B-vitamins and the ageing brain: human studies incorporating novel technologies

Katie Patricia Moore

BSc. (Hons) Dietetics

Research conducted within

The Nutrition Innovation Centre for Food and Health (NICHE)

Faculty of Life and Health Sciences

of Ulster University

A thesis submitted for the Degree of Doctor of Philosophy

May 2018

I confirm that the word count of this thesis is less than 100,000 words

For those who never gave up

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Acknowledgements

I feel lucky to have been a member of the folate research group within NICHE. Sincere thanks to my supervisors Prof Helene McNulty, Dr Catherine Hughes, Prof Mary Ward and Dr Leane Hoey. Your constant encouragement and patience made this initially challenging task possible for me. I have learnt so much from you all and I really appreciate your kind guidance throughout the PhD. Helene, your energy and passion for our work is contagious, and your generosity in sharing your knowledge of nutrition and research is boundless. Despite what only could be described as an unbelievable workload, you manage to fit in time for intriguing scientific discussions as well as supporting us in attending and presenting at conferences around the world-thank you. Catherine, thanks for your guidance throughout the PhD and with all aspects of the TUDA cohort. Thanks for your constant patience and caring support with statistics, presentations and writing tasks. Mary, from first meeting you at the University open day, right through the past 9 years, you nurtured my interest in research and provided vital advice and support in relation to dietetics and my career in general-thank you. Leane, for the millions of cognitive and dietary questions that you so patiently answered-thank you. I would also like to thank Prof Kristina Pentivea for sharing her wealth of knowledge and experience in relation to the wider area of research, and in particular, about vitamin B6. I want to thank Prof JJ Strain for his support and expert feedback on the abstracts, posters, presentations and papers. I would also like to thank Dr Leanne Doherty, who's kindness got me over the line when I needed it most-thank you! To all the team in NICHE, the friendly caring supportive working atmosphere has been so lovely to be a part of. Thanks for the all the opportunities presented to me, including being introduced to other collaborators from across the world as we climbed up mountains, attended wine receptions or swam in hot springs!

To my fellow PhD students along the way, Dr Emma McAuley, Dr Emma Cuddy and Dr Conal Strain, your advice in the first years was very helpful-thank you. Thanks to my partner in crime and best buddy, Dr Kirsty Porter, who guided and supported me from day one in every aspect of my PhD and remains to this day a close friend. Sincere thanks my FASEB roomie Dr Amy McMahon, your support, advice and laughs, even from across the sea, are much appreciated. Thanks to Dr William Crowe for sharing your scientific (and general knowledge) throughout the PhD. A massive thank you to Marie Conway, Emma O'Sullivan, Aoife Caffrey, Martina Rooney, Elina Psara, Harry Jarrett and Ben Parmenter. Having fellow supportive PhD student friends to discuss nutrition, stats, recruitment, successes and failures, all the ups and downs throughout the PhD adventure has been immensely important to me, thank you and best wishes to you all!

To all the undergraduate, Master, placement and international students that have contributed so much to our work: Aoife O'Reilly, Hayley Dignam, Hazel Fleming, Maeve O'Shea, Abigail Carthy-Walsh, Una McNaughton Aimee Kerr, Hannah Doherty, Hayley Edwards, Rachel Kennedy, Hazel Hackett, Caroline McMinn, Jordan Hardyway. Rachel Tinney, Sinead McShane, Juliette Gambaretti, Andreanne Venot, Fabrico Caoimhe Murray, Holly Neill.

The team in stores- Robert, Davey and Lisa. Your support (including moral) throughout the orders and deliveries during the PhD is really appreciated. To Alison Deehan, Liadhan McAnena, Velma Hayes, Ciara Bannon, Danny Coulter, Barry Hyland and Neil Dennison, your technical support was vital to me, thank you for all your knowledge, patience and kindness. Thanks to John Liggit and Liam McAteer for sorting the never ending IT challenges that were faced throughout the PhD, your help was so much appreciated. To Russell and all the guys in the mail room, for helping to send over 25,000 letters to our participants, no matter how many envelopes I was posting, you always met

me with a smile and helping hand -thank you! To the cleaners and security in UU, your bright and cheery attitudes made even the coldest wettest darkest day enjoyable!

Many thanks to Prof Anne Molly, Dr Eamon Laird and Tracey Claxton in the TCD B-vitamin Lab at St James Hospital. To say that I learnt at lot while working there would be an understatement. Many thanks to Dr Eamon Laird, your help, encouragement and support, not only in Dublin, but also at conferences, has been priceless. The team in CTRIC (Patricia Moran, Geraldine Horigan, Marianna Sweeney, Vanessa Devine, Michelle, Tracey and the nursing team) and the teams in the blood laboratories in Altnagelvin and Causeway hospital's, thanks to you all for your kindness and for being so accommodating with our project. Dr Ruth Price and Dr Julie Sittlington, from training me in phlebotomy, to supporting me with TUDA, ethics, supplements and crisis management, sincere thanks for your kindness and moral support. Sincere thanks to Prof Prasad and Promod Gaur for all your time, help and patience, in setting up and running the MEG component of this study. Many thanks also to Prof Maestú and Noelia from LNCyC in Madrid for their expertise and guidance in running a MEG study.

To all the TUDA participants. You have made this PhD for me. Thank you for helping us to answer our many question about the roles of B-vitamins. Your willingness to help and your readiness to do whatever it takes to help find ways of preventing chronic conditions for future generations, your diligence, kindness and care during the PhD is inspiring. I have truly learnt so much from you all- thank you.

To my friends from home, and from far and near, thank you for your patience, understanding and perseverance over the past few years, it was so important to me. To Phil, Joe, Alice, Philomena, Rob and John McAuley, all the McCann ladies and the McAuley men, your interest, support and enthusiasm has meant a lot to me. To all my aunts, uncles, god mother, god father and cousins on the Moore and Murphy side, thanks for your love and support throughout, in particular in the last few years.

Thanks to my mam and dad, you have brought me a long way from “playing fishes”. You have taught us the importance of hard work, perseverance and love. I can’t thank you enough for your incredible patience, love, encouragement and essential pep talks…keep the faith. No matter what adversities I have ever encountered, you have always supported me through them, from labelling supplements, proofing reports and servicing my car, despite the distance, you were never far, always on hand to provide support, advice, laughs, hugs and belief. You have been there every step of the way and are the biggest part of any of my successes- thank you! Carmel, you have always been my leader, your generosity, kindness and work ethic is second to none, not far behind is your intelligence, which is inspirational and has been very useful! Your calls, answers, honest advice, postal surprises and sense of humour have been precious-thank you. Ruth, despite being my younger sister, your strength and knowledge has assisted me greatly, furthermore, your fashion advice, energy and general love of life has really helped me put things into perspective and kept me focused, reminding me along the way that I still had to “..write that red book”- go raibh míle maith agat! Andrew, my little big brother, as I’m sure the rest of the Moore’s will all agree, you never have had to do much to brighten up my day or put me into fits of laughter- thank you. Louis, for all your love, support and encouragement, my heartfelt thanks. Thank you for your patience, courage, persistence, energy, phone calls and in particular, the education in farming! Despite the distance and our busy work lives, you have made the journey exciting and fun, we can now look forward to continuing our adventure in the Nanny Valley.

To those in heaven watching over me-thank you.

Summary

By 2050 the number of people aged 60 years and over is projected to reach 2 billion, of which an estimated 131 million will have dementia, therefore there is an urgent need to identify public health strategies aimed at promoting better brain health in our ageing populations. A review of the literature within this thesis identified certain dietary patterns, as well as specific nutrients, which could be beneficial in preventing or delaying the onset of brain disorders, with the strongest evidence supporting roles for folate and metabolically related B-vitamins. The biological mechanism whereby these B-vitamins are linked with the brain is likely related to their crucial roles as co-factors in one-carbon metabolism. The overall aim of the original studies in this thesis was therefore to investigate the impact of folate-related B-vitamins on brain health in older adults recruited to the Trinity, Ulster and Department of Agriculture (TUDA) cohort study. Results from new analysis of existing data from the TUDA cohort (n = 5071) in relation to mental health showed that lower biomarker status of folate (<1520nmo/L), vitamin B6 (<51.9nmol/L) or riboflavin (EGRac ≥1.46) were each associated with an increased risk of depression (by up to 80%), while an increased risk of anxiety was found in TUDA participants with a deficient status of vitamins B6 (but not any other B-vitamin). Daily intake of fortified foods was associated with improved B-vitamin biomarker status and a 50% reduction in the risk of depression. A new investigation of the TUDA cohort, the BrainHOP trial (n = 328), identified a protective effect of supplementation with combined folic acid, vitamin B12, vitamin B6 and riboflavin on visuospatial cognitive function. In a pilot study, involving a subset of the BrainHOP cohort (n = 25) who were examined post-intervention using magnetoencephalography, the results suggested a protective effect of B-vitamins on neuronal function. In conclusion, the findings of this thesis have identified the potential importance of optimising B-vitamin status as a means to help achieve better brain health in older people, which in turn could have a significant impact on public health and associated costs in ageing populations.

Keywords

B-vitamins; Brain Health; Ageing; Cognition; Depression; Mental health; Nutrition; Folate; Folic acid; Vitamin B12; Vitamin B6; Riboflavin.

Abbreviations

AChE acetylcholinesterase inhibitors

AD Alzheimer's disease

ANCOVA Analysis of covariance

ATP Adenosine Triphosphate

BMI Body Mass Index

BP Blood Pressure

B-PROOF B-Vitamins for the Prevention of Osteoporotic Fractures

BrainHOP B-vitamins and Brain Health in Older People

CES-D Centre of Epidemiological Studies Depression Scale

CHAP The Chicago Health and Aging Study

CI Confidence interval

Cm centimetre

CT Computerized Tomography

CTRIC Clinical Translational Research and Innovation Centre.

CVD Cardiovascular disease

DNA Deoxyribonucleic acid

DHA Docosahexaenoic acid

DTI Diffusion Tensor Imaging

EAR Estimated Average Requirement

EEG Electroencephalography

EGRac Erythrocyte glutathione reductase activation

EPA eicosapentaenoic acid

FAB Frontal Assessment Battery

FAD Flavin dinucleotide

FD four-day food dairy

FDA Food and Drug Administration

FFQ Food Frequency Questionnaire

FMN Flavin mononucleotide

fMRI Functional MRI

GPs General Practitioners

GI Glycaemic index glycaemic load

GL Glycaemic load

GPCOG General Practitioner Assessment of Cognition

HADS Hospital Anxiety and Depression Scale

HbA1c glycosylated haemoglobin

holoTC Holo-transcobalamin

Hz hertz

IADL Instrumental Activities of Daily Living

ILSI International Life Sciences Institute

IU International Units

LNCyC Laboratory of Cognitive and Computational Neuroscience

LRNI Lower Reference Nutrient Intake

ISRC Intelligent Systems Research Centre

Kg kilogram

Kg/m2 Kilograms pre meter squared

m2 meters squared

MCI Mild Cognitive Impairment

MEG magnetoencephalography

Mg milligram

MMSE Mini-Mental State Examination

MTHF Methylenetetrahydrofolate

MTHFR Methylenetetrahydrofolate reductase

MRI Magnetic resonance imaging

NAD Nicotinamide adenine dinucleotide

NADP Nicotinamide adenine dinucleotide phosphate

NANS National Adult Nutrition Survey

NDNS National Diet and Nutrition Survey

NI Northern Ireland

NICE National Institute of Health and Clinical Excellence

NICHE Nutrition Innovation Centre for Food and Health

NTDs neural tube defects

NuAge Quebec longitudinal study on nutrition and Aging

OR Odds ratio

ORECNI Office of Research and Ethics Committee Northern Ireland

PET Positron Emission Tomography Imaging

PPO Pyridoxine-phosphate oxidase

PLP plasma pyridoxal phosphate

PUFA polyunsaturated fatty acids

RBANS Repeatable battery for the assessment of neuropsychological status

RBC Red blood cell

RCT Randomised Controlled Trials

RDA Recommended Dietary Allowance

RNA Ribonucleic Acid

RoI Republic of Ireland

RR Relative Risk

SACN Scientific Advisory Committee on Nutrition

SAM S-adenosyl-methionine

SBP Systolic blood pressure

SD Standard deviation

SOP Standard operating procedure

SPECT Single-Photon Emission Computerised Tomography Imaging

PSMS Physical Self-Maintenance Scale

SPSS Statistical Package for the Social Sciences

SQUIDs Superconducting Quantum Interference Devices

THF Tetrahydrofolate

TIA Transient Ischemic Attack

TUDA Trinity, Ulster and Department of Agriculture

TUG Timed Up-and-Go

UCC University College Cork

UCD University College Dublin

UU Ulster University

UK United Kingdom

USA United States of America

WC Waist circumference

WHO World Health Organisation

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Statement of Collaboration

The research relating to the original Trinity, Ulster and Department of Agriculture (TUDA) Ageing Cohort Study (**Chapter 3**) was conducted within the Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, Clinical Translational Research and Innovation Centre (CTRIC), Altnagelvin Area Hospital and St James' Hospital, Dublin, in collaboration with Professor John Scott and Professor Anne Molloy, Trinity College Dublin. This project was conducted prior to commencement of the authors PhD by the TUDA team. On commencement of the authors PhD, the author contributed the following work: assisted in the management of the data, managing and transporting of blood samples, all statistical analysis relating to B-vitamins and depression and anxiety and completion of the related manuscripts (Chapter 3).

The research relating to the intervention trial (BrainHOP) (Chapter 4) was conducted within NICHE and CTRIC. The Magnetoencephalography pilot study (Chapters 5) was conducted within the Intelligent Systems Research Centre (ISRC), Ulster University Magee, in collaboration with Pramod Gaur and Professor Girijesh Prasad. The following work for the above chapters was carried out by the author: obtaining appropriate ethical approval of from ORECNI (Office of Research Ethics Committees Northern Ireland) and trust approval from the Western Health and Social Care Trust and Northern Health and Social Care Trust in Northern Ireland (under the guidance of Dr Ruth Price, Clinical Trials Manager) and ordering and organising the analysis of the combined B-vitamins and placebo. Furthermore the author was responsible for participant screening, recruitment and sampling, general study management and execution, collection and analysis of data, anthropometric and blood pressure measurements. The author also conducted the cognitive tests, blood sample collection and processing, provision of supplements to participants, monitoring compliance via capsule counting and telephone calls, data entry, statistical analysis and preparation of scientific papers and abstracts. K. Porter, L. Doherty

and C. Hughes assisted with the recruitment, blood sampling and data collection. L. Hoey assisted with the coding of the RBANS data. P. Gaur and H. Jarrett assisted with MEG appointments. F. Maestú and colleagues in Madrid provided advice and expertise in relation to the MEG analysis.

H. McNulty, C.F. Hughes, M. Ward and L. Hoey provided overall study supervision and contributed to the final thesis.

Signed …….

Katie Patricia Moore

Chapter 1

General Introduction

Burden of declining brain health in ageing

Populations around the world are ageing rapidly, with estimates that one fifth of the world's population will be 60 years or over by 2050 (United Nations Department of Economic and Social Affairs/Population Division 2015). An estimated 23% of the global burden of disease arises in older people (Prince *et al.* 2015), of which dementia and depression are reported as the leading causes (World Health Organisation 2016). Dementia currently affects 46.8 million people worldwide and is projected to affect over 131 million people by 2050 (Prince *et al.* 2016), whilst depression is anticipated to be the second leading cause of disability worldwide by 2020 (National Collaborating Centre for Mental Health 2010). The economic burden of cognitive decline and depression is profound. Experts have calculated that dementia is a trillion dollar disease (Prince *et al.* 2016). Figures for depression are currently estimated at £7.5 billion in England (National Collaborating Centre for Mental Health 2010) and over €3 billion in Ireland (including other metal health conditions such as schizophrenia, bipolar disorder) (O'Shea and Kennelly 2008). With no disease-modifying treatments for dementia (Frankish and Horton 2017) and poor response to antidepressant medication (Rush *et al.* 2006) health systems are in danger of becoming overwhelmed by the future cost of caring for people with dementia (Frankish and Horton 2017). Estimates suggest that even a delay in onset of 1 year could prevent more that 9 million cases of dementia by 2050 (Frankish and Horton 2017). There is an urgent need therefore to identify modifiable factors for targeted interventions to promote better brain health in our ageing populations.

Role of dietary patterns and nutrients in brain health in older adults

Epidemiological evidence supports a role for certain dietary components or dietary patterns in brain health, opening up new potential avenues for prevention of dementia and mental illness in ageing (Panza *et al.* 2008; Rechenberg 2016). Within this thesis and

other ageing research publications, older adults are considered to have healthy brain function when their cognitive assessments scores do not exceed the threshold limits for the testing regime being applied. For example those with a score ≥25 within the Mini Mental State Examination (MMSE; (Folstein *et al.* 1975), or >15 in the Frontal Assessment Battery (FAB; (Dubois *et al.* 2000) or >80 in the Repeatable Battery of Neuropsychological Assessment (RBANS; (Randolph 2010) are considered to have good cognitive health. Furthermore, depression scores that are <16 within the Centre for Epidemiological Studies Depression Scale (CES-D) (Radloff 1977), and anxiety scores that are <11 within the 7 item Hospital Anxiety and Depression scale (HAD) (Zigmond and Snaith 1983), are indicative of good mental health. These assessments are often used in isolation however they can be used with age-appropriate magnetic resonance imaging (MRI) brain templates, which have been gathered from healthy adults, to define a healthy brain (Fillmore *et al.* 2015). More recently however, the American Heart Association/American Stroke Association identified specific metrics to define optimal brain health in adults including ideal health behaviours (non-smoking, physical activity, healthy diet and normal body mass index) and ideal health factors (untreated blood pressure, untreated total cholesterol and fasting blood glucose) (Gorelick *et al.* 2017). The Mediterranean diet is receiving significant attention as regards its potential role in preserving cognitive health and protecting against depression in ageing (Wu and Sun 2017). There is also evidence supporting roles for specific nutrients including omega-3 polyunsaturated fatty acids (PUFAs), polyphenols, vitamin D and B-vitamins. Most studies to date in this area, however, focus on specific dietary patterns or components, making it difficult to appreciate the overall role of nutrition in brain health and disease. Therefore, a broad review of the role of diet and nutrition in the ageing brain seems timely in terms of future public health implications.

B-vitamins and the ageing brain

Folate and the metabolically related B-vitamins (vitamin B12 and vitamin B6) are nutrients which have received particular attention in relation to their role in protecting against depression and cognitive decline in ageing. The potential biological mechanisms explaining their relationship are not fully known, but most likely arise as a result of the crucial roles played by these B-vitamins in the one-carbon metabolic network. Tetrahydrofolate (THF) obtains a carbon unit from serine in the presence of B6 which results in the formation of 5,10 methylenetetrahydrofolate (MTHF). 5, 10 MTHF is catalysed to 5 MTHF by the enzyme methyltetrahydrofolate reductase (MTHFR) with flavin-adenine-dinucleotide (FAD) as a cofactor which riboflavin is the precursor. It is 5 MTHF that is the single methyl donor in the remethylation of homocysteine to methionine, which take place in the presence of B12 and is catalysed by the enzyme methionine synthase. The activation of methionine by adenosine triphosphate (ATP) results in the subsequent generation of S-adenosylmethionine, (SAM). SAM, the universal methyl donor, is involved in the methylation of DNA, phospholipids, proteins and neurotransmitters, thus reduced status of one or more of the B-vitamins involved in one-carbon metabolism may impair methylation processes (Selhub *et al.* 2000; Bottiglieri *et al.* 2000). The inhibition of methylation reactions may in turn influence cognitive impairment in ageing in various ways (Smith and Refsum 2016). Additionally, reduced tissue concentration of SAM may be linked to depression through perturbing monoamine (serotonin, dopamine and noradrenaline) synthesis and methylation (Bottiglieri *et al.* 2000).

Intake and status of B-vitamins in relation to depression and cognition in older age

Historically, clinical deficiencies of folate and vitamin B12 were associated with a range of neuropsychiatric symptoms, including depression (Carney 1967; Reynolds *et al.* 1970;

Shorvon *et al.* 1980), raising the possibility that optimising relevant B-vitamin intake and status could improve mental health. Research to date in this area has, however, focused predominantly on folate, and to a lesser extent vitamin B12 (Kim *et al.* 2008; Almeida *et al.* 2014; Almeida *et al.* 2015; Petridou *et al.* 2016) whereas related B-vitamins, i.e. vitamin B6 and riboflavin, also necessary for one-carbon metabolism, have received much less attention. Furthermore, there are important nutrient-nutrient interactions within this network of pathways, therefore, investigating the role of all four B-vitamins in depression is crucial.

Epidemiological studies have linked lower biomarker status of folate, vitamin B12 and vitamin B6 (or higher concentrations of the related metabolite homocysteine) with an increased risk of cognitive dysfunction (Smith and Refsum 2016; Porter *et al.* 2016) and depression (Hvas *et al.* 2004; Kim *et al.* 2008; Ng *et al.* 2009; Robinson *et al.* 2011). The evidence however from randomised trials, in terms of depression is weak, due to the small number of available trials (Almeida *et al.* 2015), and in the case of cognition, the evidence has not been consistent (McMahon *et al.* 2006; Durga *et al.* 2007). Furthermore numerous limitations in various trials have been highlighted including: too short in duration (Lewerin *et al.* 2005; Pathansali *et al.* 2006); inappropriate target populations (McMahon *et al.* 2006; Kwok *et al.* 2011; van der Zwaluw *et al.* 2014); too small a sample size for adequate statistical power (Pathansali *et al.* 2006). Despite these inconsistencies, the strongest evidence to date comes from the VITACOG trial where pharmacological doses of folic acid, vitamin B12 and vitamin B6 over 2-years were shown to protect cognitive function and reduce brain atrophy in older adults with Mild Cognitive Impairment (MCI) (Smith *et al.* 2010; de Jager *et al.* 2012; Douaud *et al.* 2013). Furthermore, a recent review concluded that research on dietary interventions with B-vitamins should be a high priority

(Kane RL *et al.* 2017). Therefore, further well-designed trials using a combined B-vitamin at doses within the dietary range are needed to further advance this area.

Assessing brain health and the role of novel technologies

Assessing and monitoring brain health in ageing is a complex task, thus investigating the influence of diet and/or specific nutrients on cognition or depression, present great challenges and should ideally be conducted using a multidisciplinary approach (Mak and Caldeira 2014). Most often assessments are conducted via questionnaire-based assessments (Macready *et al.* 2010), which can have limitations including inter and intra-rater variability and there is often limited evidence regarding their performance (Velayudhan *et al.* 2014). It is recommended that brain-imaging outcomes should be included as secondary outcomes to clinical and cognitive measures (de Jager and Kovatcheva 2010) in studies investigating the role of nutrition in brain health. The availability of neuro-imaging techniques has improved in recent years and they are increasingly being utilised in nutrition and brain health studies, potentially providing objective and highly robust means of assessing brain structure, function and response to nutrition (Sizonenko *et al.* 2013). Available studies using MRI and functional MRI have strengthened the evidence supporting beneficial effects of the Mediterranean Diet, vitamin D, B-vitamins and polyphenols on brain and brain blood volumes (Brickman *et al.* 2014; Hooshmand *et al.* 2014; Smith *et al.* 2015; Staubo *et al.* 2017). Magnetoencephalography (MEG) is a relatively new neuroimaging technique, which measures the magnetic fields that are generated by neuronal activity, and thus directly measures oscillatory (neural) activity. MEG is a completely non-invasive technique, has the highest temporal resolution compared to other imaging techniques and overall may be able to identify the earliest functional changes that may occur as a result of

neuropathological processes, but prior to the onset of neurological disorder dementia (Zamrini *et al.* 2011).

Public health implications

Maintaining brain health in older age is a significant public health priority. Within the current literature, there is strong evidence for the protective role of folate and the related B-vitamins in cognitive function, while there is less evidence in relation to their roles in depression. Furthermore, as older adults are at particular risk of B-vitamin deficiencies (ter Borg *et al.* 2015; Porter *et al.* 2016) the potential effect of B-vitamin fortified food consumption (which provides a highly bioavailable source of B-vitamins) (Hopkins *et al.* 2015) and B-vitamin supplement use should be explored in relation to depression and cognitive function in ageing.

Thesis Aims:

The overall aim of this thesis was to investigate the role of B-vitamins in brain health in ageing. In fulfilment of this aim, the objectives were to provide:

1. A comprehensive review exploring the influence of ageing on brain health and the emerging evidence linking diet and specific nutrients with cognitive function and depression in ageing, and to consider the use of novel imaging technologies in nutrition and brain research. (**Chapter 2**)
2. An investigation of folate, vitamin B12, vitamin B6 and riboflavin intake and status in relation to depression and anxiety in ageing, including the potential for fortified foods to optimise B-vitamin status and reduce the risk of these mental health disorders. (**Chapter 3**)
3. An investigation of the effect of B-vitamin supplementation with combined folic acid, vitamin B12, vitamin B6 and riboflavin over 2 years on cognitive function and depression in older adults. (**Chapter 4**)
4. An investigation of the role of B-vitamins in brain function in older adults using MEG. (**Chapter 5**)

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Chapter 2

Diet, nutrition and the ageing brain: current evidence and new directions’

Published in Proceedings of the Nutrition Society

Katie Moore, Catherine F. Hughes, Mary Ward, Leane Hoey, and Helene McNulty (2018) Diet, nutrition and the ageing brain: current evidence and new directions’, *Proceedings of the Nutrition Society doi:10.1017/S0029665117004177*

Abstract

Globally populations are ageing. By 2050, it is estimated that there will be 2 billion people aged 60 years or over, of which 131 million are projected to be affected by dementia, while depression is predicted to be the second leading cause of disability worldwide by 2020. Preventing or delaying the onset of these disorders should therefore be a public health priority. There is some evidence linking certain dietary patterns, particularly the Mediterranean diet, with a reduced risk of dementia and depression. Specific dietary components have also been investigated in relation to brain health, with emerging evidence supporting protective roles for omega-3 polyunsaturated fatty acids (PUFAs), polyphenols, vitamin D and B-vitamins. At this time, the totality of evidence is strongest in support of a role for folate and the metabolically related B-vitamins (vitamin B12, vitamin B6 and riboflavin) in slowing the progression of cognitive decline and possibly reducing the risk of depression in ageing. Future studies incorporating new technologies, such as magnetic resonance imaging and magnetoencephalography, offer much promise in identifying effective nutrition interventions that could reduce the risk of cognitive and mental disorders. This review will explore the ageing brain and the emerging evidence linking diet and specific nutrients with cognitive function and depression in ageing, with the potential to develop strategies that could improve quality of life in our ageing population.

Keywords: Nutrition; Cognition; Depression; Ageing; B-vitamins

Introduction

Globally the population is ageing, with predictions that the number of people aged 60 years and over will reach up to 2 billion by 2050. An estimated 23% of the global burden of disease arises in older people (≥60 years) and mental disorders are reported as the leading cause of disability and ill health (Prince *et al.* 2015). Dementia and depression are the most common of these disorders in ageing as identified by the WHO (World Health Organisation 2016). Cognitive function declines with age, ranging in severity from Mild Cognitive Impairment (MCI) to dementia, with up to 50% of those with MCI going on to develop dementia within 5 years (Gauthier *et al.* 2006). MCI can be defined as "cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life" (Gauthier *et al.* 2006), whereas dementia interferes with activities of daily living (World Health Organisation 2015). Dementia currently affects 46.8 million people worldwide and is projected to affect over 131 million people by 2050 (Prince *et al.* 2016), whilst depression is anticipated to be the second leading cause of disability worldwide by 2020 (National Collaborating Centre for Mental Health 2010), with 22% of males and 28% of females over the age of 65 years affected by depression (Craig *et al.* 2007). The economic burden of cognitive decline and depression is profound. Experts have calculated that dementia will be a trillion dollar disease by 2018 (Prince *et al.* 2016). Figures for depression are currently estimated at over €3 billion in Ireland (O'Shea and Kennelly 2008) and £7.5 billion in England (National Collaborating Centre for Mental Health 2010). With mental health considered to be one of the greatest global challenges (Livingston *et al.* 2017), there is an urgent need to identify modifiable factors (well recognised examples include early life education, hypertension, obesity, hearing loss, smoking, depression, physical inactivity, social isolation and diabetes) for targeted interventions to promote better brain health in our ageing populations. Epidemiological evidence supports a role for certain dietary factors

in brain health, opening up new potential avenues for prevention of dementia and mental illness in ageing (Panza *et al.* 2008; Rechenberg 2016).

This review will explore the influence of ageing on brain health and the emerging evidence linking diet and specific nutrients with cognitive function and depression in ageing. The use of novel imaging technologies in nutrition and brain research will be discussed, along with the potential for nutrition to play a protective role in preserving better brain health in ageing.

1. The Ageing Brain

a) Physiology and pathophysiology

The structure and metabolic pathways within a healthy brain are progressively altered with ageing, though the precise aetiologies of ageing have not been fully elucidated. As people age, there is a reduction in brain volume in both grey and white matter (Resnick *et al.* 2003), while white matter lesions increase (Peters 2006) and there is development of amyloid plaques, neurofibrillary tangles, Lewy bodies, synaptic dystrophy and neuron loss (Svennerholm *et al.* 1997; Elobeid *et al.* 2016), which have been suggested to parallel the progression of cognitive decline (Serrano-Pozo *et al.* 2011). There are also changes in the production of neurotransmitters – in particular serotonin and dopamine – which have been reported to decline by up to 10% per decade from early adulthood (Peters 2006). Additionally, there is an increase in oxidative stress response (Bishop *et al.* 2010) and more dysfunction of the blood brain barrier (Goodall *et al.* 2017). The blood brain barrier (BBB) protects the brain, by regulating the flux of nutrients and metabolites from the blood to the brain using six major transport pathways (Campos-Bedolla *et al.* 2014). These pathways control the influx of nutrients such as glucose, amino acids and specific vitamins and minerals from the blood into the brain (Camandola and Mattson 2017).

Within normal ageing, the permeability of the BBB increases and is further increased with dementia where increased permeability is suggested to be involved in the development neurodegenerative diseases, specifically those related to the cerebral vasculature (Farrall and Wardlaw 2009). Interestingly however, specific nutrients and natural compounds including omega three fatty acids, folate and plant polyphenols, have been suggested to have protective roles in maintaining BBB function in ageing (Campos-Bedolla *et al.* 2014).

Normal ageing is associated with a decline in cognitive function, with most cognitive change observed in memory during the ageing process. MCI is a recognised clinical condition where individuals have evidence of cognitive impairment but do not meet the criteria for the diagnosis of dementia (Winblad *et al.* 2004). Alzheimer's disease (AD) is the most common form of dementia, accounting for 62% of cases, with other forms including vascular dementia, mixed, Lewy body, dementia of Parkinson's disease and frontotemporal dementia (Prince *et al.* 2014). Depression in older adults (≥70 years) is often referred to as 'late–life depression' and is reported more commonly in females than males (Luppa *et al.* 2012; Büchtemann *et al.* 2012; Polyakova *et al.* 2014). The depressive symptoms of older adults (≥70 years) are thought to be different from those experienced by younger adults, as somatic and psychological symptoms are often accompanied by fatigue, hopelessness about the future, loss of appetite and sleep disturbance (Luppa *et al.* 2012).

b) Pharmaceutical treatments

Pharmacological treatment for dementia is prescribed by specialist clinicians (National Institute for Health and Care Excellence 2017b), but only a limited number of medications that target the biochemical abnormalities of neuronal loss are included within the National

Institute for Health and Care Excellence (NICE) recommendations for dementia interventions. These include acetylcholinesterase (AChE) inhibitors (donepezil, galantamine, rivastigmine) and memantine (N-methyl-D-aspartate receptor antagonists). There are however a variety of pharmacological treatment options available for depression including tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin and noradrenaline reuptake inhibitors and selective noradrenaline reuptake inhibitors (Mann 2005; Rang *et al.* 2016). Overall, poor response rates to these costly pharmacological treatments for depression have been observed (Rush *et al.* 2006; Ilyas and Moncrieff 2012), and despite significant investigation into the role of pharmacological treatments for dementia, no licenced medication can cure these diseases of the brain. Therefore, much efforts are currently focusing on options for prevention rather than treatment of brain disorders.

2. Assessment of Brain Function

The assessment of brain function for neurodegenerative diseases and depressive disorders in ageing is a developing area. There are numerous neurological tests available which are designed to assess and distinguish different individuals in their response to day-to-day cognitive tasks (de Jager *et al.* 2014) and for the detection of common mental health disorders (Ali *et al.* 2016). NICE has provided guidance on the recommended diagnostic criteria for depression (National Institute for Health and Care Excellence 2017c) and dementia (National Institute for Health and Care Excellence 2017a). For dementia, the guidelines emphasise the need to assess the following domains; attention and concentration, orientation, short- and long-term memory praxis, language and executive function. Furthermore, NICE recommends that formal tests should be conducted, including the Mini Mental State Examination (MMSE), 6-Item Cognitive Impairment Test (6-CIT), General Practitioner Assessment of Cognition (GPCOG) and 7-Minute

Screen and that other factors known to influence performance such as education level, should also be taken into account. Lastly, only healthcare professionals with expertise in differential diagnosis and using international standardised criteria (such as the National Institute of Neurological Communicative Disorders) should be responsible for diagnosing subtypes of dementia (National Institute for Health and Care Excellence 2017a).

Investigating cognitive and mental health outcomes via questionnaire-based assessments is the most common approach for assessing the effects of nutrition (Macready *et al.* 2010). For assessing brain health and function in relation to nutritional factors, studies should be aimed at prevention rather than treatment, and non-nutrition factors contributing to cognitive impairment and depression should be incorporated into studies and considered at the time of analysis (de Jager and Kovatcheva 2010). Concerning the specific tests to assess cognitive function, these should be carefully selected and should be based on a known or hypothesized relationship of a specific food/nutrient with cognitive function and not solely on their availability or ease of administration. It is also important that the tests are suitable for repeated administration, are appropriate to the population being studied, and are relatively simple to interpret and administer. More work is required using standardised tests across laboratories so that the specific tests or markers that are most sensitive to the nutrients tested can be established (de Jager and Kovatcheva 2010; de Jager *et al.* 2014). Lastly, computerised cognitive assessments have been utilised and these should be considered for use in future trials in terms of their accuracy and ability to capture reaction-time data, standardisation of administration, availability of parallel versions of tasks for testing at multiple time-points, and availability in multiple languages (de Jager and Kovatcheva 2010).

3. Food, Nutrition and Brain Health in Ageing

a) Foods and dietary patterns

Increasing evidence implicates certain dietary patterns such as higher intake of fruits and vegetables (Kang *et al.* 2005) and fish (Barberger-Gateau *et al.* 2007) as being beneficial to brain health. The Mediterranean diet is receiving significant attention as regards its role in preserving cognitive health and protecting against depression in ageing. This diet is typically characterised by higher intakes of fruit, vegetables, wholegrains, fish, unsaturated fatty acids and a regular but moderate consumption of alcohol. A recent meta-analysis (*n* 34,168) showed that the highest Mediterranean diet score was associated with reduced incidence of developing cognitive disorders (RR 0.79 95% CI 0.70, 0.90) (Wu and Sun 2017) while supplementation of the Mediterranean diet with olive oil or nuts was associated with improved cognitive function (Valls-Pedret *et al.* 2015). Of note, studies using Magnetic Resonance Imaging (MRI) have shown that adherence to the Mediterranean diet was associated with larger cortical thickness (which in turn is associated with a lower risk of cognitive impairment) (Staubo *et al.* 2017). There is also accumulating evidence to support a potential role for the Mediterranean diet in preventing depression in older adults (≥50 years; Skarupski *et al.* 2013), with cross-sectional and prospective studies showing inverse associations between Mediterranean diet score and risk of depression (Psaltopoulou *et al.* 2013; Hodge *et al.* 2013; Skarupski *et al.* 2013; Rienks *et al.* 2013; Veronese *et al.* 2016). Further well-designed intervention studies are however required to more fully investigate the potential role of the Mediterranean diet as a means of helping to preserve better brain health in ageing.

b) Specific Nutrients

Protein and carbohydrates

The role of dietary protein intake on cognitive function or mental health has not been extensively studied in ageing populations. Lower verbal memory scores were however

observed in older people (>60 years) with lower dietary protein intakes (Goodwin *et al.* 1983). Additionally, higher dietary protein intake was found to be positively correlated with nonverbal learning, verbal memory and reduced risk of mild cognitive impairment or dementia (Koehler *et al.* 1997; Roberts *et al.* 2012). One randomised controlled trial (RCT) investigating the effects of dietary protein from red meat on cognitive function in older adults (≥65 years) is in progress (ACTRN12613001153707) with results expected in 2018 (Daly *et al.* 2015). Investigations have focused on the essential amino acid tryptophan in brain health in ageing as it is the sole precursor of the monoamine neurotransmitter serotonin and therefore a plausible mechanism exists. A recent review in the area concluded that lower serotonin levels cause cognitive impairment, in specific cognitive domains associated with memory and learning such as cortex, amygdala, and hippocampus (Jenkins *et al.* 2016). Furthermore, supplementation with tryptophan appears to have beneficial effects on attention and memory in human subjects.

The association between carbohydrates and cognitive function is unclear because available evidence is scarce, with one Cochrane review identifying only one relevant RCT in older adults (≥55 years) (Ooi *et al.* 2011; Power *et al.* 2015). However, higher dietary carbohydrate and sugar intakes were associated with lower cortical thickness, which is in turn associated with high risk of late life mild cognitive impairment and dementia (Staubo *et al.* 2017). Whilst more research has focused on carbohydrates and depression, the available evidence is somewhat conflicting. One study of community dwelling adults (mean age 74.0 years in those suffering from depression) and 76.1 years (those without depression), found that those with depressive symptoms consumed a diet with a higher glycaemic index (GI) and glycaemic load (GL) (Mwamburi *et al.* 2011). A prospective investigation also reported that a high GI diet was associated with an increased risk of depression (Gangwisch *et al.* 2015). Contrary to these findings, however, institutionalised

older adults (≥ 65 years) with depression were reported to consume diets with a lower GL (Aparicio *et al.* 2013). Given the inconsistencies in this area, there is clearly a need for further well-designed studies.

Omega 3 fatty acids

The membranes of the cells within the nervous system are made of phospholipid bilayers which are mainly composed of long-chain *n*-3 polyunsaturated fatty acids (PUFA). These are directly affected by diet and thus attention has focused on the role of dietary fatty acids in brain health. There is evidence that PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have potential benefits in cognitive and mental health (Gillette-Guyonnet *et al.* 2013; Grosso *et al.* 2014a). These PUFA lipid groups are postulated to positively influence brain health by helping to maintain neuronal cell membrane functioning, by preventing hypo-perfusion and by decreasing inflammation (Janssen and Kiliaan 2014). Furthermore, DHA itself has been suggested to be neuroprotective via its' ability to increase production of brain derived neurotrophic factor and also plays roles in improving synaptic membrane fluidity and antioxidant capabilities (Cole *et al.* 2010). One meta-analysis of 10 RCT studies concluded that *n*-3 fatty acids may have a protective effect on certain cognitive domains in cognitively impaired patients, however, no effects were seen in healthy people or in AD sufferers (Mazereeuw *et al.* 2012). A recent Cochrane review, which identified three RCTs for inclusion involving 632 patients with mild to moderate AD, concluded that there was no convincing evidence that PUFA had a role in the treatment of people with existing dementia (Burckhardt *et al.* 2016).

On the other hand, systematic reviews and meta-analyses of RCTs have reported significant clinical benefits of *n*-3 PUFA intervention in the treatment of depression. The

use of predominantly EPA compared with DHA supplementation appears to have greater efficacy (Grosso *et al.* 2014b; Hallahan *et al.* 2016). Furthermore, supplementation with EPA-predominant formulas as an adjuvant therapy to antidepressants was found to have greater clinical efficacy in the treatment of depression (compared to antidepressants alone), but did not prevent depressive symptoms among populations without a diagnosis of depression (Grosso *et al.* 2014b; Hallahan *et al.* 2016). A Cochrane review in this area reported a small to modest non-clinical beneficial effect of *n*-3 PUFA in depression symptomology, but concluded that there was not enough good quality evidence to determine the effect on depression (Appleton *et al.* 2015).

Polyphenols

Phenolic compounds have at least one aromatic ring with one or more hydroxyl groups attached and are classified as flavonoids and nonflavonoids (Del Rio *et al.* 2013). Polyphenols are the biggest group of phytochemicals, and many of them have been found in plant-based foods including nuts, berries, cocoa, red wine and tea beverages. They are structurally diverse compounds, with in excess of 8000 structures having been reported. The role of these polyphenols in brain health and ageing is an emerging area (Schaffer *et al.* 2012; Brickman *et al.* 2014; Ward and Pasinetti 2016). Large prospective studies have identified associations between the dietary intakes of total or specific polyphenols and cognitive function after up to 13 years of follow-up investigation (Letenneur *et al.* 2007; Kesse-Guyot *et al.* 2012; Rabassa *et al.* 2015). Supplementation with cocoa flavanol for periods of up to 2 months was reported to improve cognitive performance in a group of cognitively intact older adults with the mean age 70 years (Mastroiacovo *et al.* 2015). Of note, Brickman and colleagues (Brickman *et al.* 2014) conducted a 3-month intervention and showed significant increases in cerebral blood volume in the dentate gyrus as measured by functional MRI (fMRI) in subjects who were assigned to a high flavanol

treatment. Research into the role of polyphenols in depression in humans has been limited (Pase *et al.* 2013), though animal studies show promise in demonstrating antidepressant-like effects of polyphenols in mouse models (Zhu *et al.* 2012).

The exact mechanisms linking polyphenols with brain health are unknown, however a recent review presented three postulated mechanisms: firstly, polyphenols may protect synaptic plasticity *via* their actions on pathways involving extracellular signal-regulated kinases; secondly, their anti-inflammatory properties are also thought to decrease neuro inflammation and finally evidence from human studies suggests that they protect brain health via improving both peripheral and cerebrovascular blood flow (Vauzour 2017).

Vitamins

Specific vitamins have been investigated in relation to brain health and disease. Oxidative stress is thought to be a major contributor to neurodegeneration and depression (Bishop *et al.* 2010), thus antioxidants have received much interest. The roles of vitamin C (Luchsinger *et al.* 2003; Devore *et al.* 2010; Hamer *et al.* 2011; Payne *et al.* 2012), β-carotene (Engelhart *et al.* 2002; Tsuboi *et al.* 2004; Kim *et al.* 2015) and vitamin E (Morris *et al.* 2002; Owen *et al.* 2005; Banikazemi *et al.* 2015; Farina *et al.* 2017) have been explored, but no clear conclusions can be made and further work in the form of intervention studies is warranted. Epidemiological studies have shown that high plasma vitamin E concentrations are associated with better cognitive outcomes. The protective mechanisms associated with high plasma vitamin E concentrations are thought to go beyond their anti-oxidant functions, as they are also postulated to regulate signal transduction between neurons, decrease expression of the Amyloid Precursor Protein binding protein as well as regulating genes involved in the formation of neuronal cells (Fata *et al.* 2014). A recent Cochrane review concluded that there is no evidence that

intervention with vitamin E (in the form of alpha-tocopherol) prevents either progression of cognitive impairment to dementia or improves cognitive function in those with MCI; however evidence from a single study suggests that it may slow functional decline in Alzheimer's disease (Farina *et al.* 2017). The postulated roles of vitamin D and B-vitamins have been more fully investigated in relation to their effects on brain health in ageing.

Following the discovery of the vitamin D receptor in the brain (Eyles *et al.* 2005), evidence for the role of vitamin D in brain health has been accumulating. Systematic reviews and meta-analyses have shown that AD sufferers have lower serum vitamin D status than healthy controls, and that low serum 25-hydroxyvitamin D (25OHD status is associated with worse cognitive outcomes (van der Schaft *et al.* 2013; Annweiler *et al.* 2013a; Annweiler *et al.* 2013b). Recent longitudinal studies with mean follow-up periods of over 4 years found that lower 25OHD status was also associated with declining MMSE scores and accelerated cognitive decline (Toffanello *et al.* 2014; Miller *et al.* 2015). Furthermore, Hooshmand *et al*. used MRI to demonstrate that higher 25OHD status was associated with greater brain volumes (2014), which is generally regarded as a valid marker of disease state and progression. A U-shaped relationship between 25OHD concentrations and cognition has been suggested within a prospective cohort of adults (>70*), where both lowest and highest concentrations of 25OHD were associated with an increased risk of cognitive impairment (Granic *et al.* 2015). Research investigating the role of vitamin D in depression is much less clear. Large cross-sectional and prospective studies reported that lower serum 25OHD status was associated with an increased risk of depression (Williams *et al.* 2014; Brouwer-Brolsma *et al.* 2016). One detailed systematic review, which included cross sectional, prospective and RCT data, concluded that lower 25OHD status may be a risk factor for late life depression (Okereke and Singh 2016).

c) One-carbon metabolism and related B-vitamins

Historically, B-vitamin deficiencies, in particular folate (Carney 1967; Reynolds *et al.* 1970) and vitamin B12 (Strachan and Henderson 1965; Shorvon *et al.* 1980), and to a much lesser extent vitamin B6 (Carney *et al.* 1982a), have been linked with poorer psychiatric wellbeing. Furthermore, Vitamin B12 deficiency is most commonly associated with peripheral neuropathy (Reynolds 2006). These B-vitamins play crucial roles in one-carbon metabolic pathways where they act as co-factors in DNA synthesis and repair, amino acid metabolism and methylation reactions, including the remethylation of homocysteine (Hcy) to methionine and subsequent generation of S-adenosylmethionine (SAM). SAM, the universal methyl donor, is involved in the methylation of DNA, phospholipids, proteins and neurotransmitters, thus reduced status of one or more of the B-vitamins (**Table 1**) involved in one-carbon metabolism may impair this metabolic pathway, thus increasing Hcy and 5-methyltetrahydrofolate concentrations and disrupting methylation processes (Selhub *et al.* 2000; Bottiglieri *et al.* 2000). The inhibition of methylation reactions may in turn influence cognitive impairment in ageing in various ways (Smith and Refsum 2016), by perturbing the regulation of gene expression in the Beta amyloid pathway, by reducing the activity of protein phosphatase-2A or by impairing the formation of phosphatidylcholine enriched omega-3 fatty acids (Smith and Refsum 2016). Additionally, reduced tissue concentration of SAM may be linked to depression through perturbing monoamine (serotonin, dopamine and noradrenaline) synthesis and methylation (Bottiglieri *et al.* 2000). Apart from folate, vitamins B12 and vitamin B6, which have well-recognised roles in these pathways, riboflavin (in its co-factor forms flavin adenine dinucleotide, FAD and flavin mononucleotide, FMN) is also essential in one-carbon metabolism but its potential role in influencing brain health has rarely been considered.

Numerous observational studies have shown that lower status of folate, vitamin B12 and vitamin B6 (and/or higher concentrations of Hcy) are associated with cognitive deficit in ageing as extensively reviewed elsewhere (Smith and Refsum 2016; Porter *et al.* 2016). Furthermore, meta-analyses of prospective studies (Wald *et al.* 2011; Nie *et al.* 2014; Beydoun *et al.* 2014) also support the view that higher Hcy concentrations (a marker of low status of folate, vitamin B12, vitamin B6 and riboflavin) is a predictor of cognitive decline . RCTs in older adults (≥50 years) that include intervention with high-dose folic acid, vitamin B12 and vitamin B6 over 2 years or more have shown, not only improved cognitive performance (Durga *et al.* 2007; Smith *et al.* 2010; de Jager *et al.* 2012; Douaud *et al.* 2013), but also a reduced rate of brain atrophy in studies which have incorporated MRI (Smith *et al.* 2010; Douaud *et al.* 2013). Notably the greatest slowing in atrophy (53%) was seen among participants with MCI and the highest Hcy concentrations at baseline (>13μmol/L), while cognitive function was preserved in those supplemented with B-vitamins and with a baseline Hcy concentration >11.3 μmol/L (de Jager *et al.* 2012). The RCT evidence is not entirely consistent, however, as one recent and rather controversial meta-analysis in this area concluded that neither folic acid nor vitamin B12 had a beneficial effect on cognition in older adults (≥50 years) (Clarke *et al.* 2014). This paper was however widely criticised at the time of publication, mainly as a result of the inclusion criteria used to select the trials for investigation, and thus the findings are in general not widely accepted by experts in this area (Garrard and Jacoby 2015; Smith *et al.* 2015). It is clear that further appropriately designed RCTs are needed, especially those targeting participants with low B-vitamin status (and in those at most risk of cognitive decline) as they are likely to benefit the most from optimising B-vitamin concentrations to achieve better cognitive health in ageing. Furthermore, research investigating the protective role of riboflavin on cognitive function is greatly lacking, albeit some evidence from older studies investigating riboflavin showed that lower biomarker status was

associated with cognitive impairment (Carney *et al.* 1982b; Xiu *et al.* 2012). Clearly there is a need for riboflavin to be considered in future RCTs.

The role of B-vitamins in depressive disorders has not received as much interest as studies of cognitive disorders, although some observational (**Table 2)** and intervention **(Table 3)** evidence exists. A meta-analysis of 19 observational studies concluded that low folate status was associated with a significantly greater risk of depression (Gilbody *et al.* 2007). Low dietary intakes (≤4.5 μg/d; <5.4 μg/d; (Sánchez-Villegas *et al.* 2009; Skarupski *et al.* 2010a) or biomarker status (lowest quintile serum vitamin B12; serum B12 <180 pmol/L; (Reynolds 2006; Kim *et al.* 2008; Ng *et al.* 2009a; Robinson *et al.* 2011) of vitamin B12 have also been linked with an increased risk of developing depression. Only a limited number of studies have considered the role of vitamin B6, but available evidence suggests an inverse association between vitamin B6 biomarker status (plasma pyridoxal 5'-phosphate) and depression (Hvas *et al.* 2004; Merete *et al.* 2008; Skarupski *et al.* 2010a). Far less evidence exists in relation to riboflavin, although one early study reported lower biomarker status of riboflavin in psychiatric inpatients (Carney *et al.* 1982a). A number of RCTs have considered the role of B-vitamin supplementation alone (Ford *et al.* 2008; Walker *et al.* 2010; Almeida *et al.* 2010; Okereke *et al.* 2015) or as an adjunct to anti-depressant medications (Coppen and Bailey 2000; Almeida *et al.* 2014). The results are somewhat conflicting, however, and no clear conclusions have emerged, partly because of major methodological differences among studies. Reviews of the available evidence in relation to depression have concluded that folate and vitamin B12 may have roles in the longer-term management of this condition (Taylor *et al.* 2004; Almeida *et al.* 2015).

Overall, there is considerable evidence to suggest that folate, vitamin B12 and vitamin B6 have protective effects on cognitive function, and potentially against depressive symptoms in ageing, however further RCTs of appropriate duration in suitable populations, and ideally interventions combining all four relevant B-vitamins, are required to support these findings.

4. Genetic Factors

Genetic factors have been identified as a non-modifiable risk factor for dementia and depression. Specifically, the APOE-ε4 allele on chromosome 19q3 is the only genetic factor that has unequivocally been identified to increase the susceptibility to late onset Alzheimer's (Brouwers *et al.* 2008); furthermore some work also suggests it is associated with an increased risk of depression (Skoog *et al.* 2015). Genome-wide association studies for Alzheimer's disease have been conducted and have identified other susceptibility genes including CLU, CR1, PICALM, BIN1, ABCA7, MS4A, CD33, EPHA1 and CD2AP (Reitz and Mayeux 2014), although to date there is no definitive association with major depression (Flint and Kendler 2014). The Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphism has also received attention as a risk factor for Alzheimer's disease and depression (Rai 2017a). As noted earlier this enzyme plays a unique role in the one carbon metabolic pathway where it irreversibly catalyses 5, 10-methylenetetrahydrofolate to 5, methyltetrahydrofolate in the presence of its cofactor flavin adenine dinucleotide (FAD). The MTHFR C677T polymorphism results in an amino acid change from alanine to valine, which results in a thermo-labile enzyme and thus those with the "TT" genotype have a 70% reduction in the activity of this enzyme compared to those with CC phenotypes (Frosst *et al.* 1995). Recent meta-analyses have suggested that the MTHFR C677T polymorphism is associated with

an increased risk of Alzheimer's disease however only insufficient evidence for its association with depression (Rai 2017a; Rai 2017b).

5. Use of Novel Imaging Technologies in Nutrition and Brain Research

Following the 2009 Nutrition and Mental Performance Task Force of the European Branch of the International Life Sciences Institute (ILSI Europe) workshop, a recommendation was developed suggesting the inclusion of brain imaging biomarkers as secondary endpoints to clinical and cognitive measures (de Jager and Kovatcheva 2010). Brain imaging techniques have been increasingly utilised in recent years and provide an objective and highly robust means of assessing brain structure, function and response to nutrition, with advantages and disadvantages associated with each of their use, as reviewed in detail elsewhere (Sizonenko *et al.* 2013) (**Table 4)**. Electroencephalography (EEG) and magnetoencephalography (MEG) are two similar techniques for functional brain imaging and have the highest temporal resolution compared to other imaging techniques.

In recent years, some of these brain-imaging techniques have been utilised to advance nutrition research in ageing. One notable study referred to earlier in this review (Smith *et al.* 2010) effectively used MRI and confirmed the beneficial effects of B-vitamins on cognition shown previously in older adults (≥ 70years) with MCI, in particular in those with good status of PUFA (Jernerén *et al.* 2015). Additionally, Brickman used fMRI and demonstrated higher brain activation in specific regions of the brain in participants who consumed high dose cocoa flavanols (2014). In a study of 239 older adults (≥ 65 years), diffusion tensor imaging (which in some cases has been suggested to be a better predictor of cognitive decline than other biomarkers) (Selnes *et al.* 2013), identified better white matter integrity in those who consumed more *n*-3 and *n*-6 PUFAs and vitamin E (Gu *et*

al. 2016). EEG has also been used, with one recent report showing improved memory and functional connectivity in the delta band in response to Souvaid®, a nutritional supplement containing PUFAs uridine, choline, phospholipids, folic acid, vitamin B6, B12, C, E and selenium in Alzheimer type patients who were not treated ACE inhibitors and/or memantine with MMSE scores between 20 -26 (Ritchie *et al.* 2014). Positron Emission Tomography Imaging (PET) has also been conducted within a 3-week intervention study, albeit in a very small study of only 11 women, leading to the conclusion that omega 3 supplementation did not affect brain glucose metabolism in healthy older people (76 ± 3 years) (Nugent *et al.* 2011).

It is clear that imaging techniques provide an objective means to improve the evidence base in this area, in particular in relation to proposed mechanisms. At this time, however, the number of studies utilising brain-imaging techniques to investigate the role of diet in brain health in ageing are limited. MEG has been approved by the US Food and Drug Administration (FDA) for use within clinical and research settings as a means to assess and investigate cognitive dysfunction (Maestú *et al.* 2008), AD (de Haan *et al.* 2012; Cheng *et al.* 2012) and depression (Kurita *et al.* 2016). However, to our knowledge, no work has been published using MEG for nutrition studies in older adults (≥60 years). The application of these new technologies in the field of nutrition, in combination with clinical and questionnaire-based assessments, could provide much potential for robust investigation in future studies, furthering knowledge and discovery.

Conclusions and Public Health Implications

Nutrition has important roles in preserving cognition and reducing the risk of late life depression. Emerging evidence in this area implicates subclinical deficiencies of certain nutrients in cognitive decline and depression in older adults. Future studies should address

the gaps in the literature, in particular in identifying of the threshold for optimal nutrient levels required to prevent or delay declining brain health. At this time, the evidence for potential protective effects is strongest in relation to B-vitamins, *n*-3 PUFAs and polyphenols. If confirmed, a public health strategy to improve status of these key nutrients may help to achieve better cognitive and mental health and thus improve quality of life in older age. Future well-designed RCTs (ideally incorporating imaging techniques such as MEG) may provide a more robust basis for confirming effective nutrition interventions, which if implemented could reduce the risk of cognitive and mental health disorders in ageing and the related burden on health services and society overall.

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Table 1. B-vitamin biomarker cut offs

B-vitamin Biomarker	Cut off for deficiency	Reference
Serum folate	<6.1nmol/L	(Molloy and Scott 1997)
Red blood cell folate	<340 nmol/L	(Molloy and Scott 1997)
Serum vitamin B12	<148 pmol/L	(Kelleher and Broin 1991)
Vitamin B6	<30 nmol/L	(Bates *et al.* 1999)
Riboflavin	>1.3	(Powers *et al.* 1983)
Homocysteine	>15µmol/L	(Leino 1999)

Table 2. Summary of observational studies investigating the association of B-vitamin intake and status with depression in older adults

Author	Country	Study Design	*n*	Assessment	B-vitamin Measurement	Outcome
Gougeon *et al* 2016	Quebec, Canada	Longitudinal	1368	GDS/ anti-depressants usage	3x 24 h-recalls	Decreased depression risk among women with higher intakes of vitamin B6.
Moorthy *et al* 2012	Boston, USA	Cross sectional	1955	MMSE CES-D	Plasma folate, vitamin B12, B6 tHcy	Low B12 concentration associated with higher depression scores.
Robinson *et al* 2011	Dublin, Ireland	Cross sectional	252	CES-D	Serum folate, B12, Holo TC	Total B12 and Holo TC concentrations inversely associated with depressive symptoms.
Beydoun *et al* 2010	USA	Cross sectional	2524	PHQ	Serum folate, B12, tHcy	Inverse association between folate concentrations and depressive symptoms; dose response relationship.
Skarupski *et al* 2010	Chicago, USA	Longitudinal	3503	CES-D	Semi quantitative FFQ	High dietary intakes of B6 and B12 protective against depressive symptoms.
Ng *et al* 2009	Singapore	Cross sectional	669	GDS	Serum folate, B12, tHcy	Lower concentrations of folate or deficient B12status associated with greater risk of depression.
Sanchez-Villegas *et al* 2009	Boston, USA	Observational	9670	Self-reported depression, anti-depressants usage	Semi quantitative FFQ	Low dietary folate intake associated with depression among men; low B12 intake associated with depression in women; no associations with vitamin B6 intake.
Murakami *et al* 2008	Japan	Cross sectional	517	CES-D	Diet history questionnaire	Dietary folate inversely associated with depressive symptoms in men. No clear association for other B-vitamins.
Kim *et al* 2008	Korea	Cross sectional & prospective	732	Geriatric Mental State	Serum folate, B12 tHcy	Lower baseline B12 concentrations associated with depression. Lower folate concentrations at baseline associated with higher risk of depression 2 years later.
Dimopoulos *et al* 2007	Greece	Observational	66	GDS	Plasma Folate, B12, tHcy,	Lower folate and vitamin B12 or higher tHcy concentrations correlated with depressive symptoms.
Ramos *et al* 2004	California, USA	Observational	1510	CES-D	Plasma folate, B12, tHcy	Participants in lowest tertile of plasma folate at increased risk of depression.
Bjelland *et al* 2003	Norway	Observational	5948	HADS	Serum folate, B12, tHcy	Elevated tHcy significantly related to depression.
Tiemeier *et al* 2002	Netherlands	Observational	3384	CES-D	Serum folate, B12, tHcy	Depressive disorder more likely with vitamin B12 deficiency.

Abbreviations: CES-D, Centre for Epidemiological Studies Depression Scale; GDS, Geriatric Depression Scale; HADS, Hospital Anxiety and Depression Scale; MMSE, Mini Mental State Examination; PHQ, Patient Health Questionnaire; tHcy, total plasma homocysteine.

Table 3. Summary of RCTs investigating the effect of B-vitamin supplementation on depression in older adults

Study	Area	Cohort	Intervention	Duration	Outcome
B-vitamin intervention alone					
Okereke et al (2015)	USA	*n* = 4331 63.6 years	FA: 2.5mg, B12: 1mg, B6: 50mg or Placebo	7 years	No effect on depression outcomes in participants without prior depression.
Walker et al (2010)	Australia	*n* = 909 65 years	FA:0.4mg, B12: 0.1mg or Placebo	2 years	FA plus B12 was not effective in reducing depressive symptoms in participants with elevated psychological distress.
Almeida et al (2010)	Australia	*n* = 273 63 years	FA: 2mg, B12: 0.5mg, B6: 25mg or Placebo	6.9-7.2 years	Reduction in risk of major depression, in participants with no previous major depressive episodes.
Ford *et al* (2008)	Australia	*n* = 299 ≥75 Years	FA: 2mg, B12: 0.4 mg, B6: 25 mg or Placebo	2 years	No effect on depressive symptoms or development of depression in participants without a prior diagnosis of depression.
B-vitamin supplement as adjunct to anti-depressant medications					
Almeida et al (2014)	Australia	*n* = 153 50+ years	20-40g Citalopram with FA: 2mg, B12: 0.5mg, B6; 25mg or placebo	52 weeks	B-vitamins did not increase 12-week efficacy of antidepressants, but enhanced and sustained antidepressant response over 1 year in participants with depression.
Coppen and Bailey (2000)	UK	*n* = 127 41.9 : 44.3 years	20 mg fluoxetine with FA: 500 µg or placebo	10 weeks	FA significantly improved the action of fluoxetine in participants with depression.

Abbreviations: FA, Folic acid.

Table 4. Brain Imagining techniques for use in nutrition research

Technique	Measurement method	Information obtained	Advantages	Disadvantages
Computerized Tomography (CT)	X-rays	Structural images of the brain	Quick, relatively inexpensive, less stringent requirement for patients.	Exposure to radiation
Magnetic Resonance Imaging (MRI)	Magnetic fields and radiofrequency pulses	Detailed structural images of brain tissue (white and grey matter, blood vessels and bone)	Safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Diffusion Tensor Imaging (DTI)	MRI-based technique using thermally induced self-diffusion of water as a probe	Mapping of the microstructures in the white and grey matter	Visualisation of microstructures, safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Functional MRI (fMRI)	MRI-based technique using blood oxygen level dependant imaging	Visualisation of changes in blood flow, identification of areas of increased cerebral blood volume	Safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Positron Emission Tomography Imaging (PET)	Radioactively labelled tracers once they begin to decay; the two gamma rays released are detected by the scanners	Measurement of the metabolic and physiological processes of the brain	Provide measures of regional cerebral metabolism, blood flow and neurotransmitter metabolism	Low availability requires intravenous injection, use of radioactive compounds, limited use in diabetic participants
Single-Photon Emission Computerised Tomography Imaging (SPECT)	Similar principles to the PET, however the radioactively labelled tracers used emit a single gamma ray	Neurotransmitter distribution and blood perfusion	Provide measures of regional cerebral metabolism, blood flow and neurotransmitter metabolism	Low availability requires intravenous injection, use of radioactive compounds, limited use in diabetic participants
Electroencephalography (EEG)	Electrodes with conductive media are used to detect electric signals	Ionic currents that flow during synaptic excitation in the apical dendrites of pyramidal cells in the cerebral cortex	Relatively inexpensive, non-invasive, good temporal resolution, widely available	Poor spatial resolution, preparation timely
Magnetoencephalography (MEG)	Specialised detectors superconducting quantum interference devices are used to record the magnetic signals	Ionic currents that flow during synaptic excitation in the apical dendrites of pyramidal cells in the cerebral cortex	Non-invasive, highest temporal and spatial resolution.	Limited availability, costly, ferromagnetic implants may interfere with scan

Chapter 3

Folate and related B-vitamins in relation to mental health in older adults from the TUDA cohort study

Abstract

Background: Mental health disorders are major contributors to disease burden in older people. Low/deficient status of folate and/or metabolically related B-vitamins may play a role.

Aim: To investigate folate, vitamin B12, vitamin B6 and riboflavin in relation to depression and anxiety in ageing. We also considered the role of fortified foods as a means of optimising B-vitamin status and potentially reducing the risk of these mental health disorders.

Methods: A cross-section analysis was conducted on community-dwelling adults (60-101 years old) who were recruited to the Trinity Ulster Department of Agriculture (TUDA) ageing cohort study (*n* = 5186); ClinicalTrials.gov Identifier: NCT02664584. Depression and anxiety were assessed using the CES-D scale and the HADS, respectively. B-vitamin biomarker status was assessed. All statistical analyses were performed using SPSS software (Statistical Package for Social Sciences, Version 23.0, SPSS UK Ltd., Chersey, United Kingdom). The risk of depression (CES-D score ≥16) and anxiety (HADS score ≥11) in relation B-vitamin biomarker status was determined using logistic regression and the model was adjusted for relevant co-variates and vitamin supplement use.

Results: Participants with a biomarker value in the lowest 20% of status for folate (OR=1.79, 95% CI 1.23-2.61), vitamin B6 (OR=1.45, 95% CI 1.01-2.06) or riboflavin (OR=1.56, 95% CI 1.10-2.00), but not vitamin B12, were at increased risk of depression (CES-D score ≥16). Correspondingly, B-vitamin fortified foods were associated with a reduced risk of depression if consumed daily (OR=0.535, 95% CI 0.41-0.70). A low/deficient status of vitamin B6 (OR=1.73, 95% CI 1.07-2.81), but not other vitamins, was associated with increased anxiety.

Conclusions: The findings suggest that a B-vitamin biomarker status above defined cut-off levels (folate ≥1520nmol/L, vitamin B6 >35.6nmol/L, riboflavin <1.46), plays a role in improving mental health in older adults. Regular intake of fortified foods can provide a means of optimising B-vitamin status and potentially reducing the risk of depression in older adults. Randomised trials are needed to confirm these observational findings.

Introduction

Globally the population is ageing and by 2050 the number of people aged ≥60 years is predicted to reach 2.1 billion (United Nations Department of Economic and Social Affairs/Population Division 2015). Mental health disorders are a leading cause of disability and ill health in older age (Andreas *et al.* 2016). and are estimated to affect 20% of adults ≥60 years worldwide (World Health Organisation 2016), Given the considerable human and economic cost of mental health conditions and the generally poor response rates to costly pharmacological treatments (Rush *et al.* 2006), there is much interest in the potential roles of certain dietary components as modifiable risk factors for depression. Folate and vitamin B12 have received particular attention in this regard (Moore *et al.* 2017). These B-vitamins have interrelated roles within one-carbon metabolism, where folate in the form of 5 MTHF and vitamin B12 in the form of methylcobalamin are required for the remethylation of Hcy to methionine which subsequently forms SAM (Bailey *et al.* 2015). SAM, in turn, is the essential methyl donor required for the production of monoamine neurotransmitters, phospholipids and nucleotides (Bottiglieri and Reynolds 2005). Historically, clinical deficiencies of folate and the related B-vitamins were associated with a range of neuropsychiatric symptoms, including depression (Reynolds *et al.* 1970), raising the possibility that optimising relevant B-vitamin intake and status could improve mental health. Research to date in this area has focused predominantly on folate, and to a lesser extent vitamin B12 (Petridou *et al.* 2016), whereas related B-vitamins, also required for one-carbon metabolism i.e. vitamin B6 and riboflavin, have received much less attention. The aim of this study therefore was to investigate biomarker status of all relevant B-vitamins - folate, vitamin B12, vitamin B6 and riboflavin - in relation to mental health in ageing. Furthermore, we considered the role of fortified foods as a means of optimising B-vitamin status and potentially reducing the risk of depression and anxiety in older adults.

Methods

Subject Recruitment:

The study involved new analysis of data from the TUDA ageing cohort study (ClinicalTrials.gov Identifier: NCT02664584). As described in detail elsewhere (McCann *et al.* 2018), 5,186 community-dwelling adults aged ≥ 60 years were recruited to the TUDA study between 2008 and 2012 from Northern Ireland (NI) and the Republic of Ireland (RoI). One of the aims of the TUDA study was to investigate the role of nutrition and lifestyle factors in the aetiology of common age-related diseases; namely, dementia, osteoporosis and cardiovascular disease (CVD). Thus, the TUDA study consists of three cohorts; cognitive, bone and hypertensive. The cognitive and bone cohort were recruited from the Department of Medicine for the Elderly at St. James's Hospital, Dublin. Those in the cognitive impairment cohort had an RBANS score <80 and were recruited from geriatric clinics or day hospital services while the bone cohort participants had diagnoses of either osteoporosis or osteopenia and were recruited from the specialist bone health services (St. James Hospital, Dublin). Those in the hypertension cohort had a diagnosis of hypertension and were recruited form Western and Northern Health and Social Care Trusts in Northern Ireland.

Participants were recruited in both jurisdictions using standardised protocols and centralised training, and deemed suitable if they (or their parents) were born on the island of Ireland and were without a diagnosis of dementia. Those who provided informed consent were deemed eligible to participate. For the current study, any participants receiving vitamin B12 injections were excluded from the analysis (**Figure 1**).

Ethical approval for the TUDA study was granted by the Office for Research Ethics Committees Northern Ireland (ORECNI; reference 08/NI/RO3113), with corresponding approvals from The Northern and Western Health and Social Care Trusts in NI, and the

Research Ethics Committee of St James Hospital and The Adelaide and Meath Hospital in Dublin.

General health, lifestyle and biophysical measures

Health and lifestyle information was gathered using a detailed, researcher-assisted, questionnaire and included information on age, smoking, alcohol, medical history, drugs including antidepressant medications, proton pump inhibitors and vitamin supplement use. Anthropometric measurements were recorded (including weight, height, waist and hip) and blood pressure (BP) measurements were taken in accordance with standard operating procedures by trained researchers. In brief, in accordance with clinical guidelines, two measurements were taken from the reference arm (the arm with the highest BP reading) with a 5-10 minute interval between each measurement; the mean of the two readings was used as the BP value. The Timed Up-and-Go (TUG) test was performed as a measure of functional mobility. Participants were asked to stand from a seated position (seat height approximately 46 cm), walk 3 m at their usual pace, turn around, walk back to the chair, and sit down. No physical assistance was given, and the time taken from command "Go" to complete the task was measured with a stopwatch (Podsiadlo and Richardson 1991). The functional abilities of the participants were also assessed using the Physical Self-Maintenance Scale (PSMS) and the Instrumental Activities of Daily Living (IADL) scale. Area-level socioeconomic deprivation was also measured by adopting a cross-jurisdictional approach, whereby geo-referenced address-based information was used to map and link participants to official socioeconomic indicators of deprivation within NI and RoI (McCann *et al.* 2018). As previously described in detail (McCann *et al.* 2018), participants in each jurisdiction were assigned a deprivation score relating to the area in which they lived and scores were categorised into comparable quintiles, which were then used to integrate the datasets from both

countries, providing deprivation data for the TUDA cohort as a whole ranging from the 20% least to 20% most deprived areas.

Dietary assessment

Dietary information on habitual intake of certain foods was collected using a researcher-assisted food frequency questionnaire (FFQ). This procedure allowed assessment of the frequency of consumption of specific foods including products known to be fortified with B-vitamins. Brand names of products were collected so that up-to-date details on relevant nutrient profiles could be obtained. Using this approach, participants were categorised according to intake of fortified foods and classed as non-consumers (0), low (1-4), medium (5-7) or high (8+) consumers based on servings of fortified foods per week. Vitamin supplement use was also recorded and participants were asked to bring specific products to their appointment so that accurate information on supplement usage including brand name, dose, frequency, and duration of use could be recorded. Where this information could not be confirmed at interview, it was followed up in a subsequent telephone call.

Neuropsychiatric assessment

Depression was assessed using the CES-D scale, which is a 20 item self-reported questionnaire, with a minimum score of 0 (no symptoms of depression) and maximum score of 60 (significant symptoms of depression). A score greater than or equal to 16 was used as a cut-off value suggestive of clinical depression (Radloff and Locke 1986). Anxiety was assessed using the 7 item HADS, with a minimum score of 0 (suggestive of no symptoms of anxiety) and a maximum score of 21 (significant anxiety). A score greater than or equal to 11 was used as a cut-off value for probable anxiety (Zigmond and Snaith 1983).

For the purpose of the current analysis, cognitive function was assessed using the Folstein MMSE (Folstein *et al.* 1975), a short, structured cognitive test. The MMSE is the most widely used screening tool in clinical settings worldwide for identifying cognitive impairment and dementia and evaluates global cognitive function by assessing the domains of orientation, registration, attention and concentration, recall and language (Sheehan 2012; Roebuck-Spencer *et al.* 2017). The maximum score achievable is 30, with a score <25 indicating a possibility of cognitive impairment and a score <20 indicating dementia.

Blood sampling and laboratory analysis

A non-fasting blood sample (50ml) was obtained and analysed for routine clinical markers of health in participating hospital laboratories. For research biomarkers, all blood processing was carried out within 4 hours of collection and B-vitamins were analysed centrally in laboratories at Trinity College Dublin (vitamin B12, folate, Hcy) or Ulster University (vitamin B6, riboflavin) using established methods. Red blood cell (RBC) folate and serum total vitamin B12 were measured by microbiological assay using Lactobacillus casei and Lactobacillus Leichmanni, respectively (Kelleher and Broin 1991; Molloy and Scott 1997). Plasma Hcy was measured by fluorescence polarization immunoassay (Leino 1999). Riboflavin status was measured by erythrocyte glutathione reductase activation coefficient (EGRac), a functional assay that measures the activity of glutathione reductase before and after in-vitro reactivation with its prosthetic group flavin adenine dinucleotide, where a higher EGRac ratio indicates a lower riboflavin status (Powers *et al.* 1983). Vitamin B6 status (plasma pyridoxal-5-phosphate, PLP) was analysed by HPLC with fluorescence detection (Bates *et al.* 1999). For each assay, quality controls were provided by the repeated analysis of pooled samples covering a wide range of values.

Participants were given instructions to swab the inside of their cheeks with the 5 cotton swabs to provide samples for DNA analysis of single nucleotide polymorphisms (SNPs). This included assessment for the common MTHFR polymorphism 677C>T (Frosst *et al.* 1995), which was identified by polymerase chain reaction followed by *Hinfl* digestion and polyacrylamide gel electrophoresis (conducted by LGC Genomics; Herts, United Kingdom).

Statistical Analysis

All statistical analysis was performed using SPSS software (Statistical Package for Social Sciences, Version 23.0, SPSS UK Ltd., Chersey, United Kingdom). Data were checked for normality and log-transformed as appropriate. Analysis of covariance with Bonferroni post hoc test was used for analysis of continuous data and chi-squared tests were used for categorical variables. Relationships of demographic, clinical and lifestyle factors with depression (CES-D score) and anxiety (HADS score) were investigated using multiple linear regression analysis. The risk of depression (CES-D score ≥16) and anxiety (HADS score ≥11) in relation B-vitamin biomarker status was determined using logistic regression and the model was adjusted for vitamin supplement use and relevant co-variates (age, gender, age finished education, area-level socioeconomic deprivation, smoking, physical frailty, living alone, antidepressant usage, and previous ischaemic attack, BMI, hypertension). For this purpose, B-vitamin biomarkers were examined in quintiles ranging from lowest 20% to highest 20% of values. For all analysis $P \leq 0.05$ was considered significant. The risk of depression (CES-D score ≥16) and anxiety (HADS score ≥11) in relation to B-vitamin fortified food intake was determined using logistic regression and the model was adjusted for relevant co-variates (age, gender, age finished education, area-level socioeconomic deprivation, smoking, physical frailty, living alone, antidepressant usage, and previous ischaemic attack, BMI, hypertension). The reference

category for logistic regression analysis were non-consumers of fortified foods, against which low (1-4 portions/week), medium (5-7 portions/week), and high consumers (8+ portions/week), were compared. These specific categories of portions of fortified food/week were utilised as they have been previously been reported in the literature in similar populations (Hoey *et al.* 2007; Hopkins *et al.* 2015).

Results

General characteristics

The general characteristics of the study population are described in **Table 1**. Participants were predominantly female (67%), the majority were fortified food consumers (71.6%) and 10.9% were B-vitamin supplement users. Overall, higher rates of depression (CES-D score ≥16.0) and anxiety (HADS score ≥11.0) were recorded in females compared to males; likewise self-reported depression and anxiety were also higher in females. Males were more likely to have a history of medical conditions such as diabetes, hyperlipidaemia, hypertension, myocardial infarction and previous stroke. B-vitamin biomarker status was generally lower, and Hcy concentrations higher, in men compared to women. Although mean B-vitamin biomarker concentrations from this TUDA cohort fell within normal reference ranges (data not shown), using accepted laboratory cut-offs some evidence of deficiencies were identified for specific B-vitamin biomarkers: folate (RBC folate ≤ 340nmol/L: 2.3%); vitamin B12 (serum B12 ≤ 145pmol/L: 11.6%); vitamin B6 (PLP ≤ 30nmol/L: 12.2%); riboflavin (EGRac ≥1.3; 48.6%).

B-vitamin intakes in relation to biomarker status

The influence of B-vitamin fortified food and supplement intake on B-vitamin biomarker status (**Table 2**), in addition to the frequency of consumption of fortified food groups (per quintiles of biomarker status) was determined (**Table 3**). Participants were categorised based on their self-reported intake of B-vitamin fortified foods as low, medium or high consumers, and as users or non-users of B-vitamin supplements; 'non-consumers' did not consume fortified foods or supplements and hence depended on natural food sources of B-vitamins only. As dietary intake of B-vitamin fortified foods increased, the biomarker status increased in a stepwise manner with the highest B-vitamin biomarker status being observed in those participants who consumed 8 or more portions of fortified foods per

week and in those taking B-vitamin supplements. B-vitamin supplement users were identified on the basis of their reported current use; of those identified as supplement users (10.8% of overall TUDA sample) the majority also consumed fortified food. A small number of participants (n = 110; 2.2%) could not be classified as regards fortified food intake and supplement use and thus were excluded from the analysis investigating the effect of B-vitamin intake on biomarker status.

Relationships of demographic, clinical and lifestyle factors with depression and anxiety

The relationship of clinical and lifestyle factors with depression (CES-D score) and anxiety (HADS score) was examined by linear regression (not shown). The following factors were significantly associated with depression; age (β -0.99; $P < 0.001$), female sex (β 0.04; $P = 0.008$), age finished education (β -0.06; $P < 0.001$), area-level socioeconomic deprivation (β 0.09; $P < 0.001$), smoking (β 0.05; $P = 0.001$), physical frailty (β 0.19; $P < 0.001$), living alone (β 0.08; $P < 0.001$), antidepressant usage (β 0.21; $P < 0.001$), and previous transient ischaemic attack (β 0.04; $P = 0.02$). For anxiety, the following factors were identified as significant determinants; age (β -0.138; $P < 0.001$), female sex (β 0.08; $P < 0.001$), age finished education (β -0.10; $P < 0.001$), area-level socioeconomic deprivation (β 0.08; $P < 0.001$), BMI (β -0.05; $P < 0.001$), hypertension (β 0.04; $P = 0.027$) and anti-depressant usage (β 0.18; $P < 0.001$).

Risk of depression and anxiety in relation to B-vitamin biomarkers, fortified food intake and supplement use

The risk of depression (CES-D score ≥16) by B-vitamin biomarker status was examined after adjustment for the above co-variates and vitamin supplement use (**Figure 2**). Each B-vitamin was examined in quintiles of biomarker status and the general characteristics of the each quintile were explored (**Table 4**). The reference category was set at the highest

20% of values and Hcy was examined in quintiles of concentration. Compared with the reference category, those in the lowest quintile of RBC folate (OR = 1.79, 95% CI 1.23-2.61; *P* = 0.002), vitamin B6 (OR = 1.45, 95% CI 1.01-2.06; *P* = 0.043) or riboflavin (OR = 1.56, 95% CI 1.10-2.00; *P* = 0.012) status were at significantly increased risk of depression. The risk of depression was found to be significantly increased in participants with the variant 677TT genotype for *MTHFR*, but only when this genotype occurred in combination with low status of riboflavin (i.e. the precursor of FAD required as a co-factor for MTHFR): OR = 1.51, 95% CI 1.02-2.22; *P* = 0.038. No significant relationship with depression scores were identified in those with the CC or CT genotype (data not shown). No statistically significant association between serum total B12 or Hcy was observed with depression.

The risk of depression was also examined in relation to B-vitamin fortified food intake (**Figure 2**); for this purpose the reference category was non-consumers. Compared to non-consumers (reliant on natural food sources of B-vitamins), among those who consumed 8 or more portions per week of fortified food (but not lower amounts) the risk of depression was reduced (OR = 0.535, 95% CI 0.41-0.70; *P* < 0.001). After adjustment for relevant co-variates including fortified food intake, B-vitamin supplement usage was not associated with depression risk (OR = 0.941, 95% CI 0.68-1.30; *P* = 0.712).

Similarly, the relationship of B-vitamins with risk of anxiety was examined in quintiles of biomarker status (data not shown). After adjustment for relevant co-variates and vitamin supplement use, only low/deficient status of B6 - but not other B-vitamins or Hcy concentrations were associated with an increased risk of anxiety (OR = 1.73, 95% CI 1.07-2.81; *P* = 0.024) and there was no significant association of the *MTHFR* 677TT genotype with anxiety (data not shown). Likewise, no significant relationship was identified between B-vitamin fortified food intake (OR = 0.97, 95% CI 0.69-1.36; *P* = 0.861) or supplement usage (OR = 0.99, 95% CI 0.64-1.54; *P* = 0.974) and anxiety.

Discussion

Main findings

This study is the first large cross sectional study to investigate all four B-vitamins involved in one-carbon metabolism in relation to depression and anxiety in older adults. The findings show that low/deficient biomarker status of folate, vitamin B6 or riboflavin, but not vitamin B12, were each independently associated with an increased risk of depression. Correspondingly, the risk of depression was lower by 50% among participants who consumed at least one portion per day of B-vitamin fortified food. Only low/deficient status of vitamin B6 (but not the other B-vitamins) was associated with the risk of anxiety, and no significant relationship of fortified food with anxiety was shown.

Comparison of findings with other studies investigating B-vitamins in relation to depression

The current results showed 78% increased risk of depression among those with the lowest 20% of RBC folate concentrations, adding to the increasing body of evidence linking low folate with depression. In one meta-analysis of 11 observational studies (n = 15,315 participants), low folate biomarker status was associated with a 42% increased risk of depression (Gilbody *et al.* 2007a). Furthermore, a more recent meta-analysis of adults ≥55 years (n = 8000) found that low serum folate concentrations were associated with a significantly higher risk of depression (OR = 1.23; 95% CI: 1.07-1.43) (Petridou *et al.* 2016). The stronger relationship of folate with depression identified in the current study when compared with the aforementioned studies (Gilbody *et al.* 2007a; Petridou *et al.* 2016), may be explained to some extent by the use of RBC folate, generally considered to be a better index of long-term folate status compared to plasma or serum concentrations (Bailey *et al.* 2015). The evidence linking folate with depression is however not entirely consistent. The Chicago Health and Aging Study (CHAP) (n = 3503) and the Quebec

longitudinal study on nutrition and Aging (NuAge) (*n* = 1368) found no association of folate with depression; however these observations were based on dietary intake data only with no corresponding folate biomarkers (Skarupski *et al.* 2010; Gougeon *et al.* 2016). Furthermore, the studies were conducted in regions with mandatory folic acid fortification, where more optimal folate biomarker status throughout the population would make a relationship with depression less likely. Despite the close metabolic relationship between folate and vitamin B12, the current study found no association of serum vitamin B12 with depression, which is in line with the findings from one large cohort study (*n* = 2,524) conducted in the USA (Beydoun *et al.* 2010), but at odds with other research which reported inverse associations of vitamin B12 intake (Skarupski *et al.* 2010; Gougeon *et al.* 2016) or biomarkers (Kim *et al.* 2008) with depression. The explanation for such discrepancy in the evidence linking vitamin B12 with depression is unclear, but may possibly relate to differences in B12 status among populations under investigation or methodological variation among studies, including the use of different B12 biomarkers to measure status, especially considering that no consensus exists as to the best biomarker for assessing B12 status in the laboratory.

The current results showed that low status of vitamin B6 or riboflavin was associated with an increased risk of depression by up to 56%. Likewise, previous studies have reported inverse associations of vitamin B6 biomarkers with depression (Merete *et al.* 2008). In contrast to the other relevant B-vitamins, riboflavin has received very little attention as regards its potential role in depression, with previous evidence limited to one early study which reported that 27% of patients admitted to a psychiatric inpatient unit had riboflavin deficiency, whilst a recent study showed no significant relationship of dietary riboflavin intake with depression (Carney *et al.* 1982; Murakami *et al.* 2008). There is a well established metabolic dependency of vitamin B6 on provision of adequate riboflavin, in that the generation of the active B6 form, PLP, in tissues (via pyridoxine 5'phosphate

oxidase) requires riboflavin in its co-factor form of flavin mononucleotide (FMN). We previously confirmed this interrelationship in humans by showing that riboflavin supplementation of older adults not only improved biomarker status of riboflavin, but also enhanced vitamin B6 concentrations, suggesting that riboflavin may be the more limiting nutrient (Madigan *et al.* 1998). Previous studies have reported a significantly increased risk of depression among individuals homozygous for the common C677T polymorphism in *MTHFR* (Bjelland *et al.* 2003), whereas the current study found that the risk of depression was significantly increased only when this genotype occurred in combination with low status of riboflavin (i.e. which acts as cofactor for MTHFR). Indeed the importance of this gene-nutrient interaction for health outcomes has previously been reported by us in human trials showing that riboflavin supplementation results in significant blood pressure lowering only in those with the variant *MTHFR* 677TT genotype (McNulty *et al.* 2002).

Given the interactions of riboflavin with vitamin B6 and with folate (via *MTHFR* genotype) as shown here, further studies (ideally randomised trials) should investigate the combined effect of these vitamins in relation to depression in older adults.

Comparison of findings with other studies investigating B-vitamins in relation to anxiety

In the current study, low/deficient vitamin B6 status was associated with an increased risk of anxiety, while no significant associations with anxiety were found for any other B-vitamin biomarkers or fortified foods. The findings are generally in line with those of the Hordaland Homocysteine Study (n = 5948) which also reported no significant relationships of folate or vitamin B12 with anxiety (Bjelland *et al.* 2003). Research in the area of vitamin B6 and anxiety has been limited and the available evidence is conflicting. Although an earlier study in 198 patients with anxiety disorders concluded that low vitamin B6 status was unlikely to be significant in the aetiology of psychiatric disorders

(Emmanuel *et al.* 1994), a more recent RCT in 60 patients, which intervened with a supplement containing combined B-vitamins (including vitamin B6, vitamin B12 and folate) in patients suffering from depression, observed short terms benefits in symptoms of anxiety (Lewis *et al.* 2013).

B-vitamin status and good mental health

The current findings suggest that maintaining good biomarker status of folate and the related B-vitamins help to maintain good mental health in older adults. Although the aim of the current study was to investigate the role of these B-vitamins in relation to depression and anxiety and not specifically good mental health, it was found that those in the top 20% of biomarker status for folate, vitamin B6 and riboflavin, appeared to have lower depression scores (CES-D) suggestive of better mental health compared to those with lower biomarker status. These findings in relation to folate status are consistent with conclusions from the meta-analysis conducted by Gilbody (2007b) where the overall trend among studies was for red cell and serum folate to be higher in healthy controls compared to those with questionnaire assessment scores indicative of clinical depression. As the current and aforementioned studies do not specifically measure 'good mental health' (rather the presence of symptoms suggestive of depression), further studies which aim to assess the role B-vitamin status in good mental health are required.

Public Health Implications

The current study not only showed that low biomarker status of specific B-vitamins was related to mental health, but importantly demonstrated the potential use of fortified foods as a means of reducing the risk of depression in older age. To our knowledge, this is the first study which has investigated the association between fortified foods and depression and suggests a reduced risk of depression (by up to 54%) in those who consumed fortified

foods on a daily basis. Fortified foods are known to provide a highly bioavailable source of B-vitamins, particularly folate, in the form of folic acid (Bailey *et al.* 2015). The impact of fortified foods in achieving more optimal B-vitamin biomarker status has also been previously reported (Hoey *et al.* 2007). The current results suggest that regular consumption of fortified foods, by improving B-vitamin biomarkers, may provide a practical means of reducing the risk of depression in older adults. Furthermore, these findings also suggest that consumption of folic acid rather than folate found in natural food sources plays a greater protective role in brain health. To our knowledge no intervention studies have investigated the role of natural food folate on depression, however the current findings are in line with the limited evidence from randomised trials in which intervention with folic acid was found to protect against depressive symptoms (Coppen and Bailey 2000; Almeida *et al.* 2014).

Possible mechanism explaining the findings

The biological mechanisms explaining the current and previous results linking folate and metabolically related B-vitamins with mental health (and most convincingly with depression) may involve perturbed monoamine synthesis. Folate deficient patients suffering from depression have been shown to have significantly lower concentrations of monoamine metabolites in cerebral spinal fluid (Bottiglieri and Reynolds 2005). As folate and related B-vitamins are required for the generation of SAM within one-carbon metabolism, lower B-vitamin status may reduce the methylation of the neurotransmitters serotonin, dopamine and noradrenaline (Bottiglieri and Reynolds 2005). Additionally, folate has a role in the synthesis of tetrahydrobiopterin (BH4), an essential co-factor in monoamine synthesis (Bottiglieri *et al.* 1992). Furthermore, the active form of vitamin B6 (PLP) is the cofactor for aromatic L-amino acid decarboxylase in the tryptophan serotonin pathway, thus suboptimal B6 status may lead to reduced concentrations of

serotonin and thus increased risk of depression (Hensler 2006). Lastly, as previously noted, riboflavin in the form of FMN is required as a coenzyme for the activation of B6 in tissues and so its relationship with depression could be mediated through vitamin B6.

Strengths and limitations of the current study

Although the TUDA study is one of the largest and most comprehensively characterised cohorts of its kind, its cross-sectional design means that the possibility of residual confounding cannot be excluded. Also, the CES-D scale used in this study to assess depression, while widely considered to have an acceptable screening accuracy in primary care settings, is not as robust as other diagnostic instruments and this may have limited the interpretation of the current findings to some extent (Vilagut *et al.* 2016). However, the current study had a number of strengths. It is the first human study to investigate the effect of all relevant B-vitamin biomarkers (including riboflavin, rarely assessed in cohort studies or nutritional surveys) on the risk of depression and anxiety in older adults, and thus allowed an in-depth examination of the role of one-carbon metabolism in mental health. Finally, this is the first study to have considered the potential role of fortified foods as a practical means of reducing depression in older age.

Implications

This study in older adults indicated that lower biomarker status of folate or vitamin B6 or riboflavin may contribute to an increased risk of depression in ageing, while deficient status of vitamin B6 was associated with an increased risk of anxiety. Higher intakes of B-vitamin fortified foods (e.g. fortified breakfast cereals) or B-vitamin supplement use resulted in the achievement of optimal B-vitamin biomarker status, while fortified foods consumed daily were associated with a reduced risk of depression. Further work in the form of well-designed RCTs, investigating the role of all B-vitamins in the one-carbon

metabolism network and in populations with sub-optimal B-vitamin status, are needed to confirm these observational findings. These results, if confirmed, may have implications for dietary recommendations and health policy involving low cost non-drug alternative means of improving mental health and thus quality of life in older adults.

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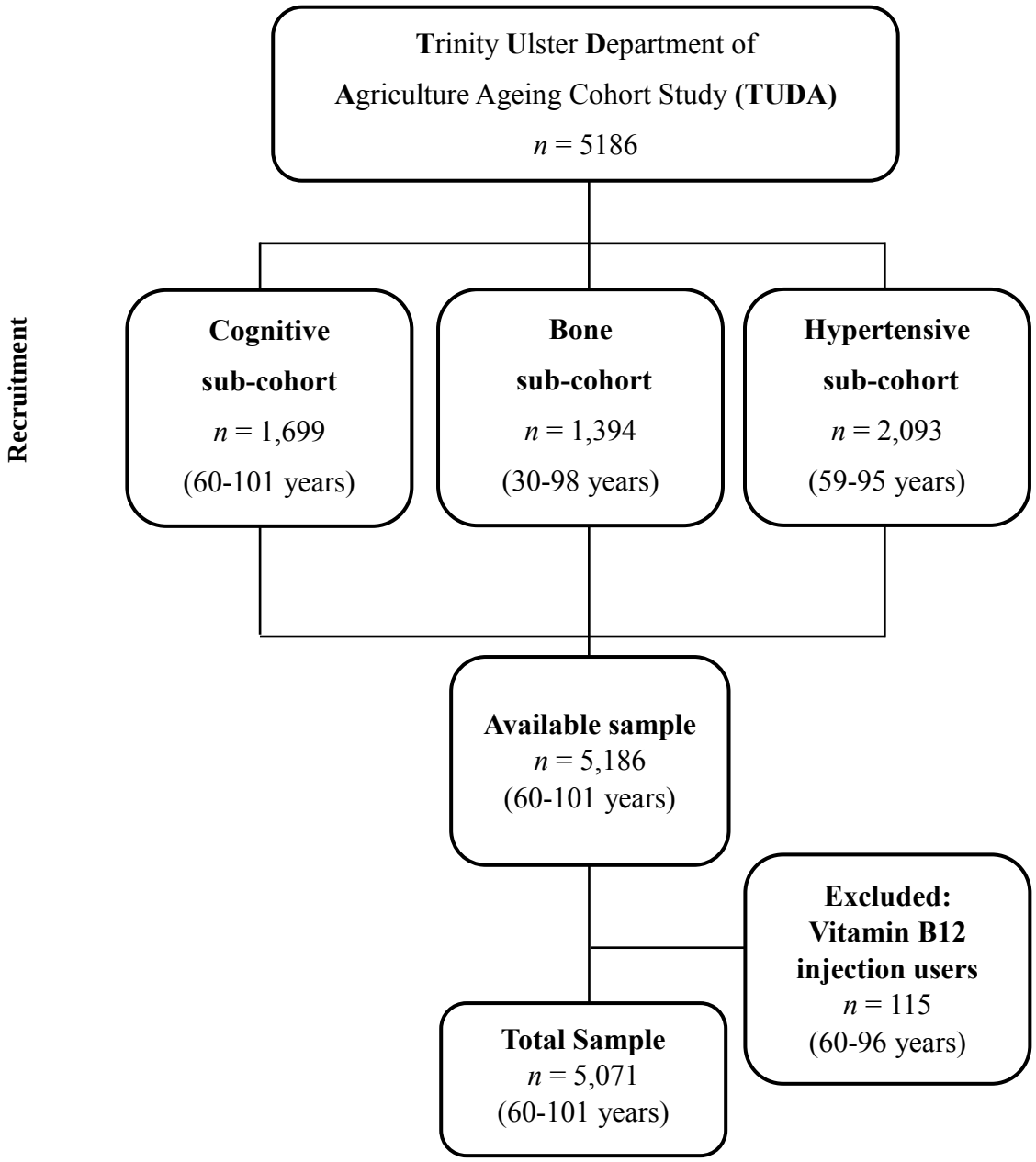

Figure.1 Flow diagram and study design of the TUDA Ageing Cohort

Table 1. General characteristics of TUDA study participants[a]

Adjusted means (s.e.m) or n (%)	Males (*n* = 1665)	Females (*n* = 3406)	*P*
Age (year)	73.4 (8.0)	74.3 (8.4)	<0.001
Health and Lifestyle			
BMI (kg/m^2)	28.4 (0.1)	27.7 (0.01)	<0.001
Waist to Hip ratio	0.97 (0.02)	0.88 (0.01)	<0.001
Timed Up and Go (seconds)	14.1 (0.2)	14.0 (0.1)	0.461
Physical Self Maintenance Score	23.1 (0.05)	22.9 (0.3)	<0.001
Instrumental Activities of Daily Living	24.1 (0.1)	24.1 (0.1)	0.895
Living alone *n* (%)	373 (22.4)	1335 (39.2)	<0.001
Area deprivation (most deprivation) *n* (%)	429 (26.4)	867 (26.2)	0.856
Current Smoker *n* (%)	193 (11.6)	411 (12.1)	0.651
Alcohol (units/week)	8.8 (0.2)	2.5 (0.2)	<0.001
Proton Pump Inhibitors *n* (%)	608 (35.8)	1367 (39.2)	0.020
Fortified Food Consumer *n* (%)	1186 (71.2)	2443 (71.7)	0.888
B-vitamin supplement user *n* (%)	163 (9.8)	382 (11.4)	0.098
Vitamin D supplement user *n* (%)	533 (32.1)	1867 (55.3)	<0.001
Medical			
Diabetes *n* (%)	311 (18.7)	327 (9.6)	<0.001
Hyperlipidaemia *n* (%)	919 (55.3)	1774 (52.1)	0.037
Hypertension *n* (%)	1318 (79.2)	2318 (68.1)	<0.001
Previous Myocardial infarction n (%)	266 (16.0)	244 (7.2)	<0.001
Previous Transient Ischemic Attack *n* (%)	135 (8.1)	286 (8.4)	0.774
Previous Stroke *n* (%)	189 (11.4)	199 (5.8)	<0.001
Brain Health*			
Anti-depressant drugs *n* (%)	169 (10.2)	542 (15.9)	<0.001
Depression score (CES-D)	5.5 (0.2)	6.3 (0.1)	0.267
Depression *n* (%)	137 (8.3)	407 (12.0)	<0.001
Self-reported depression *n* (%)	325 (19.5)	893 (26.2)	<0.001
Anxiety score (HADS)	2.8 (0.1)	3.4 (0.1)	0.513
Anxiety *n* (%)	61 (3.7)	190 (5.6)	0.004
Self-reported anxiety *n* (%)	264 (15.9)	832 (24.4)	<0.001
Cognitive Score (MMSE)	27.0 (0.1)	27.1 (0.0)	<0.001
Impairment (MMSE <25) *n* (%)	187 (11.9)	444 (13.5)	0.134
Biomarker			
Red blood cell folate (nmol/L)	1043 (13.5)	1094 (9.2)	0.001
Serum vitamin B12 (pmol/L)	263 (3.1)	288 (2.1)	<0.001
Plasma vitamin B6 (PLP; nmol/L)	65.4 (1.0)	72.0 (0.7)	<0.001
Riboflavin (EGRac)	1.34 (0.00)	1.33 (0.00)	0.146
Plasma total Homocysteine (µmol/L)	15.2 (0.1)	14.3 (0.1)	<0.001
***MTHFR* 677TT genotype *n* (%)**	192 (11.9)	405 (12.2)	0.689

TUDA, Trinity Ulster Department of Agriculture; MMSE, Mini Mental State Examination; CES-D, Centre for Epidemiologic Studies Depression; HADS, Hospital Anxiety and Depression Scale; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase activation co-efficient (a higher EGRac ratio indicates lower riboflavin status).; MTHFR methylenetetrahydrofolate reductase.

a. Differences were assessed using ANCOVA with Bonferroni post hoc tests on log-transformed data when applicable, with adjustment for age, *BMI, smoking status, alcohol, anti-depressant medication usage, vitamin supplement usage and fortified food. Categorical variables were assessed using χ^2 analysis. *P*-value <0.05 was considered significant.

Table 2. B-vitamin intakes in relation to biomarker status

	Non Consumer	**Fortified Food Consumer**			**Supplement User**
		Low consumer	Medium consumer	High consumer	
Servings of Fortified Foods/week	0	1-4	5-7	8+	0-8+
TUDA Total *n (%)*	*1164 (23.0)*	*479 (9.5)*	*1049 (20.7)*	*1724 (34.0)*	*545 (10.8)*
Vitamin Biomarker					
RBC folate (nmol/L)	691 (525, 910)[‡]	802 (612, 1089)[¶]	909 (664, 1238)[§]	1138 (809, 1577)[¥]	1554 (1034, 2023)[¯]
Serum total vitamin B12 (pmol/L)	238 (174, 318)[‡]	243 (180, 323)[‡¶]	260 (188, 336)[¶]	271 (208, 361)[§]	293 (213, 392)[§]
Plasma vitamin B6 PLP (nmol/L)	47.0 (31.9, 70.0)[‡]	54.1 (37.5, 80.0)[¶]	60.8 (41.5, 87.6)[§]	70.3 (47.5, 97.6)[¥]	70.6 (39.0, 115.0)[¥]
Riboflavin (EGRac)	1.35 (1.25, 1.47)[‡]	1.32 (1.22, 80.0)[¶]	1.28 (1.20, 1.38)[§]	1.28 (1.20, 1.39)[§]	1.24 (1.15, 1.34)[¥]
Homocysteine (µmol/L)	15.2 (12.2, 19.1)[‡]	13.7 (11.4, 16.7)[¶]	13.7 (11.3, 17.1)[¶]	12.6 (10.7, 15.7)[§]	12.2 (10.3, 15.0)[§]

Data presented as median (25^{th}, 75^{th} percentiles). Differences were assessed using ANCOVA with Bonferroni post hoc tests on log-transformed data when applicable, controlling for co-variates. Values within a row without a common superscript symbol are significantly different ($P < 0.05$)
A small number of participants ($n = 110$; 2.2%) could not be classified as regards fortified food intake and supplement use
RBC, red blood cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase activation co-efficient (a higher EGRac ratio indicates lower riboflavin status).

Table 3. Intake of fortified food groups relative to quintiles of B-vitamin status

	Q1	Q2	Q3	Q4	Q5
RBC folate	***≤623***	**623-8.25**	**825-1084**	**1084-1520**	**1520+**
Breakfast Cereals	38.7	42.6	50.7	52.9	54
Cereal bars	1.3	2.5	2.7	1.9	1.8
Bread	18.7	16.2	18.9	18.6	19.6
Fat spread	28.0	35.0	41.8	50.7	53.2
Drinks	5.3	7.5	12.0	20.0	32.2
Marmite	1.2	1.5	0.8	1.1	1.1
Serum B12	***≤177***	***177-235***	***235-290***	***290-373***	***373+***
Breakfast Cereals	44.4	45.9	47.9	51.0	48.7
Cereal bars	1.8	2.0	3.3	1.8	1.3
Bread	19.3	17.7	19.2	15.7	19.3
Fat spread	31.4	38.3	46.1	44.7	48.3
Drinks	10.3	14.0	15.5	13.7	21.8
Marmite	1.3	1.0	1.0	1.3	1.1
Vitamin B6 (nmol/L)	**≤35.9**	**35.7-51.9**	**51.8-70.0**	**70.0-96.3**	**96.4+**
Breakfast Cereals	39.9	44.6	49.0	48.7	56.5
Cereal bars	2.1	1.8	1.9	2.2	2.3
Bread	22.7	19.0	17.6	15.4	16.5
Fat spread	26.9	32.9	42.4	35.3	56.3
Drinks	9.2	12.7	15.4	18.4	18.6
Marmite	0.7	1.5	1.3	0.9	1.1
Riboflavin (EGRac)	***1.45+***	***1.34-1.45***	***1.27-1.33***	***1.20-1.26***	***≤1.19***
Breakfast Cereals	35.1	44.1	45.0	52.3	60.1
Cereal bars	2.4	2.1	1.9	2.2	1.8
Bread	20.2	16.8	18.3	16.7	18.8
Fat spread	37.9	41.9	40.9	41.7	45.1
Drinks	9.5	11.2	15.3	18.1	21.1
Marmite	1.2	0.8	1.4	0.9	1.4

Data presented as % of participants that reported to have consumed specific fortified food group within each biomarker quintile. EGRac, erythrocyte glutathione reductase activation co-efficient (a higher EGRac ratio indicates lower riboflavin status)

Table 4 .General descriptive for each quintiles of all B-vitamin biomarker status

	Q1	Q2	Q3	Q4	Q5
RBC folate	***≤623***	623-8.25	825-1084	1084-1520	1520+
Age	74.2 (8.3)	73.7 (8.4)	74.0 (8.1)	73.1 (8.1)	74.7 (8.5)
Male n (%)	337 (35)	330 (34)	332 (34)	305 (32)	267 (28)
Age Finished education	15.8 (2.6)	15.9 (2.8)	16.3 (3.4)	16.2 (3.1)	16.1 (3.0)
CEDS	5.7 (8.4)	6.1 (7.7)	5.8 (7.0)	5.9 (7.3)	5.6 (6.6)
HADS	3.3 (3.8)	3.3 (3.7)	3.1 (3.5)	3.2 (3.7)	3.0 (3.5)
MMSE	26.9 (2.8)	27.2 (2.5)	27.1 (2.5)	27.2 (2.5)	27.1 (2.6)
Serum B12	***≤177***	***177-235***	***235-290***	***290-373***	***373+***
Age	78.0 (8.5)	74.4 (7.9)	73.7 (8.0)	73.2 (8.1)	73.6 (8.5)
Male n (%)	377 (37)	376 (37)	363 (36)	313 (31)	246 (24)
Age Finished education	15.7 (2.7)	16.0 (3.0)	15.9 (2.8)	16.1 (3.0)	16.5 (3.4)
CEDS	6.4 (7.8)	6.1 (7.6)	6.0 (7.4)	6.3 (7.7)	5.6 (6.9)
HADS	3.2 (3.6)	3.0 (3.6)	3.17 (3.7)	3.3 (3.8)	3.2 (3.7)
MMSE	26.9 (2.5)	27.0 (2.7)	27.2 (2.3)	27.2 (2.5)	27.1 (2.6)
Vitamin B6 (nmol/L)	**≤35.9**	**35.7-51.9**	**51.8-70.0**	**70.0-96.3**	**96.4+**
Age	76.4 (8.5	75.2 (8.3)	73.8 (8.2)	72.9 (8.0)	72.1 (7.6)
Male n (%)	334 (33)	348 (35)	362 (36)	338 (33)	295 (29)
Age Finished education	28.2 (6.4)	15.7 (2.7)	16.1 (3.0)	16.2 (3.0)	16.6 (3.5)
CEDS	7.5 (8.5)	6.6 (7.8)	5.9 (7.2)	5.5 (6.7)	5.0 (6.8)
HADS	3.5 (4.1)	3.2 (3.7)	3.1 (3.6)	3.0 (3.5)	3.1 (3.5)
MMSE	26.4 (3.1)	26.9 (2.6)	27.1 (2.4)	27.3 (2.4)	27.6 (2.3)
Riboflavin (EGRac)	***1.45+***	***1.34-1.45***	***1.27-1.33***	***1.20-1.26***	***≤1.19***
Age	73.0 (8.3)	73.2 (8.1)	73.8 (8.3)	74.5 (8.1)	75.5 (8.3)
Male n (%)	353 (36)	352 (34)	294 (32)	325 (32)	350 (31)
Age Finished education	15.7 (2.6)	16.0 (2.9)	16.0 (2.8)	16.2 (3.1)	27.6 (5.0)
CEDS	7.1 (8.4)	6.0 (7.6)	5.8 (7.2)	5.8 (7.0)	5.6 (7.0)
HADS	3.5 (4.1)	3.3 (3.7)	3.2 (3.5)	2.9 (3.4)	3.0 (3.6)
MMSE	26.9 (2.8)	27.2 (2.4)	27.1 (2.6)	27.1 (2.6)	27.1 (2.6)

Data presented as mean (SD) unless stated otherwise.

MMSE, Mini Mental State Examination; CES-D, Centre for Epidemiologic Studies Depression; HADS, Hospital Anxiety and Depression Scale

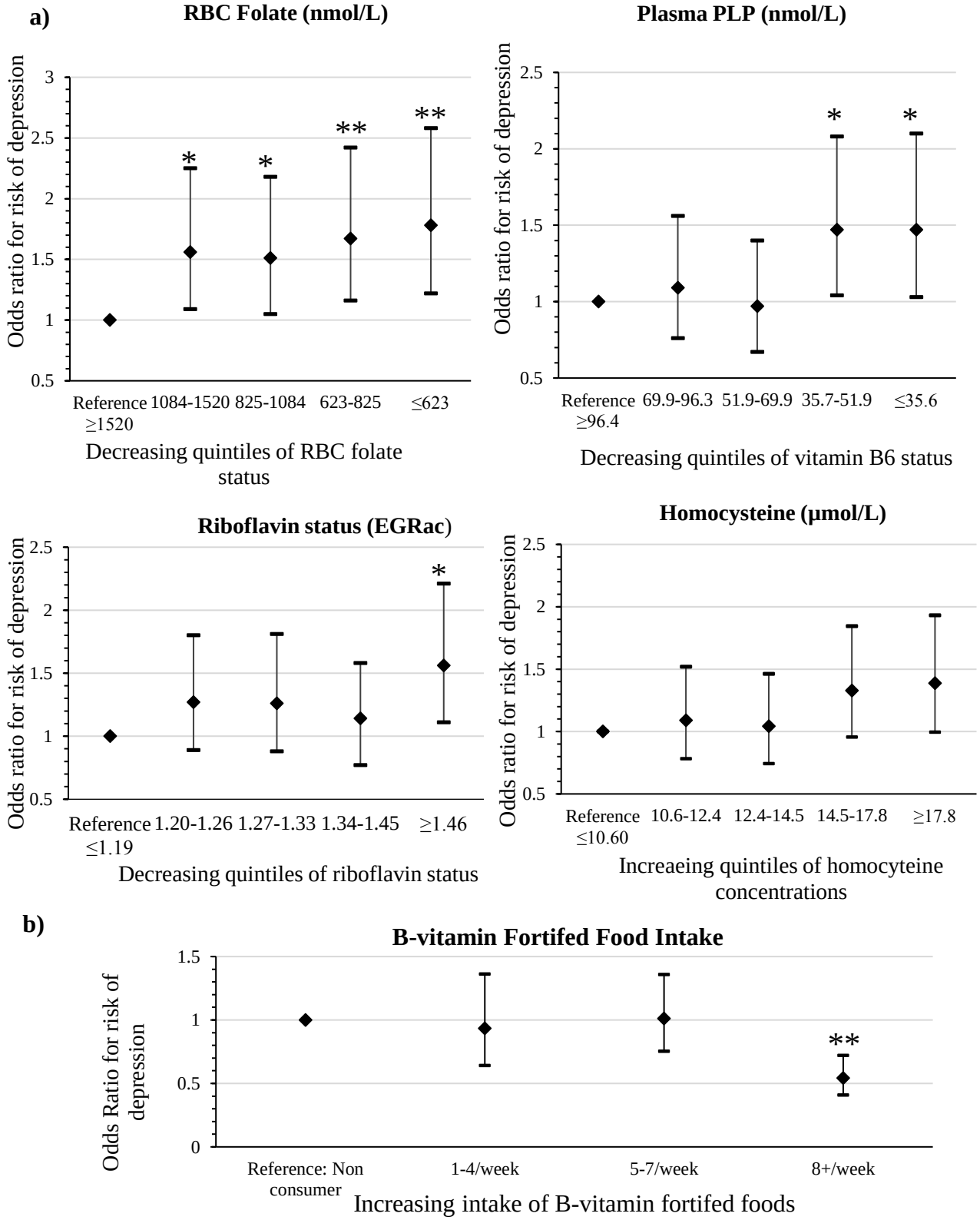

Figure. 2 Risk of depression in relation to B-vitamin a) biomarker status and b) fortified food intake [a]
RBC, red blood cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase co-efficient (Values are odds ratios for risk of CES-D score ≥16 with 95% CI relative to reference category), with adjustment for age, gender, anti-depressant drug usage, age finished education, vitamin supplement usage, smoking status, physical frailty (Timed up and go), living alone, area deprivation and transient ischaemic attack. *p < 0.05 **p < 0.01.

Chapter 4

B-vitamins and Brain Health in Older People (BrainHOP):

A randomised controlled trial (RCT) of B-vitamin

supplementation on neuropsychiatric performance.

Abstract

Background: Globally populations are ageing and mental health disorders, including dementia and depression, are reported as the leading cause of disability and ill health in older adults. Epidemiological and randomised trial evidence supports potential roles for folate and the metabolically related B-vitamins in preventing cognitive dysfunction and depression in ageing, but the evidence is inconsistent.

Objective: The objective was to investigate the effect of B-vitamin supplementation with folic acid and related B-vitamins over 2 years on cognitive function (primary outcome) and depression (secondary outcome) in older adults.

Design: A randomized controlled trial (RCT) was conducted over 2 years in adults aged 70 years and older. Participants who had previously taken part in the TUDA cohort study were invited to participate. Those eligible were randomised to receive daily, either placebo or a supplement containing folic acid (400 µg), vitamin B12 (10 µg), vitamin B6 (10 mg) and riboflavin (10 mg). Cognitive function was assessed before and after the 2-year intervention using the MMSE, FAB and the RBANS. Depression was assessed using the CES-D scale. Statistical analysis was conducted using SPSS, repeated measures ANCOVA was used to analyse the time × treatment interaction assessing the effect of treatment versus placebo over time, the main outcome of the trial.

Results: Of the 328 participants initially recruited (78 years; 44.8% male), 249 participants completed the intervention (74%). Results showed that neither frontal lobe nor global cognitive function (as measured by FAB and RBANS, respectively) showed a significant effect of B-vitamin intervention. However, when specific domains within global cognition (i.e. RBANS index scores) were examined separately, B-vitamin intervention was found to protect against visuospatial cognitive decline over the two year period, with pre and post intervention values of 98.8 (1.6) and 95.8 (1.7) for the placebo

group and 95.0 (1.7) and 99.2 (1.7) for treatment group (P = 0.001; Repeated Measures ANCOVA, time x treatment interaction). In the case of depression, although the effect of B-vitamin intervention on depression (i.e. CES-D score) did not reach statistical significance, rates of depression (i.e. % with CES-D score > 16) doubled over the 2-year period in the placebo group (from 4.0% to 8.0%) compared with a minimal increase in the B-vitamin group (from 4.0% to 4.8%).

Conclusion: Intervention with low dose B-vitamins (within the dietary range) resulted in beneficial effects on both visuospatial cognition and depression. Optimising B-vitamin status in older populations (through fortification or supplementation programs) may have important impacts on neuropsychiatric health and in turn help to preserve better quality of life in ageing.

Introduction

An estimated 23% of the global burden of disease arises in older people, with mental health disorders considered to be the leading cause of disability and ill health in ageing (Prince *et al.* 2015) and a significant global challenge of our time (United Nations Department of Economic and Social Affairs/Population Division 2015; Livingston *et al.* 2017). Dementia and depression are the most common of these disorders (World Health Organisation 2016) with predictions that dementia will affect over 131 million people by 2050 (Prince *et al.* 2016), whilst depression is anticipated to be the second leading cause of disability worldwide by 2020 (National Collaborating Centre for Mental Health 2010). The economic burden of mental health disorders is profound, with dementia estimated to be a trillion dollar disease (Prince *et al.* 2016) and response rates to antidepressant medications being typically poor (Rush *et al.* 2006). Considering such immense social and economic costs, there is an urgent need to identify modifiable factors for targeted interventions to help preserve better brain health in our ageing populations.

Evidence accumulated over many years suggests that folate and the metabolically related B-vitamins may play protective roles against depression (Carney 1967; Reynolds *et al.* 1970; Clarke *et al.* 1998; McCaddon 2006) and cognitive decline (Carney 1967; Reynolds *et al.* 1970; Clarke *et al.* 1998; McCaddon 2006) in older age. Epidemiological studies report that lower biomarker status of folate, vitamin B12 and vitamin B6 (or higher concentrations of the related metabolite Hcy) is associated with an increased risk of cognitive dysfunction (Smith and Refsum 2016; Porter *et al.* 2016) and depression (Hvas *et al.* 2004; Kim *et al.* 2008; Ng *et al.* 2009; Robinson *et al.* 2011). There is also evidence from randomised trials demonstrating the beneficial effects of B-vitamin supplementation on cognitive function (Durga *et al.* 2007; Smith *et al.* 2010), albeit the evidence is not entirely consistent (McMahon *et al.* 2006). One notable and rather controversial meta-analysis concluded that neither folic acid nor vitamin B12 had a beneficial effect on

cognition in older adults (Clarke *et al.* 2014), however, this paper was widely criticised, mainly as a result of the inclusion criteria used to select the trials for investigation, and thus the findings are in general not widely accepted by experts in this area (Garrard and Jacoby 2015; Smith *et al.* 2015). The strongest evidence to date to support the role of B-vitamins protecting cognition in ageing comes from the VITACOG trial, where intervention over 2 years with a combined high dose B-vitamin supplement (folic acid, vitamin B12 and vitamin B6) in patients with MCI was found, not only to improve cognitive performance (de Jager *et al.* 2012), but also reduced the rate of brain atrophy, as measured by MRI (Smith *et al.* 2010; Douaud *et al.* 2013). However, given that the VITACOG trial intervened with pharmacological doses of B-vitamins in MCI patients, it remains unknown whether B-vitamins at lower doses (i.e. within the dietary range) could be beneficial for cognitive function in apparently healthy (non MCI) older adults. In the case of depression, some randomised trial evidence suggests a beneficial effect of certain B-vitamins on depression (Almeida *et al.* 2010; Almeida *et al.* 2014). Although one meta-analysis concluded that neither folic acid nor vitamin B12 had a beneficial effect on depression, the number of published trials in depression for investigation was small, with a high degree of heterogeneity between studies (Clarke *et al.* 2014; Almeida *et al.* 2015).

Therefore in order to address the current gaps in the existing literature base, and to consider all the relevant B-vitamins within one-carbon metabolism, the aim of this study was to investigate the effect of B-vitamin supplementation with combined folic acid, vitamin B12, vitamin B6 and riboflavin over 2 years on cognitive function and depression in older adults. We hypothesised that B-vitamins will be beneficial in helping to maintain better cognitive function and improve depression over a 2-year period.

Methods

Participants

The B-vitamin and Brain Health in Older People (BrainHOP) trial was conducted in participants who had previously been recruited to the TUDA ageing cohort study (ClinicalTrials.gov Identifier: NCT02664584). As described in detail elsewhere, 5,186 community-dwelling adults aged ≥ 60 years were recruited to the TUDA study between 2008 and 2012 from Northern Ireland (NI) and the Republic of Ireland (RoI) (McCarroll *et al.* 2015; McCann *et al.* 2018). The TUDA study initially aimed to investigate the role of nutrition and lifestyle factors in the aetiology of common age-related diseases, namely, dementia, osteoporosis and CVD. A total of 2093 participants from the TUDA study cohort were recruited within Northern Ireland, of which 689 participants met inclusion criteria for the current trial. These were determined at the time of initial recruitment to the TUDA study and were as follows: aged ≥67 years old; not a user of B-vitamin supplement; MMSE score ≥21; normal renal function (Creatinine >130μmol/L) and plasma Hcy >12.05μmol/L. Reassessment for eligibility took place before being invited to participate in the BrainHOP trial and potential participants were excluded at this point if they had an MMSE <21 or were currently/recently on a B-vitamin supplement (**Figure 1**). The BrainHOP trial was carried out according to the principles expressed in the Declaration of Helsinki and ethical approval was granted from Office for Research Ethics Committees Northern Ireland (ORECNI) 08/NIR03/113 and each participant provided written informed consent.

The minimum sample size required for detecting an effect of B-vitamins on MMSE score was estimated to be 164 subjects in each treatment group (n = 328) accounting for a 20% dropout rate. The type 1 error rate was 0.05, with the desired power of 0.80 (Faul et al., 2007). This sample size calculations were based on the size of the effect of B-vitamin

supplementation on Mini-Mental State Examination (MMSE) score in older adults as previously reported (de Jager *et al.* 2012).

Study design and compliance

This study was conducted as a double-blinded placebo controlled randomised controlled trial (**Figure 1**). Participants who agreed to take part were stratified by baseline MMSE score and age and subsequently randomised within each stratum to receive daily for the 2 year intervention period either placebo or a low dose combined B-vitamin capsule (folic acid 400 µg, B12 10 µg, B6 10 mg and riboflavin10 mg) as provided by Healthspark Ltd, St Helier, Jersey. Once the supplements were delivered, independent analysis was conducted which confirmed that the supplements contained the target doses, albeit that there were small variations in the concentrations between the different companies (**Appendix Supplemental Table 1**). To ensure that the study was double-blinded, randomisation was conducted using the Minim software programme by the Human Interventions Studies Unit (HISU) Clinical Trials Manager at Ulster University Coleraine. The treatment and placebo were capsules administered daily and they were identical in size, shape, colour, smell, and taste and were packaged in identical blister packs. To maximise compliance, participants were provided with a 3-month supply of capsules (14 day, blister packed strips). Participants were asked to return all blister packs, including any untaken capsules, at 3 monthly intervals throughout the 2 years. Adherence to allocated treatment was measured by counting the number of tablets returned throughout the two years of the study.

Participants were invited to attend two 90 minute appointments at the start and end of the trial at a number of locations including: the Nutrition Innovation Centre for Food and Health (NICHE) at Ulster University, Coleraine; the Clinical Translational Research and Innovation Centre (CTRIC) at Altnagelvin Area Hospital or within the participants home.

At each sampling point biophysical, neuropsychiatric, cognitive, and functional assessments were completed and information on nutritional intake, demography, medical and social history was also obtained.

General health, lifestyle and biophysical measures

As described in detail elsewhere (McCarroll *et al.* 2015; McCann *et al.* 2018) Health and lifestyle information was gathered using a detailed, researcher-assisted, questionnaire and included information on age, smoking, alcohol, medical history, drugs including antidepressant medications and vitamin supplement use. Anthropometric measurements were recorded (including weight, height, waist and hip) and BP measurements were taken in accordance with standard operating procedures by trained researchers. In brief, two BP measurements were taken from the reference arm (the arm with the highest BP reading) with a 5-10 minute interval between each measurement; the mean of the two readings was used as the BP value. The TUG test was performed as a measure of functional mobility. Participants were asked to stand from a seated position (seat height approximately 46 cm), walk 3 m at their usual pace, turn around, walk back to the chair, and sit down. No physical assistance was given, and the time taken from command "Go" to complete the task was measured with a stopwatch (Podsiadlo and Richardson 1991), The functional abilities of the participants were also assessed using the PSMS and the IADL scale.

Neuropsychiatric assessment

Cognitive performance was assessed at both time points using 3 tests of cognition: the Folstein MMSE, the FAB and the RBANS. The MMSE is the most widely used screening tool in clinical settings worldwide for identifying cognitive impairment and dementia and evaluates global cognitive function by assessing the domains of orientation, registration, attention and concentration, recall and language. The maximum score achievable is 30,

with a score <25 indicating a possibility of cognitive impairment and a score <20 indicating dementia (Folstein *et al.* 1975). The FAB specifically assesses frontal lobe function, with a maximum score attainable of 18, with a score ≤15 indicative of cognitive impairment (López *et al.* 2014). RBANS assesses specific cognitive domains including immediate and delayed memory, visual-spatial, language, and attention, with a score <80 indicative of cognitive dysfunction (Radloff 1977).

Anxiety and depression were assessed using the CES-D scale and the HADS. The CES-D scale is a 20 item self-reported questionnaire, with a minimum score of 0 (no symptoms of depression) and maximum score of 60 (significant symptoms of depression). A score greater than or equal to 16 is generally indicative of clinical depression (Radloff and Locke 1986). HADS is a 7 item scale, with a minimum score of 0 (suggestive of no symptoms of anxiety) and a maximum score of 21 (significant anxiety). A score greater than or equal to 11 is generally indicative of probable anxiety (Zigmond and Snaith 1983).

Dietary assessment

Dietary intakes were assessed with the use of a 4-day food diary and a FFQ. This combined dietary method was previously validated at this Centre for the assessment of dietary intakes of folate, vitamin B12, vitamin B6 and riboflavin against each of their biomarkers (Hoey et al. 2007). The FFQ, designed specifically to investigate foods rich in the relevant B-vitamins including fortified foods, was completed by each participant. Details of brand names of the relevant products consumed were also obtained in order to establish the nutrient profile of any fortified foods. A 4-day food diary was completed by each participant in which all foods and beverages consumed over 2 week days and 2 weekend days were recorded at the time of consumption. Each participant received detailed oral and written instructions on how to complete the food diary and FFQ. Portion sizes were estimated using household measures such as bowls, spoons, etc., and counts

were taken for eggs and bread. The cooking method and brand names were also recorded. The 4-day food diaries were cross-checked using the FFQs to ensure the most accurate representation of the participants' normal diet was obtained; where inconsistencies occurred these were clarified with the participant in a follow-up telephone call.

To determine the nutritional content of the dietary records, the information from the crossed-checked food diaries was entered into a customised food composition database (Nutritics, Nutrition Analysis Software, v4.25 Research Edition, Dublin, Ireland). This software does not enable natural food folate to be distinguished from folic acid added to fortified foods by manufacturers, as it generates one value for total folate present in a food. The software was therefore customised in two ways as previously described (Hoey et al. 2007). Firstly, the software was customised to include folic acid as a new nutrient. This allowed dietary folate equivalents (DFEs) to be calculated (i.e. µg natural folate plus 1.7×µg added folic acid). Secondly, the database was updated to replace nutrient values with the most up-to date nutrient data for fortified food, obtained either from food companies where possible, otherwise the data were obtained from on-pack nutritional labelling.

Statistical analysis

All statistical analyses was performed using the SPSS statistical package for the social sciences (version 24.0, SPSS UK Ltd, Chersey, United Kingdom). Descriptive statistics are expressed as mean (SD), median (IQR) or adjusted means (SEM) throughout the article. Data were checked for normality and log transformed as appropriate. As dietary intakes of B-vitamins did not differ between treatment and placebo group at baseline, this was not controlled for within subsequent analyses. Differences in baseline characteristics between the treatment groups were analysed using independent t-tests for continuous variables and $\chi 2$ tests for categorical parameters. Response to intervention was conducted

only in participants who completed the intervention (n = 249). A change in either the cognitive function or depression scores (pre and post intervention) over the period of the study were examined using analysis of covariance (ANCOVA). When cognitive scores were being analysed the following were controlled for age, gender, baseline cognition, depression, anxiety, education, hypertension, hyperlipidaemia and smoking, while antidepressant medication usage was controlled for within the depression analysis. Repeated measures ANCOVA was used to analyse the time × treatment interaction assessing the effect of treatment versus placebo over time, the main outcome of the trial. The between-patient factor was the intervention group (placebo versus combined B-vitamin), and the within-patient factor was time (pre and post). Repeated measures ANCOVA was used to assess the change in depression scores over the 5 year period prior to the RCT. In all analyses, P values <0.05 were considered significant.

Results

A total of 328 participants were recruited from the TUDA ageing cohort study and were randomised to receive the combined B-vitamin supplement (*n* = 163) or placebo (*n* = 165) (**Figure 1**). There was a slightly higher proportion of female participants, with an average age of 78 years. Within the cohort there was a high rate of hypertension and (87%) and hyperlipidaemia (57%) and 16% suffered from diabetes (**Table 1**). Overall, the baseline characteristics of both the treatment arms were similar with the exception smoking, hyperlipidaemia and hypertension which showed small but significant differences between the two groups. The majority of the participants were B-vitamin fortified food consumers, with no significant difference in dietary intake between the treatment arms at baseline. Overall, 249 participants completed the study, with a 24% drop out rate in both arms (**Figure 1**). The participants who discontinued the intervention were similar to those that completed however those who did not complete the study had a lower IADL score, significantly higher rates of hyperlipidaemia, anxiety and depression than those that completed the intervention (**Appendix Supplemental Table 2**).

Overall, there was generally excellent compliance of participants with the intervention protocol (estimated by pill-counting to be 98.7%). There was no significant change in the global or frontal lobe cognitive function in response to B-vitamin supplementation over the 2 years (**Table 2**), as measured by MMSE *(P* = 0.337), RBANS (P = 0.105) and FAB (*P* = 0.959) respectively. B-vitamin supplementation however resulted in an improved visuospatial score by 3.4 points over the 2 year intervention period while those who received placebo declined by 2.4 points (*P* = 0.004). Likewise, when the response was examined using repeated measures ANCOVA with adjustment for relevant co-variates, a significant time × treatment interaction was observed for visuospatial score (*P* = 0.001), with pre- and post intervention values of 95.0 (1.7) and 99.2 (1.7) (treatment group) and

98.8 (1.6) and 95.8 (1.7) (placebo group) (**Table 3**). The responses detected in MMSE, FAB or in other RBANS index scores did not reach significance.

In the case of depression, although no significant time × treatment interaction was observed for depression in the cohort as a whole or when split by the median age (**Table 3**), rates of depression (i.e. % above cut point for clinical depression) doubled in the placebo group (from 4.0% to 8.0%) compared with a minimal increase in the B-vitamin group (from 4.0% to 4.8%) over the 2-year period (**Table 2**). Depression was also examined over a 7 year period in those who completed the intervention, i.e. by tracking depression data as recorded at the time of the original recruitment to the TUDA study (T1), to the start of the intervention (T2), to the completion of the intervention (T3). The results showed that whereas depression scores significantly increased over the 7 years period in those randomised to placebo ($P < 0.001$), no significant increase was observed in those randomised to B-vitamins, an effect that was particularly marked in older participants (>70 years) at time of randomisation (**Figure 2**).

Discussion

The BrainHOP trial is the first randomised trial to investigate the potential benefits of combined folic acid, vitamin B12, vitamin B6 and riboflavin supplementation on cognitive function and depression in generally healthy older adults. Low dose B-vitamin supplementation for 2 years in adults aged 78 years resulted in a significant beneficial effect on visuospatial cognitive function, but no effect on global cognition or frontal lobe function. In the case of depression, B-vitamin intervention was found to prevent the significant increase in depression observed in participants assigned to placebo, with rates of depression (i.e. % with CES-D score ≥16) doubling over the 2-year period in the placebo group compared with a minimal increase in the B-vitamin group.

B-vitamin supplementation over 2 years was found to significantly protect visuospatial cognitive function (measured using RBANS Index II); scores for this index increased by 3.4 in those provided with B-vitamins compared to 2.2 decline in participants on placebo. These results are broadly in agreement with the findings from two other large-scale B-vitamin intervention studies namely, the FACIT (Durga *et al.* 2007) and VITACOG (Smith *et al.* 2010) trials. Of particular note, the current results showing significant effects on visuospatial cognition are in line with findings from the VITACOG trial, which reported that supplementation with pharmacological doses of folic acid, vitamin B12 and vitamin B6, compared to placebo, stabilized executive function as measured by the CLOX tool (de Jager *et al.* 2012). Although the CLOX tool specifically measures executive function, it is similar to the visuospatial cognitive assessment conducted within the current study as part of the RBANS index II, with earlier studies noting significant correlations of CLOX 2 with more complex visuospatial tasks (Pena *et al.* 2008). The current study is the first to report a beneficial effect of B-vitamins on cognitive function in older adults without a diagnosis of cognitive impairment and using doses of B-vitamins within the dietary range; in contrast, the aforementioned VITACOG trial intervened in

patients diagnosed with MCI and used pharmacological doses of B-vitamins (de Jager *et al.* 2012).

In line with the findings from the current study, the VITACOG trial also reported no significant effect of B-vitamin supplementation on overall global cognitive function (as measured by MMSE) (de Jager *et al.* 2012) with the exception of those with higher Hcy concentrations (>11.3mmol/L) (de Jager *et al.* 2012), which would in turn reflect generally lower B-vitamin biomarker status. The biomarker analysis for the BrainHOP trial is currently underway and, once completed, pre-defined sub-group analysis based on B-vitamin biomarker results will be conducted in line with current literature and to address the hypothesis that participants with sub-optimal biomarker status at baseline will show greater benefits of B-vitamins intervention (de Jager 2014; Smith and Refsum 2016). Unlike the current study, the VITACOG trial also conducted brain imaging using MRI and showed that the rate of overall and grey matter brain atrophy was reduced in the B-vitamin intervention group compared with the placebo group over the 2 year intervention, an effect that was again dependant on baseline Hcy (Smith *et al.* 2010; Douaud *et al.* 2013).

The findings from the current intervention study, BrainHOP, together with the aforementioned studies VITACOG and FACIT, generally supporting a protective role of folate and the related B-vitamins on cognitive function in older adults, are in contrast with another similar RCT which did not find significant effects of B-vitamin intervention on cognition function over a 3 year period (McMahon *et al.* 2006). This discrepancy could be explained by the fact that the study cohort in the latter trial had an already optimal B-vitamin biomarker status, making it unlikely that further increases in B-vitamin biomarkers, as a result of intervention, would result in benefits on cognitive function. Furthermore, a recent meta-analysis of randomised trials concluded that "*B-vitamin supplementation had no significant effect on individual cognitive domains or global*

cognitive function or on cognitive aging" (Clarke *et al.* 2014), albeit the conclusions from this meta-analysis were not widely accepted by B-vitamins experts (Smith and Refsum 2016). One of the most significant limitations of the latter meta-analysis was in relation to the inclusion criteria used to select the trials, and that baseline cognitive assessments (an important covariate to be considered in the analysis of response to intervention) were not available for 76% of the participants included in the study (Smith *et al.* 2015; Garrard and Jacoby 2015). Since the publication of this meta-analysis there have been two further trials, one investigating folic acid and vitamin B12 supplementation (B-Vitamins for the PRevention Of Osteoporotic Fractures (B-PROOF); (van der Zwaluw *et al.* 2014)) and the other investigating vitamin B12 alone (Older People and Enhanced Neurocognitive function study; (Dangour *et al.* 2015)). Neither of these trials found significant effects of B-vitamins supplementation, albeit cognitive function was not the primary outcome for either trial. The B-PROOF trial did show a slower rate of global cognitive decline, but surprisingly this was attributed to chance (van der Zwaluw *et al.* 2014), and one notable limitation of the Dangour *et al* (2015) trial was the finding that cognitive scores in the placebo group did not decline. Of note, visuospatial cognitive function was not assessed in either trial, yet it has recently been identified to demonstrate significant diagnostic and prognostic potential for dementia (Salimi *et al.* 2018).

Over the 7 years observation of the BrainHOP cohort, intervention with B-vitamins for the last 2 years was found to prevent the significant increase in depression which occurred in participants assigned to placebo. Furthermore, rates of depression (i.e. % with CES-D score ≥16) doubled over the 2-year intervention period in the placebo group compared with a minimal increase in the B-vitamin group. These findings are somewhat in line with findings from the VITAmins TO Prevent Stroke-DEPression (VITATOPS-DEP trial; n 273, 63 years)(Almeida *et al.* 2010), which reported a non-significant trend towards a reduction in odds of depression in those randomised to high dose B-vitamins. In contrast,

however, a recent RCT in over 4331 older females found no significant reduction in the risk of depression in response to 7 years of combined B-vitamin intervention (Okereke *et al.* 2015). However, this study was conducted in female health professionals in the United States, where mandatory folic acid fortification of flour has been in place since 1996, perhaps suggesting that optimal folate biomarkers may have limited the opportunity for improving B-vitamin status and thus influencing rates of depression. Of note, systematic reviews and meta-analyses in this area which concluded that B-vitamin intervention may delay the onset of clinically significant symptoms in people with existing or at risk of depression, have focused on the use of B-vitamins as a treatment rather than a preventive measure for depression (Taylor *et al.* 2004; Almeida *et al.* 2015).

The biological mechanisms explaining the current and previous results linking folate and metabolically related B-vitamins with cognitive function and depression are likely related to their crucial roles in one-carbon metabolism. Douaud *et al.* (2013) proposed increased atrophy may be as a result of elevated plasma Hcy, which in turn has been suggested to increase phosphorylated Tau which is associated with greater deposition of neurofibrillary tangles and thus increased atrophy (Zhang *et al.* 2008). Furthermore, folate and related B-vitamins act as co-factors in DNA synthesis and repair, amino acid metabolism and methylation reactions, including the remethylation of Hcy to methionine and subsequent generation of SAM. SAM, the universal methyl donor, is involved in the methylation of DNA, phospholipids, proteins and neurotransmitters, thus reduced status of one or more of the B-vitamins involved in one-carbon metabolism may impair methylation processes. The inhibition of methylation reactions may, in turn, influence cognitive function in a number of ways, by perturbing the regulation of gene expression in the Beta amyloid pathway, by reducing the activity of protein phosphatase-2A or by impairing the formation of phosphatidylcholine enriched omega-3 fatty acids (Smith and Refsum 2016). Additionally, reduced tissue concentration of SAM may be linked to

depression through perturbing monoamine (serotonin, dopamine and noradrenaline) synthesis and methylation (Bottiglieri *et al.* 2000). In addition, folate has a role in the synthesis of tetrahydrobiopterin (BH4), an essential co-factor in monoamine synthesis (Bottiglieri *et al.* 1992). Furthermore, the active form of vitamin B6 (PLP) is the cofactor for aromatic L-amino acid decarboxylase in the tryptophan serotonin pathway, thus suboptimal B6 status may lead to reduced concentrations of serotonin and thus increased risk of depression (Hensler 2006). Lastly, as previously noted, riboflavin in the form of FMN is required as a coenzyme for the activation of B6 in tissues and so its relationship with depression could be mediated through a vitamin B6 dependent pathway.

Considering the protective effects on cognitive function following supplementation with B-vitamins observed in this study, and in earlier work from the VITACOG and FACIT trials (Durga *et al.* 2007; de Jager *et al.* 2012), the impact of optimising B-vitamin biomarker status at a public health level could be considerable. It has been estimated that a 5-year delay in the onset of dementia could reduce the population prevalence by 50% (Livingston *et al.* 2017), thus improving B-vitamin status could have an important impact on dementia prevention at a societal level. Tsiachristas and Smith (2016) conducted a decision model to calculate the lifetime costs and quality-adjusted life years of providing B-vitamin treatment to people in the UK over 60 years with plasma Hcy >13 mmol/L. It was estimated that B-vitamin supplementation would save £60,021 per quality-adjusted life year gained and could thus be highly cost-effective (Tsiachristas and Smith 2016). Optimising B-vitamin biomarker status in older populations could also potentially be achieved (perhaps more efficiently) via food fortification, as research has highlighted that regular consumption of B-vitamin fortified products can significantly benefit biomarker status (Hoey *et al.* 2007; Hopkins *et al.* 2015), however further work in this area in the form of an RCT using such food products is required.

The main strength of the BrainHOP study is that it is a randomised trial and therefore has the ability to investigate potential causal links between B-vitamins and cognitive function and depression in older adults. Furthermore, participants were recruited based on having prior Hcy concentrations suggestive of lower B-vitamin status and were at an age where cognitive decline was likely to occur (Murman 2015). Also, rather than relying on assessments of global cognitive function alone, the battery of cognitive assessments conducted within the study allowed for the analysis of both global cognition as well as a variety of sub-domains, which have been found to be sensitive to the ageing process (Howieson *et al.* 1993). The current study provided all four B-vitamins involved in one-carbon metabolism, thus fully optimising this network of metabolic pathways. Finally, the B-vitamin intervention lasted for a period of 2 years thus the likelihood of detecting changes in cognitive function was greater. This study was not however without limitations. The BrainHOP cohort were generally a healthy, well educated, older cohort and over 78% consumed fortified food products, thus the effect of B-vitamin supplementation on cognition and depression might be less than in other populations where lower levels of education are achieved and voluntary fortification is restricted. With regards to depression, the CES-D scale used in this study to assess depression, while widely considered to have an acceptable screening accuracy in primary care settings, is not as robust as other diagnostic instruments and this may have limited the interpretation of the current findings to some extent (Vilagut *et al.* 2016). Furthermore, the primary outcome of the current study was cognitive function (with sample size calculations based on MMSE scores) therefore, it is likely that the current study was underpowered to detect significant effects of B-vitamins on depression which was a secondary outcome. A final limitation of the BrainHOP trial at this time is that the biomarker results have not been included, as the analysis is currently ongoing.

In conclusion, the BrainHOP trial presents the first evidence from an RCT that supplementation with folic acid, vitamin B12, vitamin B6 and riboflavin within dietary ranges can protect against cognitive decline in healthy older adults, specifically in relation to visuospatial cognition. Results from this study appear to suggest that B-vitamin supplementation may also have a role in reducing the risk of depression in older age. The current findings are supported by the known biological mechanisms of folate and related B-vitamins within one-carbon metabolism and suggest that optimising B-vitamin status in older adults may contribute to reducing the significant social and economic global burden related to declining brain health in ageing. Further biomarker analysis from this trial will help to interpret the current findings more fully, while future trials which include objective brain imaging techniques will enable a fuller understanding of the biological and clinical nature of relationships of B-vitamins with brain health.

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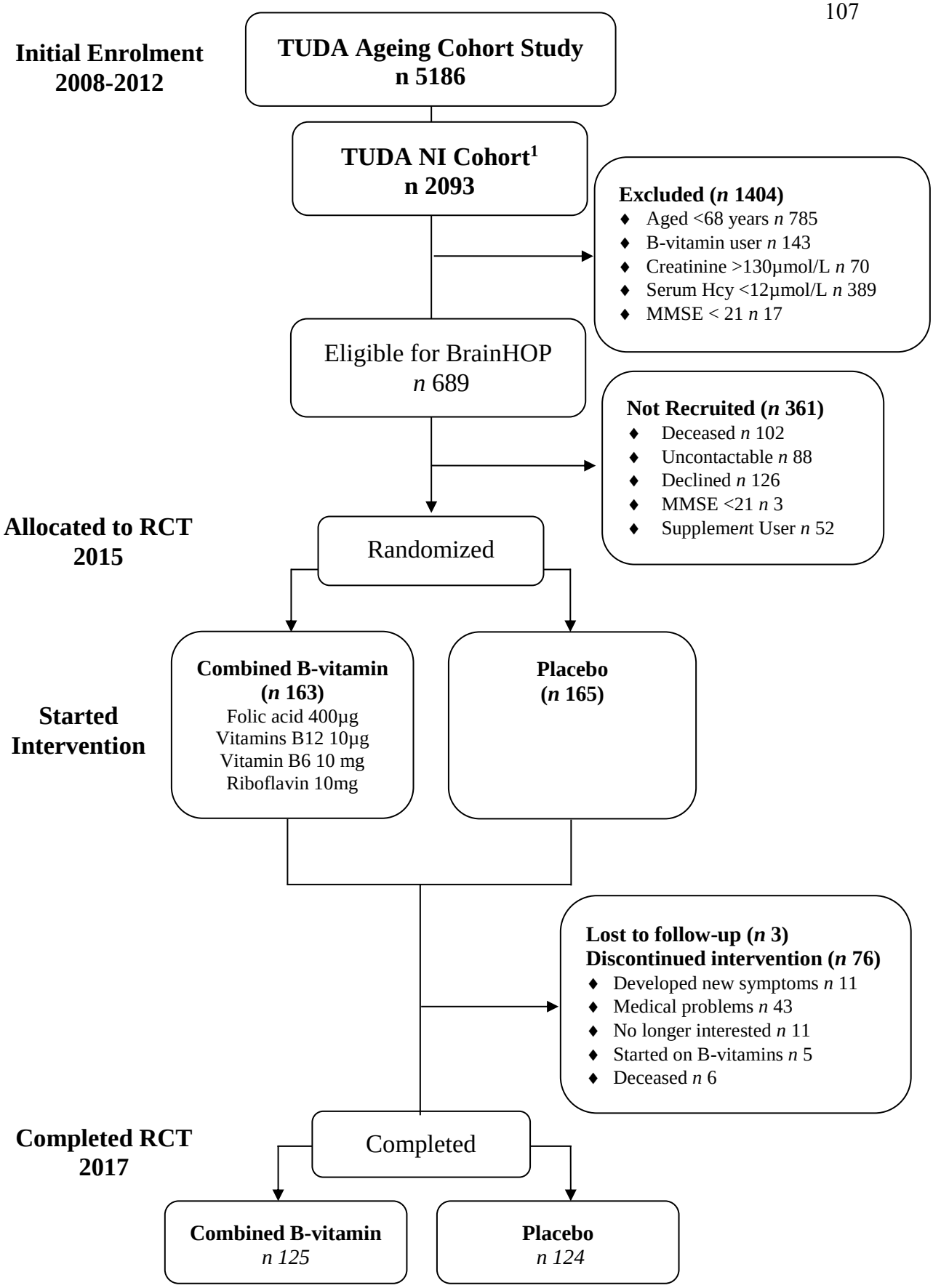

Figure 1. CONSORT flow diagram of participants from the BrainHOP trial
[1] Recruitment was from GP clinics in the Northern and Western Health and Social Care Trusts, Northern Ireland. **Abbreviations:** TUDA, Trinity Ulster Department of Agriculture; MMSE, Mini-Mental State Examination; Hcy, Homocysteine

Table 1. General characteristics of BrainHOP trial participants at baseline

	Total ***n*** **328**	**Placebo** ***n*** **165**	**B-vitamin** ***n*** **163**	***P****
Age (year)	78.0 (4.5)	78.2 (4.7)	77.9 (4.2)	0.607
Gender (male) n (%)	147 (44.8)	80 (48.5)	67 (51.1)	0.218
Education (years)	16.6 (3.1)	16.7 (3.3)	16.4 (2.8)	0.454
Area deprivation (most deprivation) n (%)	35 (10.7)	19 (11.5)	16 (9.8)	0.487
Health and Lifestyle				
BMI (kg/m^2)	29.3 (4.9)	29.2 (4.7)	29.4 (5.1)	0.713
Waist to Hip ratio	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.256
Timed Up and Go (seconds)	11.3 (6.6)	11.4 (8.5)	11.3 (4.0)	0.456
Hand Grip Strength (kg)	24.4 (10.5)	25.1 (10.6)	23.7 (10.5)	0.241
PSMS score	22.9 (1.9)	23.1 (1.9)	22.8 (1.9)	0.093
IADL score	26.4 (2.6)	26.6 (2.5)	26.3 (2.7)	0.340
Living alone *n* (%)	93 (28.4)	45 (27.3)	48 (29.4)	0.738
Current Smoker *n* (%)	17 (5.2)	14 (8.5)	3 (1.8)	**0.014**
Alcohol (units/week)	4.7 (8.4)	4.8 (7.9)	4.7 (8.9)	0.682
Medical				
Diabetes *n* (%)	53 (16.2)	25 (15.2)	28 (17.2)	0.727
Hyperlipidaemia *n* (%)	188 (57.3)	105 (63.6)	83 (51.0)	**0.027**
Hypertension *n* (%)	285 (86.9)	136 (82.4)	149 (91.4)	**0.025**
Previous MI n (%)	37 (11.3)	21 (12.7)	16 (9.8)	0.510
Previous TIA n (%)	34 (10.4)	17 (10.3)	17 (10.4)	1.000
Previous Stroke *n* (%)	12 (3.7)	8 (4.8)	4 (2.5)	0.389
Dietary intake				
Fortified Food n (%)	256 (78)	127 (77)	129 (79.1)	0.508
Energy (MJ/d)	7.5 (1.8)	7.3 (1.8)	7.7 (1.8)	0.134
DFE[§] (µg/d)	310 (108)	306 (114)	313 (103)	0.954
Vitamin B-12 (µg/d)	5.0 (2.0)	4.8 (2.1)	5.1 (2.0)	0.894
Vitamin B-6 (mg/d)	1.9 (0.6)	1.9 (0.7)	1.9 (0.6)	0.517
Riboflavin (mg/d)	1.7 (0.5)	1.6 (0.5)	1.7 (0.5)	0.212

Data presented as means (SD), unless otherwise indicated.

*Differences between treatment groups was assessed using independent sample t-tests on log transformed data where required, categorical variables were assessed using chi square test, $P < 0.005$ was considered significant.

§ Dietary Folate Equivalents were calculated as µg natural folate plus 1.7 µg × added folic acid.

Abbreviations: BrainHOP, B-vitamin and Brain Health in Older People; PSMS, Physical Self Maintenance Score; IADL, Instrumental Activities of Daily Living; MI, Myocardial infarction; TIA, Transient Ischemic Attack; DFE, Dietary Folate Equivalents.

Table 2. Change in cognitive function and depression after 2 years of B-vitamin supplementation

	Placebo *n* 125			**B-vitamin *n* 124**		
	Pre	Post	*Change*	Pre	Post	*Change*
Cognition						
MMSE						
Impairment n (%)[§]	4 (3.3)	9 (7.4)	*5 (4)*	11 (8.9)	10 (8.7)	*-1 (0.8)*
FAB						
Impairment n (%)[§]	76 (60.8)	86 (68.8)	*10 (8)*	66 (53.2)	68 (58.1)	*2 (1.6)*
RBANS						
Impairment n (%)[§]	25 (20.0)	19 (15.2)	*-6 (4.8)*	19 (15.3)	16 (12.9)	*-3 (2.4)*
Depression						
Depression n (%)[§]	5 (4.0)	10 (8.0)	*5 (4)*	5 (4.0)	6 (4.8)	*1 (0.8)*

Data presented as n (%).[§]Following cut-offs for cognitive impairment and depression were used: MMSE <25, FAB ≤15, RBANS <80, CES-D ≥16.

Abbreviations: BrainHOP, B-vitamin and Brain Health in Older People; MMSE, Mini Mental State Examination; CES-D, Centre for Epidemiologic Studies Depression; FAB, frontal Assessment Battery; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

Table 3. Response in cognitive function and depression to B-vitamin intervention over 2 years.

	Placebo n 125	**B-vitamin n 124**	**P***
Cognition			
MMSE score			
Pre	27.8 (0.2)	27.4 (0.2)	
Post	27.7 (0.2)	27.3 (0.2)	0.922
FAB score			
Pre	14.7 (0.2)	15.1 (0.2)	
Post	14.4 (0.2)	14.7 (0.2)	0.485
RBANS score			
Pre	93.3 (1.3)	93.4 (1.3)	
Post	95.5 (1.4)	97.8 (1.4)	0.117
Specific Domains			
Immediate Memory			
Pre	94.4 (1.4)	96.3 (1.4)	
Post	96.6 (1.5)	98.0 (1.5)	0.857
Visuospatial			
Pre	98.8 (1.6)	95.0 (1.7)	
Post	95.8 (1.7)	99.2 (1.7)	**0.001**
Language			
Pre	92.5 (1.0)	92.9 (0.9)	
Post	101.0 (1.0)	100.8 (1.0)	0.760
Attention			
Pre	95.8 (1.5)	97.2 (1.5)	
Post	95.3 (1.5)	96.9 (1.6)	0.948
Delayed memory			
Pre	93.8 (1.7)	93.9 (1.7)	
Post	95.3 (1.6)	96.3 (1.6)	0.602
Depression, CES-D score			
Pre	5.3 (4.5)	4.8 (0.5)	
Post	6.2 (0.6)	5.4 (0.6)	0.855
Younger (<77.0 years[¥])			
Pre	5.2 (0.6)	3.9 (0.6)	
Post	5.3 (0.7)	4.6 (0.7)	0.767
Older (≥77.0 years[¥])			
Pre	5.4 (0.7)	5.6 (0.7)	
Post	7.1 (0.9)	6.1 (0.9)	0.555

Data expressed as adjusted mean (SEM).

*Time × treatment interaction (repeated measures ANCOVA, comparing the effect of treatment vs placebo over time, controlling for age, education, depression, anxiety, hyperlipidaemia, hypertension and smoking status. Depression analysis was controlled for anti-depressant usage.

[¥] Median age pre-intervention.

Abbreviations: BrainHOP, B-vitamins and Brain Health in Older People; MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status CES-D, Centre for Epidemiologic Studies Depression

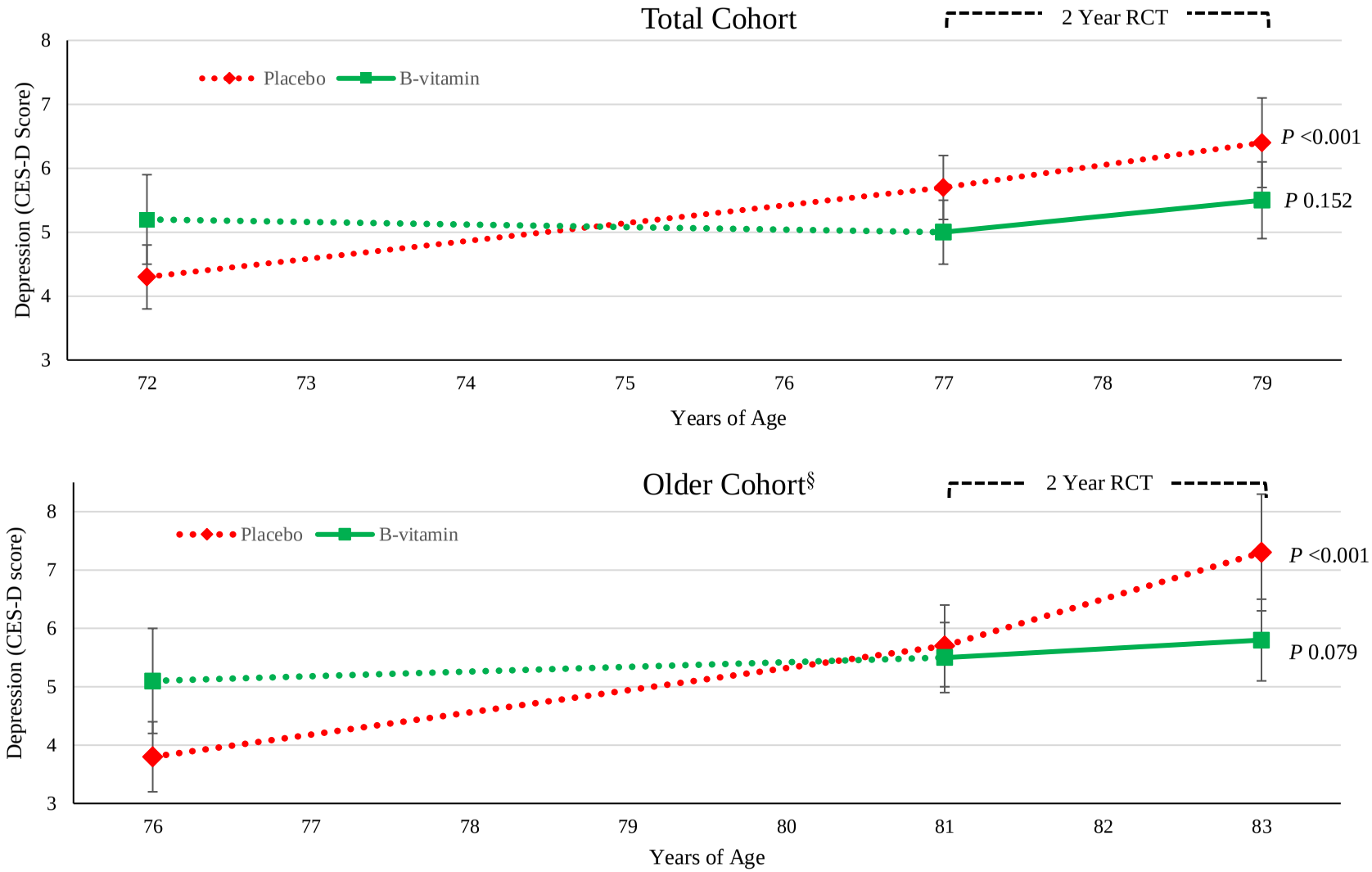

Figure 2. Depression score in BrainHOP participants over a 7 year period, including a 2 year RCT
Values are CES-D score presented as adjusted means (SEM)
§The older cohort (lower plot) was identified as age >median value of 77 years at start of RCT.

Appendix Supplemental Table 1. Manufacturing and analysis details of BrainHOP supplement

	Manufacturing		Analysis						
	Requested Dose	**Healthspark Pre-manufacturing formulation**	**Eurofins**	*% of Formulation*	**ALS**	*% of Formulation*	**Campden BRI**	*% of Formulation*	**Average**
Folic acid (µg)	400	460	375	*(81.5)*	440	*(95.7)*	572	*(124.3)*	**407.5**
B12 (µg)	10	12	9.74	*(81.2)*	10.3	*(85.8)*	6.8	*(56.7)*	**10.02**
B6 (mg)	10	13.65	18.7	*(137.0)*	12.0	*(87.9)*	9.96	*(73.0)*	**15.35**
Riboflavin (mg)	10	11.22	16.1	*(143.5)*	12.7	*(113.2)*	6.8	*(60.6)*	**14.4**

Manufacturer:
Healthspark Ltd: Healthspark Lts, Maxwell Chambers, La Colomberie, St Helier, Jersey, JE2 4QB
Analysis:

- Eurofins Food Testing UK Ltd: I54 Business Park, Valiant Way, Valiant Way, Wolverhampton, WV9 5GB
- ALS: ALS Food and Pharmaceutical, Medcalfe Way, Bridge Street, Chatteris, PE16 6QS
- Campden BRI: Campden BRI (Chipping Campden) Ltd, Station Road, Shipping Campden, Gloucestershire, GL55 6 LD

Appendix Supplemental Table 2. General characteristics of BrainHOP trial participants who completed and dropped out

	Total *n* 328	**Completed n 249**	**Drop-out n 79**	***P***
Age (year)	78.0 (4.5)	77.9 (4.4)	78.4 (4.8)	0.427
Gender (male) n (%)	147 (44.8)	117 (53.0)	31 (60.8)	0.282
Education (years)	16.6 (3.1)	16.6 (3.2)	16.3 (2.7)	0.503
Area deprivation (most deprivation) n (%)	35 (10.7)	26 (11.5)	9 (11.8)	0.886
Health and Lifestyle				
BMI (kg/m^2)	29.3 (4.9)	29.1 (4.8)	29.7 (5.0)	0.366
Waist to Hip ratio	0.94 (0.08)	0.94 (0.08)	0.93 (0.07)	0.296
Timed Up and Go (seconds)	11.3 (6.6)	10.9 (3.3)	12.8 (12.1)	0.056
Hand Grip Strength (kg)	24.4 (10.5)	24.5 (10.1)	24.2 (11.9)	0.683
PSMS score	22.9 (1.9)	23.0 (1.8)	22.8 (2.2)	0.834
IADL score	26.4 (2.6)	26.6 (2.5)	26.0 (2.8)	**0.033**
Living alone *n* (%)	93 (28.4)	70 (28.1)	23 (29.1)	0.863
Current Smoker *n* (%)	17 (5.2)	10 (4.0)	7 (8.9)	0.161
Alcohol (units/week)	4.7 (8.4)	4.6 (8.4)	5.1 (8.2)	0.837
Medical				
Diabetes *n* (%)	53 (16.2)	40 (16.1)	13 (16.5)	0.934
Hyperlipidaemia *n* (%)	188 (57.3)	133 (53.4)	55 (69.6)	**0.011**
Hypertension *n* (%)	285 (86.9)	216 (86.7)	69 (87.3)	0.891
Previous MI n (%)	37 (11.3)	24 (9.6)	13 (16.5)	0.143
Previous TIA n (%)	34 (10.4)	21 (8.4)	13 (16.5)	0.068
Previous Stroke *n* (%)	12 (3.7)	11 (3.4)	1 (0.3)	0.339
Cognition				
MMSE score	27.4 (2.0)	27.6 (1.9)	27.2 (1.9)	0.110
% impaired (MMSE <25)	33 (10.1)	15 (6.1)	8 (10.4)	0.208
FAB score	14.9 (2.2)	14.8 (2.1)	14.8 (2.2)	0.792
% impaired (FAB ≤15)	191 (58.2)	142 (57.0)	49 (62.0)	0.513
RBANS score	93.0 (14.5)	93.6 (14.3)	91.3 (15.2)	0.228
% impaired (RBANS <80)	63 (19.2)	44 (17.7)	19 (24.1)	0.276
Depression score	5.3 (5.1)	5.1 (5.0)	6.96 (7.2)	0.059
% depressed (CES-D ≥16)	20 (6.1)	10 (4)	10 (12.7)	**0.012**
Anxiety score	2.8 (2.7)	2.7 (2.7)	3.2 (2.1)	0.292
% anxious (HADS ≥11)	9.0 (2.7)	5 (2.0)	4 (5.1)	**0.026**

Data presented as means (SD), unless otherwise indicated. Differences between treatment groups was assessed using independent sample t tests on transformed data where required, categorical variables were assessed using chi square test, $P < 0.005$ was considered significant**.**

Abbreviations: BrainHOP, B-vitamin and Brain Health in Older People; PSMS, Physical Self Maintenance Score; IADL, Instrumental Activities of Daily Living; MI, Myocardial infarction; TIA, Transient Ischemic Attack; MMSE, Mini Mental State Examination; CES-D, Centre for Epidemiologic Studies Depression; FAB, frontal Assessment Battery; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; HADS Hospital Anxiety and Depression Scale.

Chapter 5

An investigation of the role of B-vitamins in brain function using Magnetoencephalography (MEG): a pilot study

Abstract

Background: Evidence, in the form of epidemiological studies and randomised trials, indicates that B-vitamins can help maintain better cognitive health in ageing. The majority of previous studies have used questionnaire-based assessment of neuropsychiatric function. To our knowledge, no study has assessed the impact of B-vitamins on brain function using Magnetoencephalography (MEG), the brain imaging technique with the highest temporal resolution.

Objective: The aim of this study was to investigate the role of B-vitamins in brain function as measured using MEG in older adults.

Design: Participants (*n* 25; 79.1 years) were healthy older adults who had just completed the B-vitamins and Brain Health in Older People (BrainHOP) randomised trial, and had received either, a daily combined B-vitamin supplement (folic acid; 400µg, vitamin B12; 10 µg, vitamin B6; 10mg and riboflavin; 10mg), or placebo, for 2 years. MEG was conducted in both groups at the end of the 2 year trial, using a working memory task and during resting state.

Results: The B-vitamin group achieved higher accuracy in the working memory task: 92.4% vs 76.7% for B-vitamin and placebo groups respectively (*P* 0.041). During resting state, MEG results showed better overall neuronal functioning in the B-vitamin group, as demonstrated by lower power in the lower frequencies [1-8 Hz]. In addition, participants that had received B-vitamin supplements for 2 years also had significantly lower power in the Delta [1-4 Hz] (*P* 0.021) and Theta [4-8 Hz] (*P* 0.011) bands, following interruption within the working memory task, suggesting better interference resolution compared with the placebo group.

Conclusion: Findings from this MEG analysis, although preliminary, suggest that better B-vitamin status is associated with improved neuronal functioning. If confirmed these

results will provide robust evidence of the role of B-vitamins in brain functioning. In addition, these findings demonstrate the potential of this novel imaging technique in future nutritional investigations.

Introduction

Normal ageing is associated with alterations in the volume, structure, metabolic and neural processes within the brain (Peters 2006; Bishop *et al.* 2010). Furthermore, cognitive function declines with age, ranging in severity from MCI to dementia, with up to 50% of those with MCI going on to develop dementia within 5 years (Gauthier *et al.* 2006). Dementia, a trillion dollar disease, currently affects 46.8 million people worldwide and is projected to affect over 131 million people by 2050 (Prince *et al.* 2016). Dementia is a complex condition of multifactorial causation (Smith 2008) with limited treatment strategies, therefore the identification of potential modifiable risk factors is a major global health priority (Livingston *et al.* 2017).

Nutrition has emerged as an important area of interest for cognitive impairment disorders and there is strong evidence to support the protective roles of certain dietary patterns and specific dietary components including B-vitamins, omega-3 PUFAs, polyphenols and vitamin D in brain health in ageing (Moore *et al.* 2018). Investigations into the influence of these nutritional factors on the ageing brain are challenging and are conducted most commonly using questionnaire-based assessments of cognitive function, however, this can be limited primarily owing to the reported Ceiling and Floor effects of the various tests.

A European workshop entitled ‘Methodologies to assess long-term effects of nutrition and brain function’ in 2009 concluded among the recommendations that brain imaging biomarkers should be included as secondary endpoints to clinical and cognitive measures (de Jager and Kovatcheva 2010). Furthermore a recent European Commission JRC report (Mak and Caldeira 2014), identified as a major priority multidisciplinary research into nutrition and brain health in older people. Neuro-imaging techniques provide an objective and highly robust means of assessing brain structure, function and potentially, response

to nutrition interventions (Sizonenko *et al.* 2013). However, only recently have these techniques been utilised to advance nutrition research in ageing. The use of MRI, functional MRI and Diffusion Tensor Imaging have significantly enhanced the evidence-base supporting protective roles for polyunsaturated fatty acids, polyphenols and vitamin D in brain health in ageing (Selnes *et al.* 2013; Brickman *et al.* 2014; Hooshmand *et al.* 2014). However, the strongest evidence to date comes from the VITACOG trial, which incorporated MRI techniques in an investigation of the effect of B-vitamin supplementation in patients with MCI. This trial of high-dose folic acid, vitamin B12 and vitamin B6 showed improved cognitive performance (de Jager *et al.* 2012) and a 30% reduction in brain atrophy (Smith *et al.* 2010), most specifically in regions identified as vulnerable in Alzheimer's disease (such as the "parieto–medial temporal pathway", hippocampus, parahippocampal gyrus, inferior parietal lobule and retrosplenial cortex). These specific brain regions have been highlighted in earlier reports to play a role in visuospatial functioning (Kravitz *et al* 2011(Douaud *et al.* 2013). These brain imaging results are ground-breaking, as they provide for the first time, objective cause and effect evidence to support the protective role of B-vitamins on brain health. MRI however, does have limitations including its relatively low temporal resolution and the fact that functional MRI relies on hemodynamic response and therefore provides only an indirect measure of neural activity (Zamrini *et al.* 2011). Both EEG and MRI studies have identified significant associations between measures of neuronal function and brain volumes (Grunwald *et al.* 2001(Grunwald *et al.* 2001; Babiloni *et al.* 2009).

Magnetoencephalography (MEG) is a relatively new neuroimaging technique, which is completely non-invasive and measures the magnetic fields that are generated by neuronal activity, and thus directly measures oscillatory (neural) activity and is reported as power (or oscillation) across the frequency (Hz) spectrum, including at specified bands delta ([1 4] Hz), theta ([4 8]Hz), alpha ([8 12] Hz), beta ([12 30] Hz) and gamma ([30 60] Hz)

(**Figure 1**). MEG provides a four-dimensional measurement of the brain- space, time, frequency and connectivity, allowing for a better description of brain function (Zamrini *et al.* 2011) and potentially improved understanding of the effect of nutrients on brain health. In order to achieve 4D accuracy, an additional neuro-imaging technique such as MRI is required to align the data with anatomical correlates (López *et al.* 2014)(review in here). MEG is somewhat similar to EEG however EEG measures electric signals and MEG has higher temporal and spatial resolution. Research to date has highlighted MEG potential ability to predict the development of dementia and MCI from normal cognitive function (Maestú *et al.* 2006; Fernández *et al.* 2006).

Confirmation of the protective role of B-vitamins in brain health, would greatly strengthen the findings of the BrainHOP trial as reported in this thesis (Chapter 4). Conducting MEG imaging in participants with optimal and sub optimal B-vitamin status would enable a more in-depth understanding of the potential protective role of folate and the metabolically related B-vitamins in brain function. The aim therefore was to investigate the role of B-vitamins in brain function using MEG in older adults.

Methods

Participants

Participants were recruited from the B-vitamin and Brain Health in Older People (BrainHOP) trial which was conducted in participants who had previously been recruited to the TUDA ageing cohort study (ClinicalTrials.gov Identifier: NCT02664584) (McCarroll *et al.* 2015; McCann *et al.* 2018). This study was conducted as a double-blinded, placebo-controlled, randomised controlled trial as described in **Chapter 4**. For this pilot study, a sample size of 48 was estimated, however this chapter presents the preliminary findings of the first 25 participants who have completed the study. Reassessment for eligibility took place once participants had completed the 2 year intervention period where they had received either placebo or a low dose combined B-vitamin capsule (folic acid 400 µg, B12 10 µg, B6 10 mg and riboflavin10 mg) as provided by Healthspark Ltd, St Helier, Jersey. Participants willing to take part were invited to participate in the BrainHOP MEG pilot study if they did not have any ferromagnetic implants (which would affect the sensitive sensors) (**Figure 2**). All participants were right handed native English speakers. The BrainHOP MEG pilot study was carried out according to the principles expressed in the Declaration of Helsinki and ethical approval was granted from Office for Research Ethics Committees Northern Ireland (ORECNI) 08/NIR03/113 and each participant provided written informed consent.

Participants completed their 90 minute appointment at the end of the 2 year trial at the Clinical Translational Research and Innovation Centre (CTRIC) at Altnagelvin Area Hospital and were invited to a 120 minute appointment in the Northern Ireland Functional Brain Mapping (NIFBM) Facility of the Intelligent Systems Research Centre (ISRC), Ulster University, Derry/Londonderry, UK. Biophysical, neuropsychiatric, cognitive, and functional assessments were completed and information on nutritional intake,

demography, medical and social history were obtained at their first 90minutes appointments.

General health, lifestyle and biophysical measures

As described in detail in Chapter 4, health and lifestyle information was gathered using a detailed, researcher-assisted questionnaire and anthropometric measurements were recorded. The functional abilities of the participants were also assessed using the Physical Self-Maintenance Scale (PSMS) and the Instrumental Activities of Daily Living (IADL) scale.

Neuropsychiatric assessment

As described in Chapter 4 cognitive performance was assessed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph 2010).

Experimental paradigm

The stimuli utilised within this experimental paradigm were kindly provided by Prof Maestú's team from The Laboratory of Cognitive and Computational Neuroscience (LNCyC) which is an interdisciplinary research group at the Center for Biomedical Technology of Madrid. It consisted of neutral unnamed male and female faces across a wide age profile. To avoid non-face specific cues affecting the participant's responses, the presented faces had both their hairlines and their ears digitally removed. The experiment was computerised through Matlab Version 24 (Mathwork, Natick, USA). The participants performed a delayed recognition working memory paradigm with two interference conditions: no interference (NI) and interruption (INT) under one memory load (**Figure 3**). All of the conditions consisted of three main phases: encoding, maintenance and recognition. Thus, each participant performed 2 conditions (NI_1,

INT_1, NI_2, INT_2). Each condition was presented in a block, which consisted of 30 randomly presented trials, resulting in a total of 120 trials per participant. Before entering the shielded room for their MEG scan, instructions regarding the experimental paradigm were discussed with the participants. Following answering any questions posed by the participants, a practice session of each experimental condition (NI & INT) was conducted using a laptop. Once it was clear participants understood the task, they were set up for their scan (further details are provided within the data acquisition and analysis section below). Once in the scanner and before each condition was commenced, the instructions were repeated to the participant *via* a microphone, after which a practice session was conducted to ensure understanding. Subsequently, in the encoding phase, a face was displayed for a period of 1000 ms. In the maintenance period, the participants were instructed to keep the encoded face in mind for a 4000 ms. In the recognition phase, a single face was displayed for 1000 ms and the participants were instructed to press the right button if it was the same or the left button if it was different to the face presented in the encoding phase. Participants were instructed to respond as quickly as possible. To ensure that all of the participants had enough time to respond, a response slide ("same face yes/no") was displayed after the recognition phase and was maintained for 4000 ms. In the NI condition, a fixation cross was displayed in the centre of the screen for the 4000 ms of maintenance, and the participants were instructed to remember the encoded face during this period. The INT condition had an additional face stimulus (a face presented between two question marks, indicating the additional requirement to process the stimulus) as an interrupter after the encoding phase, which was displayed for 1000 ms after the first 1500 ms of the maintenance period. The participants were instructed to decide if the interruption face was aged over 60 years, while maintaining the encoded face. Twenty-five per-cent of the interruption faces were aged over 60.

Data acquisition and analysis

The MEG signal was recorded with an Elekta Neuromag 306-channel MEG system in the Northern Ireland Functional Brain Mapping (NIFBM) Facility of the Intelligent Systems Research Centre (ISRC) (**Figure 4**). The signal was recorded with a sampling rate of 1 kHz using 204 planar gradiometers and 102 magnetometers. Participants were encouraged to sit comfortably and requested to keep as still as possible. Head movement was recorded using head-position information (HPI) with coils attached to the scalp. HPI coils position and participants head shape were defined using a 3D digitizer (Fastrak Polhemus, Colchester, USA) referenced to three anatomical locations (the crus of helix of the right and left ear and the bridge of the nose). Eye movement was monitored by two bipolar electrodes attached above and below the right eye, two bipolar electrodes either side of the eyes and one ground electrode which was placed on the cheek (**Figure 4 a**). The heartbeat was monitored by two bipolar electrodes attached on the left and right collar bones. The Neuromag software Maxfilter 2.2 (Elekta-Neuromag Oy, Stockholm, Sweden) that implements Signal-Space Separation (SSS) was used to pre-process the signal. This method separates magnetic signals coming from within the brain from those coming from outside the brain. Finally, the signal was bandpass-filtered between 0.1 Hz and 330 Hz.

Resting state was recorded with eyes open (5 minutes) and eyes closed (5 minutes).The analysis of the signals and the time-windows of interest were the following: the called pre- interference period (including a time-window of 1000 ms, from 1500 ms to 2500 ms after the onset of the encoding stimulus) and the called post-interference period (including a time-window of 1000 ms, from 3500 ms to 4500 ms after the onset of the encoding stimulus). The frequency range of the band power was averaged in standard spectral bands: delta ([1 4] Hz), theta ([4 8]Hz), alpha ([8 12] Hz), beta ([12 30] Hz) and gamma ([30 60] Hz). The band power and time-window of interest (pre-interference and

post-interference) was selected and thereafter the trials were averaged in Matlab. The main analysis involved creating a subband of 1 Hz frequency and computing band power in each subband. For this analysis, the frequency started from 1 Hz to 60 Hz so the number of bands obtained is 59. Additionally, the band power was also computed in the delta, theta, alpha, beta, and gamma bands. The analysis focused on the eyes open resting state and the INT condition and the Power (oscillation) across the frequency spectrum and within the aforementioned bands.

Statistical analysis was conducted using SPSS (Statistical Package for Social Sciences, Version 23.0, SPSS UK Ltd., Chersey, United Kingdom) where non-parametric approaches based on the Mann Whitney Test were applied to evaluate potential statistical differences of the power between the B-vitamin and placebo group and a Wilcoxon Signed Rank test was conducted to assess differences pre- and post- interruption within each group. The following comparisons were performed: pre- vs. post-interruption in placebo and B-vitamin participants separately, and pre-interference period of placebo vs. pre-interference period of B-vitamin; post-interference period of placebo vs. post - interference period of those on B-vitamins. Power was normalised for presentation purposes to E*25.

Results

Preliminary analysis of the BrainHOP participants (n = 25) that have completed the Magnetoencephalography (MEG) scan was conducted **(Table 1**). The average age of the cohort was 79 years and there was no significant difference between the treatment groups in terms of general health, cognitive function, or depression and anxiety scores.

Analysis of the classification accuracy of the cohort was conducted for each condition **(Table 2).** Participants that had received B-vitamins for 2 years had a trend towards higher accuracy than those within the placebo group for each condition (No interference and Interruption), albeit a significant difference was detected only in the interruption condition, whereby those in the B-vitamin group reached a higher accuracy (92.4%) compared to those on placebo (76.7%; P = 0.041).

Average band power vs frequency spectrum for all channels in the placebo and B-vitamin groups was conducted for eyes opened resting state and pre and post interruption in the working memory task. Increased power (oscillations) at lower frequencies was seen for those in the placebo group compared to the B-vitamin group under all conditions (**Figure 5 and Figure 6**).

Power (oscillatory activity) of each frequency band, pre and post interruption, within the working memory task (INT), were analysed within the treatment groups (**Table 3**). The placebo group had significantly lower power in the alpha, beta and gamma bands post interruption, while the B-vitamin cohort had significantly lower power across the delta, alpha and beta bands.

Analysis comparing the difference in power post interruption between the placebo and B-vitamin groups identified a significant difference in the delta (P = 0.021) and theta (P =

0.011) bands, with the placebo group having significantly higher power (oscillation) compared to the B-vitamin group (**Figure 7**).

Discussion

The preliminary findings of this pilot study suggest that optimising B-vitamin status (via a combined B-vitamin supplement over 2 years) may protect neuronal functioning in older adults as measured by MEG during resting state. Furthermore, using a working memory task better interference resolution was observed in the B-vitamin supplemented group. In addition, a significantly higher rate of accuracy within a working memory task was seen in those supplemented with B-vitamins for 2 years.

These preliminary results suggest that B-vitamin supplementation for 2 years resulted in protected neuronal functioning, as shown by lower power across the frequency spectrum, in particular in the lower frequencies [1-8Hz] during resting state and during the working memory tasks in those in the B-vitamin supplemented group compared to those on placebo. Notably, it has been previously reported during resting state MEG scans, that power in the theta band [4-8 Hz] was lower in healthy controls compared to those with poorer cognitive function (i.e. patients with MCI) (López *et al.* 2014). Evidence also suggests increases in the Delta power [1-4 Hz] in MCI patients when compared to healthy controls, and that spectral changes in Alzheimer's disease start with an increase in the Theta power [4-8 Hz] (López *et al.* 2014). Preliminary results from the current study suggest that those supplemented with B-vitamins over 2 years appear to be protected from this increased Theta and Delta power [1-8 Hz] previously reported in patients with MCI and Alzheimer's disease, while those in the placebo group appear to experience higher power in the lower frequencies. These are preliminary results, therefore no firm conclusions can be made. If however these results are confirmed, the power observed in the delta and theta band within the current study would suggest that those who received B-vitamins for 2 years appear to be demonstrating similar MEG patterns to healthy

controls while the placebo group appear (in relative terms) seem to be following a similar MEG pattern to that observed previously in MCI patients (López *et al.* 2014).

The preliminary findings also suggest that B-vitamins may have a role in maintaining successful interference resolution in ageing, as shown by the significant difference between the placebo and B-vitamin groups in the post-interference power in the Theta band [4-8Hz], where the placebo group has a significantly higher power than the B-vitamin group. Research conducted using Electroencephalography (EEG), which like MEG, also measures neuronal activity but has poorer spatial resolution (as the electric fields are distorted by the skull and scalp), has specifically investigated the effects of interference on working memory and frequency band power. Interestingly a previous study using EEG suggested that higher levels of interferences are seen via higher power in the Theta band and that successful interference resolution causes a reduction in power in Theta band (Staudigl *et al.* 2010). These findings, although preliminary, suggest that B-vitamins may have a role in maintaining successful interference resolution in ageing, and thus a lower Theta value post interruption in the working memory task (**Figure 5**).

Preliminary results from the current MEG study, specifically those relating to the Theta power [4-8 Hz], are somewhat in line with MRI outcomes of the VITACOG study where supplementation for 2 years appear to the protect against atrophy in the hippocampus (Smith *et al.* 2010; Douaud *et al.* 2013). Theta band power was higher in the placebo group compared to the B-vitamin group. Earlier evidence from an EEG study suggested that increased power in Theta band (as seen in those in the placebo group) is negatively correlated with hippocampal volumes (Grunwald *et al.* 2001). Likewise, in the VITCAOG trial reduced atrophy particularly of the hippocampal regions were seen after

2 years of intervention with B-vitamins (Smith *et al.* 2010; Douaud *et al.* 2013). These preliminary findings appear to support the earlier results from the VITACOG trial and provide new evidence further linking optimised B-vitamin status with improved brain health in ageing.

Interestingly the participants investigated in the current study and the aforementioned VITACOG trial differ somewhat, in that the current participants are generally healthy while the VITACOG participants all suffered from MCI (Smith *et al.* 2010; Douaud *et al.* 2013). Despite these differences, the imaging results from both studies suggest similar protective effects of B-vitamins on brain health. MEG however, has the ability to detect the earliest functional changes that may occur as a result of neuropathological processes, but prior to the onset of the neurological disorder has been reported elsewhere (Zamrini *et al.* 2011). This highlights a significant benefit of utilising MEG within studies investigating the effect of nutrition on brain health and also its use in monitoring brain health before the onset of a significant disease. Other practical advantages of MEG which are important to consider in older cohorts are that MEG is non-invasive, does not require significant participant preparation and can be conducted in longitudinal or intervention studies as it a safe procedure (Braeutigam 2013). This pilot study highlights the potential for this imaging technique to be used in future studies investigating the role of nutrition in brain health.

As mentioned in earlier chapters within this thesis, the postulated mechanism linking B-vitamins with brain health in ageing are invariably associated with the one carbon metabolic pathways. Smith and Refsum (2016) have provided a detailed summary of the potential mechanisms including the potential role of B-vitamins in the epigenetic

regulation of gene expression in the Beta amyloid pathway via s-adenosylmethionine. Interestingly amyloid Beta peptides have also been reported to contribute to the destabilisation of the neuronal networks (Palop and Mucke 2010) and thus may provide a biological explanation for the current results. Lopez et al (2014) have also highlighted the potential for MEG as a candidate "neuronal degeneration" marker of Alzheimer's disease. Future studies exploring B-vitamins in relation to brain health may benefit from using MEG as a means to help elucidate the biological mechanism involved.

Several studies have used MEG to measure neuronal activity in response to consumption of dietary components; these include interventions with alcohol, L-Theanine and water and studies investigating the effects of hydration (Müller *et al.* 2002; Müller *et al.* 2003; Marinkovic *et al.* 2014; White *et al.* 2016). Furthermore, combined nutrient interventions have been conducted; including a 24 week trial in mild Alzheimer's disease patients (Van Straaten *et al.* 2016) using a specific oral nutritional supplement (Souvenaid which contains the nutrient combination Fortasyn® Connect by Nutricia Research). A further 6 month intervention (Scholey *et al.* 2013) using Lacprodan® in a larger cohort of mild-Alzheimer's disease patients was reported, however, to date no results for this trial are available. These aforementioned studies examined brain region specific analysis, functional connectively, network connectively and peak frequency analysis and were able to incorporate other imaging techniques such as fMRI. Once data collection from our study cohort is completed a more definitive comparison between correlates of brain function and nutritional interventions will be possible.

A significant strength of this study is that it explores the effect of a combined B-vitamin supplement on brain function as measured using MEG and provides objective observational evidence for a protective role of B-vitamins in supporting brain health. This study also had limitations. A cause and effect relationship cannot be assessed as the scans

were only conducted after the 2-year intervention. Additionally, B-vitamin biomarker data were not available at the time of writing; once available, it will enable a more robust analysis of the results in relation to B-vitamin status. Finally, as previously noted the current findings are preliminary, therefore, no firm conclusion can be made, however the ultimate outcomes may help to support and guide further research in this area.

In conclusion, findings from this MEG analysis, although preliminary, suggest that better B-vitamin status is associated with improved neuronal functioning. If confirmed these results will provide robust evidence of the role of B-vitamins in brain functioning. In addition, these findings support the potential of employing this novel imaging technique to determine if functional changes in neuronal activity can be achieved through nutritional intervention. Ideally, studies that utilise a combination of imaging techniques to assess structural anatomy (such as MRI) and the direct measurement of 4D neuronal activity (MEG) rather than indirect measures of neuronal activity via hemodynamic response alone (fMRI) should yield stronger mechanistic insights.

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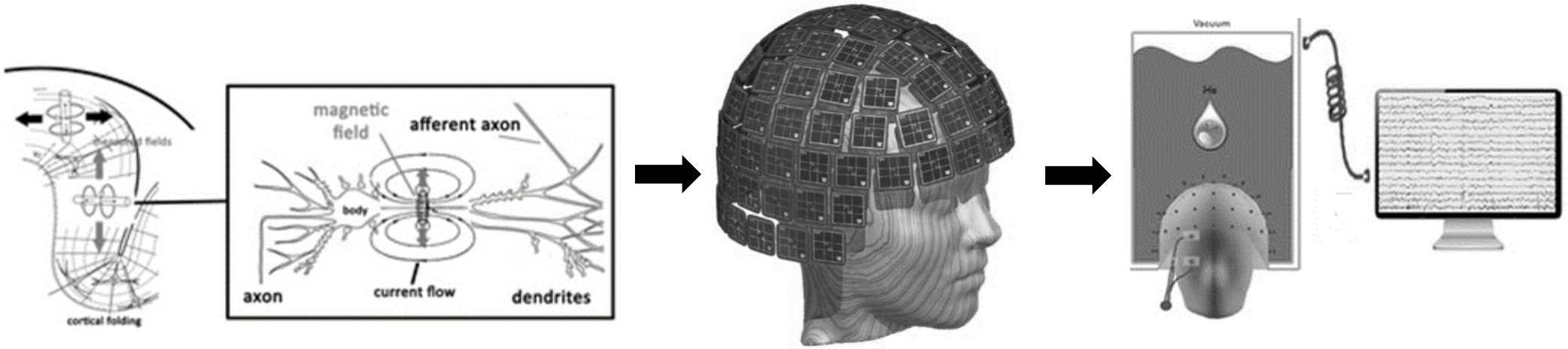


Figure 1. When an excitatory or inhibitory potential is generated in the neuronal dendrites, it depolarises, causing a current flow inside and outside the neuron, which creates a magnetic field. These magnetic field currents are very small, however the superconducting quantum interference device is sensitive enough to detect and amplify the signal from these magnetic fields (adapted from a variety of sources). (López *et al.* 2014; Stokes 2015)

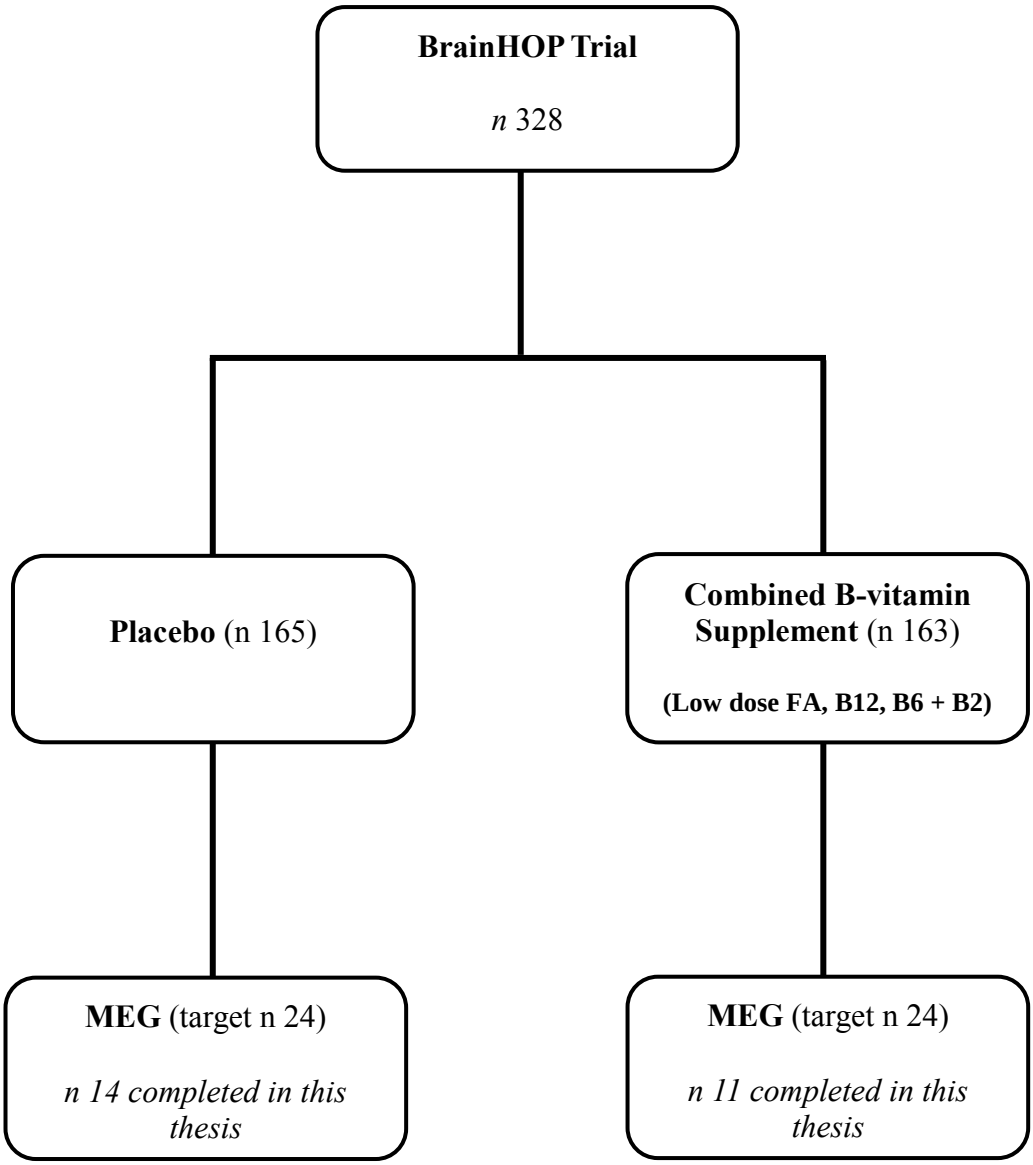


Figure 2. Flow diagram of the BrainHOP MEG pilot study

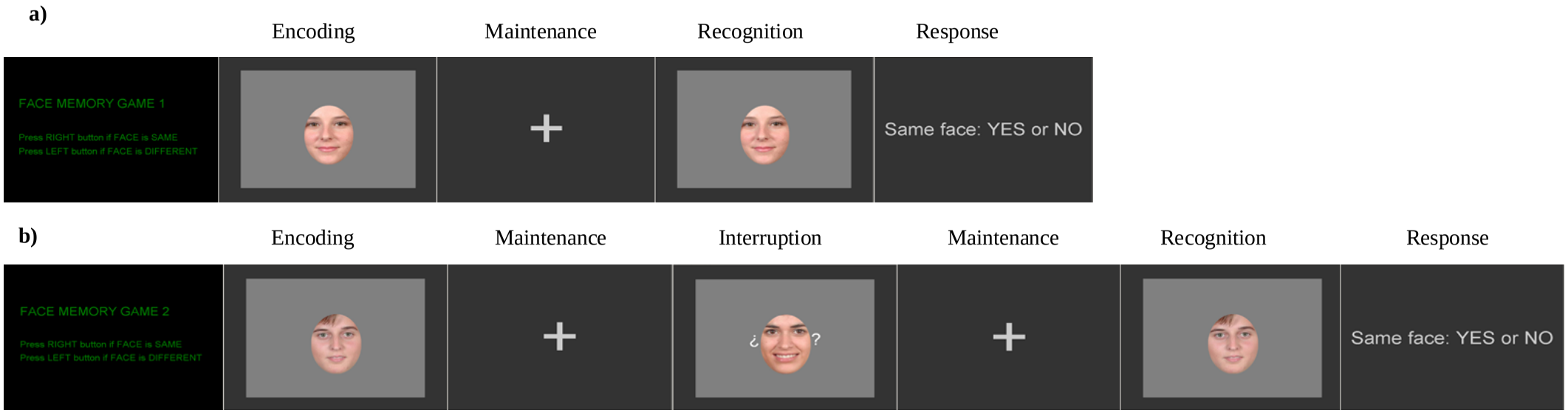


Figure 3. Representation of the experimental paradigm. The experimental paradigm consisted of two conditions a) No-interference and b) Interruption, Both conditions were structured in three main phases: encoding, maintenance and recognition, and involved one memory load.

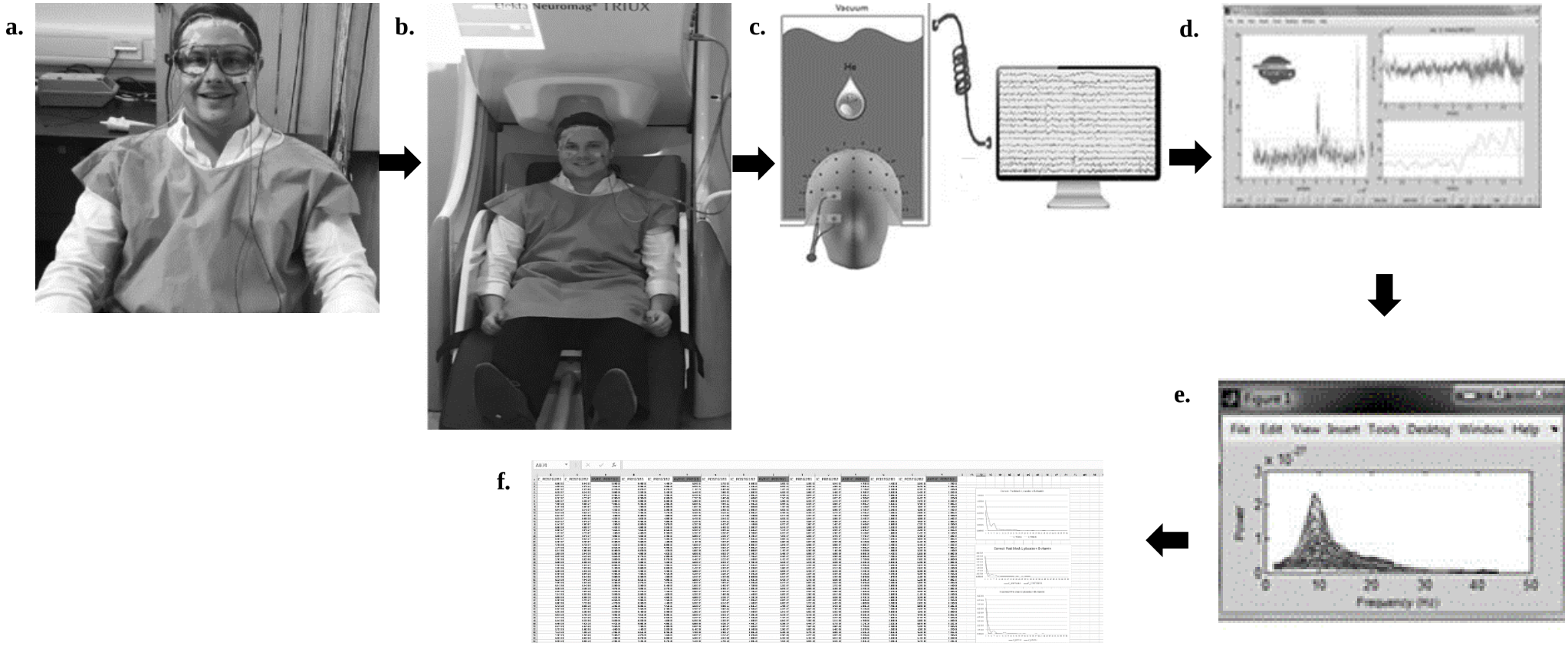


Figure 4. Work flow for MEG scanner
a.Placing head-position information coils to scalp and electrodes to monitor eye movement and heartbeat, head then digitised using a 3D digitizer; b. Participant seated in the Elekta Neuromag chair within the shielded room, before being positioned within the helmet where the SQUIDS are held; c. Elekta Neuromag 306 channel MEG system recording output; d. The Neuromag software Maxfilter 2.2 that implements Signal-Space Separation (SSS) was used to pre-process the signal; e. The signal was bandpass-filtered between 0.1 Hz and 330 Hz; f. Statstical analysis of the data. (López *et al.* 2014)

Table 1. General characteristics of participants in the BrainHOP MEG pilot study

	Total n 25	**Placebo** n 14	**B-vitamin** n 11	***P***
Age (year)	79.1 (3.9)	78.6 (4.0)	79.9 (3.9)	0.453
Gender (male) n (%)	11 (44)	8 (57)	6 (55)	1.00
Education (years)	16 (4.0)	16.9 (4.6)	15.8 (3.1)	0.570
Health and Lifestyle				
BMI (kg/m^2)	29.4 (4.6)	30.8 (4.3)	27.7 (4.7)	0.143
Timed Up and Go (seconds)	10.3 (3.0)	10.8 (3.7)	9.7 (1.9)	0.530
PSMS score	23.8 (0.5)	23.9 (0.3)	23.6 (0.7)	0.504
IADL score	27.8 (0.6)	27.9 (0.5)	27.6 (0.7)	0.211
Living alone *n* (%)	5 (20.0)	3 (21.4)	2 (18.2)	1.000
Alcohol (units/week)	5.4 (8.0)	6.3 (9.8)	4.3 (5.4)	0.431
Medical				
Diabetes *n* (%)	6 (24.0)	3 (21.4)	3 (27.3)	1.000
Hyperlipidaemia *n* (%)	10 (40)	7 (50.0)	3 (27.3)	0.459
Hypertension *n* (%)	20 (80)	11 (78.6)	9 (81.8)	1.000
Previous MI n (%)	2 (8.0)	2 (14.3)	0 (0)	0.573
Previous TIA n (%)	1 (4.0)	0 (0)	1 (9.1)	0.902
Cognition				
RBANS score	95.2 (11.8)	94.0 (12.6)	96.7 (11.0)	0.570
Immediate Memory	100.4 (14.1)	97.7 (15.1)	104.1 (12.6)	0.287
Visuospatial	93.4 (15.4)	92.5 (18.3)	94.5 (11.2)	0.760
Language	100.8 (10.0)	101.4 (10.1)	99.9 (10.5)	0.722
Attention	94.3 (14.6)	90.9 (15.9)	99.1 (11.7)	0.160
Delayed Memory	96.4 (10.9)	97.0 (8.9)	95.6 (13.6)	0.751

Data presented as means (SD), unless otherwise indicated. Differences between treatment groups were assessed using independent sample t-tests on transformed data where required, categorical variables were assessed using chi square test, $P < 0.005$ was considered significant.

Abbreviations: BrainHOP, B-vitamin and Brain Health in Older People; PSMS, Physical Self-Maintenance Score; IADL, Instrumental Activities of Daily Living; MI, Myocardial infarction; TIA, Transient Ischemic Attack; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

Table 2. Accuracy in working memory task after 2 years of intervention with B-vitamins or placebo

	Classification Accuracy		***P****
	Placebo	Treatment	
No interference			
Overall	84.9 (15.1)	90.3 (8.5)	0.415
Block 1	83.1 (17.7)	89.4 (7.7)	0.698
Block 2	87.7 (13.9)	91.2 (12.0)	0.346
Interruption			
Overall	76.4 (22.1)	93.3 (7.5)	0.080
Block 1	80.6 (21.5)	88.8 (8.3)	0.445
Block 2	76.7 (20.9)	92.4 (7.3)	**0.041**

Data presented as means (SD), unless otherwise indicated.

*Data analysed using Mann-Whitney Test.

Table 3. Oscillation Power by MEG after 2 years of intervention with B-vitamin or placebo

	Placebo					B-vitamins				
Hz	Pre		Post		P^1	Pre		Post		P^1
Delta [1 4]	2.96	(3.46)	2.88	(4.57)	0.398	1.87	(0.32)	1.13	(0.19)	**0.036**
Theta [4 8]	0.13	(0.17)	0.12	(0.09)	0.310	0.06	(0.01)	0.05	(0.01)	0.093
Alpha [8 12]	0.09	(0.08)	0.07	(0.07)	**0.028**	0.06	(0.01)	0.05	(0.01)	**0.017**
Beta [12 30]	0.15	(0.12)	0.13	(0.13)	**0.018**	0.09	(0.01)	0.08	(0.01)	**0.036**
Gamma [30 60]	0.04	(0.04)	0.02	(0.02)	**0.018**	0.02	(0.00)	0.01	(0.00)	0.093

Date presented as mean (SD) Data*E+25

[1]Differences between pre and post interference within each treatment group were assessed using Wilcoxon Signed Ranks Test.

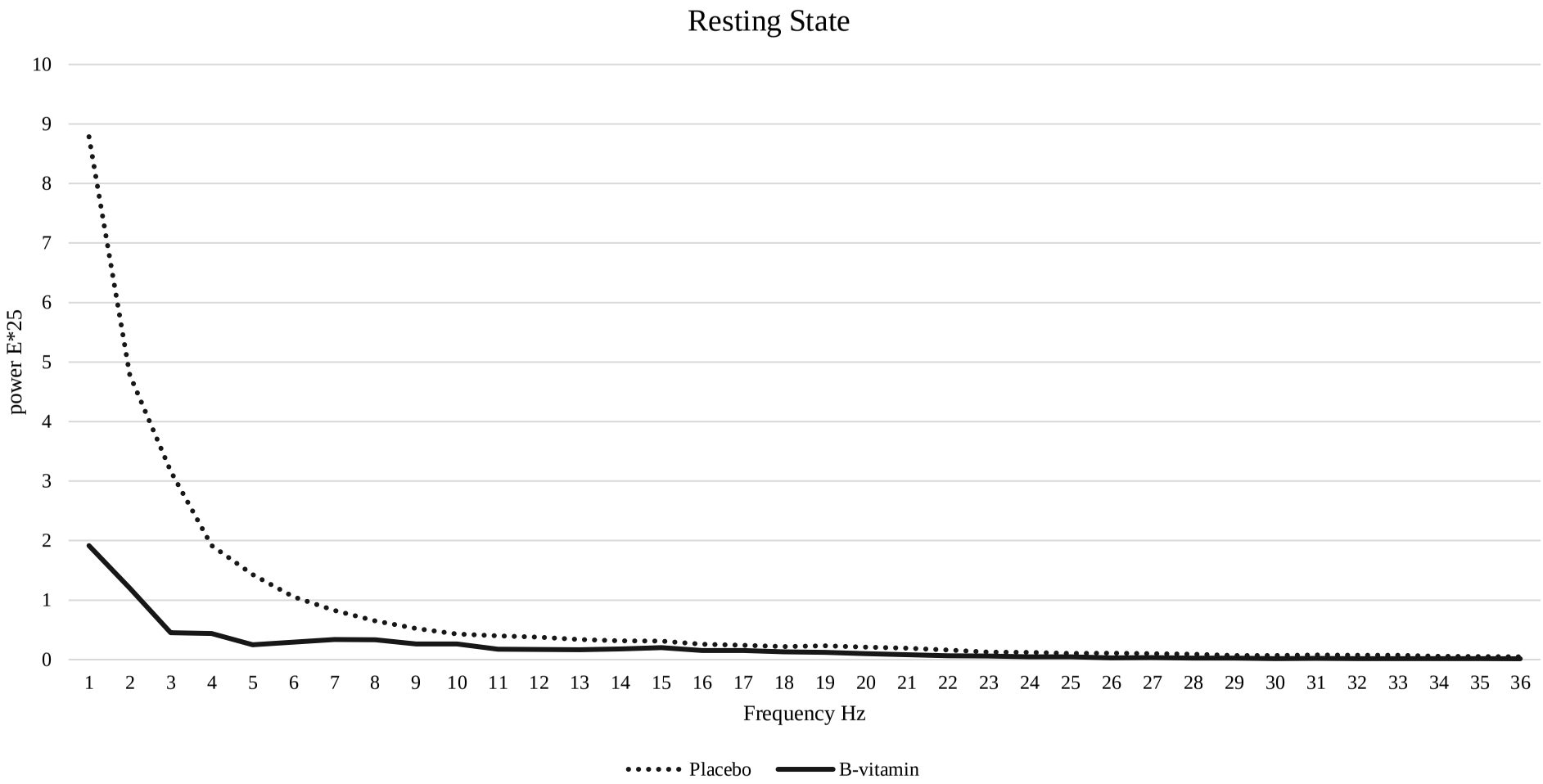


Figure 5. Average Power vs Frequency spectrum for all channels in the B-vitamin and placebo group during 5 minutes (Eyes open resting state)

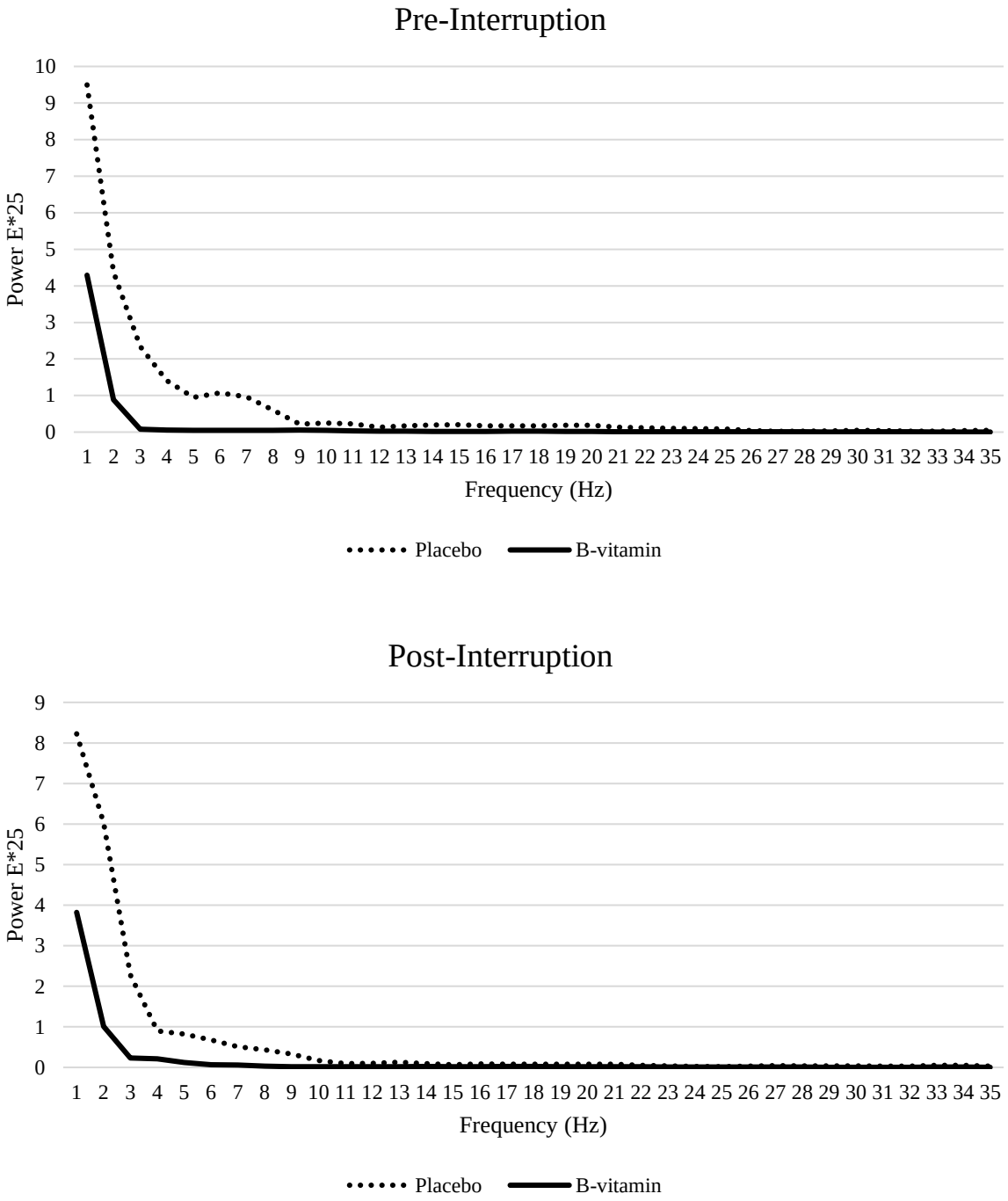
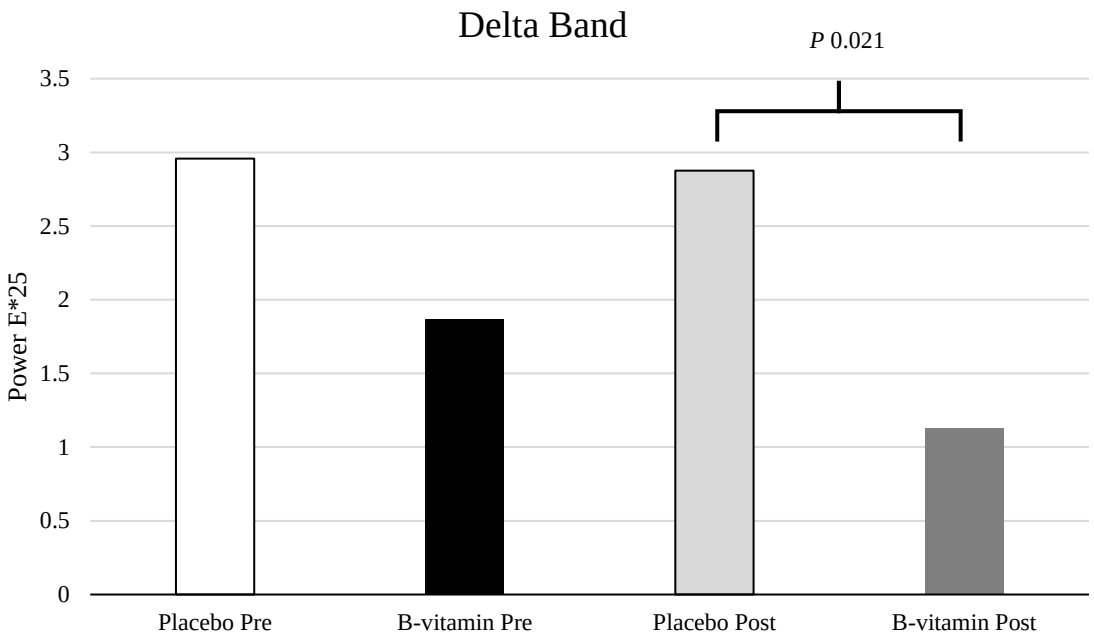


Figure 6. Average Power vs Frequency spectrum for all channels in the B-vitamin and placebo group (pre and post interruption within the working memory task)

a)



b)

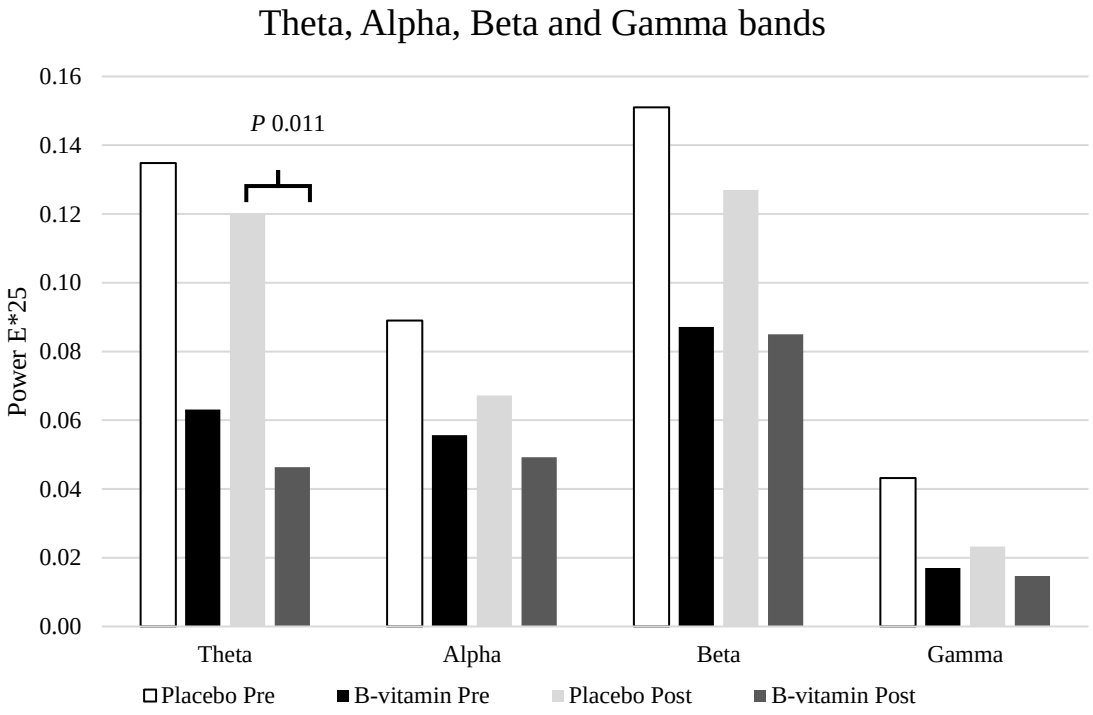


Figure 7. a) Delta b) Theta, Alpha, Beta and Gamma bands, pre and post-interference within the working memory task after 2 years of intervention with B-vitamin or placebo

Difference between placebo and B-vitamin assessed using Mann-Whitney test.

Chapter 6

General Discussion

The overall aim of this thesis was to investigate the role of B-vitamins on brain health in in the Trinity, Ulster and Department of Agriculture (TUDA) ageing study cohort. In fulfilment of this aim, the following objectives were addressed; a comprehensive review which explored the emerging evidence linking diet and specific nutrients with mental health in ageing (**Chapter 2**); an investigation of folate, vitamin B12, vitamin B6 and riboflavin in relation to depression and anxiety in older adults and to consider the role of fortified foods as a means of improving B-vitamin status and reducing the risk of these mental health disorders (**Chapter 3**); a randomised trial, BrainHOP, conducted in TUDA participants to investigate the effect of B-vitamin supplementation with folic acid and related B-vitamins over 2 years on cognitive function and depression in older adults (**Chapter 4**); an investigation of B-vitamins in brain function using MEG in older adults (**Chapter 5**).

Summary of the current findings

Declining brain health in older age is one of the greatest health challenges we face, given the significant increase in life expectancy. There is an urgent need to identify modifiable risk factors that could be targeted to help maintain brain health in ageing, with much interest in dietary patterns and specific nutrients which may have protective roles against mental health disorders. In **Chapter 2** an extensive review of the available literature showed that the strongest evidence, in the form of randomised controlled trials, supports roles for the Mediterranean diet and B-vitamins, *n*-3 PUFAs and polyphenols, as having a beneficial effect on brain health in ageing. The majority of research in the nutrition and mental health sphere has relied on questionnaire-based assessment of brain health, however, studies that have also incorporated brain imagining technologies have provided the most objective and robust evidence to date. In the case of B-vitamins, the VITACOG trial which used MRI showed that supplementation with B-vitamins over 2 years resulted

not only in improved cognitive performance (de Jager *et al.* 2012), but also reduced brain atrophy (Douaud *et al.* 2013). Although the evidence in this area is accumulating, it is not entirely consistent, and there are a limited number of published trials with considerable methodological heterogeneity between the available studies. The review indicated that there is an urgent need for future work in the form of randomised trials, ideally incorporating novel brain imaging technologies, to support and confirm the current evidence and also to enhance our understanding of the biological mechanism linking these nutrients with brain health in ageing.

In relation to depression the review (**Chapter 2**) also identified that the majority of the research conducted to date involving B-vitamins has focused predominantly on folate and vitamin B12 with evidence that deficiency of either of these vitamins is associated with an increased risk of depression. There was however limited evidence available in relation to the roles of vitamin B6 and riboflavin in depression despite both B-vitamins functioning as co-factors within one carbon metabolism. An examination, therefore, of the impact of all four B-vitamins involved in one carbon metabolism on depression and anxiety in older adults was identified as a research priority. Analysis within **Chapter 3** showed that lower biomarker status of folate or vitamin B6 or riboflavin were each associated with an increased risk of depression in ageing (by up to 78%), while vitamin B6 deficiency was associated with an increased risk of anxiety (by up to 73%). Further investigations considered the impact of B-vitamin fortified food consumption on the risk of depression. B-vitamin fortified foods and/or B-vitamin supplement use was found to optimise B-vitamin biomarker status of older adults and importantly daily consumption of B-vitamins fortified foods (e.g. fortified breakfast cereals) was associated with a 50% reduction in the risk of depression in older adults. The biomarker results presented within **Chapter 3** for folate, vitamin B6 and riboflavin were determined using what are considered to be the gold standard analysis methods, however as of yet the gold stand for

assessing vitamin B12 biomarker status is not clear (McNulty and Hughes 2017).As highlighted by Porter *et al* (2016) and Bailey *et al* (2015) there are direct and functional biomarkers for each B-vitamin, which may explain the discrepancy between B-vitamin status of participants in the TUDA cohort and other published work in the area.

It was apparent, from the current evidence reviewed in **Chapter 2** and the observational results found in **Chapter 3,** that further work in the form of a well-designed randomised trial are required to more fully investigate the role of B-vitamins on mental health in older age. The B-vitamins and Brain Health in Older People (BrainHOP) randomised trial of low dose B-vitamin supplementation over 2 years, was conducted in a sub-set of the TUDA ageing cohort, who were generally healthy adults aged 70 years and older at time of randomisation. To our knowledge this is the first trial to demonstrate that supplementation with folic acid, vitamin B12, vitamin B6 and riboflavin (at doses within the dietary range) could protect against visuospatial cognitive decline in healthy older adults (**Chapter 4**). These results build significantly on results from the VTIACOG trial which reported similar findings albeit using a different cognitive tool. Of note, visuospatial cognitive function has recently been identified to have diagnostic and prognostic potential for dementia (Salimi *et al.* 2018), but to date evidence of the role for nutrition influencing this cognitive domain is limited. Furthermore, the findings of the BrainHOP trial suggest that B-vitamin supplementation may also have a role in reducing the risk of depression in older age, albeit the study was not powered to detect the effect B-vitamin supplementation on depression (i.e. a secondary outcome) and therefore results in relation to depression need to be confirmed. In addition, the results of the BrainHOP trial as a whole are somewhat preliminary at this time given that the B-vitamin biomarker analysis has not been completed and thus final conclusions cannot be made. Once B-vitamin biomarker data become available, sub-group analysis will be conducted to

examine responses (in relation to cognition and depression) in participants with the lowest B-vitamin status (and/or higher Hcy concentrations).

Considering the novel conclusions from the recent VITACOG trial where beneficial effects of B-vitamin supplementation were observed, not only in cognitive function (de Jager *et al.* 2012), as per the current findings, but also in brain atrophy (as measured using MRI) (Smith *et al.* 2010; Douaud *et al.* 2013), a pilot study utilising a novel brain imaging technique MEG was undertaken (**Chapter 5**). A subset of participants from the 2-year BrainHOP trial were investigated using MEG in order to further explore the role of B-vitamins in influencing neuronal functioning. Initial results confirmed the cognitive function findings from the main BrainHOP trial, in that significantly higher rates of accuracy (i.e. based on correct response within the working memory interruption task) were observed in those that had received B-vitamin supplementation (compared to placebo) over the previous 2 years. For the first time, findings from the MEG analysis suggested that the B-vitamin supplement group had better neuronal functioning shown by their lower power in the lower frequencies of activity [1-8 Hz]. Furthermore, differences in relative power (at a given frequency, particularly lower frequencies) between the B-vitamin and placebo groups in this study were similar to previous observations of differences in the relative power between healthy controls and MCI patients (López *et al.* 2014). In addition, those that had received B-vitamin supplements also had significantly lower power in the Delta [1-4 Hz] ($P = 0.021$) and Theta [4-8 Hz] ($P = 0.011$) bands following interruption within the working memory task, suggesting better interference resolution compared with the placebo group.

Mechanism linking B-vitamins with brain health

The biological mechanisms explaining the findings of this thesis showing a protective effect of folate and the metabolically relate B-vitamins (vitamin B12, vitamin B6 and

riboflavin) on brain health although unknown, are however likely to relate to the crucial roles of these nutrients within one carbon metabolism. Within this metabolic network, the aforementioned B-vitamins act as co-factors in DNA synthesis and repair, amino acid metabolism and methylation reactions, including the remethylation of Hcy to methionine and subsequent generation of SAM. Insufficient supply of any of these B-vitamins may impair methylation processes (Selhub *et al.* 2000; Bottiglieri *et al.* 2000). The findings within this thesis are consistent with the hypothesis that inhibition of methylation reactions influences cognitive impairment during ageing as reviewed by Smith and Refsum (2016). Another possibility relating to the protective role of B-vitamins on brain health could be via maintaining vascular health, as B-vitamins may be associated with lower risks of vascular events such as stroke (Spence *et al.* 2017). The role of vascular dysfunction (cerebrovascular disease) in the pathophysiology of declining cognitive function is more generally accepted (Gorelick *et al.* 2016) The role that B-vitamins play in protection of vascular function (BP, cardiovascular history and measures of vascular inflammation) and improving cognitive health will be further explored in the TUDA cohort. In the case of depression the results from this thesis are consistent with Bottiglieri's work where reduced tissue concentrations of SAM are suggested to be linked to depression through perturbing monoamine (serotonin, dopamine and noradrenaline) synthesis and methylation (Bottiglieri *et al.* 2000). Furthermore the active form of vitamin B6 (plasma pyridoxal-5-phosphate; PLP), is the important co-factor for aromatic L-amino acid decarboxylase in the tryptophan serotonin pathway, thus lower vitamin B6 biomarker status may lead to reduced concentrations of serotonin and thus increased risk of depression (Hensler 2006).

Wider public health implications

Dementia and depression are the leading causes of disease burden in older adults in our society which is ageing rapidly (World Health Organisation 2016). Without any disease-modifying treatments for dementia (Frankish and Horton 2017) and with poor response rates to antidepressant medication (Rush *et al.* 2006), there is an urgent need to identify targeted interventions to promote better brain health in our ageing populations. It has been estimated that even a delay of one year in the onset of dementia could prevent more than 9 million cases of dementia by 2050 (Frankish and Horton 2017). Results from this thesis support the potential benefits of optimising B-vitamin status using dietary doses, which would in turn be achievable on a population level via mandatory or voluntary fortification of food, as a measure to help delay the onset of dementia and potentially reduce the risk of depression in ageing. Mandatory fortification, which was introduced to reduce the incidence of neural tube defects, is currently implemented in 80 countries around the world and this measure has resulted in substantially reducing the prevalence of folate deficiency in these populations (Pfeiffer *et al.* 2005). Many European countries that do not have mandatory folic acid fortification, for example Ireland and the UK, have quite liberal voluntary fortification policies. Findings from the current thesis have highlighted the significant benefits of regular consumption of B-vitamin fortified foods on biomarker status as previously reported in Ireland (Hoey *et al.* 2007; Hopkins *et al.* 2015) and in The Newcastle 85+ Study in the UK (Mendonça *et al.* 2016). There are however certain concerns in relation to an adverse interaction of high dose folic acid and low vitamin B12 in potentially leading to increased risk of cognitive impairment (Porter *et al.* 2016). From a population perspective, and to avoid potential adverse effects of one vitamin at high levels, a balanced approach to optimising the status of all relevant B-vitamins may be the most appropriate approach and this in turn could be achieved through food fortification or supplementation. Tsiachristas and Smith (2016) conducted a decision model where it was estimated that B-vitamin supplementation would save £60,021 per quality-adjusted

life year gained and could thus be a highly cost-effective means of prevention of brain disorders. Clinical practice and recommendations in other countries, including the United States and Sweden, proactively address low/deficient B-vitamins status by recommending older adults to consume the majority of their vitamin B12 in crystalline forms (Institute of Medicine Panel 2000) and offering B-vitamins to those with concerns regarding their memory and elevated Hcy concentrations (Lőkk 2013). Overall, the results from this thesis, if confirmed, will have important implications for dietary recommendations and future health policies targeting low cost non-drug strategies to improve brain health and thus quality of life in older adults.

Strengths and limitations

The main strength of this thesis is that the findings reported are based on analysis of data from the TUDA cohort, a large, well characterised cohort of 5,186 older adults. The inclusion of a highly controlled 2 year randomised trial of this older adult cohort enabled an investigation of potential causal links between B-vitamins and cognitive function, and to a lesser extent depression, in older adults. The 2 year trial followed-up TUDA participants found to have higher Hcy concentrations (i.e. indicative of lower B-vitamin status) and who were at an age where cognitive decline is more likely to occur (Murman 2015). A further strength of this thesis was that the methods used to assess brain health included a battery of cognitive assessment tests which enabled the analysis of both global cognition as well as a number of sub-domains which have been reported to be sensitive to ageing process (Howieson *et al.* 1993). This thesis also included a novel and robust imaging technique (MEG) which, to our knowledge, is the first to investigate the effect of a combined B-vitamin supplement on cognitive function in cognitively intact older adults, although it has been utilised to investigate the effect of a nutritional supplement

(containing B-vitamins among other nutrients) in cohorts suffering from mild Alzheimer's disease (Van Straaten *et al.* 2016). Finally, this thesis investigated the effect of all four B-vitamins involved in one-carbon metabolism and at doses within the dietary range, thus enabling the role of B-vitamins within this metabolic network to be more fully explored compared with previous studies in this area. However, this thesis was not without limitations. Overall, the TUDA cohort was in general a healthy, well educated, older cohort and thus the effect of B-vitamin supplementation on cognition and depression might be less than expected in other populations. One of the important risk factors for Alzheimer's disease is the APOEε4 polymorphism (Brouwers *et al.* 2008), however, this genetic information was not available for the TUDA cohort and could not be investigated. Also the CES-D scale used to assess depression, may not be as robust as other diagnostic instruments and this may have limited to some extent the interpretation of the current findings in relation to depression (Vilagut *et al.* 2016). A significant limitation of the BrainHOP trial findings, is that the final B-vitamin biomarker results were not available at the time of writing this thesis and therefore no sub-group analysis by B-vitamin status at baseline could be performed. For the chapter reporting the MEG analysis, the MEG scans were taken at one time point only and were not conducted prior to the B-vitamin intervention thus limiting the interpretation of the results with respect to verifying any beneficial effects of B-vitamins that were observed.

Conclusions and future work

In summary, the research presented in this thesis provides evidence to show that:

1. Better B-vitamin biomarker status, specifically of folate, vitamin B6 and riboflavin, may each be associated with protective effects against depression in ageing.

2. Regular B-vitamin fortified food consumption and B-vitamins supplement usage can significantly improve B-vitamin biomarker status in older adults.
3. The consumption of B-vitamin fortified foods consumed on a daily basis was associated with a 50% reduction in the risk of depression in older adults.
4. Supplementation with folic acid, vitamin B12, vitamin B6 and riboflavin at doses within the dietary range, over 2 years was associated with protecting visuospatial cognition and reducing the risk of depression in older adults.
5. Preliminary findings suggest that optimisation of B-vitamin status (following 2 years of supplementation with combined B-vitamins) was associated with better brain function as measured using MEG.

This thesis has also identified areas that require further investigation:

1. Further randomised trials investigating brain health and disease in older age are warranted. Future trials should ideally target:
    a. Participants who have suboptimal B-vitamin biomarker status at baseline (and thus most likely to benefit from B-vitamin supplementation)
    b. Use of brain imaging technologies to provide structural (MRI) and functional (MEG) measurements of the brain pre and post intervention with B-vitamins
    c. Use of a sensitive validated tool for assessing depression
    d. Multidisciplinary approach including experts in nutrition, psychology, bioengineering, radiology and genetics
    e. A large multi-centre approach (across Europe) with standardised protocols for assessment of cognition and depression and centralised biomarker analysis.

2. A randomised controlled trial investigating the effect of intervention with B-vitamin fortified foods on B-vitamin biomarker status and cognition and depression outcomes in older adults.
3. Mechanistic studies to investigate the effect of B-vitamin supplementation in older adults on DNA methylation using a multi-disciplinary approach incorporating nutrition and genetic expertise.

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Appendix 1:

Confirmation of ethical approval (Chapter 3)

For Geraldine it's file

Office for Research Ethics Committees Northern Ireland (ORECNI)

HSC REC 3

Office Suite 3
1-4 Haslems Lane
Lisburn
Co. Antrim BT28 1TW
Tel: + 44 (0) 28 9260 3107
Fax: + 44 (0) 28 9260 3619
www.orecni.org.uk

26 March 2009

Professor Sean Strain
Professor of Human Nutrition and Co-Director of Centre for Molecular Biosciences
Northern Ireland Centre for Food and Health (NICHE)
University of Ulster
Cromore Road
Coleraine
BT52 1SA

Dear Professor Strain

Full title of study: **The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study**
REC reference number: **08/NIR03/113**

Thank you for your letter of 12 March 2009, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form. Confirmation of approval for other sites listed in the application will be issued as soon as local assessors have confirmed they have no objection.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Response to Request for Further Information Cover letter, Dr G Horigan		
Participant Consent Form: Bone Densitometer Scan	Revised Jan 09	
Participant Consent Form	2.0	05 January 2009
Participant Information Sheet: Done densitometer scan	Revised Jan 09	
Participant Information Sheet: Cohort study	3.0	05 January 2009
Questionnaire: TUDA Questionnaire	2	05 January 2009
Letter from Sponsor Dr A Kilgallen		07 January 2009
Summary/Synopsis	2	05 January 2009
Protocol	2	05 January 2009
Application	Parts B & C	21 January 2009
Compensation Arrangements Email, Ms B Rutherford		09 February 2009
Summary/Synopsis		06 November 2008
Covering Letter Professor J J Strain		21 November 2008
Protocol	1	06 November 2008
Investigator CV Professor J J Strain		
Application		25 November 2008
Investigator CV and Honorary Contract, Miss C Wilson		
Investigator CV and Honorary Contract, Dr G Horigan	1.0	06 November 2008
Investigator CV, Dr M Ward	1.0	03 December 2007
Participant Consent Form: (Appendix 5)	1.0	11 November 2008
Participant Information Sheet: and consent form, Bone Densitometer Scan (Appendix 6)		01 November 2004
Participant Information Sheet: and Invitation (Appendix 4)	2.0	21 November 2008
Peer Review RG2 Dr V Naughton, RG2 Dr E Duffy, RG3		
Investigator CV Dr M O'Kane		
Questionnaire: TUDA questionnaire	1	10 November 2008
Letter from Sponsor Dr M O'Kane		07 November 2008
Response to Request for Further Information Email, Dr G Horigan		12 March 2009
Letter from Sponsor Ms M McDonald		11 March 2009
Investigator CV Dr T Trouton		01 January 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review –guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

08/NIR03/113	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Jan Daley

pp

Dr Stanley Hawkins
Alternate Vice Chair, HSC REC 3

Email: daleyj@orec.n-i.nhs.uk

Enclosures: "After ethical review – guidance for researchers"
Site approval form

Copy to: Nick Curry
Senior Administrative Officer
Research Governance
Research Office
University of Ulster Jordanstown
Shore Road
Newtownabbey
Co Antrim
BT37 0QB

Bridgeen Rutherford
R&D Office
Western HSC Trust
Altnagelvin Hospital
Glenshane Road
Londonderry
BT47 6SB

HSC REC 3

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	08/NIR03/113	**Issue number:**	0	**Date of issue:**	26 March 2009
Chief Investigator:	Professor Sean Strain				
Full title of study:	The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study				

This study was given a favourable ethical opinion by HSC REC 3 on 13 April 2009. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes (1)*
Dr Maurice O'Kane	Consultant Clinical Chemist	Western HSC Trust Altnagelvin Hospital Glenshane Road Londonderry BT47 6SB	HSC REC 3	26/03/2009	

Approved by the Chair on behalf of the REC:

Ian Daley ..... (Signature of Chair/Co-ordinator)

(delete as applicable)

Ian Daley ..... (Name)

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

Appendix 2:

Research protocol (Chapter 3)

Research Protocol

Title

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study.

Background

Cardiovascular disease (CVD), osteoporosis and Alzheimer's disease are chronic diseases of ageing that impact adversely on the lives of those affected and have major health, social and economic consequences.

A number of factors are considered to be implicated in these diseases, ranging from the more established factors to those that are less well recognised. Lifestyle factors such as diet, body weight, smoking, physical activity and years of education are acknowledged as risk factors for the development of these chronic diseases. Of the nutritional factors, calcium and vitamin D are well-established as important nutrients for the maintenance of bone mineral density (BMD) and the prevention of osteoporosis (Lanham-New, 2008). However, new research now suggests that elevated homocysteine and/or sub-optimal status of the metabolically related B-vitamins (folate, vitamin B12) may be associated not only with a higher risk of osteoporosis (Cagnacci et al, 2003; Dhonukshe-Rutten et al, 2005; Gjesdal et al, 2006), but also of CVD, in particular stroke (Hcy studies collaboration, 2002; Wald et al, 2002; Wang et al, 2007), and of cognitive decline and dementia (Quadri et al, 2004; Kado et al, 2005; Haan et al, 2007). In addition, genetic factors are increasingly recognised as playing a critical role in disease risk. Individuals who are homozygous (TT genotype) for the common 677C→T polymorphism in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR) that plays an important role in folate/homocysteine metabolism, are reported to have a higher risk of hypertension (Jiang et al, 2004), CVD (Wald et al, 2002; Klerk et al, 2002) and osteoporosis (Riancho et al, 2006) compared to those without the 677C →T polymorphism. Likewise, polymorphisms of the vitamin D receptor (VDR) gene have been associated with an increased risk of osteoporosis (Uitterlinden et al, 2006; Moffett et al, 2007). However, there are inconsistencies in the literature as to the importance of such genetic variants in disease risk. It is possible that the detrimental health effects associated with these genetic factors may only occur in particular population subgroups, such as those with low nutritional status. There is evidence to suggest that the typical phenotype (i.e. elevated homocysteine concentrations) associated with homozygosity for the MTHFR 677C→T polymorphism can be modified by folate (Klerk et al, 2002) and riboflavin status (McNulty et al, 2006), and that the risk of osteoporosis associated with polymorphisms of the VDR gene only occurs in those with low calcium intakes (MacDonald, 2003).

Although the interplay between relevant genetic and nutrient factors (gene-nutrient interactions) is considered to be highly relevant in the development (and prevention) of chronic diseases of ageing, this relatively new area of research is as yet poorly understood. The collection of clinical, lifestyle, nutritional and genetic data on large numbers of patients would permit the investigation of those nutrients which interact with specific genes to increase the likelihood of a person developing chronic diseases of ageing. Funding has recently been secured from both Governments (Irish and UK) for a large cross-border project to investigate this research area.

Study Aim

The aim of this study is to collect detailed clinical, lifestyle, dietary, genetic and biochemical data to investigate gene-nutrient interactions (particularly from the perspective of the B-vitamins and vitamin D/calcium) in the development of CVD, osteoporosis and Alzheimer's disease by studying older adults exhibiting the early stages of these common diseases, namely hypertension, low bone mineral density, and early memory loss, respectively.

Study Design and Methods

The current study (**T**rinity, **U**lster and **D**epartment of **A**griculture; TUDA) is one of three studies being undertaken as part of a cross-border collaborative project (the National Nutrition Phenotype Database Project), involving four universities: University College Dublin, Trinity College Dublin, University College Cork and the University of Ulster. The TUDA study is an observational study of a convenience sample of 6000 patients recruited from both the North and South of Ireland with phenotypic early evidence of Alzheimer's disease, osteoporosis and CVD. Ethical approval has been applied for, and obtained in the South for the cognitively impaired (n=2000) and the osteoporotic cohorts (n=2000) who will be recruited from St James's Hospital Dublin. Ethical approval is being sought here only for the recruitment of the 2000 hypertensive patients who are under the care of Consultant Cardiologists at two separate Trusts in Northern Ireland (Western Health and Social Care Trust and, Northern Health and Social Care Trust) and for the examination of data from all 6000 participants.

Patients under the care of the Consultant Cardiologists will be approached. Those patients who give their permission will be contacted by telephone by the researchers who will fully explain the study to them and then invite them to participate once current suitability is established according to the inclusion/exclusion criteria. The inclusion criteria are: patients over 60 years of age who attend (or have attended) a cardiology clinic and have been diagnosed with hypertension defined as a blood pressure of greater than 140/90 mmHg (British Hypertension Society, 2006) or are currently taking hypertensive medication. The exclusion criteria are as follows: patients under 60 years of age, patient (or their parent) not born in either the North or South of Ireland and those with severe dementia. Patients that are suitable and that verbally agree to participate will be given a participant information sheet and consent form and will be allowed at least 48 hours to consider the written information and decide if they wish to participate. If agreeable and after informed consent is received, participants will be asked to do the following:

- complete a detailed health and lifestyle questionnaire to obtain information on diet, general health, drug and supplement use.
- complete physiological function tests (e.g. blood pressure, DXA scans and a battery of cognitive function tests). The researchers will be trained in how to administer the cognitive tests by a consultant physician (Dr. F. Tracey CHSST).
- have their height, weight and waist/hip measurements taken.
- provide a non-fasting blood sample (50ml). Blood samples will be taken by a qualified phlebotomist.
- Provide a buccal swab

Blood samples will be analysed for the following:

- routine clinical markers of health (e.g. renal function, liver function, lipids, full blood count, glucose, glycosylated haemoglobin) will be performed at the participating hospital laboratory;

- vitamin B12 biomarkers - serum total vitamin B12 (microbiological assay), serum transcobalamin (holoTC, microparticle enzyme immunoassay) and plasma methylmalonic acid (GCMS) at Trinity College Dublin;
- plasma homocysteine (immunoassay), serum folate and red cell folate (microbiological assay) at Trinity College Dublin;
- riboflavin status (erythrocyte glutathione reductase activation coefficient; EGRac) at the University of Ulster;
- vitamin B6 status (pyridoxal-5-phosphate, PLP by HPLC) at the University of Ulster;
- bone biomarkers including serum 25-hydroxyvitamin D (ELISA) and serum intact parathyroid hormone (PTH; ECIA) at the University of Ulster; and

Blood samples and buccal swabs will be anlaysed for

- single nucleotide polymorphisms (SNPs) from the perspective of folate/vitamin B12 and calcium/vitamin D metabolism.

Blood samples/buccal swabs for the TUDA study will be stored at both Trinity College Dublin and the University of Ulster.

Statistical analysis

As this is a collaborative all-Ireland study, statistical analysis will be approached centrally. A working group on data management and analysis is currently being established which will include the task leaders and all PIs in the field of bioinformatics, statistics and epidemiology. A workshop will be held to ascertain the possible options for the best architecture and warehousing of the data and subsequent centralised training will be arranged in Dublin to cover the collection, entry and statistical analysis of data. Dr Cathal Walsh, Lecturer in Statistics at Trinity College Dublin, will act as statistician to the overall TUDA cohort.

Data management

The research associates will be responsible for the collection, input and analysis of all data. All data collected for the study will be kept strictly confidential, and subjects will be assigned a unique indemnification code. The file containing personal details and study indenification numbers will be kept under secure conditions in accordance with the NHS Guidelines (HSC99/053) and will be kept for a minimum of 25 years. The data will be archived securely in a restricted access room for the duration. The main database involved in the study will be stored on a password-protected computer. Any hard copies will be kept under locked conditions within the University of Ulster.

Ethical considerations

These are the main ethical issues in relation to this study.

i) Informed Consent – all potential participants will be given both written and oral information about the study before providing informed consent. It is envisaged that some of the participants will have difficulties in reading, hearing and writing. Participants will be provided with enlarged copies of the information sheet and consent form on request and they will be informed that they may nominate someone who can sign the consent form on their behalf. Every effort will be made to provide a translation of the information relevant to the study for those who do not understand written or verbal English.

ii) Participant Confidentiality – all participants will be assigned a unique identification study code.
iii) Blood Sampling – blood samples will be taken by a fully trained phlebotomist with a first aider on site.
iv) DXA Scanning – the radiation which the volunteer will be subjected to will be the minimum possible dose. Those individuals performing the scans will be fully trained and will adhere to all current health and safety regulations (IRMER) and DXA performance protocols.
v) Sample storage – all human samples will be stored as per requirements under the Human Tissue Act (HTA).
vi) Data Records – all participant data will be stored on a password protected computer. Hard copies of data will be stored in locked cabinets under the custodial care of the Chief Investigator.
vii) Inclusion and exclusion criteria will be implemented.

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Appendix 3:

Participant information sheet (Chapter 3)

Participant Information Sheet

Northern Ireland Centre for Food and Health (NICHE)
School of Biomedical Science
University of Ulster

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study

(A study to examine the link between diet, genetics and health)

Invitation

You are being invited to take part in research being conducted at the University of Ulster in collaboration with two Health and Social Care Trusts in Northern Ireland (Western and Northern Trusts). Before you decide whether or not to participate, it is important that you understand what the research is for and what you will be asked to do. Please read the following information and do not hesitate to ask any questions about anything that is unclear to you. Take time to consider whether or not you want to take part.

Thank you for taking the time to consider this invitation.

What is the purpose of the study?

High blood pressure, low bone density and mild memory loss are common health problems among older adults that can lead to more serious problems if left untreated. A number of

factors such as diet, genetic make-up and lifestyle are known to influence the likelihood of a person developing these diseases. The purpose of this study is to collect information on these factors from adults over 60 years of age who live in the North or South of Ireland, and who attend an outpatient clinic for treatment of these conditions. The information gathered will be used to try to more fully understand the link between diet, genetic make-up and health that is likely to help in the prevention of these common diseases.

Why have I been chosen?

You have been chosen because you have been diagnosed with high blood pressure or currently take medication to lower your blood pressure, and/or you are an outpatient at a clinic in either the Northern or Western Health and Social Care Trust and you are over 60 years of age. If you accept you will be one of a possible 2000 people participating in this study. However, there are several factors that may affect the study results, so the following individuals would **not** be suitable to take part in the study:

Anyone under 60 years of age

If you or your parents were not born in the North or South of Ireland.

Anyone with severe dementia.

Do I have to take part?

It is up to you to decide whether or not you take part. If you decide to participate, you will be given this information sheet to keep and you will be asked to sign a consent form. You have

the right to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to participate, will not affect the standard of care that you receive.

What will happen to me if I take part?

You will be asked to:

- Have your blood pressure measured
- Have your height, weight and waist circumference measured
- Provide one 50ml blood sample

- Provide answers to some questions about your diet, lifestyle, use of vitamin supplements and medications, your own general health and family history of disease
- Undergo some tests of memory and mood

(The above measurements will take place at a clinic within your Health Trust, which is convenient to you.)

- Have your bone density measured using a bone scanner (this will take place at either Altnagelvin Hospital or University of Ulster Coleraine, whichever is more convienent). You will have to lie still on a bed for approximately 15 minutes whilst the machine takes measurements of your bones.

Your participation in the study will involve one or two appointments which will take no more than 1 hour 30 minutes in total (excluding travel time).

We will use your blood sample to test for some general markers of health such as cholesterol, kidney and liver function, and to test your vitamin levels. If you are willing we would also like to take a blood sample from you that we will use for DNA analysis. We would also ask for your permission to retain your blood samples for use in future studies.

We also ask you if you agree to be contacted by researchers from the University of Ulster at a later date and invited to take part in similar nutrition related studies. You will only be agreeing to receive information and will not be under any obligation to take part in any future studies. If you decide not to consent to being contacted in the future it will have no influence on your involvement in this research study and will not affect any standard of care that you receive.

Risks and/or disadvantages of taking part?

There is a very small risk of bruising when giving a blood sample, but a fully trained phlebotomist will take your blood sample to ensure that any discomfort is kept to a minimum The bone scan involves the use of extremely small doses of x-ray. The dose you will be exposed to is equivalent to the background radiation you would be exposed to naturally if outside for approximately three hours. In the unlikely event that your results show something abnormal you will be contacted and the information will be forwarded to your GP.

Are there any possible benefits in taking part?

There are no direct benefits from taking part in this study although the information gained may prove extremely useful in understanding the link between diet, health and related genetic factors in people born in the North and South of Ireland. The results of the DNA tests would

have no direct relevance to you but may help us understand why Irish people get certain chronic diseases. By taking part in the study you will also find out how you perform on a range of physical and psychological tests and some clinical blood tests. If we detect any abnormal clinical results we will notify your GP.

What if something goes wrong?

It is very unlikely that something will go wrong during this research. However, you should know that the University take complaints and concerns seriously and has procedures in place for reporting, investigating, recording and handling them. The University is insured for its staff and students to carry out research involving people however, this does not extend to non-negligent harm. The University knows about this research project and has approved it. Further details on insurance can be found in the University's research indemnity statement. Ask us if you would like a copy.

Will my information be kept confidential?

Your doctor will be aware of your participation in the study. Besides that, all information collected about you for the study will be kept strictly confidential, in accordance with the NHS Guidelines (HSC99/053) all data will be kept for a minimum of 25 years. The data will be archived securely in a restricted access room for the duration.

Any information that leaves the University of Ulster will have your name and address removed so that you cannot be identified. All samples collected from you will be coded so that you cannot be identified from them and will be stored in a locked freezer until they are analysed. Information will be safely destroyed once it is no longer required.

What will happen to the findings of the research study?

It is intended that the findings from this study will be published in scientific or medical journals and presented at conferences. You will not be identified in any report or publication. Once the study is complete we will send you a summary of the results.

Who is organising and funding the research?

Funding for this study was obtained initially from the Irish Department of Food and Agriculture with additional funding obtained from The Department of Employment and Learning (DEL) in Northern Ireland.

Thank you for taking the time to read this information.

If you have any questions or would like more information, contact:

Dr Geraldine Horigan researcher on 028 70323516; gb.horigan@ulster.ac.uk

Dr Leane Hoey researcher on 028 70323516; l.hoey@ulster.ac.uk

Professor Sean Strain Chief investigator on 028 70324795; jj.strain@ulster.ac.uk

Appendix 4:

Study consent form (Chapter 3)

UNIVERSITY OF ULSTER **RESEARCH GOVERNANCE**

Consent Form for studies involving the use of human tissue **Participant Identification no:**

Title of Study	The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study (A study to examine the link between diet, genetics and health)
Chief Investigator	Professor Sean Strain Tel: 028 70324795; email: jj.strain@ulster.ac.uk

Please confirm, by ticking the boxes provided, that you agree with the following statements:

1. I have been given and have read and understood the information sheet dated 07/05/09 (version 4.0) for the above study and have asked and received answers to any questions raised ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my rights being affected in any way ☐
3. I understand that the researchers will hold all information and data collected during the study securely and in confidence and that all efforts will be made to ensure that I cannot be identified as a participant in the study (except as might be required by law) and I give permission for the researchers to hold relevant personal data ☐
4. I understand that my blood or other tissues are required for the purposes of this study and confirm that I have been given details of the amount(s) to be taken and how it will be stored, used and the method of disposal ☐
5. I agree to take part in the above study ☐
6. The potential benefits of keeping my blood or other tissues for future research studies have been explained to me and: ☐

   a. I consent to their indefinite storage and use in any future study including genetic studies, or ☐

   b. I consent to their indefinite storage and use in any future study that does not involve the isolation of my genetic material ☐

   c. I do not wish my blood or tissues to be used for any purpose other than this study ☐
7. I agree to being contacted at a later date and invited to take part in future studies of a similar nature. I understand that I am agreeing only to receive information and I am under no obligation to take part in any future studies. ☐

Name of Participant (please print)	Signature	Date (dd/mmm/yy)

1 copy for participant; 1 copy for researcher

Name of person taking consent (if different from researcher)	Signature	Date (dd/mmm/yy)
Name of Researcher	Signature	Date (dd/mmm/yy)

1 copy for participant; 1 copy for researcher

Appendix 5:

TUDA Health and lifestyle questionnaire (Chapters 3-5)

TUDA Questionnaire

Date: **Time:** **Rater :** **/**

ID:

Name:

Address:

Phone:

D.O.B.

G.P.

Time blood collected:

Sex: ☐ Male ☐ Female **Timed up and go:**
Age (deciage):

Waist (cm):
Hip (cm):

Hand grip strength (kg): (patient to stand upright and use non dominant hand x 3 measures)
L / R 1)
2)
3)
Av:

Hours since your last meal (to the nearest whole hour)
Minutes to blood sample

Weight (kg):
(without shoes but in normal clothes)

Height (cm)
(without shoes)

BP (sitting with arm supported) (circle reference arm)

	L	**R**
1st Reading	2nd Reading	
(3rd Reading)	(4th Reading)	(5th Reading)
(6th Reading)	**Average:**	

(Please allow 5 minutes in seated position with back and arm supported before recording first reading and a further 1-2 minutes before subsequent readings. Continue to take readings until two are within 5 mmHg Systolic of each other and take mean of these two as actual reading). In the event that no two readings are within 5mmhg of each other (after 6 readings) then note this and take mean of last four.

Lives
☐ Alone
☐ Spouse
☐ Children
☐ Other

Marital Status:
☐ Single
☐ Married
☐ Common-law
☐ Separated
☐ Widow(er)

Driving:
☐ Currently
☐ Past
☐ Never

Past Occupation

Medications *(N.B. tablets, inhalers, injections):* ***All > 6/12 Y/N***

Have you started any of these medication within the past (tick all that apply):

1	1 ☐ 2-6 ☐ >6 ☐ months
2	1 ☐ 2-6 ☐ >6 ☐ months
3	1 ☐ 2-6 ☐ >6 ☐ months
4	1 ☐ 2-6 ☐ >6 ☐ months
5	1 ☐ 2-6 ☐ >6 ☐ months
6	1 ☐ 2-6 ☐ >6 ☐ months
7	1 ☐ 2-6 ☐ >6 ☐ months
8	1 ☐ 2-6 ☐ >6 ☐ months
9	1 ☐ 2-6 ☐ >6 ☐ months
10	1 ☐ 2-6 ☐ >6 ☐ months
11	1 ☐ 2-6 ☐ >6 ☐ months
12	1 ☐ 2-6 ☐ >6 ☐ months
13	1 ☐ 2-6 ☐ >6 ☐ months
14	1 ☐ 2-6 ☐ >6 ☐ months
15	1 ☐ 2-6 ☐ >6 ☐ months
16	1 ☐ 2-6 ☐ >6 ☐ months
17	1 ☐ 2-6 ☐ >6 ☐ months
18	1 ☐ 2-6 ☐ >6 ☐ months
19	1 ☐ 2-6 ☐ >6 ☐ months
20	1 ☐ 2-6 ☐ >6 ☐ months

PPI H2 blockers BP Metformin Other DM Med Statin Anti-depressant

Specific Cardiovascular Risk Factors:

- **Hypertension** Yes ☐ No ☐ Don't Know☐
  If YES, when diagnosed?_____ Age started medication____
  Anti-hypertensive medication last taken:
  <4 hours _____ 4-12 hours _____ >12 hours_____
- **Diabetes** Yes ☐ No ☐ Don't Know☐
  If YES, when diagnosed? _____
  Compliance with diet? Yes ☐ No ☐
  Compliance with testing? Yes ☐ No ☐
- **Hyperlipidaemia/Hypercholestrolaemia**
  Yes ☐ No ☐ Don't Know☐
  If YES, when diagnosed? _____
- Ishaemic Heart Disease Yes ☐ No ☐ Don't Know☐
- Angina Yes ☐ No ☐ Don't Know☐
- Heart attack Yes ☐ No ☐ Don't Know☐
- Heart failure Yes ☐ No ☐ Don't Know☐
- Atrial Fibrillation Yes ☐ No ☐ Don't Know☐
- Angioplasty/CABG Yes ☐ No ☐ Don't Know☐
- Stroke Yes ☐ No ☐ Don't Know☐
  If YES, how many and when?_____
- TIA Yes ☐ No ☐ Don't Know☐
  If YES, how many and when?_____
- Peripheral artery disease, e.g. Intermittent claudication?
  Yes ☐ No ☐ Don't Know☐

- ABI/Carotid Artery Dopplers?
  Yes ☐ No ☐ Don't Know☐
- Carotid Endarterectomy Yes ☐ No ☐ Don't Know☐
- Bypass Operations Yes ☐ No ☐ Don't Know☐

Has a doctor ever told you that you had:

Anxiety	☐ Yes ☐ No
Depression	☐ Yes ☐ No
Other serious disease (e.g. cancer)	☐ Yes ☐ No

If yes for other serious disease, please specify:

Operations:

FALLS

1) Have you fallen in the last year? Yes☐ No ☐
(Note: "A fall is an event in which an individual comes to rest on the ground or another lower level with or without loss of consciousness," (Oxford textbook of Geriatric Medicine 2nd Edition 2000).

***If yes*: a) How many times in the past year?** _____
b) Did you sustain any injuries? Yes☐ No ☐

***If yes,* what type of injury?**
☐Soft tissue injury (bruise / laceration)
State location: _____
☐Fracture
State location: _____

c) Did you need to see your General Practitioner as a result?
Yes☐ No ☐

d) Did you need to go to an Emergency department (A/E) as a result?
Yes☐ No ☐

e) Did you need to be admitted to hospital as a result?
Yes☐ No ☐

2) Do you have to be careful not to stand up too quickly when rising from a sitting or lying position? Yes☐ No ☐

3) Do you feel dizzy if you stand up too quickly? Yes☐ No ☐

***If yes,* how often?**	Several times a day	☐
	Several times a week	☐
	Several times a month	☐
	Several times a year	☐
	Less than once a year	☐

4) Do you feel dizzy if you stand for a prolonged period (other than just after standing up)? Yes☐ No ☐

***If yes,* how often?**	Several times a day	☐
	Several times a week	☐
	Several times a month	☐
	Several times a year	☐
	Less than once a year	☐

5) Have you ever fainted (i.e. lost consciousness)? Yes☐ No ☐

If yes: a) How many times? _____
b) How many times in the past year? _____

6) Have you ever felt that you were going to faint but did not?
Yes ☐ No ☐

If yes: a) How many times? _____
b) How many times in the past year? _____

7) Are you afraid of falling? Yes☐ No☐

8) Do you limit any household activities because you are afraid you might fall? Yes☐ No☐

9) Do you limit any outside activities because you are frightened you may fall? Yes☐ No☐

EXERCISE

Have you done any exercise in the past 2 weeks?

If YES: How many times did you exercise? _____
On average, how long did you exercise for on each occasion? _____

Please place a tick activities partaken in, in the past 2 weeks:

☐ Walking for exercise
☐ Housework
☐ Jogging/Running
☐ Gardening
☐ Dancing
☐ Calisthenics or General Exercise
☐ Golf
☐ Cycling
☐ Swimming
☐ Other, please specify _____

BONE HISTORY

A)

- Have you ever had a fractured bone? Yes ☐ No ☐
- Have you ever had a hip fracture? Yes ☐ No ☐
- Have parent(s) ever had hip fracture? Yes ☐ No ☐
- Do you suffer with Rheumatoid Arthritis? Yes ☐ No ☐
- Do you suffer with Osteoporosis? Yes ☐ No ☐
- Have you ever taken Glucocorticoids for more than 3 months? Yes ☐ No ☐
  If yes, Duration_____
  Mean daily dose_____
- Have you ever suffered from Epilepsy? Yes ☐ No ☐
- Have you ever taken anti-epileptic medication? Yes ☐ No ☐
  If yes, for how long (in months) _____
  Name of medication(s)_____

B) Osteoporotic Medication: Duration (months):

- Protelos Yes☐ No ☐ _____
- Bisphosphonates Yes☐ No ☐ _____
  (Alendronate ☐ / Risedronate ☐ / Ibandronic Acid ☐ / Etidronate ☐ Zoledronic acid ☐)

C) Have you ever taken: Duration (months):

1. Aromatase Inhibitors: Yes☐ No ☐ _____
   (Arimidex ☐ / Femara ☐)
2. GnRH / LHRH analogues: Yes☐ No ☐ _____
   (Zoladex ☐ / Gonapeptyl ☐ / Prostap ☐)
3. Anti-androgen: Yes☐ No ☐ _____
   (Casodex ☐)

SMOKING

Smoking status

Current ☐ (i.e. Smoked in last month)
Past ☐
Never ☐

Have you ever smoked cigarettes regularly (at least 1/day) for a period longer than 6 months? Yes ☐ No ☐

If YES:
At what age did you start smoking?_____

Are you still smoking? Yes ☐ No ☐

If YES:

How many cigarettes do you smoke per day? _____
Or if ROLL, how quickly do you go through a 25g pack of tobacco (in days)?_____

If NO:

At what age did you stop smoking?_____
How many cigarettes did you smoked on average (per day)?_____
Or if ROLL, how quickly do you go through a 25g pack of tobacco (in days)?_____

DIET & SUPPLEMENTS

1. Do you eat any fortified foods? *(Researcher, please refer to Aide Memoire)* Yes ☐ No ☐

If YES, please specify:
☐ Fortified Breakfast Cereals
☐ Fortified Cereal Bars
☐ Fortified Bread
☐ Fortified Fat Spreads
☐ Fortified Drinks
☐ Marmite or other yeast extracts
☐ Other _____

For each fortified product ticked, name the product and brand below and state how often.

Product 1:	Product 2:	Product 3:
_____	_____	_____
☐ Twice/day or more	☐ Twice/day or more	☐ Twice/day or more
☐ Once/day	☐ Once/day	☐ Once/day
☐ 5-6 times/week	☐ 5-6 times/week	☐ 5-6 times/week
☐ 3-4 times/week	☐ 3-4 times/week	☐ 3-4 times/week
☐ 1-2 times/week	☐ 1-2 times/week	☐ 1-2 times/week
☐ Other _____	☐ Other _____	☐ Other _____

If YES, when did you last eat these products?

Product 1:	Product 2:	Product 3:
_____	_____	_____

2. Do you take milk? Yes ☐ No ☐

If YES:

As a drink ☐	In tea/coffee ☐	With cereal ☐
How often?		
☐ Twice/day or more	☐ Twice/day or more	☐ Twice/day or more
☐ Once/day	☐ Once/day	☐ Once/day
☐ 5-6 times/week	☐ 5-6 times/week	☐ 5-6 times/week
☐ 3-4 times/week	☐ 3-4 times/week	☐ 3-4 times/week
☐ 1-2 times/week	☐ 1-2 times/week	☐ 1-2 times/week
☐ Other _____	☐ Other _____	☐ Other _____

If YES: Name the brand of milk you typically take _____

Do you typically take -

Skimmed ☐ Soya ☐ Semi-skimmed ☐ Whole ☐

If YES, when did you last take milk? _____

3. Do you eat –

Meat? Yes ☐ No ☐

Poultry? Yes ☐ No ☐

If YES, how often?

☐ Twice/day or more
☐ Once/day
☐ 5-6 times/week
☐ 3-4 times/week
☐ 1-2 times/week
☐ Other _____

☐ Twice/day or more
☐ Once/day
☐ 5-6 times/week
☐ 3-4 times/week
☐ 1-2 times/week
☐ Other _____

4. Do you eat –

White fish? Yes ☐ No ☐
(e.g. cod/haddock/plaice/fishfingers)

Oily fish? Yes ☐ No ☐
(e.g. salmon/trout/mackerel)

If YES, how often?

☐ Twice/day or more
☐ Once/day
☐ 5-6 times/week
☐ 3-4 times/week
☐ 1-2 times/week
☐ Other _____

☐ Twice/day or more
☐ Once/day
☐ 5-6 times/week
☐ 3-4 times/week
☐ 1-2 times/week
☐ Other _____

5. Do you eat –

Eggs? Yes ☐ No ☐ **Cheese?** Yes ☐ No ☐ **Yoghurt?** Yes ☐ No☐

If YES, how often?

☐ Twice/day or more	☐ Twice/day or more	☐ Twice/day or more
☐ Once/day	☐ Once/day	☐ Once/day
☐ 5-6 times/week	☐ 5-6 times/week	☐ 5-6 times/week
☐ 3-4 times/week	☐ 3-4 times/week	☐ 3-4 times/week
☐ 1-2 times/week	☐ 1-2 times/week	☐ 1-2 times/week
☐ Other _____	☐ Other _____	☐ Other _____

6. Do you take any vitamin supplements (e.g. vitamins in tablet form, cod liver oil, etc)? Yes ☐ No ☐

If YES, how often?

☐ Twice/day or more
☐ Once/day
☐ 5-6 times/week
☐ 3-4 times/week
☐ 1-2 times/week
☐ Other _____

If on **FOLIC ACID** when last taken?_____

If YES, name the supplement(s) *(Researcher please put product name in capitals)* -

(i)_____ (ii)_____ (iii)_____

How long have you been taking each supplement?

(i)_____ (ii)_____ (iii)_____

Was the name of the supplement(s) verified by researcher *(Researcher please tick)* -

At interview (examination of product)	☐
In a follow-up phone call	☐
Not verified	☐

7. When you are outdoors during the sunny months, do you stay in the sun or do you seek the shade?

I try to avoid staying in direct sunshine ☐
I stay sometimes in the sunshine ☐
I enjoy staying often in the sunshine ☐

8. During the sunny months, how often would you apply sun protection factor?

Never ☐ Rarely ☐ Sometimes ☐ Usually ☐ Always ☐ Other ☐

What sun protection factor do you usually apply? _____

9. Have you been on a sunny holiday in the last 6 months?

Yes ☐ No ☐

If YES, please specify:

(i) where you went, (ii) during which month(s) and (iii) how long (no of days) (*Researcher, ensure response to (iii) is the total number of days of ALL breaks in sunnier climates in the last 6 months*)

_____

_____

10. Apart from the last 6 months, do you generally tend to go on sunny holidays?

Yes ☐ No ☐

If YES, specify how often (e.g. once a year, twice a year, etc)

_____

11. Do you use a sun-lamp or sun-bed regularly?

Yes ☐ No ☐

If YES, specify how often (e.g. weekly, monthly, several times a year, etc)

ALCOHOL

Do you drink alcohol

☐ Yes, currently (within the past year)
☐ No, but I have in the past (more than 1 year ago)
☐ No, never

How often do you drink alcohol? _____ (days per month)

How many units (of each type) of alcohol do you consume per week?
(1 unit = ½ pint of beer, 1 glass of wine, 1 measure of spirits; 1 bottle of wine=10 units)

Beer _____

Wine _____

Spirits _____

Total units _____

MEMORY CONCERNS

1) Do you have any concerns with regard to your memory?
Yes ☐ No ☐

2) Does your family have any concerns with regard to your memory?
Yes ☐ No ☐

DEPRESSION

Below is a list of the ways you might have felt or behaved. Please respond on how often you have felt this way during the <u>PAST WEEK,</u> by ticking the most appropriate box.

I was bothered by things that usually don't bother me	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I did not feel like eating; my appetite was poor	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt that I could not shake off the blues even with help from my family or friends	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt that I was just as good as other people	3 Never or Rarely (less than 1 day) 2 Some of the time (1-2 days) 1 Occasionally (3-4 days) 0 Most of the time (5-7 days)
I had trouble keeping my mind on what I was doing	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt depressed	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt that everything I did was an effort	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt hopeful about the future	3 Never or Rarely (less than 1 day) 2 Some of the time (1-2 days) 1 Occasionally (3-4 days) 0 Most of the time (5-7 days)
I thought my life had been a failure	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt fearful	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)

My sleep was restless	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I was happy	3 Never or Rarely (less than 1 day) 2 Some of the time (1-2 days) 1 Occasionally (3-4 days) 0 Most of the time (5-7 days)
I talked less than usual	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt lonely	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
People were unfriendly	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I enjoyed life	3 Never or Rarely (less than 1 day) 2 Some of the time (1-2 days) 1 Occasionally (3-4 days) 0 Most of the time (5-7 days)
	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt sad	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I felt that people disliked me	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 day) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)
I could not get “going”	0 Never or Rarely (less than 1 day) 1 Some of the time (1-2 days) 2 Occasionally (3-4 days) 3 Most of the time (5-7 days)

TOTAL SCORE:

ANXIETY (HADS)

In general do you ever feel

I feel tense or wound up…	Most of the time 3	A lot of the time 2	Occasionally 1	Not at all 0
I get a sort of frightened feeling as if something awful is about to happen…	Quite badly 3	Not too badly 2	A little 1	Not at all 0
Worrying thoughts go through my mind…	A great deal of the time 3	A lot of the time 2	From time to time 1	Only occasionally 0
I can sit at ease and feel relaxed…	Definitely 0	Usually 1	Not often 2	Not at all 3
I get a sort of frightened feeling like butterflies in the stomach…	Not at all 0	Occasionally 1	Quite often 2	Very often 3
I feel restless as if I have to be on the move…	Very much 3	Quite a lot 2	Not very much 1	Not at all 0
I get sudden feelings of panic…	Very often 3	Quite often 2	Not often 1	Not at all 0

TOTAL SCORE:

PHYSICAL SELF MAINTENANCE SCALE

1) Do you eat:

3 Without any help
2 With some help (e.g. cutting food, etc.)
1 Someone must feed me

2) Do you dress and undress yourself:

3 Without help (able to pick out clothes & dress)
2 With some help
1 No, someone must dress and undress me

3) Do you take care of your own appearance? (e.g. combing your hair, or for *men*, shaving)

3 Yes, without help
2 Yes, with some help
1 No, someone must help me with this type of thing

4) Are you able to get around your house/apartment without any help?

3 Yes, without help of any kind (except a cane)
2 Yes, with some help (from a person, walker, crutches, or chair)
1 No, I cannot get around my home unless someone moves me

5) Are you able to get in and out of bed yourself?

3 Yes, without help or aid
2 Yes, with some help (from a person or device)
1 No, I cannot get out of bed unless someone lifts me

6) Are you able to bathe, --that is, take a bath, shower, or sponge bath by yourself?

3 Yes, without help
2 Yes, with some help (from a person or device)
1 No, someone must bathe me and lift me in and out of the bath

7) a. Do you ever have trouble getting to the bathroom on time?

1 Yes
2 No

b. About how often would you wet or soil yourself during the day or night?

4 Never
3 Less than once a week
2 Once or twice a week
1 Three times a week or more

TOTAL SCORE:

INSTRUMENTAL ACTIVITIES OF DAILY LIVING

1) Can you the use the telephone?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to use the telephone

2) Can you get to places out of walking distance?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to travel unless special arrangements are made

3) Can you go shopping for groceries?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do any shopping

4) Can you prepare your own meals?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to prepare meals

5) Can you do your own housework?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do my own housework

6) Can you do your own handyman work?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do my own handyman work

7) Can you do your own laundry?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do any laundry at all

8) a. Do you take or use any medications?

1 Yes
2 No

b. Do you take your own medicine?

c. If you had to take medicine, can you do it:

3 Without help, taking the right dose at the right time
2 With some help (e.g. someone prepares it for you, or reminds you)
1 I am completely unable to take my own medicines

9) Can you manage your own money?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to handle money TOTAL SCORE:

Appendix 6:

MMSE (Chapters 3-5)

MINI-MENTAL STATE EXAMINATION

Name:
Examiner:
Date:

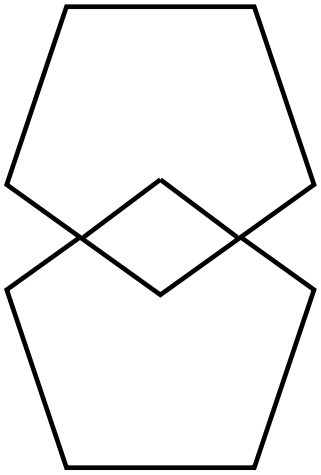
Maximum Score	Score	
		ORIENTATION
5	( )	What is the (year) (season) (date) (day) (month)?
5	( )	Where are we: country, county, town, street, room?
		REGISTRATION
3	( )	Name 3 objects: 1 second to say each. Then ask the volunteer to repeat all three after you have said them. Give 1 point for each correct answer. Then repeat the trials until he/she either learns all 3 or has had 6 trials. Count all trials and record them.
		No of Trials.....
		ATTENTION AND CALCULATION
5	( )	Begin with 100 and count backwards by subtracting 7. Stop after 5 answers. Score 1 point for each correct answer. Alternatively spell "world" backwards.
		RECALL
3	( )	Ask for the three objects learned above. Give 1 point for each correct answer.
		LANGUAGE
2	( )	Name a pencil and a watch.
1	( )	Repeat the following "No ifs ands or buts".
3	( )	Follow a 3-stage command: "Take a sheet of paper in your right hand, fold it in half, and put it on the floor".
1	( )	Read and obey the following: CLOSE YOUR EYES
1	( )	Write a sentence.
1	( )	Copy a design.

CLOSE

YOUR

EYES

.....
.....
.....
.....
...



Appendix 7:

FAB (Chapters 3-5)

FAB
FRONTAL ASSESSMENT BATTERY

1. Similarities (conceptualization)

"In what way are they alike?"

A banana and a orange (In the event of total failure: "they are not alike" or partial failure "both have a peel", help the patient by saying "both a banana and an orange are…."; but credit 0 for the item; do not help the patient for the tow following items)

A table and a chair

A tulip, a rose, and a daisy

Score: only category responses (fruits, furniture, flowers) are considered correct.
- Three correct: 3
- Two correct: 2
- One correct: 1
- None correct: 0

2. Lexical fluency (mental flexibility)

"Say as many words as you can beginning with the letter 'S', any words except surnames or proper nouns."

If the patient gives no response during the first 5 seconds, say: "For instance, snake." If the patient pauses 10 seconds, stimulate him by saying: "any word beginning with the letter 'S'. The time allowed is 60 seconds.

Score: word repetitions or variations (shoe, shoemaker), surnames, or proper nouns are not counted as correct responses.
- More than nine words: 3
- Six to nine words: 2
- Three to five words: 1
- Less than three words: 0

3. Motor series (programming)

"Look carefully at what I'm doing."

The examiner, seated in front of the patient, performs alone three times with his left hand the series of Luria "fist-edge-palm." "Now, with your right hand do the same series, first with me, then alone." The examiner performs the series three times with the patient, and then says to him/her: "Now, do it on your own."

Score:
- Patient performs six correct, consecutive series alone: 3
- Patient performs at least three correct consecutive series alone: 2
- Patient fails alone, but performs three correct consecutive series with the examiner: 1
- Patient cannot perform three correct consecutive series even with the examiner: 0

4. Conflicting instructions (sensitivity to interference)

"Tap twice when I tap once."

To be sure that the patient has understood the instructions, a series of three trials is run: 1 – 1 – 1. "Tap once when I tap twice." To be sure the patient has understood the instructions, a series of three trials is run: 2 – 2 – 2. The examiner performs the following series: 1 – 1 – 2 – 1 – 2 – 2 – 2 – 1 – 1 – 2.

Score:
- No error: 3
- One or two errors: 2
- More than 2 errors: 1
- Patient taps like the examiner at least four consecutive times: 0

FAB (cont)
FRONTAL ASSESSMENT BATTERY

5. Go-No-Go (inhibitory control)

"Tap once when I tap once."

To be sure that the patient has understood the instructions, a series of three trials is run: 1 – 1 – 1. "Do not tap when I tap twice." To be sure the patient has understood the instructions, a series of three trials is run: 2 – 2 – 2. The examiner performs the series: 1 – 1 – 2 – 1 – 2 – 2 – 2 – 1 – 1 – 2.

Score:

No errors: 3
One or two errors: 2
More than two errors: 1
Patient taps like the examiner at least four consecutive times: 0

6. Prehension behavior (environmental autonomy)

"Do not take my hands."

The examiner is seated in front of the patient. Place the patient's hands palm up on his/her knees. Without saying anything or looking at the patient, the examiner brings his/her hands close to the patient's hands and touches the palms of both the patient's hand, to see if he/she will spontaneously take them. If the patient takes the hands, the examiner will try again after asking him/her: "Now, do not take my hands."

Score:

Patient does not take the examiner's hands: 3
Patient hesitates and asks what he/she has to do: 2
Patient takes the hands without hesitation: 1
Patient takes the examiner's hands even after he/she has been told not to do so: 0

SCORE:_____

EXAMINER:_____ DATE:_____

Appendix 8:

RBANS (Chapters 3-5)

RBANS UPDATE

Repeatable Battery for the Assessment of Neuropsychological Status

Christopher Randolph

Record Form b

UK Adaptation

Name _____ Age_____ Sex_____ Education Level _____

Examiner_____ Date of Testing _____ Ethnicity _____

	Immediate Memory	Visuospatial/ Constructional	Language	Attention	Delayed Memory		TOTAL SCALE	
Index Score								
Confidence Interval ____%								
Percentile								
Index Score						Percentile Rank		Total Scale Index Score
160						>99.9		160
155						>99.9		155
150						>99.9		150
145						99.9		145
140						99.6		140
135						99		135
130						98		130
125						95		125
120						91		120
115						84		115
110						75		110
105						63		105
100						50		100
95						37		95
90						25		90
85						16		85
80						9		80
75						5		75
70						2		70
65						1		65
60						0.4		60
55						0.1		55
50						<0.1		50
45						<0.1		45
40						<0.1		40

Observations: _____

_____

_____

PEARSON

15 16 17 18 19 A B C D E

PsychCorp

ISBN 978 0 749169 14 5

1 List Learning

Trial 1

Say ***I am going to read you a list of words. I want you to listen carefully and, when I finish, repeat back as many words as you can. You don't have to say them in the same order that I do—just repeat back as many words as you can remember, in any order. Okay?***

Trials 2–4

Say, ***I am going to read the list again. When I finish, repeat back as many words as you can, even if you have already said them before. Okay?***

Record responses in order.

Scoring: 1 point for each word correctly recalled on each trial.

List	Trial 1	Trial 2	Trial 3	Trial 4
Candle				
Sugar				
Wagon				
Hotel				
Farmer				
Village				
Sandwich				
Feather				
Artist				
Paper				

	+		+		+		=	
Total Trial 1		Total Trial 2		Total Trial 3		Total Trial 4		Total Score Range=0–40

PEARSON

PsychCorp is an imprint of Pearson Clinical Assessment.

Published by Pearson Assessment, 80 Strand, London WC2R 0RL. Printed in the United Kingdom.
www.pearsonclinical.co.uk

Printed in the United Kingdom.

2 Story Memory

Trial 1

Say ***I am going to read you a short story. I'd like you to listen carefully and, when I finish, repeat back as much of the story as you can remember. Try and use the same wording, if you can. Okay?***

Read the story below, then say ***Now repeat back as much of that story as you can.***

Trial 2

Say ***I am going to read that same story again. When I finish, I want you to again repeat back as much of the story as you can remember. Try to repeat it as exactly as you can.***

Read the story below, then say ***Now repeat back as much of that story as you can.***

Scoring: 1 point for *verbatim* recall of bold, italic words or alternatives, shown below in colour within parentheses. Record intrusions or variations in the Responses column.

Story	Trial 1 Responses	Trial 1 Score (0 or 1)	Trial 2 Responses	Trial 2 Score (0 or 1)	Item Score (0–2)
1. On ***Monday,***					
2. ***Fifth***					
3. of ***March,***					
4. in ***Brighton,*** Sussex,					
5. a ***storm*** hit.					
6. Although ***2 million pounds***					
7. in ***damage*** was done					
8. to the ***waterfront,***					
9. only ***seven people***					
10. were ***injured,*** ***(hurt)***					
11. and ***nobody*** ***(no one)***					
12. was ***killed.***					
				Total Score (Trial 1 + Trial 2) Range=0–24	

3 Figure Copy

Time Limit: 4 minutes

Fold this page back and present the Figure Copy Drawing Page along with the stimulus. Ask the examinee to make an exact copy of the figure. Tell the examinee that he or she is being timed, but that the score is based *only* on the exactness of his or her copy.

Scoring: 1 point for correctness and completeness (drawing), and 1 point for proper placement. See Appendix 1 in Stimulus Booklet B for complete scoring criteria and scoring examples.

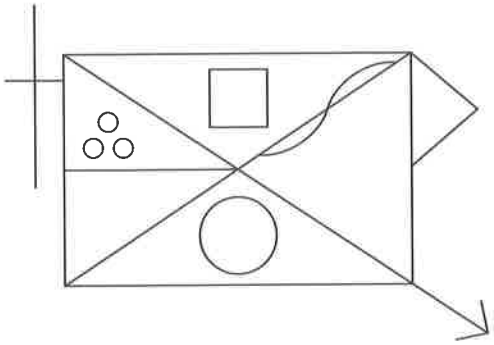


Figure Copy Criteria

(Fold back for use.)

Item	Drawing (0 or 1)	Placement (0 or 1)	Score (0, 1, or 2)	Scoring Criteria
1. rectangle				**Drawing:** lines are unbroken and straight; angles 90 degrees; top/bottom lines 25% longer than sides **Placement**: not rotated more than 15 degrees
2. diagonal cross				**Drawing:** lines are unbroken and straight and should approximately bisect each other **Placement**: ends of lines should meet corners of the rectangle without significant overlap or measurable distance between the ends of the lines and the corners
3. horizontal line				**Drawing:** line is unbroken and straight; should not exceed 1/2 the length of the rectangle **Placement:** should bisect left side of the rectangle at approximately a right angle and intersect the diagonal cross
4. circle				**Drawing:** round, unbroken and closed; diameter should be approximately 1/4–1/3 height of rectangle **Placement**: placed in appropriate segment; not touching any other part of figure
5. 3 small circles				**Drawing:** round, unbroken and closed; equal size; triangular arrangement; not touching each other **Placement**: in appropriate segment; not touching figure; triangle formed not rotated more than 15 degrees
6. square				**Drawing:** must be closed; 90 degree angles; lines straight and unbroken; height is 1/4–1/3 height of rectangle **Placement**: in appropriate segment; not touching any other part of figure; not rotated more than 15 degrees
7. curving line				**Drawing:** 2 curved segments are approximately equal in length and symmetrical; correct direction of curves **Placement**: ends of line touch diagonal; do not touch corner of rectangle or intersection of diagonal lines
8. outside cross				**Drawing:** vertical line of the outside cross is parallel to side of rectangle; >1/2 the height of rectangle; horizontal line crosses vertical at 90 degree angle and is between 20–50% of length of vertical line **Placement**: horizontal line of outside cross touches rectangle higher than 2/3 the height of rectangle, but below top; does not penetrate the rectangle
9. triangle				**Drawing:** angle formed by 2 sides of triangle is between 60–100 degrees; sides are straight, unbroken and meet in a point; distance on vertical side of rectangle subsumed by triangle is approximately 50% of the height of vertical side **Placement**: no measurable distance between the top of the rectangle and the triangle
10. arrow				**Drawing:** straight and unbroken; lines forming arrow are approximately equal in length and not more than 1/3 length of staff **Placement**: must protrude from appropriate corner of rectangle such that staff appears to be continuation of diagonal cross
		Total Score Range=0–20		

Figure Copy Drawing Page

(Fold back for use.)

4 Line Orientation

Time Limit: 20 seconds/item

Present the sample item, and say ***These two lines down here*** (indicate) ***match two of the lines on top. Can you tell me the numbers, or point to the lines that they match?*** Correct any errors and make sure the examinee understands the task. Continue with Items 1–10.

Scoring: 1 point for each line correctly identified.

Item	Responses	Correct Responses	Score (0, 1, or 2)
Sample		1, 7	
1.		2, 5	
2.		4, 12	
3.		6, 11	
4.		7, 10	
5.		9, 12	

Item	Responses	Correct Responses	Score (0, 1, or 2)
6.		2, 10	
7.		6, 12	
8.		3, 8	
9.		4, 7	
10.		2, 8	
		Total Score Range=0–20	

5 Picture Naming

Time Limit: 20 seconds/item

Ask the examinee to name each picture. Give the semantic cue only if the picture is obviously misperceived.

Scoring: 1 point for each item that is correctly named spontaneously or following semantic cue, or alternatives, shown below in colour within parentheses. Record intrusions or variations in the Responses column.

Item	Semantic Cue	Responses	Score (0 or 1)
1. bed	a piece of furniture		
2. mushroom	something that grows; can be eaten		
3. flower	grows in the garden		
4. iron	used to get wrinkles out of clothes		
5. barn	a type of building *(garage, shed)*		
6. anchor	used on a boat		
7. hammer	a tool		
8. scissors	used for cutting		
9. dice	used in games		
10. sea horse	animal found in the ocean		
		Total Score Range=0–10	

6 Semantic Fluency

Time Limit: 60 seconds

Say ***Now I'd like you to tell me the names of all of the different kinds of animals you would find in a zoo that you can think of. I'll give you one minute to come up with as many as you can. Ready?***

Scoring: 1 point for each correct response.

1. _____	11. _____	21. _____	31. _____
2. _____	12. _____	22. _____	32. _____
3. _____	13. _____	23. _____	33. _____
4. _____	14. _____	24. _____	34. _____
5. _____	15. _____	25. _____	35. _____
6. _____	16. _____	26. _____	36. _____
7. _____	17. _____	27. _____	37. _____
8. _____	18. _____	28. _____	38. _____
9. _____	19. _____	29. _____	39. _____
10. _____	20. _____	30. _____	40. _____

Number Correct Range=0–40 [ ] + 4 = Total Score Range=4–40 [ ]

7 Digit Span

Say ***I am going to say some numbers, and I want you to repeat them after me. Okay?***
Read the numbers at the rate of 1 per second. Only read the second string in each set if the first string was failed.
Discontinue after failure of both strings in any set.

Scoring: 2 points for the first string correct, 1 point for the second string correct, and 0 points for both strings failed.

Item	First String	String Score (0 or 2)	Second String	String Score (0 or 1)	Item Score (0–2)
1.	**9—4**		**3—5**		
2.	**5—3—8**		**1—4—2**		
3.	**6—4—2—7**		**8—3—6—1**		
4.	**4—2—9—3—5**		**1—9—4—8—3**		
5.	**5—3—9—2—4—6**		**6—3—7—5—1—9**		
6.	**6—3—9—5—1—8—2**		**2—9—4—7—1—3—5**		
7.	**4—2—6—9—1—7—3—8**		**8—3—7—9—1—4—2—5**		
8.	**3—1—7—8—2—9—5—4—6**		**2—1—9—5—4—7—3—8—6**		

Total Score Range=0–16 [ ]

8 Coding

Time Limit: 90 seconds

Say ***Look at these boxes*** (indicate key)***. For each one of these marks there is a number that goes with it. Down here there are marks, but no numbers. I want you to fill in the number that goes with each mark.***

Demonstrate the first three. Say ***Now I would like you to fill in the rest of these boxes up to the double lines*** (indicate) ***for practice.*** Correct any errors as they are made. Make sure that the examinee understands the task and has correctly completed the sample items before you begin timing.

Say ***Now I would like you to continue to fill in the numbers that match the marks. Go as quickly as you can without skipping any. When you reach the end of the line, go on to the next one. Ready? Go ahead.***

Redirect the examinee to the task if he or she becomes distracted. If the examinee is unable to comprehend the task, the subtest score is 0.

Scoring: 1 point for each item correctly coded within 90 seconds (*do not* score the sample items).

Note: Familiarise yourself with these instructions before administering this subtest.

Total Score
Range=0–89

9 List Recall

Say ***Do you remember the list of words that I read to you in the beginning? Tell me as many of those words as you can remember now.***

Scoring: 1 point for each word correctly recalled.

List (Do not read.)	Response	Score (0 or 1)
Candle		
Sugar		
Wagon		
Hotel		
Farmer		
Village		
Sandwich		
Feather		
Artist		
Paper		
	Total Score Range=0–10	

10 List Recognition

Say ***I'm going to read you some words. Some of these words were on that list, and some of them weren't. I want you to tell me which words were on the list.*** For each word, ask ***Was _____ on the list?***

Scoring: 1 point for each word correctly identified. Circle the letter corresponding to examinee's response (y = yes, n = no); bold, capitalised (**Y, N**) letter indicates correct response.

List	Circle One		List	Circle One		List	Circle One		List	Circle One	
1. **Candle**	**Y**	n	6. season	y	**N**	11. **Hotel**	**Y**	n	16. **Sandwich**	**Y**	n
2. metal	y	**N**	7. building	y	**N**	12. pupil	y	**N**	17. **Feather**	**Y**	n
3. **Farmer**	**Y**	n	8. **Wagon**	**Y**	n	13. **Artist**	**Y**	n	18. summer	y	**N**
4. **Paper**	**Y**	n	9. mirror	y	**N**	14. party	y	**N**	19. **Village**	**Y**	n
5. purple	y	**N**	10. **Sugar**	**Y**	n	15. castle	y	**N**	20. insect	y	**N**
									Total Score Range=0–20		

11 Story Recall

Say ***Do you remember that story about a storm I read to you earlier? Tell me as many details from the story as you can remember now.***

Scoring: 1 point for each *verbatim* recall of bold, italic words or alternatives, shown below in colour within parentheses. Record intrusions or variations in the Responses column.

Story (Do not read.)	Responses	Item Score (0 or 1)
1. On ***Monday,***		
2. ***Fifth***		
3. of ***March,***		
4. in ***Brighton,*** Sussex,		
5. a ***storm*** hit.		
6. Although ***2 million pounds***		
7. in ***damage*** was done		
8. to the ***waterfront,***		
9. only ***seven people***		
10. were ***injured,*** ***(hurt)***		
11. and ***nobody*** ***(no one)***		
12. was ***killed.***		
	Total Score Range=0–12	

12 Figure Recall

Say ***Do you remember that figure that I asked you to copy? I want you to draw as much of it as you can remember now. If you remember a part, but you're not sure where it goes, put it anywhere. Try to draw as much of it as you can.***

Now, present the Figure Recall Drawing Page.

Scoring: 1 point for correctness and completeness (drawing), and 1 point for proper placement. See Appendix 1 in Stimulus Booklet B for complete scoring criteria and scoring examples.

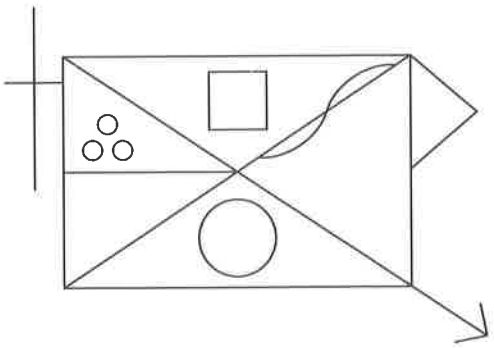


Figure Recall Criteria

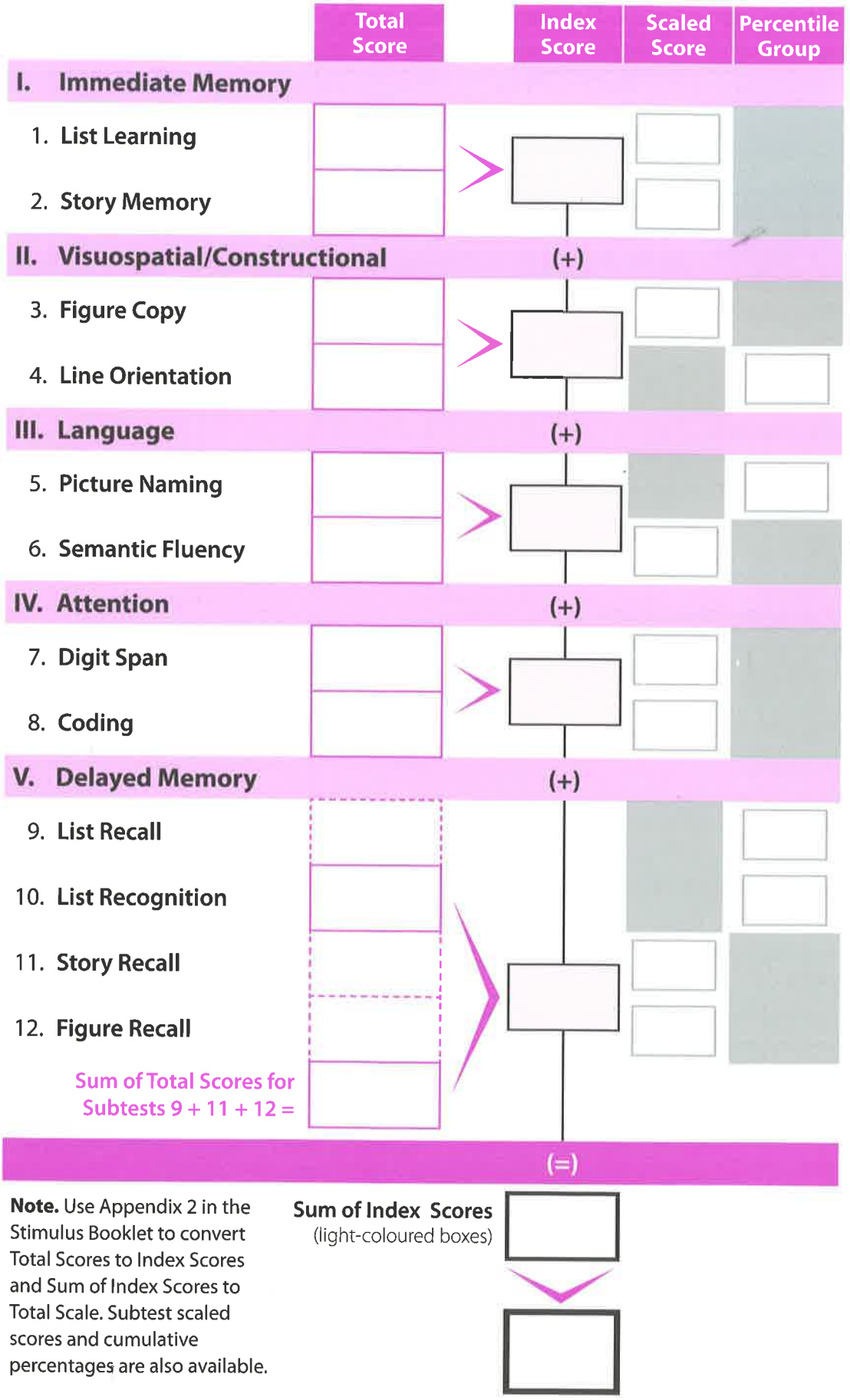
(Fold back for use.)

Item	Drawing (0 or 1)	Placement (0 or 1)	Score (0, 1, or 2)	Scoring Criteria
1. rectangle				**Drawing:** lines are unbroken and straight; angles 90 degrees; top/bottom lines 25% longer than sides **Placement**: not rotated more than 15 degrees
2. diagonal cross				**Drawing:** lines are unbroken and straight and should approximately bisect each other **Placement**: ends of lines should meet corners of the rectangle without significant overlap or measurable distance between the ends of the lines and the corners
3. horizontal line				**Drawing:** line is unbroken and straight; should not exceed 1/2 the length of the rectangle **Placement:** should bisect left side of the rectangle at approximately a right angle and intersect the diagonal cross
4. circle				**Drawing:** round, unbroken and closed; diameter should be approximately 1/4–1/3 height of rectangle **Placement**: placed in appropriate segment; not touching any other part of figure
5. 3 small circles				**Drawing:** round, unbroken and closed; equal size; triangular arrangement; not touching each other **Placement**: in appropriate segment; not touching figure; triangle formed not rotated more than 15 degrees
6. square				**Drawing:** must be closed; 90 degree angles; lines straight and unbroken; height is 1/4–1/3 height of rectangle **Placement**: in appropriate segment; not touching any other part of figure; not rotated more than 15 degrees
7. curving line				**Drawing:** 2 curved segments are approximately equal in length and symmetrical; correct direction of curves **Placement**: ends of line touch diagonal; do not touch corner of rectangle or intersection of diagonal lines
8. outside cross				**Drawing:** vertical line of the outside cross is parallel to side of rectangle; >1/2 the height of rectangle; horizontal line crosses vertical at 90 degree angle and is between 20–50% of length of vertical line **Placement**: horizontal line of outside cross touches rectangle higher than 2/3 the height of rectangle, but below top; does not penetrate the rectangle
9. triangle				**Drawing:** angle formed by 2 sides of triangle is between 60–100 degrees; sides are straight, unbroken and meet in a point; distance on vertical side of rectangle subsumed by triangle is approximately 50% of the height of vertical side **Placement**: no measurable distance between the top of the rectangle and the triangle
10. arrow				**Drawing:** straight and unbroken; lines forming arrow are approximately equal in length and not more than 1/3 length of staff **Placement**: must protrude from appropriate corner of rectangle such that staff appears to be continuation of diagonal cross
		Total Score Range=0–20		

Figure Recall Drawing Page

(Fold back for use.)

Score Conversion Page



Note. Use Appendix 2 in the Stimulus Booklet to convert Total Scores to Index Scores and Sum of Index Scores to Total Scale. Subtest scaled scores and cumulative percentages are also available.

Supplemental Discrepancy Analysis Page

Index Differences

Score 1–Score 2	Score 1	Score 2	Difference	Statistical Significance _____Level	Frequency of Difference in Standardisation Sample
Immediate Memory—Visuospatial/Constructional					
Immediate Memory—Attention					
Immediate Memory—Language					
Immediate Memory—Delayed Memory					
Immediate Memory—Total Scale					
Visuospatial/Constructional—Attention					
Visuospatial/Constructional—Language					
Visuospatial/Constructional—Delayed Memory					
Visuospatial/Constructional—Total Scale					
Attention—Language					
Attention—Delayed Memory					
Attention—Total Scale					
Language—Delayed Memory					
Language—Total Scale					
Delayed Memory—Total Scale					

⊃	⌋	∧	⊥	⊂	⊢	=	∨	+
1	2	3	4	5	6	7	8	9

SAMPLE______________________________

=	⊢	⊂	∧	+	⌋	⊥	⊃	∨	=	⊢	∧	⊃	+

⊥	⊃	∨	⊢	=	∧	⊂	+	⌋	∧	⊥	⊂	+	⌋

⊃	⊢	∧	=	∨	⊂	⌋	+	⊥	=	⊃	∧	⊢	⊂

+	⊂	⊢	⌋	=	⊢	+	∧	⊃	⊂	⌋	⊥	+	⊢

⊂	+	⊢	⊃	∧	=	⊥	⌋	⊂	=	+	∨	⊥	∧

∧	=	⌋	⊢	+	∨	⊥	⌋	∧	⊃	∨	⊥	⊂	⌋

+	⊂	⌋	⊃	∧	=	⊂	+	⊥	∨	⌋	∧	⊃	=

Appendix 9:

TUDA Food dairy (Chapter 4)

Northern Ireland Centre for Food and Health (NICHE)

Date:

Dear

Thank you for agreeing to take part in this TUDA follow up study. We are interested in relating your dietary intake of B-vitamins to your blood levels.

Enclosed within this letter is a 4 day food diary. The diary should be **recorded on 4 consecutive days including a Saturday and a Sunday.** Instructions on how food and drink are to be recorded are explained at the front of the food diary so please read thoroughly before starting to keep your food diary. You don't need to weigh everything but simply describe the amount eaten using household measures, for example, *1 small bowl of Kellogg's Corn Flakes*. **Brand names, type and portion sizes of each food must be recorded**. It is important to **record all food and drink over the 4 day period** including alcohol and snacks. Do not change your eating habits as we are not going to judge your diet and it is crucial that we get an accurate picture of your habitual (usual) eating patterns.

Please bring the completed diary to your scheduled appointment or if it is not completed in time please return the completed diary as soon as possible in the self-addressed envelope enclosed. If you have any queries or questions please do not hesitate to leave us a message on the office phone: 028 7012 3529.

Yours sincerely
Kirsty Porter/Katie Moore

For Office Use Only
D.O.B: ……
Height: ……...
Weight:……

The TUDA cohort study

FOOD DIARY

Volunteer Identification Code: ☐☐☐☐

NAME: ……

DATE: ……

Please keep this diary for 4 days in total:
2 consecutive weekdays and 2 consecutive weekend days

Contact details:
Miss Kirsty Porter/Miss Katie Moore
Room W2020
Biomedical Sciences
University of Ulster
Cromore Road
Coleraine
BT52 1SA
Tel: 028 70323529

FOOD DIARY INSTRUCTIONS

Please record **ALL** the foods you eat on the days requested.

For each food please describe:
1. The **method of cooking** e.g. fried, boiled, grilled, stewed, microwaved etc.
2. If possible please give the **brand and name** of the product used and the weight given on the packet were appropriate.
3. For **milk** please state type i.e. whole milk, semi-skimmed or skimmed.
4. For **butter, margarine, cooking oil** etc please give exact brand name.
5. Describe the **portion size** according to the instructions below.

PORTION SIZES

To estimate a portion size use the following household measurements:

RICE/PASTA
Estimate the portion size as small/ medium/ large, or give the number of tablespoons of cooked food eaten.

BREAD
State the number of slices/ rolls/ crisp breads eaten.

BREAKFAST CEREALS
Estimate the portion size as small/ medium/ large, or give the number of tablespoons eaten.

MEAT & FISH
Estimate the portion size as small/ medium/ large. For fish fingers, state the number eaten.

FRUIT
For whole fruit give the number eaten and estimate the size of the fruit as small/ medium/ large.
For tinned fruit estimate the number of tablespoons eaten or state the size of the can if the whole tin is consumed.
For dried fruit estimate the amount consumed in tablespoons.
For grapes estimate the number eaten.

VEGETABLES
Estimate the portion size as small/medium/large or give the number of tablespoons eaten.

NUTS
Estimate the number eaten or give the size of the bag.

MILK
Estimate the amount consumed in pints.

CHEESE
Estimate the number of slices eaten and state whether the slices were small medium or large.

EGGS
State the number eaten.

BISCUITS
State the number eaten.

CAKES
Estimate the size of the slice as small, medium or large.

CRISPS, SWEETS, CHOCOLATE, FIZZY DRINKS ETC
Report the brand name and weight as stated on the packet.

DRINKS
State whether cup or mug. For glasses state whether small, medium ($^1/_2$ pint) or large (pint).

SAUCES, CHUTNEYS ETC
Estimate the number of tablespoons consumed.

SUGAR, JAMS ETC
Estimate the amount consumed in teaspoons.

OTHER FOOD
Estimate the number of teaspoons consumed.

An example of how to complete a food diary is provided for you on the next page.

THANK YOU FOR YOUR HELP !

EXAMPLE

Day: Monday Date: 12/10/14

FOR OFFICE USE ONLY

	CODE	WT (g)
BREAKFAST 1 cup coffee (1 teaspoon sugar & Dale farm semi-skimmed milk) 6 tablespoons Kelloggs cornflakes with ¼ pint semi-skimmed milk 1 slice of Brennans white bread toasted with flora margarine thinly spread 1 teaspoon strawberry jam		
LUNCH Cheese and tomato sandwich 2 slices Hovis wholemeal bread, spread thinly with flora, 3 medium thick slices of Coleraine cheddar cheese, 1 small tomato, 2 teaspoons HP brown sauce) 1 packet cheese and onion crisps (Tayto) 1 small orange and 1 medium sized banana 1 can of coca-cola (330ml)		
EVENING MEAL 1 medium sized chicken breast (without skin) fried in vegetable oil 3 medium sized potatoes 5 tablespoons boiled carrots 3 tablespoons bisto gravy (made with bisto gravy granules) 1 small glass white wine medium slice of appletart (homemade) & coffee made as above		
BETWEEN MEAL SNACKS 2 cups of tea (each cup 1 teaspoon sugar and semi-skimmed milk) 1 mars bar 1 pot yoplait full fat strawberry yoghurt 4 McVities chocolate digestive biscuits		

Day: Date:

FOR OFFICE USE ONLY

	CODE	WT (g)
BREAKFAST		
LUNCH		
EVENING MEAL		
BETWEEN MEAL SNACKS		

Day: Date:

FOR OFFICE USE ONLY

	CODE	WT (g)
BREAKFAST		
LUNCH		
EVENING MEAL		
BETWEEN MEAL SNACKS		

Day: Date:

FOR OFFICE USE ONLY

	CODE	WT (g)
BREAKFAST		
LUNCH		
EVENING MEAL		
BETWEEN MEAL SNACKS		

Day: Date:

FOR OFFICE USE ONLY

	CODE	WT (g)
BREAKFAST		
LUNCH		
EVENING MEAL		
BETWEEN MEAL SNACKS		

Appendix 10:

Confirmation of ethical approval (Chapter 4)

HSC Business Services Organisation

Office for Research Ethics Committees Northern Ireland (ORECNI)

Customer Care & Performance Directorate
Office Suite 3
Lisburn Square House
Haslem's Lane
Lisburn
Co. Antrim BT28 1TW
Tel:+44 (0) 28 9260 3107
www.orecni.hscni.net
HSC REC B

27 November 2014

Prof Sean Strain
Director of Northern Ireland Centre for Food and Health (NICHE)
University of Ulster
Room W2097, CMB
Cromore Road
Coleraine
BT52 1SA

Dear Prof Strain

Study title:	**The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study**
REC reference:	**08/NIR03/113**
Amendment number:	**Substantial Amendment 10 – 24/10/2014**
Amendment date:	**24 October 2014**
IRAS project ID:	

The above amendment was reviewed at the meeting of the Sub-Committee held on 26 November 2014.

Ethical opinion

The members of the Committee taking part in the review gave a **favourable ethical opinion** of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	IRAS Version 3.5	24 October 2014
Other [Email from C Hughes]		24 October 2014
Other [CI Signature]		24 October 2014
Other [CV - Katie Moore (Appendix 12)]		
Participant information sheet (PIS) [Clean]	Version 7.0	07 March 2014
Participant information sheet (PIS) [Tracked]	Version 8.0	22 October 2014
Participant information sheet (PIS) [Clean]	Version 8.0	22 October 2014

Research protocol or project proposal [Tracked Changes]	Version 6.0	22 October 2014
Research protocol or project proposal [Clean]	Version 6.0	22 October 2014
Research protocol or project proposal	Version 5.0	07 March 2014

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

08/NIR03/113: Please quote this number on all correspondence

Yours sincerely

pp **Ms Sue Trouton**
Alternate Vice-Chair – Chair of the meeting
E-mail: RECB@hscni.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mr Nick Curry, University of Ulster*
Mrs Sally Doherty, Western HSC Trust

HSC REC B

Attendance at Sub-Committee of the REC meeting on 26 November 2014

Committee Members:

Name	*Profession*	*Present*	*Notes*
Ms Marilyn Trimble		Yes	
Ms Sue Trouton	Community Midwifery Sister	Yes	AVC – Chair of the meeting

Also in attendance:

Name	*Position (or reason for attending)*
Dr Siobhan McGrath	Head of ORECNI – minuted meeting

Appendix 11:

Research protocol (Chapter 4)

Research Protocol

Title

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study

Background

Cardiovascular disease (CVD), osteoporosis and Alzheimer's disease are chronic diseases of ageing that impact adversely on the lives of those affected and have major health, social and economic consequences.

A number of factors are considered to be implicated in these diseases, ranging from the more established factors to those that are less well recognised. Lifestyle factors such as diet, body weight, smoking, physical activity and years of education are acknowledged as risk factors for the development of these chronic diseases. Of the nutritional factors, calcium and vitamin D are well-established as important nutrients for the maintenance of bone mineral density (BMD) and the prevention of osteoporosis (Lanham-New, 2008). However, new research now suggests that elevated homocysteine and/or sub-optimal status of the metabolically related B-vitamins (folate, vitamin B12) may be associated not only with a higher risk of osteoporosis (Cagnacci et al, 2003; Dhonukshe-Rutten et al, 2005; Gjesdal et al, 2006), but also of CVD, in particular stroke (Hcy studies collaboration, 2002; Wald et al, 2002; Wang et al, 2007), and of cognitive decline and dementia (Quadri et al, 2004; Kado et al, 2005; Haan et al, 2007). In addition, genetic factors are increasingly recognised as playing a critical role in disease risk. Individuals who are homozygous (TT genotype) for the common 677C→T polymorphism in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR) that plays an important role in folate/homocysteine metabolism, are reported to have a higher risk of hypertension (Jiang et al, 2004), CVD (Wald et al, 2002; Klerk et al, 2002) and osteoporosis (Riancho et al, 2006) compared to those without the 677C →T polymorphism. Likewise, polymorphisms of the vitamin D receptor (VDR) gene have been associated with an increased risk of osteoporosis (Uitterlinden et al, 2006; Moffett et al, 2007). However, there are inconsistencies in the literature as to the importance of such genetic variants in disease risk. It is possible that the detrimental health effects associated with these genetic factors may only occur in particular population subgroups, such as those with low nutritional status. There is evidence to suggest that the typical phenotype (i.e. elevated homocysteine concentrations) associated with homozygosity for the MTHFR 677C→T polymorphism can be modified by folate (Klerk et al, 2002) and riboflavin status (McNulty et al, 2006), and that the risk of osteoporosis associated with polymorphisms of the VDR gene only occurs in those with low calcium intakes (MacDonald, 2003).

Although the interplay between relevant genetic and nutrient factors (gene-nutrient interactions) is considered to be highly relevant in the development (and prevention) of chronic diseases of ageing, this relatively new area of research is as yet poorly understood. The collection of clinical, lifestyle, nutritional and genetic data on large numbers of patients would permit the investigation of those nutrients which interact with specific genes to increase the likelihood of a person developing chronic diseases of ageing. Funding has recently been secured from both Governments (Irish and UK) for a large cross-border project to investigate this research area.

Study Aim

The aim of this study was to collect detailed clinical, lifestyle, dietary, genetic and biochemical data to investigate gene-nutrient interactions (particularly from the perspective of the B-vitamins and vitamin D/calcium) in the development of CVD, osteoporosis and Alzheimer's disease by studying older adults exhibiting the early stages of these common diseases, namely hypertension, low bone mineral density, and early memory loss, respectively.

Secondary aims (follow up investigation)

- To re-assess clinical, nutritional, genetic and biochemical factors in relation to neuropsychiatric function in a subset of TUDA study participants, five years after initial investigation.
- To investigate the effect of low does B-vitamin supplementation for 2 years on brain health.

Study Design and Methods

Initial investigation (recruitment and sampling completed by October 2012)
The current study (**T**rinity, **U**lster and **D**epartment of **A**griculture; TUDA) was one of three studies being undertaken as part of a cross-border collaborative project (the National Nutrition Phenotype Database Project), involving four universities: University College Dublin, Trinity College Dublin, University College Cork and the University of Ulster. The TUDA study is an observational study of a convenience sample of 5,201 patients recruited from both the North and South of Ireland with phenotypic early evidence of Alzheimer's disease, osteoporosis and CVD. Ethical approval has been applied for, and obtained in the South for the cognitively impaired (n=2000) and the osteoporotic cohorts (n=2000) who will be recruited from St James's Hospital Dublin. Ethical approval is being sought here only for the recruitment of the 2100 hypertensive patients who are under the care of Consultant Cardiologists at two separate Trusts in Northern Ireland (Western Health and Social Care Trust and, Northern Health and Social Care Trust) and for the examination of data from all 5,201 participants.

Patients under the care of the Consultant Cardiologists will be approached. Those patients who give their permission will be contacted by telephone by the researchers who will fully explain the study to them and then invite them to participate once current suitability is established according to the inclusion/exclusion criteria. The inclusion criteria are: patients over 60 years of age who attend (or have attended) a cardiology clinic and have been diagnosed with hypertension defined as a blood pressure of greater than 140/90 mmHg (British Hypertension Society, 2006) or are currently taking hypertensive medication. The exclusion criteria are as follows: patients under 60 years of age, patient (or their parent) not born in either the North or South of Ireland and those with severe dementia. Patients that are suitable and that verbally agree to participate will be given a participant information sheet and consent form and allowed at least 48 hours to consider the written information and decide if they wish to participate. If agreeable and after informed consent is received, participants will be asked to do the following:

- complete a detailed health and lifestyle questionnaire to obtain information on diet, general health, drug and supplement use.

- complete physiological function tests (e.g. blood pressure, DXA scans and a battery of cognitive function tests). The researchers will be trained in how to administer the cognitive tests by a consultant physician (Dr. F. Tracey CHSST).
- have their height, weight and waist/hip measurements taken.
- provide a non-fasting blood sample (50ml). Blood samples will be taken by a qualified phlebotomist.
- Provide a buccal swab

Blood samples will be analysed for the following:

- routine clinical markers of health (e.g. renal function, liver function, lipids, full blood count, glucose, glycosylated haemoglobin) will be performed at the participating hospital laboratory;
- vitamin B12 biomarkers - serum total vitamin B12 (microbiological assay), serum transcobalamin (holoTC, microparticle enzyme immunoassay) and plasma methylmalonic acid (GCMS) at Trinity College Dublin;
- plasma homocysteine (immunoassay), serum folate and red cell folate (microbiological assay) at Trinity College Dublin;
- riboflavin status (erythrocyte glutathione reductase activation coefficient; EGRac) at the University of Ulster;
- vitamin B6 status (pyridoxal-5-phosphate, PLP by HPLC) at the University of Ulster;
- Pepsinogen 1 and 2 will be measured using Biohit Pepsinogen I and II ELISA kits at the University of Ulster
- bone biomarkers including serum 25-hydroxyvitamin D (ELISA) and serum intact parathyroid hormone (PTH; ECIA) at the University of Ulster; and

Blood samples and buccal swabs will be anlaysed for

- single nucleotide polymorphisms (SNPs) from the perspective of folate/vitamin B12 and calcium/vitamin D metabolism and the Apolipoprotein E gene.

Blood samples/buccal swabs for the TUDA study will be stored at both Trinity College Dublin and the University of Ulster.

In addition, volunteers who have previously had a bone scan at the University of Ulster as part of a research study (and have agreed to be recontacted) were invited to participate. If agreeable and after informed consent was received, participants were asked complete the study protocol as above with the omission of the DXA scan.

Five to seven year follow-up investigation

We propose to conduct a follow-up investigation in a subset of participants (n=444). Only patients that consented to be re-contacted for further research will be contacted via the telephone by the researchers. In addition a subset of participants *n* 222 will be randomised to receive either placebo or low dose B vitamin for two years. All participants are eligible except those with a MMSE score <25 (i.e. indicative of mild cognitive impairment). Those that verbally agree to participate will be given a participant information sheet and will be allowed at least 48 hours to consider this. If agreeable, participants will be asked to complete procedures identical to those outlined above at both time points with the following exception. A DXA scan will not be performed.

Statistical analysis

As this is a collaborative all-Ireland study, statistical analysis will be approached centrally. A working group on data management and analysis is currently being established which will include the task leaders and all PIs in the field of bioinformatics, statistics and epidemiology. A workshop will be held to ascertain the possible options for the best architecture and warehousing of the data and subsequent centralised training will be arranged in Dublin to cover the collection, entry and statistical analysis of data. Dr Cathal Walsh, Lecturer in Statistics at Trinity College Dublin, will act as statistician to the overall TUDA cohort.

Data management

The study investigators (please see revised research team) will be responsible for the collection, input and analysis of all data.
All data collected for the study will be kept strictly confidential, and subjects will be assigned a unique identification code. The file containing personal details and study identification numbers will be kept under secure conditions in accordance with the NHS Guidelines (HSC99/053) and will be kept for a minimum of 25 years. The data will be archived securely in a restricted access room for the duration. The main database involved in the study will be stored on a password-protected computer. Any hard copies will be kept under locked conditions within the University of Ulster.

Ethical considerations

These are the main ethical issues in relation to this study.

i) Informed Consent – all potential participants will be given both written and oral information about the study before providing informed consent. It is envisaged that some of the participants will have difficulties in reading, hearing and writing. Participants will be provided with enlarged copies of the information sheet and consent form on request and they will be informed that they may nominate someone who can sign the consent form on their behalf. Every effort will be made to provide a translation of the information relevant to the study for those who do not understand written or verbal English.
ii) Participant Confidentiality – all participants will be assigned a unique identification study code.
iii) Blood Sampling – blood samples will be taken by a fully trained phlebotomist with a first aider on site.
iv) DXA Scanning – the radiation which the volunteer will be subjected to will be the minimum possible dose. Those individuals performing the scans will be fully trained and will adhere to all current health and safety regulations (IRMER) and DXA performance protocols.
v) Sample storage – all human samples will be stored as per requirements under the Human Tissue Act (HTA).
vi) Data Records – all participant data will be stored on a password protected computer. Hard copies of data will be stored in locked cabinets under the custodial care of the Chief Investigator.
vii) Inclusion and exclusion criteria will be implemented.

References

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Appendix 12:

Participant information sheet (Chapter 4)

Participant Information Sheet

Northern Ireland Centre for Food and Health (NICHE)
School of Biomedical Science
University of Ulster

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study

(A study to examine the link between diet, genetics and health)

Invitation

You are being invited to take part in research being conducted at the University of Ulster in collaboration with two Health and Social Care Trusts in Northern Ireland (Western and Northern Trusts). Before you decide whether or not to participate, it is important that you understand what the research is for and what you will be asked to do. Please read the following information and do not hesitate to ask any questions about anything that is unclear to you. Take time to consider whether or not you want to take part.

Thank you for taking the time to consider this invitation.

What is the purpose of the study?

High blood pressure, low bone density and mild memory loss are common health problems among older adults that can lead to more serious problems if left untreated. A number of factors such as diet, genetic make-up and lifestyle are known to influence the likelihood of a person developing these diseases. The purpose of this study is to collect information on these factors from adults over 60 years of age who live in the North or South of Ireland. The information gathered will be used to try to more fully understand the link between diet, genetic make-up and health that is likely to help in the prevention of these common diseases.

This study is a follow up from the original TUDA study conducted by the University of Ulster in which you took part. The purpose on this occasion is to re-examine the same health and lifestyle measurements 5-7 years later.

Why have I been chosen?

You have been chosen because you previously took part in the original TUDA study conducted at the University of Ulster and agreed to be re contacted by researchers. If you agree to take part, you will be one of 444 people taking part in this study.

Do I have to take part?

It is up to you to decide whether or not you take part. If you decide to participate, you will be given this information sheet to keep and you will be asked to sign a consent form. You have the right to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to participate, will not affect the standard of care that you receive.

What will happen to me if I take part?

The assessment will be very similar to the previous assessment that you took part in
You will be asked to:

- Have your blood pressure measured
- Have your height, weight and waist circumference measured
- Provide one 50ml blood sample
- Provide a buccal swab (a cotton swab is rubbed gently on the inside of your cheek)
- Provide answers to some questions about your diet, lifestyle, use of vitamin supplements and medications, your own general health

- Undergo some tests of memory and mood
- Take a low dose B vitamin or dummy capsule for 2 years (sub-set *n* 222)

(The above measurements will take place at the University of Ulster or a clinic within your Health Trust, which is convenient to you.)

Your participation in the study will involve no more than two appointments each of which will take approximately 1 hour 15 minutes in total (excluding travel time).

We will use your blood sample to test for some general markers of health such as cholesterol, kidney and liver function, and to test your vitamin levels. If you are willing we would also like to take a blood and buccal swab sample from you that we will use for DNA analysis. We would also ask for your permission to retain your blood samples for use in future studies.

We also ask you if you agree to be contacted by researchers from the University of Ulster at a later date and invited to take part in similar nutrition related studies. You will only be agreeing to receive information and will not be under any obligation to take part in any future studies. If you decide not to consent to being contacted in the future it will have no influence on your involvement in this research study and will not affect any standard of care that you receive.

Risks and/or disadvantages of taking part?

There is a very small risk of bruising when giving a blood sample, but a fully trained phlebotomist will take your blood sample to ensure that any discomfort is kept to a minimum. In the unlikely event that your results show something abnormal you will be contacted and the information will be forwarded to your GP.

Are there any possible benefits in taking part?

There are no direct benefits from taking part in this study although the information gained may prove extremely useful in understanding the link between diet, health and related genetic factors in people born in the North and South of Ireland. The results of the DNA tests would have no direct relevance to you but may help us understand why Irish people get certain chronic diseases. By taking part in the study you will also find out how you perform on a range of physical and psychological tests and some clinical blood tests. If we detect any abnormal clinical results we will notify your GP.

What if something goes wrong?

It is very unlikely that something will go wrong during this research. However, you should know that the University take complaints and concerns seriously and has procedures in place for reporting, investigating, recording and handling them. The University is insured for its staff and students to carry out research involving people however, this does not extend to non-negligent harm. The University knows about this research project and has approved it. Further details on insurance can be found in the University's research indemnity statement. Ask us if you would like a copy.

Will my information be kept confidential?

Your doctor will be aware of your participation in the study. Besides that, all information collected about you for the study will be kept strictly confidential, in accordance with the NHS Guidelines (HSC99/053) all data will be kept for a minimum of 25 years. The data will be archived securely in a restricted access room for the duration.

Any information that leaves the University of Ulster will have your name and address removed so that you cannot be identified. All samples collected from you will be coded so that you cannot be identified from them and will be stored in a locked freezer until they are analysed. Information will be safely destroyed once it is no longer required.

What will happen to the findings of the research study?

It is intended that the findings from this study will be published in scientific or medical journals and presented at conferences. You will not be identified in any report or publication. Once the study is complete we will send you a summary of the results.

Who is organising and funding the research?

Funding for this study was obtained initially from the Irish Department of Food and Agriculture with additional funding obtained from The Department of Employment and Learning (DEL) in Northern Ireland.

Thank you for taking the time to read this information.

If you have any questions or would like more information, contact:

Miss Kirsty Porter PhD student on 028 7012 3529; Porter-K7@email.ulster.ac.uk

Miss Katie Moore PhD student on 02870123529: Moore-K@ulster.ac.uk

Dr Catherine Hughes researcher on 028 7012 3516; c.hughes@ulster.ac.uk

Dr Leane Hoey researcher on 02870124781; l.hoey@ulster.ac.uk

Professor Sean Strain Chief investigator on 028 70124795; jj.strain@ulster.ac.uk

Appendix 13:

Study consent form (Chapter 4)

ULSTER UNIVERSITY **RESEARCH GOVERNANCE**

Consent Form for studies involving the use of human tissue **Participant Identification no:**

Title of Study	The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study (A study to examine the link between diet, genetics and health)
Chief Investigator	Professor Sean Strain Tel: 028 70324795; email: jj.strain@ulster.ac.uk

Please confirm, by marking the boxes provided, that you agree with the following statements:

1. I have been given and have read and understood the information sheet dated 22/10/2014 (version 8.0) for the above study and have asked and received answers to any questions raised ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my rights being affected in any way ☐
3. I understand that the researchers will hold all information and data collected during the study securely and in confidence and that all efforts will be made to ensure that I cannot be identified as a participant in the study (except as might be required by law) and I give permission for the researchers to hold relevant personal data ☐
4. I understand that my blood or other tissues are required for the purposes of this study and confirm that I have been given details of the amount(s) to be taken and how it will be stored, used and the method of disposal ☐
5. I agree to take part in the above study ☐
6. The potential benefits of keeping my blood or other tissues for future research studies have been explained to me and: ☐

   a. I consent to their indefinite storage and use in any future study including genetic studies, or ☐

   b. I consent to their indefinite storage and use in any future study that does not involve the isolation of my genetic material ☐

   c. I do not wish my blood or tissues to be used for any purpose other than this study ☐
7. I agree to being contacted at a later date and invited to take part in future studies of a similar nature. I understand that I am agreeing only to receive information and I am under no obligation to take part in any future studies. ☐

Name of Participant (please print)	Signature	Date (dd/mmm/yy)
Name of Researcher	Signature	Date (dd/mmm/yy)

1 copy for participant; 1 copy for researcher

Appendix 14:

Confirmation of ethical approval (Chapter 5)

HSC Business Services Organisation

Office for Research Ethics Committees Northern Ireland (ORECNI)

Customer Care & Performance Directorate
Unit 4, Lissue Industrial Estate West
Rathdown Walk
Moira Road
Lisburn
BT28 2RF
Tel: 028 95361400
www.orecni.hscni.net
HSC REC B

09 May 2017

Professor Sean Strain
Director of the centre for molecular biosciences
University of Ulster
w2010, CMB, University of Ulster
Cromore Road,Coleraine
County Derry
BT52 1SA

Dear Professor Strain

Study title:	**The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study**
REC reference:	**08/NIR03/113**
Amendment number:	**Amendment 14**
Amendment date:	**28 April 2017**
IRAS project ID:	**N / A**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a **favourable ethical opinion** of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	Amendment 14	28 April 2017
Other [Hubert Cecotti CV]		
Other [Professor Girijesh Prasad CV]		
Participant information sheet (PIS) [Appendix 6 Participant	1	14 April 2017

Information Sheet and consent form for MEG scan]		
Research protocol or project proposal [Appendix 1 Research Protocol tracked changes]	9	14 April 2017
Research protocol or project proposal [Appendix 1 Research Protocol clean]	9	14 April 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

08/NIR03/113: Please quote this number on all correspondence

Yours sincerely

Jane Keenan

pp Dr Sarah Anne Moorhead
HSC REC B Alternative Vice-Chair (Chair of the Sub-Committee)

E-mail: recb@hscni.net

Enclosures: *List of names and professions of members who took part in the review*

HSC REC B

Sub-Committee of the REC

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Sarah Anne Moorhead	Lecturer in Health & Interpersonal Communication	Yes	Chair
Mr Leon O'Hagan	Pharmacist	Yes	

Appendix 15:

Research protocol (Chapter 5)

Research Protocol

Title

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study

Background

Cardiovascular disease (CVD), osteoporosis and Alzheimer's disease are chronic diseases of ageing that impact adversely on the lives of those affected and have major health, social and economic consequences.

A number of factors are considered to be implicated in these diseases, ranging from the more established factors to those that are less well recognised. Lifestyle factors such as diet, body weight, smoking, physical activity and years of education are acknowledged as risk factors for the development of these chronic diseases. Of the nutritional factors, calcium and vitamin D are well-established as important nutrients for the maintenance of bone mineral density (BMD) and the prevention of osteoporosis (Lanham-New, 2008). However, new research now suggests that elevated homocysteine and/or sub-optimal status of the metabolically related B-vitamins (folate, vitamin B12) may be associated not only with a higher risk of osteoporosis (Cagnacci et al, 2003; Dhonukshe-Rutten et al, 2005; Gjesdal et al, 2006), but also of CVD, in particular stroke (Hcy studies collaboration, 2002; Wald et al, 2002; Wang et al, 2007), and of cognitive decline and dementia (Quadri et al, 2004; Kado et al, 2005; Haan et al, 2007). In addition, genetic factors are increasingly recognised as playing a critical role in disease risk. Individuals who are homozygous (TT genotype) for the common 677C→T polymorphism in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR) that plays an important role in folate/homocysteine metabolism, are reported to have a higher risk of hypertension (Jiang et al, 2004), CVD (Wald et al, 2002; Klerk et al, 2002) and osteoporosis (Riancho et al, 2006) compared to those without the 677C →T polymorphism. Likewise, polymorphisms of the vitamin D receptor (VDR) gene have been associated with an increased risk of osteoporosis (Uitterlinden et al, 2006; Moffett et al, 2007). However, there are inconsistencies in the literature as to the importance of such genetic variants in disease risk. It is possible that the detrimental health effects associated with these genetic factors may only occur in particular population subgroups, such as those with low nutritional status. There is evidence to suggest that the typical phenotype (i.e. elevated homocysteine concentrations) associated with homozygosity for the MTHFR 677C→T polymorphism can be modified by folate (Klerk et al, 2002) and riboflavin status (McNulty et al, 2006), and that the risk of osteoporosis associated with polymorphisms of the VDR gene only occurs in those with low calcium intakes (MacDonald, 2003).

Although the interplay between relevant genetic and nutrient factors (gene-nutrient interactions) is considered to be highly relevant in the development (and prevention) of chronic diseases of ageing, this relatively new area of research is as yet poorly understood. The collection of clinical, lifestyle, nutritional and genetic data on large numbers of patients would permit the investigation of those nutrients which interact with specific genes to increase the likelihood of a person developing chronic diseases of ageing. Funding has recently been secured from both Governments (Irish and UK) for a large cross-border project to investigate this research area.

Study Aim

The aim of this study was to collect detailed clinical, lifestyle, dietary, genetic and biochemical data to investigate gene-nutrient interactions (particularly from the perspective of the B-vitamins and vitamin D/calcium) in the development of CVD, osteoporosis and Alzheimer's disease by studying older adults exhibiting the early stages of these common diseases, namely hypertension, low bone mineral density, and early memory loss, respectively.

Secondary aims (follow up investigation)

- To re-assess clinical, nutritional, genetic and biochemical factors in relation to neuropsychiatric function in a subset of TUDA study participants, five years after initial investigation.
- To investigate the effect of low does B-vitamin supplementation for 2 years on brain health.
- To investigate brain function using Magnetoencephalography (MEG) in a subset participants with optimal versus sub-optimal B-vitamins status.

Study Design and Methods

Initial investigation (recruitment and sampling completed by October 2012)
The current study (**T**rinity, **U**lster and **D**epartment of **A**griculture; TUDA) was one of three studies being undertaken as part of a cross-border collaborative project (the National Nutrition Phenotype Database Project), involving four universities: University College Dublin, Trinity College Dublin, University College Cork and the University of Ulster. The TUDA study is an observational study of a convenience sample of 5,201 patients recruited from both the North and South of Ireland with phenotypic early evidence of Alzheimer's disease, osteoporosis and CVD. Ethical approval has been applied for, and obtained in the South for the cognitively impaired (n=2000) and the osteoporotic cohorts (n=2000) who will be recruited from St James's Hospital Dublin. Ethical approval is being sought here only for the recruitment of the 2100 hypertensive patients who are under the care of Consultant Cardiologists at two separate Trusts in Northern Ireland (Western Health and Social Care Trust and, Northern Health and Social Care Trust) and for the examination of data from all 5,201 participants.

Patients under the care of the Consultant Cardiologists will be approached. Those patients who give their permission will be contacted by telephone by the researchers who will fully explain the study to them and then invite them to participate once current suitability is established according to the inclusion/exclusion criteria. The inclusion criteria are: patients over 60 years of age who attend (or have attended) a cardiology clinic and have been diagnosed with hypertension defined as a blood pressure of greater than 140/90 mmHg (British Hypertension Society, 2006) or are currently taking hypertensive medication. The exclusion criteria are as follows: patients under 60 years of age, patient (or their parent) not born in either the North or South of Ireland and those with severe dementia. Patients that are suitable and that verbally agree to participate will be given a participant information sheet and consent form and allowed at least 48 hours to consider the written information and decide if they wish to participate. If agreeable and after informed consent is received, participants will be asked to do the following:

- complete a detailed health and lifestyle questionnaire to obtain information on diet, general health, drug and supplement use.

- complete physiological function tests (e.g. blood pressure, DXA scans and a battery of cognitive function tests). The researchers will be trained in how to administer the cognitive tests by a consultant physician (Dr. F. Tracey CHSST).
- have their height, weight and waist/hip measurements taken.
- provide a non-fasting blood sample (50ml). Blood samples will be taken by a qualified phlebotomist.
- Provide a buccal swab
- Hand grip strength
- Undergo Magnetoencephalography (MEG) (Elekta Neuroimag Truix) brain scan to assess brain function by passively measuring the magnetic fields produced by neurons in the brain. It is a safe, non invasive technique that measures electo-physiological responses in terms of brain magnetic activities recorded from extra-cranial sensors. This will take place at the Intelligent Systems Research Centre (ISRC) at Ulster University Magee.

Blood samples will be analysed for the following:

- routine clinical markers of health (e.g. renal function, liver function, lipids, full blood count, glucose, glycosylated haemoglobin) will be performed at the participating hospital laboratory;
- vitamin B12 biomarkers - serum total vitamin B12 (microbiological assay), serum transcobalamin (holoTC, microparticle enzyme immunoassay) and plasma methylmalonic acid (GCMS) at Trinity College Dublin;
- plasma homocysteine (immunoassay), serum folate and red cell folate (microbiological assay) at Trinity College Dublin;
- riboflavin status (erythrocyte glutathione reductase activation coefficient; EGRac) at the University of Ulster;
- vitamin B6 status (pyridoxal-5-phosphate, PLP by HPLC) at the University of Ulster;
- Pepsinogen 1 and 2 will be measured using Biohit Pepsinogen I and II ELISA kits at the University of Ulster
- bone biomarkers including serum 25-hydroxyvitamin D (ELISA) and serum intact parathyroid hormone (PTH; ECIA) at the University of Ulster; and

Blood samples and buccal swabs will be anlaysed for

- single nucleotide polymorphisms (SNPs) from the perspective of folate/vitamin B12 and calcium/vitamin D metabolism and the Apolipoprotein E gene.

Blood samples/buccal swabs for the TUDA study will be stored at both Trinity College Dublin and the University of Ulster.

In addition, volunteers who have previously had a bone scan at the University of Ulster as part of a research study (and have agreed to be recontacted) were invited to participate.
If agreeable and after informed consent was received, participants were asked complete the study protocol as above with the omission of the DXA scan.

Five to seven year follow-up investigation
We propose to conduct a follow-up investigation in a subset of participants (n=600-650). Only patients that consented to be re-contacted for further research will be contacted via the telephone by the researchers. In addition a subset of TUDA participants (and their partners) $n \leq 300$ will be randomised to receive either placebo or low dose B vitamin for two years, of which a selection of participants (n 50) will undergo brain function assessments using

magnetoencephalography (MEG). All participants are eligible except those with a MMSE score <25 (i.e. indicative of mild cognitive impairment). Those that verbally agree to participate will be given a participant information sheet and will be allowed at least 48 hours to consider this. If agreeable, participants will be asked to complete procedures identical to those outlined above at both time points with the following exception. A DXA scan will not be performed.

Statistical analysis

As this is a collaborative all-Ireland study, statistical analysis will be approached centrally. A working group on data management and analysis is currently being established which will include the task leaders and all PIs in the field of bioinformatics, statistics and epidemiology. A workshop will be held to ascertain the possible options for the best architecture and warehousing of the data and subsequent centralised training will be arranged in Dublin to cover the collection, entry and statistical analysis of data. Dr Cathal Walsh, Lecturer in Statistics at Trinity College Dublin, will act as statistician to the overall TUDA cohort.

Data management

The study investigators (please see revised research team) will be responsible for the collection, input and analysis of all data.
All data collected for the study will be kept strictly confidential, and subjects will be assigned a unique identification code. The file containing personal details and study identification numbers will be kept under secure conditions in accordance with the NHS Guidelines (HSC99/053) and will be kept for a minimum of 25 years. The data will be archived securely in a restricted access room for the duration. The main database involved in the study will be stored on a password-protected computer. Any hard copies will be kept under locked conditions within the University of Ulster.

Ethical considerations

These are the main ethical issues in relation to this study.

i) Informed Consent – all potential participants will be given both written and oral information about the study before providing informed consent. It is envisaged that some of the participants will have difficulties in reading, hearing and writing. Participants will be provided with enlarged copies of the information sheet and consent form on request and they will be informed that they may nominate someone who can sign the consent form on their behalf. Every effort will be made to provide a translation of the information relevant to the study for those who do not understand written or verbal English.
ii) Participant Confidentiality – all participants will be assigned a unique identification study code.
iii) Blood Sampling – blood samples will be taken by a fully trained phlebotomist with a first aider on site.
iv) DXA Scanning – the radiation which the volunteer will be subjected to will be the minimum possible dose. Those individuals performing the scans will be fully

trained and will adhere to all current health and safety regulations (IRMER) and DXA performance protocols.

v) Sample storage – all human samples will be stored as per requirements under the Human Tissue Act (HTA).

vi) Data Records – all participant data will be stored on a password protected computer. Hard copies of data will be stored in locked cabinets under the custodial care of the Chief Investigator.

vii) Inclusion and exclusion criteria will be implemented.

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Moffett SP, Zmuda JM, Cauley JA, et al. Association of the VDR translation start site polymorphism and fracture risk in older women. *J Bone Miner Res* 2007;22:730-6.

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Uitterlinden AG, Ralston SH, Brandi ML, et al. The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. *Ann Intern Med* 2006;145:255-64.

Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* 2002;325:1202-6

Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007;369:1876-82.

Appendix 16:

Participant information sheet and consent form (Chapters 5)

Participant Information Sheet

Northern Ireland Centre for Food and Health (NICHE)
School of Biomedical Science
University of Ulster

The Trinity, Ulster and Department of Agriculture (TUDA) Cohort Study
Magnetoencephalography Pilot Study

Invitation

You are being invited to take part in research being conducted at the University of Ulster in collaboration with two Health and Social Care Trusts in Northern Ireland (Western and Northern Trusts). Before you decide whether or not to participate, it is important that you understand what the research is for and what you will be asked to do. Please read the following information and do not hesitate to ask any questions about anything that is unclear to you. Take time to consider whether or not you want to take part.

Thank you for taking the time to consider this invitation.

What is the purpose of the study?

Declining brain health including mild memory loss, depression and anxiety are common health problems among older adults that can lead to more serious problems if left untreated. A number of factors such as diet, genetic make-up and lifestyle are known to influence the likelihood of a person developing these diseases. The purpose of this pilot study is to carry out the novel, safe and non-invasive Magnetoencephalography (MEG) (Elekta Neuroimag Truix) brain scan to assess brain function in those with optimised verses non-optimised B-vitamin status. The information gathered will be used to try to more fully understand the link between diet, genetic make-up and health that is likely to help in the prevention of these common diseases.

What is MEG?

Magnetoencephalography (MEG) is a modern non-invasive technique for measuring magnetic fields generated by the brain in the space above the scalp. It is a completely safe and non-invasive brain imaging technique which records the small magnetic fields produced by the brain using delicate sensors. MEG is housed in a magnetic shielded room (MSR) for reducing noise from the surrounding environment. It allows very fast measurement of ongoing brain activity. As seen in Fig. 1, study participants will be seated on a comfortable chair with their head placed inside the helmet of MEG sensors that are able to detect extremely small magnetic signals produced by the brain.

Fig. 2: A participant seated in the MEG scanner

Do I have to take part?

As with the wider study it is up to you to decide whether or not you take part. If you decide to participate, you will be given this information sheet to keep and you will be asked to sign a consent form. You have the right to withdraw from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to participate, will not affect the standard of care that you receive.

What will happen to me if I take part?

You will be invited to come for an appointment at the Intelligent Systems Research Centre (ISRC), Ulster University, Magee campus. Experiments will take place in ISRC's Northern Ireland Functional Brain Mapping (NIFBM) facility where your Magnetoencephalography (MEG) brain scan will be recorded. The MEG brain scan is a modern method of measuring signals produced by the brain.

This will involve some preparation consisting of attaching a few electrodes and converting the signals into a digital image of your head following a standard operating procedure (SOP). Once ready, you will be seated in an arm-chair in front of a projector or computer screen. Please note that currents or voltages will not be applied to your head at any stage.

A typical experiment will consist of about 30 minutes of scanning. Your participation in the study will involve no more than one appointment which will take approximately 1 hour in total (excluding travel time).

Will my information be kept confidential?

As with the wider study all information obtained during this investigation will be kept strictly confidential using your unique ID number and held securely on a password protected computer.

Thank you for taking the time to read this information.

If you have any questions or would like more information, contact:

Miss Katie Moore PhD student on 02870123529: Moore-K@ulster.ac.uk

Dr Catherine Hughes researcher on 028 7012 3516; c.hughes@ulster.ac.uk

Dr Leane Hoey researcher on 02870124781; l.hoey@ulster.ac.uk

Professor Sean Strain Chief investigator on 028 70124795; jj.strain@ulster.ac.uk

ULSTER UNIVERSITY **RESEARCH GOVERNANCE**

Consent Form for Magnetoencephalography (MEG) study **Participant Identification no:**

Title of Study	The Trinity, Ulster and Department of Agriculture (TUDA) Cohort study Magnetoencephalography Pilot Study
Chief Investigator	Professor Sean Strain

Please confirm, by marking the boxes provided, that you agree with the following statements:

1. I have been given and have read and understood the information sheet dated 14/04/17 (Version 1.0) for the above study and have asked and received answers to any questions raised ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my rights being affected in any way ☐

3. I understand that the researchers will hold all information and data collected during the study securely and in confidence and that all efforts will be made to ensure that I cannot be identified as a participant in the study (except as might be required by law) and I give permission for the researchers to hold relevant personal data ☐

4 I agree to take part in the above study ☐

Name of Participant (please print)	Signature	Date (dd/mmm/yy)

Name of Researcher	Signature	Date (dd/mmm/yy)

Appendix 17:

Publications

i. List

ii. Copies

List of Publications

Full papers

Adrian McCann, Helene McNulty, Jan Rigby, Catherine F Hughes, Leane Hoey, Anne M Molloy, Conal J Cunningham, Miriam C Casey, Fergal Tracey, Maurice O'Kane, Kevin McCarroll, Mary Ward, **Katie Moore**, JJ Strain and Adrian Moore. (2018) Impact of area-level socioeconomic deprivation on the risk of cognitive dysfunction in older adults. *Journal of the American Geriatrics Society.* DOI: 10.1111/jgs.15258.

Katie Moore, Catherine F. Hughes, Mary Ward, Leane Hoey and Helene McNulty (2018) Diet, nutrition and the ageing brain: current evidence and new directions'. *Proceedings of the Nutrition Society.* doi.org/10.1017/S0029665117004177

Katie Moore, Maeve O'Shea, Catherine F. Hughes, Leane Hoey, Mary Ward and Helene McNulty (2017) Current evidence linking nutrition with brain health in ageing *Nutrition Bulletin*. 42, 1, 61-68

Abstracts

Moore K., Hughes C. F., Hoey L., Ward M., Porter K., Strain JJ., Pentieva K., Molloy A., and McNulty H. (2017) Role of fortification and supplementation in achieving optimal biomarker status of B-vitamins for better mental health in older adults *Proceedings of the Nutrition Society*, 76 (OCE3), E49

Moore K., Hughes C. F., Hoey L., Ward M., Porter K., Strain JJ., Molloy A., Cunningham C., Pentieva K., and McNulty H. 144/2371 Role of folate and the metabolically related B-vitamins in Brain Health in older adults: *Annals of Nutrition and Metabolism Journal* 2017;71(suppl 2):1–1433

Porter K., Hughes C. F., Hoey L., Ward M., **Moore K.,** Strain J.J., Molloy A., Cunningham C., Casey M., Pentieva K., and McNulty H. (2017). Biomarkers of folate and related B-vitamins as predictors of cognitive decline in older Irish adults over a 5 year follow up period. *Proceedings of the Nutrition Society*. 76 (OCE1), E4.

Porter K., Hughes C. F., Hoey L., Ward M., **Moore K.,** Strain J.J., Molloy A., Cunningham C., Casey M., Pentieva K., and McNulty H. (2016). Investigation of the role of riboflavin, vitamin B6 and MTHFR genotype as determinants of cognitive health in ageing. *Proceedings of the Nutrition Society*, 75 OCE3.

Moore K., Hughes C. F., Hoey L., Ward M., Porter K., Strain J.J., Molloy A., Cunningham C., Casey M., Pentieva K., and McNulty H. (2016). Role of B-vitamins as determinants of neuropsychiatric health in ageing. *Proceedings of the Nutrition Society*, 75 OCE3.

CLINICAL INVESTIGATION

Effect of Area-Level Socioeconomic Deprivation on Risk of Cognitive Dysfunction in Older Adults

Adrian McCann, PhD, Helene McNulty, PhD,* Jan Rigby, PhD,† Catherine F. Hughes, PhD,* Leane Hoey, PhD,* Anne M. Molloy, PhD,‡ Conal J. Cunningham, MD,§ Miriam C. Casey, MD,§ Fergal Tracey, MD,¶ Maurice J. O'Kane, MD,** Kevin McCarroll, MD,§ Mary Ward, PhD,* Katie Moore, BSc,* J.J. Strain, PhD,* and Adrian Moore, PhD††*

OBJECTIVES: To investigate the relationship between area-level deprivation and risk of cognitive dysfunction.

DESIGN: Cross-sectional analysis.

SETTING: The Trinity, Ulster, and Department of Agriculture (TUDA) study from 2008 to 2012.

PARTICIPANTS: Community-dwelling adults aged 74.0 ± 8.3 without dementia (N = 5,186; 67% female).

MEASUREMENTS: Adopting a cross-jurisdictional approach, geo-referenced address-based information was used to map and link participants to official socioeconomic indicators of deprivation within the United Kingdom and the Republic of Ireland. Participants were assigned an individual deprivation score related to the smallest administrative area in which they lived. These scores were categorized into comparable quintiles, that were then used to integrate the datasets from both countries. Cognitive health was assessed using the Mini-Mental State Examination (MMSE); cognitive dysfunction was defined as a MMSE score of 24 or less.

RESULTS: Approximately one-quarter of the cohort resided within the most-deprived districts in both countries. Greater area-level deprivation was associated with significantly lower MMSE scores; fewer years of formal education; greater anxiety, depression, smoking and alcohol use, and obesity; and more adverse outcomes, including higher blood pressure and diabetes risk. After adjustment for relevant covariates, area deprivation was associated with significantly higher risk of cognitive dysfunction (odds ratio =1.40, 95% confidence interval = 1.05–1.87, P = .02, for most vs least deprived).

CONCLUSION: This analysis combining data from two health systems shows that area deprivation is an independent risk factor for cognitive dysfunction in older adults. Adults living in areas of greatest socioeconomic deprivation may benefit from targeted strategies aimed at improving modifiable risk factors for dementia. Further cross-national analysis investigating the impact of area-level deprivation is needed to address socioeconomic disparities and shape future policy to improve health outcomes in older adults. **J Am Geriatr Soc 2018.**

Key words: older adults; cross-jurisdictional; geo-referencing; area-level deprivation; cognition

Health, well-being, and socioeconomic status are closely linked, with considerable evidence showing poorer health[1] and earlier morbidity and death[2–4] in persons at lower socioeconomic levels. There has been growing interest in whether the area in which a person lives can influence health in addition to the effects of individual socioeconomic factors.[5] Area deprivation indices represent a geographic area-based composite measure of the socioeconomic deprivation of neighborhoods. They are typically constructed from a range of domains relating to deprivation such as income, employment, education, proximity to services, living environment, and crime and disorder and are presented as a single value or score for each neighborhood or area. Higher index values represent greater levels of deprivation in an area, and available evidence suggests that higher area deprivation is associated with greater risk

From the *Nutrition Innovation Centre for Food and Health, Ulster University, Coleraine, Northern Ireland, United Kingdom; †National Centre for GeoComputation, Maynooth University, Maynooth, Ireland; ‡Institute of Molecular Medicine, School of Medicine, Trinity College Dublin, Dublin, Ireland; §Mercer's Institute for Research on Ageing, St James's Hospital, Dublin, Ireland; ¶Causeway Hospital, Northern Health and Social Care Trust, Coleraine, Northern Ireland, United Kingdom; **Clinical Chemistry Laboratory, Altnagelvin Hospital, Western Health and Social Care Trust, Londonderry, Northern Ireland, United Kingdom; and ††School of Environmental Sciences, Ulster University, Coleraine, Northern Ireland, United Kingdom.

Address correspondence to Professor Helene McNulty, Nutrition Innovation Centre for Food and Health, Ulster University, Coleraine, Northern Ireland BT52 1SA, United Kingdom.
E-mail: h.mcnulty@ulster.ac.uk

See related editorial by Soo Borson.

DOI: 10.1111/jgs.15258

0002-8614/18/$15.00

of chronic disease and premature mortality[6,7] independent of individual socioeconomic circumstances.[6,8–10]

Cognitive function in aging has become a global public health priority because it has important implications for independence and quality of life of older adults.[11] With the prevalence of dementia predicted to triple by 2050, it is important to identify individuals at greatest risk of developing cognitive dysfunction, an early predictor of dementia.[11] Few previous studies have examined the influence of area-level deprivation on cognitive health in older adults, although one study from England reported that greater area-level deprivation was not significantly associated with cognitive impairment and dementia after accounting for individual-level factors.[12] To our knowledge, no previous study has investigated area-level deprivation in relation to cognitive health in cross-national research.

Some progress has been made in advancing the development of standardized deprivation indicators at a European Union level[13] and more detailed comparisons of small area-level deprivation for a selection of countries within Europe,[14] demonstrating the potential for using such measures in cross-national health inequality research. The aim of this study was to investigate the effect of area-level socioeconomic deprivation on the risk of cognitive dysfunction and related health and lifestyle factors in older adults by combining data from two jurisdictions within the island of Ireland: Northern Ireland (NI), United Kingdom, and the Republic of Ireland (RoI).

METHODS

Participants and Study Design

This investigation was conducted as an observational study using data from the Trinity, Ulster, and Department of Agriculture (TUDA) cohort, as described in detail elsewhere.[15] The TUDA study included 5,186 community-dwelling, noninstitutionalized adults aged 60 and older recruited between 2008 and 2012 from NI and RoI and initially sought to investigate the role of nutritional and related lifestyle factors in the development of three common diseases of aging—cardiovascular disease, osteoporosis, and dementia. TUDA participants were recruited using standardized protocols from general practice or hospital outpatient clinics and deemed suitable if they (or their parents) were born on the island of Ireland and did not have a diagnosis of dementia. Participants who were able to provide informed consent were considered eligible. The final study cohort was composed of 2,093 participants recruited in NI and 3,093 in RoI.

The Office for Research Ethics Committees Northern Ireland granted ethical approval (reference 08/NIR03/113), with corresponding approvals from the Northern and Western Health and Social Care Trusts in NI and the Research Ethics Committee of St James Hospital and The Adelaide and Meath Hospital in Dublin.

Health and Lifestyle Data

A health and lifestyle questionnaire was administered to participants to collect general information relevant to medical history, medication use, smoking status, and alcohol consumption. Weight and height were recorded to the nearest 0.01 kg and 0.01 m, respectively, using portable scales and stadiometer (Seca; Brosch Direct Ltd, Peterborough, United Kingdom), and body mass index (BMI; kg/m^2) was calculated. Waist and hip measurements were recorded to the nearest 0.1 cm using a flexible tape measure and standardized protocols. The Timed Up-and-Go (TUG) test was administered as a measure of functional mobility.[16] Participants were asked to stand from a seated position (seat height approximately 46 cm), walk 3 m at their usual pace, turn around, walk back to the chair, and sit down. No physical assistance was given, and the time taken from command "Go" to completion of the task was measured using a stopwatch. Blood pressure (BP) measurements were taken in accordance with standard operating procedures. In brief, two measurements were taken from the reference arm (the arm with the highest BP reading), with a 5- to 10-minute interval between each measurement, and the mean of the two values was used as the BP value. Nonfasting blood samples were collected, stored, and analyzed using standard operating procedures and routine hospital laboratory assays.

Cognitive health was assessed using the Mini-Mental State Examination (MMSE),[17] a short, structured cognitive test that evaluates global cognitive function by assessing the domains of orientation, registration, attention and concentration, recall and language and is the most widely used screening tool in clinical settings worldwide for identifying cognitive impairment or dementia. The maximum score achievable is 30, with a score less than 25 indicating a possibility of cognitive impairment and a score less than 20 indicating dementia. Anxiety and depression were also assessed, using the Hospital Anxiety and Depression Scale[18] and the Center for Epidemiologic Studies Depression Scale, respectively.[19]

Measurement of Area Deprivation

Deprivation indices are used in the United Kingdom and Ireland on a factor analytical approach that reduces a large number of indicator variables to a smaller number of underlying domains or factors that are presented as a single value or score. Participants from NI were initially mapped using their house number, street name, unit postcode, town information, and the Land and Property Services Ordnance Survey of Northern Ireland POINTER Geo-referencing database.[20] After cleaning and verification of the address information, 1,982 participants (94.7%) were geo-referenced and linked to an area deprivation score based on the Census Output Area (COA) in which they lived using data from the Northern Ireland Multiple Deprivation Measure 2010,[21] which comprises 7 domains of deprivation, each developed to measure a distinct form or type of deprivation: income, employment, health, education, proximity to services, living environment, and crime. These domains were then presented as a single value or area deprivation score, which was then categorized according to quintile (each quintile representing 20% of all COAs in NI) ranging from least (Q1) to most (Q5) deprived. This was the preferred measure of deprivation because it was calculated at the smallest area-level

available (with a mean number of households of 125 and a mean population of 340).

In the absence of a comparable postal code reference system in the RoI, an alternative geocoding method using Irish Grid X and Y co-ordinates[22] was used to map and link participants to the appropriate socioeconomic indicators of area-level deprivation using the 2011 Pobal HP Deprivation Index for Small Areas in the Republic of Ireland,[23] which conceptualizes underlying indicators of deprivation based on earlier deprivation indices for Ireland and analyses from other countries to identify three domains of affluence or disadvantage: demographic profile, social class composition, and labor market situation. After address information was cleaned and verified, 3,066 participants (99.1%) were allocated to a Small Area Population Statistics (SAPS) area (with a mean number of households of 107 and mean population of 248) and given an area deprivation score. Again, the single area deprivation scores were categorized according to quintile, with each quintile representing 20% of all SAPS areas in RoI, ranging from least (Q1) to most (Q5) deprived.

Using local area deprivation data and the appropriate geo-referencing methods for each jurisdiction, comparable area deprivation scores categorized as quintiles could be generated, allowing for TUDA study data from two different countries within the island of Ireland (NI, RoI) to be effectively linked and integrated. One hundred thirty-eight (2.7%) participants of the study cohort were not allocated an area deprivation quintile because of incomplete address information.

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY). Before statistical analysis was performed, tests for normality were performed, and variables were log-transformed as appropriate. Between-group differences were analyzed using analysis of covariance (controlling for relevant covariates) with Bonferroni correction for multiple comparisons or chi-square tests using standardized residuals. The effect of area deprivation on the main study outcome, cognitive dysfunction, was investigated using logistic regression. In line with current clinical practice in the United Kingdom and Ireland, participants were classified according to MMSE score into two groups—normal cognitive function (MMSE score ≥25, reference) and cognitive dysfunction (MMSE score ≤24). Area deprivation was categorized into quintiles, and the model was adjusted for factors relevant to cognitive dysfunction. These covariates included age, sex, education, depression, anxiety, BMI, TUG test, smoking, alcohol, blood pressure, blood lipids, and diabetes.

RESULTS

Relevant characteristics of the TUDA study cohort are shown in Table 1. Of the 5,186 TUDA study participants, 5,048 (97.3%) were allocated to an area deprivation quintile. Figure 1 shows the geographical distribution of TUDA participants in NI and RoI according to area deprivation category. Marginally higher proportions of TUDA participants were found in the least- (21.2%) and most- (26.2%) deprived quintiles than in the other quintiles.

Table 1. Relevant Characteristics of Trinity, Ulster, and Department of Agriculture Study Participants (N = 5,186)

Characteristic	Value
General characteristics	
Age, mean ± SD	74.0 ± 8.3
Female, n (%)	3,487 (67)
Age finished education, mean ± SD	16.0 ± 3.0
Mini-Mental State Examination score, mean ± SD	27.1 ± 2.6
Center for Epidemiologic Studies Depression Scale score, mean ± SD	6.1 ± 7.5
Hospital Anxiety and Depression Scale score	3.2 ± 3.7
Lifestyle and clinical risk factors	
BMI, kg/m^2, mean ± SD	27.9 ± 5.4
Obese (BMI ≥30 kg/m^2), n (%)	341 (34)
Waist-to-hip ratio, mean ± SD	0.91 ± 0.08
Timed Up-and-Go time, seconds, mean ± SD	14 ± 9
Current smoker, n (%)	623 (12)
Alcohol intake, units/wk, mean	7.8 ± 12.5
Systolic blood pressure, mmHg, mean ± SD	144 ± 21
Diastolic blood pressure, mmHg, mean ± SD	78 ± 11
Total cholesterol, mmol/L, mean ± SD	4.6 ± 1.0
Low-density lipoprotein, mmol/L, mean ± SD	2.4 ± 0.9
High-density lipoprotein, mmol/L, mean ± SD	1.5 ± 0.5
Triglycerides, mmol/L, mean ± SD	1.6 ± 0.9
HbA_{1c}, %, mean ± SD	5.9 ± 0.8
With or at risk of diabetes, n (%)[a]	1,145 (23)

SD = standard deviation; BMI = body mass index.
[a]Glycosylated hemoglobin (HbA_{1c}) ≥ 6.1% or antidiabetic medication use.

Participant characteristics and disease risk factors were then examined in relation to area-level socioeconomic deprivation (Table 2). MMSE score was significantly lower at the highest levels of area deprivation (Q4, Q5). With increasing area deprivation, the number of years spent in formal education decreased, and anxiety, depression, smoking, alcohol use, and obesity all increased, along with disease risk factors, including blood pressure and diabetes risk.

Table 3 shows determinants of cognitive dysfunction in older Irish adults, calculated using logistic regression analysis. Area-level socioeconomic deprivation was associated with greater risk of cognitive dysfunction (odds ratio = 1.40; 95% confidence interval = 1.05–1.87; $P = .02$ for most vs least deprived) after adjustment for other relevant factors. A comparison of logistic regression analysis with and without area deprivation showed that the inclusion of area deprivation significantly strengthened the model (likelihood ratio test; see Table 3 footnote).

DISCUSSION

This study showed that area-level socioeconomic deprivation in older people is associated with poor cognition and an adverse general health profile. Older adults living in areas with the greatest socioeconomic deprivation in NI and the RoI had a 40% greater risk of cognitive dysfunction than those living in areas of least deprivation, after adjustment for other relevant risk factors.

There is accumulating evidence that the place where a person lives influences their disease risk, even after

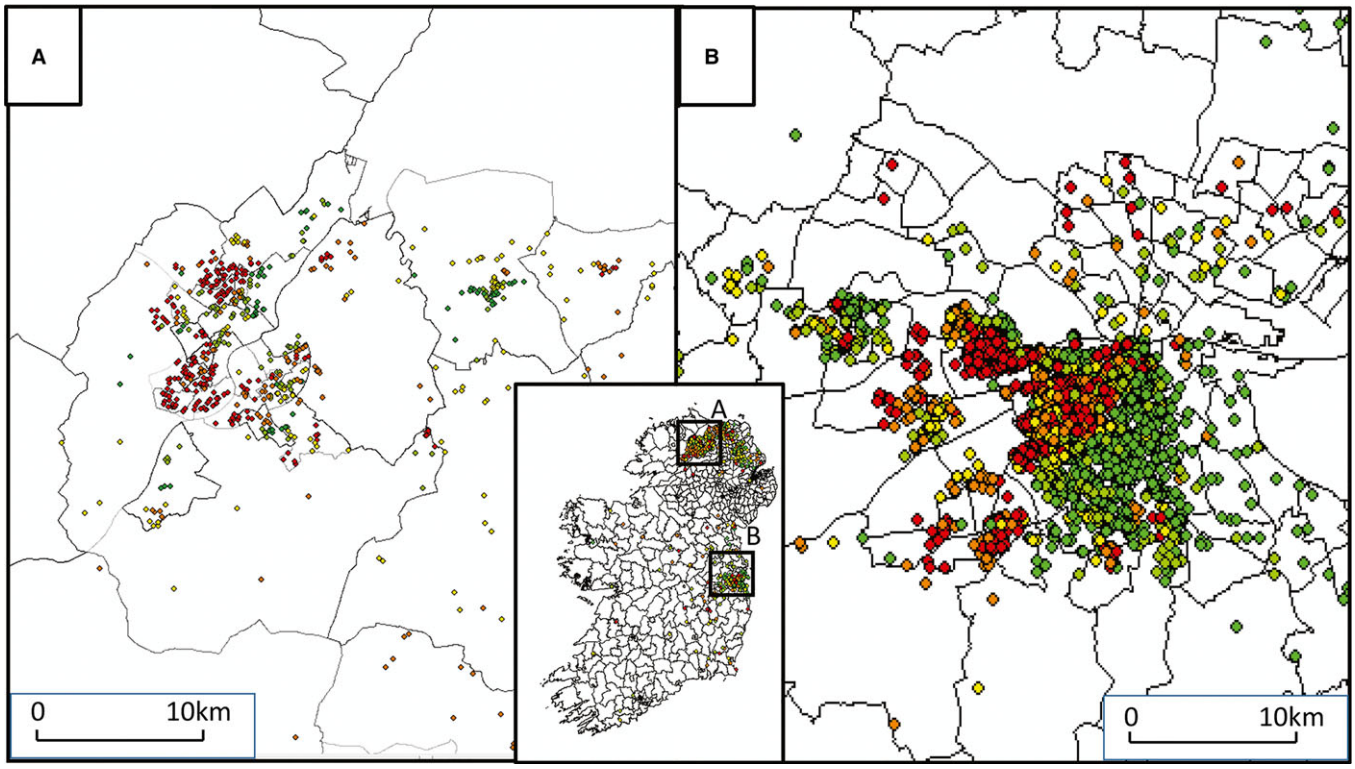

Figure 1. Map showing the distribution of Trinity, Ulster, and Department of Agriculture study participants from (A) Northern Ireland, United Kingdom and (B) the Republic of Ireland, color-coded according to area deprivation category, ranging from least-deprived 20% of areas (GREEN) to most-deprived 20% of areas (RED) in each jurisdiction.

accounting for individual factors.[6–10,24–26] Area deprivation refers to a geographical measure of the socioeconomic deprivation in an area or region. In the current cross-jurisdictional study, greater area deprivation was associated with significantly lower MMSE scores, fewer years spent in formal education, poorer mental health, higher alcohol use, and smoking (e.g., 8% vs 18% smokers in least vs most deprived), along with a greater range of disease risk factors, notably blood pressure, diabetes risk, and obesity (e.g., 27% vs 35% obese in least vs most deprived). These observations are generally consistent with findings from other studies undertaken in Sweden, Australia, England, and the United States.[27–30] Most previous studies investigating area-based deprivation have focused on mortality[7] and cardiovascular diseases or diabetes,[6,31] and there is a paucity of research investigating the influence of area deprivation on cognitive dysfunction; in particular, no previous study has addressed this relationship across different jurisdictions. In the current study, consistent with previous reports,[32,33] older people living in areas of higher deprivation were found to have higher rates of depression and anxiety. Poor mental health (in particular depression) is in turn an established risk factor for cognitive dysfunction, and the current results showed that depression was an independent factor contributing to cognitive dysfunction.

The mechanisms underlying the relationship between area deprivation and cognitive dysfunction in the present study are not clear, but educational attainment[34] and depression[35] may be two important mediators in this complex relationship. One recent population-based longitudinal survey of more than 10,000 older adults in the United States concluded that higher educational attainment was associated with significantly lower risk of dementia.[36] Likewise, a 2-decade comparison of the prevalence of dementia in the United Kingdom suggests that education is a strong modifiable factor in dementia,[37] further supporting the view that poor education may be one of the drivers of the relationship between area deprivation and cognition shown here. We found significantly lower educational attainment in older adults living in areas of greatest deprivation, who had 3 fewer years of formal education than those in the least deprived areas. In addition, depression has been shown to increase cognitive dysfunction, and poorer cognitive health can also predispose older adults to depression,[38,39] suggesting a bidirectional relationship between the two conditions. Also, in agreement with the current findings, it was previously observed that depression in older adults was associated with socioeconomic disadvantage, poverty, and deprivation related to place of residence.[40] The association between area deprivation and cognitive dysfunction observed in the current study remained significant even after adjustment for education, depression, and other factors, suggesting that other social determinants of health could also contribute to this relationship. These include contextual poverty, income inequality, social cohesion, access to resources, and relationships with the built and natural environment.[1,12,41]

Despite expectations that aging populations globally would lead to large increases in the number of adults with dementia, recent studies from the United States and Europe suggest that the prevalence of dementia in some

Table 2. Participant Characteristics and Disease Risk Factors According to Quintile (Q) of Area Deprivation

Characteristic	Q1, n = 1,069	Q2, n = 859	Q3, n = 919	Q4, n = 877	Q5, n = 1,324	*P*-Value
General characteristics						
Age, mean ± SD	75.4 ± 8.7	74.0 ± 8.3	73.3 ± 7.9	73.6 ± 8.0	73.9 ± 8.3	<.001
Female, n (%)	734 (69)	585 (68)	591 (64)	597 (68)	886 (67)	.36
Age finished education, mean ± SD	17.8 ± 3.8	16.6 ± 3.1	15.9 ± 2.7	15.3 ± 2.2	14.8 ± 1.9	<.001
Mini-Mental State Examination score, mean ± SD	27.4 ± 2.5	27.4 ± 2.3	27.4 ± 2.3	26.9 ± 2.7	26.5 ± 2.8	.008
Center for Epidemiologic Studies Depression Scale score, mean ± SD	5.2 ± 6.8	5.4 ± 6.8	5.5 ± 7.4	6.3 ± 7.6	7.5 ± 8.2	.001
Hospital Anxiety and Depression Scale score	2.7 ± 3.2	2.8 ± 3.4	3.1 ± 3.6	3.4 ± 3.8	3.7 ± 4.0	.01
Lifestyle and clinical risk factors						
BMI, kg/m^2, mean ± SD	27.1 ± 5.0	27.5 ± 5.1	28.0 ± 5.1	28.7 ± 5.8	28.1 ± 5.8	<.001
Obese (BMI ≥30 kg/m^2), n (%)	291 (27)	274 (32)	324 (35)	348 (40)	469 (35)	<.001
Waist-to-hip ratio, mean ± SD	0.90 ± 0.08	0.90 ± 0.09	0.91 ± 0.08	0.92 ± 0.09	0.91 ± 0.08	.001
Timed Up-and-Go time, seconds, mean ± SD	14 ± 10	14 ± 9	13 ± 9	14 ± 9	15 ± 9	.009
Current smoker, n (%)	86 (8)	76 (9)	100 (11)	108 (12)	242 (18)	<.001
Alcohol intake, units/wk, mean	6.6 ± 10.9	6.7 ± 10.9	8.0 ± 12.8	8.1 ± 12.9	9.3 ± 14.5	.004
Systolic blood pressure, mmHg, mean ± SD	142 ± 20	144 ± 21	147 ± 21	145 ± 21	144 ± 21	.006
Diastolic blood pressure, mmHg, mean ± SD	77 ± 11	78 ± 11	79 ± 11	79 ± 11	78 ± 12	.03
Total cholesterol, mmol/L, mean ± SD	4.7 ± 1.0	4.7 ± 1.0	4.7 ± 1.1	4.6 ± 1.1	4.6 ± 1.0	.01
Low-density lipoprotein, mmol/L, mean ± SD	2.5 ± 0.9	2.4 ± 0.8	2.5 ± 0.9	2.4 ± 0.9	2.4 ± 0.9	.06
High-density lipoprotein, mmol/L, mean ± SD	1.6 ± 0.5	1.5 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	<.001
Triglycerides, mmol/L, mean ± SD	1.5 ± 0.8	1.5 ± 0.9	1.7 ± 0.9	1.6 ± 0.9	1.6 ± 0.9	.001
HbA_{1c}, %, mean ± SD	5.8 ± 0.8	5.8 ± 0.8	5.8 ± 0.8	6.0 ± 0.9	5.9 ± 0.8	.20
With or at-risk of diabetes, n (%)[a]	209 (20)	157 (18)	189 (21)	244 (28)	315 (24)	.001

Participants were allocated to a deprivation quintile (1–5) based on the deprivation score of the area in which they lived, with 1 being the least deprived and 5 the most deprived.

Statistical tests: Between-group analysis of covariance (controlling for age) with Bonferroni correction for multiple comparisons or chi-square test using standardized residuals. $P < .05$ was considered significant.

SD = standard deviation; BMI = body mass index.

[a]Glycosylated hemoglobin (HbA_{1c}) ≥ 6.1% or antidiabetic medication use.

Table 3. Determinants of Cognitive Dysfunction in Older Adults (n = 4,554)

Determinant	Beta Value	OR (95% Confidence Interval)	*P*-Value
Age	0.059	1.06 (1.05–1.08)	<.001
Female sex	−0.078	0.93 (0.74–1.15)	.49
Age at which finished education	−0.151	0.86 (0.82–0.90)	<.001
Center for Epidemiologic Studies Depression Scale score	0.040	1.04 (1.03–1.06)	<.001
Hospital Anxiety and Depression Scale score	−0.016	0.98 (0.96–1.01)	.27
Body mass index	−0.024	0.98 (0.96–1.00)	.02
Timed Up-and-Go test	0.029	1.03 (1.02–1.04)	<.001
Current smoker	0.109	1.12 (0.83–1.49)	.46
Alcohol consumption in past year	0.084	1.09 (0.83–1.43)	.54
Systolic blood pressure	−0.002	1.00 (0.99–1.00)	.52
Diastolic presssure	0.003	1.00 (0.99–1.01)	.50
Total cholesterol	−0.025	0.98 (0.88–1.08)	.64
Triglycerides	−0.074	0.93 (0.81–1.06)	.28
With or at risk of diabetes[a]	0.023	1.02 (0.81–1.29)	.85
Country: Republic of Ireland	0.421	1.52 (1.16–1.99)	.002
Quintile of area deprivation[b]			
2	−0.112	0.89 (0.64–1.25)	.51
3	−0.246	0.78 (0.55–1.11)	.17
4	0.209	1.23 (0.89–1. 70)	.20
5	0.337	1.40 (1.05–1.87)	.02

Cognitive dysfunction defined as Mini-Mental State Examination (MMSE) score ≤24.

Results obtained from a fully adjusted logistic regression model.

Odds ratio (OR) given as exponentiation of the beta coefficient (Exp B), where Exp B = change in the OR).

Likelihood ratio test indicates a significant improvement in the logistic regression model with the inclusion of area deprivation (with area deprivation: log-likelihood = 2,907.8, chi-square = 519.2; degrees of freedom (*df*) = 20, $P < .001$; without area deprivation: log-likelihood = 2,926.7; chi-square = 500.4, $df = 16$, $P < .001$).

[a]Glycosylated hemoglovin ≥6.1% or antidiabetic medication use.

[b]Based on the score of the area in which participants live (reference Q1: least deprived).

countries may be stabilizing (or even declining), possibly as a result of improved health in midlife and potential protection that better educational attainment in early life affords.[36,37] The findings of a recent investigation of epidemiological data over time from 5 studies in western Europe suggest that primary prevention aimed at increasing cognitive reserve, along with better treatment of vascular and chronic conditions, could have the greatest effect on future dementia.[37] In addition, deprived social environments are known to breed social isolation and psychosocial stress and limit access to resources and health services, all of which can potentially interact with individual susceptibility to cognitive dysfunction. The current findings linking area deprivation with not only greater risk of cognitive dysfunction and lower educational attainment, but also a range of adverse lifestyle and cardiovascular disease risk factors, points to the living environment as an important component in dementia risk and thus a worthwhile target for efforts to reduce dementia occurrence and disability. A comprehensive report recently highlighted the potential for effective dementia prevention through targeted interventions to modify risk factors that could transform the future for society.[42]

The current study benefited from the use of a large, well-characterized cohort of older adults. It used individual-level data on health and disease status and area-level data on deprivation to determine whether living in a deprived area increases the risk of poor health, specifically cognitive dysfunction. A further strength was the use of novel country-specific geocoding approaches that facilitated the integration of regionally independent ethnically homogenous (Caucasian) datasets, enabling the TUDA cohort as a whole to be readily described in relation to the underlying socioeconomic profile of the base population of the two countries within the island of Ireland. This novel cross-jurisdictional approach provided a unique opportunity to link area-level deprivation with cognitive outcomes in older adults from two health systems; to our knowledge, this is the first time this has been achieved. As such, this work sets a precedent for future research initiatives seeking to integrate comparable data from cross-national studies (e.g., Survey of Health, Ageing and Retirement in Europe[43]) to investigate area-level deprivation in relation to health. The limitations of our approach relate to geographical coverage, geo-referencing accuracy, scale, and the direct comparability of measures of deprivation across jurisdictions. The study also used unique composite measures of deprivation from each jurisdiction made up of different combinations of univariate socioeconomic indicators; thus the measures of small area deprivation are not exactly comparable, but for the purposes of this study, the measures were categorized into quintiles ranging from the most- to the least-deprived areas in each jurisdiction, providing a meaningful measure for comparison across the deprivation spectrum. In addition, although area-based income deprivation indicators are associated with health outcomes, the effect is less pronounced than that of individual income measures,[44] and as such, the lack of specific data relating to personal income has a potentially confounding residual influence in this analysis. Nevertheless, this study further underscores the value of using area deprivation indices (that include domains related to personal income and wealth), particularly in situations in which socioeconomic data for individuals are not readily available. Finally, although the MMSE as a measure of cognition has sometimes been criticized for its ceiling and floor effects, it is the most widely used screening cognitive test in clinical settings and in epidemiological studies worldwide.[45]

In conclusion, the novel, cross-jurisdictional approach of the current study provides a unique insight into the relationship between area deprivation and cognitive performance and suggests that older Irish adults living in areas with the greatest level of deprivation are at significantly higher risk (40%) of cognitive dysfunction. Although further research is needed to fully elucidate the mechanisms explaining our observations, this work represents a first step toward identifying the specific aspects of area-level socioeconomic deprivation connected with cognitive health in older adults. Given the widening health and socioeconomic disparities seen globally, the current findings identify the potential for effective dementia prevention through targeted interventions to modify risk factors in communities with the greatest area-level socioeconomic deprivation.

ACKNOWLEDGMENTS

The TUDA study was supported by government funding from the Irish Department of Agriculture, Food and the Marine and the Health Research Board (under its Food Institutional Research Measure) and from the Northern Ireland Department for Employment and Learning (under its strengthening the all-Ireland research base initiative). Funding to support the geo-referencing methodologies described in this article was obtained through a small grant from the Centre for Ageing Research and Development in Ireland and further facilitated by the Health Research Board of Ireland (HRA_PHS/2010/19).

Conflict of Interest: None declared.

Author Contributions: AM, HMN, LH, AMM, JR, AMC: study concept and design, funding. AMC, AM, JR: data analysis. HMN, AM, JR, AMC, CFH, LH, KM, AMM, CC, MCC, FT, MOK, KMC, MW, JJS: data interpretation. AMC, AM, CFH, HMN: drafting the manuscript. All authors: critical revision of manuscript for important intellectual content, approval of content. HMN and AM had full access to the data and take responsibility for the data analysis.

Sponsor's Role: The funders of this research had no role in the design or conduct of the study, data collection, management, analysis, or preparation of the paper.

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NEWS AND VIEWS
DOI: 10.1111/nbu.12250

Current evidence linking nutrition with brain health in ageing

K. Moore, M. O'Shea, C. F. Hughes, L. Hoey, M. Ward and H. McNulty
Northern Ireland Centre for Food and Health, Ulster University Coleraine, Coleraine, UK

Abstract

Cognitive dysfunction and depression are significant problems of ageing with major health and socio-economic impacts; therefore, preventing or delaying the onset of these disorders should be a public health priority. In particular, there is a need to identify modifiable factors that could be targeted to promote better brain health in ageing. Epidemiological studies indicate a protective role for certain dietary patterns, in particular the Mediterranean diet, and for specific nutrients, including *n*-3 polyunsaturated fatty acids, polyphenols and B vitamins. Although, the evidence to date from randomised controlled trials is generally inconsistent, there is clearer evidence to support a role for folate and related B vitamins in slowing the progression of cognitive decline and possibly reducing the risk of depression in ageing. Future studies incorporating new technologies offer much promise for the development of effective nutrition strategies that could reduce the risk of cognitive and mental disorders and improve quality of life in our ageing population.

Keywords: ageing, B vitamins, cognitive function, depression, fatty acids, nutrition

Introduction

The global population is ageing with the number of those aged 60 years and over predicted to reach two billion by 2050 (United Nations Department of Economic and Social Affairs/Population Division 2009). An estimated 46.8 million people are living with dementia worldwide, and this figure is predicted to double in the next 20 years, with associated costs to the global economy currently estimated at over $818 billion/year (Prince *et al.* 2015). Depression is a leading cause of disability, currently costing approximately £7.5 billion annually in England alone, and projected to increase by 67% by 2026 (National Collaborating Centre for Mental Health 2010). Given the significant impact of these conditions, there is a need to identify modifiable factors that could be targeted to promote better brain health in ageing populations. Epidemiological evidence supports a role for certain dietary factors in brain health, opening up new potential avenues for prevention of dementia and mental illness in ageing (Panza *et al.* 2008; Rechenberg 2016).

Correspondence: Prof. Helene McNulty, Director, Northern Ireland Centre for Food and Health, Ulster University Coleraine, Coleraine BT52 1SA, UK.
E-mail: h.mcnulty@ulster.ac.uk

The ageing brain

Ageing affects the brain from a cellular to a functional level. The brain has a high metabolic rate; therefore, oxidative stress and inflammation are common in ageing neural tissue (Bishop *et al.* 2010). On average, brain size decreases with age in later life and although there is no loss of cortical volume, there is white matter atrophy (Silverman *et al.* 1997). The rate of this atrophy is known to be a marker of cognitive decline (Fox *et al.* 1999) and white matter lesions in ageing are linked with late onset depression (Wang *et al.* 2014). Neurodegeneration is the loss of neuronal

processes and nerve cells. This can lead to neurodegenerative disorders which are characterised by selective loss of neurons in the motor, sensory and cognitive systems. This affects memory, cognition, language, personality and mood and can have sporadic and/or hereditary origins (Gelb 2016).

Mild cognitive impairment can be described as a rate of cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life (Gauthier *et al.* 2006). Of note, however, is that an estimated 50% of those with mild cognitive impairment will go on to develop dementia within 5 years (Gauthier *et al.* 2006). Dementia is characterised by progressive deterioration of multiple cognitive domains leading to impaired daily functioning, with Alzheimer's disease being the most common cause of dementia in older adults (Prince *et al.* 2014). The World Health Organization (2014) defines mental health as a state of 'complete physical, mental and social wellbeing and not merely the absence of disease or infirmity'; mental illness includes depression and anxiety as common disorders. A number of modifiable risk factors for declining cognitive and mental health have been identified, including dietary patterns and specific nutrients (Smith 2008; Stahl *et al.* 2014; Gallagher *et al.* 2016; Kennedy 2016; Rechenberg 2016). Ageing itself is associated with a reduction in the quantity of food consumed, altered metabolism and decreased absorption of nutrients, all of which can increase the risk of malnutrition (Wakimoto & Block 2001). Therefore, nutritional approaches to prevent or slow the progression of cognitive decline are of much current research interest.

Foods and brain health

Increasing evidence implicates certain dietary patterns, such as high intake of fruit and vegetables (Kang *et al.* 2005) and fish consumption (Barberger-Gateau *et al.* 2007), as being beneficial to brain health. Compliance with a Mediterranean diet (typically characterised by higher intakes of fruit, vegetables, wholegrains, olive oil and fish) has been associated with a lower risk of Alzheimer's disease and slower progression of symptoms from mild cognitive impairment to Alzheimer's disease (Scarmeas *et al.* 2006; Solfrizzi *et al.* 2010; Singh *et al.* 2014). Adherence to a Mediterranean diet also has been associated with a protective effect against depression (Psaltopoulou *et al.* 2013). These findings were recently confirmed in a study of 4470 participants, aged from 45 to 79 years, which found significantly lower depression scores in those with greater adherence to a Mediterranean diet (Veronese *et al.* 2016). Although there is some biological basis for the protective role of the Mediterranean diet in brain health, the findings do not prove causality as the studies to date have predominantly been observational.

Specific nutrients and brain health

Fatty acids

The fatty acid composition of the brain membrane is directly affected by diet, and this has focused attention on the role of dietary fatty acids in brain health. There is evidence that long-chain *n*-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have potential benefits in cognitive and mental health. The suggested mechanism for the effects of these fatty acids is via their antithrombotic and anti-inflammatory properties (Gillette-Guyonnet *et al.* 2013) and their potential interactions with neurotransmitters via phospholipid metabolism and regulation of signal transduction (Grosso *et al.* 2014a). Regular consumption of dietary *n*-3 polyunsaturated fatty acids has been associated with reduced risk of cognitive decline and reduced development of incident dementia in epidemiological studies (Jicha & Markesbery 2010). Likewise, higher dietary DHA has been associated with lower incidence of dementia (Schaefer *et al.* 2006; Lopez *et al.* 2011). Randomised controlled trials (RCTs) of *n*-3 polyunsaturated fatty acid supplementation have, however, shown somewhat inconsistent results with regard to cognitive function (Freund-Levi *et al.* 2006; Quinn *et al.* 2010; Yurko-Mauro *et al.* 2010). Furthermore, the relationship between fatty acids and cognitive outcomes is complex since higher intakes of *n*-6 fatty acids, irrespective of *n*-3 polyunsaturated fatty acid intake, seem to negate any of the aforementioned beneficial effects of *n*-3 polyunsaturated fatty acids (Jicha & Markesbery 2010). One meta-analysis of RCTs intervening with supplemental *n*-3 polyunsaturated fatty acids concluded that the beneficial effects on brain health may be confined only to patients with mild cognitive impairment (Mazereeuw *et al.* 2012); however, this interpretation requires further investigation.

In two recent meta-analysis of RCTs, EPA supplementation was found to benefit patients with a diagnosis of depression; however, in those with non-clinical depression, the findings were far less clear (Grosso *et al.* 2014b; Hallahan *et al.* 2016). A recent

Cochrane review concluded that there was insufficient evidence to support increased *n*-3 polyunsaturated fatty acid intake as a treatment for depression, highlighting the need for further investigation in this area (Appleton *et al.* 2015).

Protein and carbohydrates

The role of dietary protein in cognitive function and mental health has not been extensively studied in ageing populations. Lower verbal memory scores were, however, observed in older people with lower dietary protein intakes (Goodwin *et al.* 1983). Additionally higher dietary protein intake was found to be positively correlated with non-verbal learning and verbal memory (Koehler *et al.* 1997) and reduced risk of mild cognitive impairment or dementia (Roberts *et al.* 2012). One RCT investigating the effects of dietary protein from red meat on cognitive function in older adults is ongoing (Daly *et al.* 2015).

The association between carbohydrates and cognitive function is unclear because available evidence is scarce (Power *et al.* 2015), with a Cochrane review identifying only one RCT in older adults (Ooi *et al.* 2011). While more research has focused on carbohydrates and depression, the available evidence is somewhat conflicting. One study of community dwelling older adults found that those with depressive symptoms had a diet with a higher glycaemic index (GI) and glycaemic load (GL) (Mwamburi *et al.* 2011). A prospective investigation also reported that a high GI diet was associated with an increased risk of depression (Gangwisch *et al.* 2015). Contrary to these findings, research in institutionalised older adults reported that those suffering from depression had diets with a lower GL (Aparicio *et al.* 2013). Given the inconsistencies within the evidence base in this area, there is clearly a need for further well-designed studies.

Polyphenols and vitamins

Inflammation is thought to be involved in the neurodegenerative cascade resulting in cognitive decline (Gorelick 2010), and evidence suggests a protective anti-inflammatory role for flavonoids against cognitive decline and Alzheimer's disease (Commenges *et al.* 2000; Letenneur *et al.* 2007; Schaffer *et al.* 2012). In one large prospective study of 5395 older adults in The Netherlands, polyphenol intake was associated with a reduced risk of dementia in smokers (Engelhart *et al.* 2002). Likewise, dietary polyphenol intake was also associated with cognitive performance in 2574 middle-aged French participants (Kesse-Guyot *et al.* 2012). Of greater note, new evidence from a recent RCT of healthy older adults reported that intervention with high doses of cocoa-flavanol enhanced dentate gyrus function, a brain area essential for learning and memory, which was measured by magnetic resonance imagining (MRI) and cognitive tests (Brickman *et al.* 2014). In the case of depression, animal studies have shown anti-depressant effects of numerous polyphenols, postulated to be related to their anti-inflammatory and neurotransmitter modulation roles (Pathak *et al.* 2013). Although positive effects of cocoa polyphenols on mood were reported in one small study of 87 middle-aged participants (Pase *et al.* 2013), the evidence from human studies is generally scarce and expert groups have called for further coordinated research in this area (Ward & Pasinetti 2016).

Oxidative stress is thought to be a major contributor to neurodegeneration and depression; thus, antioxidants have received particular attention in the study of older adults. Although large prospective studies have failed to demonstrate a protective effect of either dietary or supplemental ascorbic acid on cognitive function in older adults (Luchsinger *et al.* 2003; Laurin *et al.* 2004; Devore *et al.* 2010), the studies had significant limitations including the lack of a biomarker measure of vitamin C status. With regard to mental health, an early RCT with an antioxidant supplement containing vitamins A, E and C in 205 participants reported substantial increases in plasma ascorbic acid concentrations and correspondingly better mood outcomes after one year (Smith *et al.* 1999). Likewise, more recent cross-sectional and prospective studies have reported lower dietary intakes or plasma concentrations of ascorbic acid among older adults with depression (Merrill *et al.* 2008; Hamer *et al.* 2011; Payne *et al.* 2012). More robust RCTs, which include both biomarker and dietary intake measures, are required to further explore the role of vitamin C in mental health.

There is extensive literature in the area of vitamin D and the ageing brain. Of note, a 7-year follow-up study of 498 women aged 75 years and older identified significantly lower risk of Alzheimer's disease for those participants in the highest quintile of dietary vitamin D intake at baseline (Annweiler *et al.* 2012). In a recent meta-analysis of observational studies, patients suffering from Alzheimer's disease were found to have significantly lower serum vitamin D concentrations compared with matched cognitively healthy controls (Annweiler *et al.* 2013). However, combined

supplementation with vitamin D and calcium was not found to decrease the risk of dementia or cognitive decline in a RCT involving 4143 older women from the US (Rossom *et al.* 2012). In the case of depression, large cross-sectional ($n = 2598$) and prospective ($n = 2839$) studies have linked lower serum vitamin D status with an increased risk of depression (Williams *et al.* 2014; Brouwer-Brolsma *et al.* 2016). The available evidence from intervention studies, however, has not confirmed a causative relationship for vitamin D in relation to the risk of depression (Li *et al.* 2014; van den Berg *et al.* 2016).

B vitamins and brain health in ageing

The role of folate and related B vitamins in brain health in ageing, and related mechanisms involving one-carbon metabolism, is an area of active research worldwide. Within one-carbon metabolism, folate, along with vitamins B_{12} and B_6, and riboflavin are cofactors in DNA synthesis and repair, amino acid metabolism and methylation reactions, including the remethylation of homocysteine to methionine and subsequent generation of S-adenosylmethionine. The latter metabolite is essential for neurotransmitter synthesis and thus vital for brain function and activity (McGarel *et al.* 2015).

A number of observational studies have shown that lower status of folate, vitamin B_{12} and vitamin B_6 (and/or higher concentrations of homocysteine) are associated with cognitive deficit in ageing (Smith & Refsum 2016). RCTs have addressed the hypothesis that optimising B vitamin status by supplementing with folate alone, or in combination with vitamins B_{12} and/or B_6, will delay cognitive decline in older adults. Available RCTs in older adults that include intervention with high-dose folic acid, vitamin B_{12} and vitamin B_6 over 2 years or more have shown not only improved cognitive performance (Durga *et al.* 2007; de Jager *et al.* 2012) but also a reduced rate of brain atrophy in those incorporating MRI (Smith *et al.* 2010; Douaud *et al.* 2013). The RCT evidence is not entirely conclusive, however, with one notable trial from New Zealand failing to observe a significant beneficial effect of folic acid, vitamin B_{12} and vitamin B_6 on cognition (McMahon *et al.* 2006), possibly owing to the inclusion of participants with generally high baseline folate status and thus unlikely to benefit from intervention aimed at improving status. One recent and rather controversial meta-analysis in this area concluded that neither folic acid nor vitamin B_{12} had a beneficial effect on cognition in older adults (Clarke *et al.* 2014). The article was widely criticised at the time of publication, mainly as a result of the inclusion criteria used to select the trials for investigation, and thus, the findings are not generally accepted by the scientific community in this area (Garrard & Jacoby 2015; Smith *et al.* 2015). It is clear that further well-designed RCTs are needed, especially those targeting participants with low B vitamin status as they are likely to benefit the most from increasing B vitamin concentrations to achieve better cognitive health in ageing.

The role of B vitamins in depressive disorders has not received as much interest as in cognitive function. However, one meta-analysis of 19 observational studies reported a significant relationship between low folate status and risk of depression (Gilbody *et al.* 2007). Low dietary intake and status of vitamin B_{12} have also been linked with an increased risk of depression (Reynolds 2006; Kim *et al.* 2008; Ng *et al.* 2009; Sánchez-Villegas *et al.* 2009; Robinson *et al.* 2011; Moorthy *et al.* 2012). RCTs of B vitamin supplementation as an adjunct to anti-depressant medications (Coppen & Bailey 2000; Almeida *et al.* 2014) or alone (Almeida *et al.* 2010; Walker *et al.* 2010) have provided mixed results and no clear conclusions, partly because of major methodological differences among studies. Reviews of the available evidence linking B vitamins with depression have concluded that folate and vitamin B_{12} may have roles in the longer term management of this condition (Taylor *et al.* 2004; Almeida *et al.* 2015).

Use of novel technologies in nutrition and brain research

Validated questionnaire-based assessments are the most common means of investigating cognitive and mental health outcomes and indeed are most informative in regard to the effect of nutrition on behaviour (Macready *et al.* 2010). However, in order to study the role of nutrition in brain function, the emerging use of brain imaging techniques in recent years provides an objective and highly robust means of assessing brain function and response (de Jager & Kovatcheva 2010). A number of such techniques are available and have been reviewed in detail elsewhere (Sizonenko *et al.* 2013). MRI is a structural imaging technique that provides detailed pictures of brain tissue (white and grey matter, blood vessels and bone) using magnetic fields and radiofrequency pulses. Functional MRI (fMRI) uses blood oxygen level-dependant imaging to visualise changes in blood flow, in order to

identify areas of neural activity within the brain. Electroencephalography (EEG) and magnetoencephalography (MEG) are two techniques for functional brain imaging that record electric and magnetic activities of the brain at the scalp and have the highest temporal resolution compared to other imaging techniques.

In recent years, these imaging techniques have been utilised in nutrition research. One notable study, referred to earlier in this review, effectively used MRI to confirm the beneficial effects of B vitamins on cognition in older adults over a 2-year intervention period (Smith *et al.* 2010). Another study used fMRI to demonstrate higher brain activation in specific regions of the brain in participants who consumed a nutritionally balanced breakfast (Akitsuki *et al.* 2011). EEG has also been used to investigate the effects of diet on brain function, with one recent report showing improved memory and functional connectivity in the delta band in response to Souvenaid®, a nutritional supplement, in mild Alzheimer-type patients (Ritchie *et al.* 2014). In recent years, MEG has been approved by the US Food and Drug Administration (FDA) and is being used for clinical and research purposes, including investigating cognitive dysfunction, Alzheimer's disease and depression (Maestú *et al.* 2008; Cheng *et al.* 2012; de Haan *et al.* 2012; Kurita *et al.* 2016). The application of these new technologies in the field of nutrition, in combination with clinical and questionnaire-based assessments, provides much potential for robust investigation in future studies, furthering knowledge and discovery, in an effort to reduce the burden of declining brain health in ageing.

Conclusions

Undoubtedly, nutrition has an important role in preserving cognitive and mental health and thus improving quality of life in older age. The impact of specific nutritional factors on the brain in ageing is an area of active investigation worldwide. Emerging evidence implicates subclinical deficiencies of certain nutrients in cognitive decline and poor mental health in older adults; however, the threshold for nutrient levels to prevent or delay declining brain function is still unknown. If the findings of studies described in this review, which show promise in relation to B vitamins, *n*-3 polyunsaturated fatty acids and polyphenols, are confirmed, a public health strategy to improve status of these key nutrients may help to achieve better cognitive and mental health in ageing. Future studies incorporating imaging techniques offer a robust basis for confirming effective nutrition interventions that could reduce the risk of cognitive and mental decline in ageing and the related burden on health services.

Acknowledgements

The PhD studentship of K.M. was funded by the Northern Ireland Department for Employment and Learning.

Conflicts of interest

The authors declare no conflict of interest.

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Proceedings of the Nutrition Society, Page 1 of 12 doi:10.1017/S0029665117004177

The Nutrition Society Irish Section Meeting was held at the Queens University, Belfast on 21–23 June 2017

Conference on 'What governs what we eat?' Irish section postgraduate meeting

Diet, nutrition and the ageing brain: current evidence and new directions

Katie Moore, Catherine F. Hughes, Mary Ward, Leane Hoey and Helene McNulty*
Nutrition Innovation Centre for Food and Health, School of Biomedical Sciences, Ulster University Coleraine, Coleraine, Northern Ireland

Globally populations are ageing. By 2050, it is estimated that there will be two billion people aged 60 years or over, of which 131 million are projected to be affected by dementia, while depression is predicted to be the second leading cause of disability worldwide by 2020. Preventing or delaying the onset of these disorders should therefore be a public health priority. There is some evidence linking certain dietary patterns, particularly the Mediterranean diet, with a reduced risk of dementia and depression. Specific dietary components have also been investigated in relation to brain health, with emerging evidence supporting protective roles for *n*-3 PUFA, polyphenols, vitamin D and B-vitamins. At this time, the totality of evidence is strongest in support of a role for folate and the metabolically related B-vitamins (vitamin B_{12}, vitamin B_6 and riboflavin) in slowing the progression of cognitive decline and possibly reducing the risk of depression in ageing. Future studies incorporating new technologies, such as MRI and magnetoencephalography, offer much promise in identifying effective nutrition interventions that could reduce the risk of cognitive and mental disorders. This review will explore the ageing brain and the emerging evidence linking diet and specific nutrients with cognitive function and depression in ageing, with the potential to develop strategies that could improve quality of life in our ageing population.

Nutrition: Cognition: Depression: Ageing: B-vitamins

Globally the population is ageing, with predictions that the number of people aged 60 years and over will reach up to two billion by 2050[1]. An estimated 23 % of the global burden of disease arises in older people, and mental disorders are reported as the leading cause of disability and ill health[2]. Dementia and depression are the most common of these disorders in ageing as identified by the WHO[3]. Cognitive function declines with age, ranging in severity from mild cognitive impairment (MCI) to dementia, with up to 50 % of those with MCI going on to develop dementia within 5 years[4]. MCI can be defined as cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life[4], whereas dementia interferes with activities of daily living[5]. Dementia currently affects 46·8 million people worldwide and is projected to affect over 131 million people by 2050[6], while depression is anticipated to be the second leading cause of disability worldwide by 2020[7], with 22 % of males and 28 % of females over the age of 65 years affected by depression[8]. The economic burden of cognitive decline and depression is profound. Experts have calculated that dementia will be a trillion dollar disease by 2018[6]. Figures for depression are currently estimated at over €3 billion in Ireland[9] and £7·5 billion in England[7]. With mental health considered to be one of the greatest global challenges[10], there is an urgent need to identify modifiable factors for targeted interventions to promote better brain health in our ageing populations. Epidemiological evidence supports a role for certain dietary factors in brain health, opening up new potential avenues for prevention of dementia and mental illness in ageing[11,12].

This review will explore the influence of ageing on brain health and the emerging evidence linking diet and specific nutrients with cognitive function and depression in ageing. The use of novel imaging technologies in

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; RCT, randomised controlled trial.

***Corresponding author:** H. McNulty, email h.mcnulty@ulster.ac.uk

nutrition and brain research will be discussed, along with the potential for nutrition to play a protective role in preserving better brain health in ageing.

The ageing brain

Physiology and pathophysiology

The structure and metabolic pathways within the brain are progressively altered with ageing, although the precise aetiologies of ageing have not been fully elucidated. As people age, there is a reduction in brain volume in both grey and white matter[13], while white matter lesions increase[14] and there is development of amyloid plaques, neurofibrillary tangles, Lewy bodies, synaptic dystrophy and neuron loss[15,16], which have been suggested to parallel the progression of cognitive decline[17]. There are also changes in the production of neurotransmitters, in particular serotonin and dopamine, which have been reported to decline by up to 10 % per decade from early adulthood[14]. Additionally, there is an increase in oxidative stress response[18] and more dysfunction of the blood–brain barrier[19].

Normal ageing is associated with a decline in cognitive function, with most cognitive change observed in memory during the ageing process. MCI is a recognised clinical condition where individuals have evidence of cognitive impairment but do not meet the criteria for the diagnosis of dementia[20]. Alzheimer's disease (AD) is the most common form of dementia, accounting for 62 % of cases, with other forms including vascular dementia, mixed, Lewy body and frontotemporal dementia[21]. Depression in older adults is often referred to as late-life depression and is reported more commonly in females than males[22–24]. The depressive symptoms of older adults are thought to be different from those experienced by younger adults, as somatic and psychological symptoms are often accompanied by fatigue, hopelessness about the future, loss of appetite and sleep disturbance[22].

Pharmaceutical treatments

Pharmacological treatment for dementia is prescribed by specialist clinicians[25], but only a limited number of medications that target the biochemical abnormalities of neuronal loss are included within the National Institute for Health and Care Excellence recommendations for dementia interventions. These include acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine) and memantine (*N*-methyl-D-aspartate receptor antagonists). There are however a variety of pharmacological treatment options available for depression including tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin and noradrenaline reuptake inhibitors and selective noradrenaline reuptake inhibitors[26,27]. Overall, poor response rates to these costly pharmacological treatments for depression have been observed[28,29], and despite significant investigation into the role of pharmacological treatments for dementia, no licenced medication can cure these diseases of the brain. Therefore, much effort is currently focusing on options for prevention rather than treatment of brain disorders.

Assessment of brain function

The assessment of brain function for neurodegenerative diseases and depressive disorders in ageing is a developing area. There are numerous neurological tests available which are designed to assess and distinguish different individuals in their response to day-to-day cognitive tasks[30] and for the detection of common mental health disorders[31]. The National Institute for Health and Care Excellence has provided guidance on the recommended diagnostic criteria for depression[32] and dementia[33]. For dementia, the guidelines emphasise the need to assess the following domains: attention and concentration, orientation, short- and long-term memory praxis, language and executive function. Furthermore, the National Institute for Health and Care Excellence recommends that formal tests should be conducted, including the mini mental state examination, six-item cognitive impairment test, general practitioner assessment of cognition and 7-min screen, and that other factors known to influence performance such as education level, should also be taken into account. Lastly, only healthcare professionals with expertise in differential diagnosis and using international standardised criteria (such as the National Institute of Neurological Communicative Disorders) should be responsible for diagnosing subtypes of dementia[33].

Investigating cognitive and mental health outcomes via questionnaire-based assessments is the most common approach for assessing the effects of nutrition[34]. For assessing brain health and function in relation to nutritional factors, studies should be aimed at prevention rather than treatment, and non-nutrition factors contributing to cognitive impairment and depression should be incorporated into studies and considered at the time of analysis[35]. Concerning the specific tests to assess cognitive function, these should be carefully selected and should be based on a known or hypothesised relationship of a specific food/nutrient with cognitive function and not solely on their availability or ease of administration. It is also important that the tests are suitable for repeated administration, are appropriate to the population being studied and are relatively simple to interpret and administer. More work is required using standardised tests across laboratories, so that the specific tests or markers that are most sensitive to the nutrients tested can be established[30,35]. Lastly, computerised cognitive assessments have been utilised and these should be considered for use in future trials in terms of their accuracy and ability to capture reaction-time data, standardisation of administration, availability of parallel versions of tasks for testing at multiple time points and availability in multiple languages[35].

Food, nutrition and brain health in ageing

Foods and dietary patterns

Increasing evidence implicates certain dietary patterns such as higher intake of fruit and vegetables[36] and fish[37] as being beneficial to brain health. The Mediterranean diet is receiving significant attention as regards its role in preserving cognitive health and

protecting against depression in ageing. This diet is typically characterised by higher intakes of fruit, vegetables, wholegrains, fish, unsaturated fatty acids and a regular but moderate consumption of alcohol. A recent meta-analysis (*n* 34 168) showed that the highest Mediterranean diet score was associated with reduced incidence of developing cognitive disorders (RR 0·79, 95 % CI 0·70, 0·90)[38] while supplementation of the Mediterranean diet with olive oil or nuts was associated with improved cognitive function[39]. Of note, studies using MRI have shown that adherence to the Mediterranean diet was associated with larger cortical thickness (which in turn is associated with a lower risk of cognitive impairment)[40]. There is also accumulating evidence to support a potential role for the Mediterranean diet in preventing depression in older adults, with cross-sectional and prospective studies showing inverse associations between Mediterranean diet score and risk of depression[41–45]. Further well-designed intervention studies are however required to more fully investigate the potential role of the Mediterranean diet as a means of helping to preserve better brain health in ageing.

Specific nutrients

Protein and carbohydrates. The role of dietary protein intake on cognitive function or mental health has not been extensively studied in ageing populations. Lower verbal memory scores were however observed in older people with lower dietary protein intakes[46]. Additionally, higher dietary protein intake was found to be positively correlated with non-verbal learning, verbal memory and reduced risk of MCI or dementia[47,48]. One randomised controlled trial (RCT) investigating the effects of dietary protein from red meat on cognitive function in older adults is in progress (ACTRN12613001153707) with results expected in 2018[49].

The association between carbohydrates and cognitive function is unclear because available evidence is scarce, with one Cochrane review identifying only one relevant RCT in older adults[50,51]. However, higher dietary carbohydrate and sugar intakes were associated with lower cortical thickness, which is in turn associated with high risk of late-life MCI and dementia[40]. While more research has focused on carbohydrates and depression, the available evidence is somewhat conflicting. One study of community-dwelling older adults found that those with depressive symptoms consumed a diet with a higher glycaemic index and glycaemic load[52]. A prospective investigation also reported that a high glycaemic index diet was associated with an increased risk of depression[53]. Contrary to these findings, however, institutionalised older adults with depression were reported to consume diets with a lower glycaemic load [54]. Given the inconsistencies in this area, there is clearly a need for further well-designed studies.

n-*3 Fatty acids.* The fatty acid composition of the brain membrane is directly affected by diet and this has focused attention on the role of dietary fatty acids in brain health. There is evidence that long-chain *n*-3 PUFA, EPA and DHA, have potential benefits in cognitive and mental health[55,56]. One meta-analysis of ten randomised trials concluded that *n*-3 fatty acids may have a protective effect on certain cognitive domains in cognitively impaired patients, however, no effects were seen in healthy people or in AD sufferers[57]. A recent Cochrane review, which identified three randomised trials for inclusion involving 632 patients with mild to moderate AD, concluded that there was no convincing evidence that PUFA had a role in the treatment of people with existing dementia[58].

Conversely, systematic reviews and meta-analyses of randomised trials have reported significant clinical benefits of *n*-3 PUFA intervention in the treatment of depression. The use of predominantly EPA compared with DHA supplementation appears to have greater efficacy[59,60]. Furthermore, supplementation with EPA-predominant formulas as an adjuvant therapy to antidepressants was found to have greater clinical efficacy in the treatment of depression (compared with antidepressants alone), but did not prevent depressive symptoms among populations without a diagnosis of depression[59,60]. A Cochrane review in this area reported a small to modest non-clinical beneficial effect of *n*-3 PUFA in depression symptomology, but concluded that there was not enough good quality evidence to determine the effect on depression[61].

Polyphenols. The role of these phytochemicals in brain health and ageing is an emerging area[62–64]. Large prospective studies have identified associations between the dietary intakes of total or specific polyphenols and cognitive function after up to 13 years of follow-up investigation[65–67]. Supplementation with cocoa flavanol for periods of up to 2 months was reported to improve cognitive performance in a group of cognitively intact older adults[68]. Of note, Brickman *et al.*[64] conducted a 3-month intervention and showed significant increases in cerebral blood volume in the dentate gyrus as measured by functional MRI in subjects who were assigned to a high flavanol treatment. Research into the role of polyphenols in depression in human subjects has been limited[69], although animal studies show promise in demonstrating antidepressant-like effects of polyphenols in mouse models[70].

Vitamins. Specific vitamins have been investigated in relation to brain health and disease. Oxidative stress is thought to be a major contributor to neurodegeneration and depression[18], thus antioxidants have received much interest. The roles of vitamin C[71–74], β-carotene[75–77] and vitamin E[78–81] have been explored, but no clear conclusions can be made and further work in the form of intervention studies is warranted. The postulated roles of vitamin D and B-vitamins have been more fully investigated in relation to their effects on brain health in ageing.

Following the discovery of the vitamin D receptor in the brain[82], evidence for the role of vitamin D in brain health has been accumulating. Systematic reviews and meta-analyses have shown that AD sufferers have lower serum vitamin D status than healthy controls, and that low serum vitamin D status is associated with

worse cognitive outcomes[83–85]. Recent longitudinal studies with mean follow-up periods of over 4 years found that lower vitamin D status was also associated with declining mini mental state examination scores and accelerated cognitive decline[86,87]. Furthermore, Hooshmand *et al.* used MRI to demonstrate that higher vitamin D status was associated with greater brain volumes[88], which is generally regarded as a valid marker of disease state and progression. Research investigating the role of vitamin D in depression is much less clear. Large cross-sectional and prospective studies reported that lower serum vitamin D status was associated with an increased risk of depression[89,90]. One detailed systematic review, which included cross-sectional, prospective and RCT data, concluded that lower vitamin D status may be a risk factor for late-life depression[91].

One-carbon metabolism and related B-vitamins

Historically, B-vitamin deficiencies, in particular folate[92,93] and vitamin B_{12}[94,95], and to a much lesser extent vitamin B_6[96], have been linked with poorer psychiatric wellbeing. These B-vitamins play crucial roles in one-carbon metabolic pathways where they act as co-factors in DNA synthesis and repair, amino acid metabolism and methylation reactions, including the remethylation of homocysteine to methionine and subsequent generation of *S*-adenosylmethionine. *S*-adenosylmethionine, the universal methyl donor, is involved in the methylation of DNA, phospholipids, proteins and neurotransmitters, thus reduced status of one or more of the B-vitamins involved in one-carbon metabolism may impair methylation processes[97,98]. The inhibition of methylation reactions may in turn influence cognitive impairment in ageing in various ways[99], by perturbing the regulation of gene expression in the β-amyloid pathway, by reducing the activity of protein phosphatase-2A or by impairing the formation of phosphatidylcholine-enriched *n*-3 fatty acids[99]. Additionally, reduced tissue concentration of *S*-adenosylmethionine may be linked to depression through perturbing monoamine (serotonin, dopamine and noradrenaline) synthesis and methylation[98]. Apart from folate, vitamins B_{12} and vitamin B_6, which have well-recognised roles in these pathways, riboflavin (in its cofactor forms flavin adenine dinucleotide and FMN) is also essential in one-carbon metabolism but its potential role in influencing brain health has rarely been considered.

Numerous observational studies have shown that lower status of folate, vitamin B_{12} and vitamin B_6 (and/or higher concentrations of homocysteine) are associated with cognitive deficit in ageing as extensively reviewed elsewhere[99,100]. Randomised trials in older adults that include intervention with high-dose folic acid, vitamin B_{12} and vitamin B_6 over 2 years or more have shown, not only improved cognitive performance[101–104], but also a reduced rate of brain atrophy in studies which have incorporated MRI[103,104]. Notably the greatest slowing in atrophy (53 %) was seen among participants with MCI and the highest homocysteine concentrations at baseline (>13 μmol/l), while cognitive function was preserved in those supplemented with B-vitamins and with a baseline homocysteine concentration >11·3 μmol/l[102]. The RCT evidence is not entirely consistent, however, as one recent and rather controversial meta-analysis in this area concluded that neither folic acid nor vitamin B_{12} had a beneficial effect on cognition in older adults[105]. This paper was however widely criticised at the time of publication, mainly as a result of the inclusion criteria used to select the trials for investigation, and thus the findings are in general not widely accepted by experts in this area[106,107]. It is clear that further appropriately designed randomised trials are needed, especially those targeting participants with low B-vitamin status (and in those at most risk of cognitive decline) as they are likely to benefit the most from optimising B-vitamin concentrations to achieve better cognitive health in ageing. Furthermore, research investigating the protective role of riboflavin on cognitive function is greatly lacking, albeit some evidence from older studies investigating riboflavin showed that lower biomarker status was associated with cognitive impairment[108]. Clearly there is a need for riboflavin to be considered in future randomised trials.

The role of B-vitamins in depressive disorders has not received as much interest as studies of cognitive disorders, although some observational (Table 1) and intervention (Table 2) evidence exists. A meta-analysis of nineteen observational studies concluded that low folate status was associated with a significantly greater risk of depression[109]. Low dietary intakes[110,111] or biomarker status[112–115] of vitamin B_{12} have also been linked with an increased risk of developing depression. Only a limited number of studies have considered the role of vitamin B_6, but available evidence suggests an inverse association between vitamin B_6 biomarker status (plasma pyridoxal 5′-phosphate) and depression[111,116,117]. Far less evidence exists in relation to riboflavin, although one early study reported lower biomarker status of riboflavin in psychiatric inpatients[96]. A number of randomised trials have considered the role of B-vitamin supplementation alone[118–121] or as an adjunct to anti-depressant medications[122,123]. The results are somewhat conflicting, however, and no clear conclusions have emerged, partly because of major methodological differences among studies. Reviews of the available evidence in relation to depression have concluded that folate and vitamin B_{12} may have roles in the longer term management of this condition[124,125].

Overall, there is considerable evidence to suggest that folate, vitamin B_{12} and vitamin B_6 have protective effects on cognitive function, and potentially against depressive symptoms in ageing, however further randomised trials of appropriate duration in suitable populations, and ideally interventions combining all four relevant B-vitamins, are required to support these findings.

Use of novel imaging technologies in nutrition and brain research

Following the 2009 Nutrition and Mental Performance Task Force of the European Branch of the

Table 1. Summary of observational studies investigating the association of B-vitamin intake and status with depression in older adults

Author	Country	Study design	*n*	Assessment	B-vitamin measurement	Outcome
Gougeon *et al.*[136]	Quebec, Canada	Longitudinal	1368	GDS/ anti-depressants usage	3 × 24 h-recalls	Decreased depression risk among women with higher intakes of vitamin B_6
Moorthy *et al.*[137]	Boston, USA	Cross-sectional	1955	MMSE CES-D	Plasma folate, vitamin B_{12}, B6 tHcy	Low B_{12} concentration associated with higher depression scores
Robinson *et al.*[112]	Dublin, Ireland	Cross-sectional	252	CES-D	Serum folate, B_{12}, Holo TC	Total B_{12} and Holo TC concentrations inversely associated with depressive symptoms
Beydoun *et al.*[138]	USA	Cross-sectional	2524	PHQ	Serum folate, B_{12}, tHcy	Inverse association between folate concentrations and depressive symptoms; dose response relationship
Skarupski *et al.*[111]	Chicago, USA	Longitudinal	3503	CES-D	Semi quantitative FFQ	High dietary intakes of B_6 and B_{12} protective against depressive symptoms
Ng *et al.*[139]	Singapore	Cross-sectional	669	GDS	Serum folate, B_{12}, tHcy	Lower concentrations of folate or deficient B_{12} status associated with greater risk of depression
Sánchez-Villegas *et al.*[110]	Boston, USA	Observational	9670	Self-reported depression, anti-depressants usage	Semi quantitative FFQ	Low dietary folate intake associated with depression among men; low B_{12} intake associated with depression in women; no associations with vitamin B_6 intake
Murakami *et al.*[140]	Japan	Cross-sectional	517	CES-D	Diet history questionnaire	Dietary folate inversely associated with depressive symptoms in men. No clear association for other B vitamins
Kim *et al.*[114]	Korea	Cross-sectional and prospective	732	Geriatric mental state	Serum folate, B_{12} tHcy	Lower baseline B_{12} concentrations associated with depression. Lower folate concentrations at baseline associated with higher risk of depression 2 years later
Dimopoulos *et al.*[141]	Greece	Observational	66	GDS	Plasma folate, B_{12}, tHcy	Lower folate and vitamin B_{12} or higher tHcy concentrations correlated with depressive symptoms
Ramos *et al.*[142]	California, USA	Observational	1510	CES-D	Plasma folate, B_{12}, tHcy	Participants in lowest tertile of plasma folate at increased risk of depression
Bjelland *et al.*[143]	Norway	Observational	5948	HADS	Serum folate, B_{12}, tHcy	Elevated tHcy significantly related to depression
Tiemeier *et al.*[144]	The Netherlands	Observational	3384	CES-D	Serum folate, B_{12}, tHcy	Depressive disorder more likely with vitamin B_{12} deficiency

CES-D, centre for epidemiological studies depression scale; GDS, geriatric depression scale; HADS, hospital anxiety and depression scale; MMSE, mini mental state examination; PHQ, patient health questionnaire; Holo TC, holo-transcobalamin; tHcy, total plasma homocysteine.

Table 2. Summary of randomised controlled trials investigating the effect of B-vitamin supplementation on depression in older adults

Study	Area	Cohort	Intervention	Duration	Outcome
B-vitamin intervention alone					
Okereke *et al.*[120]	USA	*n* 4331 63·6 years	FA: 2·5 mg, B_{12}: 1 mg, B_6: 50 mg or placebo	7 years	No effect on depression outcomes in participants without prior depression
Walker *et al.*[118]	Australia	*n* 909 65 years	FA:0·4 mg, B_{12}: 0·1 mg or placebo	2 years	FA plus B_{12} was not effective in reducing depressive symptoms in participants with elevated psychological distress
Almeida *et al.*[119]	Australia	*n* 273 63 years	FA: 2 mg, B_{12}: 0·5 mg, B_6: 25 mg or placebo	6·9–7·2 years	Reduction in risk of major depression, in participants with no previous major depressive episodes
Ford *et al.*[121]	Australia	*n* 299 ≥75 years	FA: 2 mg, B_{12}: 0·4 mg, B_6: 25 mg or placebo	2 years	No effect on depressive symptoms or development of depression in participants without a prior diagnosis of depression
B-vitamin supplement as adjunct to anti-depressant medications					
Almeida *et al.*[123]	Australia	*n* 153 50+ years	20–40 g Citalopram with FA: 2 mg, B_{12}: 0·5 mg, B_6; 25 mg or placebo	52 weeks	B vitamins did not increase 12-week efficacy of antidepressants, but enhanced and sustained antidepressant response over 1 year in participants with depression
Coppen and Bailey[122]	UK	*n* 127 41·9:44·3 years	20 mg fluoxetine with FA: 500 mg or placebo	10 weeks	FA significantly improved the action of fluoxetine in participants with depression

FA, folic acid.

International Life Sciences Institute workshop, a recommendation was developed suggesting the inclusion of brain-imaging biomarkers as secondary endpoints to clinical and cognitive measures[35]. Brain-imaging techniques have been increasingly utilised in recent years and provide an objective and highly robust means of assessing brain structure, function and response to nutrition, with advantages and disadvantages associated with each of their use, as reviewed in detail elsewhere[126] (Table 3). Electroencephalography and magnetoencephalography are two similar techniques for functional brain imaging and have the highest temporal resolution compared with other imaging techniques.

In recent years, some of these brain-imaging techniques have been utilised to advance nutrition research in ageing. One notable study referred to earlier in this review[103] effectively used MRI and confirmed the beneficial effects of B-vitamins on cognition shown previously in older adults with MCI, in particular in those with good status of PUFA[127]. Additionally, Brickman *et al.* used functional MRI and demonstrated higher brain activation in specific regions of the brain in participants who consumed high-dose cocoa flavanols[64]. In a study of 239 older adults, diffusion tensor imaging (which in some cases has been suggested to be a better predictor of cognitive decline than other biomarkers)[128], identified better white matter integrity in those who consumed more *n*-3 and *n*-6 PUFA and vitamin E[129]. Electroencephalography has also been used, with one recent report showing improved memory and functional connectivity in the δ band in response to Souvaid®, a nutritional supplement containing PUFA uridine, choline, phospholipids, folic acid, vitamin B_6, B_{12}, C, E and selenium in mild Alzheimer-type patients[130]. Positron emission tomography imaging has also been conducted within a 3-week intervention study, albeit in a very small study of only eleven women, leading to the conclusion that *n*-3 supplementation did not affect brain glucose metabolism in healthy older people[131].

It is clear that imaging techniques provide an objective means to improve the evidence base in this area, in particular in relation to proposed mechanisms. At this time, however, the number of studies utilising brain-imaging techniques to investigate the role of diet in brain health in ageing are limited. Magnetoencephalography has been approved by the US Food and Drug Administration for use within clinical and research settings as a means to assess and investigate cognitive dysfunction[132], AD[133,134] and depression[135]. However, to our knowledge, no work has been published using magnetoencephalography for nutrition studies in older adults. The application of these new technologies in the field of nutrition, in combination with clinical and questionnaire-based assessments, could provide much potential for robust investigation in future studies, furthering knowledge and discovery.

Conclusions

Nutrition has important roles in preserving cognition and reducing the risk of late-life depression. Emerging evidence in this area implicates subclinical deficiencies of certain nutrients in cognitive decline and depression in older adults. Future studies should address the gaps in the literature, in particular in identifying of the threshold for optimal nutrient levels required to prevent or delay declining brain health. At this time, the evidence for potential protective effects is strongest in relation to B-vitamins, *n*-3 PUFA and polyphenols. If confirmed, a public health strategy to improve status of these key nutrients may help to achieve better cognitive and mental health and thus improve quality of life in older age. Future well-designed randomised trials (ideally

Table 3. Brain-imagining techniques for use in nutrition research

Technique	Measurement method	Information obtained	Advantages	Disadvantages
Computerised tomography	X-rays	Structural images of the brain	Quick, relatively inexpensive, less stringent requirement for patients	Exposure to radiation
MRI	Magnetic fields and radiofrequency pulses	Detailed structural images of brain tissue (white and grey matters, blood vessels and bone)	Safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Diffusion tensor imaging	MRI-based technique using thermally induced self-diffusion of water as a probe	Mapping of the microstructures in the white and grey matters	Visualisation of microstructures, safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Functional MRI	MRI-based technique using blood oxygen level-dependant imaging	Visualisation of changes in blood flow, identification of areas of increased cerebral blood volume	Safe, non-invasive, good availability, repeatable	Unsuitable if participants have ferromagnetic implants or claustrophobia, costly to conduct
Positron emission tomography imaging (PET)	Radioactively labelled tracers once they begin to decay; the two γ rays released are detected by the scanners	Measurement of the metabolic and physiological processes of the brain	Provide measures of regional cerebral metabolism, blood flow and neurotransmitter metabolism	Low availability requires intravenous injection, use of radioactive compounds, limited use in diabetic participants
Single-photon emission CT imaging	Similar principles to the PET, however the radioactively labelled tracers used emit a single γ-ray	Neurotransmitter distribution and blood perfusion	Provide measures of regional cerebral metabolism, blood flow and neurotransmitter metabolism	Low availability requires intravenous injection, use of radioactive compounds, limited use in diabetic participants
Electroencephalography	Electrodes with conductive media are used to detect electric signals	Ionic currents that flow during synaptic excitation in the apical dendrites of pyramidal cells in the cerebral cortex	Relatively inexpensive, non-invasive, good temporal resolution, widely available	Poor spatial resolution, preparation timely
Magnetoencephalography	Specialised detectors superconducting quantum interference devices are used to record the magnetic signals	Ionic currents that flow during synaptic excitation in the apical dendrites of pyramidal cells in the cerebral cortex	Non-invasive, highest temporal and spatial resolution	Limited availability, costly, ferromagnetic implants may interfere with scan

incorporating imaging techniques such as magnetoencephalography) may provide a more robust basis for confirming effective nutrition interventions, which if implemented could reduce the risk of cognitive and mental health disorders in ageing and the related burden on health services and society overall.

Acknowledgements

The PhD studentship of K. M. was funded by the Northern Ireland Department for Employment and Learning who had no role in the design, analysis or writing of the present paper.

Financial Support

This work was supported by funding from the Northern Ireland Department for Employment and Learning, which funded the PhD studentship for K. M. The Northern Ireland Department for Employment and Learning had no role in the design, analysis or writing of the present paper.

Conflicts of Interest

None.

Authorship

K. M. drafted the manuscript. H. McN., C. F. H., L. H. and M. W. critically revised the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

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Proceedings of the Nutrition Society (2017), **76 (OCE3)**, E49 doi:10.1017/S0029665117001215

Irish Section Meeting, 21–23 June 2017, What governs what we eat?

Role of fortification and supplementation in achieving optimal biomarker status of B-vitamins for better mental health in older adults

K. Moore[1], C.F. Hughes[1], L. Hoey[1], M. Ward[1], K. Porter[1], J.J. Strain[1], K. Pentieva[1], A. Molloy[2] and H. McNulty[1]

[1]*Northern Ireland Centre for Food and Health, Ulster University, Coleraine, BT52 1SA and*
[2]*Institute of Molecular Medicine, Trinity College, Dublin 2, Ireland*

Worldwide the number of adults aged 60 years and over is predicted to reach 2 billion by 2050,[(1)] and hence the associated chronic diseases of ageing will continue to increase in the coming decades. Mental health disorders such as depression are common in older age and are major contributors to disability and poor quality of life.[(2)] Folate and the related B-vitamins (vitamin B12, vitamin B6 and riboflavin) involved in one-carbon metabolism are required for normal brain function and thus may have a protective role against depression. We have recently reported that low biomarker status of the relevant B-vitamins was associated with an increased risk of depression:[(3)] however it is unclear whether protective biomarker status can be achieved through natural food sources alone or if fortification and/or supplementation with B-vitamins is required. The aim of this study was to investigate the role of fortified food and supplements in optimising B-vitamin biomarker status in older adults, to levels associated with a reduced risk of depression.

This investigation was conducted as part of the Trinity Ulster Department of Agriculture (TUDA) Ageing cohort study (*n* 5186), where detailed health and lifestyle information was collected along with measurements of cognitive and mental health. Some 70 % of participants were fortified food consumers while 11 % were B-vitamin supplement users. Participants were categorised on the basis of dietary sources of B-vitamins as: natural food sources only; fortified food consumers; or supplement users (with and without concurrent consumption of fortified foods) (Table). B-vitamin biomarkers were examined in relation to each dietary category.

Data presented as medians. Differences were assessed using ANCOVA with Bonferroni post hoc tests on log-transformed data when applicable, controlling for age, gender, BMI and smoking. Values across a row without a common superscript letter are significantly different ($P < 0{\cdot}05$). **Abbreviations:** RBC, red blood cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase co-efficient.

	Natural Food Sources	Fortified Food Consumer			Supplement User
		Low	Medium	High	
Servings/week	0	1–4	5–7	8+	0–8+
TUDA Total %	*23·0*	*9·5*	*20·7*	*34·0*	*10·8*
RBC folate (nmol/L)	691^{a}	802^{b}	909^{c}	1138^{d}	1554^{e}
Serum Total Vitamin B12 (pmol/L)	238^{a}	243^{ab}	260^{b}	271^{c}	293^{c}
Plasma Vitamin B6 PLP (nmol/L)	$47{\cdot}0^{a}$	$54{\cdot}1^{b}$	$60{\cdot}8^{c}$	$70{\cdot}3^{d}$	$70{\cdot}6^{d}$
Riboflavin status (EGRac)	$1{\cdot}35^{a}$	$1{\cdot}32^{b}$	$1{\cdot}28^{c}$	$1{\cdot}28^{c}$	$1{\cdot}24^{d}$
Homocysteine (μmol/L)	$15{\cdot}2^{a}$	$13{\cdot}7^{b}$	$13{\cdot}7^{b}$	$12{\cdot}6^{c}$	$12{\cdot}2^{c}$

Biomarker status of each B-vitamin increased significantly with increasing intakes of fortified foods while non-consumers of fortified foods or supplements had the lowest status of all B-vitamin biomarkers. As previously reported,[(3)] lowest biomarker status of folate (p 0·003), vitamin B6 (p 0·034) and riboflavin (p 0·011) were, in turn, associated with an increased risk of depression by 47–78 %.

In conclusion, natural food sources alone appear to be insufficient in achieving a biomarker concentration of B-vitamins associated with lowest risk of depression, while regular consumption of fortified foods or supplements appear to be associated with biomarker concentrations that may protect mental health in ageing. These findings, if confirmed through randomised controlled trials, may have implications for dietary recommendations or fortification policy.

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taining the importance of home based interventions. Schools also have a substantial role to play in bringing about positive changes and can be considered as another action point for intervention.

Keywords: Waist circumference, school age, siblings, multi-level model

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ROLE OF FOLATE AND THE METABOLICALLY RELATED B-VITAMINS IN BRAIN HEALTH IN OLDER ADULTS: THE TUDA STUDY

[1]Moore, Katie;[2]Hughes, Catherine;[2]Hoey, Leane;[2]Ward, Mary;[2]Porter, Kirsty;[2]Strain, Sean JJ;[3]Molloy, Anne;[4]Cunningham, Conal;[3]Pentieva, Kristina;[2]McNulty, Helene.

[1]BSc. Registered Dietitian. Northern Ireland Centre for Food and Health. Ulster Univeristy Coleraine. Northern Ireland; [2]PhD. Northern Ireland Centre for Food and Health. Ulster University Coleraine. Northern Ireland.; [3]PhD. Institute of Molecular Medicine. Trinity College Dublin. Ireland; [4]MD. Mercers Institute for Research on Ageing. St James's Hospital Dublin. Ireland; [5]MD. Northern Ireland Centre for Food and Health. Ulster University Coleraine. Northern Ireland.

Background and objectives: Folate and the metabolically related B-vitamins involved in one-carbon metabolism may be important for maintaining brain health in aging but few studies have investigated the biomarker status of all relevant B-vitamins. The aim of this study was to examine the role of folate, vitamin B12, vitamin B6 and riboflavin in mental and cognitive health in older adults.

Methods: Participants were recruited (n 5186) to the Trinity, Ulster, Department of Agriculture (TUDA) Ageing Cohort study in 2008-2012, from across the Island of Ireland; of these, a sub-sample (n 587) of participants were reinvestigated 5 years after the initial TUDA study. Cognition was assessed using the Mini-Mental State Examination (MMSE), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and the Frontal Assessment Battery (FAB). Depression and anxiety were assessed using the Centre for Epidemiologic Studies Depression (CES-D) and the Hospital Anxiety and Depression (HADS) scales. Biomarkers of all the relevant B-vitamins were determined.

Results: At baseline, poor B-vitamin status (i.e. lowest 20%) was associated with an increased risk of depression (by 47-78%) for folate (p 0.003), vitamin B6 (p 0.034) and riboflavin (p 0.011), whereas only vitamin B6 deficiency was associated with anxiety (p 0.010) and cognitive dysfunction (p ≤0.001). Furthermore, at the 5 year follow up assessment, lower biomarker status at baseline (i.e. below median value) of vitamin B6 and riboflavin, but not folate or vitamin B12, were significant predictors of the rate of cognitive decline, as measured by change in MMSE (vitamin B6 p 0.020) and RBANS (vitamin B6 p 0.008; riboflavin p 0.018). Concentrations of all B-vitamin biomarkers increased significantly with increasing intake of fortified foods and with supplement use, while non-consumers of fortified foods or supplements had the lowest status of all B-vitamin biomarkers.

Conclusions: These results suggest that better B-vitamin status can have a positive impact on mental and cognitive health older adults. Optimization of B-vitamin status, via fortified foods or supplements, may offer a means of protecting brain health in aging.

Keywords: B-vitamins. One-carbon metabolism. Cognition. Depression. Aging.

Further collaborators:

Miriam Casey. MD. Mercers Institute for Research on Ageing. St James's Hospital Dublin. Ireland

Eamon Laird. PhD. Institute of Molecular Medicine. Trinity College Dublin. Ireland

Kevin McCarroll. MD. Mercers Institute for Research on Ageing. St James's Hospital Dublin. Ireland

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PROTEIN INTAKE AND THE DEVELOPMENT OF TYPE 2 DIABETES

[1]Voortman, Trudy;[2]Chen, Zhangling;[2]Franco, Oscar;[2]Voortman, Trudy.

[1]Postdoctoral scientist. Department of Epidemiology. Erasmus University Medical Center Rotterdam. The Netherlands; [2]Department of Epidemiology. Erasmus University Medical Center. Rotterdam. The Netherlands.

Background and objectives: Short-term trials suggest a beneficial effect of high protein intake on obesity and other diabetes risk factors, however, epidemiological studies observed positive associations of dietary protein with type 2 diabetes (T2D) risk. We aimed to examine, in a large prospective cohort, associations of intake of different sources of protein with insulin resistance, prediabetes, and T2D; and the role of obesity in these associations.

Methods: This study has been performed in the Rotterdam Study, a prospective cohort of subjects ≥45 years in the Netherlands. Prediabetes and T2D were diagnosed on the basis of medical records and fasting glucose and insulin concentrations, which were measured every few years in our research. We included 6814 subjects who were free of T2D at baseline, of whom 5795 were without prediabetes. Protein intake was assessed with the use of validated food-frequency questionnaires and expressed in energy percentages (E%). We used multivariable cox proportional hazard regression to analyze the associations between protein intake and risk of prediabetes and T2D; multivariable linear mixed models to analyze the associations with insulin resistance; and joint models to examine the role of repeatedly measured obesity data in this association . For all models, we used nutrient density substitution models to examine macronutrient substitution effects.

Results: We documented 643 cases of T2D during a median 7.2 years of follow up; and 931 cases of prediabetes during 5.7 years. In multivariable models, higher intakes of total protein were positively associated with insulin resistance, risk of prediabetes (hazard ratio (HR) =1.35 (95%CI 1.20-1.51) per 5 energy-percent), and risk of T2D (HR=1.38 (95%CI 1.20-1.60)). Additional adjustment for repeatedly measured BMI or waist circumference atten-

Proceedings of the Nutrition Society (2017), **76 (OCE1)**, E4 doi:10.1017/S0029665117000040

Winter Meeting, 6–7 December 2016, Diet, Nutrition and Mental Health and Wellbeing

Biomarkers of folate and related B-vitamins as predictors of cognitive decline in older Irish adults over a 5 year follow up period.

K. Porter[1], C.F. Hughes[1], L. Hoey[1], M. Ward[1], K. Moore[1], J.J. Strain[1], A. Molloy[2], C. Cunningham[3], M.C. Casey[3], E. Laird[2], K. McCarroll[3], K. Pentieva[1] and H. McNulty[1]

[1]*Northern Ireland Centre for Food and Health, Ulster University, Coleraine, BT52 1SA,*
[2]*Institute of Molecular Medicine, School of Medicine, Trinity College, Dublin 2, Ireland and*
[3]*Mercers Institute for Research on Ageing, St James's Hospital, Dublin, Ireland*

This abstract was awarded the student prize for best original communication presentation

Cognitive dysfunction and dementia are important public health issues in ageing, while optimal nutrition may be important in their prevention. One-carbon metabolism and the related B-vitamins may be important for maintaining cognitive health in ageing[(1)] but few studies have investigated biomarker status of all relevant B-vitamins and furthermore studies in this area are typically observational cohort studies at one time point. The primary aim of this investigation was to examine the role of baseline status of folate and the metabolically related B-vitamins (vitamins B12, B6 and riboflavin) as predictors of subsequent cognitive decline over a five-year follow-up period in healthy older people.

From the total sample recruited to the Trinity, Ulster, Department of Agriculture (TUDA) Ageing Cohort Study (≥60 years; n 5186), as previously described[(2)], a sample of 2093 participants in Northern Ireland were potentially available, from which a sub-set (n 587) meeting the selection criteria (aged >67 years at baseline, MMSE score >21, no B12 injections) were recruited for reinvestigation 5 years after the initial TUDA study. The rate of cognitive decline was evaluated by re-assessment of cognition using the original battery of tests including the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Mini-Mental State Examination (MMSE) and the Frontal Assessment Battery (FAB). Accelerated cognitive decline was defined as the highest quartile of change in cognitive score over the 5 year follow up period; i.e. ≥7 RBANS; ≥ 2 MMSE; ≥3 FAB points. At follow up, lower vitamin B6 status (PLP below median) and lower riboflavin status (EGRac above median) at baseline were each associated with an accelerated rate of cognitive decline, as measured by RBANS (Table). A similar relationship was shown with MMSE for vitamin B6 (OR: 1·485, CI: 1·002–2·200, *p* 0·049), but not for any other B-vitamin, while no significant relationships were observed for any B-vitamin biomarker with cognition as assessed by FAB.

B-vitamin Biomarker status[1]	Accelerated cognitive decline RBANS		
	OR	95 % CI	p value
Plasma Homocysteine (μmol/L) Reference <13·0 vs (13·0–27·5)	0·967	0·660–1·416	0·862
RBC Folate (nmol/L) Reference > 868 vs lower status (185–865)	1·126	0·759–1·670	0·556
Serum Total B12 (pmol/L) Reference > 252 vs lower status (58–251)	0·828	0·564–1·215	0·334
Plasma PLP (B6) (nmol/L) Reference > 61·3 vs lower status (11·6–61·3)	1·537	1·039–2·273	**0·032**
EGRac (B2)* Reference < 1·30 vs lower status (1·30–2·03)	1·493	1·013–2·201	**0·043**

Binary logistic regression was performed with adjustment for age (years) and education (years).
Abbreviations: OR, odds ratio; CI, confidence interval; RBC, Red Blood Cell; PLP, Plasma Pyridoxal Phosphate; *EGRac, Erythrocyte Glutathione Reductase Activation Coefficient- higher EGRac ratio indicates lower riboflavin status.
[1] For each biomarker, participants with lower status were identified using the median value as the cut off point.

The results indicate that vitamin B6 and the metabolically related B-vitamin riboflavin, may have important roles in helping to maintain better cognitive health in ageing. Further research including RCTs targeted at those with lower B-vitamin status are warranted to investigate the role of all relevant B-vitamins in cognitive function.

1 Smith D, Refsum H (2016) *Annual Review of Nutrition* **36**:211–39.
2 McCarroll K, Beirne A, Casey M *et al.* (2015) *Age & Ageing* **44**(5):847–853.

Proceedings of the Nutrition Society (2017), **76 (OCE1)**, E4 doi:10.1017/S0029665117000040

Winter Meeting, 6–7 December 2016, Diet, Nutrition and Mental Health and Wellbeing

Biomarkers of folate and related B-vitamins as predictors of cognitive decline in older Irish adults over a 5 year follow up period.

K. Porter[1], C.F. Hughes[1], L. Hoey[1], M. Ward[1], K. Moore[1], J.J. Strain[1], A. Molloy[2], C. Cunningham[3], M.C. Casey[3], E. Laird[2], K. McCarroll[3], K. Pentieva[1] and H. McNulty[1]

[1]*Northern Ireland Centre for Food and Health, Ulster University, Coleraine, BT52 1SA,*
[2]*Institute of Molecular Medicine, School of Medicine, Trinity College, Dublin 2, Ireland and*
[3]*Mercers Institute for Research on Ageing, St James's Hospital, Dublin, Ireland*

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Cognitive dysfunction and dementia are important public health issues in ageing, while optimal nutrition may be important in their prevention. One-carbon metabolism and the related B-vitamins may be important for maintaining cognitive health in ageing[(1)] but few studies have investigated biomarker status of all relevant B-vitamins and furthermore studies in this area are typically observational cohort studies at one time point. The primary aim of this investigation was to examine the role of baseline status of folate and the metabolically related B-vitamins (vitamins B12, B6 and riboflavin) as predictors of subsequent cognitive decline over a five-year follow-up period in healthy older people.

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The results indicate that vitamin B6 and the metabolically related B-vitamin riboflavin, may have important roles in helping to maintain better cognitive health in ageing. Further research including RCTs targeted at those with lower B-vitamin status are warranted to investigate the role of all relevant B-vitamins in cognitive function.

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Proceedings of the Nutrition Society (2016), **75 (OCE3)**, E115 doi:10.1017/S0029665116001300

Summer Meeting, 11–14 July 2016, New technology in nutrition research and practice

Role of B-vitamins as determinants of neuropsychiatric health in ageing

K. Moore[1], C. F. Hughes[1], L. Hoey[1], M. Ward[1], K. Porter[1], J.J. Strain[1], A. Molloy[2], C. Cunningham[3], M. Casey[3], E. Laird[2] and H. McNulty[1]

[1]*Northern Centre for Food and Health, Ulster University Coleraine, Cromore Road, Coleraine, BT52 1SA,* [2]*Institute of Molecular Medicine, School of Medicine, Trinity College Dublin, Dublin 2, Ireland and* [3]*Mercers Institute for Research on Ageing, St James's Hospital, Dublin, Ireland*

The number of adults aged 60 years and over is predicted to reach up to 2 billion by 2050 and hence the associated health and socio-economic costs will continue to increase. Cognitive dysfunction, depression and anxiety are significant problems of ageing. Preventing or delaying the onset of these disorders should therefore be a public health priority. Accumulating evidence suggests that low status of folate and the related B-vitamins (B12, B6 and riboflavin) are linked to an increased risk of these conditions[1,2,3]. The aim of this study is to investigate whether these B-vitamins are determinants of neuropsychiatric health in ageing.

Participants for this investigation were recruited to the Trinity Ulster Department of Agriculture (TUDA) Ageing cohort study and health, clinical, medication, lifestyle and nutritional details were collected (*n* 5186). A non-fasting blood sample was taken for the analysis of B-vitamin biomarkers. Cognitive function was assessed using the Mini Mental State Examination (MMSE), and depression and anxiety were assessed by the Centre for Epidemiologic Studies Depression scale (CES-D) (a score ⩾16·0 suggestive of depression) and the Hospital Anxiety and Depression (HADS) scale (a score ⩾11·0 suggestive of anxiety).

		Depression[3] (CES-D)			Anxiety[4] (HADS)		
B-Vitamin Biomarker[2]		OR	95 % CI	P[1]	OR	95 % CI	P
RBC folate (nmol/l)	Q5: *Reference ⩾1520*						
	Q1: ⩽623	1·571	1·120–2·202	**0·009**	0·850	0·543–1·330	0·476
Serum B12 (pmol/l)	Q5: *Reference ⩾373*						
	Q1: ⩽177	1·443	1·047–1·990	**0·025**	0·783	0·510–1·200	0·261
Plasma PLP (nmol/l)	Q5: *Reference ⩾96·4*						
	Q1: ⩽35·6	1·406	1·005–1·967	**0·047**	1·457	0·943–2·251	0·090
Riboflavin (EGRac)	Q5: *Reference ⩽1·19*						
	Q1: ⩾1·46	1·574	1·141–2·170	**0·006**	1·130	0·746–1·713	0·563

[1]Binary logistic regression was performed with adjustment for confounding factors as appropriate.
[2]The highest quintile (Q) of B-vitamin biomarker status was set as the reference category and was compared to the lowest quintile as shown for all biomarkers. P-value <0·05 was considered significant.
[3]Depression defined as CES-D score ⩾16·0 [4] Anxiety defined as HADS score ⩾11·0.
Abbreviations: RBC, Red Blood Cell; PLP, pyridoxal 5-phosphate; EGRac, erythrocyte glutathione reductase activation coefficient.

Those in the lowest quintile of status for each of the four B-vitamin biomarkers were at the greatest risk of depression, with a 40–57 % increased risk compared to those with the best status, after adjustment for confounding factors. No significant association was found between any B-vitamin and anxiety. Likewise, when similar analysis was performed to examine the impact of fortified food consumption, those with the highest intake (at least one portion of fortified food a day) had a significantly lower risk of depression than those who depended on natural sources of B vitamins (OR = 0·542, 95 % CI = 0·409-–·718, P ⩽ 0·001). These results suggest that a better status of B-vitamins can have a positive impact on mental health. Confirmation of these findings must await the outcomes of randomised controlled trials.

1. Reynolds E (2002) Folic acid, ageing, depression, and dementia *Br Med J* **324**, 1512–5.
2. Hooshmand B, Solomon A, Kareholt I, *et al*. (2012) Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study *J Intern Med* **271** 204–212.
3. Smith D (2008) The worldwide challenge of the dementias: A role for B vitamins and homocysteine? *Food Nutr Bull* **29** S143–S172.

Appendix 18: Presentations

List of Presentations

October 2017 OC	'*Role of folate and the metabolically related B-vitamins in brain health in older adults: The TUDA Study'* **International Congress of Nutrition,** Buenos Aires, Argentina.
June 2017 OC	"*Nutrition and the ageing Brain*" **Nutrition Society Summer Meeting**, Review Competition Winner; Queens University Belfast, Northern Ireland.
June 2017 OC	'*Role of fortification and supplementation in achieving optimal biomarker status of B-vitamins for better mental health in older adults'* **Nutrition Society Summer Meeting**, Queens University Belfast, Northern Ireland.
May 2017 P	*"Biomarker status of folate and related B-vitamins as predictors of cognitive decline in older adults over a 5-year follow-up period: The TUDA+5 Study*" **International Conference on Homocysteine and One Carbon Metabolism**, Best Poster; Aarhus, Denmark.
April 2017 OC	*"B-vitamins and Mental Health in Ageing"* **The Irish Gerontology Society Study Day** 2017, Our Lady's Hospice, Dublin, Ireland.
February 2017 OC	*"B-vitamins and the Ageing Brain"* **The Nutrition Society Irish Section Postgraduate Meeting**, Talbot Hotel Stillorgan, Dublin, Ireland.
January 2017 *P*	*"Impact of B-vitamin status on mental health in older Irish adults***" The Irish Nutrition & Dietetic Institute (INDI) Research evening**, Convention Centre, Dublin, Ireland.
November 2016 OC	*"Dietary intake and biomarker status of B-vitamins as determinants of mental health in ageing"* **British Dietetic Association Research Symposium**, Ulster University Jordanstown, Northern Ireland.
October 2016 OC	*"One-carbon metabolism, related B-vitamins and the ageing brain*" **Mini-Symposium on Nutrition: Implications to Ageing Processes and People, The Rank Prize Fund**, Grasmere, England.

August 2016 *P*	*"One-Carbon metabolism, related B-vitamins and MTHFR genotype as determinants of psychiatric health in older Irish adults"* **Folic acid, vitamin B12 and One Carbon Metabolism Conference, Federation of American Societies for experimental Biology**; Colorado, USA.
July 2016 OC	*"Role of B-vitamins as determinants of neuropsychiatric health in ageing"* **Nutrition Society Summer Meeting**, UCD, Dublin Ireland.
May 2016 OC	*"Depression, anxiety and ageing; do B-vitamins have a role?"* **Faculty of Health and Life Sciences Postgraduate Conference**; Shaping Healthier Communities, UU Coleraine, Northern Ireland.
February 2016 OC	*"B-vitamin determinants of neuropsychiatric health in aging"* **Nutrition Society, Irish Section Postgraduate Meeting,** Radisson Blue, Little Island, Cork, Ireland.
November 2015 OC	"*BrainHOP; B-vitamins and Brain Health in older adults".* **DSM visit to Northern Ireland Centre of Food and Health**, UU Coleraine, Northern Ireland.

OC, Oral Communication

P, Poster.