in US older adults, according to Fan *et al.*⁽¹⁷⁹⁾. Another study using the 'dietary inflammatory index', a dietary pattern marker of foods and nutrient intakes related with inflammation, which may contribute to frailty, found that among 1948 participants who were tracked for up to 4 years, those with the highest adherence to the index had a higher risk of frailty and slower gait speed⁽¹⁸⁰⁾.

The majority of observational studies examining cognitive impairment point to an association between higher adherence to the Mediterranean diet and slower performance decline on various cognitive test batteries, as well as a decreased risk of dementia, mild cognitive impairment or progression from mild cognitive impairment to dementia. Two clinical trials that compared the Mediterranean diet pattern with nuts or olive oil to recommendations to limit dietary fat confirm these findings^(181,182).

These approaches have the benefit of capturing potential interactions between microconstituents of diet, whether they are additive, antagonistic or synergistic⁽¹⁸³⁾. Applying similar approaches to the study of frailty and cognitive impairment may yield more informative insights than focusing on single nutrients alone.

Summary and way forward

In this review, we have focused on selected micronutrients that have been demonstrated to have a high prevalence of insufficiency and/or deficiency among older adults. We have reviewed the evidence, from TILDA and other studies, for their impact on age-related pre-frailty, frailty and cognitive decline. We have shown that low concentrations of folate and carotenoids are implicated in poorer cognitive health and that the co-occurrence of multiple nutrient deficiencies confers greatest risk for pre-frailty and frailty in the TILDA cohort of older adults. These findings are largely, if not consistently, supported by other epidemiological studies internationally. While the results from RCT often fail to support these relationships, there may be design reasons for this, such as relatively short follow-up times during RCT and the exclusion of those older adults with morbidities, frailty and cognitive problems. Inconsistent relationships for individual micronutrients with these outcomes in older adults may be overcome by assessing sup-optimal levels of several micronutrients simultaneously and using the accumulation of micronutrient insufficiencies/deficiencies, as demonstrated by TILDA data (Fig. 1). Single measurements can lead to misclassification, and the cut-off points routinely used to define deficiency may identify an acute rather than a chronic deficiency in older age groups. The complex synergistic interactions between nutrients are also important to consider. Vitamins and nutrients often function as a collection of co-factors, therefore interventions using singular or closely related compounds may have too narrow a focus. Also, given that older adults tend to experience malabsorption of nutrients, some micronutrients may be absorbed differently or less efficiently among older age groups. This may mean that definitions of micronutrient insufficiency and deficiency may be less accurate at older ages and chronic low/sub-optimal status could have less well-understood negative impacts on health. These biological changes coupled with diminution of appetite may partly explain the consistent observation that older adults struggle to maintain sufficient dietary intakes and circulating levels of several micronutrients with advancing age. Among previous TILDA studies, our group has demonstrated that the current custom of voluntary micronutrient fortification in Ireland is not effective in maintenance of sufficient micronutrient status among older age groups^[96,122].

Sustaining the health of a globally ageing population requires strategies that will prolong healthspan by delaying the onset of age-related diseases until later in the life course. Therefore, it is important to focus on modifiable factors, such as micronutrients, that can be intervened upon, particularly in 'at-risk' groups if they can be identified early, i.e. those who have pre-frailty and/or indications of cognitive decline. Testing for micronutrient insufficiencies/deficiencies in these 'at-risk' groups may be used to both monitor the health of older adults and as an intervention target to support biological function and decelerate biological ageing and the onset of physical and cognitive decline. To achieve this, public health

policies and awareness programmes are required that highlight the importance of maintaining micronutrient sufficiency via mandatory fortification and/or supplementation to support health as we age.

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Conflict of Interest

None.

Authorship

The authors had sole responsibility for all aspects of preparation of this paper.

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Appendix 11.1.2 - Paper 2 - Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: Evidence from the Irish Longitudinal Study on Ageing (TILDA).

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Voluntary fortification is ineffective to maintain the vitamin B₁₂ and folate status of older Irish adults: evidence from the Irish Longitudinal Study on Ageing (TILDA)

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Abstract

Mandatory fortification of staple grains with folic acid and/or vitamin B₁₂ (B₁₂) is under debate in many countries including Ireland, which has a liberal, but voluntary, fortification policy. Older adults can be at risk of both deficiency and high folate status, although little is known on the actual prevalence and the major predictors. Population prevalence estimates from older adults (*n* 5290 ≥50 years) from the Irish Longitudinal Study on Ageing (TILDA) (Wave 1) are presented here. Measures included plasma total vitamin B₁₂ and folate, whereas predictors included detailed demographic, socio-economic, geographic, seasonal and health/lifestyle data. The prevalence of deficient or low B₁₂ status (<185 pmol/l) was 12%, whereas the prevalence of deficient/low folate status was 15%. High folate status (>45 nmol/l) was observed in 8.9%, whereas high B₁₂ status was observed in 3.1% (>601 pmol/l). The largest positive predictor of B₁₂ concentration was self-reported B₁₂ injection and/or supplement use (coefficient 51.5 pmol/l; 95% CI 9.4, 93.6; *P*=0.016) followed by sex and geographic location. The largest negative predictor was metformin use (−33.6; 95% CI −51.9, −15.4; *P*<0.0001). The largest positive predictor of folate concentration was folic acid supplement use (6.0; 95% CI 3.0, 9.0 nmol/l; *P*<0.001) followed by being female and statin medications. The largest negative predictor was geographic location (−5.7; 95% CI −6.7, −4.6; *P*<0.0001) followed by seasonality and smoking. B-vitamin status in older adults is affected by health and lifestyle, medication, sampling period and geographic location. We observed a high prevalence of low B₁₂ and folate status, indicating that the current policy of voluntary fortification is ineffective for older adults.

Key words: B-vitamins: Biomarkers: Fortification: Supplements: Folate: Vitamin B₁₂

National surveys have shown that the proportion of older adults within most populations (defined as those >60 years) is increasing, and by 2050 global projections indicate that the number of older adults worldwide will be more than 2 billion (21% of the population)⁽¹⁾. With such profound shifts in demographics also comes the corresponding increase in the prevalence of the chronic diseases of ageing including CVD, cognitive impairment and associated co-morbidities⁽²⁾. Maintenance of optimal nutritional status has been cited as an achievable goal in preventing or delaying the development of chronic disease⁽³⁾, and recent emphasis has been placed on the protective roles of micronutrients, including the B-vitamins.

Both vitamin B₁₂ (B₁₂) and folate are essential co-factors in intracellular one-carbon metabolism, which is involved in DNA synthesis, cellular regulation processes and amino acid metabolism⁽⁴⁾. Both vitamins are thought to be associated with

cardiovascular health and cognitive function. A recent meta-analysis (*n* 82 334) indicated a 10% lower risk of stroke and a 4% lower risk of overall CVD with folic acid (FA) supplementation⁽⁵⁾, whereas in a longitudinal study of older adults (>60 years, *n* 501) higher concentrations of vitamin B₁₂ were associated with a decreased rate of brain volume loss over 6 years⁽⁶⁾. Estimates of vitamin B₁₂ deficiency in older populations range from 5 to 40%, depending on the marker of measurement and the deficiency cut-off selected⁽⁷⁾. The consequences of a B₁₂-deficient state can include the development of megaloblastic anaemia, irreversible demyelinating neurological disease/paresthesias and the potential for impaired immune and cognitive function⁽⁸⁾. Hence, the early detection of deficiency and awareness of the factors that can affect B₁₂ status is important. In terms of folate, since the introduction of mandatory FA fortification of grains in the USA to reduce the

Abbreviations: B₁₂, vitamin B₁₂; FA, folic acid; NANA, National Adult Nutrition Study; NHANES, National Health and Nutritional Examination Survey; TILDA, Irish Longitudinal Study on Ageing.

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incidence of neural tube defects, the prevalence of deficient and low folate status in the US population has substantially decreased⁽⁹⁾. Conversely, there is an accompanying increased incidence of high blood folate concentrations (>45 nmol/l), which has led to concerns of potential adverse effects, especially when combined with low vitamin B₁₂ status, as frequently observed in older adults⁽¹⁰⁾.

To date, mandatory FA fortification does not occur in the European Union (EU). Uniquely within the EU, however, both Ireland and the UK have a voluntary but liberal food fortification policy that includes FA. The foods most commonly fortified with FA are breakfast cereals, but not all are fortified^(11,12). In addition, there are reports of varying degrees of FA enrichment of foods, and enrichment also appears to be inconsistent across time and across products⁽¹³⁾. Moreover, foods that are fortified or rich in B₁₂ are not necessarily consumed by the older population. For instance, dairy foods, which are a particularly good source of B₁₂, are only consumed in the recommended amounts by only 4% of older Irish adults⁽¹⁴⁾.

A recent Irish National Survey that previously examined vitamin B₁₂ and folate status was limited to a small number of older adults and had a narrow range of demographic and clinical data⁽¹⁵⁾. Therefore, the aims of this study were (i) to report the B₁₂ and folate status of a large, nationally representative sample of the older Irish population (*n* >5000) and (ii) to investigate the demographic, socio-economic, geographic, seasonal, health and lifestyle determinants of these vitamin concentrations in this population, which is subject to a voluntary, but liberal, food fortification policy.

Methods

Study population

Participants were recruited as part of The Irish Longitudinal Study on Ageing (TILDA), a nationally representative cohort of community-dwelling adults aged ≥50 years, designed to investigate how the health, social and economic circumstances of the older Irish population interact in the determination of 'healthy' ageing. Full details of the study design, sampling and methodology have been published previously^(16,17). The first wave of data collection (Wave 1) was conducted between October 2009 and July 2011, using a stratified clustered procedure to randomly sample postal addresses from the Irish Geo-Directory (a listing of all residential addresses in the Republic of Ireland). All postal addresses in Ireland were assigned to one of 3155 geographic clusters using RANSAM (a random sampling design for Ireland) where all household residents aged ≥50 years were eligible to participate; a sample of 640 of these clusters was selected, stratified by socio-economic group and geography to maintain a population representative sample. Clusters were selected with a probability proportional to the number of individuals aged ≥50 years in each cluster. At Wave 1, 8175 adults completed a computer-aided personal interview (CAPI) (response rate of 62%). Approximately 72.1% (*n* 5895) consented to, and participated in, a health assessment that included donating a blood sample. In total, folate measurements were available for 90.7% (*n* 5350)

and B₁₂ measurements were available for 88.5% (*n* 5219) of these participants. Missing data were randomly dispersed across clusters and were generally because of sample assay failures or insufficient sample volume. The study was conducted according to the guidelines set out in the Declaration of Helsinki, and ethical approval was granted by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin.

Lifestyle and supplement use

During the CAPI, data were collected regarding sex, age, province of residence (Leinster – East/South East; Munster – South/South West; Ulster and Connacht – North and West), habitation status (living alone yes/no), current smoker status (yes/no), current alcohol status (yes/no) and household housing wealth (measure of economic resource 'asset wealth' coded above or below the average for the sample). A list of up to twenty medications including both prescription and non-prescription medications and dietary/vitamin supplements were recorded by interviewers. Once entered into the data set, they were assigned WHO Anatomical Therapeutic Chemical Classification codes and binarised to yes/no for FA supplement use (single tablet or multi-vitamin) and for use of vitamin B₁₂ (injection or single tablet or B₁₂ in multi-vitamin). Classification codes were also binarised to yes/no for the commonly prescribed medications of older adults, which have been associated with B-vitamin concentrations including metformin, proton pump inhibitors (PPI), statins and thyroxine medications^(18,19). Self-reported physical activity was assessed by the International Physical Activity Questionnaire (IPAQ). Physical activity levels were also dichotomised and defined according to the IPAQ categories: physically active = minimally or health enhancing physically active; not physically active = inactive or insufficiently active. During the health assessment, anthropometric measurements including height (to the nearest 0.01 m) and weight (to the nearest 0.01 kg) were measured with SecaTM (Seca Ltd) using a standardised protocol. BMI was then calculated and obesity was classified as a BMI >30 kg/m². Seasonality was examined and defined as winter (December through February), spring (March through May), summer (June through August) and autumn (September through November).

Blood samples

A non-fasting blood sample was collected by venepuncture into one 10-ml K₂EDTA tube (BD, Becton, Dickinson Limited) by a trained phlebotomist. Samples were kept chilled and centrifuged (3000 rpm for 15 min) and aliquots were labelled and stored at –80°C until required for analysis. Plasma total B₁₂ and folate concentrations were determined by microbiological assays, as previously described^(20,21). The inter-assay CV for plasma B₁₂ and folate were <10.9%. B₁₂ status profiles (pmol/l) were defined as follows: <148, deficient; 148–<185, low; 185–<258, low normal; >258–601, normal; and >601, high^(22,23). Folate status profiles (nmol/l) were defined as follows: <6.8, deficient; 6.8–10.0, low; >10–23.0, low normal; >23.0–45.0, normal; >45.0, high⁽²⁴⁾. These cut-offs were selected to allow for comparison with previous studies and to give an indication of the population range.

Statistical analyses

Base population weights were calculated by comparing TILDA with the 2011 Irish Census with respect to age, sex, educational attainment, marital status and urban *v.* rural residence (weights reflected the reciprocal of the selection probability). The staged stratified clustering sample design of TILDA was accounted for when computing CI and standard errors. Prevalence estimates and descriptive statistics were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether or not respondents provided a blood sample. TILDA participants with plasma total B₁₂ and folate concentrations more than 3 SD above the mean or who were missing data on other covariates were excluded from the analysis, resulting in 5158 results for total B₁₂ and 5290 results for plasma folate (the +3 SD cut-off was applied to remove outlying high values, likely attributable to measurement error). Weighted prevalence estimates are reported as percentages with 95% CI. Weighted geometric means are presented along with B₁₂ and folate concentrations categorised by the stated biomarker cut-offs, stratified by the population characteristics. Pairwise comparisons of proportions by B₁₂ and folate status cut-offs were computed across variables of interest, and false-discovery rate correction for multiple comparisons was applied ($P < 0.041$, achieving $q < 0.05$). Multiple linear regression estimated the effects of health, lifestyle, geographic and socio-economic factors on B₁₂ and folate concentrations. Multiple imputation (twelve cycles) was used to impute missing information on asset wealth in the final statistical

models (information was missing for <5% of participants). All analyses were carried out using STATA 14 (StataCorp).

Results

The population characteristics of the TILDA cohort have been published comprehensively elsewhere^(16,17). In short, of the participants included in this combined analysis, the mean age was 63.6 years (age range 50–98) and 53% were female. The proportion who reported currently consuming alcohol was 76.8%, whereas 15.6% were current smokers. The prevalence of reported combined B₁₂ supplementation (injection and/or supplements) was 2.6%, with women reporting significantly higher usage than men (3.5 *v.* 1.7%; $P < 0.001$, respectively). The prevalence of FA supplementation was 2.8%, with significantly more women than men reporting supplementation use (3.6 *v.* 2.0%; $P < 0.001$, respectively).

Prevalence of biomarker status

The estimated distribution of both B₁₂ and folate status by age group is displayed in Fig. 1. The prevalence of deficient or low B₁₂ status was 12%, whereas 15% had deficient or low folate status. High B₁₂ status (>601 pmol/l) was observed in 3.1% of the population, whereas 8.9% had high folate status (>45 nmol/l). The prevalence estimates by demographic and lifestyle factors are displayed in Table 1 for total B₁₂ and Table 2 for plasma folate. Participants reporting supplement use had significantly higher concentrations of both total B₁₂ and

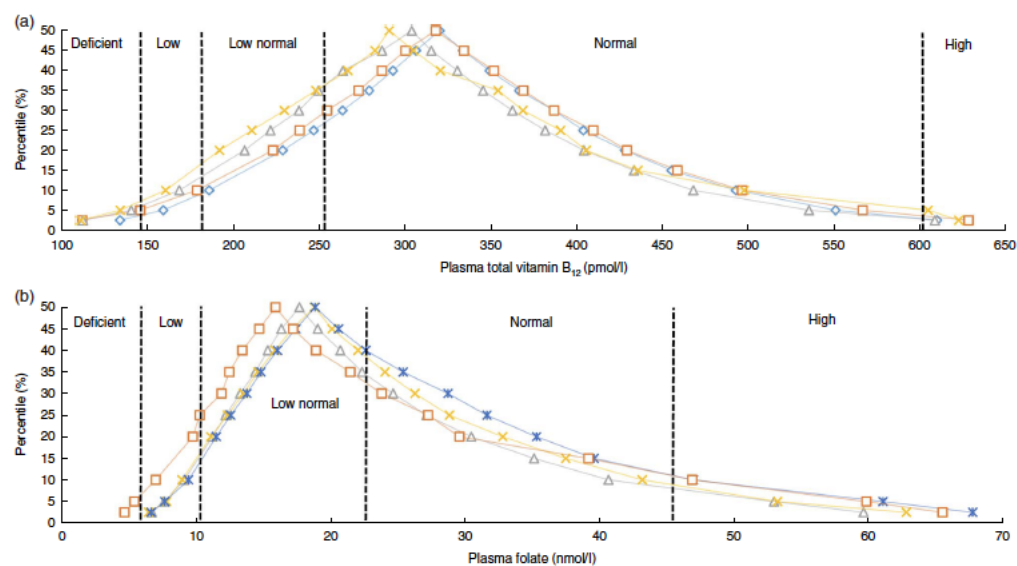


Fig. 1. Frequency distribution of plasma total vitamin B₁₂ (a) and folate (b) of older Irish adults from the Irish Longitudinal Study on Ageing study by age group. a: —◇—, 50–59; —□—, 60–69; —△—, 70–79; —×—, >80; b: —△—, 50–59; —×—, 60–69; —◇—, 70–79; —□—, >80.

**Table 1.** Mean plasma total vitamin B₁₂ with weighted prevalence of status by demographic and lifestyle characteristics† (Weighted means and prevalence estimates and 95% confidence intervals)

Characteristics	Subjects (n)	Vitamin B ₁₂									
		Average (pmol/l)	95% CI	<148 pmol/l (n 237)		148–<185 pmol/l (n 320)		185–<258 pmol/l (n 994)		>258 pmol/l (n 3627)	
				%	95% CI	%	95% CI	%	95% CI	%	95% CI
Supplements and/or B ₁₂ injection	147	352.3	326.8, 378.3	2.9	1.0, 7.8	3.9	1.7, 8.5	14.7	9.7, 21.5	78.5	70.7, 84.5
No supplements or B ₁₂ injection	5011	312.5	308.3, 316.4	5.1	4.4, 5.9	7.0	6.1, 7.8	19.6	18.4, 20.7	68.3	66.8, 69.7
<i>P</i>			0.0022*		<0.0001*		<0.0001*		<0.0001*		<0.0001*
Sex											
Male	2428	303.4	298.5, 308.7	5.1	4.1, 6.2	7.5	6.3, 8.8	20.2	18.5, 22.0	67.2	65.0, 69.1
Female	2730	323.2	317.5, 329.0	5.0	4.1, 6.1	6.3	5.2, 7.5	18.7	17.1, 20.3	70.0	67.8, 71.8
<i>P</i>			<0.0001*		0.8516		0.3239		0.5619		0.0147*
Age (years)											
50–59	2196	320.7	314.7, 326.5	4.0	3.0, 5.1	5.7	4.6, 6.9	17.9	16.2, 19.6	72.4	70.3, 74.4
60–69	1757	317.1	310.4, 324.0	5.4	4.2, 6.7	6.1	4.8, 7.6	19.7	17.8, 21.8	68.8	66.3, 71.1
70–79	929	299.2	290.0, 308.3	6.4	4.8, 8.5	8.9	6.9, 11.3	22.6	19.4, 25.9	62.1	58.2, 65.7
>80	276	297.2	281.2, 313.2	6.6	4.2, 10.1	11.1	7.7, 15.6	19.8	15.2, 25.2	62.5	56.3, 68.2
<i>P</i>			<0.0001*		0.0431		0.0013*		0.1011		<0.0001*
Obesity											
BMI >30 kg/m ²	1734	298.9	292.7, 305.5	5.9	4.6, 7.3	8.2	6.6, 9.9	21.6	19.6, 23.7	64.3	61.7, 66.7
BMI <30 kg/m ²	3410	321.1	315.7, 326.1	4.7	3.8, 5.6	6.2	5.3, 7.2	18.3	16.8, 19.7	70.8	68.9, 72.5
<i>P</i>			<0.0001*		0.0053*		0.0032*		<0.0001*		<0.0001*
Smoking status											
Smoker	803	296.8	287.3, 306.2	6.9	5.0, 9.4	7.3	5.5, 9.6	21.3	18.4, 24.4	64.5	60.7, 68.0
Non-smoker	4355	317.5	313.2, 322.2	4.7	3.9, 5.4	6.8	5.9, 7.7	19.0	17.7, 20.3	69.5	67.9, 71.0
<i>P</i>			0.0002*		<0.0001*		<0.0001*		<0.0001*		<0.0001*
Alcohol consumption											
Alcohol consumer	3638	314.3	309.7, 319.3	4.8	4.0, 5.8	6.3	5.4, 7.2	19.0	17.5, 20.3	69.9	68.1, 71.6
Non-consumer	1103	315.7	306.6, 324.7	5.4	4.0, 7.1	8.1	6.3, 10.2	19.5	17.0, 22.2	67.0	63.7, 70.1
<i>P</i>			0.8346		<0.0001*		<0.0001*		<0.0001*		<0.0001*
Physical activity											
Physically active	3637	312.5	305.2, 320.0	5.5	4.6, 6.5	6.1	5.2, 7.0	18.9	17.5, 20.2	69.5	67.8, 71.1
Not physically active	1479	313.9	309.0, 318.6	4.2	3.1, 5.5	8.6	7.0, 10.5	20.9	18.5, 23.3	66.3	63.4, 69.0
<i>P</i>			0.8000		<0.0001*		0.0008*		<0.0001*		<0.0001*
Habitation											
Lives alone	988	304.8	295.4, 314.3	6.5	4.8, 8.7	7.8	6.1, 10.0	20.2	17.5, 23.0	65.5	62.0, 68.7
Does not live alone	4170	316.4	311.8, 320.7	4.6	3.9, 5.3	6.6	5.7, 7.5	19.2	17.9, 20.5	69.6	68.0, 71.1
<i>P</i>			0.0319*		<0.0001*		<0.0001*		<0.0001*		<0.0001*
Asset wealth											
<Mean asset wealth	2622	309.0	303.4, 314.3	5.9	4.8, 6.9	7.2	6.1, 8.4	19.1	17.6, 20.5	67.8	65.7, 69.7
>Mean asset wealth	2270	320.0	314.7, 326.1	4.0	3.0, 4.9	6.4	5.1, 7.5	19.9	18.1, 21.7	69.7	67.6, 71.8
<i>P</i>			0.0080*		<0.0001*		0.0004*		0.0004*		<0.0001*
Season											
Summer	1290	304.5	296.8, 312.2	5.2	4.0, 6.6	7.8	6.2, 9.7	22.4	19.8, 25.1	64.6	61.5, 67.6
Autumn	1057	311.5	302.4, 320.7	6.3	4.7, 8.2	8.4	6.6, 10.5	17.7	15.1, 20.5	67.6	64.4, 70.6
Winter	1113	317.9	309.4, 326.5	4.6	3.3, 6.1	5.7	4.3, 7.5	20.6	18.0, 23.3	69.1	65.8, 72.2
Spring	1695	318.9	312.5, 325.8	4.6	3.4, 6.0	6.0	4.7, 7.4	17.6	15.8, 19.6	71.8	69.4, 74.0
<i>P</i>			<0.0001*		0.3126		0.0520		0.0173*		0.0031*
Location											
East/South East	2553	310.4	305.2, 316.1	5.5	4.4, 6.6	7.1	5.9, 8.4	19.5	17.9, 21.1	67.9	65.9, 69.9
South/South West	1562	311.5	304.5, 318.9	4.5	3.3, 5.8	7.0	5.6, 8.7	20.5	18.3, 22.9	68.0	65.2, 70.6
North and West	1043	324.0	314.7, 333.7	5.1	3.8, 6.8	6.1	4.7, 7.8	17.7	15.5, 20.1	71.1	67.8, 74.0
<i>P</i>			<0.0001*		0.4841		0.6132		0.2476		0.2387

* Statistically significant after adjustment for multiple comparisons ($P < 0.041$).† *P* values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

folate and had lower rates of deficiency compared with non-supplement users. For both biomarkers, men had significantly lower concentrations as did the oldest old (>80 years) ($P < 0.001$). For both total B₁₂ and folate, those who were obese, smoked or reported lower mean asset wealth had significantly lower biomarker concentrations and higher rates of deficiency/low status. Seasonality was also significantly associated with both biomarker concentrations ($P < 0.001$).

Concentrations of total B₁₂ were the highest in spring and the lowest in summer (318.9 *v.* 304.5 pmol/l), whereas plasma folate concentrations were also the highest in spring but lowest in autumn (19.5 *v.* 16.4 nmol/l) (but we note that in the regression analyses, the effects of seasonality were only robust for folate concentrations in autumn *v.* winter). When examined by geographic area, participants residing in the North and West of the Island (Connaught and Ulster) had lower plasma

**Table 2.** Mean plasma folate with weighted prevalence of status by demographic and lifestyle characteristics† (Weighted means and prevalence estimates and 95% confidence intervals)

Characteristics	Subjects (n)	Plasma folate									
		Average		<10 nmol/l (n 731)		10–23 nmol/l (n 2610)		>23–45 nmol/l		>45 nmol/l (n 479)	
		(nmol/l)	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Supplements	162	24.5	20.7, 29.1	8.0	3.9, 15.3	35.5	28.0, 43.7	29.3	21.7, 38.1	27.2	19.9, 35.9
No supplements	5128	18.5	18.1, 19.0	15.1	13.8, 16.4	49.8	48.2, 51.4	26.7	25.2, 28.1	8.4	7.4, 9.4
<i>P</i>		0.0013*		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Sex											
Male	2476	17.9	17.4, 18.4	15.9	14.2, 17.6	51.9	49.7, 54.0	25.0	23.1, 26.9	7.2	6.0, 8.5
Female	2814	19.5	18.9, 20.1	14.0	12.4, 15.6	47.0	44.9, 49.1	28.4	26.5, 30.2	10.6	9.8, 12.1
<i>P</i>		<0.00001*		0.3284		0.2314		0.0009*		<0.00001*	
Age (years)											
50–59	2256	18.3	17.8, 18.9	14.3	12.6, 16.1	52.2	49.9, 54.4	26.0	23.9, 28.0	7.5	6.3, 8.9
60–69	1784	19.1	18.4, 19.7	14.5	12.7, 16.5	47.8	45.2, 50.3	28.8	26.5, 31.0	8.9	7.3, 10.7
70–79	968	19.8	18.9, 20.9	12.7	10.5, 15.3	48.0	44.2, 51.6	28.0	24.8, 31.4	11.3	9.0, 14.0
>80	282	16.7	15.2, 18.4	23.1	17.7, 29.6	45.1	38.4, 51.8	21.0	16.1, 26.6	10.8	7.6, 15.2
<i>P</i>		<0.00001*		0.0004*		0.041		0.0309*		0.0235*	
Obesity											
BMI >30 kg/m ²	1782	17.6	17.0, 18.2	16.8	14.7, 19.0	51.8	49.0, 54.4	24.4	22.1, 26.7	7.1	5.8, 8.6
BMI <30 kg/m ²	3493	19.2	18.7, 19.8	14.0	12.5, 15.4	48.3	46.4, 50.2	27.8	26.1, 29.5	9.9	8.7, 11.3
<i>P</i>		<0.00001*		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Smoking status											
Smoker	828	16.7	15.9, 17.6	19.2	16.5, 22.2	51.5	47.7, 55.2	22.6	19.4, 26.1	6.7	4.9, 8.8
Non-smoker	4462	19.2	18.7, 19.7	13.9	12.5, 15.2	48.9	47.2, 50.6	27.7	26.2, 29.2	9.5	8.3, 10.7
<i>P</i>		<0.00001		<0.00001		<0.00001		<0.00001		<0.00001*	
Alcohol consumption											
Alcohol consumer	3738	19.0	18.5, 19.5	14.0	12.6, 15.4	49.1	47.3, 50.9	27.7	26.0, 29.4	9.2	8.0, 10.4
Non-consumer	1123	18.1	17.4, 19.0	16.8	14.2, 19.6	48.9	45.7, 52.1	25.3	22.6, 28.0	9.0	7.2, 11.1
<i>P</i>		0.056		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Physical activity											
Physically active	3728	18.7	18.2, 19.3	14.5	13.1, 16.0	49.8	47.9, 51.5	26.6	25.0, 28.3	9.1	7.9, 10.3
Not physically active	1516	18.5	17.8, 19.2	15.7	13.5, 18.1	48.8	45.9, 51.6	27.0	24.5, 29.6	8.5	6.8, 10.4
<i>P</i>		0.571		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Habitation											
Lives alone	1011	18.2	17.3, 19.1	17.9	15.2, 20.9	46.8	43.2, 50.3	25.2	22.2, 28.3	10.1	8.1, 12.4
Does not live alone	4279	18.8	18.4, 19.3	13.9	12.6, 15.2	50.3	48.5, 51.9	27.2	25.7, 28.7	8.6	7.5, 9.7
<i>P</i>		0.2069		<0.00001*		<0.00001*		<0.00001*		<0.00001*	
Asset wealth											
<Asset wealth	2683	17.6	17.1, 18.2	16.3	14.6, 18.2	51.9	49.7, 54.00	24.7	22.8, 26.6	7.1	5.9, 8.3
> Asset wealth	2335	20.3	19.6, 21.1	12.3	10.6, 14.2	46.3	43.9, 48.6	30.0	27.8, 32.2	11.4	9.6, 11.3
<i>P</i>		<0.00001*		<0.00001*		<0.00001*		0.0633		0.2547	
Season											
Summer	1335	19.1	18.3, 19.8	13.1	11.0, 15.4	49.6	46.6, 52.5	29.7	26.9, 32.6	7.6	6.0, 9.5
Autumn	1069	16.4	15.6, 17.2	19.8	16.7, 23.3	51.5	47.7, 55.1	25.7	22.6, 29.0	3.0	1.9, 4.3
Winter	1145	19.3	18.4, 20.3	13.6	11.4, 16.0	49.4	45.9, 52.8	24.7	21.9, 27.6	12.3	10.2, 14.7
Spring	1738	19.5	18.8, 20.3	14.0	12.0, 16.0	48.1	45.3, 50.8	26.5	24.2, 28.9	11.4	9.5, 13.6
<i>P</i>		<0.00001*		0.0004*		0.5235		0.0852		<0.00001*	
Location											
East/South East	2604	21.1	20.4, 21.9	10.5	8.9, 12.0	46.4	44.2, 48.5	30.1	28.2, 32.1	13.0	11.3, 14.8
South/South West	1398	16.2	15.7, 16.8	20.3	17.7, 22.9	52.2	49.4, 54.9	23.2	20.9, 25.7	4.3	3.3, 5.4
North and West	1088	17.0	16.3, 17.7	18.0	15.4, 20.6	52.7	49.1, 56.2	23.6	20.7, 26.8	5.7	4.3, 7.5
<i>P</i>		<0.0001*		<0.0001*		0.0008*		<0.0001*		<0.0001*	

* Statistically significant after adjustment for multiple comparisons ($P < 0.041$).† *P* values indicate statistically significant pairwise comparisons of the difference in proportion of column criteria across row variables. For variables with more than two levels linear regression was used.

folate compared with those living in the East and South East (Leinster region) (16.2 *v.* 21.1 nmol/l). For medications, only metformin use was associated with lower total B₁₂ concentrations (272.9 *v.* 315.7 pmol/l; $P < 0.0001$) and a higher prevalence of B₁₂ deficiency (11.0 *v.* 4.8%; $P < 0.0001$). There were no statistically significant differences in the concentration of total B₁₂ or folate by statin, PPI or thyroid medication use (data not shown).

Regression analyses

In a multiple linear regression analysis (Table 3), the largest positive predictor of total B₁₂ concentration was self-reported B₁₂ injection and/or supplement use (coefficient 51.5 pmol; 95% CI 9.4, 93.6; $P = 0.016$). This was closely followed by being female and geographic location (North and West *v.* East and South East). The largest negative predictor was metformin

Table 3. Determinants of plasma total vitamin B₁₂ concentrations (pmol/l) in older Irish adults (Regression coefficients and 95 % confidence intervals)

Characteristics	Coefficient	Linearised		
		SE	P	95 % CI
Age (years)	-0.94	0.25	<0.0001	-1.44, -0.45
Sex				
Female	17.91	4.13	<0.0001	9.80, 26.03
BMI				
Obese*	-21.70	4.33	<0.0001	-30.21, -13.20
Smoking status				
Current	-22.39	5.62	<0.0001	-33.43, -11.34
Physical activity				
Inactive†	0.69	5.72	0.584	-8.11, 14.39
B ₁₂ injection/supplement use				
User‡	51.53	21.42	0.016	9.45, 93.61
Folate supplement use				
User	-20.59	16.92	0.224	-53.82, 12.63
Season sampled				
Spring	3.13	5.72	0.584	-8.11, 14.39
Summer	-9.10	6.31	0.150	-21.49, 3.29
Autumn	-1.46	6.51	0.822	-14.25, 11.31
Alcohol use				
Consumer	-7.04	5.18	0.175	-17.23, 3.13
Living arrangement				
Alone	7.17	5.65	0.205	-3.93, 18.28
Province				
Ulster and Connacht	15.60	5.85	0.008	4.10, 27.11
Munster	3.15	4.86	0.517	-6.93, 12.69
Asset wealth				
> Mean asset wealth	8.89	4.35	0.042	0.33, 17.45
Metformin use				
User	-33.69	9.29	<0.0001	-51.94, -15.43
Statin use				
User	3.46	4.52	0.444	-5.41, 12.34
PPI use				
User	6.48	6.05	0.285	-5.41, 18.37
Thyroxine use				
User	6.05	8.64	0.484	-10.93, 23.03

PPI, proton pump inhibitors.

* Obesity = BMI >30 kg/m².

† Physical activity levels were defined by IPAQ categories.

‡ Vitamin B₁₂ injections or vitamin B₁₂-containing supplements (including multi-vitamins) were defined by the use of the World Health Organization's Anatomical Therapeutic Chemical codes.

medication use (-33.6; 95 % CI -51.9, -15.4; $P < 0.001$). Other smaller negative predictors included smoking and obesity. FA supplement use was not a significant predictor of total B₁₂ concentrations. The largest positive predictor of plasma folate concentration was FA supplement use (6.0 nmol/l; 95 % CI 3.0, 9.0; $P < 0.001$) followed by being female and statin medication use (Table 4). The largest negative predictor was geographic location (North, West and South *v.* East and South East; $P < 0.001$) followed by data being sampled in the autumn, smoking and obesity.

Discussion

To our knowledge, we are the first to present prevalence data for B₁₂ and folate status and their determinants in a large representative sample of the older Irish population. In a country with a voluntary fortification policy, the estimated prevalence of folate deficiency/low folate status is 15 % or one in seven people aged >50 years. These data are in stark contrast to countries with mandatory fortification (of enriched cereal grains

or flour) such as the US where rates of low folate status (<10 nmol/l) have been reported in only 1.2 % of those aged ≥60 years (post-fortification)⁽²⁵⁾. Such a high rate of low folate status is important given the implications of sub-optimal concentrations, particularly for older adults. Deficiencies in folate can result in impaired biosynthesis of DNA with clinical symptoms including the development of megaloblastic anaemia, alopecia, achromotrichia and neuropathy^(26,27). Longer-term, low folate status has been associated with higher rates of cognitive impairment, CVD and cancer mortality^(28,29). For instance, in the US National Health and Nutritional Examination Survey (NHANES) (1999–2010), women with folate concentrations of approximately 20 nmol/l had a 1.5-fold increased all-cause mortality risk in comparison with those >20 nmol/l folate concentrations⁽²⁹⁾. In terms of B₁₂, the estimated prevalence of B₁₂ deficiency and low B₁₂ status (<185 pmol/l) in older Irish adults is 12 % or one in eight. Our data are similar to observations across other older adult surveys (aged >60 years). In NHANES (1999–2002), the prevalence of B₁₂ deficiency (<148 pmol/l) was about 3 %, whereas 7 % had a low B₁₂ status

Table 4. Determinants of plasma folate concentrations (nmol/l) in older Irish adults (Regression coefficients and 95 % confidence intervals)

Characteristics	Coefficient	Linearised		
		SE	P	95 % CI
Age (years)	0.35	0.29	0.237	–0.23, 0.93
Sex				
Female	2.04	0.43	<0.0001	1.19, 2.90
BMI				
Obese*	–2.16	0.47	<0.0001	–3.10, –1.23
Smoking status				
Current	–2.27	0.61	<0.0001	–3.49, –1.05
Physical activity				
Inactive†	–0.28	0.54	0.607	–1.35, 0.79
Folate supplement use				
User‡	6.02	1.53	<0.0001	3.01, 9.03
Season sampled				
Spring	–0.09	0.74	0.900	–1.54, 1.36
Summer	–1.37	0.72	0.059	–2.79, 0.05
Autumn	–4.81	0.69	<0.0001	–6.18, –3.44
Alcohol use				
Consumer	0.72	0.55	0.192	–0.36, 1.81
Living arrangement				
Alone	–0.33	0.66	0.616	–1.64, 0.97
Province				
Ulster and Connacht	–4.86	0.67	<0.0001	–6.17, –3.54
Munster	–5.70	0.55	<0.0001	–6.79, –4.61
Asset wealth				
> Mean asset wealth	1.59	0.52	0.003	0.56, 2.63
Metformin use				
User	0.61	1.17	0.598	–1.68, 2.91
Statin use				
User	1.36	0.57	0.019	0.22, 2.49
PPI use				
User	–1.11	0.70	0.114	–2.50, 0.26
Thyroxine use				
User	0.90	0.92	0.332	–0.92, 2.72

PPI, proton pump inhibitors.

* Obesity = BMI >30 kg/m².

† Physical activity levels were defined by IPAQ categories.

‡ Folate-containing supplements (including multi-vitamins) were defined by the use of the World Health Organization's Anatomical Therapeutic Chemical codes.

(<185 pmol/l)⁽³⁰⁾. In three combined UK surveys (*n* 3511), one in twenty people (aged 65–74 years) and one in ten people (aged >75 years) had B₁₂ deficiency (defined as <150 pmol/l)^(31,32). These observed high rates of B₁₂ deficiency in older adults are of concern given that early diagnosis is important to avoid irreversible neurologic damage.

Both vitamin B₁₂ and folate status have been examined previously in a much smaller, representative study of older Irish adults (*n* 358)⁽¹⁵⁾. In the National Adult Nutrition Study (NANS), the average B₁₂ concentration for adults >50 years was 281 pmol/l, which is close to the value of 308 pmol/l observed in the current study. Our data for folate are somewhat lower than in NANS, which may be explained by using the matrix of plasma instead of serum⁽³³⁾. Interestingly, our plasma folate data were closely aligned with a study from Northern Ireland (*n* 662) where the folate concentrations of low-medium consumers of FA fortified foods were similar (16.2–22.6 nmol/l)⁽³⁴⁾. However, unlike the NANS or Hoey *et al.* study, which were both limited to a small sample number and a narrow range of potential determinants, using the TILDA data allowed for the examination of a wide range of possible health and lifestyle determinants. We observed that the oldest old (>80 years) had

the highest rates of both B₁₂ deficiency and low folate status (6.6 and 23.1 %, respectively). Older adults can be at a particular risk of deficiency owing to increased malabsorption from atrophic gastritis, infection or medication interactions⁽⁸⁾. Interestingly, we also observed that men and those with a lower asset wealth also had lower folate and B₁₂ concentrations. It has been reported that men often display a lower B-vitamin status than women^(15,35), which in some studies has been attributed to a higher intake of supplements (which also appears to be a factor in the current study). In addition, it has been observed that older men tend to have a poorer awareness of healthy diet recommendations compared with women who were also more likely to have healthier dietary patterns^(36,37). Few studies have examined measures of economic wealth and B-vitamins. Pfeiffer *et al.*⁽³⁵⁾ in NHANES (2003–2006) reported that those with a lower poverty income ratio had a significantly lower serum folate concentration. Similar dietary mechanisms may explain the decrease in B-vitamin status with age and lower asset wealth including compromised food quantity, quality and variety and lower fruit and vegetables intakes in those with lower incomes^(36–38).

Obesity and smoking were also observed as risk factors for lower B-vitamin status in the current study. Obesity has been



associated previously with higher erythrocyte folate and lower serum folate in 3767 adults from NHANES. The suggested mechanisms included volumetric dilution, lower folate intakes, lower folate intestinal absorption and higher folate uptake by red blood cells⁽³⁹⁾. This is of concern for Ireland, as over half of the over 50s population are classified as centrally obese, with only 16% of men and 26% of women having a normal BMI⁽⁴⁰⁾. Smoking has also been associated with lower B-vitamin concentrations in both the British National Diet and Nutrition Survey and in the US NHANES (2003–2006)^(35,41). It has been hypothesised that nitric oxide or hydrogen sulphide could oxidise or compete for the B₁₂ cobalt atom, whereas tetrahydrofolates could react with cyanates to form biologically inactive derivatives⁽⁴²⁾.

Uniquely, we were also able to examine the associations with geographic location and with seasonality. We observed that concentrations of folate were lowest in autumn and concentrations of B₁₂ were lowest in summer compared with winter. Some^(43–46) but not all studies^(47,48) examining folate concentrations after exposure to UV light from sun-bed use or outdoor exposure have observed a lower status. For example, in a small study of forty-five Australian women (aged 18–47 years), UV exposure appeared to reduce the effectiveness of FA supplementation⁽⁴⁵⁾. Other studies have also observed that UV exposure may affect B₁₂ status. In a cohort of 1013 elderly Chileans, solar radiation was associated with B₁₂ deficiency⁽⁴⁹⁾. Similar mechanisms for both B₁₂ and folate have been proposed including direct and indirect photo-degradation by UV radiation and by the formation of free radicals^(50,51). These observations may not only be important for B-vitamin status but also for other micronutrients as UV exposure is the main determinant of vitamin D status.

It is important to note that FA supplement use was not a contributor to B₁₂ concentrations or status in our population, as there have been some concerns that high folate status could exacerbate the clinical effects of B₁₂ deficiency⁽⁵²⁾. Within TILDA, 8.9% had a folate concentration >45 nmol/l. This is lower than the frequency observed either in NANS (19%) or in post-fortification NHANES in the USA (38%)^(15,53). The same methodology was used in all three studies and the folate measurements for this study and for NANS were carried out in the same laboratory. It is unclear what the clinical implications are of high folate status. Previously, high folate concentrations have been correlated with unmetabolised FA in plasma⁽⁵⁴⁾, which has been suggested to be a driver of some adverse health effects⁽⁵⁴⁾. In a cross-sectional study of 219 older adults (>65 years), higher folate concentrations were associated with lower brain volumes⁽⁵⁵⁾, whereas a higher cancer incidence was reported in the folate and B₁₂ intervention group of the B-PROOF study of older adults, with the effect on cancer incidence more pronounced in the oldest old (>80 years)⁽⁵⁶⁾. FA has been suggested to be a preventive agent for cancer initiation but a promoter of established neoplastic cells⁽⁵⁷⁾. However, this effect is still not clear as higher levels of folate have been associated with a lower risk of lung cancer diagnosis in a case-control study of 366 adults⁽⁵⁸⁾, whereas lower folate status in comparison with high status has been associated with a higher risk of all-cause mortality⁽²⁹⁾.

The major strength of this study is that it is the largest nationally representative older population sample reported with

detailed data on a wide range of potential determinants. A limitation is that no dietary intake data were available including the consumption of fortified food products or the dosage of B-vitamin supplements taken. However, previous studies have shown a weak relationship between intake and blood biomarker data for these micronutrients^(15,34). One recent study has indicated that dietary intake data and biomarkers reflect the same underlying folate status with a systematic under-estimation bias in intake data⁽⁵⁹⁾. Furthermore, our study was limited to the use of plasma total B₁₂ and folate as indicators of status with the corresponding benefits and drawbacks associated with each of these biomarkers (i.e. total B₁₂ measurement in blood is known to have relatively low sensitivity for estimating B₁₂ deficiency⁽⁶⁰⁾, whereas plasma folate is subject to variations from recent dietary intakes). It is also important to note that because of the recruitment sampling criteria these findings may not be applicable to non-Caucasians, older adults in institutional care or those with severe cognitive impairment.

In summary, we have observed high rates of low B₁₂ and folate status coupled with a much smaller rate of high folate status in the older Irish population. Ideally, encouraging older persons to improve their normal eating practices and to follow the food pyramid has the potential to enhance the B₁₂ and folate status of this population group. However, compliance to healthy eating recommendations, as well as practical issues such as cooking practices, bioavailability, medication intakes and age-related changes to the gastrointestinal tract, means that the holistic unfortified approach can be ineffectual. Our data (and others previously) indicate that the current policy of voluntary fortification is ineffective at preventing the occurrence of deficiency and low blood status. Serious consideration must now be given to the implementation of mandatory FA and B₁₂ fortification. Such a policy would ensure that the requirements of the 'at-risk' older adult population are met while also helping to reduce the prevalence of folate-responsive neural tube defects in the unborn child. The continued delay in consideration of this implementation could put >180 000 and 145 000 older Irish adults at risk of the health consequences of low folate and B₁₂ status, respectively.

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The authors contribution are as follows: A. M. M., R. A. K., A. M. O. H. and E. J. L. designed the research; E. J. L., A. M. M. and A. M. O. H. conducted the research; D. C., D. O. C. and E. J. L. analysed the data; and all authors contributed in the final preparation of the manuscript.

The authors declare that there are no conflicts of interest.



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Appendix 11.1.3 - Paper 3 - Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA).

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Plasma concentrations of vitamin B₁₂ and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA)

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Abstract

The uncertainty surrounding high intakes of folic acid and associations with cognitive decline in older adults with low vitamin B₁₂ status has been an obstacle to mandatory folic acid fortification for many years. We estimated the prevalence of combinations of low/normal/high vitamin B₁₂ and folate status and compared associations with global cognitive function using two approaches, of individuals in a population-based study of those aged ≥50 years in the Republic of Ireland. Cross-sectional data from 3781 men and women from Wave 1 of The Irish Longitudinal Study on Ageing were analysed. Global cognitive function was assessed by the Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Prevalence estimates for combinations of vitamin B₁₂ (plasma vitamin B₁₂ < or ≥258 pmol/l) and folate (plasma folate ≤ or >45.3 nmol/l) concentrations were generated. Negative binomial regression models were used to investigate the associations of vitamin B₁₂ and folate status with global cognitive function. Of the participants, 1.5% (n 51) had low vitamin B₁₂ (<258 pmol/l) and high folate (>45.3 nmol/l) status. Global cognitive performance was not significantly reduced in these individuals when compared with those with normal status for both B-vitamins (n 2433). Those with normal vitamin B₁₂/high folate status (7.6%) had better cognitive performance (MMSE: incidence rate ratio (IRR) 0.82, 95% CI 0.68, 0.99; P=0.043; MoCA: IRR 0.89, 95% CI 0.80, 0.99; P=0.025). We demonstrated that high folate status was not associated with lower cognitive scores in older adults with low vitamin B₁₂ status. These findings provide important safety information that could guide fortification policy recommendations in Europe.

Key words: Vitamin B₁₂; Folate; Ageing; Older people; Cognitive function; Epidemiology

Cognitive impairment has been associated with low vitamin B₁₂⁽¹⁾ and low folate status⁽²⁾. In addition, some previous observational data have suggested that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia and cognitive impairment or decline^(3–5); however, the causal relevance of these associations remains uncertain.

Vitamin B₁₂ and folate participate in one carbon metabolism, critical for the formation of DNA and RNA, neurotransmitters, membrane lipids and proteins. Because of these functions, there has been growing interest in the possibility that age-related conditions, in particular loss of cognitive function, may partly be explained by inadequate status of these vitamins^(6–9). Vitamin B₁₂ deficiency (plasma vitamin B₁₂ < 148 pmol/l) is associated with severe neurological consequences, including sensory and

motor neuropathy, altered mental status and cognitive decline⁽¹⁾. Vitamin B₁₂ deficiency also reduces the availability of folate for DNA synthesis⁽¹⁾.

Since the introduction of mandatory fortification of staple foods with folic acid to reduce neural tube defects (NTD), high folate status (>45.3 nmol/l) is frequently observed in countries such as the USA and Canada. Recent research has raised concerns regarding the adverse implications of simultaneous low vitamin B₁₂ and high folate status given that high-dose folic acid treatment was shown to temporarily mask clinical symptoms in persons with pernicious anaemia⁽¹⁰⁾.

While some studies have reported that older adults with low vitamin B₁₂ and high folate status had higher risks of anaemia, cognitive impairment and decline^(3–5), other large studies

Abbreviations: MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; TILDA, The Irish Longitudinal Study on Ageing.

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conducted in diverse populations have not replicated such adverse associations^(11–13). There is considerable heterogeneity among the published studies, including differences in vitamin status cut-offs, biochemical methodologies and the instruments used to assess cognitive function⁽¹⁴⁾. Likewise, large-scale randomised controlled trials have failed to demonstrate any beneficial effects of supplementation with vitamin B₁₂ or folic acid on global or domain-specific cognitive function⁽¹⁵⁾.

Ireland has one of the highest rates of neural tube defects in Europe⁽¹⁶⁾, and supplementation initiatives have been shown to be ineffective⁽¹⁷⁾, yet one major obstacle impeding population-based food fortification is the concern that high folate status in the presence of low vitamin B₁₂ status could have a detrimental effect on cognitive function in older people. In our population study, The Irish Longitudinal Study on Ageing (TILDA), the prevalence of older people with deficient or low vitamin B₁₂ status (<185 pmol/l) was 12%⁽¹⁸⁾, while high folate status (>45.3 nmol/l) was observed in 9% of the population⁽¹⁹⁾. TILDA affords a unique opportunity to investigate whether there is an association between cognitive performance and variations of vitamin B₁₂ and folate in a large, nationally representative population sample of older adults. We hypothesise that a low vitamin B₁₂ status coupled with high blood concentrations of folate does not confer greater risk to global cognitive performance in this older population group.

Methods

Study population

Cross-sectional data from Wave 1 of TILDA were analysed. TILDA is a nationally representative prospective cohort study on ageing

of community-dwelling adults aged 50 years and older, living in the Republic of Ireland. Details of the study design have been reported previously^(19–21). Briefly, Wave 1 took place between October 2009 and February 2011. Eight thousand hundred and seventy-four adults aged ≥50 years from 6279 households completed a computer-aided personal interview, representing a response rate of 62%. Of the total sample, 84.6% completed the self-completion questionnaire (*n* 7196) and 72.1% (*n* 5895) participated in a health assessment which included blood measurements. Plasma vitamin B₁₂ measurements were available for 88.5% (*n* 5219) and plasma folate for 90.7% (*n* 5350) of participants. Missing data were due to insufficient sample and/or assay failures. Individuals who did not complete both standardised assessments of cognitive function, the Mini Mental State Examination (MMSE)⁽²²⁾ and Montreal Cognitive Assessment (MoCA)⁽²³⁾, were not included in this analysis, giving a final analytical sample of 3871 (Fig. 1). Ethical approval was obtained from the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin. All participants were fully informed of the study and gave written consent.

Assessment of cognitive function

Global cognitive function was assessed using the MMSE and MoCA. The MMSE is a brief 30-point test that is used to screen for cognitive impairment, commonly used in clinical practice to screen for dementia and assesses orientation, recall, attention, calculation, language abilities and visuospatial ability. The MoCA, also used to screen for dementia, is particularly sensitive to mild cognitive deficits^(23,24), with additional assessments of executive function, abstraction and visuospatial ability. Both tools have scores ranging from 0 to 30.

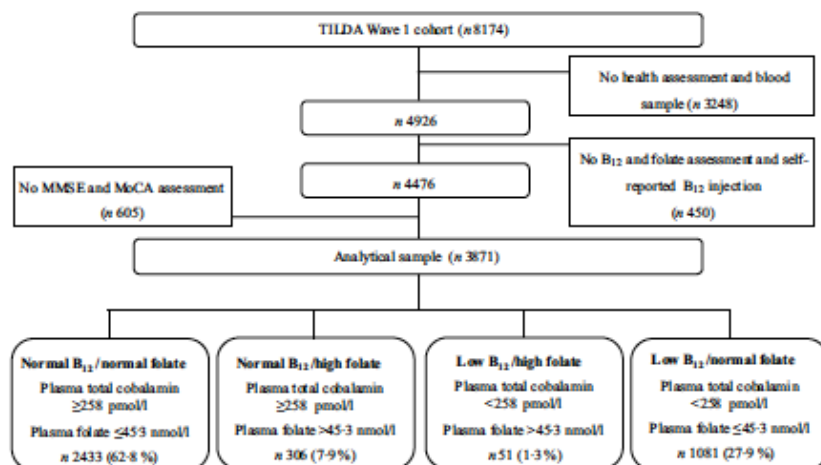


Fig. 1. Flow diagram – analytical sample design and selection. TILDA, The Irish Longitudinal Study on Ageing; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; B₁₂, vitamin B₁₂.

Analysis of B-vitamins

A non-fasting blood sample was collected by venepuncture into one 10 ml K₂EDTA tube (BD, Becton, Dickinson Limited) for immediate analysis and two 10 ml EDTA (BD, Becton, Dickinson Limited) tubes for long-term storage (Sarstedt). Samples were kept chilled and were centrifuged (3000 rpm for 15 min). Aliquots of plasma were labelled and stored at -80°C within 48 h of blood collection and stored until required for analysis. The TILDA protocol for blood sample collection, processing, and storage is detailed elsewhere⁽²⁰⁾. Plasma vitamin B₁₂ and folate concentrations were determined by microbiological assay; *Lactobacillus leichmannii* (*L. leichmannii* (delbrueckii) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/l⁽²⁰⁾ and *Lactobacillus casei* (*L. rhamnosus* (NCIB 10463; ATCC 27773)), limit of detection 0.6 nmol/l⁽²⁰⁾, respectively. Inter-assay CV for both methods were <10.9%.

Other covariates

Sociodemographic characteristics examined included age, sex, educational attainment, mean asset wealth and habitation status. Lifestyle characteristics comprised self-reported physical activity (International Physical Activity Questionnaire), smoking status, alcohol consumption, depressive symptoms (Center for Epidemiologic Studies Depression Scale)⁽²⁷⁾ and history of stroke. Use of medications (up to twenty prescription and non-prescription medications, including food supplements) was classified by World Health Organization, Anatomical Therapeutic Chemical classification codes.

Anthropometric measurements included height, weight, BMI and handgrip strength (average two recordings from dominant hand using a dynamometer). Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg and/or antihypertensive medication use. Diabetes was defined as diabetes or prediabetes by measurement of glycated Hb (HbA_{1c})⁽²⁸⁾, self-reported diabetes diagnosis and/or use of anti-glycaemic medications.

Exclusion criteria

Individuals without a health assessment and blood sample were excluded (*n* 3248). Those for whom vitamin B₁₂ and/or folate concentrations could not be measured, or whose measurement values exceeded the 99th percentiles and participants in receipt of vitamin B₁₂ injections, were omitted from the analysis (*n* 450). In order to rule out participants with possible mild cognitive impairment, additional exclusionary criteria were applied to both MMSE and MoCA results. Those with MMSE scores of <24⁽²⁹⁾ and MoCA below the age and education normative cut-offs of Rossetti *et al.*⁽³⁰⁾ were also omitted (*n* 605).

Statistical methods

While there is no international consensus regarding the cut-offs for both vitamin B₁₂ and folate status, we adopted the widely used cut-offs identified in the literature. For plasma vitamin B₁₂ (pmol/l), these included <148 (deficient), 148–<185 (low status), 185–<258 (low/normal status), >258–>601 (normal)

and >601 (high)^(31,32). For plasma folate (nmol/l), the cut-offs included 10–23.0 (low/normal) and >23.0–45.3 (normal) >45.3 (high)^(33,34). To compare with previous studies, a four-level categorical variable was created on the basis of vitamin B₁₂ (< or ≥ 258 pmol/l)^(31,32) and folate concentrations ($\leq$ or >45.3 nmol/l)⁽³⁴⁾ and was defined as (i) normal vitamin B₁₂/normal folate (≥ 258 pmol/l, ≤ 45.3 nmol/l), (ii) normal vitamin B₁₂/high folate (≥ 258 pmol/l, >45.3 nmol/l), (iii) low vitamin B₁₂/high folate (<258 pmol/l, >45.3 nmol/l) and (iv) low vitamin B₁₂/normal folate (<258 pmol/l, ≤ 45.3 nmol/l).

Non-normally distributed continuous variables were described as medians and interquartile ranges for each group, stratified by sample characteristics, biomarkers and cognitive tests. These were compared using Kruskal–Wallis and Mann–Whitney *U* tests for pairwise comparisons. Categorical variables were compared using χ^2 tests or Fisher's exact test, as appropriate. A false discovery rate correction for multiple comparisons was applied ($P < 0.013$, achieving false discovery rate $q < 0.05$) to adjust for the number of individual tests carried out (i.e. *q* indicates that the average alpha level across the family of tests does not exceed 0.05, following correction).

Associations of vitamin B₁₂ and folate with cognitive function and other covariates were explored in a series of regression analyses. The relationship between cognitive function and groups of vitamin B₁₂ and folate status was analysed with models appropriate for the distribution of the outcome variables, MMSE (continuous) and MoCA (continuous). Visualisation of the data and detailed inspection of model residuals demonstrated that neither outcome was normally distributed after appropriate removal of outliers. Therefore, MMSE and MoCA were analysed using negative binomial regression models (due to overdispersion) with the outcome representing the count of errors made during the test (30 minus the raw score) due to their left-skewed distributions, consistent with previous reports using TILDA data⁽³⁵⁾.

The results of these analyses are presented as the incidence rate ratio for each model, relative to the reference category, normal vitamin B₁₂/normal folate, as previously performed in this cohort⁽³⁶⁾. For both cognitive tests, three models were investigated: unadjusted (model 0); adjusted for sociodemographic covariates – age, sex, educational attainment, mean asset wealth and habitation status (model 1) and adjusted for sociodemographic, lifestyle, health indicators and medications – physical activity, smoking, alcohol consumption, BMI, mean grip strength, hypertension, diabetes, depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of previous stroke (model 2).

These analyses were then repeated in the sample aged ≥ 60 years (*n* 2070), for more direct comparison with other studies in this field. Further, a sensitivity analysis was performed including the participants with lower cognitive scores, aged ≥ 50 and ≥ 60 years, omitted from the main analysis.

Additionally, quintiles of vitamin B₁₂ concentrations were created based on plasma vitamin B₁₂ concentrations in those aged ≥ 60 years. An interaction term was included to examine whether the association between plasma folate (as a continuous variable) differed between quintiles of vitamin B₁₂, using the highest quintile of vitamin B₁₂/folate (Q5) as the reference



category (the reverse instantiation was also analysed, that is, continuous vitamin B₁₂ and quintiles of folate).

Population weights were calculated for the regression analyses by comparing the TILDA population with the 2011 Irish Census with respect to age, sex, educational attainment, marital status and urban *v.* rural residence (weights reflected the reciprocal of the selection probability). Prevalence estimates, descriptive statistics and model coefficients were adjusted using modified base weights that accounted for survey non-response, non-attendance at the health assessment component of the study and whether a blood sample was provided. The staged, stratified and clustered sample design of TILDA was considered when estimating confidence intervals and standard errors. Analyses were carried out using STATA 15 (StataCorp).

Results

Baseline characteristics of the study sample

The study design and exclusion criteria are detailed in Fig. 1. The mean age was 61.8 years (sd 8.8) (range 50–98 years); 50.7% were female (*n* 3871). Median (interquartile ranges) MMSE and MoCA scores were 29 (28–30) and 26 (24–28), respectively. Vitamin B₁₂ concentrations ranged from 45.4 to 719 pmol/l, with 4.8% (95% CI 4.1, 5.7) classified as deficient (<148 pmol/l) and 25.5% (95% CI 24.1, 27.0) with low vitamin B₁₂ status (148–258 pmol/l). Folate concentrations were 0.9–82 nmol/l, 5.7% (95% CI 4.9, 6.7) had high folate status (45–60 nmol/l), with an additional 3.3% (95% CI 2.8, 4.0) classified as very high folate status (>60 nmol/l), similar to rates observed in our full population⁽¹⁰⁾. The prevalence of normal vitamin B₁₂/normal folate status was 62% (95% CI 60.2, 63.8), normal vitamin B₁₂/high folate was 7.6% (95% CI 6.6, 8.8), low vitamin B₁₂/high folate was 1.5% (95% CI 1.1, 2.0) and low vitamin B₁₂/normal folate was 28.9% (95% CI 27.3, 30.6).

Sample characteristics, by vitamin B₁₂ and folate groups

Sample characteristics, stratified by vitamin B₁₂ and folate groups, are detailed in Table 1, with additional sample characteristics displayed in online Supplementary Table S1. All groups were older than the reference category, normal vitamin B₁₂/normal folate, and were more likely to be hypertensive. When compared with the reference category, subjects with normal vitamin B₁₂/high folate were more likely to be female, have above average household wealth, lower mean grip strength and BMI and were less likely to be categorised as obese. This group was also more likely to report taking a folic acid supplement and had better global cognitive scores than the reference group. Those with low vitamin B₁₂/high folate status were more likely to be obese, have above average household wealth and be hypertensive and diabetic relative to the reference category. Subjects with low vitamin B₁₂/normal folate status had lower levels of educational attainment, higher overall BMI and were more likely to smoke and to be obese, hypertensive, diabetic and thus to use metformin (online Supplementary Table S1). These groups were further examined by age group (online Supplementary Table S2), though no additional relevant significant findings were observed.

Associations with combinations of vitamin B₁₂ and folate status

Fig. 2 and online Supplementary Table S3 display the associations between the groups of vitamin B₁₂ and folate status in those aged ≥50 and ≥60 years for MMSE. Relative to normal vitamin B₁₂/normal folate, having low vitamin B₁₂/high folate was not associated with a significant increase in MMSE error rates for models 0, 1 or 2. Further, for all three models, there were statistically significant decreases in MMSE error rates for those in the normal vitamin B₁₂/high folate group in both the ≥50 and ≥60 years groups (model 2: 0.84 (95% CI 0.71, 1.00); *P* = 0.047 and 0.82 (95% CI 0.68, 0.99); *P* = 0.043, respectively). Fig. 3 and online Supplementary Table S3 show the associations for MoCA. Similarly, low vitamin B₁₂/high folate status was not associated with increases in error rates in the three models tested. However, having normal vitamin B₁₂/high folate was associated with significant decreases in MoCA error rates for models 0, 1 and 2, respectively, in those aged ≥50 years (0.91 (95% CI 0.83, 0.99); *P* = 0.031, 0.92 (95% CI 0.85, 1.00); *P* = 0.040 and 0.91 (95% CI 0.85, 1.00); *P* = 0.042) and ≥60 years (0.87 (95% CI 0.78, 0.97); *P* = 0.014, 0.91 (95% CI 0.83, 1.00); *P* = 0.044 and 0.89 (95% CI 0.80, 1.00); *P* = 0.025). Results of the full multivariate analyses for models 1 and 2 are presented in online Supplementary Tables S4 and S5. Sensitivity analysis indicated that those in the low vitamin B₁₂/high folate groups for each model and in the subgroup aged ≥60 years were not more likely to make errors with the MMSE or MoCA compared with those within the reference category (online Supplementary Table S6).

Associations with quintiles of vitamin B₁₂ and continuous folate concentrations

Additional models, in subjects aged ≥60 years, examined folate concentrations on a continuum, interacted with quintiles (Q1–Q5) of vitamin B₁₂ (Q1 vitamin B₁₂, 45.4–225 pmol/l (*n* 409); Q2 225–289 pmol/l (*n* 427); Q3 289–350 pmol/l (*n* 408); Q4 350–428 pmol/l (*n* 415); Q5 428–719 pmol/l (*n* 412)). These demonstrated there was no evidence of interactions with respect to MMSE and MoCA error rates (Fig. 4). For MoCA, model 2 revealed a small yet statistically significant decrease in the rate of errors for those with the highest vitamin B₁₂ concentrations (online Supplementary Table S7). The reverse instantiation was also tested (i.e. vitamin B₁₂ as the continuous variable, interacted with quintiles of folate). This analysis showed no association of error rates with decreasing concentrations of vitamin B₁₂ (online Supplementary Table S8).

Discussion

The present study estimated the prevalence of groups of vitamin B₁₂ and folate status in a nationally representative sample of older adults in Ireland and investigated the hypothesis that low vitamin B₁₂ status in conjunction with high folate status is associated with increased risk of cognitive decline in older people^(3–5). We demonstrated that the 1.5% of the population with low vitamin B₁₂/high folate status did not have any adverse associations with cognitive performance compared with those with normal status for both B-vitamins. In contrast, the present study

Table 1. Basic sample characteristics, grouped by concentrations of vitamin B₁₂ (B₁₂) and folate (Numbers and percentages; medians and interquartile ranges (IQR))

	n	Vitamin B ₁₂ ≥ 25.8 pmol/l						Vitamin B ₁₂ < 25.8 pmol/l						P for comparison across all groups
		Folate ≤ 45.3 nmol/l			Folate > 45.3 nmol/l			Folate > 45.3 nmol/l			Folate ≤ 45.3 nmol/l			
		Normal B ₁₂ /normal folate			Normal B ₁₂ /high folate			Low B ₁₂ /high folate			Low B ₁₂ /normal folate			
		Median	IQR		Median	IQR		Median	IQR		Median	IQR		
Subjects (n)	3871	2433		306		66		51		61		1081		
Age (years)	3871	55.67		56.68		57.73		57.73		55.68		55.68		0.0001
P		Reference		0.0050*		0.0015*		0.0015*		0.0105*				
Over 80 years	95/3871													0.017
n		52		5		4		4		34		34		
%		2.1		1.6		7.8		7.8		3.2		3.2		
P		Reference		0.0204		0.1347		0.1347		0.0967		0.0967		
Females	2045/3871													0.0000
n		1292		198		23		23		531		531		
%		53.1		64.7		45.1		45.1		49.1		49.1		
P		Reference		0.0001*		0.2602		0.2602		0.0294		0.0294		
Obese	1274/3866													0.0000
n		777		72		24		24		401		401		
%		31.9		23.5		47.1		47.1		37.1		37.1		
P		Reference		0.0013*		0.0338*		0.0338*		0.0032*		0.0032*		
Vitamin B ₁₂ (pmol/l)	3871	361.3	308.7, 435.2	421.0	344.7, 514.0	214.2	160.1, 241.3	205.9	170.3, 235.4	205.9	170.3, 235.4	205.9	170.3, 235.4	0.0001
P		Reference		0.0000*		0.0000*		0.0000*		0.0000*		0.0000*		
Folate (nmol/l)	3871	18.8	13.1, 26.9	56.4	50.0, 65.5	55.4	49.1, 61.5	14.7	10.7, 22.0	14.7	10.7, 22.0	14.7	10.7, 22.0	0.0001
P		Reference		0.0000*		0.0000*		0.0000*		0.0000*		0.0000*		
Folic acid supplement use	123/3871													0.0000
n		68		22		7		7		26		26		
%		2.8		7.2		13.7		13.7		2.4		2.4		
P		Reference		0.0038*		0.0251		0.0251		0.4969		0.4969		
MMSE (global cognition)	3871	29	28.0, 30.0	29	29.0, 30.0	29	28.0, 30.0	29	28.0, 30.0	29	28.0, 30.0	29	28.0, 30.0	0.0170
P		Reference		0.0003*		0.9448		0.9448		0.7175		0.7175		
MoCA (global cognition)	3871	26	24.0, 28.0	26	25.0, 28.0	26	24.0, 27.0	26	24.0, 27.0	26	24.0, 27.0	26	24.0, 28.0	0.0054
P		Reference		0.0243*		0.5625		0.5625		0.0338		0.0338		

MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

* Statistically significant after adjustment for multiple comparisons ($P < 0.013$).

† Non-normally distributed continuous variables are described as medians and IQR (all such values) and compared using the Kruskal–Wallis test as appropriate, and the Mann–Whitney U-test for pairwise comparisons with the reference category. Categorical variables were compared using χ^2 tests of Fisher's exact test, as appropriate.

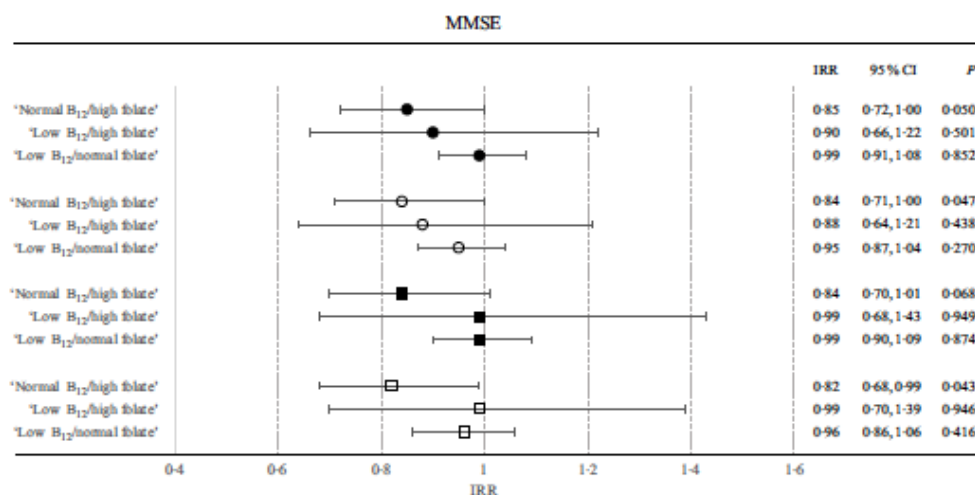


Fig. 2. Associations of vitamin B₁₂ (B₁₂) and folate status and global cognition (Mini Mental State Examination (MMSE)) – aged ≥ 50 and ≥ 60 years (incidence rate ratios (IRR) and 95% confidence intervals). (●), Aged ≥ 50 years, model 1 (Sociodemographic) (MMSE error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (○), Aged ≥ 50 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). (■), Aged ≥ 60 years, model 1 (Sociodemographic) (MMSE error rate and B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥ 60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

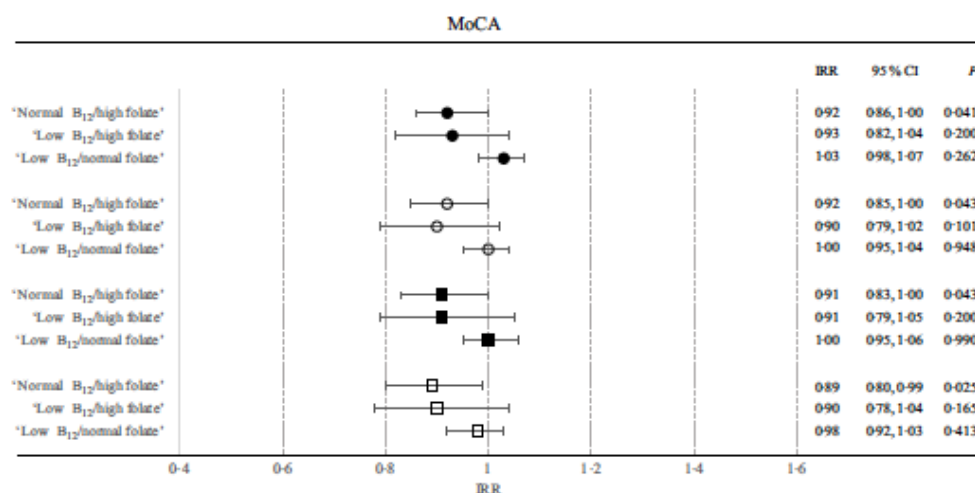


Fig. 3. Associations of vitamin B₁₂ (B₁₂) and folate status and global cognition (Montreal Cognitive Assessment (MoCA)) – aged ≥ 50 and ≥ 60 years (incidence rate ratios (IRR) and 95% confidence intervals). (●), Aged ≥ 50 years, model 1 (Sociodemographic) (MoCA error rate and vitamin B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (○), Aged ≥ 50 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). (■), Aged ≥ 60 years, model 1 (Sociodemographic) (MoCA error rate and B₁₂ and folate groups with controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥ 60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke).

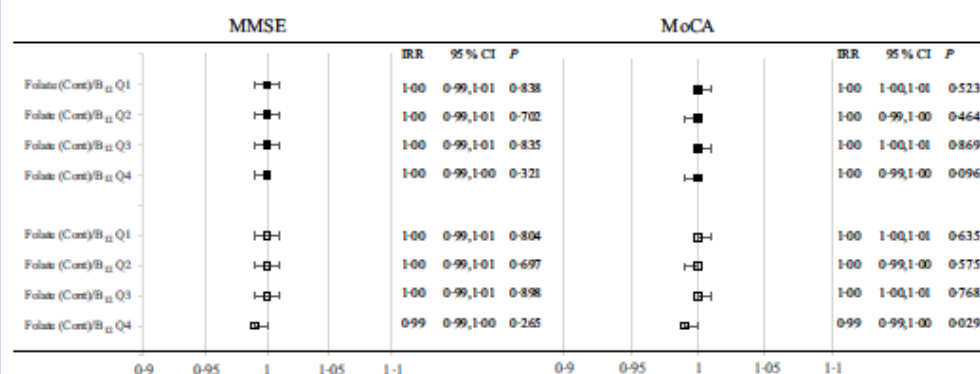


Fig. 4. Associations between concentrations of folate (continuous (Cont)) and quintiles (Q) of vitamin B₁₂ and global cognition – aged ≥60 years (incidence rate ratios (IRR) and 95% confidence intervals). (■), Aged ≥60 years, model 1 (Sociodemographic) (Global cognition measure error and vitamin B₁₂ and folate concentrations with controls for age, sex, educational attainment, mean asset wealth and habitation status). (□), Aged ≥60 years, model 2 (Sociodemographic, Lifestyle, Health Indicators and Medications) (model 1 + controls for physical activity, smoking status and alcohol consumption, BMI, grip strength, hypertension status, diabetes status and depressive symptoms, folic acid supplement use, proton pump inhibitor, statin and thyroid medication use and history of stroke). MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

demonstrated that higher concentrations of folate were associated with small, but statistically significant higher scores for MMSE and MoCA performance. In contrast to previous research using single measures of global cognition in older cohorts, we employed two clinically validated methods of global cognitive function measurement simultaneously.

There are a number of possible explanations as to why our findings diverge with those of the National Health and Nutrition Examination Survey (NHANES), the Framingham Heart Study and three combined cohorts of Moore and colleagues (2014)^(5–7). In NHANES (*n* 1457), concentrations of high plasma folate (>59 nmol/l) with low vitamin B₁₂ (plasma B₁₂ <148 pmol/l or methylmalonic acid >0.21 nmol/l) (*n* 42) were associated with poorer cognitive performance cross-sectionally. The latter study was conducted in the USA, a region implementing mandatory folic acid fortification and where the use of supplements containing folic acid was common, leading to extremely high concentrations of plasma folate and the presence of circulating unmetabolised folic acid^(5,7,30). Thus, very high concentrations (plasma folate >59 nmol/l) were observed in 20.7% of the NHANES cohort, compared with just 3.3% (plasma folate ≥60 nmol/l) in the present study. The use of folic acid supplements was uncommon amongst older adults in Ireland, ranging between 7.2 and 13.7% amongst the two groups with the highest status. In addition, the Digit Symbol-Substitution subtest of the Wechsler Adult Intelligence Scale III was used in NHANES to examine cognitive performance in attention and processing speed, while the present study assessed global cognitive performance using both MMSE and MoCA. From the Framingham Heart Study⁽⁶⁾, the data of which pertained to pre-mandatory fortification in the USA (1986–1990), Morris *et al.*⁽⁴⁾ reported faster rates of decline in MMSE were associated with vitamin B₁₂ concentrations of <257 pmol/l and plasma folate of >20.2 nmol/l – after 8-year follow-up (*n* 24). While the vitamin B₁₂ cut-off used was similar to the present study, we applied a

higher threshold to define high folate status and used a cognitively intact sample. Furthermore, there were twice as many participants included in the present analysis.

Moore *et al.*⁽⁵⁾ also suggested that high folate or folic acid supplements may be detrimental to cognition in older people with low vitamin B₁₂ concentrations (*n* 1354)⁽⁵⁾. Compared with the present study, a similar vitamin B₁₂ cut-off (<250 pmol/l) was used, and red cell folate of >1594 nmol/l, which approximate to those employed in the Framingham Heart Study investigation and had thirty-nine participants in the low vitamin B₁₂/high folate group. However, the sample was recruited from three separate clinical cohorts in Australia, a region also implementing mandatory folic acid fortification. In addition, 480 subjects had established Alzheimer's disease and 187 had confirmed mild cognitive impairment. This is in contrast with our study, all of whom were free living and cognitively robust (*n* 3871). In addition, Moore *et al.*⁽⁵⁾ used the binary outcome of MMSE of <24 to define cognitive impairment, whereas our study used MMSE and MoCA as a continuous outcome to investigate the range of risk to cognitive performance.

The findings of the present study are consistent with other studies using European population data; those of Clarke *et al.*⁽¹¹⁾ and Doets *et al.*⁽³⁵⁾ showing no association of low vitamin B₁₂/high folate status with an increased risk to cognitive function. In addition, the Sacramento Area Latino Study on Aging in America⁽³²⁾ did not demonstrate a negative association with cognitive function (*n* 1535) using a vitamin B₁₂ deficiency cut-off of <148 pmol/l and high folate of >45.3 nmol/l, although biochemical anomalies were observed.

To our knowledge, this is the largest such study to use a nationally representative sample of community-dwelling older adults that were cognitively unimpaired, employing rigorous exclusion criteria for cognitive performance to ensure accurate investigation of early cognitive changes and to mitigate potential confounding. Another strength of the present study is the use of



two tests of global cognitive performance in parallel. Most other studies in this field have investigated the MMSE or variations of it to determine cognitive performance or impairment. We also measured global cognitive performance using the MoCA, an arguably better screening tool for subtle cognitive decline and mild cognitive deficits^(33,34). In addition, we are the first study to explore interactions of vitamin B₁₂ and folate concentrations with respect to MoCA and we controlled for a large extent of covariates to account for factors that may affect B-vitamin status, particularly medications and food supplement use.

Consistent with previous studies, the present study was limited by the low numbers of participants in the 'at-risk' group. This determined the cut-off for vitamin B₁₂ (<258 pmol/l) utilised in the present study, as there were too few individuals with vitamin B₁₂ concentrations in the deficient range. The present study had only fifty-one participants in the low vitamin B₁₂/high folate group, albeit this number exceeded previous studies addressing this hypothesis. The low number in the group of interest may reflect the large proportion of the older population of Ireland captured by 'sub-optimal' but not deficient status, and the inherent selection bias in analysis of the most robust participants in a study population. However, the vitamin B₁₂ cut-off used in the present study is comparable with those used by the Framingham and Moore *et al.* studies^(4,5). Further, the small number of such individuals included in the present study reflected the application of strict exclusion criteria, and the authors are satisfied that this is representative of the proportion of those aged ≥50 years in this interest group. Similarly, our investigation using continuous concentrations of plasma folate was conducted to compensate for the low proportions of participants with vitamin B₁₂ concentrations below 148 pmol/l.

Another limitation was the cross-sectional nature of the present study, and therefore we cannot predict decline in the at-risk group. Moreover, the present study was limited to the use of plasma vitamin B₁₂ and folate as indicators of status, acknowledging the advantages and disadvantages associated with these biochemical measures (i.e. total vitamin B₁₂ in blood has relatively low sensitivity for estimating vitamin B₁₂ deficiency⁽³⁵⁾ when compared with other biomarkers, whereas plasma folate can be subject to variations from recent dietary intakes). In addition, dietary intake data at Wave 1 were not ascertained, therefore we could not estimate dietary exposure of both vitamin B₁₂ and folate. It is also important to note that because of the recruitment sampling criteria and rigorous exclusion criteria in the present study, these findings may not be generalisable to non-Caucasians, older adults in institutional care or those with cognitive impairment. Finally, we acknowledge the potential reverse causality and bidirectional relationship between global cognitive performance and vitamin B₁₂ and folate status and that the possible association between B-vitamin status and cognitive impairment in older adults might reflect effects of such vitamins on Alzheimer's disease or cerebrovascular disease, which are the primary pathogenic processes underlying cognitive impairment in older adults.

In summary, we undertook the present study to explore the possible hazards for cognitive function in older people associated with voluntary folic acid fortification in Ireland and found no evidence that high blood folate concentrations affected the

associations of low vitamin B₁₂ status with cognitive impairment. Our inability to detect differences in cognitive performance using these instruments implies that elevated folate status should not cause an increased risk to the present study population. In contrast, our data suggest that higher folate status may be protective of global cognitive function irrespective of vitamin B₁₂ status, in a representative sample of community-dwelling population of older people. This is particularly relevant, as our group has previously determined that in addition to 12% of older adults in the Republic of Ireland having low or deficient vitamin B₁₂ status, up to 15% also had deficient or low folate status⁽¹⁰⁾ – demonstrating that the policy of voluntary fortification with folic acid is ineffective for older people in the community.

These findings have significant implications for public policy on folic acid fortification in Ireland and elsewhere in Europe and provide reassurance to both healthcare providers and policy makers that high folate status is not associated with increased risk of cognitive impairment in older people.

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The author responsibilities were as follows: D. M. A. O., E. J. L., R. A. K. and A. M. M. designed the research; D. M. A. O., E. J. L., D. C. and A. M. O. collected and analysed the data; D. M. A. O., E. J. L. and D. C. performed statistical analysis; D. M. A. O., E. J. L., D. C., R. A. K. and A. M. M. wrote the initial draft of the manuscript; D. M. A. O., E. J. L., D. C., A. M. O., R. C., R. A. K. and A. M. M. critically revised the manuscript; and all authors had responsibility for accuracy of the final content and read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520001427>

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Appendix 11.1.4 - Paper 4 – Low folate predicts accelerated cognitive decline: 8-year follow-up of 3140 older adults in Ireland.

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ARTICLE



Health issues and nutrition in the elderly

Low folate predicts accelerated cognitive decline: 8-year follow-up of 3140 older adults in Ireland

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OBJECTIVE: To examine associations of plasma folate concentrations and risk of global and domain-specific cognitive decline in older people.**METHODS:** Data of 3140 participants from The Irish Longitudinal Study on Ageing (TILDA), a nationally-representative cohort of adults aged ≥ 50 years were used over 8-year follow-up. Biannual cognitive assessments included the Mini-Mental State Examination (MMSE), verbal fluency and immediate and delayed word recall tests (Waves 1–5) and the Montreal Cognitive Assessment, (MoCA) (Waves 1 and 3). Plasma folate concentrations were measured in stored blood collected at baseline. Mixed effects Poisson and linear regression determined associations between baseline folate concentrations and cognition.**RESULTS:** In multivariable-adjusted models of those aged ≥ 50 years at baseline, low folate at baseline (<11.2 nmol/L) was associated with higher proportions of MMSE errors (incidence rate ratio [IRR] = 1.10; 95% confidence interval [CI] (1.00, 1.21), lowest vs. highest quintile) over 8 years. Plasma folate <21.8 nmol/L predicted declines in episodic memory for immediate (beta [β] = -0.26 ; 95% CI (-0.48 , -0.03), β = -0.29 ; 95% CI (-0.50 , 0.08) and β = -0.29 ; (-0.50 , -0.08), for lowest three vs. highest quintile) and delayed recall (β = -0.20 ; 95% CI (-0.38 , -0.01), β = -0.18 ; 95% CI (-0.37 , -0.01) and β = -0.19 ; (-0.36 , -0.01) lowest three vs. highest quintile). There were no significant associations in a subsample aged ≥ 65 years.**CONCLUSION:** In those aged ≥ 50 years, lower concentrations of folate may have differential relationships with cognitive domains. Folate <11.2 nmol/L predicted a decline in global cognitive function, while <21.8 nmol/L predicted poorer episodic memory. Low folate was associated with accelerated decline in cognitive function and is an important marker for cognitive decline among older people.*European Journal of Clinical Nutrition*; <https://doi.org/10.1038/s41430-021-01057-3>

IMPACT STATEMENT

We certify that this work is novel. This is the largest sample to date to investigate longitudinal associations of folate and cognitive function, using repeated assessments of cognitive tests in a deeply phenotyped cohort. These data pertain to community-dwelling individuals that were cognitively robust at baseline and demonstrate evidence of an accelerated decline in cognitive function over an 8-year follow-up period in those with lower folate levels at baseline. The results are more pronounced for tests of episodic memory—memory being a sensitive indicator for early cognitive decline and mild cognitive impairment, and this means that low folate may serve as a useful early marker of risk of accelerated cognitive decline. Low folate status is a modifiable risk factor for cognitive decline, but is an omni-present issue for older adults in Ireland and other countries where fortification with folic acid is not mandated.

WHY DOES THIS PAPER MATTER?

This paper matters as it demonstrates that even among a relatively health and cognitively robust community-dwelling sample, that

low folate status predicts accelerated cognitive decline across several cognitive domains, over an 8-year time period.

INTRODUCTION

Approximately 7% of the world's population aged ≥ 65 years have dementia, a major cause of disability and mortality, that increases exponentially with age [1]. Cognitive impairment, a precursor to dementia, can range from mild to severe and is characterized by a deterioration in attention, executive function, learning and memory [2]. Maintenance of cognition is essential for functional independence in ageing. The optimization of nutritional status has been suggested to be a possible modifiable factor for prevention of cognitive impairment [3] and may be involved in the delay or prevention of chronic diseases including dementia [4, 5], cardiovascular disease [6] and structural changes in the brain [7–9].

Folate and vitamin B₁₂ (B₁₂) are essential co-factors in one-carbon metabolism, DNA-methylation and nucleotide synthesis, processes that occur in all tissues in the body including the brain and have been linked to neurodegeneration [10]. Both vitamins

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Table 1. Details of cognitive tests.

Domain	Test	Assessed in waves	Scoring	Administration	Effect measure	Interpretation of effects
Global cognition	MMSE	1, 2, 3, 4, 5	Total number of errors (0–30)	Participants were asked questions comprising attention, memory, orientation, language, and spatial ability	Rate ratio	> 1 = worse cognition
	MoCA	1, 3		Participants were asked questions comprising attention, memory, orientation, language, executive function and spatial ability	Rate ratio	> 1 = worse cognition
Semantic memory	Verbal fluency	1, 2, 3, 4, 5	Total number of words	Participants were asked to name as many animals as possible in 60 s	β coefficient	< 0 = worse cognition
Episodic memory	Immediate recall	1, 2, 3, 4, 5	Total number of words (0–20)	Participants were read a list of ten words on two occasions and asked to recall as many words as possible from the list	β coefficient	< 0 = worse cognition
	Delayed recall	1, 2, 3, 4, 5	Total number of words (0–10)	Participants were asked to recall as many words as possible from the list read previously (~15 min delay)	β coefficient	< 0 = worse cognition

Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall. CAP/ computer-assisted personal interview, MMSE Mini-Mental State Examination, MoCA Montreal Cognitive Assessment.

are required for efficient metabolism of homocysteine (HCY), a cytotoxic intermediary amino acid produced by the methionine cycle [10] that has been associated with cardiovascular disease and stroke, as well as cognitive decline [11]. The importance of folate insufficiency in cognitive impairment and dementia has been extensively studied in recent years due to its key role in HCY regulation [12]. Several epidemiological studies have shown cross-sectional and prospective associations between low folate and the risk of cognitive impairment and dementia [13–21].

Overt folate deficiency (<7 nmol/L) has been associated with accelerated ageing processes in the brain [22]. While rarely observed in countries where mandatory folic acid fortification (MFAF) of staple grains is in place to reduce the incidence of neural tube defects [23], deficiency and low folate is frequently observed in countries without MFAF such as the UK [24] and Ireland [25]. The Irish Longitudinal Study on Ageing (TILDA) demonstrated a 15% prevalence of low-to-deficient folate status (<10 nmol/L) among older adults living in a setting of voluntary fortification, highlighting its lack of effectiveness for maintenance of folate status in older people [26].

Using TILDA data, we investigated the longitudinal associations of folate concentrations with changes in cognitive performance in community-dwelling people, including sequential assessments of cognitive function at 2, 4, 6- and 8 years follow-up. We hypothesized that low folate was associated with accelerated cognitive decline in older people living in Ireland.

METHODS

TILDA is a nationally-representative prospective cohort study of community-dwelling adults aged ≥ 50 years living in the Republic of Ireland. The study design and sampling procedure have been described previously [27]. Briefly, participants were asked to complete a structured interview at each wave, capturing information on their social, financial and health circumstances. At baseline (2009–2011), 8504 participants were recruited and data were collected every two years thereafter, with objective health assessments conducted at alternate waves. Data for this study were obtained from Waves 1 to 5 of TILDA, collected between 2009 and 2018. The median interval between interviews for participants between Wave 1 to Wave 2 was 2.0 years, Wave 2 to Wave 3 was 2.3 years, Wave 3 to Wave 4 was 1.7 years and Wave 4 to Wave 5 was 2.0 years. Ethical approval was obtained at each data collection from the Faculty of Health Sciences Research Committee, Trinity College Dublin. Written consent was obtained from all participants.

Assessment of cognitive function

Participants underwent a battery of cognitive assessments at each wave which included the Mini-Mental State Examination [28] (MMSE; Global Cognition), a verbal fluency test (Semantic Memory) and immediate and delayed word recall tests (Episodic Memory). The Montreal Cognitive Assessment, (MoCA; Global Cognition) was measured at Waves 1 and 3 [29]. Table 1 describes the details of the cognitive tests. Data are presented by the cognitive domains for each test. Both the MMSE and MoCA scores are subject to ceiling effects, therefore the numbers of errors were calculated with the outcome representing the count of errors made during the test (30 minus the raw score) and used in analyses rather than total score.

Measurement of B vitamins

In Wave 1 during the health assessment, a non-fasting blood sample was collected by venipuncture into one 10 ml K2EDTA tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10 ml ethylene diamine tetra-acetic acid (EDTA; BD, Becton, Dickinson Limited) tubes for long term storage (Sarstedt; Numbrecht, Germany). Samples were kept chilled and were centrifuged (3000 rpm for 15 min). Aliquots of plasma were labeled and stored at -80°C within 48 h of blood collection and stored until required for analysis. The protocol for blood sample collection, processing, and storage used is detailed elsewhere [30]. Blood samples were analysed for plasma folate and B_{12} determined by microbiological assay; *Lactobacillus casei* (*L. rhamnosus* (NCIB 10463; ATCC 27733)), limit of detection 0.6 nmol/L [31] and *Lactobacillus leichmannii*

(*L. leichmannii* (delbrueckii) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/L [32], respectively. Inter-assay coefficients of variability (CV) for both methods were <10.9%.

Covariates

Socioeconomic covariates included were age, sex and educational attainment (primary, secondary or tertiary/higher); lifestyle characteristics including physical activity (Short-Form International Physical Activity Questionnaire) [33], smoking status, alcohol use and use of folic acid (FA) supplements; and health characteristics included weight and height (Body Mass Index (BMI) derived), (kg/m²), depressive symptoms (Center for Epidemiologic Studies Depression Scale (CES-D) scale [34], hypertension (doctor diagnosis or prescribed medications) and diabetes (doctor diagnosis or prescribed medications). Plasma B₁₂, (categorized as < or ≥258 pmol/L, vitamin D (plasma 25-hydroxyvitamin D) and lutein concentrations were also included. Medications (up to 20 prescription and non-prescription medications, including food supplements) were classified by World Health Organization, Anatomical Therapeutic Chemical (ATC) classification codes. These covariates were chosen as they have previously been or were expected to be associated with cognitive performance or folate metabolism.

Analytical sample

Of the 8504 participants interviewed in Wave 1, 3871 were eligible at baseline after exclusion criteria (Supplementary Fig. S1). Individuals aged <50 years (*n* = 330), without health assessment data (*n* = 3140) and blood samples (*n* = 108), in receipt of B₁₂ injections (*n* = 5), and for whom folate and/or B₁₂ measurements exceeded the 99th percentiles (*n* = 74 and *n* = 371, respectively) were excluded from the analysis. Individuals with MMSE scores of <24 [35] and MoCA below established age and education normative cut-offs [36] at baseline were also excluded (*n* = 605) to remove participants with possible mild cognitive impairment (MQI). Prospective analyses used eligible baseline participants with two or more follow-up cognitive measurements and those who did not receive B₁₂ injections in subsequent waves, yielding a final analytical sample of 3140 participants. Data on missing samples are presented in Supplementary Table S1.

Statistical analysis

Baseline characteristics of the sample were summarized by quintiles of plasma folate. Categorical variables were examined using percentages and continuous variables were explored using means and standard deviations, or medians and interquartile ranges, where appropriate. Performance scores for all cognitive tests were obtained for each wave. Histograms and Q-Q plots were used to assess marginal distributions of cognitive variables. Mixed effects models were used to examine the longitudinal associations between baseline folate status and cognitive function and take account for clustering of repeated measures within the same individual [37]. Further details of the methodology used are presented in Supplementary

Appendix S1. Cognitive function scores for MMSE and MoCA errors were right-skewed and Poisson models were used. Verbal fluency, immediate and delayed recall were modeled using linear regression models. Separate models were run for each cognitive outcome with the theoretically most favorable folate quintile (highest) used as the reference category. The analysis allowed us to examine if the effect of the association across quintiles of folate was apparent over the full exposure distribution (or was limited to the extremes). Model 1 are unadjusted regression models. Model 2 adjusts for age, age squared, sex, sociodemographic, behavioral, lifestyle and medical covariates. Statistical significance was defined as *P* < 0.05 and were performed using Stata 15.1 (StataCorp, College Station, TX).

RESULTS

Baseline characteristics

The mean (standard deviation) age of the baseline sample was 62.1 years (7.2), 52.7% were female. Cognitive scores for participants at each wave show small differences between waves, with the most notable decline evident with verbal fluency (Table 2). Sample characteristics were compared by quintiles of folate concentrations at Wave 1 and are summarized in Table 3. Relative to those in the highest quintile, participants in the lowest quintile of folate at baseline (<11.2 nmol/L) were more likely to be male, smoke, classified as obese (BMI ≥ 30), have higher levels of creatinine, report depressive symptoms and have lower mean B₁₂ and vitamin D. Furthermore, they were less likely to use a FA supplement. Cognitive function scores did not differ across quintile groups at Wave 1. Supplementary Table S2 summarizes the sample characteristics that were compared by B₁₂ groups (Deficient <148 pmol/L, Low 148–<258 pmol/L and Normal ≥258 pmol/L).

Regression analyses

Regression analyses examining associations between baseline folate and cognitive function are presented in Fig. 1. Multivariable-adjusted models demonstrated that folate levels of <11.2 nmol/L (lowest quintile) at baseline were associated with increases of MMSE errors (Incidence Rate Ratio [IRR] = 1.10; 95% confidence interval [95% CI] 1.00, 1.21), over eight years when compared to the reference category, the highest quintile (32.6–81.3 nmol/L). Baseline folate levels <21.8 nmol/L (quintiles 1–3) predicted an overall decline in episodic memory for both immediate (beta [β] = −0.26; (95% CI −0.48, −0.03), β = −0.29; (−0.50, −0.08) and β = −0.29; (−0.50, −0.08), for quintiles 1–3 vs. highest quintile) and delayed recall (β = −0.20; 95% CI = −0.38 to −0.01, β = −0.18; 95% CI −0.37, 0.01 and β = −0.19; (−0.36, −0.01) for

Table 2. Unadjusted cognitive function scores for all eligible and available participants at each wave.

	Wave 1	Wave 2	Wave 3	Wave 4	Wave 5
<i>Global cognition</i>					
MMSE errors ^a	1 (0–2)	0 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)
MoCA errors ^a	4 (2–5)	–	3 (2–5)	–	–
<i>Semantic memory</i>					
Verbal fluency ^b	22.4 ± 6.9	20.5 ± 5.9	19.9 ± 5.7	19.6 ± 5.7	19.5 ± 5.7
<i>Memory</i>					
Immediate recall ^b	14.1 ± 2.8	14.5 ± 2.8	14.3 ± 2.9	14.2 ± 3.0	14.1 ± 3.0
Delayed recall ^b	6.6 ± 2.1	6.6 ± 2.3	6.5 ± 2.3	6.4 ± 2.4	6.4 ± 2.4

Available cognitive data varies for each test depending on whether it was included in the interview or health assessment; data from Wave 1 (collected October 2009 to July 2011; *N* = 3123–3140), Wave 2 (collected February to December 2012; *N* = 3077–3095), Wave 3 (collected March 2014 to December 2015; *N* = 2962–3136), Wave 4 (collected January to December 2016; *N* = 2919–2939) and Wave 5 (collected January 2018 to December 2018; *N* = 2664–2691). Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall.

MMSE Mini-Mental State Examination, MoCA Montreal Cognitive Assessment.

^aMedian (IQR) (IQR interquartile range).

^bMean ± SD (SD Standard deviation).

Table 3. Characteristics of sample at baseline (2009–2011), according to plasma folate concentrations in quintiles.

Characteristic	All participants	Plasma folate level, nmol/L					p value ^a
		Quintile 1 0.9–11.2 nmol/L	Quintile 2 11.2–15.6 nmol/L	Quintile 3 15.7–21.8 nmol/L	Quintile 4 21.8–32.5 nmol/L	Quintile 5 32.6–81.3 nmol/L	
<i>n</i>	3140	573	638	629	646	654	
Age, mean $\pm$ SD	61.4 $\pm$ 8.0	61.6 $\pm$ 8.4	60.7 $\pm$ 8.0	60.6 $\pm$ 7.7	61.7 $\pm$ 8.0	62.3 $\pm$ 7.9	<0.001
Female, <i>N</i> (%)	1661 (52.9)	290 (50.6)	303 (48.4)	330 (52.5)	345 (53.4)	387 (59.2)	<0.01
Educational attainment, <i>N</i> (%)							
Primary	639 (20.4)	121 (21.1)	139 (21.8)	132 (21.0)	131 (20.3)	116 (17.7)	0.418
Secondary	1377 (43.9)	264 (46.1)	272 (42.6)	277 (44.0)	284 (44.0)	280 (42.8)	
Tertiary	1124 (35.8)	188 (32.8)	227 (35.6)	220 (35.0)	231 (35.8)	258 (39.5)	
Lives with someone, <i>N</i> (%)	2639 (83.7)	94 (16.4)	106 (16.6)	106 (16.9)	91 (14.1)	114 (17.4)	0.537
Smoke, <i>N</i> (%)	426 (13.6)	91 (15.9)	113 (17.7)	75 (11.9)	75 (11.6)	72 (11.0)	<0.01
Alcohol use, <i>N</i> (%)	2379 (80.4)	415 (78.6)	474 (79.8)	486 (81.5)	495 (80.1)	509 (81.7)	0.658
BMI, mean $\pm$ SD	27.5 (4.7)	27.8 (4.8)	27.9 (5.2)	27.6 (4.4)	27.6 (4.4)	27.0 (4.5)	<0.05
Obese, <i>N</i> (%)	1013 (32.4)	191 (33.5)	218 (34.3)	219 (35.0)	215 (33.5)	170 (26.1)	<0.001
Exercise group, <i>N</i> (%)							
Low	836 (26.6)	158 (27.6)	170 (26.7)	164 (26.1)	174 (26.9)	170 (26.0)	0.855
Moderate	1116 (35.5)	207 (36.1)	209 (32.8)	227 (36.1)	232 (35.9)	241 (36.9)	
High	1188 (37.8)	208 (36.3)	259 (40.6)	238 (37.8)	240 (37.2)	243 (37.2)	
Creatinine, mean (95% CI)*	78.9 [78.4, 79.6]	81.4 [79.9, 82.9]	79.1 [77.7, 80.4]	79.1 [78.1, 81.1]	78.2 [76.9, 79.6]	76.9 [75.6, 78.1]	<0.01
Women, mean (95% CI)*	70.4 [69.8, 71.1]	71.8 [70.3, 73.3]	70.4 [68.8, 71.9]	70.5 [69.1, 72.0]	69.8 [68.4, 71.2]	69.9 [68.5, 71.3]	0.883
Men, mean (95% CI)*	88.8 [87.9, 89.7]	91.6 [89.4, 93.8]	87.4 [85.6, 89.1]	89.5 [87.2, 91.8]	87.9 [86.0, 89.9]	87.5 [85.8, 89.3]	<0.01
Folic acid supplement use, <i>N</i> (%)	107 (3.4)	13 (2.3)	8 (1.3)	20 (3.2)	22 (3.4)	44 (6.7)	<0.001
Number of medications, mean $\pm$ SD	2.3 $\pm$ 2.4	2.3 $\pm$ 2.6	2.2 $\pm$ 2.6	2.0 $\pm$ 2.1	2.4 $\pm$ 2.4	2.4 $\pm$ 2.4	0.061
High blood pressure, <i>N</i> (%)	1259 (40.3)	245 (43.0)	259 (40.7)	224 (35.8)	257 (40.1)	274 (42.0)	0.102
Diabetes, <i>N</i> (%)	192 (6.2)	32 (5.6)	36 (5.7)	36 (5.8)	48 (7.6)	40 (6.2)	0.588
History of stroke, <i>N</i> (%)	30 (1.0)	6 (1.1)	5 (0.8)	9 (1.4)	2 (0.3)	8 (1.2)	0.278
CES-D, mean $\pm$ SD	5.2 $\pm$ 6.5	5.3 $\pm$ 7.0	5.2 $\pm$ 6.7	5.4 $\pm$ 6.5	5.3 $\pm$ 6.5	4.8 $\pm$ 6.0	0.561
Folate nmol/L, mean (95% CI)*	23.1 [22.6, 23.6]	8.8 [8.7, 9.0]	13.4 [13.2, 13.5]	18.6 [18.5, 18.7]	26.4 [26.2, 26.6]	46.5 [45.6, 47.3]	<0.001
B ₁₂ pmol/L, mean (95% CI)*	333.5 [330.6, 337.5]	290.8 [282.2, 299.5]	300.2 [292.3, 308.2]	335.6 [327.2, 344.0]	349.2 [340.8, 357.6]	386.5 [377.2, 395.0]	<0.001
Vitamin D nmol/L, mean (95% CI)*	53.4 [52.5, 54.2]	46.4 [44.6, 48.3]	49.6 [47.9, 51.5]	51.7 [50.0, 53.5]	56.8 [55.0, 58.7]	62.8 [61.0, 64.6]	<0.001
Lutein μ mol/L, mean (95% CI)*	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	<0.001
MMSE errors, median (IQR)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–1)	0.094
MoCA errors, median (IQR)	4 (2–5)	4 (2–6)	4 (2–5)	4 (2–5)	4 (2–5)	3.5 (2–5)	<0.05
Verbal fluency, mean $\pm$ SD	22.4 $\pm$ 6.9	22.3 $\pm$ 6.8	22.4 $\pm$ 7.2	22.5 $\pm$ 6.7	22.3 $\pm$ 6.8	22.5 $\pm$ 6.8	0.979
Immediate recall, mean $\pm$ SD	14.1 $\pm$ 2.8	14.0 $\pm$ 2.9	14.0 $\pm$ 2.9	14.1 $\pm$ 0.8	14.3 $\pm$ 2.8	14.3 $\pm$ 2.8	0.102
Delayed recall, mean $\pm$ SD	6.6 $\pm$ 2.1	6.4 $\pm$ 2.2	6.5 $\pm$ 2.1	6.5 $\pm$ 2.1	6.5 $\pm$ 2.2	6.7 $\pm$ 2.1	0.289

SD standard deviation, CI confidence interval

*Geometric mean.

Significance testing used one-way ANOVAs for normally distributed continuous characteristics, Kruskal–Wallis for non-normally distributed continuous characteristics and chi-square tests for categorical characteristics.

quintiles 1–3 vs. highest quintile) over eight years. In Model 1, significant increases in the number of MoCA errors amongst those in the lowest quintile of folate (<11.2 nmol/L) were observed (IRR = 1.10; 95% CI 1.03 to 1.18) over four years, however, this effect was attenuated with adjustment for additional covariates (Model 2). Results for Models 1 and 2 are presented in Supplementary Tables S3, S4, respectively.

Sensitivity analysis

Regression estimates of the sensitivity analysis conducted on subsamples of participants are presented in Supplementary Table S5. Among the subsample aged ≥ 65 years, there were no significant associations between baseline folate and changes in cognitive function.

DISCUSSION

We investigated the hypothesis that low folate status was associated with accelerated cognitive decline by examining the longitudinal associations between baseline folate status and repeated measures of five tests of cognitive function. To our knowledge, this is the largest longitudinal study to examine this issue using repeated measurements of tests exploring multiple cognitive domains. The results indicate that over an 8-year follow-up period, low folate (<11.2 nmol/L) was associated with decline in global cognitive function and a modest insufficiency (<21.8 nmol/L) predicted declines in both immediate and delayed recall.

Folate insufficiency is often observed among older Europeans and increases in prevalence with age [24]. Causes of deficiency include decreased dietary intake and malabsorption, age-related impairments in folate transport and metabolism, use of anti-folate medications, genetic factors and excessive alcohol intake [38]. Our findings are consistent with other studies showing low folate status was associated with higher risks of cognitive impairment or dementia [13–21]. In one study of older adults (≥ 60 years), those with MCI or dementia had significantly lower serum folate levels than healthy controls. Subjects in the lowest tertile (<13.5 nmol/L) had an adjusted Odds Ratio (OR) of 3.8 (95% CI: 1.3, 11.2; $P = 0.018$) for dementia [13]. Further, a meta-analysis examining thirteen studies (≥ 60 years) demonstrated low folate was associated with general and specific impairments in cognition such as attention, episodic and visuospatial memory or abstract reasoning (OR = 1.66, 95% CI: 1.40, 1.96) [19]. In the current study, the results for episodic memory add to emerging evidence that folate may have differential relationships with specific domains of cognition. This may be due to its role in hippocampal neurogenesis, as shown in animal models [39, 40]. The hippocampus is among the most important structures in the brain involved in memory [41] and is critical in the pathogenesis of diseases such as Alzheimer's and dementia. High concentrations of HCY have been associated with hippocampal atrophy [42]. Adequate folate is essential for balanced HCY levels and normal protein methylation processes in the brain, needed for cell signaling pathways, therefore it is conceivable that B-vitamin-related memory deficits could be due to changes of hippocampal neuronal processes linked to encoding information in episodic memory. Our results also suggest that there may be a continuum of folate levels related to performance even within the 'low' and 'low-normal' ranges established in literature [26]. Over eight years, low folate was associated with declining MMSE, a routine screening test often regarded as a crude indicator of cognitive performance. While MMSE was a key test in many studies assessing B vitamins and cognitive function, we included additional tests to add granularity to our study and to explore subtle changes across separate domains of cognition. Using folate quintiles, a modest insufficiency at baseline predicted a decline in episodic memory, suggesting it may be subtle enough to predict deleterious effects among more sensitive cognitive tests.

Evidence from randomized controlled trials (RCTs) using FA and other B vitamins to lower HCY have shown no consistent benefit of supplementation over approximately five years on cognitive outcomes. FA supplementation was associated with improved domain-specific cognitive performance in RCTs with relatively large samples and ≥ 2 years follow-up [43–45]. Another trial examined individuals with high HCY to exclude causes other than low folate concentrations and FA supplementation (0.8 mg oral FA per day) was associated with improved memory, processing speed and sensorimotor speed after 3 years [44]. Furthermore, supplementation of daily oral 0.4 mg FA and 100 μ g B₁₂ promoted improvement in cognitive function after 2 years, particularly in immediate and delayed recall [45]. While FA supplementation (0.4 mg per day for 2 years) for individuals with MCI ($n = 180$) was associated with better performance in tests of both memory and attention [20]. However, a meta-analysis of 11 RCTs involving 37,485 individuals by Clarke et al. concluded that HCY-lowering by supplementation with B vitamins for 5 years had no significant effect on individual domains or global cognitive function [46]. Further, a recent systematic review and meta-analysis of 31 RCTs of B-vitamin supplementation in individuals with and without cognitive impairment also concluded that B vitamins did not generally improve cognitive performance or slow cognitive decline compared to placebo [47]. The interpretation of such findings should however consider the limitations of available data and lack of an obvious benefit of lowering HCY using B vitamins, rather than evidence of no effect. Inconsistencies among RCT results may be explained by chance, bias, power issues or possible methodological differences between the studies, including FA fortification and trial duration, thereby making it difficult to draw firm conclusions.

It is plausible that biological mechanisms other than hyperhomocysteinemia may underlie the associations between low folate and cognitive impairment, with proposed mechanisms including impaired methylation and mis-incorporation of uracil into DNA. The complex synergistic interactions between nutrients are also important to consider. Vitamins and nutrients often function as a collection of co-factors, therefore interventions using singular or closely-related compounds may have too-narrow a focus. This is illustrated by another trial using FA, B₆ and B₁₂, showing treatment was effective only in those with high baseline omega-3 (n-3) fatty acid concentrations. In fact, n-3 fatty acid status was protective against brain atrophy only in the presence of B-vitamin supplementation, suggesting that both are needed for effectiveness [48].

The major dietary source of folate is green leafy vegetables and lower incidence of Alzheimer's disease or dementia has been associated with higher dietary folate intake [49]. Thus, even if low folate status is a surrogate for a less healthy diet and consequently is associated with poorer cognitive function, it is important nonetheless to consider what it tells us about individuals with lower folate levels. The present study shows that those with lower baseline folate were more likely to be male, smokers and obese, to have lower mean B₁₂ and vitamin D levels, but also had higher creatinine levels. They were less likely to use a FA supplement, which may be an indicator of lower socioeconomic status. While it is possible that those taking dietary supplements engage in healthier behaviors which may positively impact cognitive performance, FA-containing supplement use was low, ranging from 3 to 6% in the whole sample (Table 3).

Limitations were present. The analysis was limited to the use of non-fasting plasma folate and tHcy and B₁₂-biomarkers such as methyl-malonic acid and holotranscobalamin were not measured. The B-vitamin analysis was conducted at one time point, thus there is a possibility of regression dilution. Longitudinal studies are subject to attrition, therefore we cannot rule out selection bias leading to under- or over-estimation of observed associations. This, and the likelihood

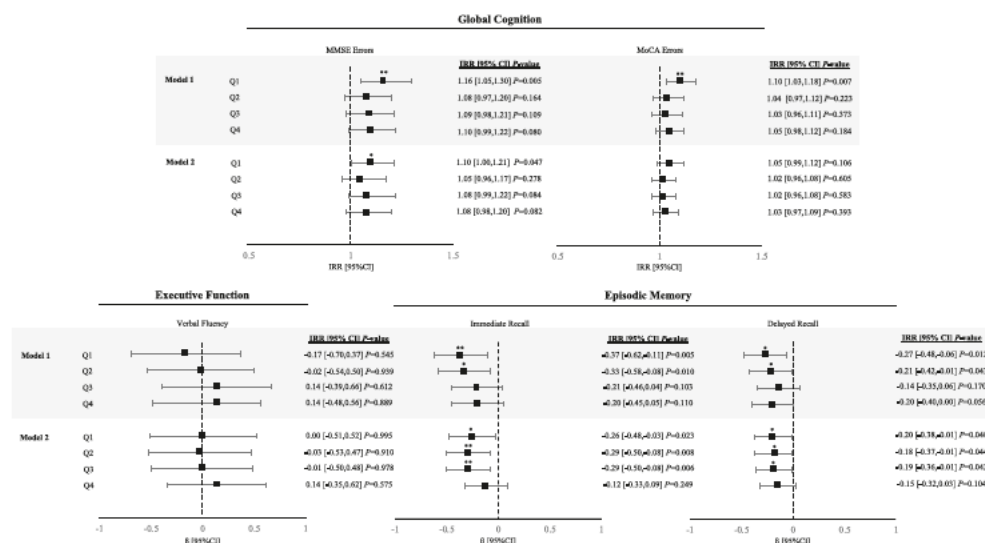


Fig. 1 Univariate and multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, verbal fluency, immediate recall, and delayed recall). Reference category: Q5 Quintile 5 (Plasma folate 32.6–81.3 nmol/L). Q1 Quintile 1 (Plasma folate 0.9–11.2 nmol/L); Q2 Quintile 2 (Plasma folate 11.2–15.6 nmol/L); Q3 Quintile 3 (Plasma folate 15.7–21.8 nmol/L); Q4 Quintile 4 (Plasma folate 21.8–32.5 nmol/L). ^aIRR Incident Rate Ratio. ^bβ Beta Coefficient. $^{**}P < 0.01$; $^{*}P < 0.05$. Model 1 = Basic model. Longitudinal cognitive variable and quintile of baseline folate controlling for time (wave). Model 2 = Sociodemographic, Health Indicators, Lifestyle, and Medical model. Model 1+ additional adjustment for age, age², sex, educational attainment, baseline vitamin B₁₂ status (< or ≥258 pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, antihypertensive medications.

that cognitive decline commenced earlier than age 65, may explain the lack of associations observed in the subsample aged ≥65 years. While our analysis controlled for a wide range of known covariates it is impossible to eliminate the risk of residual confounding. The factors controlled for in the statistical analyses were chosen a priori to be consistent with previous publications using folate and cognitive function in this population [50]. Finally, the sample consisted of high-functioning, largely healthy and younger community-dwelling participants and may not represent the older population living in residential care.

Our study has several strengths. The five cognitive tests examined encompassed three domains, global cognition, semantic and episodic memory. The longitudinal design included multiple assessments over 8 years, objective assessments of vitamin biomarkers using gold standard methods and detailed phenotypic data pertaining to sociodemographic and lifestyle factors, diseases and medication use in a large, well-characterized sample from one population. The use of mixed effects models in addition to the consideration of relevant covariates and interactions in the analyses, adds to the rigor of the current study. This study was performed in Ireland, a country that does not have MFAF, and we consider this to be a strength. With a high prevalence of folate insufficiency observed in the population, it allows reliable assessment of the associations with cognitive function over time.

In summary, we have shown that a decline in global cognitive performance and episodic memory among older adults that were cognitively normal can be predicted by low baseline folate levels and therefore it may be an important indicator for risk of early decline. This could have implications for clinical and public health policy recommendations for prevention of cognitive decline in older people.

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AUTHOR CONTRIBUTIONS

DMAO'C, CAMcG, RAK: Conception of research and study design. DMAO'C, SS, CDL, CAMcG: Analysis and interpretation of data. DMAO'C, SS, CAMcG: Preparation of paper. DMAO'C, SS, CDL, AMO'H, EL, AMM, RC, CAMcG, RAK: Critical revision of paper. DMAO'C, CAMcG, RAK: Final approval for publication.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Plasma concentrations of vitamin B12 and folate and global cognitive function in an older population: cross-sectional findings from The Irish Longitudinal Study on Ageing (TILDA)

Author:
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Appendix 11.3. Food Frequency Questionnaire

<https://tilda.tcd.ie/data/documentation/doc/wave3/Wave%203%20Self-Completion%20Questionnaire.pdf>

31. THINKING ABOUT THE FOOD THAT YOU EAT, WE WOULD LIKE YOU TO TELL US HOW OFTEN YOU USUALLY EAT THE FOLLOWING FOODS.

FOR EACH FOOD THERE IS AN AMOUNT SHOWN, EITHER WHAT WE THINK IS A "MEDIUM SERVING" OR A COMMON HOUSEHOLD UNIT SUCH AS A SLICE OR TEASPOON. PLEASE PUT A TICK IN THE BOX TO INDICATE HOW OFTEN, ON AVERAGE, YOU HAVE EATEN THE SPECIFIED AMOUNT OF EACH FOOD (TO THE NEAREST WHOLE NUMBER) DURING THE PAST YEAR, I.E. FROM WHEN YOU RECEIVE THIS QUESTIONNAIRE TO THE SAME MONTH THE PREVIOUS YEAR.

Examples:

The following are examples on how to estimate how often and how much bread and potatoes you ate over the past year. Please estimate your food intake for all foodstuffs in the same way.

Potatoes: If you ate a medium serving of potatoes 3 times per week over the past year, put a tick in the box "2-4 per week". If you think you usually ate more or less than a medium serving, please try to estimate which box suits best.

EXAMPLE 1:

AVERAGE USE LAST YEAR

Potatoes, Rice, Pasta (medium serving)	Never/ less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Potatoes, including boiled, mashed, baked potatoes, but excluding roast potatoes, chips or potato products (e.g. waffles)	☐	☐	☐	☒	☐	☐	☐	☐	☐

For white bread a medium serving is one medium-sized slice. Therefore if you usually ate 1 medium slice 4 or 5 times per day, then you should put a tick in the column headed "4-5 per day". If you ate 2 medium slices 4-5 times per day, then you should put a tick in the column "6+ per day".

EXAMPLE 2:

AVERAGE USE LAST YEAR

Cereals and Breads (one bowl or one slice)	Never/ less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread	☐	☐	☐	☐	☐	☐	☐	☒	☐

PLEASE ESTIMATE YOUR AVERAGE FOOD USE AS BEST YOU CAN. PLEASE ANSWER EVERY QUESTION, DO NOT LEAVE ANY LINES BLANK.

	Never/less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Meat and meat alternatives (medium serving)									
Beef or Lamb-including roast, steak stew, mince	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pork-including roast, chops, slices	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ham, Bacon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken or Turkey portion -including breast, thigh, leg	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken products- including chicken nuggets or breaded chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish, including breaded, battered, or fish fingers	☐	☐	☐	☐	☐	☐	☐	☐	☐
Processed meat - including meat pies, pasties, sausage rolls, burgers, sausages,	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lentils, tofu, soya meat, vegeburger	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cereals and Breads (one bowl or one slice)									
White bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brown bread	☐	☐	☐	☐	☐	☐	☐	☐	☐
Porridge, readybrek	☐	☐	☐	☐	☐	☐	☐	☐	☐
High fibre cereal e.g Weetabix, all bran branflakes, bran buds, muesli	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other cereal e.g. cornflakes, rice crispies	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Never/less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Potatoes, Rice, Pasta (medium serving)									
Potatoes, including boiled, mashed, baked potatoes, but excluding roast potatoes, chips or potato products eg waffles	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chips, roast potatoes, and potato products, eg potato waffles, smiles	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dairy Products and Fats									
Yoghurt (carton)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese-including cheddar, cheese slices, soft cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eggs (one) including boiled, scrambled, poached, fried	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream (tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salad dressings (tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Butter (teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low fat spread (teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cholesterol lowering spread e.g. benecol, flora pro active	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit and Vegetables									
Fruit including fresh, frozen, dried, tinned	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green vegetables, including cabbage, broccoli, peas, green beans	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Never/less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Orange/Yellow vegetables, including carrots, turnips, cauliflower	☐	☐	☐	☐	☐	☐	☐	☐	☐

Salad or other vegetables, including leeks, onions, garlic, sweet peppers, mushrooms, sweetcorn, tomatoes, beetroot	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sweets and snacks

Plain biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐

Chocolate Biscuits, including wrapped chocolate biscuits, eg Twix, Kit-Kat, Penguin	☐	☐	☐	☐	☐	☐	☐	☐	☐

Confectionary, including sweets and chocolate bars	☐	☐	☐	☐	☐	☐	☐	☐	☐

Cakes, buns, desserts, eg cheesecakes, apple tart	☐	☐	☐	☐	☐	☐	☐	☐	☐

Savoury snacks, eg crisps, tortilla chips	☐	☐	☐	☐	☐	☐	☐	☐	☐

Soups, sauces, spreads

Vegetable soup (homemade/carton)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Vegetable soup (packet, cup-a-soup)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sauces e.g. white sauce, cheese sauce, gravy (tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Marmite, Bovril	☐	☐	☐	☐	☐	☐	☐	☐	☐

Jam, marmalade	☐	☐	☐	☐	☐	☐	☐	☐	☐

Drinks

Water (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Never/less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cocoa, hot chocolate (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Horlicks, Ovaltine (cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wine (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer (half pint)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spirits (single measure)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low Calorie or Diet Fizzy drinks (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fizzy drinks (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pure fruit juice (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit squash, diluted orange (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐

32. WHAT TYPE OF MILK DO YOU USE MOST OFTEN?

PLEASE TICK ONE BOX

- ☐ None
- ☐ Whole/full fat
- ☐ Low fat
- ☐ Skimmed
- ☐ Super/fortified
- ☐ Soya
- ☐ Other

IF 'NONE' GO TO QUESTION 34

33. HOW MUCH MILK DO YOU USE EACH DAY?

PLEASE TICK ONE BOX

- ☐ Less than half a pint
- ☐ 250ml (half pint)
- ☐ 568ml (1 pint)
- ☐ One litre
- ☐ More than one litre