Vitamin D in the elderly and its relationship to inflammation and cognitive health

A thesis submitted for the degree of Master of Science (M.Sc)

2020

By

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1. Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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Brian Mullen
2. Summary

Introduction

The increasing prevalence of the ageing population is paralleled by a subsequent rise in age-related chronic diseases such as dementia. Dietary factors have being implicated as modifiable risk factors in the development of cognitive decline, with one such micronutrient identified as vitamin D. Vitamin D status in the population living at far northern latitudes are poorly maintained, with older adults at even high risk of deficiency due to physiological changes that accompany the ageing process. Furthermore, inflammation in the elderly is at an increasingly heightened state and is a known contributing factor in cognitive decline. Vitamin D food fortification and/or supplementation to maintain constant sufficient status has the potential to alleviate some of the economic burden resulting from age-related disease by reducing inflammation and attenuating the rate of cognitive decline.

Aims

The aim of this study was to explore the association between vitamin D, and cognition in community dwelling older Irish adults both cross – sectionally and longitudinally. A secondary aim was to investigate whether vitamin D mediates cognitive integrity through inflammatory pathways or if it exerts an association independent of inflammation. This will be addressed by making adjustments in the statistical model to account for the influence of systemic inflammation on changes in cognitive function.

Methods
This body of research is an observational cross–sectional and longitudinal cohort study by design. It involved enrolment of 1000 older Irish adults (>60 yrs at baseline) 5 years following baseline (n=5186). Recruitment took place in Northern Ireland and Dublin as part of the Trinity, Ulster, Department of Agriculture (TUDA) ageing cohort follow-up study. The baseline cohort comprised of three disease defined groups, namely hypertension, bone, and cognition. At baseline detailed data was collected regarding psychosocial, medical history and medication use, lifestyle, and neuropsychological (MMSE, FAB, RBANS). Biological samples were also taken in the form of blood from the cubital vein in order to examine inflammatory markers and cytokine concentrations (CRP, IL-6, TNF-alpha, IL-10), and vitamin D status by liquid chromatography tandem mass spectrometry (LC-MS/MS). This study then followed-up with a sub-cohort of participants 5+ years later.

**Results**

Analyse was performed on 378 participants at follow-up and partial correlation has shown that 25(OH)D is positively associated with domain specific cognition when assessed by MMSE and RBANS Index IV score (P<0.05 for both). Furthermore inflammatory status was found to correlate with domain specific cognition. Specifically, IL-10 negatively correlates with RBANS index III (P<0.05), index V (P<0.05) and total scale (P<0.05), no further correlation was found between inflammatory cytokines and cognition. However, analysis could not be progressed due to lack of power.

**Conclusion**

This study highlights that vitamin D is positively correlated with domain specific cognition, even after adjustment for well-established covariates. The results of this
study do not rule out the possibility of a protective role of vitamin D in cognition decline. Although this study was lacking sufficient power to further the analysis, there is still much agreement with the existing literature.
3. Dedications

I dedicate this work to my supportive family

“Love is patient, love is kind. It does not envy, it does not boast, it is not proud. It does not dishonour others, it is not self-seeking, it is not easily angered, it keeps no record of wrongs. Love does not delight in evil but rejoices with the truth. It always protects, always trusts, always hopes, always perseveres. Love never fails.” (Corinthians 13:4-8)
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<td>1,25(OH)2D</td>
<td>1,25 dihydroxyvitamin D</td>
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<td>25(OH)D</td>
<td>25-hydroxyvitamin</td>
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<tr>
<td>3MSE</td>
<td>Modified Mini Mental State Examination</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
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<td>ADAS-cog</td>
<td>Alzheimer’s Disease Assessment Scale</td>
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<td>AMT</td>
<td>Abbreviated Mental Test</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<tr>
<td>CANTAB</td>
<td>Cambridge Neuropsychological Testing Automated Battery</td>
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<td>CDR</td>
<td>Clinical Dementia Rating Scale</td>
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<td>CDT</td>
<td>Clock Drawing Test</td>
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<tr>
<td>CES-D</td>
<td>Centre of Epidemiologic Studies Depression Scale</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CSF-Aβ</td>
<td>Cerebral Spinal Fluid Amyloid Beta</td>
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<td>CSI</td>
<td>Cognitive Style Index</td>
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<td>CT</td>
<td>Coding Task</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<td>DEQAS</td>
<td>Vitamin D External Quality Assessment Scheme</td>
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<tr>
<td>DS-F</td>
<td>Digit Symbol Forward</td>
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<tr>
<td>DXA</td>
<td>Dual-energy X-ray Absorptiometry</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>EBMT</td>
<td>Eastern Boston Memory Test</td>
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<td>FAB</td>
<td>Frontal Assessment Battery</td>
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<td>FTSS</td>
<td>Five Time Sit to Stand</td>
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<td>GDS</td>
<td>Geriatric Depression Scale</td>
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<td>GOS-E</td>
<td>Glasgow Outcome Scale</td>
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<td>HADS</td>
<td>Hospital Anxiety Depression Scale</td>
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<tr>
<td>HTN</td>
<td>Hypertension</td>
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<tr>
<td>IADL</td>
<td>Instrumental Activities of Daily Living</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
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<tr>
<td>LCMS</td>
<td>Liquid Chromatography Mass Spectroscopy</td>
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<tr>
<td>MAC</td>
<td>Membrane Attack Complex</td>
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<td>MCS</td>
<td>Memory Composite Score</td>
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<td>MFQ</td>
<td>Mood and Feelings Questionnaire</td>
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<td>MHI</td>
<td>Mental Health Inventory</td>
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<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<td>NAART</td>
<td>North American Adult Reading Test</td>
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<td>NAS</td>
<td>Neuropsychological Assessment Scale</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drugs</td>
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<tr>
<td>OTS</td>
<td>One Touch Stocking of Cambridge</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<td>PSMS</td>
<td>Physical Self-Maintenance Scale</td>
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<td>RBANS</td>
<td>Repeatable Battery for the Assessment of Neuropsychological Status</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RCPM</td>
<td>Raven’s Coloured Progressive Matrices</td>
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<td>RCT</td>
<td>Randomised Controlled Trial</td>
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<td>RDA</td>
<td>Recommended Daily Allowance</td>
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<td>RI-48</td>
<td>Rappel Indice-48 item cued recall test</td>
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<td>Short Portable Mental State Questionnaire</td>
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<td>SWM</td>
<td>Spatial Working Memory</td>
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<td>Th</td>
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<td>TMA&amp;B</td>
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<tr>
<td>TUDA</td>
<td>Trinity, University of Ulster, Dept of Agriculture</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed Up and Go</td>
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<tr>
<td>VDBP</td>
<td>Vitamin D binding protein</td>
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<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
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<tr>
<td>VF</td>
<td>Verbal Fluency</td>
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<tr>
<td>VRM-R</td>
<td>Verbal Working Memory - Recall</td>
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<tr>
<td>WAIS</td>
<td>Wechsler Adult Intelligence Scale</td>
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<tr>
<td>WMS</td>
<td>Wechsler Memory Scale</td>
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9. Abstract

**Background:** There is a major shift in ageing demographics as individuals are experiencing increased longevity. This shift may be beneficial for the individual who will live into more advanced year than their ancestors. However, this demographic shift also has an economic health burden in the form of age-related chronic diseases healthcare costs. The healthcare costs are estimated to increase significantly from the €3 billion currently being spent annually for the treatment of dementia and experts estimate the global cost will soon reach over one trillion dollars (Prince et al. 2016). In 2017 PHE reported that dementia was the leading cause of mortality in older adults, exceeding CVD, stroke and lung cancer for the first time. The most common form of dementia is Alzheimer disease accounting for approximately 62% of cases, while other forms include vascular dementia, mixed Lewy body dementia, and frontotemporal dementia. There is currently no cure for dementia and few treatments. Although there is relatively little known of the aetiology of dementia, it is thought to be largely multifactorial with a long preclinical phase prior to symptoms presenting themselves. Some research has shown inflammation to contribute to cognitive decline, and certain dietary nutrients have also being identified as possible modifiable risk factors. One such nutrient identified is vitamin D. Many previous studies have suggested that vitamin D exerts its beneficial effects on cognitive health through anti-inflammatory and immunomodulatory pathways. However, no studies have examined vitamin D for effects exclusive to these pathways. **Objective:** The aim of this study was to explore the association between vitamin D status and cognition in community dwelling older Irish adults. A secondary aim was to investigate if vitamin D mediates cognitive integrity through inflammatory
pathways or if it exerts an association independent of inflammation. This will be addressed through statistical adjustment for the presence of inflammation. This study also aims to assess whether vitamin D can predict incidence cognitive decline over 5+ years follow-up period. **Design, setting, and participants:** This observational cross-sectional and longitudinal cohort study enrolled 1000 older Irish adults (>60 yrs at baseline) 5 years following baseline (n=5186). Recruitment took place in Northern Ireland and Dublin as part of the Trinity, Ulster, Department of Agriculture (TUDA) ageing cohort follow-up study. **Outcomes measured:** Participants were examined for anthropometric measures, lifestyle factors, medical history, current medication, cognition (MMSE, FAB, RBANS), dietary, inflammatory markers and cytokine concentrations (CRP, IL-6, TNF-alpha, IL-10), and vitamin D status by liquid chromatography tandem mass spectrometry (LC-MS/MS). **Results:** Partial correlation shows that 25(OH)D is positively associated with cognition when assessed by MMSE and RBANS Index II score (P<0.05 for both). Furthermore, IL-6 significantly negatively correlates with RBANS index III (P<0.05 ) index V (P<0.05), and total scale (P<0.05 ). No other correlations were observed between inflammatory cytokines and cognition. **Conclusion:** This study highlights that vitamin D is positively correlated with domain specific cognition, even after adjustment for well-established covariates. The results of this study do not rule out the possibility of a protective role of vitamin D in cognition decline. Although this study was lacking sufficient power to further the analysis, there is still much agreement with the existing literature.
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10. Chapter 1: Cognitive health: A vitamin D and inflammation perspective
10.1. Public health concern with ageing

As life expectancy continues to increase, so does the corresponding proportion of older adults in population. It is estimated that by the year 2050 the proportion of those aged >60yrs will reach more than two billion (Moore et al. 2018). With such large increases, there is the subsequent matching rise in the number of chronic conditions of aging such as cardiovascular disease (CVD), arthritis, cancer, falls and fractures etc. Currently, it is estimated that 23% of the global burden of disease can be attributed to older adults, with cognitive dysfunction topping the list of causes of ill health (Prince et al. 2015). The World Health Organisation (WHO) reported that the two most common dysfunction disorders in the ageing population are dementia and depression (Prince et al. 2015). The projection for dementia prevalence worldwide for 2050 is over 131 million (Prince et al. 2016) while depression is predicted to be the second most common cause of disability in the ageing (>65yrs) population by 2020 (National Collaborating Centre for Mental Health 2010). Irish Government figures currently estimate the economic cost at over €3 billion and €1.69 billion for depression and dementia respectively (Shea and Kennelly 2008). Additionally, a Public Health England (PHE) report in 2017 found dementia as the leading cause of mortality in the elderly, for the first time exceeding mortality rate by CVD, stroke, and lung cancer (Public Health England 2017). These trends are not unique to England, but seen globally (Public Health England 2017), largely a result of the changes in age demographics, increased life expectancy and advances in diagnostic procedures (Lutz et al. 2008). The severity of cognitive dysfunction can range from mild cognitive impairment (MCI) to full dementia, with up to half of those diagnosed with MCI expected to become demented within 5 years (Gauthier et al. 2006). Cognitive dysfunction is assessed using various cognitive function assessment tools, and MCI is
defined as cognitive function decline greater than that expected for age, adjusting for individual’s education level (Gauthier et al. 2006).

The exact ethology of cognitive impairment and dementia have not yet fully elucidated. However, it is thought to be largely multifactorial by nature, resulting from complex interactions between many risk factors through different pathophysiological mechanisms (Moore et al. 2018). Some known risk factors are non-modifiable such as genetics, gender, and age, while other modifiable risk factors include, lifestyle habits (smoking, alcohol, obesity), and diet (Prince et al. 2014). Many of these risk factors are prevalent at midlife, which is consistent with what is known regarding the long preclinical phase of dementia. Interestingly, a recent study also associated midlife systemic inflammation with neurodegeneration in late life (Walker et al. 2017). Many of the modifiable risk factors have a commonality with increased levels of systemic inflammation (Wichmann et al. 2014). Inflammation is a normal biological function that the employs as a result of insult, injury, or invasion from foreign pathogens. Under normal circumstance the body deals with such insult and inflammation subsides. However, the ageing process is associated with an increased and prolonged state of dysregulation of inflammation which can cause damage to tissues and cells and interfere with biological functional homeostasis (Walker et al. 2017). Increased inflammation has also being associated with many chronic diseases of ageing, such as CVD, metabolic disorders, cancers, frailty, and mortality (Cannell et al. 2014). Furthermore, inflammation has being implicated in the development of mild cognitive impairment (MCI) and dementia independently as well as on a secondary level through its integral involvement in atherosclerosis, a known mechanism for the development of MCI and dementia (Wichmann et al. 2014).

In terms of dietary risk factors, a recent longitudinal population based study reported that adherence to the Mediterranean diet or similar healthy dietary patterns was associated
with a lower risk of Alzheimer disease (Scarmeas et al. 2006). Consistent with these findings, further studies have found that similar dietary patterns were found to be associated with greater brain volume and cognitive performance (Gu et al. 2015, 2016), both of which are known predictors of cognitive decline. A cross-sectional study of 330 non-demented older adults that performed a reduced-rank regression (RRR) model using inflammatory markers as response variable identified vitamin D, among other nutrients, as being associated with reduced inflammation (Gu et al. 2018). Similarly, a longitudinal study using a RRR model identified those consuming an inflammatory diet patterns as having elevated serum Interleukin - 6 (IL-6) and experience accelerated cognitive decline in old age (Ozawa et al. 2017). IL-6 is a proinflammatory T-helper 2 cell (Th2) cytokine with a central role in the immune response (Michaud et al. 2013). This new evidence opens the potential to identify novel therapeutic dietary protocols in order to aid in the attenuation of cognitive function decline. However, in the absence of significant gains in understanding the pathology of cognitive decline, and increased public health strategies, it is reasonable to assume that these figures will rise with the increasing ageing population. Therefore it is imperative to identify lifelong modifiable risk factors in order to reduce the incidence of or attenuate the progression of such cognitive disorders. One such modifiable risk factor that has being highlighted in the literature, and the subject of this review, as showing promise in terms of protective effects on cognitive health is vitamin D (Aspell et al. 2018; Moore et al. 2018; Marino and Misra 2019).

10.2. Vitamin D: The history

Historically, vitamin D has been known for its role in bone health and for its curative role for rickets by the 1930’s. It was discovered that both sunlight and cod liver oil, which contains vitamin D could be successfully administered to combat and prevent rickets in
children and osteomalacia in adults (Zhang et al. 2016). Rickets was not a disease of the poor or under-privileged, in fact, rickets was prevalent in all layers of society regardless of socio-economic status, evidenced by historical information spanning in excess of two millennia (Zhang et al. 2016). It is a disease characterised by the classical presentation of skeletal deformities of the lower limbs such as “bowed” or ”knock knees” (Figure 1). There is evidence that rickets was described back in the 1st and 2nd century by Greek physician Soranus (Σωρανός) of Ephesus who practiced in Rome and Claudius Galenus (Κλαυδίος Γαληνός) a physician of the Roman Empire (Zhang et al. 2016). Both described conditions in children that are similar to rickets but attributed the cause of the deformities to improper childcare and swaddling clothes (Zhang et al. 2016). Furthermore, evidence of the knowledge of rickets was also discovered in pre-Cristian ancient China (Gwei-Djen and Needham 1976). However, it was not until 1645, when Dr David Whistler (1619-1684) (Figure 2) defended his Thesis, at the University of Leiden, titled “De morbo puerile anglorum, quem patrio idiomate indigenae vocant the Rickets” (Concerning the disease of English children, which in English it is called “Rickets”), that the scientific literature on rickets was opened. This however, was superseded five years later when one Dr Francis Gleeson (1597-1677) (Figure 3) published his book on rickets titled “De Rachitide Sive Morbo Puerili, qui Vulgo The Rickets dicitur, Tractatus” in 1650 (Zhang et al. 2016). Although cod liver oil had being used for health benefits for a long time, it was not until 1824 when it was first prescribed for rickets by Dr Scheutte and 1906 before a possible dietary component of the disease was postulated by Sir Frederick Gowland Hopkins (Wolf 2004). In 1936 Adolf Windaus (1876-1959) (Figure 4) correctly established the structure of calciferol after winning the Nobel prize for chemistry in 1928 for his studies on the properties sterols and their connection with vitamins (Wolf 2004). Rickets has being virtually eradicated since the mid 1950’s and
there was being a milliard of research into the effect of vitamin D for skeletal health benefits. However, recent discoveries have elucidated the fact that vitamin D’s role in the human body extends far from its classical role.

*Figure 1. Skeleton deformed by Rickets, 1879 (Burns & Burns, 2014)*
Figure 2. Daniel Whistler, 1619–1684.

Portrait in the Royal College of Physicians, London.

Figure 3. Francis Glisson, 1597–1677.

Portrait in the Royal College of Physicians, London.
10.3. Vitamin D in older adults

Vitamin D is a fat soluble pro-hormone essential for various physiological roles in the human body. Vitamin D is known for its role in bone metabolism through the regulation of calcium and phosphorus homeostasis (Anandabaskar et al. 2018). However, it is now accepted that vitamin D exhibits pleiotropic actions through lesser understood biological routes that may be of importance for informing public health policies, disease prevention, and the ageing process (Herrmann et al. 2017; Anandabaskar et al. 2018). As a result, vitamin D has become the target of much interest in recent years in an effort to understand the biological mechanisms linking it to brain function, as well as its neuroprotective properties (Perna et al. 2014). Vitamin D is primarily (80%) obtained endogenously when 7-dehydrocholesterol, or pro-vitamin D (present in the stratum basale and stratum spinosum of the epidermis) in the skin is exposed to ultraviolet B sunlight (UVB, 290-320nm) to form pre-vitamin D prior to isomerization to vitamin D (Zhang and Naughton 2010; Sassi et al. 2018). Exogenous forms of vitamin D (20%) include vitamin D2/ergocalciferol (Figure 5) which is photosynthesised by plants and fungi and consumed
in the diet, while vitamin D3/cholecalciferol (Figure 5) can be obtained through the consumption of oily fish and other foods, albeit very few food sources are naturally rich in vitamin D (Zhang and Naughton 2010; Sassi et al. 2018). Within Ireland, meat intakes are actually the largest source of vitamin D but one of the poorest sources (Cashman et al. 2013). Vitamin D is also obtained in the diet though Ireland has a very liberal, non-mandatory food fortification policy with very few food products actually fortified with vitamin D in contrast to other far latitude countries such as Finland (Jääskeläinen et al. 2017)

Vitamin D acquisition through dermal irradiation is influenced by skin pigmentation, clothing coverage or the use of sunscreen, seasonal changes (Figure 6), zenith angle, pollution, obesity, and sedentary behaviours such as limited outdoor activities (Zhang and Naughton 2010; Clifford and Rosen 2011; Hossein-nezhad and Holick 2013). As a result of ageing, sustaining adequate levels of vitamin D becomes even more of a challenge as a result of malabsorption or deceased appetite, diseases of the kidneys or liver, as well as medication use (Yu et al. 2010; Pérez-López et al. 2011; Van Der Schaft et al. 2013) or generally spending more daylight hours indoors due to reduced mobility/ frailty or institutionalisation (Yu et al. 2010; McCarroll et al. 2015). Furthermore, skin integrity affects the efficiency of UV-B production of vitamin D in the elderly as skin become thin with reduced epidermal concentration of 7-dehydrocholestrol, therefore, despite similar sunshine exposure, vitamin D production can be up to 75% less (McCarroll et al. 2015).

To compound factors further, countries residing at high latitudes north of the equator (>50°N) experience an increased zenith angle of the sun (reduced UVB reaching the earth’s surface) 6-months of the year (October – March), which is insufficient for cutaneous synthesis of vitamin D (Hossein-nezhad and Holick 2013; O'Sullivan et al. 2017). As a result of the lack of fortification and issues in dermal synthesis, deficiency
rates for Ireland and far latitude countries are high. For instance, recent research has demonstrated that approximately 1 in 8 older Irish adults are deficient in vitamin D rising to 1 in 4 during the winter while only 8.5% of the older Irish population take vitamin D supplements (Laird et al. 2017). This corresponds to other populations of older adults in far latitude countries including the UK and Europe though with one exception, Finland where deficiency rates are now <1% due to an aggressive policy of vitamin D food fortification (Jääskeläinen et al. 2017). However, interpreting the literature in terms of vitamin D status of a population is not without difficulty. For example, vitamin D deficiency rates reported in the literature varies greatly, with methodological discrepancies, including that of serum/plasma 25(OH)D status, population, ethnic groups, sex, sample size, geographical location, season and method of vitamin D assay measurement (Aspell et al. 2018). For instance, the Institute of Medicine (IOM) guidelines for vitamin D for deficiency, insufficiency, and sufficiency are defined as <30, 30–50, and >50 nmol/L, respectively (Institute of medicine 2010). However, some studies define deficiency as <25nmol/L (Buell et al. 2009) or <50nmol/L (Wilson et al. 2014; Meghil et al. 2019), while others suggest adequate levels of 50-124nmol/L (Miller et al. 2015), or high vitamin D levels at ≥125nmol/L (Miller et al. 2015). Although toxicity is thought only to occur at levels >375 nmol/L (Hathcock et al. 2007). In terms of method of measurement, the gold standard is LC-MS/MS (Herrmann et al. 2017) though many studies do not utilise this test, but use a variety of assays including electrochemiluminescence immunoassays (ECLIA) (Codoñer-Franch et al. 2012; Perna et al. 2014; Ahn and Kang 2015), radioimmunoassay (RIA) (Buell et al. 2009; Llewellyn et al. 2010; Codoñer-Franch et al. 2012; Littlejohns et al. 2014; Wilson et al. 2014; Ahn and Kang 2015; Meghil et al. 2019; Sakuma et al. 2019), or competitive protein binding assay (Van Schoor et al. 2016). Finally, commonly used cut-off criteria for vitamin D are
those developed for the purpose of bone health maintenance, no cut-off levels exist to reflect adequate intake for cognitive health (Pettersen 2016).

Figure 5. Vitamin D2 (Ergocalciferol); Vitamin D3 (Cholecalciferol); 1,25(OH)2D (1,25-Dihydroxycholecalciferol)
Figure 6. Geographic & seasonal variation in the percentage of Irish older adults who are 25(OH)D deficient (<30nmol/L) according to Laird et al. (2017)
10.4. **Vitamin D metabolism**

Vitamin D, whether ingested or synthesised by solar irradiation goes through a two-step hydroxylation process in order to become the biologically active form known as 1,25-dihydroxycholecalciferol [1,25(OH)2D3] (Figure 5) (Laird *et al.* 2014). Vitamin D enters the liver where it is converted to 25-hydroxyvitamin D [25(OH)D3], the major circulating form of vitamin D typically used to determine vitamin D status (Deluca and Cantorna 2001), a reaction catalysed by the enzyme vitamin D 25-hydroxylase (CYP2R1) (Hossein-nezhad and Holick 2013). In order to be converted to the biologically active form, 25(OH)D3 undergoes additional hydroxylation in the kidneys (Deluca and Cantorna 2001) catalysed by the vitamin D activating enzyme 1α-hydroxylase (CYP27B1) to form the secosteroid hormone 1,25(OH)2D3 (Hossein-nezhad and Holick 2013). This second hydroxylation is strictly controlled by a renal negative feedback loop where high 1,25(OH)2D3 and fibroblast growth factor 23 (FGF-23) halt CYP27B1 synthesis while simultaneously activating 24-hydroxylase (CYP24A1) which is expressed in nearly all cell types, thereby inactivating 1,25(OH)2D3 to 1,24,25(OH)3D3 (Van Etten *et al.* 2008; Sassi *et al.* 2018). 1,24,25(OH)3D3 can subsequently be further processed to the excretion product calcitriol acid (Van Etten *et al.* 2008). This biological protection produced by a 24-hydroxylase hydroxylation prevents endogenous 1,25(OH)2D3 overstimulation (Pérez-López *et al.* 2011). However, recent discoveries have acknowledged that various other tissues of the body exhibit mechanistic properties that facilitate local 1,25(OH)2D3 creation, including the brain (Hossein-nezhad and Holick 2013). This provides a plausible biological role of vitamin D in brain health.
10.5. Vitamin D and cognition

As mentioned earlier, there is mounting evidence suggesting that dietary factors have a role to play in the development of cognitive impairment and dementia (Luchsinger and Mayeux 2004; Buell and Dawson-Hughes 2008; Moore et al. 2018; Rutjes et al. 2018), Low circulating vitamin D levels are commonly cited in AD patients (Buell and Dawson-Hughes 2008; Van Der Schaft et al. 2013; Lopes Da Silva et al. 2014; Goodwill and Szoeke 2017) and vitamin D has been associated with the pathways involved in Alzheimer disease development, such as Aβ-induced neurotoxicity, Aβ-generation/degradation, oxidative stress and inflammation (Grimm et al. 2014, 2017; Guo et al. 2016; Pierucci et al. 2017). In addition, vitamin D has also being implicated in brain macrostructure such as brain atrophy (as assessed by magnetic resonance imagining (MRI)) (Hooshmand et al. 2014; Mosconi et al. 2014; Gu et al. 2015), increased ventricle size (Annweiler et al. 2014), increased amyloid-β (Aβ) clearance and Tau pathology (Hooshmand et al. 2014). Tau is a structural protein that stabilise microtubules, and found in abundance in neurons of the CNS (Mandelkow and Mandelkow 2012). In dementia, Tau protein aggregate due to hyperphosphorylation to form neurofibrillary tangles, a primary marker of Alzheimer disease (Vasilica Pîrşcoveanu et al. 2017). Although, Aβ build-up/clearance and Tau pathology’s are not exclusive to Alzheimer’s disease patients, as in the normal population large numbers of non-demented individuals also can exhibit significant Aβ and Tau pathology, asymptomatic of dementia (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study. 2001). However, ventricle size has being linked to mental disorders commonly observed in the elderly population such as depression and mild cognitive impairment, known risk factors for dementia (Annweiler et al. 2014). Global cognitive function is commonly assessed using the mini mental state examination (MMSE) or similar modified versions of the test.
It is a 30-point cognitive assessment tool (Buell et al. 2009) however, the score that determines cognitive impairment differs from study to study. Some use a cut-off score of <24 (Toffanello et al. 2014; Ahn and Kang 2015; Sakuma et al. 2019), while other use a score of <18 (Matchar et al. 2016; Palacios et al. 2019). Furthermore, some longitudinal studies examine delta (Δ) value (change from baseline) rather than absolute score, and define cognitive decline with more agreement (decline of ≥3 points) (Llewellyn et al. 2010; Slinin et al. 2012; Olsson et al. 2017), albeit follow-ups are performed over differing time periods ranging from 2 – 11 years.

Vitamin D has being frequently, but not always, associated with cognitive decline as measured by global function. Miller and colleagues noted that low vitamin D status was associated with an accelerated decline in cognitive function domains following adjustment for known confounders (Miller et al. 2015). This study was one of few that explored domain specific cognitive function. Following cross-sectional analysis of the 382 community dwelling multi-ethnic cohort, lower vitamin D levels were linked to poor cognitive performance for semantic memory (P=.02), visuospatial ability (P=.04), and executive function (P=.01), but not for episodic memory performance (Miller et al. 2015). The same study analysed a subset (n=318) on longitudinal cognitive performance where significance held true (Miller et al. 2015). Furthermore, a study of 1658 healthy elderly found that the odds of all-cause dementia or AD at baseline in participants who were severely 25(OH)D deficient (<25 nmol/L) was 3-6 time that of 25(OH)D adequate participants at follow-up (mean= 5.6 years) (Littlejohns et al. 2014). Not all studies observed associations though, in a study of 1182 Swedish men over 18 years, no
association was found between baseline vitamin D status and long-term risk of dementia or cog impairment, as tested using the MMSE (Olsson et al. 2017). However, vitamin D cut points were higher than other studies at ≤50, >50–75, and >75 nmol/L (Olsson et al. 2017). In addition, 25(OH)D was measured at baseline only with cognitive assessment 18 years later, which may not reliably ascertain long-term exposure to inflammation (Olsson et al. 2017), although longer follow-up periods do limit the risk of reverse causation, as dementia is thought to have a long preclinical phase (Prince et al. 2014). However, none of these studies measured blood markers for inflammation, which is thought to be a key contributing factor in the development of neurological diseases (Gorelick 2010). Therefore these studies do not necessarily highlight vitamin D as having a primary role in neurological disease development, as affects could be through secondary mechanisms such as anti-inflammatory pathways. Only five randomised controlled trials (RCTs) have being performed to determine the association between vitamin D and cognition (table 1). However, only one of these RCT’s demonstrated a protective effect of high dose (4000IU/day) vitamin D on cognition over an 18-week supplementation period (Pettersen 2017). This dose is well above what is recommended for maintenance of skeletal health (400-800IU/day) (IOM 2010). Furthermore, of the other four RCT’s, one recruited from a young population (18-24yrs) with high baseline 25(OH)D (Dean et al. 2011), one used a low dose (400IU/day) which may only provide skeletal health benefits (Rossom et al. 2013), another used vitamin D2 supplements (Stein et al. 2011), which may be less effective at increasing levels of 25(OH)D (Armas et al. 2004; Guo et al. 2018), while the most recent study supplemented weekly rather than daily and used limited cognitive assessment tools (Jorde et al. 2019).
10.6. Inflammation and ageing (Inflammageing)

Inflammation is a necessary process, and a natural response by the immune system to injury, pathogen, irritant or oxidative stress (Sartori et al. 2012). Although immune modulated inflammation is crucial to the healing process and necessary for sufficient cell and tissue stabilization following an insult, prolonged dysregulated inflammation can be detrimental to the body causing tissue damage (Sartori et al. 2012). The ageing process is associated with increasingly heightened and prolonged dysregulated inflammation throughout the body, including inflammation of the brain (Raz and Rodrigue 2006; Russo et al. 2011). Furthermore, ageing with the absence of other comorbidities, or so called successful ageing, is still associated with low-grade chronic inflammation (Krabbe et al. 2004). This chronic low-grade inflammation is characterised by cytokine dysregulation causing increased proinflammatory cytokines and reduced anti-inflammatory cytokines (Michaud et al. 2013). These changes in the immune system that accompany the ageing process is a phenomenon known as “immunosenescence” (Michaud et al. 2013).

Inflammation with ageing is considered a major contributor for the development of age related diseases, such as frailty, vascular diseases, neurological diseases, cancers, CVD, and cognitive dysfunction (Michaud et al. 2013). Inflammatory status can be evaluated though blood biomarkers. In the literature there is a wide range of inflammatory biomarkers including C-reactive protein (CRP) (Blasko et al. 2007; Jordanova et al. 2007; Holmes et al. 2009; Marioni et al. 2009; Economos et al. 2013; Laird et al. 2014; Lima et al. 2014), interleukin (IL)-6 (Jordanova et al. 2007; Codoñer-Franch et al. 2012; Economos et al. 2013; Laird et al. 2014; Sharma et al. 2016; Chi et al. 2017; Meghil et al. 2019), tumour necrosis factor (TNF)-alpha (Jordanova et al. 2007; Codoñer-Franch et al. 2012; Economos et al. 2013; Laird et al. 2014; Sharma et al. 2016; Chi et al. 2017; Meghil et al. 2019), IL-2 (Sharma et al. 2016; Chi et al. 2017; Meghil et al. 2019), IL-10...
(Laird et al. 2014; Sharma et al. 2016; Chi et al. 2017; Meghil et al. 2019), fibrinogen (Marioni et al. 2009), serum amyloid A & P (Jordanova et al. 2007; Sharma et al. 2016; Chi et al. 2017), pentraxin-3 (PTX-3), and receptor for advanced glycation end products (RAGE) (Sharma et al. 2016; Chi et al. 2017). The three most frequently used inflammatory markers in studies of cognitive health in ageing are CRP, IL-6, and TNF-α, with varying levels of association found, as discussed later.

10.7. Vitamin D, Inflammation and immunity

The relationship between vitamin D and inflammation is reported as being through the immunomodulating effects of 1,25(OH)₂D, and exerts its affect through the vitamin D receptors (VDR), a member of the superfamily of nuclear receptors (Okereke and Singh 2016). It was the discovery that the VDR is found throughout the human body that generated the interest to elucidate the pleiotropic nature of vitamin D (Deluca and Cantorna 2001; Mathieu and Adorini 2002). In terms of immune function, the VDR has been found to be expressed in circulating macrophages, dendritic cells, activated T-lymphocyte cells, monocytes, and can express influence over both adaptive and innate immunity through cytokine secretion and via its effects on toll-like receptor (Guillot et al. 2010; Zhang et al. 2012; Cannell et al. 2014; Laird et al. 2014). In addition, the VDR can be found in most organs of the body including the brain, heart, gonads, prostate, breasts, gut, and skin (Marino and Misra 2019). Furthermore, the gene encoded for 1α-hydroxylase D (CYP27B1) has being identified in immune cells and various organs, allowing for local 1,25(OH)₂D activation (Adams and Hewison 2008). It has also being evidenced in previous studies that vitamin D can increase secretion of anti-inflammatory cytokine IL-10 while also causing a decrease in pro-inflammatory cytokines such as TNF-α.
α and IL-6 (Deluca and Cantorna 2001). Vitamin D has previously been linked to various inflammatory conditions such as rheumatoid arthritis (Song et al. 2012; Lee and Bae 2016; Wang 2016), dermatomyositis (Smith et al. 1986; Disphanurat et al. 2019), inflammatory bowel disease (Cantorna et al. 2018; Janssen et al. 2019), hepatitis (Suneetha et al. 2006), asthma and respiratory infections (Hall and Agrawal 2017; Jolliffe et al. 2017; Reinehr et al. 2018), as well as some auto-immune diseases, for instance multiple sclerosis (Van Der Mei et al. 2003; Islam et al. 2007; Dalmay et al. 2010), type-1 diabetes (Zipitis et al. 2016), systemic lupus erythematosus and dermatomyositis (Amital et al. 2010; Robinson et al. 2012). Although for each condition listed above there are studies that have observed conflicting results and conclude a null effect of vitamin D on outcome measures (reviewed by Marino and Misra 2019). For example, an RCT of 45 patients from Thailand who suffer with mild psoriasis were supplemented with either 20,000IU vitamin D2 or placebo twice monthly for 6 months (Disphanurat et al. 2019). Although there was a significant increase in serum 25(OH)D in the supplementation group, there was no significant between group differences for C-reactive protein (CRP) (Disphanurat et al. 2019). Perhaps the use of further inflammatory markers such as cytokines secreted from T-helper cells (Th-cells) might produce different results. Furthermore, vitamin D has been suggested as a selective immunosuppressant by modulating the immune response from a proinflammatory (Th-1 cell mediated) to an anti-inflammatory (Th-2 cell mediated) response (Deluca and Cantorna 2001; Correale et al. 2009; Laird et al. 2014). Similarly, an RCT of 35 obese adolescent patients (mean age 14.1yrs) who received 4000IU D3/day for 6 months did not differ significantly from the placebo group for inflammatory status when tested for serum IL-6, TNF-α, or CRP (Belenchia et al. 2013). Conversely, supplementation of 580 healthy Iranian adolescent girls with a high dose (50,000IU D3/wk) vitamin D did significantly improve
inflammatory status when high sensitivity CRP (hs-CRP) was examined (Tabatabaeizadeh et al. 2017). Although confliction exists in the literature, there is evidence to suggest that vitamin D may have beneficial effect on the immune system and inflammation. However, such benefits may affected by age, dose response, and disease state. Therefore, further research is required utilising a wide demographic spread, at various stages of disease progression, and diverse dosage of vitamin D to further the knowledge of immune responses to vitamin D status.

10.8. Inflammation and cognition

As with many chronic diseases of ageing, inflammation has being implicated in the pathogenesis of cognitive impairment and dementia (Cunningham and Hennessy 2015; McGeer et al. 2017). However, such studies have reported conflicting results. This may be resulting from varied methodological approaches such as sample size, apolipoprotein E genotype (APOE 4) data availability, or use of global function or domain specific cognitive function for analysis. An important discovery by Roger et al (1992) found that an accumulation of amyloid-β (Aβ) in the brain can stimulate activation of the complement immune system, which in term promotes opsonization (Rogers et al. 1992). Through the release of membrane attack complex (MAC), host cells are at risk of attack through bystander lysis (McGeer et al. 2017). For this reason, Aβ has being the focus of much drug trials in the development of anti-neurodegenerative medication, albeit without much success (Cummings et al. 2014). A longitudinal study carried out on 2422 participants over 20 years concluded that exposure to an elevated or high inflammatory state during midlife significantly increased the likelihood of cognitive impairment in later life (Wichmann et al. 2014). Although findings were inconsistent for CRP, while higher
IL-6 was associated with increased likelihood of cognitive impairment (Wichmann et al. 2014). Furthermore, medication use has been associated with cognitive function. For example, drugs used to combat inflammatory disease that has being linked to reduced risk of dementia. Those who suffer from rheumatoid arthritis (RA) regularly consume anti-inflammatory agents, and this group have being observed having much less incident dementia than the normal population (McGeer et al. 2011). Likewise, other studies have noticed a sparing effect ranging from 36% - 80% of NSAID users of more than 24 months (Stewart et al. 1997; In’t Veld et al. 2001; Zandi et al. 2002; Yip et al. 2005; Szekely et al. 2008). However, in 2014 Lima and colleagues challenged previous findings and found that elevated plasma CRP levels was associated with slower decline in global cognitive function in the very old (>75yrs) (Lima et al. 2014). However, this protective effect was only observed in those who were not carriers of the APOE e4 allele. In agreement, the epidemiology of hearing loss study found that high CRP levels collected 10 years apart had a lower risk of developing cognitive impairment than those who had low CRP at both time points (Wichmann et al. 2014). However, it was concluded that inconsistent CRP finding might be due to statin use, hardy survivor effect, or harmful effects of chronically low CRP levels (Wichmann et al. 2014). The same study however, in line with what is previously reported in the literature, found that repeatedly high levels of IL-6 was associated with greater risk of cognitive impairment (Wichmann et al. 2014).

In terms of brain volumes, the ARIC study (ongoing) analysed inflammation and brain volume over a 24 years period on 1633 middle-aged (45-65 years at baseline) adults found that mid-life inflammation (fibrinogen, albumin, von Willebrand factor and FVIII, and WBC) was indicative of reduced hippocampal (p=0.013) and occipital lobe (p=0.009) volume and increased ventricular volume (p=0.013) (Walker et al. 2017). However, the ARIC study did not see a significant reduction in total brain volume like other studies.
such as the Framingham offspring study, where IL-6 was associated with reduced total brain volume (Jefferson et al. 2007). However, similar to previous finding there was no significant association between CRP and any marker of cognition (Jefferson et al. 2007). A strength of both studies are that they both recruited participants at mid-life and who are generally healthy, limiting the chances of observations resulting from reverse causation.

10.9. Vitamin D, Inflammation and cognition

As highlighted previously, vitamin D is associated with favourable inflammatory outcomes, and inflammation has being associated with the development of neurological disorders. Therefore, conclusions have being made to suggest vitamin D exerts its neuroprotective effects through inflammatory mechanisms. However, no study has examined the effects of vitamin D on cognitive decline over time independent of inflammation. For example, a study of 16 male Sprague Dawley adult rats with hepatosteatosis, those fed 0.3µg/kg/day vitamin D3 exhibit reduced inflammation and improved cognition when compared to rats no few vitamin D3 (Erbaş et al. 2014). However, no significance was observed for inflammation or cognition when compared to rats without hepatosteatosis. Indicating that the VDR becomes more active in the presence of inflammation. Furthermore, using a two-way analysis of variance, no independent effect of vitamin D on cognition was established. Similarly, male aged F344 rats who were supplemented with 43IU/kg/day 1,25(OH)2D3 for 3-weeks experienced less cognitive decline and less inflammation compared to their non-supplemented counterparts (Briones and Darwish 2012). However, unlike in animal studies, human studies have the advantage of domain specific cognitive function tools, and to this authors knowledge no analysis has being done to identify cognitive function domain specific
effects of vitamin D independent of inflammation. The answer to this question will establish whether or not vitamin D acts on neurological function through mechanisms other than that of the immune system and inflammation.

10.10. Conclusion

In conclusion, there seems to be moderate evidence to suggest a link between vitamin D and cognition, and strong evidence of a link between inflammation and cognitive health. However, until a vitamin D normative data range has being developed for cognitive health, such research will continue to use normative data for the maintenance of bone health, which may not be appropriate. In terms of inflammation, further longitudinal studies are required to further elucidate how and when inflammation affects cognitive health. Dementia has a long preclinical phase where evidence of onset may be evident at midlife regardless of patients presenting asymptomatic. Therefore, further well designed clinical trials and prospective studies are warranted, utilising healthy participants from an early age with regular follow-ups into old age to fully elucidate how and to what extent vitamin D and inflammation can effect cognitive decline. Furthermore, studies of ageing are all categorising participants according to chronological age. However, recent evidence suggest that individuals of the same chronological ages exhibit different trajectories in terms of age-related decline, whereas biological age may be a better indicator of ageing (Jylhävä et al. 2017; Khan et al. 2017). However, to date there does not exist a validated method to determine biological age, but as reviewed by Jylhävä and colleagues, there are few promising developments with the strongest method being the epigenetic clock (Jylhävä et al. 2017).
Table 1. Randomised Controlled Trials of Vitamin D and Cognition

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Country</th>
<th>Cohort</th>
<th>Age (Yrs)</th>
<th>Length</th>
<th>Cognitive outcome</th>
<th>Intervention</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorde et al. 2019</td>
<td>Norway</td>
<td>422 healthy participants</td>
<td>51.8</td>
<td>4 months</td>
<td>Verbal recall, The Digit Symbol-Coding Test, tapping test</td>
<td>100,000IU once + 20,000IU D3/wk, or placebo</td>
<td>No effect</td>
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<tr>
<td>Pettersen 2017</td>
<td>Canada</td>
<td>82 healthy adults 25(OH)D ≤100nmol/L</td>
<td>56.7</td>
<td>18 weeks</td>
<td>SDMT, VF, DS-F/B, CANTAB® battery</td>
<td>4000IU vit D3/day, or 400IU vit D3/day</td>
<td>Positive effect, high dose only</td>
<td></td>
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<tr>
<td>Rossom et al. 2013</td>
<td>USA</td>
<td>4143 healthy community dwelling females</td>
<td>≥65</td>
<td>7.8 years</td>
<td>Incidence probable dementia, Mild cognitive impairment</td>
<td>Calcium 1000mg + 400IU Vit D3/day, or placebo</td>
<td>No effect</td>
<td>Low vit D dose, own supplement usage, female only</td>
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<tr>
<td>Stein et al. 2011</td>
<td>Australia</td>
<td>32 community dwelling mild/moderate AD (MMSE 12-24)</td>
<td>≥60</td>
<td>16 weeks</td>
<td>MMSE, ADAS-cog, WMS-R LM</td>
<td>1000IU vit D2 throughout, after 8 wks either additional: 1. 6000IU daily + nasal insulin 2. Placebo + nasal insulin</td>
<td>No effect</td>
<td>Vit D2 supplements used</td>
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<tr>
<td>Dean et al. 2011</td>
<td>Queensland</td>
<td>128 healthy adults</td>
<td>18-24</td>
<td>6 weeks</td>
<td>Working memory – N back, Response inhibition - Stop signal task, Cognitive flexibility</td>
<td>5000IU D3/day</td>
<td>No effect</td>
<td>Young adult cohort, 25(OH)D high at baseline</td>
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<tr>
<td>ADAS-cog</td>
<td>Alzheimer Disease Assessment Scale</td>
<td></td>
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<tr>
<td>FAB</td>
<td>Frontal Assessment Battery</td>
<td></td>
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<tr>
<td>DS-F/B</td>
<td>Digital Span Forward/Backwards</td>
<td></td>
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<tr>
<td>AD</td>
<td>Alzheimer Disease</td>
<td></td>
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<tr>
<td>VF</td>
<td>Phonemic fluency</td>
<td></td>
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</table>
Table 2. Cross-sectional studies of vitamin D and cognition

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Country (countries)</th>
<th>No. &amp; age</th>
<th>Outcome measures</th>
<th>25(OH)D Cut-offs</th>
<th>Association</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahn and Kang 2015</td>
<td>Korea</td>
<td>467 74.4yrs</td>
<td>MMSE, TUG, GDS</td>
<td>Not listed</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Min Lee et al. 2019</td>
<td>Korea</td>
<td>345</td>
<td>GOS-E, MMSE, CDR</td>
<td>&lt;75nmol/L, 75-125nmol/L, &gt;125nmol/L</td>
<td>Yes</td>
<td>Retrospective study</td>
</tr>
<tr>
<td>Llewellyn et al. 2011</td>
<td>USA (NHANES III)</td>
<td>3325 &gt;65yrs</td>
<td>MMSE, EBMT, WAIS</td>
<td>&lt;25, 25-&lt;50, 50-&lt;75, ≥75 nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Slinin et al. 2012</td>
<td>USA</td>
<td>6257 &gt;64</td>
<td>3MSE, Trails B</td>
<td>&lt;25, 25-49, 50-74, ≥75nmol/L</td>
<td>Yes</td>
<td>Women only</td>
</tr>
<tr>
<td>Palacios et al. 2019</td>
<td>USA</td>
<td>967 45-75yrs</td>
<td>MMSE, 16-word list learning test, Stroop test, clock drawing test, figure copy test, DS-F, DS-B, verbal fluency</td>
<td>&lt;20nmol/L, 20-30nmol/L, &amp; &gt;30nmol/L</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Przybelski and Binkley 2007</td>
<td>USA</td>
<td>32 &gt;60yrs</td>
<td>MMSE, 25(OH)D, vitamin B12</td>
<td>Vitamin D used as continuous variable</td>
<td>Yes</td>
<td>No adjustment for confounders</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Age Range</td>
<td>Cognitive Tests &amp; Assessment Tools</td>
<td>Homocysteine Level</td>
<td>Outcome</td>
<td></td>
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</tr>
<tr>
<td>Pettersen 2016</td>
<td>Canada</td>
<td>142-56yrs</td>
<td>VF, DS-F, DS-B, CANTAB SWM &amp; VRM-R and OTS</td>
<td>&lt;50, 50-74, 75-99, ≥100 nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Hooshmand et al. 2014</td>
<td>Sweden</td>
<td>75-61.6yrs</td>
<td>MMSE, CSF Aβ, total tau, brain tissue volume</td>
<td>&lt;25, 25-&lt;50, ≥50 nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Annweiler et al. 2011</td>
<td>France</td>
<td>7421-&gt;75yrs</td>
<td>FTSS, SPMSQ</td>
<td>Vit D deficiency &lt;25nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Sakuma et al. 2019</td>
<td>Japan</td>
<td>740-68.1yrs</td>
<td>MMSE-J</td>
<td>≤50nmol/L, 50-75nmol/L, ≥75nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Van Schoor et al. 2016</td>
<td>Netherlands</td>
<td>1320-≥55yrs</td>
<td>MMSE, RCPM, CT, 15WT</td>
<td>&lt;30, 30-49, 50-74, ≥75 nmol/L</td>
<td>Yes</td>
<td></td>
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</table>

### Table 3. Longitudinal studies of vitamin D and cognition

<table>
<thead>
<tr>
<th>Author &amp; Year</th>
<th>Country</th>
<th>No. &amp; age</th>
<th>Follow-up Length (yrs)</th>
<th>Outcome measures</th>
<th>25(OH)D Cut-offs</th>
<th>Association</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson et al. 2014</td>
<td>USA</td>
<td>2234 73.6yrs</td>
<td>4</td>
<td>3MSE, DSST</td>
<td>&lt;50, 50-75, &amp; &gt;75nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Littlejohns et al. 2014</td>
<td>USA</td>
<td>1658 5.6</td>
<td></td>
<td>Incident all cause dementia and AD</td>
<td>&lt;25, 25-&lt;50 ≥50 nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Miller et al. 2015</td>
<td>USA</td>
<td>382 4.8</td>
<td></td>
<td>NAS</td>
<td>&lt;30, 30-49 50-124, ≥125nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Slinin et al. 2010</td>
<td>USA</td>
<td>1604 74.6yrs at follow-up</td>
<td>4.6</td>
<td>3MSE and Trails B</td>
<td>&lt;50, 50-63, &gt;63-74, ≥75nmol/L</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Slinin et al. 2012</td>
<td>USA</td>
<td>6257 &gt;64yrs</td>
<td>4</td>
<td>MMSE, Trails B</td>
<td>&lt;25, 25-&lt;50, 50-&lt;75, ≥75nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Palacios et al. 2019</td>
<td>USA</td>
<td>967 45-75yrs</td>
<td>2</td>
<td>MMSE, 16-word list learning, Stroop test, Clock drawing, Figure copy, DS-F, DS-B, VF</td>
<td>&lt;20, 20-30, &amp; &gt;30nmol/L</td>
<td>No</td>
<td>Short follow-up period</td>
</tr>
<tr>
<td>Panwar et al. 2016</td>
<td>USA</td>
<td>474 ≥45yrs</td>
<td>3.5</td>
<td>6-item screener, VF, word list learning, &amp; list recall</td>
<td>&lt;50, 50-74, ≥75nmol/L</td>
<td>Yes</td>
<td>Race specific association</td>
</tr>
<tr>
<td>Toffanello et al. 2014</td>
<td>Italy</td>
<td>1927 74.1yrs</td>
<td>4.4</td>
<td>MMSE</td>
<td>&lt;50, 50-75, ≥75nmol/L</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Cohort</td>
<td>Sample Size</td>
<td>Age at follow-up</td>
<td>Outcome Measures</td>
<td>Cut-offs</td>
<td>Results</td>
</tr>
<tr>
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</tr>
<tr>
<td>Llewellyn et al. 2010</td>
<td>Italy</td>
<td>inCHIANTI study</td>
<td>1154</td>
<td>74yrs at follow-up</td>
<td>MMSE, Trails A &amp; B</td>
<td>&lt;25, 25–&lt;50, 50–&lt;75, ≥75 nmol/L</td>
<td>Yes</td>
</tr>
<tr>
<td>Van Schoor et al. 2016</td>
<td>Netherlands</td>
<td>LASA cohort</td>
<td>1320</td>
<td>79.7yrs at follow-up</td>
<td>MMSE, RCPM, CT, 15-Words Test</td>
<td>&lt;30, 30–&lt;50, 50–&lt;75, ≥75 nmol/L</td>
<td>No</td>
</tr>
<tr>
<td>Perna et al. 2014</td>
<td>Netherlands</td>
<td>ESTHER cohort</td>
<td>527</td>
<td>&gt;70yrs</td>
<td>COGTEL</td>
<td>&lt;30, 30–&lt;50, ≥50 nmol/L</td>
<td>Yes</td>
</tr>
<tr>
<td>Matchar et al. 2016</td>
<td>China</td>
<td></td>
<td>1202</td>
<td>80.3yrs</td>
<td>MMSE-C</td>
<td>25(OH)D used as continuous variable</td>
<td>Yes</td>
</tr>
<tr>
<td>Breitling et al. 2012</td>
<td>Germany</td>
<td>ESTHER cohort</td>
<td>1639</td>
<td>&gt;65yrs</td>
<td>COGTEL</td>
<td>Sex specific quintiles used</td>
<td>Yes</td>
</tr>
<tr>
<td>Olsson et al. 2017</td>
<td>Sweden</td>
<td></td>
<td>1182</td>
<td></td>
<td>MMSE</td>
<td>≤50, &gt;50–75, &amp; ≥75 nmol/L</td>
<td>No</td>
</tr>
<tr>
<td>Assmann et al. 2015</td>
<td>France</td>
<td></td>
<td>1009</td>
<td>&gt;45yrs at baseline</td>
<td>VF, RI-48 cued recall test, DS-F, DS-B, Delis–Kaplan Trail Making Test</td>
<td>&lt;25, 25–49, 50–74, ≥75nmol/L</td>
<td>Yes</td>
</tr>
</tbody>
</table>

MMSE – Mini Mental State Examination, MMSE-C – Mini Mental State Examination Chinese version, VF – Verbal Fluency, DS-F – Digital Span Forwards, DS-B – Digital Span Backwards, 3MSE – Modified Mini Mental State Examination, RI-48 cued recall test - rappel indice-48 items cued recall test, RCPM - Raven’s Colored Progressive Matrices, NAS - Neuropsychological Assessment Scales, CT – Coding Task
11. Chapter 2: TUDA Study

Introduction
11.1. TUDA background

This prospective cohort study utilises the large genotype/phenotype database from the Trinity, Ulster, Department of Agriculture (TUDA) ageing cohort study. The TUDA study was designed to investigate nutritional factors, gene-nutrient interactions and a range of health and lifestyle factors related to the pathogenesis of certain chronic diseases of ageing in community-dwelling adults. The TUDA study (Figure 6) was originally undertaken as a cross-border collaboration (the National Nutrition Phenotype Database Project), comprising of the collaborative efforts of; Trinity college Dublin, Ulster University and the Department of Agriculture, Food and the Marine. The TUDA study is observational by design and includes community-dwelling participants recruited from both Northern Ireland and the Republic of Ireland who exhibit phenotypic evidence of early stages of Alzheimer disease, osteoporosis, or CVD. Ethic approval was sought from the relevant authorities and approval was granted in the Republic of Ireland for the recruitment of a cognitively impaired sub-cohort (n=2000) and an osteoporotic sub-cohort (n=2000) at St. James hospital, Dublin by the Research Ethics Committee of St. James’s Hospital and the Adelaide and Meath Hospital, Dublin. While in Northern Ireland approval was granted for the recruitment of a sub-cohort of hypertensive patients (n=2000) who were under the care of consultant cardiologists at two separate trusts in Northern Ireland (Altnagelvin and Causeway Hospital’s in Northern Ireland) by the Office for Research Ethics Committees Northern Ireland (ORECNI; Ref 08/NI/RO3113). All participants provided written informed consent at the time of enrolment with all blood samples and questionnaire data coded and made anonymous prior to analysis and relevant GDPR guidelines were adhered to.
Once ethical approval was granted, patients under the care of consultant geriatricians at St. James Hospital Dublin were approached. Those patients who met the initial criteria were subsequently contacted by a member of the TUDA research team for further details regarding the study, and an official invitation to participate, once screening for all inclusion/exclusion criteria was met. The inclusion criteria were: patients must be >60 years of age and attend (or have attended) either the memory or bone clinic and have been diagnosed with mild-moderate cognitive dysfunction or osteopenia. The exclusion criteria were anything contrary to the aforementioned inclusion criteria, and the participant (or their parent) not born in either Northern or Republic of Ireland and those with severe dementia. Once suitability was established and patients verbally agreed to participate, each participant was given a study information sheet and consent form (Appendix). Following a minimum consideration period of 48 hour, participants were obliged to sign a written informed consent form prior to data collection. Appointments were made for each participant for which the relevant data and biological samples could be collected, these include as follows:

- Detailed health, medical, and lifestyle questionnaire (Appendix) was used in order to obtain information on diet, general health, drug and supplement use.
- Assessment of mental state and self-maintenance using Center for Epidemiology Studies Depression scale (CES-D), Hospital Anxiety and Depression Scale (HADS), Physical Self-Maintenance Scale (PSMS) & Instrumental Activates of Daily Living (IADL) (Appendix)
- Complete physiological and cognitive function assessment, including blood pressure, mobility/frailty, Dual X-ray absorptiometry (DXA), battery of cognitive function tests (MMSE, FAB, RBANS) (Appendix).
• Anthropometric measurements including height, weight, waist: hip ratio, and body mass index (BMI) was taken.

• Biological samples, non-fasting blood was taken (50ml approx.)

Each appointment was carried out in its entirety by a single TUDA researcher, who had previously being trained to perform all takes required (eg. phlebotomy, cognitive assessments, anthropometry). Clinical blood samples were analysed at participating hospital laboratories Trinity college Dublin, or by Ulster University. Each participant’s blood was analysed for the following parameters:

• Routine clinical bloods including renal function, liver function, lipids, full blood count, glucose, glycosylated haemoglobin, and were 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 Ulster university.

• Plasma homocysteine (immunoassay), serum folate and red cell folate (microbiological assay) at Ulster University.

• Bone biomarkers including serum 25-hydroxyvitamin D (ELISA) and serum intact parathyroid hormone (PTH; ECIA) at St. James hospital laboratory.

Blood samples are stored at both Trinity College Dublin and the University of Ulster. Recruitment of baseline commenced in December 2008 and was complete in September 2012 with 5186 participants processed.
11.2. **The TUDA 5+ follow-up study:**

TUDA 5+ is a follow-up study (Figure 7) of the original TUDA cohort, five plus years after the initial investigation. As previously described (Section 11.1), all of the previous parameters from the original study were reassessed in order to investigate the progression of risk factors and the potential to identify optimal nutritional status to prevent such progression. Of the original cohort a random sample of 1000 (Ulster n=600; Trinity n=400) participant were invited back to the follow-up study to form a sub-cohort for longitudinal analysis. All participants were held to the same inclusion/ exclusion criteria as outlined in the original study. To that end, 1000 suitable community-dwelling individuals aged >60 years at baseline were recruited, using standardised protocols, from outpatient services at the department of medicine for the elderly, St James hospital and from General Practitioner practices in the Western and Northern Health and Social Care Trusts, Northern Ireland. Participants were sent, via postal mail, an invite letter (Appendix) and a study information letter (Appendix) and a follow-up phone call was made in the weeks following to identify interested individuals. Once interest and suitability was established, an appointment was made for each participant to attend
The Trinity, Ulster, Department of Agriculture (TUDA) Study

\[ n \ 5,186 \]

- Bone Cohort\(^1\)
  \[ n \ 1,394^a \]
  (Dublin)

- Cognitive Cohort\(^2\)
  \[ n \ 1,699^a \]
  (Dublin)

- Hypertensive Cohort\(^3\)
  \[ n \ 2,093^a \]
  (Northern Ireland)

The Trinity, Ulster, Department of Agriculture (TUDA 5+) Follow-up Study

\[ n \ 987 \]

- Bone Cohort\(^1\)
  \[ n \ 366^{a,b} \]
  (Dublin)

- Cognitive Cohort\(^2\)
  \[ n \ 34^{a,b} \]
  (Dublin)

- Hypertensive Cohort\(^3\)
  \[ n \ 587^a \]
  \[ n \ 587 \text{ missing } 25(\text{OH})D \text{ follow-up & baseline inflammatory data} \]
  (Northern Ireland)

Figure 7. TUDA & TUDA 5+ study population. 1. Recruitment of the Bone cohort was from specialist bone health service at St. James’ Hospital, Dublin. 2. Recruitment of the cognitive cohort was at St. James Hospital, Dublin. 3. Recruitment of the hypertensive cohort was from GP clinics in Northern Ireland. \(^a\) Represents participants eligible to participate in study 2, Chapter 4 of this Thesis. \(^b\) Represents participants eligible to participate in study 1, Chapter 3 of this Thesis.
St James hospital for processing. Participants were assessed for all outcomes measured in the original TUDA study including cognitive assessment, anthropometric data, biological sample collection, metabolic health markers, and comprehensive health and lifestyle questionnaire. Unfortunately due to funding constraints it was not possible to repeat DXA scans at follow-up. The author of this thesis was part of the research team that processed all 400 participants from the Trinity cohort, and contributed specifically to all aspects of data collection and collection/processing of biological samples. Biochemistry analysis’ were carried out by the wider research team, as listed in the appropriate section of text, at various sites within the criteria outlined by the Human Tissue Act (HTA, 2004).

11.3. Blood processing

Prior to blood draw, aliquots of 1% solution of ascorbic acid (450 µl; 2 per participant) was placed on a roller to defrost and cover with foil as ascorbic acid is photosensitive. Virakon solution was made up and left ready at the workstation. Cryovials (22 per participant) were placed, pre-labelled with tubee labels in racks. Labels contain details regarding sample type, study identifier, unique participant identifier and volume of sample, no personal identifiers where placed on biological samples. Non-fasted blood was drawn from the antecubital vein for each participant during their visit. Subsequently, serum (8ml) samples were let sit for a minimum of 30 minutes at room temperature to ensure complete separation. Two EDTA (9ml each) samples were drawn from each participant, one was kept at room temperature (RT) and the other kept refrigerated (RS). The RT sample was placed on a roller for at least 10 minutes prior to aliquoting. Lithium heparin samples were left to sit at room temperature until all samples were prepared for
centrifuge. Fifty microliters of RT plasma was aliquot into each of the 450µl 1% ascorbic acid, capped and inverted to ensure adequate mixture. All samples were then placed in a centrifuge and spin for 15 minutes at 3000rpm. When centrifuge run was complete all samples were removed back to the workstation. Plasma from lithium heparin tubes were separated into two 1.5ml tubes and capped. Serum samples were separated into three tubes in 0.5ml, 1.0ml and 0.25ml aliquots respectively. The remainder was divided between two 1.5ml tubes, and capped. Plasma RT samples were separated equally between three 1.5ml cryovials, and buffy coat removed to another 1.5ml cryovial. Plasma RS samples were separated into two 1.5ml cryovials in aliquots of 0.5ml and 0.75ml, while the remainder was transferred into two 1.5ml tubes using a pastuer pipette and capped. Plasma RS buffy coat was removed as above. Red blood cells from plasma RT and RS were washed using PBS and spun initially at 3000rpm for 15 minutes before two runs at 2000rpm for 10 minutes each. PBS was refreshed after each centrifugal spin. Washed red blood cells were subsequently removed to two 1.5ml cryovials (per sample) and capped. All samples were capped with colour coded cryovial screw caps and placed in freezer sample boxes (Starstedt) prior to deep freeze at -80°C until analysis. All expired evacuated blood tubes were disposed of into a sharps box for incineration. Bloods for clinical assessment were drawn into one 4ml serum, one 4ml fluoride, and two 4ml EDTA evacuated tubes and analysed at St James hospital laboratory.

11.4. 25(OH)D analysis

Vitamin D was analysed in the same fashion as at baseline. Non-fasting blood samples were collected from the antecubital vein into the evacuated clotting tubes (Starstedt) as previously described (section 10.3). Following blood processing, serum aliquots were
labelled and stored at -80°C in Trinity Translational Medical Institute (TTMI), St. James Hospital prior to batch analysis at the Biochemistry Department of St. James Hospital, Dublin, Ireland. Stored samples were analysed for total serum 25(OH)D (D2 & D3) using fully validated methods (Chromsystems Instruments and Chemicals GmbH; MassChrom 25-OH-Vitamin D3/D2) employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) (API 4000; AB SCIEX). The quality and accuracy of methods used were continually monitored by internal quality controls and through participation in the vitamin D external quality assessment scheme and use of the national institute of standards and technology 972 vitamin D standard reference material. Inter and intra-assay coefficient of variance was 5.7% & 4.5% respectively.

11.5. Biomarkers of inflammation

Pro-inflammatory cytokines IL-6, TNF-α, inflammatory marker CRP and anti-inflammatory cytokine IL-10 were processed in an identical fashion as at baseline in all 1000 follow-up plasma samples. All cytokines were batch analyses and quantified using high-sensitivity ELISA kits (R&D Systems) and a Triturus ELISA analyser (Grifols) (Laird et al. 2014). For the inflammatory marker CRP, analysis was performed using high-sensitivity ELISA CRP kits (Roche Diagnostics, UK). Non-fasted blood samples were drawn from the antecubital vein into lithium heparin evacuated tubes (Starstedt) by a trained phlebotomist and handled as previously described (section 10.3) prior to analysis. Samples were delivered to Ulster University, Coleraine by a member of the research team or courier for analysis by an Ulster researcher. Samples were defrosted at room temperature prior to 25μl of plasma samples being set for a 2-hour incubation with vigorous shaking (900 rpm) at room temperature. The samples subsequently
removed from the plate and the wells washed with PBS (0.05% Tween-20) and repeated thrice. Following the wash protocol, 25 µl of detection antibody was added to each well and subsequently incubated for 2 hours with vigorous shaking (900 rpm) at room temperature. The detection antibody was emptied from the wells are then washed with PBS (0.05% Tween-20) and repeated three times. Prior to reading, 150 µl of read buffer was added to each well and the plate was immediately read using the Triturus ELISA analyser (Grifols). Each plasma sample was measured in duplicate, the quality and accuracy of the method was continuously monitored using internal quality controls. All kits were stored at 4°C, and all reagents prepared according to the manufacturer’s instructions. The immune profile of Th-1/Th-2 for all samples was calculated in addition to cytokine levels alone by dividing the pro-inflammatory cytokine/CRP by the anti-inflammatory cytokine IL-10. This ratio has being previously described as being more informative regarding inflammatory status than using absolute cytokine or inflammatory markers in isolation (Dodoo et al. 2002).

11.6. Cognitive assessment

Cognitive health was comprehensively assessed in the TUDA cohort study. There was three cognitive assessments utilized in the battery, including the Mini-Mental State Examination (MMSE), the Repeated Battery for the Assessment of Neuropsychological Status (RBANS), and the Frontal Assessment Battery (FAB). The MMSE is the most frequently used tool worldwide to assess global cognitive function and examines orientation, registration, attention and concentration, and recall and language (Prince et al. 2016). The maximum score in the MMSE is 30, while scores below 24 are indicative of cognitive dysfunction, and a score of less than 20 would indicate dementia. The FAB
is a tool that specifically assesses frontal lobe function, with a focus on mental flexibility, conceptualisation, interference sensitivity, programming, inhibitory control and environmental autonomy (Dubois et al. 2000). This assessment has 6 sections, a maximum attainable score of 18 points, with cognitive dysfunction indicated at scores of ≤15 points. The RBANS is the most comprehensive of all the cognitive assessment tools used. It is an age-adjusted tool that can assess specific cognitive domains, including immediate and delayed memory, visual-spatial, language, and attention, with scores <80 points indicating cognitive impairment (Ntholang et al. 2018). Depression and anxiety was also assessed using the Centre of Epidemiological Studies Depression (CES-D) Scale and the Hospital Anxiety and Depression Scale (HADS) as these are potential confounders of cognitive performance. HADS assesses anxiety with a maximum score of 21, with a score ≥11 indicating the presence of probable anxiety. CES-D is a screening tool, with a maximum attainable score of 60, a score 16 is the clinical cut-off for depression.

11.7. Statistical analysis

All data was double entered by different members of the TUDA research team and cross checked for consistency purposes and error identification. Once a final database had being developed in each respective recruitment district, all data was subsequently amalgamated into a complete database shared between centres.

Statistical analysis was completed by this author using Statistical Package for Social Sciences version 26.0 (IBM, SPSS UK Ltd). Normality was checked across variables required for analysis by employing the Kolmogorov-Smirnov test and Q-Q plots and non-normally distributed data was log-transformed or square-rooted where appropriate.
Expression of normally distributed data will take the form of mean and standard deviations (SD), with non-normal data expressed as median (25th and 75th interquartile range). Where appropriate, ANOVA was applied to normally distributed and transformed data with post hoc as required, or chi-squared to assess differences between sexes and 25(OH)D tertiles. Partial correlation was used to identify variables associated with cognitive decline prior to multinomial regression model to determine the predictors of cognitive impairment. All observed $P$ values were significant and values $\leq 0.05$. 
12.1. Introduction

In the past decade there has been a resurgence of research into the biological effects of vitamin D (Van Der Schaft et al. 2013). Although it has been long accepted that vitamin D is an essential nutrient in terms of bone health (Hathcock et al. 2007; Pérez-López et al. 2011), it seems that calcium and phosphorus homeostasis is only what is presently known about the biological roles of vitamin D, while in fact it is now thought to serve a much broader range of roles within the body (Annweiler 2017).

Much recent research (as previously discussed) has endeavoured to elucidate the extent of the pleiotropic nature of vitamin D (Lai and Fang 2013; Anandabaskar et al. 2018; Sassi et al. 2018), and a protective role in age-related cognitive decline has being highlighted as one of the potential biological roles of vitamin D (Annweiler 2017). There is good biological plausibility to this hypothesis, for instance, 25(OH)D is known to pass the blood-brain barrier (BBB) and the VDR is found in multiple areas of the brain, including the frontal, parietal and temporal lobes, and hippocampus (Guo et al. 2016). Furthermore, 25(OH)D-1α-hydroxylase (CYP27B1) is present locally in many cells of the brain which facilitates local 1,25 dihydroxyvitamin D production (Grimm et al. 2017).

However, it is not yet clear as to if and how vitamin D expresses its effect on cognition, and whether it has a primary role in protecting against cognitive decline or whether it mediates an effect through secondary mechanisms.

It is thought that vitamin D exerts its cognitive protective effects through immune modulation and anti-inflammatory pathways, and modulation of vascular risk factors (Aspell et al. 2018). Moreover, it has being particularly linked to the amelioration of amyloid Beta aggregation and Tau pathology (Grimm et al. 2017).

Much research has being performed to establish a link between vitamin D and cognition, with most cross-sectional studies finding associations with global or domain specific
cognition (Buell et al. 2009; Ahn and Kang 2015; Van Schoor et al. 2016). Much inconsistency exists in longitudinal studies, but it is suggested that vitamin D deficiency is a risk factor for cognitive decline, albeit at a higher cut-off than that used for the assessment of skeletal health (<50nmol/L).

Therefore the purpose of this study is to assess whether serum 25(OH)D concentrations are associated with cognitive impairment in a community-dwelling elderly (≥60yrs) Irish cohort, and whether that effect is expressed independently of inflammation.

12.2. Study population

This study comprised of participants from the TUDA 5+ follow-up study which has previously being described (Section 11.2). This is a large collaborative study, cross-sectional and longitudinal by design. Participants for the follow-up study were recruited from the original cohort of 1,394, 1699, 2093 (n=5186) from the bone, cognitive and hypertensive cohorts, respectively. Four hundred participants were followed-up from the bone and cognitive cohort, with 600 being followed-up from the hypertensive cohort. At the time of writing, vitamin D analysis had only being performed on the 400 samples obtained at TCD, while of the 400 participants complete study data was available for 383 participants.

12.3. Methodology

Data was collected as previously described (chapter 2) for cognition using a neuropsychological battery consisting of the MMSE for global cognition (Folstein et al.
1975), FAB for assessment of executive function (Folstein et al. 1975), and the RBANS to assess five cognitive domains: immediate memory, visuospatial, language, attention and delayed memory, which can be scored separately or combined to give an overall index score.

Inflammatory status assessed through the analysis of blood samples for IL-6, IL10, TNFα and CRP. The immune profiles of TH1/TH2 were computed to create new variables by dividing the proinflammatory cytokine (IL-6) and CRP by the anti-inflammatory cytokine IL-10, and categorised as anti-inflammatory (<1:1), neutral (1:1 – 2:1), and proinflammatory (>2:1).

Vitamin D was assessed from non-fasted serum samples as previously described (chapter 2) using the gold-standard method of Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS).

12.4. **Statistical analysis**

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 26 (SPSS UK Ltd). Data was assessed for normality using Kolmogorov-Smirnov test and transformed as appropriate using log-transformation or square root. Data are expressed as mean and standard deviation (SD), or median (25th and 75th interquartile ranges) for non-normal data. Where appropriate ANOVA was used to determine statistical differences (P<0.05) for normal and non-normal data with Dunnett post-hoc to determine between group differences while categorical data was assessed using a chi-square analysis. Data was further explored using a Partial correlation analysis adjusting for age, gender, BMI, smoking status, and the presence of inflammatory conditions to determine significant interaction between markers/ratios of inflammation and 25(OH)D.
Multinomial logistic regression was then performed to determine the predictors of cognitive decline using age, gender, BMI, smoking status, education, and inflammatory markers included in the model. All P values <0.05 observed were considered statistically significant.

12.5. Results

Characteristics of 987 participants (325 male and 662 female) from TUDA 5+ follow-up study population are presented in Table 5. Males had a significantly higher BMI (p<0.0001) and waist: hip ratio (p<0.0001), while the prevalence of any inflammatory condition (rheumatoid arthritis, ulcerative colitis, coeliac disease) was significantly higher in females (p=0.006), coeliac disease was where the greatest difference was seen (p=0.019). Females had statistically significantly higher 25(OH)D levels of 84.33 (P25-75; 81.5-87) compared to men 69.89 (P25-75; 62.5-77.2) (p<0.0001), attributed to the higher uptake of vitamin D supplementation and/or medication within the female population (p<0.0001). No other between sex differences were observed except for the inflammatory cytokine IL-6 were men had significantly higher levels at 1.85pg/ml compared to 1.38pg/ml (p=0.028).

Complete data was available for only 383 participants at the time of writing. Results were stratified by 25(OH)D status (Table 6) and analysed core variables and variables known to effect vitamin D and/or inflammation. There were more females at follow-up than male (p<0.0001), but no difference in educational level across 25(OH)D status. BMI was significantly higher in those who were 25(OH)D deficient compared to the sufficient group (p<0.0001) and similarly for waist to hip ratio those who were deficient were significantly larger than the sufficient group (p<0.0001).
Participants who took metformin or proton pump inhibitors had statistically significantly lower 25(OH)D levels than those who did not (p<0.0001), but those taking statin did not differ significantly (p=0.078). No significant difference was observed for any inflammatory marker or cytokine ratio with the exception of IL-6 where those who were 25(OH)D deficient had significantly higher levels of plasma IL-6 than those who were categorised as 25(OH)D sufficient (p<0.0001).

Table 7 presents results of the partial correlation analysis where relationships between 25(OH)D, inflammatory markers, cytokines and ratios, and cognitive impairment (as assessed by MMSE, FAB, RBANS [total & index scores]) were explored while controlling for age, gender, years of education, BMI, presence of Inflammatory condition, and smoking status. 25(OH)D as a continuous variable was significantly negatively correlated with the inflammatory cytokine IL-6 (P<0.001), the proinflammatory marker CRP (P<0.005), but not for any of the inflammatory ratios IL-6:IL-10 (P=0.694), CRP:IL-10 (P=0.120), or TNFα:IL-10 (P=0.673).

Partial correlation also eluted to significant correlations between 25(OH)D and MMSE score (dichotomised; <24 =impaired) with a positive correlation (P<0.05), and for RBANS index IV score (P<0.05). Some of the inflammatory markers also negatively correlate with cognition, IL-10 correlates significantly with RBANS index III and index V, and total scale(P<0.05). When considering 25(OH)D status (deficient, insufficient, sufficient) as a categorical variable rather than continuous, partial correlation analysis reveals positive correlation between 25(OH)D and RBANS index I score and total scale (P<0.001 for both) and positive correlation between 25(OH)D status and RBANS index II score (P<0.05).

Following partial correlation a multinomial regression analysis was performed using dichotomised cognitive assessment scores as the dependant variable and 25(OH)D as the
predictor. However, due to low numbers of participants (n=1) who were 25(OH)D sufficient and cognitively impaired there was not enough power to proceed with the regression analysis. Crosstabulation of 25(OH)D status versus cognitive impairment by MMSE are present in Table 4.

Table 4. Crosstabulation of cognitive impairment (MMSE) versus 25(OH)D status

<table>
<thead>
<tr>
<th>Vitamin D status (&lt;50, 50-75, &gt;75nmol/L)</th>
<th>Cognitively impaired (MMSE)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Deficient</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Insufficient</td>
<td>115</td>
<td>8</td>
</tr>
<tr>
<td>Sufficient</td>
<td>221</td>
<td>1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>372 (97.12)</td>
<td>11 (2.87)</td>
</tr>
</tbody>
</table>

12.6. Discussion

It is promising that the results of the partial correlation suggests some association between vitamin D and cognitive impairment assessed by MMSE, RBANS index scores I, II, and IV and RBANS total scale. This suggests that however vitamin D exerts its influence on cognition may be domain specific. These finding could not be explored further due to lack of power but the association between visuospatial domains and 25(OH)D has being observed in other cross-sectional studies (Lee et al. 2009).

Importantly, this study shows that vitamin D status could be a possible determinant of cognitive decline, with those who were 25(OH)D deficient more likely to have an MMSE score of less than 24 compared to those who were 25(OH)D sufficient, even after adjusting for covariates known to affect cognition. These finding suggest that vitamin D
may have the potential to protect against cognitive impairment within the older adult population. However, it is important to note that within this cohort nearly 60% of participants were vitamin D sufficient and less than 10% in the deficient category (Table 4). This is most likely as a result of participants being recruited from a bone clinic where nearly 50% are taking either medication or supplementing with vitamin D. Furthermore, at the time of writing only 40% of the 25(OH)D analysis had being completed, which might serve to increase the power of the study so that interactions can be analysed through regression analysis.

This study is in agreement with many other cross sectional studies that examine the relationship between vitamin D and cognition. Ahn et al (2015) found that vitamin D was a significant predictor of global cognitive performance as assessed by MMSE-J in an elderly Korean cohort after controlling for age, sex, education, GDS and body fatness (partial $R^2 = 0.210$, $p = 0.012$). However, unlike the present study, they did not have any objective measures of systemic inflammation, therefore could not control for inflammation mediated effects. Likewise, Buell et al (2009) analysed results from an ethnically diverse American cohort of 65 – 99 year old adults, and found that vitamin D was associated with both executive function and attention/processing speed, but not for memory.

The present study had a relatively small sample size ($n=383$) and therefore analysis on associations of vitamin D on cognition stratified by gender could not be done. However, previous large cross sectional studies such as a study by Slinin et al (2012) analysed 6,257 older women (>64yrs) concluded that women with very low vitamin D had increased odds of global impairment (OD 1.60, CI 1.05 – 2.42). Further illustrating the association between vitamin D and cognition is a study by Petterson et al (2016) where supratherapeutic (>100nmol/L) vitamin D was associated with significantly better
performance on verbal fluency for 142 Canadians (average age 56.3yrs). This author could not find any studies where analysis had being done on a male cohort only.

Partial regression shows no association between 25(OH)D and inflammation, with the exception of IL-6 (P<0.001) and CRP (P<0.05). However neither of these shows any correlation with cognition. However, the anti-inflammatory cytokine IL-10 was significantly negatively correlated with RBANS index III, Index V, and total scale (P<0.05 for each), suggesting that perhaps 25(OH)D and inflammation effects on cognition are domain specific. Further analysis would need to be performed to test this hypothesis.

12.7. Conclusion

The primary aim of this study was to assess whether serum 25(OH)D concentrations and/or status was associated with cognition. Analysis did highlight positive partial correlations between 25(OH)D and cognitive impairment for global function as assessed by MMSE and domain specific cognitive function as assessed by RBANS.

The second aim of this study could not be answered due to lack of sufficient power to proceed further with statistical analysis. However, negative partial correlation was observed between inflammatory cytokine IL-10 and RBANS index III, index V, and total scale. This suggests that inflammation also correlates with cognitive function and therefore the need to control for inflammation when assessing for independence of effects of 25(OH)D on cognition.

Although this study was under powered and as a result further exploration of such correlations between vitamin D, inflammation and cognitive function could not proceed, it is still in agreement with the existing literature. That is 25(OH)D is associated cognition
in older adults, but the question still remains whether vitamin D status is causative or as a result of cognitive changes.
### Table 5. Characteristics of TUDA 5+ follow-up participants (n=987)

| Characteristic                        | Total sample n=987 | Male n=325 | Female n=662 | P Value
|---------------------------------------|--------------------|------------|--------------|---------|
| Age (Yrs)$^2$                         | 75.8 (75.5 - 76.1) | 76.1 (75.6 - 76.6) | 75.7 (75.3 - 76.1) | 0.231
| BMI (kg/m$^2$)$^2$                    | 28.1 (27.7 - 28.4) | 29.0 (28.5 - 29.5) | 27.6 (27.2 - 28.0) | <0.0001
| Waist: Hip ratio$^2$                  | 0.92 (0.92 - 0.93) | 0.97 (0.97 - 0.98) | 0.90 (0.89 - 0.90) | <0.0001
| % Smokers (n)                         | 5.7 (57)           | 5.2 (17)    | 6.0 (40)    | 0.608
| **Inflammatory conditions**           |                    |            |              |         |
| % Autoimmune disease (n)              | 21.3 (211)         | 16.3 (53)   | 23.8 (158)  | 0.006
| % Rheumatoid arthritis (n)            | 17.4 (171)         | 19.4 (93)   | 16.4 (108)  | 0.243
| % Ulcerative colitis (n)              | 1.7 (17)           | 1.8 (6)     | 1.6 (11)    | 0.834
| % Coeliac disease (n)                 | 2.6 (26)           | 0.92 (3)    | 3.4 (23)    | 0.019
| **Vitamin D concentration**           |                    |            |              |         |
| 25(OH)D, nmol/L (n=383)               | 82.44 (79.8 - 85.0)| 69.89 (62.5 - 77.2)| 84.33 (81.5 - 87.0)| <0.0001
| % Vitamin D supplements/medication (n)| 48.3 (477)         | 28.6 (93)   | 58.0 (384)  | <0.0001
| **Inflammatory markers**              |                    |            |              |         |
| TNF-α pg/ml                           | 3.73 (3.2 - 4.2)   | 3.34 (3.0 - 3.6) | 3.9 (3.1 - 4.6) | 0.29
| IL-6, pg/ml                           | 1.53 (1.3 - 1.7)   | 1.85 (1.3 - 2.3) | 1.38 (1.2 - 1.5) | 0.028
| CRP, mg/L                             | 7.35 (6.2 - 8.5)   | 7.25 (5.4 - 9.0) | 7.39 (5.9 - 8.8) | 0.908
| IL-10, pg/ml                          | 0.85 (0.4 - 1.2)   | 0.72 (0.2 - 1.2) | 0.92 (0.3 - 1.5) | 0.673

$^1$ Between sex differences were assessed using ANOVA for continuous variables or chi-square analysis for categorical data

$^2$ Values displayed are median (25, 75th percentile) or n(%)
Table 6. Demographic characteristics of the TUDA 5+ follow-up cohort by 25(OH)D profiles (n=383)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (25(OH)D &lt;50nmol/L)</th>
<th>Group 2 (25(OH)D 50-75nmol/L)</th>
<th>Group 3 (25(OH)D &gt;75nmol/L)</th>
<th>P for comparison between all 3 groups&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants (%)</td>
<td>38 (9.9)</td>
<td>123 (32.1)</td>
<td>222 (57.9)</td>
<td></td>
</tr>
<tr>
<td>Age (Yrs)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>73.51 (72.1 - 74.9)</td>
<td>74.51 (73.6 - 75.3)</td>
<td>73.46 (72.9 - 74.0)</td>
<td>0.087</td>
</tr>
<tr>
<td>Female n(%)</td>
<td>27 (71)</td>
<td>102 (82.9)</td>
<td>204 (91.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.014</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Education (Yrs)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>16.7 (15.4 - 17.9)</td>
<td>17.3 (16.6 - 18.1)</td>
<td>17.7 (17.3 - 18.2)</td>
<td>0.234</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>28.99 (26.8 - 31.1)</td>
<td>26.90 (26.0 - 27.7)</td>
<td>25.43 (24.8 - 26.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist: Hip ratio (cm)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.95 (0.92 - 0.98)</td>
<td>0.90 (0.89 - 0.92)</td>
<td>0.88 (0.87 - 0.89)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25(OH)D, nmol/L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>37.57 (33.74 - 41.39)</td>
<td>64.97 (63.62 - 66.32)</td>
<td>99.81 (97.52 - 102.09)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke or TIA n(%)</td>
<td>6 (15.7)</td>
<td>8 (6.5)</td>
<td>19 (8.5)</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>0.169</td>
<td>0.497</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI diseases n(%)</td>
<td>7 (18.4)</td>
<td>33 (26.8)</td>
<td>70 (31.5)</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td>0.107</td>
<td>.0361</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lifestyle n(%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>6 (15.7)</td>
<td>8 (6.5)</td>
<td>14 (6.3)</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>0.054</td>
<td>0.862</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current alcohol use</td>
<td>29 (76.3)</td>
<td>92 (74.7)</td>
<td>165 (74.3)</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>0.733</td>
<td>0.655</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>44.59 (42.79)</td>
<td>43.15 (149.45)</td>
<td>64.14 (59.82)</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>0.443</td>
<td>0.107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Reference
<table>
<thead>
<tr>
<th>Medicine</th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>Reference P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statins</td>
<td>24 (63.1)</td>
<td>60 (48.7)</td>
<td>97 (43.6)</td>
<td>0.078</td>
</tr>
<tr>
<td>Metformin</td>
<td>6 (15.7)</td>
<td>5 (4.06)</td>
<td>4 (1.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>23 (60.5)</td>
<td>43 (34.9)</td>
<td>62 (27.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D med/Supp</td>
<td>13 (34.2)</td>
<td>81 (65.8)</td>
<td>195 (87.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>5.01 (3.87)</td>
<td>3.92 (3.75)</td>
<td>4.85 (15.76)</td>
<td>0.777</td>
</tr>
<tr>
<td><strong>IL-6, pg/ml</strong></td>
<td>3.08 (4.48)</td>
<td>1.56 (1.83)</td>
<td>1.22 (1.88)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>IL-10, pg/ml</strong></td>
<td>0.43 (0.53)</td>
<td>0.53 (1.3)</td>
<td>0.62 (2.3)</td>
<td>0.836</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>14.47 (26.96)</td>
<td>7.99 (17.62)</td>
<td>7.82 (21.38)</td>
<td>0.191</td>
</tr>
<tr>
<td><strong>Cytokine ratios</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α/IL-10</td>
<td>17.17 (10.73)</td>
<td>14.20 (10.17)</td>
<td>16.57 (24.70)</td>
<td>0.531</td>
</tr>
<tr>
<td>IL-6/IL-10</td>
<td>8.83 (9.73)</td>
<td>6.22 (10.70)</td>
<td>7.91 (56.27)</td>
<td>0.923</td>
</tr>
<tr>
<td>CRP/IL-10</td>
<td>50.93 (105.96)</td>
<td>36.46 (96.90)</td>
<td>38.73 (128.77)</td>
<td>0.803</td>
</tr>
</tbody>
</table>
Differences between all 3 groups were assessed using ANOVA for continuous variables or chi-square analysis for categorical data.

Values displayed are median (25, 75th percentile) or n(%).

Groups 1 & 2 were compared to group 3 (reference) and differences were assessed using ANOVA followed by a Dunnett test, or logistic regression for categorical variables.
Table 7. Partial Correlations of Vitamin D, Inflammation and Immunity

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>Vitamin D statusb</th>
<th>CRP</th>
<th>IL-10</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>CRP:IL10 Ratio</th>
<th>IL6:IL10 Ratio</th>
<th>TNFa:IL10 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D statusb</td>
<td>0.41**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>-0.147*</td>
<td>-0.020</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>-0.001</td>
<td>-0.032</td>
<td>0.004</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.169**</td>
<td>0.007</td>
<td>0.145**</td>
<td>-0.002</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.019</td>
<td>0.068*</td>
<td>0.077*</td>
<td>0.019</td>
<td>0.073*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP:IL10</td>
<td>-0.084</td>
<td>-0.017</td>
<td>0.696**</td>
<td>-0.042</td>
<td>0.013</td>
<td>-0.027</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6:IL10 Ratio</td>
<td>-0.023</td>
<td>-0.013</td>
<td>0.026</td>
<td>-0.022</td>
<td>0.387**</td>
<td>-0.019</td>
<td>0.075*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>TNFa:IL10 Ratio</td>
<td>-0.025</td>
<td>0.049</td>
<td>0.045</td>
<td>-0.087*</td>
<td>0.008</td>
<td>0.656**</td>
<td>0.183**</td>
<td>0.317**</td>
<td>1.000</td>
</tr>
<tr>
<td>MMSE</td>
<td>0.072*</td>
<td>0.012</td>
<td>0.012</td>
<td>0.011</td>
<td>-0.062</td>
<td>-0.040</td>
<td>0.025</td>
<td>-0.034</td>
<td>-0.028</td>
</tr>
<tr>
<td>FAB</td>
<td>0.084</td>
<td>0.051</td>
<td>-0.033</td>
<td>0.002</td>
<td>-0.029</td>
<td>-0.038</td>
<td>0.029</td>
<td>0.032</td>
<td>0.022</td>
</tr>
<tr>
<td>RBANS index score I</td>
<td>0.080</td>
<td>0.091**</td>
<td>-0.017</td>
<td>-0.049</td>
<td>-0.045</td>
<td>0.003</td>
<td>0.004</td>
<td>0.025</td>
<td>0.026</td>
</tr>
<tr>
<td>RBANS index score II</td>
<td>0.082</td>
<td>0.07*</td>
<td>-0.039</td>
<td>0.002</td>
<td>-0.059</td>
<td>-0.058</td>
<td>0.026</td>
<td>0.016</td>
<td>-0.011</td>
</tr>
<tr>
<td>RBANS index score III</td>
<td>-0.069</td>
<td>0.043</td>
<td>-0.044</td>
<td>-0.085*</td>
<td>-0.023</td>
<td>-0.002</td>
<td>-0.027</td>
<td>0.024</td>
<td>0.043</td>
</tr>
<tr>
<td>RBANS index score IV</td>
<td>0.105*</td>
<td>0.033</td>
<td>-0.049</td>
<td>0.024</td>
<td>-0.058</td>
<td>-0.055</td>
<td>-0.015</td>
<td>-0.001</td>
<td>-0.012</td>
</tr>
<tr>
<td>RBANS index score V</td>
<td>0.037</td>
<td>0.056</td>
<td>0.011</td>
<td>-0.09*</td>
<td>-0.025</td>
<td>-0.014</td>
<td>0.037</td>
<td>0.019</td>
<td>0.024</td>
</tr>
<tr>
<td>RBANS total score</td>
<td>0.077</td>
<td>0.086**</td>
<td>-0.036</td>
<td>-0.05*</td>
<td>-0.063</td>
<td>-0.036</td>
<td>0.009</td>
<td>0.021</td>
<td>0.014</td>
</tr>
</tbody>
</table>

*aAdjusted for age, gender, years of education, BMI, presence of Inflammatory condition, & smoking.

*bVitamin D status: deficient = <50 nmol/L, insufficient = 50 – 75 nmol/L, sufficient = >75 nmol/L

*P<0.05

**P<0.001
13. Chapter 4: Does 25(OH)D predict cognitive decline in the elderly?
13.1. Introduction

There are many factors associated with cognitive impairment, and it is known that cognitive decline and neurodegenerative conditions including Alzheimer’s and dementia have a long preclinical phase. This means that pathological markers of such conditions could possibly be identified at mid-life (Assmann et al. 2015; Walker et al. 2017).

Cognitive dysfunction is known to be multifactorial and can be seen directly, using techniques such as Magnetic Resonance Imaging (MRI), in changes such as reduced brain volume in both white and grey matter, aggregation of amyloid plaque, and Tau pathology leading to neurofibrillary tangles (Cunningham and Hennessy 2015; Moore et al. 2018) and indirectly using cognitive assessments. Many other factors are thought to play a role in cognitive decline over time, such as vitamin D or inflammation, however proving a factor plays a causal role is not so straightforward. Furthermore, cross-sectional studies can only make association between variables and outcomes measured, they cannot prove causality. For example, in the case of cognitive decline, cross-sectional studies cannot argue that vitamin D plays a causal factor in brain changes as it is equally as possible that lifestyle changes as a result of cognitive dysfunction effects vitamin D levels. However, longitudinal study can help to bridge that gap by observing changes in lifestyle and markers of health and cognitive change over time. Such studies will serve to identify factors that do play a causal role and to eliminate those that are caused by cognitive impairment. In addition, longitudinal studies can provide key knowledge to better inform and design RCT’s to further test such observation. Therefore the purpose of this study is to determine if vitamin D can predict cognitive decline in the TUDA 5+ cohort of older Irish adults.
13.2. Study population

This study was made up of the participants from the original TUDA study and of those who were followed-up 5+ years later. As previously described, the TUDA study is a large observational study of elderly Irish adults aged ≥60 years at baseline and therefore ≥65 years at follow-up. The follow-up study commenced recruitment of the Northern Irish cohort in 2016 with completion in 2018, while recruitment of the Irish cohort commenced in March 2018, with completion in December of the same year. Exclusion/inclusion criteria was consistent with the original study as previously outlined (section 10.1) and written informed consent was gained prior to enrolment onto the study. The study was conducted in accordance to the guidelines laid down in the declaration of Helsinki, and ethical approval was granted from the relevant authorities from respective cohorts (previously described). All biological samples and data relating to the questionnaire was coded and anonymised prior to analysis and all relevant GDPR guidelines were adhered to.

13.3. Methodology

Data collection and methods used have being extensively described previously (Chapter 2: TUDA Study Introduction). Briefly, a questionnaire was developed for the purpose of the TUDA study and included questions relating to health, lifestyle, medical status, family medical history, self-maintenance, depression & anxiety, mobility and physical stature were also tested using standard methods. Cognitive assessments were also given, and biological samples were obtained and treated as previously described. Neurophysiological exams consisted of the Mini Mental State Examination (MMSE), Frontal Assessment Battery (FAB), and the Repeated Battery for the Assessment of Neuropsychological Status RBANS. Where available, alternative versions of each exam
was used at follow-up to reduce the risk of bias by residual memory. All exams were carried out by trained professionals. Biological samples collected were collected in the form of blood by trained phlebotomists and treated as previously described. Samples were then batch analysed for 25-hydroxyvitamin D [25(OH)D] (D₂ & D₃) at the Biochemistry Department of St. James Hospital, Dublin, Ireland, using fully validated methods (Chromsystems Instruments and Chemicals GmbH; MassChrom 25-OH-Vitamin D3/D2) employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) (API 4000; AB SCIEX).

13.4. Statistical analysis

The statistical analysis was performed by this author using the Statistical Package for Social Sciences Version 26.0 (SPSS, IBM UK Ltd.). All data was assessed for normality by means of the Kolmogorov-Smirnov test and Q-Q plots. Where skewness occurred, data was log transformed as required for normalisation purposes. Expression of normal data are means and SDs, or with median (25th – 75th interquartile ranges) for non-normal data. Study characteristics were assessed using ANOVA with post hoc test for continuous variable of chi-squared test for categorical data. Partial correlation adjusted for age, sex, years of education BMI, and smoking status was applied to ascertain statistically significant relationships between baseline 25(OH)D and follow-up measures of cognition (MMSE, FAB, RBANS index I-V, RBANS total scale). A multinomial logistic regression model was used to analyse the predictive value of vitamin D for future cognition, adjusting for previously identified covariates and baseline cognition. All observations were considered significant at P values ≤.05).
13.5. Results

Four thousand two hundred and one subjects were excluded from the analysis as there was missing or incomplete follow-up data for cognition. Therefore, 985 were included with complete data for baseline 25(OH)D, and cognitive assessments at follow-up as well as relevant covariates. Full cohort characteristics at baseline are shown in Table 8. Significant differences were observed between 25(OH)D status for gender ($P < .0001$), education ($P < .0001$), BMI ($P < .0001$), for those whom have had a stroke or Transient Ischaemic Attack (TIA) ($P < .0001$), for those who had gastrointestinal (GI) disease ($P = .048$), smokers ($P < .0001$), alcohol users ($P < .0001$), those who exercise regularly ($P < .0001$), or are taking any medications such as statins ($P < .0001$), or proton pump inhibitors (PPI) ($P < .0001$), and for those taking vitamin D medication or supplements ($P < .0001$), when compared against sufficient status ($>75 \text{nmol/L}$) as a reference.

Partial correlation analysis showed a significant positive correlation between baseline 25(OH)D and follow-up RBANS total scale and Index I score ($P < .05$). While baseline vitamin D status correlates with follow-up RBANS index I and index II score, and RBANS total scale ($P < .05$ for all). No other significant correlations were observed between 25(OH)D and other follow-up measures of cognition (Table 9).

These results were used to inform the multinomial logistic regression analysis to assess whether baseline serum vitamin D levels can predict cognitive outcomes 5+ years later. Analysis was performed for cognitive measure highlighted from partial correlation, controlling for commonly cited covariate with the addition of baseline cognitive scores. Goodness of fit was non-significant (NS) when running analysis for RBANS index I ($\chi^2 = 101.05$; df=179; $P = 0.505$), RBANS index II ($\chi^2 = 191.58$; df=185; $P = 0.355$), and for
Baseline 25(OH)D status versus cognition at follow-up is graphically represented in Figure 8. The results were similar across all cognitive measures, those who were 25(OH)D sufficient had a higher percentage of normal cognition, and as 25(OH)D status decreased to deficient status there was a higher percentage of cognitively impairment. When using sufficient status as a reference group we find significant difference in all but one (RBANS index III; \( P=0.342 \)) cognitive outcomes.

13.6. Discussion

In this large cohort study of older Irish adults, baseline vitamin D status was correlated with follow-up cognitive measures after adjustment for covariates. Specifically, we observed a positive association between vitamin D status and two index scores of the RBANS, index I and index II scores which represent immediate memory and visuospatial/constructional memory respectively. However these findings did not hold in the multinomial logistic regression model to assess predictability of baseline vitamin D to future cognitive decline.

The findings of this study are in agreement with other longitudinal studies. For example a study by Olsson et al. (2017) found that vitamin D status (sufficient \( \geq 75 \text{nmol/L} \)) did not predict incident dementia in 1182 Swedish male participants over and 18 year period. Likewise, another study that assessed cognition with a broad range of measures also concluded no relationship between vitamin D and incident cognitive impairment (Van Schoor et al. 2016). Both of these studies included populations similar to that described in the current study, namely an elderly cohort (>60yrs) and independent living individuals. However, the largest longitudinal study this author has retrieved did associate
vitamin D levels at baseline with higher risk of global cognitive decline 4-years later (Slinin et al. 2012). This study involved 6,257 community dwelling adults and were reassessed after 4 years. However, this population consisted of only elderly (mean age 76.6yrs) women and could not be generalised to the other populations. Furthermore, direct measures of inflammation was not adjusted for in the statistical model, while inflammation in a known contributory factor in cognitive dysfunction (Walker et al. 2017; Ntlholang et al. 2018). RCT’s have also being used to investigate the effects of vitamin D on cognition, but only one out of five conducted has found significant effects (Pettersen 2017). The largest of the RCT studies conducted on 4143 elderly (≥65yrs) women concluded that vitamin D (400IU vitamin D₃/day) had no effect on cognition (Rossom et al. 2013). However, all participants were allowed to continue with their habitual supplementation use, which may include vitamin D, although baseline dietary total (dietary plus supplement) did not significantly differ between groups (Rossom et al. 2013). These RCT’s seem to agree with the current study in that vitamin D may not significantly influence cognition, or cognitive dysfunction.

This study would suggest that vitamin D status is correlated with cognition. Although the aim of the study was to test if vitamin D status could predict cognitive decline, it was not possible to further the analysis as the model did not hold. These results should be interpreted with caution, as it may have being effected by selection bias. All participants included in the analysis were recruited from a bone clinic where they were either medicated or supplementing vitamin D. Therefore, it is only possible to draw conclusions in respect of this particular group and should not be generalised to the rest of the population. Furthermore, drawing on these results to inform clinical practice or public policy may not be appropriate. It is not possible to say that vitamin D fortification is not required in Ireland as this study consisted of a clinical population and does not reflect the
widespread issue of vitamin D deficiency in Ireland. Furthermore, with what is known about the long preclinical phase of neuropsychological condition, perhaps a single 5-year follow-up may be insufficient to capture such effect.

13.7. Conclusion

These findings in relation to the protective role of vitamin D in cognitive function with ageing suggests that vitamin D may play a part in cognitive maintenance in late-life. However, our findings in relation to vitamin D as playing a causative role in cognitive decline with ageing are not supported in the current study.

Having said that, it is possible that vitamin D exerts a greater effect on cognitive function early in life, and would be in support of the long preclinical phase of Alzheimer’s and dementia. However, that would require robust and standardised assessment throughout the lifecycle and is not within the scope of the current work.
Table 8. Demographic characteristics of the TUDA baseline cohort by 25(OH)D profiles (n=5186)

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (25(OH)D &lt;50nmol/L)</th>
<th>Group 2 (25(OH)D 50-75nmol/L)</th>
<th>Group 3 (25(OH)D &gt;75nmol/L)</th>
<th>P for comparison between all 3 groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>2326</td>
<td>1355</td>
<td>1505</td>
<td></td>
</tr>
<tr>
<td>Age (Yrs)²</td>
<td>74.0 (73.6 - 74.3)</td>
<td>73.5 (73.1 - 74.0)</td>
<td>74.4 (74.0 - 74.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>Female n(%)</td>
<td>1441 (61.9)</td>
<td>864 (63.7)</td>
<td>1182 (78.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Education (Yrs)²</td>
<td>15.7 (15.6 - 15.8)</td>
<td>16.1 (15.9 - 16.3)</td>
<td>16.4 (16.2 - 16.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 (28.6 - 29.1)</td>
<td>27.8 (17.5 - 28.1)</td>
<td>26.3 (26.0 - 26.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke or TIA n(%)</td>
<td>394 (17.0)</td>
<td>183 (13.5)</td>
<td>188 (12.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gl diseases n(%)</td>
<td>467 (20.0)</td>
<td>235 (17.3)</td>
<td>313 (20.7)</td>
<td>0.048</td>
</tr>
<tr>
<td>Lifestyle n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>335 (14.4)</td>
<td>138 (10.1)</td>
<td>150 (9.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current alcohol use</td>
<td>1257 (54.0)</td>
<td>792 (58.4)</td>
<td>926 (61.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Exercise</td>
<td>1646 (70.7)</td>
<td>1091 (80.5)</td>
<td>1226 (81.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Medication n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>N (n%)</td>
<td>P</td>
<td>N (n%)</td>
<td>P</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------</td>
<td>-----</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>Statins</td>
<td>696 (51.3)</td>
<td>0.247</td>
<td>682 (45.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Metformin</td>
<td>85 (6.2)</td>
<td>0.056</td>
<td>54 (3.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>538 (39.7)</td>
<td>0.093</td>
<td>553 (36.7)</td>
<td>0.265</td>
</tr>
<tr>
<td>Vitamin D med/Supp</td>
<td>757 (58.7)</td>
<td>&lt;0.0001</td>
<td>1193 (84.4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Differences between all 3 groups were assessed using ANOVA for continuous variables or chi-square analysis for categorical data
2 Values displayed are median (25, 75th percentile) or n(%)
3 Groups 1 & 2 were compared to group 3 (reference) and differences were assessed using ANOVA followed by a Dunnett test, or logistic regression for categorical variables
Table 9. Partial correlations of baseline 25(OH)D and cognition at follow-up (n=985) a

<table>
<thead>
<tr>
<th>Control Variables</th>
<th>25(OH)D (nmol/L)</th>
<th>Vitamin D Status b</th>
<th>MMSE</th>
<th>FAB</th>
<th>RBANS Index I</th>
<th>RBANS Index II</th>
<th>RBANS Index III</th>
<th>RBANS Index IV</th>
<th>RBANS Index V</th>
<th>RBANS total scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D Status</td>
<td>0.856</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>0.009</td>
<td>0.003</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAB</td>
<td>0.025</td>
<td>0.043</td>
<td>0.313**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>0.069*</td>
<td>0.075*</td>
<td>0.432**</td>
<td>0.357**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>0.051</td>
<td>0.069*</td>
<td>0.316**</td>
<td>0.320**</td>
<td>0.284**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>0.047</td>
<td>0.055</td>
<td>0.250**</td>
<td>0.300**</td>
<td>0.353**</td>
<td>0.282**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>0.007</td>
<td>0.017</td>
<td>0.386**</td>
<td>0.445**</td>
<td>0.416**</td>
<td>0.381**</td>
<td>0.262**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>0.016</td>
<td>0.056</td>
<td>0.473**</td>
<td>0.370**</td>
<td>0.661**</td>
<td>0.346**</td>
<td>0.393**</td>
<td>0.328**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RBANS total scale</td>
<td>0.056*</td>
<td>0.078*</td>
<td>0.521**</td>
<td>0.492**</td>
<td>0.769**</td>
<td>0.666**</td>
<td>0.575**</td>
<td>0.669**</td>
<td>0.783**</td>
<td>1</td>
</tr>
</tbody>
</table>

*aAdjusted for age, sex, years of education BMI, and smoking status

bVitamin D status: deficient = <50 nmol/L, insufficient = 50 – 75 nmol/L, sufficient = >75 nmol/L

*p<0.05
**MMSE**

- MMSE <24: Deficient
  - P = .021
  - Percentage: 7.5%

- MMSE >24: insufficient
  - P = .159
  - Percentage: 4.8%

- MMSE >24: sufficient
  - P = .973
  - Percentage: 2.7%

**FAB**

- FAB <15: Deficient
  - P < .001
  - Percentage: 10.4%

- FAB >15: insufficient
  - P = .347
  - Percentage: 4.8%

- FAB >15: sufficient
  - P = .961
  - Percentage: 3.9%

**RBANS Index I**

- Index I <80: Deficient
  - P < .001
  - Percentage: 20.1%

- Index I >80: insufficient
  - P = .001
  - Percentage: 15.5%

- Index I >80: sufficient
  - Percentage: 12.4%
Figure 8. 25(OH)D at baseline as a determinant of cognitive impairment at follow-up across all measures of cognition (MMSE, FAB, RBANS Index I–V, & RBANS total scale). P-value assessed by ANOVA with Dunnett post hoc with sufficient as reference value (n=985).
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15. Appendix
Participant Invite Letter

(insert name/ address) (insert date)

Re: Participation in The Trinity, University of Ulster and Department of Agriculture (TUDA) Cohort Phenotype / Genotype database study

Dear (insert name)

We are writing to you because in (2008-12), when you were attending the outpatients’ clinic in the Department of Medicine for the Elderly at St James’s Hospital, you took part in the TUDA study, which was being conducted at the Hospital between 2008 and 2012. The purpose of the TUDA study was to collect dietary and medical information plus blood data from Irish adults over 60 years old, to explore some of the factors contributing to the development of chronic diseases of ageing such as bone disease, impaired cognitive function and hypertension. The Study was sponsored by the Irish Department of Agriculture, Food and the Marine (DAFM) and the data that we collected are helping us to understand associations between diet and health that are likely to be of benefit in the prevention of these common diseases.

We now wish to conduct a follow-up study on a group of the original TUDA participants. If you agree to come back for re-assessment, you will be one of 400 people taking part in this 5-year follow-up study. You may remember that the assessment took about 90 minutes and included a detailed questionnaire of your dietary and medical history, plus some memory and mood tests. We also took a non-fasting blood sample looking at your blood count, kidney function, glucose, cholesterol and vitamin levels. For this follow-up study, the procedure would be the same as before and the assessment will be carried out by an experienced researcher. In the unlikely event that we find anything wrong in any tests we would also contact your General Practitioner and / or arrange further tests ourselves as appropriate.

One of our researchers will ring you in the next few weeks to find out whether you are interested in participating or not. Remember you are free to bring a relative or friend with you on the day of the appointment. Participation is completely voluntary, and we will
continue to look after you in the future regardless of whether you choose to participate or not.

If you would like to know more about the study, please phone one of our study researchers at (insert number).

Signed on behalf of the TUDA team
Patient Information and Consent

Title of study:
The Trinity, University of Ulster and Department of Agriculture (TUDA) Cohort Phenotype / Genotype database – Five-year follow-up study

Introduction:
The Department of Medicine for the Elderly at St. James’s Hospital sees many patients each year who suffer from memory problems or osteoporosis (i.e. brittle bones). To try to more fully understand the link between diet and health in Irish people, we are involved in research programmes that aim to explore the relationship between our diet, our genetic make-up and our risk of developing chronic diseases of ageing, including cognitive decline and bone disease. The purpose of the TUDA study is to collect dietary and medical information and blood data from Irish adults over 60 years old, to explore some of the factors contributing to the development of chronic diseases of ageing such as bone disease, impaired cognitive function and hypertension. The original TUDA study was sponsored by the Department of Food, Agriculture and the Marine (DAFM). The data that we collected will help us to understand associations between diet and health that are likely to be of benefit in the prevention of these common diseases.

We now wish to conduct a follow-up study on a group of the original TUDA participants. This follow-up study will help us to understand how our diet and genetic make-up can influence the rates at which changes occur in our physical and mental health as we age. You are one of the original TUDA participant, which was conducted at the Hospital between 2008 and 2012. If you agree to come back for re-assessment, you will be one of 400 people taking part in this 5-year follow-up study.

Procedures;
You may remember that the assessment took about 90 minutes and included a detailed questionnaire of your dietary and medical history, some memory and mood tests. We also took a blood sample to look at your blood count,
kidney function, glucose, cholesterol and vitamin levels. For this follow-up study, the procedure would be the same as before and the assessment will be carried out by an experienced researcher. We will measure an expanded list of biochemicals in blood and will carry out a more detailed investigation of your diet.

Your participation requires that you are:

(i) Willing to answer some questionnaires about your diet, physical and mental health and use of vitamin supplements
(ii) Willing to have some blood tests
(iii) Willing to undergo some tests of memory and mood

Benefits:
The main benefit of this study will be to help your doctors better understand the association between the link between diet and health in Irish people. By taking part in the study you will also find out how you perform on a battery of physical and psychological tests and get some clinical blood tests. In the unlikely event that we find any abnormal clinical results we will notify your GP and arrange for you to get treatment as appropriate.

Risks:
An experienced phlebotomist will take a blood sample from you. If you tend to faint after a blood test, please tell the person taking the sample and he/she will make sure you are sitting down. The blood test may be a little uncomfortable, and may, in a small number of cases, result in some bruising. The study does not involve taking any medications.

Exclusion from participation:
You cannot be in this study if you suffer from severe cognitive impairment and are unable to fully appreciate the implications of participation.

Confidentiality:
Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the hospital.

**Compensation:**
The medical practitioners involved in this study have current medical malpractice insurance cover. Nothing in this document restricts or curtails your rights.

**Voluntary Participation:**
You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits which you had before entering the study.

**Stopping the study:**
You understand that your doctor may stop your participation in the study at any time without your consent.

**Permission:**
Approval will be sought from the hospital Research Ethics Committee

**Further information:**
You can get more information or answers to your questions about the study, your participation in the study, and your rights, from our researchers who can be telephoned at (insert number). If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.
Title of research study: The Trinity, University of Ulster and Department of Agriculture (TUDA) Cohort Phenotype / Genotype database – Five-year follow-up study

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe that I understand what will happen if I agree to be part of this study.

I have read, or have had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement.

PARTICIPANT’S NAME: ________________________________

PARTICIPANT’S SIGNATURE: __________________________

DATE: __________________________

DATE ON WHICH PARTICIPANT WAS FIRST FURNISHED WITH THIS FORM: _____________________

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:

NAME OF CONSENTOR, PARENT or GUARDIAN: __________________________

SIGNATURE: __________________________

RELATION TO PARTICIPANT: __________________________

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness: -

NAME OF FIRST WITNESS: __________________________

SIGNATURE: __________________________
NAME OF SECOND WITNESS: _________________________

SIGNATURE: _______________________

Statement of investigator’s responsibility:
I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands by explanation and has freely given informed consent.

Consent form for participation in genetic research

Protocol Number: 
To be filled in

Participant Identification Number:
………………………………………

Title of Protocol: The Trinity, University of Ulster and Department of Agriculture (TUDA) Cohort Phenotype / Genotype database -Five Year Follow up study

Name of Institution leading the Research: TUDA Consortium – St James Hospital

Research Director: Dr. Conal Cunningham

Phone Number and Contact Details: 01 4162616  Dr Conal Cunningham, Consultant Geriatrician, Robert Mayne Day Hospital, St. James’s Hospital, Dublin 8

Please initial boxes

1. I have read the attached information sheet on the above project dated……………. and have been given a copy to keep. The information has been fully explained to me and I have had an opportunity to ask questions about the project and understand why the research is being done and any foreseeable risks or consequences involved. I also understand that no guarantee can be given about the possible results.

2. I agree to give a sample(s) of blood / other bodily sample / DNA for research in the above project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason. If I withdraw my consent I understand that my sample will be destroyed
unless I otherwise authorise. I understand that I may ask for my samples to be destroyed and that this will be without my medical treatment or legal rights being affected. I agree that the samples I have given and the information gathered by me can be stored and looked after by the **TUDA investigators** in Trinity College Dublin. I understand that any genetic information obtained will not be made available to me.

3. I give permission for my medical records to be looked at and information taken from them to be analysed in the strictest confidence by the relevant and responsible people from the **TUDA study team** or from organisations supervising the research. I have been told that all medical information / data pertaining to me will be protected by the principles of confidentiality and both national and E U data protection legislation. I have further been told of / shown assurances that this also applies to all medical information / data pertaining to me that are utilised in any non-E U state.

4. I understand that the confidentiality of the sample(s) I donate and information derived therefrom will be protected. I have been told that all medical information / data pertaining to me and derived from the sample(s) will be protected by the principles of confidentiality and both national and E U data protection legislation. I have further been told of / shown assurances that this also applies to all medical information / data pertaining to me and derived from the sample(s) that are utilised in any non-E U state.

**FOR OTHER GENETIC RESEARCH:**

(Note: New research should be submitted for approval by the Research Ethics Committee before proceeding)

5. I understand that future research using the sample I give may include genetic research aimed at understanding the genetic influences in disease but that such tests will not be of predictive / clinical value and that the results of these investigations are unlikely to have any implications for me personally.

6. I understand that I will not benefit financially in any way if this research leads to the development of a new treatment or medical test.
7. I know how to contact the research team if I need to.

Name of participant (BLOCK CAPITALS)  Date
Signature

Name of researcher  Date
Signature

Name of witness  Date  Signature
Repeated Battery for the Assessment of Neuropsychological Status (RBANS)

<table>
<thead>
<tr>
<th>Immediate Memory</th>
<th>Visuospatial/Constructual</th>
<th>Language</th>
<th>Attention</th>
<th>Delayed Memory</th>
<th>TOTAL SCALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Confidence Interval %</td>
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<tr>
<td>Percentile</td>
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</tr>
<tr>
<td>Total Scale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Index Scores:
- 160
- 155
- 150
- 145
- 140
- 135
- 130
- 125
- 120
- 115
- 112
- 110
- 105
- 100
- 95
- 90
- 85
- 80
- 75
- 70
- 65
- 60
- 55
- 50
- 45
- 40

Observations:

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Manuscript not shown due to copyright law
Mini Mental State Examination (MMSE)

MMSE

Mini Mental State Examination

Name: __________________________________________

Date: __________________________________________

ID No.: _________________________________________

MMSE Assessor: _________________________________
MINI-MENTAL STATE EXAMINATION

Name: 
Examiner: 
Date: 

<table>
<thead>
<tr>
<th>Maximum Score</th>
<th>Score</th>
<th>ORIENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>(     )</td>
<td>What is the (year) (season) (date) (day) (month)?</td>
</tr>
<tr>
<td>5</td>
<td>(     )</td>
<td>Where are we: country, county, town, street, room?</td>
</tr>
</tbody>
</table>

REGISTRATION

3 | (     ) | Name 3 objects: (APPLE, TABLE, PENNY) 

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
Frontal Assessment Battery (FAB)

1. Similarities (conceptualization)
   “In what way are they alike?”
   - A banana and an orange

(In the event of total failure: “they are not alike” or partial failure: “both have peel,” help the patient by saying: “both a banana and an orange are fruit”; but credit 0 for the item; do not help the patient for the two 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)

> 9 words: 3  6 -9 words: 2  3 -5 words: 1  < 3 words: 0

3. Motor series “Luria” test (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 “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, 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 ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Tap once when I tap twice."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-1-1-2.

<table>
<thead>
<tr>
<th>Score</th>
<th>No errors: 3</th>
<th>1-2 errors: 2</th>
<th>&gt; 2 errors: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient taps like the examiner at least four consecutive times: 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Go–No Go (inhibitory control)

"Tap once when I tap once."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Do not tap when I tap twice."  
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-1-1-2.

<table>
<thead>
<tr>
<th>Score</th>
<th>No errors: 3</th>
<th>1-2 errors: 2</th>
<th>&gt; 2 errors: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient taps like the examiner at least four consecutive times: 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Prehension behaviour (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 knees. Without saying anything or looking at the patient, the examiner brings his own hands close to the patient’s hands and touches the palms of both the patient’s hands, to see if he will spontaneously take them. If the patient takes the examiner’s hands, try again after asking the patient: “Now, do not take my hands.”

<table>
<thead>
<tr>
<th>Score</th>
<th>Patient does not take the examiner’s hands: 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Patient hesitates and asks what he/she has to do: 2</td>
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<tr>
<td></td>
<td>Patient takes the hands without hesitation: 1</td>
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<tr>
<td></td>
<td>Patient takes the examiner’s hand even after he/she has been told not to do so: 0</td>
</tr>
</tbody>
</table>
TUDA Questionnaire

Date: 
Time: 
Rater: /

ID:

Name:

Address:

Phone:
D.O.B.
G.P.

Time blood collected:

Comments:
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)

Have you had any unintentional weight loss in the last 6 months?

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

Education
### 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):

<p>| | | | | |</p>
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<td>20</td>
<td></td>
<td>2-6</td>
<td>&gt;6</td>
</tr>
</tbody>
</table>

**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/Hypercholesterolaemia**
  - 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

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

If yes for other serious disease, please specify:

Operations:
Family History of cancer:

Family history means occurrence before the age of 60 years in a first degree relative (Mother, father, brother or sister).

Family History of Stroke:

Family history means occurrence before the age of 60 years in a first degree relative (Mother, father, brother or sister).

Family History of Heart disease:

Family history means occurrence before the age of 60 years in a first degree relative (Mother, father, brother or sister).

Family History of Pre Senile Dementia (onset under 65years):

Family history means occurrence before the age of 60 years in a first degree relative (Mother, father, brother or sister).

Family History of Senile Dementia (onset at or after 65years):

Family history means occurrence before the age of 60 years in a first degree relative (Mother, father, brother or sister).
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 ☐)
<table>
<thead>
<tr>
<th>Smoking status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current (i.e. Smoked in last month)</td>
</tr>
<tr>
<td>Past</td>
</tr>
<tr>
<td>Never</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Product 1:</th>
<th>Product 2:</th>
<th>Product 3:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- [ ] 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 _________

- [ ] Twice/day or more
- [ ] Once/day
- [ ] 5-6 times/week
- [ ] 3-4 times/week
- [ ] 1-2 times/week
- [ ] Other _________

If YES, when did you last eat these products?

<table>
<thead>
<tr>
<th>Product 1:</th>
<th>Product 2:</th>
<th>Product 3:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- [ ] 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 _________

- [ ] Twice/day or more
- [ ] Once/day
- [ ] 5-6 times/week
- [ ] 3-4 times/week
- [ ] 1-2 times/week
- [ ] Other _________

**2. Do you take milk?** Yes [ ] No [ ]

If YES:

- As a drink [ ]
- In tea/coffee [ ]
- With cereal [ ]

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 _________

- [ ] Twice/day or more
- [ ] Once/day
- [ ] 5-6 times/week
- [ ] 3-4 times/week
- [ ] 1-2 times/week
- [ ] Other _________
If YES: Name the brand of milk you typically take ________________

Do you typically take -

<table>
<thead>
<tr>
<th>Whole</th>
<th>Low-fat</th>
<th>Skimmed/ Slimline</th>
<th>Soya</th>
<th>Other</th>
</tr>
</thead>
</table>

If YES, when did you last take milk? ________________

3. Do you eat –

**Meat?** Yes □ No □

If YES, how often?

| □ Twice/day or more | □ Twice/day or more |
| □ Once/day | □ Once/day |
| □ 5-6 times/week | □ 5-6 times/week |
| □ 3-4 times/week | □ 3-4 times/week |
| □ 1-2 times/week | □ 1-2 times/week |
| □ Other _________ | □ Other _________ |

**Poultry?** Yes □ No □

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 | □ Twice/day or more |
| □ Once/day | □ Once/day |
| □ 5-6 times/week | □ 5-6 times/week |
| □ 3-4 times/week | □ 3-4 times/week |
| □ 1-2 times/week | □ 1-2 times/week |
| □ Other _________ | □ 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 (CES-D)

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 PAST WEEK, by ticking the most appropriate box.

<table>
<thead>
<tr>
<th></th>
<th>0 Never or Rarely (less than 1 day)</th>
<th>1 Some of the time (1-2 days)</th>
<th>2 Occasionally (3-4 days)</th>
<th>3 Most of the time (5-7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I was bothered by things that usually don’t bother me</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I did not feel like eating; my appetite was poor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt that I could not shake off the blues even with help from my family or friends</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt that I was just as good as other people</td>
<td>3 Never or Rarely (less than 1 day)</td>
<td>2 Some of the time (1-2 days)</td>
<td>1 Occasionally (3-4 days)</td>
<td>0 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I had trouble keeping my mind on what I was doing</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I felt depressed</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I felt that everything I did was an effort</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I felt hopeful about the future</td>
<td>3 Never or Rarely (less than 1 day)</td>
<td>2 Some of the time (1-2 days)</td>
<td>1 Occasionally (3-4 days)</td>
<td>0 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I thought my life had been a failure</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I felt fearful</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>My sleep was restless</td>
<td>0 Never or Rarely (less than 1 day)</td>
<td>1 Some of the time (1-2 days)</td>
<td>2 Occasionally (3-4 days)</td>
<td>3 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>I was happy</td>
<td>3 Never or Rarely (less than 1 day)</td>
<td>2 Some of the time (1-2 days)</td>
<td>1 Occasionally (3-4 days)</td>
<td>0 Most of the time (5-7 days)</td>
</tr>
<tr>
<td>Description</td>
<td>Score Options</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I talked less than usual</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt lonely</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>People were unfriendly</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I enjoyed life</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I had crying spells</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt sad</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt that people disliked me</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I could not get “going”</td>
<td>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)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL SCORE:**
**Anxiety (HADS)**

**In general do you ever feel**

<table>
<thead>
<tr>
<th><strong>I feel tense or wound up…</strong></th>
<th>Most of the time</th>
<th>A lot of the time</th>
<th>Occasionally</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>I get a sort of frightened feeling as if something awful is about to happen…</strong></th>
<th>Quite badly</th>
<th>Not too badly</th>
<th>A little</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Worrying thoughts go through my mind…</strong></th>
<th>A great deal of the time</th>
<th>A lot of the time</th>
<th>From time to time</th>
<th>Only occasionally</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>I can sit at ease and feel relaxed…</strong></th>
<th>Definitely</th>
<th>Usually</th>
<th>Not often</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>I get a sort of frightened feeling like butterflies in the stomach…</strong></th>
<th>Not at all</th>
<th>Occasionally</th>
<th>Quite often</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>I feel restless as if I have to be on the move…</strong></th>
<th>Very much</th>
<th>Quite a lot</th>
<th>Not very much</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>I get sudden feelings of panic…</strong></th>
<th>Very often</th>
<th>Quite often</th>
<th>Not often</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**TOTAL SCORE:**
Instrumental activities of daily living (IADL)

1) Can you 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