Mild cognitive impairment associated with higher serum 25-hydroxyvitamin D levels in a middle aged population living at a moderately high latitude in New Zealand.

by

Andrea Strydom

A thesis submitted in fulfilment of the requirements for the degree of

Master of Science in Human Nutrition

At the University of Otago,
Christchurch, New Zealand

August 2014
Abstract

Objective: The association between serum 25-hydroxyvitamin D (s25OHD) concentrations and cognitive function has been investigated in only a few studies of middle aged adults (40-60 years); with inconsistent results found. The main objective of this Canterbury-based study was to examine the association between s25OHD levels and mild cognitive impairment (MCI) in participants in their early fifties, while adjusting for a number of confounders. The secondary objective was to assess if this association would differ when stratified by gender.

Design: Cross-sectional study participants, 366 adults aged 49-53 years living within the Canterbury District Health Board region from the Canterbury Health, Aging and Life Course study, were divided into two cognitive function groups: MCI and normal cognitive function (NCF). A score of <26 on the Montreal Cognitive Assessment test was defined as MCI and NCF was defined as a score ≥26 out of 30. Logistic regression was used to investigate the relationship between the cognitive function groups (outcome variable) and two s25OHD groups: insufficient s25OHD (≤50 nanomoles per litre (nmol/L)) and sufficient s25OHD (>50 nmol/L). Age, gender, education, ethnicity, annual household income, body mass index, depression, season of blood draw, alcohol intake, current smoking status, serum intact parathyroid hormone, serum creatinine, general health, vitamin D food intake, calcium food intake, vitamin D supplement use and chronic diseases were considered as potential confounders.

Results: In the univariate and multivariate logistic regression with cognitive function as the binary outcome variable (MCI (n=89) and NCF (n=277)) and vitamin D as a continuous predictor variable, the results did not reach statistical significance (crude odds ratio (OR) 1 (0.99, 1.01) p=0.306, adjusted OR 0.99 (0.98, 1.00) p=0.084). However, an inverse association
between s25OHD levels and cognitive function were observed in the univariate and multivariate logistic regression with vitamin D as a categorical variable. Compared to those with MCI, participants with NCF belonged more often to the insufficient s25OHD group (≤50 nmol/L, n=124) than the sufficient s25OHD group (>50 nmol/L, (n=242)) (crude OR 0.52 (0.30, 0.89) p=0.020, adjusted OR 0.44 (0.24, 0.79) p=0.008). In the analyses stratified by gender, the effect observed was similar, however, the association reached statistical significance only for female participants (adjusted OR 0.43 (0.18, 0.94) p=0.041) and not for male participants (adjusted OR 0.48 (0.18, 1.19) p=0.127). Further adjusted for vitamin D supplement use and season, did not change the effect size, however was just statistically significant, for female participants (adjusted OR 0.43 (0.17, 0.99) p=0.053).

Conclusions: Those with MCI belonged more often to the s25OHD group >50 nmol/L than the s25OHD group ≤50 nmol/L in a middle aged community-dwelling cohort, mostly for women, and appeared to be due to vitamin D supplement use. However, causality cannot be concluded due to the cross-sectional design of this study.
Preface:

The Canterbury Health, Aging and Life Course (CHALICE) study is a prospective longitudinal study of 50 year old adults living in the Canterbury District Health Board region. One of the aims of the CHALICE data is to increase knowledge of the factors associated with physiological ageing. Planning of the study started in 2009, participant recruitment and baseline data collection began in August 2010, 400 assessments were completed in September 2013 and a follow-up is intended every 5 years. This thesis is a cross-sectional study based on the 400 CHALICE study participants’ baseline data.

As part of this thesis, the candidate:

- Observed a full typical assessment day at the CHALICE offices.
- Checked participant estimated four day food and beverage diaries (FBDs) for any important information omitted, regarding the type, quantity and detail regarding their food or beverage intake, and provided the interviewers with this information.
- Rechecked and amended FBDs transferred from an old nutrient analysis programme (Diet Cruncher) to a current nutrient analysis programme (Kai-culator).
- Entered new FBDs into Kai-culator.
- Entered new recipes into Kia-culator.
- Provided the Kia-culator administrators with feedback on system errors.
- Collaborated with colleagues and updated the existing assumptions list to enable consistency among diet record data capturers.
- Entered some physical function data.
- Completed the statistical analyses included in this thesis.
Acknowledgements

I am eternally grateful to many people, without their help this thesis would not be possible, a big thank-you goes out to:

Dr Paula Skidmore and Associate Professor Richard Gearry, who have been amazing supervisors working as a great team. Thank-you for constantly believing in me, being patient, always accommodating, encouraging, supportive, offering valuable advice, giving prompt useful feedback and giving up your time to read and correct this thesis.

John Pearson who shared his knowledge, gave much needed advice and assistance regarding statistical analyses and interpretation, and who always pointed out something I had overlooked, sending me back to the drawing board.

The CHALICE interview team: Anna Thorpe, Ester Vierck and Janet Spittlehouse; who made this data possible. Janet Spittlehouse who promptly answered endless questions regarding CHALICE protocol, supplied relevant and useful documentation, supplied the cleaned data and was always accommodating and available. Monica Johnstone for ensuring I had all the important data before she left.

Andre Strydom, my husband, who has always been incredibly supportive and patient, encouraging and a great father. Who has sacrificed much and has worked hard so that I could complete this thesis. Xavier (4 years) and Alexander (6 years) Strydom who gave up time with their mum, who at their young age tried to understand when I was not available, and who prayed on many occasions that I finish the thesis on time.
All my friends and family who supported, encouraged and helped me with babysitting the boys, especially my mum (Ruza Spratek), Mato Strbac, Lara Potgieter, Yvette Jacque, Melanie Abbott and the wonderful teachers at kindergarten who made it easier to leave Xavier in their care: Sandra Laffey, Maria Calderwood, Samantha Hopkins, Kelly Parker and Hannah Scott.
# Table of Contents

1 Introduction .................................................................................................................. 1

2 Literature review .......................................................................................................... 4

2.1 Cognitive function ..................................................................................................... 4

2.1.1 Cognitive function domains and age of decline ................................................. 5
2.1.2 Mild cognitive impairment ..................................................................................... 7
2.1.3 Diagnosis ............................................................................................................... 8
2.1.4 Other potential confounders affecting cognitive function .................................. 12
2.1.5 Treatment of dementia ......................................................................................... 13

2.2 Vitamin D ................................................................................................................ 14

2.2.1 Vitamin D metabolism ....................................................................................... 15
2.2.2 Vitamin D sources ............................................................................................. 16
2.2.3 The role of Vitamin D in the human body ............................................................ 26
2.2.4 Measures of Vitamin D status ............................................................................ 27
2.2.5 Vitamin D status ................................................................................................ 31
2.2.6 Factors affecting serum 25-hydroxyvitamin D levels ........................................ 32

2.3 Cognitive function and vitamin D .......................................................................... 34

2.3.1 Vitamin D’s role in the brain ............................................................................ 34
2.3.2 Association between cognitive function and vitamin D .................................... 36
2.3.3 Possible reasons for inconclusive results .......................................................... 50
2.3.4 Vitamin D supplementation random control trials .......................................... 54
2.3.5 Conclusion .......................................................................................................... 54

3 Objective ................................................................................................................... 57

3.1 Hypotheses .............................................................................................................. 57

4 Methods ...................................................................................................................... 58

4.1 Study design ............................................................................................................ 58

4.1.1 Participant selection and recruitment ................................................................. 59
4.1.2 Participant assessment and follow up ................................................................. 60

4.2 Data collection procedures and variable coding .................................................... 62

4.2.1 Physical measurements and blood samples ....................................................... 62
4.2.2 Demographics ................................................................................................... 67
4.2.3 Risk Factors ....................................................................................................... 69
References .............................................................................................................................................. 112
Appendices ................................................................................................................................................ 134
8.1 Appendix 1: Ethical approval letter ........................................................................................................ 134
8.2 Appendix 2: Participant consent form ....................................................................................................... 136
8.3 Appendix 3: CKD-EPI formula .................................................................................................................. 138
8.4 Appendix 4: CHALICE study module 2 questionnaire ............................................................................. 139
8.5 Appendix 5: The Alac straight up guide to standard drink ................................................................. 177
8.6 Appendix 6: Calculating typical alcohol consumption per day in grams .............................................. 179
8.7 Appendix 7: MoCA test score sheet, tasks and point allocation ......................................................... 180
8.8 Appendix 8: Mini-International Neuropsychiatric Interview .............................................................. 182
8.9 Appendix 9: Medical Outcomes Study Short Form-36 version 2 ....................................................... 183
8.10 Appendix 10: The CHALICE four day food and beverage diary ...................................................... 187
8.11 Appendix 11: Reasons for missing MoCA test scores ......................................................................... 195
8.12 Appendix 12: Baseline characteristics of participants included and excluded from this thesis ......... 196
8.13 Appendix 13: Distribution of MoCA scores and the transformed data .............................................. 197
8.14 Appendix 14: Scatterplot with BMI and 25OHD levels according to BMI categories ...................... 199
8.15 Appendix 15: Scatterplot with creatinine and 25OHD according to gender ...................................... 200
8.16 Appendix 16: Other variables added to model 2 to see if there was a significant effect on the model parameters ......................................................................................................................................................... 201
8.17 Appendix 17: Scatterplot of BMI (kg/m²) and MoCA test scores ......................................................... 202
8.18 Appendix 18: Model 2 logistic regression rerun with data amended by adding additional point to MoCA scores for low education .......................................................................................................................................................... 202
List of Tables

Table 1: Description of cognitive domains and the estimated age at which it deteriorates. .................. 6
Table 2: Criteria to diagnose mild cognitive impairment. ................................................................. 8
Table 3: Description, advantages and disadvantages of three commonly used neuropsychological tests. .............................................................................................................................. 10
Table 4: Adult vitamin D dietary recommended intake values in Australia, New Zealand, Canada and the United States .................................................................................................................. 17
Table 5: Examples of New Zealand food containing vitamin D and the average price. .................. 18
Table 6: Regulations regarding voluntary and mandatory vitamin D food fortifications in various countries .................................................................................................................................. 20
Table 7: Average vitamin D dietary intake from food and supplements in various countries ....... 22
Table 8: Summary of the Osteoporosis Australia sun exposure recommendations for vitamin D production ....................................................................................................................................... 26
Table 9: Differences between serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D as measures of vitamin D status ........................................................................................................................................ 28
Table 10: Categories of current assay methods measuring serum 25-hydroxyvitamin D, their description, advantages and disadvantages. ................................................................................ 29
Table 11: Vitamin D status in selected countries .............................................................................. 32
Table 12: Reviews and meta-analyses assessing the association between serum 25-hydroxyvitamin D and cognitive function. .................................................................................................... 35
Table 13: Cross-sectional studies investigating the association between cognitive function and serum 25-hydroxyvitamin D .................................................................................................. 38
Table 14: Prospective studies investigating the association between serum 25-hydroxyvitamin D and cognitive function ........................................................................................................ 44
Table 15: Random control trials investigating the effect of vitamin D supplementation on cognitive function ..................................................................................................................................... 55
Table 16: A brief description of the CHALICE study assessment modules ......................................................................................................................................................... 63
Table 17: Ethnic categories and the associated CHALICE study ethnic groups .................................. 68
Table 18: Baseline characteristics for participants in the study and difference between gender and self-reported ethnicity (Māori and non-Māori). ................................................................. 82
Table 19: Baseline characteristics of participants with mild cognitive impairment and normal cognitive function. .................................................................................................................. 83
Table 20: Baseline characteristics of study participants according to serum vitamin D status ........ 85
Table 21: Determinants of cognitive function and serum 25-hydroxyvitamin D levels: univariate logistic regression ..................................................................................................................................... 88
Table 22: Multivariate logistic regression showing the association between normal cognitive function (dependent variable) and serum 25-hydroxyvitamin D (independent variable) ........................................ 90
Table 23: Logistic regression models illustrating the odds of normal cognitive function according to s25OHD status separated by gender ........................................................................ 93
List of Figures
Figure 1: Typical CHALICE study assessment day flow chart. ................................................................. 61
Figure 2: Flow chart of the participants included in this thesis .......................................................... 80
Figure 3: Scatterplots investigating the relationship between serum 25-hydroxyvitamin D and Montreal Cognitive Assessment test scores with an added linear regression line (left) and a smoother (right). ... 86
Figure 4: Boxplot of Montreal Cognitive Assessment (MoCA) test scores according to serum 25-hydroxyvitamin D insufficient (≤50 nmol/L) and sufficient levels (>50 nmol/L)......................................................... 87
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>$</td>
<td>New Zealand Dollar</td>
</tr>
<tr>
<td>µg</td>
<td>Micrograms</td>
</tr>
<tr>
<td>χ²</td>
<td>Chi-squared</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>25OHD</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>-2LL</td>
<td>Deviance</td>
</tr>
<tr>
<td>Aβ</td>
<td>Beta-amyloid protein</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>β</td>
<td>Beta coefficients</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CHALICE</td>
<td>Canterbury Health, Ageing and Life Course</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence intervals</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variance</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D binding protein</td>
</tr>
<tr>
<td>DHB</td>
<td>District Health Board</td>
</tr>
<tr>
<td>DRIs</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>EOL</td>
<td>End of line</td>
</tr>
<tr>
<td>FBD</td>
<td>Food and beverage diary</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>ID</td>
<td>Identification</td>
</tr>
<tr>
<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography combined with mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>Meters</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>MFD</td>
<td>Manufactured Food Database</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>MINI</td>
<td>Mini-International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
</tbody>
</table>
NCF  Normal cognitive function
nmol/L  Nanomoles per litre
NRVs  Nutrient Reference Values
NZ  New Zealand
NZFCD  NZ Food Composition Database
OH  Hydroxyl
OR  Odds ratio
pmol/L  Picomoles per litre
PTH  Parathyroid hormone
RCTs  Randomized control trials
RDA  Recommended dietary allowance
RIA  Radioimmunoassay
s25OHD  Serum 25-hydroxyvitamin D
SD  Standard deviation
SE  Standard error
SF-36  Short Form-36 version 2
Std res  Standardized residuals
µmol/L  Micromoles per litre
US  United States
UV  Ultraviolet
VDR  Vitamin D receptors
Vitamin D  Vitamin D₃ and Vitamin D₂
Vitamin D₂  Ergocalciferol
Vitamin D₃  Cholecalciferol
WHO  World Health Organisation
1 Introduction

Globally there is a high prevalence of vitamin D deficiency (1). Vitamin D is known for its role in maintaining bone health (2). However, the recent discovery of vitamin D receptors (VDR) in tissues (3-5) other than those responsible for bone health has led to numerous studies associating vitamin D deficiency with several other illnesses (6), including cognitive impairment.

Convincing evidence suggests vitamin D plays an important role in the brain, such as: the discovery of VDR and metabolites in the human brain (7-9); animal experiments and in vitro studies show vitamin D plays a vital role in brain development and functioning (10, 11); and low serum 25-hydroxyvitamin D (s25OHD) levels are commonly observed in psychiatric inpatients (10, 12, 13), people with Alzheimer’s disease (AD) and Parkinson’s disease (14).

Subsequently, this evidence has led to several epidemiological studies investigating the association between s25OHD levels and cognitive function (13, 15-33). Even though results are not all in agreement (12, 21, 28, 30-32), there are a number of studies (17-20, 26, 27) and reviews (34, 35) that have found low s25OHD levels associated with cognitive impairment or an increased risk of dementia.

Cognitive function gradually declines with age (36); some domains deteriorate from between 20 and 30 years of age and others are unaffected till later life (36-38), but generally the decline speeds up after the age of 60 (37). The World Health Organization (WHO) (39) reported that globally people are generally living longer, and that in high income countries, such as New Zealand (NZ), the life expectancy at birth in 2012 for men is 75.8 years and in women 82 years. In NZ the percentage of the population over the age of 60 years has grown from 16 % in 2001 (n=604,995) to 20% in 2013 (n=840,198) (40).
The increase in the aging population is accompanied by an increase in the prevalence of dementia. In 2010 it was estimated that 35.6 million people were living with dementia globally (41). Presently, in NZ, approximately 50,000 people have been diagnosed with dementia (42). These numbers are likely to be underestimated due to unreported cases (43-45). Dementia is a financial burden, as considerable care is required especially at the later stages (46). In 2010, the total estimated worldwide cost of dementia was US$ 604 billion (44), and in 2011 in NZ, the estimated total financial cost was $954.8 million (47).

Currently there is no known cure for dementia (48) and only a few preventive strategies have been identified (49). However, early detection can assist in prolonging the onset, and cognitive strengths can be identified that can compensate for the domains that have deteriorated (49, 50). Evidence has shown that vitamin D₃ has a neuroprotective role (10, 11) and may possibly have a therapeutic effect on brain aging (51) and AD (52). The effect of vitamin D supplementation on cognitive function is unknown, as only a few randomized control trials (RCTs) are available and are inconclusive and underpowered (53). More RCTs are required, with one currently underway (52).

In the interim, the increase in cross sectional and prospective studies, investigating the relationship between cognitive function and vitamin D are mostly finding lower s25OHD levels associated with worse cognition. However, most of these published papers investigated the association in an older population. In order to detect dementia early, studies may need to focus on a middle aged sample using neuropsychological tests that can identify early cognitive function decline (50).
Very few studies have investigated the association between vitamin D and cognitive function in a middle aged population, thus the aim of this thesis is to examine this relationship in a middle aged population in the Canterbury District Health Board (DHB) region. Compared to the rest of NZ, this region has more people aged 65 years and over (54) and lies at a latitude of 44 degrees south, considered a vulnerable region for vitamin D deficiency (55).
2 Literature review

This section is an overview of the current systematic reviews, meta-analyses and epidemiological studies assessing the association between vitamin D status and cognitive function. To better understand this relationship, this section also looks at cognitive function and vitamin D in more detail.

Cognitive aging, predictors associated with cognition and mild cognitive impairment (MCI) are reviewed. As only MCI is assessed in this study population, dementia will not be discussed in detail. Prevalence, diagnostics and current treatment of MCI and dementia are briefly considered. With regards to Vitamin D this section looks at its sources, metabolism, measurement, its role in the body and the brain, its status globally and in NZ and the different factors that can affect its circulating levels.

The literature search was completed using EMBASE and MEDLINE via PubMed and Ovid. Keywords included: “mild cognitive impairment”, “vitamin D”, “25-hydroxyvitamin D”, “cognitive function”, “cognition” and “middle age”. Searches were performed from 1 January 2013 to 18 December 2014, and where appropriate, preference was given to articles published after 2008 for the latest current information. A manual check was also done on the reference lists of articles.

2.1 Cognitive function

Cognitive function is defined as ‘….an intellectual process by which one becomes aware of, perceives, or comprehends ideas. It involves all aspects of perception, thinking, reasoning, and remembering’ (56). Certain cognitive domains gradually decline with age (36, 38) and the aging
brain is accompanied by structural and functional changes (38) which may lead to brain disorders (49). The stage between normal cognition and dementia is MCI (50).

‘Dementia is a loss of mental ability severe enough to interfere with normal activities of daily living... is a group of symptoms caused by gradual death of brain cells..... leads to impairments in memory, reasoning, planning, and behaviour..... is caused by specific brain diseases.’(57)

Approximately 28% (58) to 46% (59) of those with MCI develop dementia after 3 years, however results are inconsistent (60, 61). The pathology of dementia is complicated and multifaceted (62). The changes seen in dementia start about 20-30 years before its onset (37, 63), and with an aging population there is a need to detect dementia early (47). A postponement of dementia for a few years may substantially reduce the cases of AD (62), which is one of the most frequent causes of dementia (62, 64).

2.1.1 Cognitive function domains and age of decline

Normal aging studies are limited by methodological problems such as different assessment methods, misdiagnoses, heterogeneity of people and their rate of cognitive decline (37, 38), differences between those taking part and those not, and cohorts born decades apart having different characteristics (38). These issues make agreement on the age of cognitive decline and the domains affected difficult (38). Although there is no consensus as to when age-related cognitive decline begins (65), evidence shows cognitive decline, in certain domains, begins gradually between the age of 20 and 30 years (36-38) and accelerates after the age of 60 years (37).

Table 1, based on a review by Harada et al. (38), lists the various cognitive domains, describes the various aspects of the domain and gives a rough estimate of when cognitive decline takes
place. General knowledge and vocabulary are overlearned, familiar and regularly practiced and thus are generally stable throughout life (38). Some cognitive abilities that are not so familiar tend to decline from an early age, such as processing speed, attention, memory, language, visuospatial ability and executive functioning (38). Mental processing speed is a core function.

Table 1: Description of cognitive domains and the estimated age at which it deteriorates.

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Type and Description</th>
<th>Age of Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>Speed of motor responses.</td>
<td>30 years</td>
</tr>
<tr>
<td></td>
<td>Speed of cognitive activities.</td>
<td>30 years</td>
</tr>
<tr>
<td>Attention</td>
<td>Auditory attention span / immediate memory.</td>
<td>Late life</td>
</tr>
<tr>
<td></td>
<td>Selective attention (ability to focus on specific information &amp; ignore irrelevant information).</td>
<td>Early life</td>
</tr>
<tr>
<td></td>
<td>Divided attention (ability to focus on several tasks at the same time).</td>
<td>Early life</td>
</tr>
<tr>
<td>Memory</td>
<td>Declarative / explicit memory such as episodic memory (personal experience memory).</td>
<td>Early life</td>
</tr>
<tr>
<td></td>
<td>Declarative / explicit memory such as semantic memory (knowing word meaning and using language).</td>
<td>Late life</td>
</tr>
<tr>
<td></td>
<td>Non-declarative memory / implicit memory (remembering familiar things like the ‘happy birthday’ song).</td>
<td>Stable</td>
</tr>
<tr>
<td></td>
<td>Non-declarative memory / implicit memory such as procedural memory (remembering how to ride a bike).</td>
<td>Stable</td>
</tr>
<tr>
<td></td>
<td>Acquisition (new information is encoded into memory).</td>
<td>Early life</td>
</tr>
<tr>
<td></td>
<td>Retention of information.</td>
<td>Stable</td>
</tr>
<tr>
<td></td>
<td>Retrieval of new information.</td>
<td>Early life</td>
</tr>
<tr>
<td>Language</td>
<td>Vocabulary.</td>
<td>Late life &amp; stable</td>
</tr>
<tr>
<td></td>
<td>Verbal fluency (generate a few words for a category).</td>
<td>Early life</td>
</tr>
<tr>
<td></td>
<td>Visual confrontation naming (naming objects).</td>
<td>Late life (70 years)</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>Construction (put together parts to make a whole).</td>
<td>Early life</td>
</tr>
<tr>
<td></td>
<td>Abilities (recognize faces or household items and physical location of objects).</td>
<td>Stable</td>
</tr>
<tr>
<td>Executive functioning</td>
<td>Self-monitor, plan, organise, problem solving &amp; mental flexibility.</td>
<td>Early</td>
</tr>
<tr>
<td></td>
<td>Automatic response inhibition.</td>
<td>Early</td>
</tr>
<tr>
<td></td>
<td>Reasoning (especially with unfamiliar material).</td>
<td>45 years</td>
</tr>
</tbody>
</table>

The information in this table is a summary from the review by Harada et al. (38)
that decreases with age and affects other cognitive domains such as recall, working memory and reasoning (50, 66).

2.1.2 Mild cognitive impairment

Numerous terms have been used to describe the state between age-related normal cognitive decline and early diagnosis of dementia (67), such as MCI, questionable dementia, isolated memory impairment, cognitive impairment not demented, and aging-associated cognitive decline (50). Currently the most commonly used term in research studies is MCI (48, 50). There is a lack of consensus on the definition of MCI, and a working group for the Alzheimer’s Association and the National Institute on Aging, have reviewed the criteria to diagnose MCI. Their recommendations are summarized in Table 2 (68) and are similar to the regularly referred to Petersen’s criteria (50).

The prevalence of MCI has been found to range from 0.5% in Austria (n=592; mean age=75 years) to 42% in France (n=6892, ≥65 years) (67). There are no recent NZ MCI epidemiology data; however, a small pilot study (n=45; 56-87 years) in Wellington found that 4.4% of the sample were cognitively impaired (n=2) (69). In Australia, an older sample (n=767, 70-90 years) had a prevalence of MCI of 39% (70).

There are few MCI prevalence studies assessing middle aged adults. In a review by Ward et al. (67) only two studies assessed this age group. In the one no MCI was found among 40 to 54 year olds (n=152), and 2.1% was found among those 55 to 64 year olds (n=141) (71). In the other study, 12% of the 50-59 years olds in India (n=77) had MCI (72).
Table 2: Criteria to diagnose mild cognitive impairment.

<table>
<thead>
<tr>
<th>No.</th>
<th>Criteria</th>
<th>Comment/Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Concern about change in cognition compared to prior level.</td>
<td>Concern can be from patient, someone who knows the patient well or from a clinician.</td>
</tr>
<tr>
<td>2</td>
<td>Compared to the same age &amp; education group they perform worse in one or more cognitive domains.</td>
<td>A decline should be seen in repeated assessments, in domains such as executive function, memory, attention, visuospatial skills and language.</td>
</tr>
<tr>
<td>3</td>
<td>No disruption to daily functional abilities.</td>
<td>Those with MCI may be less efficient at performing some tasks, but they can still perform them.</td>
</tr>
<tr>
<td>4</td>
<td>Without diagnosis of dementia.</td>
<td>Changes should be mild, without affecting normal functioning.</td>
</tr>
</tbody>
</table>

This table is a summary of the Alzheimer’s Association and the National Institute on Aging criteria for diagnosing MCI (68). Abbreviations: MCI = mild cognitive impairment, no. = number.

A recent study, used various MCI assessment criteria to assess a sample of middle aged men (mean=55.4 years; n=1237), and found those with MCI to range from 1% to 65%, depending on which assessment criteria they used (73). Determining MCI prevalence and incidence is difficult due to the lack of information (69), the various cognitive function tests that have varying results (30, 73), various definitions, and various sample characteristics such as sample size, age group and country being assessed (67, 74).

2.1.3 Diagnosis

Currently there is no gold standard instrument or cut-off point to assess MCI (61), thus diagnosis of normal cognitive function (NCF), MCI and dementia is challenging (68). For an accurate diagnosis a clinical assessment is required (68). First neuropsychological tests should be performed and if there are signs of MCI, the cause needs to be ascertained (degenerative,
vascular, depressive, traumatic, medical comorbidities, or mixed disease) (68), and other biomarkers (Section 2.1.3.2) should be assessed (75).

2.1.3.1 Neuropsychological tests

Numerous tests are available to assess cognitive function and dementia (75). Standard neuropsychological test batteries measure five to seven cognitive domains (75): non-verbal and verbal IQ, executive function, attention, motor speed, and two separate memory domains (learning or encoding and delayed recall) (50).

Neuropsychological tests have limitations. For example, very sensitive tests safeguard against missing early cases but may diagnose a false positive case (45). Very specific tests safeguard against false diagnoses but can miss early cases (45). Some cognitive domains are affected by the retest effect, where people perform better when retested, which masks deterioration in cognition (37). Tests can also have a ceiling or floor effect, depending on education, age and ethnicity of a participant (45).

Some neuropsychological tests are not comprehensive and only assess a few of the usual five to seven cognitive domains (34, 76, 77). Too many tests, however, can produce a false positive result (50). Some tests have validated cut-off scores for MCI (78), others (25, 30) do not have cut-off scores but use 1 to 1.5 standard deviation (SD) below the mean of the study sample, making them less accurate (30, 68). To improve accuracy, researchers have created norms for age, education and even ethnicity (79), but when tests are administered norms are often overlooked (75).
## Table 3: Description, advantages and disadvantages of three commonly used neuropsychological tests.

<table>
<thead>
<tr>
<th>Test &amp; Description</th>
<th>Domains Measured</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mini-Mental State Examination (MMSE)</strong>&lt;sup&gt;(80)*&lt;/sup&gt;:</td>
<td>Orientation, registration, attention or calculation (serial sevens or spelling), recall, naming, repetition, comprehension (verbal &amp; written), writing &amp; construction.</td>
<td>1. Assesses a variety of cognitive disorders.</td>
<td>1. Not a diagnostic tool.</td>
</tr>
<tr>
<td>Most commonly used test.</td>
<td></td>
<td>2. Test-retest reliability is good.</td>
<td>2. Sensitivity for identifying MCI is 18% &amp; AD is 78%.</td>
</tr>
<tr>
<td>8 - 15 minutes in length.</td>
<td></td>
<td>3. Accurate first step screening tool.</td>
<td>2. High scores lead to ± 10 % false negative rate.</td>
</tr>
<tr>
<td>30-point test.</td>
<td></td>
<td>4. Reasonable performance in detecting MCI among otherwise unimpaired individuals.</td>
<td>3. Has a floor effect in those with advanced dementia, severe language problems &amp; those less educated.</td>
</tr>
<tr>
<td>Higher score = better cognition.</td>
<td></td>
<td></td>
<td>4. Likely to have a ceiling effect in those with early dementia but extensive education.</td>
</tr>
<tr>
<td>A score of 26 = impairment (78).</td>
<td></td>
<td></td>
<td>4. MMSE items are influenced by age, education &amp; ethnicity. Available tables of adjustment are often overlooked.</td>
</tr>
<tr>
<td>Other suggested cut off is ≤24 (81).</td>
<td></td>
<td></td>
<td>6. Validation studies are underpowered.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7. A score of 26 = impairment is inaccurate (78).</td>
</tr>
<tr>
<td><strong>Modified Mini-Mental State Examination (mMMSE)</strong>&lt;sup&gt;(82)*&lt;/sup&gt;:</td>
<td>Same as MMSE (above) and also concentration &amp; visuospatial function (19).</td>
<td>1. Assesses more cognitive domains than the MMSE.</td>
<td></td>
</tr>
<tr>
<td>Commonly used test.</td>
<td></td>
<td>2. Extended ceiling &amp; floor effects.</td>
<td></td>
</tr>
<tr>
<td>Scores: 0-100.</td>
<td></td>
<td>3. A brief test.</td>
<td></td>
</tr>
<tr>
<td>Higher score = better cognition (30).</td>
<td></td>
<td>4. At a cut-off score of 77/78: highly sensitive (0.88) and specific (0.90) for identifying MCI.</td>
<td></td>
</tr>
<tr>
<td>&lt;80 = impairment.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 - 15 minutes in length.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Montreal Cognitive Assessment (MoCA)</strong>&lt;sup&gt;(83)*&lt;/sup&gt;:</td>
<td>Attention, memory, executive functions, language, visuospatial, naming, recall, orientation &amp; abstract (84).</td>
<td>1. Has excellent sensitivity in identifying MCI (90%) &amp; AD (100 %) from healthy controls.</td>
<td>1. Not a diagnostic tool.</td>
</tr>
<tr>
<td>Screening tool developed to detect MCI (score of &lt;26).</td>
<td></td>
<td>2. Globally used in 36 dialects and languages.</td>
<td>2. Created in a memory clinic venue and norm-referenced test scored in a highly educated population.</td>
</tr>
<tr>
<td>10 minutes in length.</td>
<td></td>
<td>3. Validated extensively (84).</td>
<td></td>
</tr>
<tr>
<td>30-point test.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The information in this table was summarised from various chapters in the book edited by Larner (45, 80, 82, 83) & information added from other sources is referenced within the table. Abreviations: AD = Alzheimer’s Disease, MCI= mild cognitive impairment, MMSE = Mini Mental State Examination, mMMSE = modified MMSE, MoCA = Montreal Cognitive Assessment*
Even with their limitations neuropsychological tests have advantages (75). They serve as biomarkers and can identify general cognitive degeneration and ascertain the cognitive domains affected, which along with other biomarkers can predict the type of dementia, as different brain regions are affected by different dementias (75). Follow up tests can assist in tracking progression, and identify functioning domains that can be used to compensate for those that have deteriorated (75).

Two of the most commonly used tests in the studies assessing the association between cognitive function and vitamin D are shown in Table 3, along with the widely used Montreal Cognitive Assessment (MoCA) test which was used to assess MCI in this study. Table 3 lists the domains covered by each test and includes the advantages and disadvantages. The Mini Mental State Examination (MMSE) is not as effective as the modified Mini Mental State Examination or the highly sensitive MoCA test at identifying earlier stages of cognitive decline (78).

2.1.3.2 Other biomarkers

Dementia and MCI have similar genetic and neuroimaging features (21, 50), such as: hippocampus degeneration, metabolic pattern changes in the cerebrum and similar ε4 alleles in apolipoprotein E (APOE) ε4 genes (50). The possibility of cerebral amyloid angiopathy and AD developing are affected by APOE ε2, ε3, and ε4 alleles. For example, APOE ε4 is associated with an increased risk, but APOE ε2 is associated with a decreased risk of developing AD (85).

Build-up of specific proteins in the brain, such as tau protein and amyloid plaques, is an indication of neuronal damage (68). Amyloid plaques are assessed by measuring the beta-
amyloid protein (Aβ) in plasma and cerebrospinal fluid (CSF) (68). Deposits of tau protein in neurofibrillary tangles (50, 68) are correlated with CSF tau or phosphorylated-tau concentrations (50, 68). Neuronal damage can also be picked up with neuroimaging methods such as magnetic resonance imaging assessing structure (50) and single-photon emission computed tomography imaging (58, 68).

2.1.4 Other potential confounders affecting cognitive function

Various factors can influence cognition, including achieved education (17, 65), possibly by: increasing the “cognitive reserve”, where a higher education means a higher point from which cognitive decline starts (32, 50); reflecting environmental situations influencing cognition, such as a higher income status (86) and better health behaviours (87); and those with a high education perform better in tests because they are more familiar with being tested (32, 50). Childhood education is also shown to affect cognitive decline in mid-life (65).

Certain age-related diseases may affect cognition (37) and explain some age-related decline (16) such as cardiovascular disease, metabolic diseases (88), obesity, hypertension and diabetes (49, 89). Common medications taken by the elderly may also affect cognitive function, such as those prescribed for diabetes and chronic obstructive pulmonary disease (50).

Neuropsychiatric symptoms may explain age-related cognitive decline (13, 16, 58), such as apathy, anxiety, irritability (90), loneliness (32) and depression (24, 70); however, studies show inconsistent results (24, 32, 58, 90). Many studies have found that body mass index (BMI) is associated with lower cognitive function (32), and physical activity has a protective effect against cognitive decline and dementia, as well as cardiovascular health (16, 48).
Other factors that are possibly associated with cognitive function are: early life traumatic brain injury (91), number of previous concussions (92), ethnicity (68), cortisol (93), high serum calcium concentrations (17), hyperparathyroidism (17, 88), menopause (37), retirement from paid employment (37), loss of smell (58), antipsychotic drug treatment (13), location and setting (90), sunlight exposure, alcohol intake (32, 94), smoking (32), low protein intake (95), subjective memory impairment (96), and s25OHD levels (section 2.3). For many of these there are inconsistent results.

2.1.5 Treatment of dementia

Neurofibrillary tangles and deposits of amyloid plaques in the brain cannot be reversed (50, 95), and currently there is no treatment available to cure or stop the development of dementia. Therefore, it is important to concentrate on methods to prevent or delay cognitive decline (50, 95). Increasing “cognitive reserves” delays dementia in those with MCI (48). Even though there are mixed results (48), two recent systematic reviews concluded that cognitive training appears to have a beneficial effect on cognition (97, 98). Neuropsychological tests can identify cognitive strengths which can be utilized to assist patients in compensating for the cognitive domains that are deteriorating (50).

Even though a few RCT interventions have found some improvement in cognitive function or a reduction in the risk of dementia, a systematic review of 41 studies (48) found that few treatments were repeated and thus no conclusion could be drawn as to their effectiveness. Improvement in cognitive function was seen with donepezil; galantamine; nicotine patches; docosahexaenoic acid-rich fish oil; Huannao Yicong containing ginseng (48); and Gingko biloba with choline (99). No improvements were found with vitamin E, Gingko biloba, rivastigmine non-steroidal anti-inflammatory drug, triflusal, docosahexaenoic acid,
eicosapentaenoic acid, fluoxetine, Shenyin oral liquid, ginseng, Wuzi Yanzong, grape juice, green tea and lithium. While rofecoxib worsened symptoms (48).

Inconsistent results were found with exercise (48). Emotional support (32), and an active social and stimulating lifestyle in later life is proposed as protective of cognitive decline (62). Management of chronic diseases from middle age may also postpone or prevent dementia (62). However, Cooper et al. (48) concluded that only pharmacological trials offered robust evidence.

2.2 Vitamin D

In 1840, Śniadecki commented that open air, especially in the sun, was the most effective way of preventing and curing rickets in children (100). Yet only in 1919 did Huldschinsky discover that rickets could be cured using artificial sun lamps (101), and Mellanby found that certain dietary fats prevented rickets in puppies (102). Due to its “anti-rachitic factor” (102, 103), vitamin D was eventually recognised in 1922, as a “fourth vitamin” (104).

In 1923 Hess and Weinstock found that only a certain wave-length from the sun cured rickets (105), and in 1925 they hypothesized that ultra-violet rays converted the cholesterol in the skin, making it anti-rachitic (106). All these discoveries led to the isolation of vitamin D$_2$ in 1930 (107), and by 1936, the structure of vitamin D$_3$ had also been discovered (108).

Vitamin D is a fat-soluble vitamin (109). The term “vitamin D” generally (110) refers to the many forms of vitamin D, about 37 (111), which have a basic structure similar to steroid hormones (112). With enough sun exposure vitamin D can be synthesized in the body, making it non-essential (112, 113) and thus many do not identify it as a vitamin (112, 114). Some describe
it as a hormone (113), as it is made in one organ and taken by the blood to act on another. Others refer to it as a prohormone (2, 114) (a precursor of a hormone (115)).

Two parent forms of vitamin D are found. Cholecalciferol (vitamin D₃) is made from sunlight in the body (113) and is found in some plants (116, 117). Ergocalciferol (vitamin D₂) is made from irradiated (118) plant sterols (113, 116). The only difference in structure, between the two parent forms, is an extra double bond and methyl group in vitamin D₂ (113).

Throughout this thesis, the “D” in “vitamin D” will refer to both D₂ and D₃, and the specific name will be used when referring to a particular form of vitamin D.

2.2.1 Vitamin D metabolism

Vitamin D₃ produced in the skin binds to a vitamin D binding protein (DBP) and enters the peripheral circulation (119, 120). Dietary vitamin D along with dietary fats is absorbed in the small intestine (121) where it is incorporated into chylomicrons and enters the peripheral circulation via the lymphatic system (120, 122). Regardless of origin, vitamin D in the peripheral circulation is inactive, and within a few hours is taken up by the liver or adipose tissue (2, 123).

In the liver, vitamin D is converted to its inactive storage form, 25OHD, where a hydroxyl (OH) group is added at the carbon-25 position by cytochrome P450 (CYP) enzyme, believed to possibly be CYP2R1 (124). Serum 25OHD circulates attached to a DBP (2); it does not seem to be regulated (2) and increases with intake (125, 126) and UV light exposure (127). When the biologically active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), also called calcitriol (128), is required, another OH group is added to 25OHD, this time at the carbon-1α
position by another CYP enzyme, called 1α-hydroxylase enzyme in the kidney (124, 129, 130). Serum 1,25(OH)2D also circulates attached to a DBP (131).

Levels of serum 1,25(OH)2D are strictly regulated, mainly by a complex relationship between serum calcium, phosphate, parathyroid hormone (PTH), 1,25(OH)2D levels (129) and fibroblast growth factor (2, 132). When there is enough 1,25(OH)2D in circulation 25-hydroxyvitamin D-24-hydroxylase breaks down the 1,25(OH)2D and 25OHD to calcitroic acid, which is excreted in the bile (132).

2.2.2 Vitamin D sources
2.2.2.1 Dietary intake from food

Nutrient Reference Values (NRVs) are recommended intake values for each nutrient, published by the Australian and NZ governments (133). The NRVs are based on the 1997–2005 United States (US) and Canadian Dietary Reference Intakes (DRIs), but also on recommendations from the United Kingdom, the European Union and Germany; Australian and NZ dietary survey data; Australasian conditions and scientific data (133).

The adult vitamin D NRVs are listed in Table 4 and are based on the required vitamin D intake needed, with minimal sun exposure, to keep s25OHD levels at 28 nanomoles per litre (nmol/L) (133). In 2010, the vitamin D DRIs were revised (2)(Table 4) and, in 2012, the Australian and NZ Bone and Mineral Society and Osteoporosis Australia put forward a position statement, recommending higher vitamin D values (134)(Table 4). The NZ Ministry of Health is also currently funding a project to assist with a future review of the vitamin D NRVs (135).
Meeting the US or NZ recommended dietary intake for over 50 year olds is difficult as vitamin D occurs naturally in small quantities in few foods (136), that are generally expensive (137) and not consumed regularly (138)(Table 5). Vitamin D$_2$ is found in sun exposed mushrooms (139) and yeast (130). Vitamin D$_3$ can be found in certain meats (136, 140, 141), dairy products, animal liver, egg yolk and in larger amounts in oily fish (136) and their oils (109). In the US, food with naturally occurring vitamin D contributed only on average $1.9 \pm 0.4 \mu g$/day to an individual’s total dietary vitamin D intake, which includes fortified food and supplements (142). Table 5 lists examples of NZ foods containing vitamin D (143) and their average prices, listed from the highest vitamin D content (micrograms (µg)/100 grams (g)) to the lowest.

Table 4: Adult vitamin D dietary recommended intake values in Australia, New Zealand, Canada and the United States.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Australia and New Zealand</th>
<th>United States and Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin D Nutrient Reference Values (2006) (133)</td>
<td>Vitamin D values from the position statement (2012)*</td>
</tr>
<tr>
<td></td>
<td>AI (µg/d)</td>
<td>UL (µg/d)</td>
</tr>
<tr>
<td>19–30</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>31–50</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>51–70 ‡</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>&gt;70 ‡</td>
<td>15</td>
<td>80</td>
</tr>
</tbody>
</table>

Definition of terms

- **EAR** Meets the need of half of the healthy population (2).
- **RDA** Derived from EAR, which meets the need of about 97.5% of the healthy population (2).
- **AI** Used when there was insufficient evidence to develop EAR/RDA and is the average daily intake level based on observed or experimental intakes (133).
- **UL** The upper intake level, which if exceeded may possibly cause harm (2).

*The position statement was put forward by the Australian and New Zealand Bone and Mineral Society and Osteoporosis Australia recommending higher vitamin D values (134).

µg/d = micrograms per day, AI = adequate intake, EAR = Estimated average requirement, RDA = Recommended dietary allowance, UL = tolerable upper intake level.

‡ This age group has a higher recommended value which takes into account the reduced ability to synthesize vitamin D that comes with aging and the fact that elderly have a higher prevalence of vitamin D deficiency (133).
Table 5: Examples of New Zealand food containing vitamin D and the average price.

<table>
<thead>
<tr>
<th>Food item</th>
<th>Vitamin D content (µg/100g)</th>
<th>Average price (NZD/100g) *</th>
<th>Average serving size</th>
<th>Vitamin D content (µg / serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon, king, New Zealand. fillet, raw</td>
<td>20</td>
<td>$2.50</td>
<td>1 serving (110g)</td>
<td>22</td>
</tr>
<tr>
<td>Margarine, light, polyunsaturated, 50% fat, Flora **</td>
<td>16</td>
<td>$0.75</td>
<td>1 teaspoon (5g)</td>
<td>0.8</td>
</tr>
<tr>
<td>Tarakihi, flesh, baked</td>
<td>11</td>
<td>$2.80</td>
<td>1 fillet (140g)</td>
<td>16</td>
</tr>
<tr>
<td>Snapper, flesh, baked</td>
<td>10</td>
<td>$3.90</td>
<td>1 fillet (107g)</td>
<td>11</td>
</tr>
<tr>
<td>Yoghurt, Greek, composite</td>
<td>7.3</td>
<td>$0.62</td>
<td>1 cup (250ml / 263g)</td>
<td>19</td>
</tr>
<tr>
<td>Milk, condensed, sweetened, whole</td>
<td>5.4</td>
<td>$0.80</td>
<td>1 tbsp (19g)</td>
<td>1</td>
</tr>
<tr>
<td>Butter, salted or unsalted, composite</td>
<td>5.2</td>
<td>$0.92</td>
<td>1 teaspoon (5g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Sardines, canned, drained</td>
<td>4.8</td>
<td>$1.65</td>
<td>1 sardine (12g)</td>
<td>0.6</td>
</tr>
<tr>
<td>Cheese, cottage, light, 1% fat, composite</td>
<td>4</td>
<td>$1.06</td>
<td>1 tbsp (16.5g)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pork, bacon, rashers, lean 73% &amp; fat 27%, grilled</td>
<td>3</td>
<td>$2.02</td>
<td>1 cup, cooked, diced (120g)</td>
<td>3.6</td>
</tr>
<tr>
<td>Tuna, canned in brine, drained</td>
<td>2</td>
<td>$1.32</td>
<td>1 can (185g)</td>
<td>3.7</td>
</tr>
<tr>
<td>Egg, chicken, white &amp; yolk, boiled</td>
<td>1.8</td>
<td>$0.57</td>
<td>1 x medium egg (49g)</td>
<td>0.9</td>
</tr>
<tr>
<td>Mussels, green, flesh, boiled</td>
<td>1.6</td>
<td>$1.44</td>
<td>1 serving (40g)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pork, loin chops, lean, grilled</td>
<td>0.8</td>
<td>$2.00</td>
<td>1 chop (68g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ham, sliced</td>
<td>0.7</td>
<td>$1.59</td>
<td>1 slice (10x10x0.25cm)(29g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Milk, high calcium, 0.1% fat, composite **</td>
<td>0.7</td>
<td>$0.26</td>
<td>1 cup (250ml / 258g)</td>
<td>1.9</td>
</tr>
<tr>
<td>Hoki, flesh, baked</td>
<td>0.6</td>
<td>$1.59</td>
<td>half fillet (122g)</td>
<td>0.7</td>
</tr>
<tr>
<td>Lamb, midloin chop, separable lean, grilled</td>
<td>0.6</td>
<td>$2.50</td>
<td>1 chop (32g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Bread, multigrain, light, sliced, prepacked, composite</td>
<td>0.5</td>
<td>$0.45</td>
<td>1 slice sandwich (11.4x10.3x1.07cm) 32g</td>
<td>0.1</td>
</tr>
<tr>
<td>Milk, standard, 3.3% fat, composite</td>
<td>0.5</td>
<td>$0.19</td>
<td>1 cup (250ml / 258g)</td>
<td>1.2</td>
</tr>
<tr>
<td>Cheese, cheddar, composite</td>
<td>0.3</td>
<td>$1.05</td>
<td>1 cup shredded (118g)</td>
<td>0.4</td>
</tr>
<tr>
<td>Chicken, breast, lean &amp; fat, roasted</td>
<td>0.3</td>
<td>$0.17</td>
<td>1 single breast (161g)</td>
<td>0.5</td>
</tr>
<tr>
<td>Milk, lite, 1.5% fat, composite</td>
<td>0.3</td>
<td>$0.19</td>
<td>1 cup (250ml / 258g)</td>
<td>0.9</td>
</tr>
<tr>
<td>Beef, fillet steak, lean &amp; fat, grilled</td>
<td>0.2</td>
<td>$3.15</td>
<td>1 steak (173g)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Apart from the prices, the information in this table was obtained from the concise New Zealand food composition tables (143) *Prices were obtained from Countdown supermarket online shopping website. The prices are based on medium sized items and an average of three different brands were taken if more than one option was available (147). **Fortified with vitamin D (143) µg = micrograms, g = grams, NZD = New Zealand dollar, tbsp. = tablespoon

In order to increase population vitamin D intake, several countries permit voluntary vitamin D fortification of certain foods and some enforce mandatory fortification (144). Table 6 lists vitamin D food fortification regulations for selected countries. In NZ, in 2010, approximately
172 different vitamin D fortified products were voluntarily listed on the Manufactured Food Database (MFD) (145), and two examples are included in Table 5. In the US about 55% of the total dietary vitamin D intake came from fortified foods (142), where a variety of foods can be fortified with vitamin D (Table 6).

Even though a wealth of literature (125, 126, 148-153) confirms that fortified foods increase s25OHD levels significantly and can be done easily (145), food fortification is not the panacea one might imagine. For example, fortification of a staple food will not benefit non-users (150, 154); manufacturers may find it easier not to fortify foods (145), limiting their availability; and fortified products are usually more expensive than unfortified products (155).

There is also significant variability between declared and actual vitamin D content, for a number of reasons, including the difficulty faced by manufacturers in obtaining the most stable supplement form of vitamin D and adding the correct quantity that will withstand processing and storage (145). For example in NZ, 28% of various fortified samples\(^1\) contained less and 39% contained more of the declared vitamin D value (156). In the US, fortified milk samples contained 19% less and 42% contained more than 125% of the declared vitamin D value (157).

To enhance the vitamin D content in food, alternative methods are also being used or currently investigated. For example, some Canadian soups are made from irradiated mushrooms (139, 158). Vitamin D supplemented chicken feeds produce eggs with a higher vitamin D content (159), and the unfeathered skin of a hen’s leg exposed to ultraviolet (UV) B radiation has effectively increased the vitamin D content in the eggs, as well as the meat (160).

\(^{1}\) Food drinks, fruit drinks, margarine, baby food and milk products

19
### Table 6: Regulations regarding voluntary and mandatory vitamin D food fortifications in various countries.

<table>
<thead>
<tr>
<th>Country and regulating body</th>
<th>Regulations</th>
<th>Mandatory Fortification</th>
<th>Voluntary Fortification</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand (Food Standards Australia New Zealand)</td>
<td>Vitamin D must not be added to a food unless it is on the permitted list.</td>
<td>No mandatory fortification.</td>
<td>Edible oils &amp; spreads</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia (Food Standards Australia New Zealand)</td>
<td>Vitamin D must not be added to a food unless it is on the permitted list.</td>
<td>Edible oils and spreads</td>
<td>Edible oil spreads &amp; margarine.*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada (Canadian Food Inspection Agency)</td>
<td>Subject to section D.03.003, no person shall sell a food to which a vitamin... has been added unless the food is listed in Column I... and... Column II of the Table.</td>
<td>Dairy</td>
<td>Milk (fluid, sterilized, evaporated or powdered, whole, skim..., partly skimmed or naming the flavour milk) &amp; products with added milk solids.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA (US Food and Drug association)</td>
<td>Most food can be voluntarily fortified. 'The Food &amp; Drug Administration does not encourage indiscriminate addition of nutrients to foods, nor does it consider it appropriate to fortify fresh produce; meat, poultry, or fish products; sugars; or snack foods such as candies and carbonated beverages.... manufacturers ...are urged to utilize these principles...’</td>
<td>Dairy</td>
<td>Evaporated milk, nonfat dry milk that is labeled 'fortified with vitamins A &amp; D'.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe (European Union Regulation of the European Parliament and of the Council)</td>
<td>Vitamins and minerals may not be added to: (a) unprocessed foodstuffs, including, but not limited to, fruit, vegetables, meat, poultry and fish; (b) beverages containing more than 1.2 % by volume of alcohol...’</td>
<td>Some Member States require the mandatory addition of some vitamins and minerals to certain ordinary foods, for reasons dictated by public health considerations. These reasons may be pertinent at national or even regional level, but would not currently justify harmonisation of the mandatory addition of nutrients across the Community. However, if and when this became appropriate, such provisions could be adopted at Community level.’</td>
<td>Vitamins and minerals may be added to foods voluntarily by food manufacturers ‘...’</td>
</tr>
</tbody>
</table>

**References:** * (164), *(165), *(166, 167), *(154)

*A spreadable food composed of edible oils & water in the form of an emulsion of the type water-in-oil & margarine is an edible oil spread containing no less than 800g/kg of edible oils.*
Even though dietary sources of vitamin D are enhanced, most people do not consume enough dietary vitamin D to meet the national recommended guidelines (126, 151, 161-163), especially those in the US. Table 7 summarizes the average adult vitamin D dietary intake from selected countries and compares it to the US recommended dietary allowance (RDA) of 15µg/day. The diet alone supplies between 14%-56% of the vitamin D RDA (2.1-8.4 µg), with the US having the highest dietary intakes (142, 144). The NZ adult nutrition survey (2008/09) excluded vitamin D intake (168) due to the incomplete NZ Food Composition Database (NZFCD). However, a recent assessment of the NZ Total Diet Study, for vitamin D content, using the NZFCD and the fortified data from MFD, estimated that NZ typical vitamin D intake was about 2.7-5.8 µg/day (145).

Though, the accuracy of vitamin D dietary intake data is affected by a number of factors. Different dietary intake assessment methods are used (Table 7), and some (diet record and 24 hour recall) are more accurate than others (food frequency questionnaires) (169). Most food composition databases are not complete. The US only recently updated their databases with more accurate vitamin D values (170-172), and currently the European Food Information Resource project, aims to harmonise food composition databases across Europe (173). The recent assessment of the NZFCD found few vitamin D values originating from NZ and that fortified food data was outdated (145).

The vitamin D content in food composition tables are sometimes missing, taken from manufacturers’ food labels (145, 173) or borrowed from other countries (145, 173), even though the vitamin D content of the same food varies from country to country (171, 174, 175). Some values are based on one or two samples (145, 176), but samples of the same food may vary. For example, the vitamin D content in fish depends on the season, the species (136) and whether it is
Table 7: Average vitamin D dietary intake from food and supplements in various countries.

<table>
<thead>
<tr>
<th>Study name &amp; reference</th>
<th>Country (period)</th>
<th>Number of Subjects</th>
<th>Age group (years)</th>
<th>Dietary assessment method</th>
<th>Vitamin D intake (µg/day)†</th>
<th>Food only</th>
<th>Supplements only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittaway et al. 2013 (180)</td>
<td>Australia (2009-2010)</td>
<td>91</td>
<td>60–85</td>
<td>113-item SQFQ</td>
<td>4.4-4.5 (30%)</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>US NHANES (170)</td>
<td>United States (2009-2010)</td>
<td>1,930</td>
<td>40-59</td>
<td>2 x MP 24-h DR (in person &amp; telephone).</td>
<td>8.4 (56%)</td>
<td>10.9 (73%)</td>
<td></td>
</tr>
<tr>
<td>Anderson et al. 2010 (175)</td>
<td>Canada (2002-2003)</td>
<td>3,393</td>
<td>25-74</td>
<td>Modified 178 items mailed FFQ</td>
<td>5.3 (35%)</td>
<td>4.4 (29%)</td>
<td></td>
</tr>
<tr>
<td>CCHS Cycle 2.2 (182)</td>
<td>Canada (2004)</td>
<td>15,810</td>
<td>19-70</td>
<td>24-h DR</td>
<td>5.8 (39%)</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>NDNS (183)</td>
<td>United Kingdom (2008-2010)</td>
<td>807</td>
<td>19-64</td>
<td>4-day food diary</td>
<td>2.9 (19%)</td>
<td>0.9 (6%)</td>
<td></td>
</tr>
<tr>
<td>EPIC study (184)</td>
<td>Scotland &amp; England (2006-2008)</td>
<td>481</td>
<td>55–70</td>
<td>7 &amp; 4 day food diary</td>
<td>3 (20%)</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>NDNS (185)</td>
<td>Republic of Ireland (2008-2010)</td>
<td>1,132</td>
<td>18 – 84</td>
<td>4-day food diary</td>
<td>4.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>EPIC study (186)* (1992 – 2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>28,694</td>
<td>SQFQ</td>
<td>4.7 (31%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>67,696</td>
<td>QDQ</td>
<td>2.7 (18%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>27,864</td>
<td>QDQ</td>
<td>3.6 (24%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>30,434</td>
<td>Mostly 35-70</td>
<td>QDQ &amp; SQFQ</td>
<td>2.1 (14%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>35,226</td>
<td>SQFQ</td>
<td>4.2 (28%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>26,287</td>
<td>SQFQ &amp; 14-day hot meal record</td>
<td>7.2 (48%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>26,432</td>
<td>QDQ</td>
<td>4.3 (29%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain (2009)</td>
<td>418</td>
<td>18-60</td>
<td>2 x 24-h DR</td>
<td>3.2 (21%)</td>
<td>0.3 (2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† The percentage (%) in these columns is the % of the US and Canadian recommended dietary allowance (DRIs) for 19-70 year olds (15µg/d) supplied by either food or supplements.

24-h DR = 24-hour dietary recall, µg = micrograms, CCHS = Canadian Community Health Survey, EPIC = European Prospective Investigation into Cancer, FFQ = food-frequency questionnaire, MP= multiple-pass, NDNS = National Diet and Nutrition Survey, NHANES = Nutritional Health and Nutrition Examination Survey, NR = not recorded, QDQ = quantitative dietary questionnaire, SQFQ= semi-quantitative food frequency questionnaire.

*This EPIC study assessed a number of European countries and the dates, age and supplement information is the same for all these countries.
farmed or wild (177). The vitamin D content in fish is halved when cooked in vegetable oil (136, 177) and reduced in milk when exposed to light (178). Vitamin D content may vary in the same sample across different laboratories and with different assessment methods (179).

2.2.2.2 Supplements

Dietary vitamin D intake can be increased with supplements (180, 181). However, in many countries, including NZ, non-prescription dietary supplement regulations are more lenient than prescription products (187). A study of selected NZ non-registered and non-prescribed supplements found that the actual vitamin D content ranged from 8% to 201% of the declared value (187).

Vitamin D$_3$ supplements are seen as more effective in raising s25OHD levels compared to vitamin D$_2$ supplements (188, 189), especially when given in large doses (190), but not always (191). Vitamin D$_2$ was also found to reduce s25OHD$_3$ concentration (189). Regardless of form, users of vitamin D supplements are found to have higher s25OHD concentrations compared to nonusers (19, 153, 180, 185).

Table 7 shows that the total daily dietary vitamin D intake from supplements ranged from 2% (0.3 μg/d) in Spain, where 3.8% of the sample used supplements (162), to 73% (10.9μg/d) in the US, where 37% of the US population used vitamin D supplements (192). In NZ 3.9% of the NZ population is prescribed a monthly 1250 μg (1.25 milligrams (mg)) vitamin D$_3$ tablet (135), but use of other vitamin D supplements was not recorded (193).

A NZ survey indicated that 87% of general practitioners were worried about their patients’ vitamin D status (194). In response to this concern, a consensus statement was issued by the
Ministry of Health and Cancer Society, recommending vitamin D supplementation only for people at high risk of vitamin D deficiency (135, 195). Those at high risk are not exposed to the sun or avoid it, are dark skinned and have specific medical conditions (135, 195). In contrast to this, in Canada, Health Canada recommends all adults over 50 years take a daily 400 international units (IU) (10 µg) vitamin D supplement (196), and the Canadian Cancer Society suggest adults take a 1000 IU (25µg) vitamin D supplement in winter (197).

2.2.2.3 Sunlight

With the aid of the sun, the body produces most of the required vitamin D (180, 198-200). The sun’s UVB (280-320 nm) rays (55, 201), convert 7-dehydrocholesterol, a precursor in the skin to the inactive previtamin D₃ which is converted to vitamin D₃ in a heat-dependent process (119). Increased s25OHD is seen with increased sun exposure, which eventually reaches a plateau (127). This may be because sunlight also breaks down the previtamin D₃ and vitamin D₃ into inactive metabolites, and thus vitamin D toxicity from the sun is not possible (184).

Exposing the skin to UV radiation is the main risk factor for the most hazardous form of skin cancer; melanoma (202, 203). Melanoma cases have increased from 1968 to 2007 in Australia and more so in NZ (202). Provisionally 2332 cases of melanoma were registered in NZ in 2012 (204). Thus it is not surprising that some dermatologists concluded dietary or supplemental intake is the best approach to maintaining ideal s25OHD levels (205).

Certain sun protection behaviour affects s25OHD levels (206-208). Even though sunscreen with sun protection factor 8, applied thickly (2 mg cm⁻²), significantly reduced s25OHD levels (209, 210), a review concluded that in a realistic situation sunscreen use was not likely to reduce vitamin D status (208, 211, 212), possibly due to limited knowledge of proper application (213).
Other factors affecting sun exposure include: clothing (180, 208, 214); shade (208); air pollution (55, 118, 215); ozone; aerosols (55, 216); altitude; surface reflectivity (55); and heavy or partly cloudy conditions (216).

Seasonal difference in s25OHD levels, which are normally at their lowest during winter and spring (153, 180, 200, 217-220), are due to reduced sun exposure, as more clothing is worn (221, 222), people go outdoors less and the zenith angle increases (55, 123, 223). The zenith angle is also affected by the time of day (55, 223) and latitude (55, 193, 220, 223), where endogenous vitamin D production is reduced above and below latitudes of approximately 40° north and south, respectively (55).

Establishing the amount of sun exposure needed to produce enough vitamin D with minimal risk of skin cancer is difficult (201). The NZ Ministry of Health suggests some sun exposure for the general population, preferably while being active (135). It specifies the best time of day and year, but length of exposure is not specified (135). McKenzie et al. (224) provide NZ guidelines on time needed in the sun to avoid sunburn and produce vitamin D, and Osteoporosis Australia guidelines (Table 8) recommend the duration of sun exposure according to skin colour (225).

Most recommendations are based on studies which use artificial UV light (226), found to be more effective at increasing s25OHD levels (227, 228). Thus sun exposure recommendations may be too low for sufficient vitamin D production (229); and suggestions have been made to increase s25OHD levels using sun beds (228), however, these are also a risk factor for melanoma (230).
### Table 8: Summary of the Osteoporosis Australia sun exposure recommendations for vitamin D production

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>Season</th>
<th>Skin Exposed</th>
<th>Time of Day</th>
<th>Sun Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately Fair</td>
<td>Summer</td>
<td>arms or equivalent</td>
<td>Mid-morning or mid afternoon</td>
<td>5 - 10 min</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>arms or equivalent</td>
<td>Midday</td>
<td>7 - 30 min</td>
</tr>
<tr>
<td>Darker skin</td>
<td>Summer</td>
<td>arms or equivalent</td>
<td>Mid-morning or mid afternoon</td>
<td>15 - 60 min**</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>arms or equivalent</td>
<td>Midday</td>
<td>20min - 3hrs**</td>
</tr>
</tbody>
</table>

* This table contains the original information from the Osteoporosis Australia website (225); however the presentation has been slightly modified.
** depends on location within Australia

### 2.2.3 The role of Vitamin D in the human body

Serum 1,25(OH)2D maintains bone health (2) by regulating serum calcium and phosphorus levels which are necessary for bone mineralization (114, 231). Serum 1,25(OH)2D promotes calcium and phosphate absorption in the intestine (2). If serum calcium levels are low, PTH is secreted, which increases 1,25(OH)2D production (114). Together PTH and 1,25(OH)2D facilitate the removal of calcium and phosphorus from the bone (232) and stimulate the kidneys to retain more calcium (233). Mineralization cannot occur with low serum calcium levels, eventually resulting in osteopenia, rickets (children) and osteomalacia (adults) (6, 234).

The discovery of VDR in tissues and organs not involved in calcium regulation implies that 1,25(OH)2D has other important roles (3-5). Since this observation, there have been numerous inconsistent studies investigating the association between s25OHD levels and several illnesses (2, 6). Vitamin D deficiency is associated with muscle weakness, falls (6), fractures (131) and physical function (235). In the immune system, it reduces infections and is associated with better outcomes in tuberculosis, acute respiratory infection, multiple sclerosis, dental caries, influenza, HIV and hepatitis C (6).
Low vitamin D levels are associated with stroke (130), cardiovascular disease (236) and its associated risk factors such as diabetes mellitus, arterial hypertension (2) and dyslipidaemia (6). Breast (237, 238), colon (239) and prostate (6) cancer are also associated with lower s25OHD levels. Vitamin D may also play a role in human fertility, pregnancy (6) and cognitive function (section 2.3). Some studies have also found a J or U shaped relationship with s25OHD and the risk of all-cause mortality (6, 240).

### 2.2.4 Measures of Vitamin D status

Serum 25OHD is the most commonly used (118, 131) and generally accepted as the most reliable marker of vitamin D status (241, 242). Serum 1,25(OH)2D should not be used to assess vitamin D status, but in some cases it is, incorrectly so, as it can be misleading. For example, some studies have found that those with the highest 1,25(OH)2D concentrations had the lowest levels of s25OHD and poorer bone health (243). This may be due to low s25OHD levels increasing the secretion of PTH, which in turn increases production of 1,25(OH)2D (243, 244). Also locally produced tissue 1,25(OH)2D is not reflected in the serum 1,25(OH)2D (132, 245, 246). Table 9 summarises the reasons why s25OHD concentrations are a better measure of vitamin D status than 1,25(OH)2D serum levels.

#### 2.2.4.1 Assay Methods

Measuring s25OHD is challenging (247) as it is highly protein bound (126), vitamin D has many molecular forms, it is reduced by direct sunlight (247) and is prone to matrix interference(16, 250). Different laboratories (126, 247) and assays produce inconsistent results (195, 247), for example, assays compared to standard reference material varied from -13.5% to 40% (251).
Table 9: Differences between serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D as measures of vitamin D status.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>25-hydroxyvitamin D</th>
<th>1,25-dihydroxyvitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life</td>
<td>2-3 weeks (244).</td>
<td>4-7 hours (244, 248).</td>
</tr>
<tr>
<td>Ease of measure and price</td>
<td>Nmol/L measure is easier and requires 1 assay method &amp; thus is less expensive (219, 247).</td>
<td>Picomole per litre is difficult to measure (247), requires 2 assay methods (normally RIA &amp; HPLC), is time consuming (243), requires more experience &amp; thus is expensive (219).</td>
</tr>
<tr>
<td>Fewer fluctuations in the serum levels</td>
<td>s25OHD does not seem to be regulated (2).</td>
<td>Serum 1,25(OH)2D is regulated by serum calcium, phosphate, PTH, 1,25(OH)2D levels (129) &amp; fibroblast growth factor (2). Only when s25OHD levels are ± 10 nmol/L does 1,25(OH)2D decrease significantly (249).</td>
</tr>
</tbody>
</table>

1,25(OH)2D = 1,25-dihydroxyvitamin D, HPLC = high performance liquid chromatography, nmol/L = nanomole per litre, PTH = parathyroid hormone, RIA = radioimmunoassay, s25OHD = serum 25-hydroxyvitamin D

The three assay categories, their descriptions, disadvantages and advantages are summarised in Table 10. Methods are constantly being improved and thus disadvantages and advantages are always changing (247). Currently immunoassay methods are the most commonly used (247) followed by, the current “gold standard” method for measuring s25OHD levels (247, 252), liquid chromatography combined with mass spectrometry (LC-MS) (247, 250). The Food and Drug Administration approved DiaSorin’s radioimmunoassay (RIA) for clinical use in the US (242), which was found to agree with LC-MS across a wide concentration range (7.7-425 nmol/L) (253).

The accuracy of results should be improved significantly with the availability of the new reference method procedure and the international reference standard material (247). Also laboratories are registering in proficiency programs, such as the Vitamin D External Quality
Table 10: Categories of current assay methods measuring serum 25-hydroxyvitamin D, their description, advantages and disadvantages.

<table>
<thead>
<tr>
<th>Assay category and description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>

25OHD = 25 hydroxyvitamin D, CPBA = competitive protein binding assay, DBP = vitamin D binding protein, FDA = Food and Drug Administration, HPLC = high performance liquid chromatography, LC-MS = liquid chromatography tandem mass spectrometry, nmol/L = nanomole per litre, RIA = radioimmunoassay.
Assessment Scheme, which enables them to compare their performance with other laboratories (134).

2.2.4.2 Serum 25-hydroxyvitamin D reference range

There is still no agreement as to the optimal s25OHD levels (252). With recent literature implicating vitamin D in a number of chronic diseases (6), new optimal levels have been proposed ranging from 75-100 nmol/L (130). Insufficient or suboptimal levels are categorised by some between 51 and 74 nmol/L (27, 254, 255). Sufficient levels are defined as >75 nmol/L (148, 255, 256) mainly because serum PTH decreases with increasing levels of s25OHD and reaches a plateau at approximately 75 nmol/L (257). Serum 25OHD levels over 75 nmol/L have not always proven to be beneficial (2), and a few studies show a J- or U- shaped relationship, where risk of death or a disease are increased at low and high levels (240). The trough varies between studies (40-120 nmol/L) (240, 259, 260) and a meta-analysis concluded a non-linear optimal level of 75-87.5 nmol/L (261).

The revised DRIs recommend s25OHD levels of 50 nmol/L to meet the need of 97.5% of the healthy population; and are based on s25OHD levels that will maintain bone health (maximize calcium absorption, and reduce risk of rickets, fractures, and osteomalacia) (2). The levels required for vitamin D’s other roles are unclear (252). As 50 nmol/L has been the most commonly used cut-off (2, 21-23, 29, 30), and as there is insufficient good quality evidence linking s25OHD to other chronic diseases (2), the NZ Ministry of Health recommends aiming for s25OHD levels of 50 nmol/L or above (135).

Vitamin D deficiency also has various cut-offs across the literature, such as <25nmol/L (131), <30 nmol/L (128), <50nmol/L (255) and <80 nmol/L (262). Typically s25OHD levels of <25
nmol/L are seen as deficient (13, 131, 135), as calcium absorption and serum 1,25(OH)2D levels are reduced significantly at this level (257).

The safety of s25OHD levels above 125 nmol/L for long periods are unknown and thus not recommended (2, 135). An overdose with oral vitamin D can occur (263) and excessive intake results in hypercalcemia and hypercalciuria (2, 109). High serum calcium is not seen with levels below 220 nmol/L and was found in normal adults at levels ≥700 nmol/L (263); it can form stones in soft tissue especially the kidneys and can harden blood vessels (2, 109). Hypercalciuria can lead to a loss of bone mineral density (264) due to urinary calcium wasting (263).

2.2.5 Vitamin D status

A recent systematic review concluded a high prevalence of vitamin D deficiency (<30 nmol/L) across all ages, and was most pronounced in the Middle East (1). Deficient adults ranged from 0.2% in Mexico (males) to 36% in Bangladesh (females), with the highest prevalence among the elderly (1). Serum 25OHD levels <50nmol/L ranged from 1% in Vietnam (males) to 80% in Bangladesh (females) (1). Examples of vitamin D status in selected countries are presented in Table 11.

In NZ the average s25OHD levels were 63 nmol/L (193), which were higher than previously reported levels of 50 nmol/L (265) and 48nmol/L (266). Most of the countries listed in Table 11 reported similar mean s25OHD levels. These higher levels do not reflect a high prevalence of vitamin D deficiency, and may be due to higher vitamin D supplement use (29, 153, 270).
Table 11: Vitamin D status in selected countries.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country &amp; Period assessed</th>
<th>Sample size &amp; age (years)</th>
<th>s25OHD assessment method</th>
<th>Mean s25OHD (nmol/L)</th>
<th>&lt;25 nmol/L (%)</th>
<th>&lt;30 nmol/L (%)</th>
<th>&lt;50 nmol/L (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mason et al., 2012 (193)</td>
<td>New Zealand 2008-2009</td>
<td>3,099 ≥ 15</td>
<td>LC-MS</td>
<td>63</td>
<td>5%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Daly et al., 2012 (267)</td>
<td>Australia 1999-2000</td>
<td>11,247 ≥ 25</td>
<td>CLIA</td>
<td>63</td>
<td>4%</td>
<td>31%</td>
<td></td>
</tr>
<tr>
<td>Looker et al., 2011 (268)</td>
<td>US 2001-2006</td>
<td>24,411 ≥1</td>
<td>RIA</td>
<td>± 60</td>
<td>8%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Cashman et al., 2013 (185)</td>
<td>Ireland 2008-2010</td>
<td>1,132 18–84</td>
<td>ELISA</td>
<td>60</td>
<td>7%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Bates et al., 2012 (269)</td>
<td>UK 2008-2011</td>
<td>582 19-64</td>
<td>CLIA</td>
<td>48</td>
<td>18%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Janz et al., 2013 (153)</td>
<td>Canada 2009-2011</td>
<td>6,395 3–79</td>
<td>CLIA</td>
<td>64</td>
<td>10%</td>
<td>32%</td>
<td></td>
</tr>
</tbody>
</table>

s25OHD = serum 25-hydroxyvitamin D, CLIA = competitive chemiluminescent immunoassay (Liaison 25OHD), ELISA = enzyme immunoassay, LC-MS = liquid chromatography and tandem mass spectrometry, nmol/L = nanomoles per litre & RIA = radioimmunoassay.

2.2.6 Factors affecting serum 25-hydroxyvitamin D levels

Numerous factors can affect circulating vitamin D levels, the ones most commonly adjusted for in the literature, not already mentioned will be discussed here. Serum 25OHD levels are generally lower among the elderly (21, 32, 218, 235, 271), however, this is not always the case (32, 181, 193) and may be due to the elderly taking more supplements (153, 270).

The lower s25OHD levels associated with age (21, 25, 33) may be due to: the skin’s reduced ability to convert the precursor to vitamin D (130, 235); the liver (131, 235) and kidney’s (131) diminished ability to add an OH group to 25OHD (219) due the reduced 1α-hydroxylase
activity; a decline in VDR (131); the elderly spend less time outdoors (235, 272); and they eat less food, thus possibly less vitamin D rich food (235).

A meta-analysis of 394 studies concluded that women had a borderline significantly higher mean s25OHD level (p=0.05) compared to men (215). However, this is not always the case, others have found either women had lower s25OHD levels (19, 25, 218, 235, 273) or there was no significant difference between genders (181, 193), however this may be due to women taking more supplements (23, 153). A higher family income (33, 181, 218, 271, 274) and a higher education (19, 25, 33) are also associated with higher s25OHD concentrations. But some studies found no association between socioeconomic status and s25OHD (217).

Ethnicity and skin pigmentation are associated with s25OHD concentrations (19, 275, 276), yet these finding are not always consistent (23, 215, 217, 275, 277). Increased sun exposure does not increase s25OHD levels significantly in black people (275, 276), unless exposed for long periods (278). Melanin absorbs and deflects UV rays (279), making them unavailable for 7-dehydrocholesterol (276), affecting the ability to produce pre-vitamin D₃ (130). Darker skin types have a higher melanin content which increases with sun exposure (279). In NZ skin pigmentation may explain lower vitamin D levels found in Pacific People (193), Māori (13) and Māori women (193).

Numerous studies found lower s25OHD levels associated with a higher BMI (32, 33, 153, 180, 181, 218, 271, 273) and total body fat percentage (180, 273), but not all (193, 280). The reason for this relationship remains unclear and proposed reasons include: increased storage of s25OHD in adipose tissues decrease its bioavailability (281, 282); VDR in adipose tissue indicate that this tissue may regulate or may be regulate by s25OHD (282), those with higher
BMI have poorer dietary habits (283); and get less sunlight exposure due to clothing and lower physical activity (274, 282). Those less active tend to also have lower s25OHD levels (19, 33, 218, 235, 284, 285), but not all (275). Possible reasons include the association with BMI, spending less time outdoors (284), and physical activity may have an effect on vitamin D’s absorption, synthesis or metabolism (284).

Associations have also been found, even though not consistently (19, 32, 217), between lower s25OHD and depression (88), smoking (32, 218, 235, 271, 273), lower alcohol intake (25, 32, 33, 218, 235), and higher serum creatinine (218) (an indicator of renal function known to influence s25OHD levels (219)). Lower s25OHD levels are also associated with some medication taken for chronic diseases which seem to interfere with vitamin D metabolism, such as, some anticonvulsants (286) and oral steroids (287). Serum 25OHD was inversely related to PTH (217, 243, 288, 289) and positively association with phosphate, calcium (217)(section 2.2.3), oral contraception and exogenous oestrogen (217).

2.3 Cognitive function and vitamin D

2.3.1 Vitamin D’s role in the brain

Recently VDR and 1α-hydroxylase were found in glial cells and neurons in the human brain (7-9). Not only is 1,25(OH)2D produced locally in the brain but vitamin D metabolites cross the blood brain barrier (290) and are also present in cerebrospinal fluid (291, 292). Reviews on animal experiments and in vitro studies show convincing evidence that vitamin D plays an important role in the brain (10, 11). There is suggestion that 1,25(OH)2D aids in brain cell growth and degeneration (10, 293); and controls transcription for numerous genes (10, 294).
Table 12: Reviews and meta-analyses assessing the association between serum 25-hydroxyvitamin D and cognitive function.

<table>
<thead>
<tr>
<th>Reference &amp; type of study</th>
<th>No.*</th>
<th>Period</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| van der Schaft et al., 2013 (34) Systematic review | 28 | Up to 2012 | Positive association  
Hypovitaminosis D is associated with worse outcome on 1 or more cognitive function tests or a higher frequency of dementia in cross-sectional as well as prospective studies. |
| Pludowski et al., 2013 (6) Review | 12 | 2007 - 2012 | Inconclusive  
Trend appearing with lower s25OHD and worse cognitive function however heterogeneity of study characteristics making it inconclusive. |
| Annweiler et al., 2013 (300) Systematic review & meta-analysis | 10 | Up to 2012 | Positive association  
AD cases had lower s25OHD levels compared to their controls. |
| Etgen et al., 2012 (53) Systematic review & meta-analysis | 15 | 1980 – 2012 | Inconclusive  
Suggestion of an association between cognitive impairment & vitamin D deficiency supported by meta-analysis but inconclusive due to heterogeneity of studies. |
| Ballion et al., 2012 (35) Systematic review & meta-analysis | 37 | up to 2010 | Positive association  
Lower s25OHD levels were associated with worse cognitive function and a higher risk of AD. |
| Barnard et al., 2010 (298) Review | 5 | 2006-2009 | Inconclusive  
Cognitive function assessed by MMSE is not associated with s25OHD but other cognitive function tests found an association with higher s25OHD levels & better cognitive function performance. |
| Annweiler et al., 2009 (299) Systematic review | 5 | 1979-2008 | Inconclusive  
Association between s25OHD and cognitive performance is not yet clear. |

*number of studies reviewed  
s25OHD = serum 25-hydroxyvitamin D, AD = Alzheimer’s disease, MMSE = Mini Mental Status Examination
Evidence also suggests Vitamin D has a neuroprotective role through various mechanisms, such as acting as an antioxidant (10) and anti-inflammatory agent (295). It protects against toxicity by regulating neuronal calcium levels and reducing the toxicity when exposed to 6-hydroxydopamine (10). It plays a helpful role in neurotransmitter expression (10). Decreases Aβ deposits by blocking protein transcription of the amyloid precursor (294) and increases Aβ clearance (296). All of these roles suggest vitamin D may have a therapeutic effect (51) and may possibly be used as a treatment or prevention for dementia (297). However, well-designed RCTs in humans are required to confirm the findings found in animal experiments and in vitro studies (10).

2.3.2 Association between cognitive function and vitamin D

Recently there have been a number of papers reviewing studies examining the relationship between vitamin D and cognitive function (Table 12). The two earlier reviews (298, 299) were inconclusive, but were mainly based on a few small cross sectional studies that did not always adjust for confounders, and had different sample characteristics (for example, memory clinic visitors, secondary hyperthyroidism and mild dementia). Subsequently, more studies and reviews (6, 10, 34, 35, 53, 300, 301) were published.

The more recent reviews were based on larger, nationally representative cross-sectional and prospective studies. Three (34, 35, 300) out of the five more recent reviews concluded that low s25OHD levels were associated with cognitive impairment or an increased risk of dementia. The other two reviews (6, 53) were inconclusive due to the heterogeneity of the study characteristics, but both acknowledged the positive association seen in many of the papers. In the review by van der Schaft et al. (34), four out of the six prospective studies concluded that overtime there was a
decline in cognitive function or an increase in the number of dementia cases with lower s25OHD levels or vitamin D intake (34).

Recent studies investigating the association between s25OHD levels and cognitive function are summarized in Table 13 (cross-sectional studies) and Table 14 (prospective studies), and include some new studies (18, 19, 21, 31, 33, 302) not included in the reviews. With some exceptions (20, 24, 28, 30), most of the papers in Table 13 and Table 14 (15, 17-20, 22, 23, 26, 27, 29, 30, 33, 88, 302, 303) found an association with lower s25OHD levels and worse cognitive function or an increased risk of MCI or dementia. The studies in Table 13 and Table 14 show that the association with worse cognitive function and s25OHD concentrations were mainly found at levels below 25 or 30 nmol/L (15-17, 20, 25-27, 33, 88, 302), where measured, with a few exceptions (24, 28). The association above this level was inconsistent, and was either positively (15, 20, 26, 33), inversely (26, 28) or no longer associated with cognitive function (19, 20, 25, 29, 32). For example, Afzal et al. (33) found low s25OHD levels associated with the risk of AD at levels of 25 nmol/L or below, but the association did not reach statistical significance above this level. Llewellyn et al. (26) found that memory was positively associated with s25OHD levels below 25 nmol/L but at 50-74 nmol/L it was negatively associated with s25OHD (OR 0.6 (0.4, 0.8)). These results suggest a non-linear relationship.

Maddock et al. (31) found no association between s25OHD and cognition when performing a linear regression. However, they did find a significant non-linear association, where s25OHD levels <25 and ≥75 nmol/L were associated with worse cognitive function and levels between 50-75 nmol/L were associated with better cognitive function. Others also found a non-linear
Table 13: Cross-sectional studies investigating the association between cognitive function and serum 25-hydroxyvitamin D.

<table>
<thead>
<tr>
<th>Reference &amp; Study</th>
<th>Sample characteristics</th>
<th>Cognitive performance tests and measure ‡</th>
<th>Cognitive domains assessed</th>
<th>s25OHDAssessment method &amp; categories *</th>
<th>Confounders considered &amp; exclusions</th>
<th>Analytical method &amp; adjusted results**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annweiler et al., 2012 (15). Gait &amp; Alzheimer Interaction Tracking study.</td>
<td>95 non-demented Caucasian community-dwellers with memory complaint. France 2009-2010 ≥60 years</td>
<td>MCI diagnosis: specialist assessment, MMSE, physical examination, blood tests &amp; MRI brain imaging. MCI = 45% (n=43).</td>
<td>Orientation, registration, attention, recall, naming, repetition, comprehension writing &amp; construction.</td>
<td>RIA Quartiles: Q1: 10-40 (25%), Q2: 41-59 (27%), Q3: 60-79 (24%), Q4: 80-189 (23%)</td>
<td>Age, gender, BMI, chronic diseases, education, MMSE score, depression, creatinine &amp; season.</td>
<td>Logistic regression: MCI associated with lower s25OHD. Continuous s25OHD: OR 0.96 (0.93, 0.98) p = 0.002. Categorical s25OHD: vs Q4: Q1: OR 50 (4.6, 533.3) p = 0.002 Q2: OR 7.4 (1.2, 46.9) p = 0.03 Q3: OR 10.2 (1.2, 85.9) p = 0.02.</td>
</tr>
<tr>
<td>Annweiler et al., 2010 (17). Epidémiologie de l’Ostéoporose cohort.</td>
<td>752 community dwelling women (multicentre). France 1992-1994 ≥ 75 year</td>
<td>Pfeifer Short Portable Mental State Questionnaire. Cognitive impairment = &lt;8 (n=78)</td>
<td>Orientation (306).</td>
<td>RIA Categories: Def.: &lt;25 (17%) Non-def.: ≥25 (83%)</td>
<td>Age, BMI, chronic diseases, depression, psychoactive drug use, education, PA, PTH &amp; calcium.</td>
<td>Linear regression: No association between cognitive scores &amp; s25OHD (p=0.512). Logistic regression: vs ≥25 s25OHD &lt; 25 associated with cognitive impairment: OR 1.99 (1.1, 3.5) p=0.017.</td>
</tr>
<tr>
<td>Brouwer-Brolsma et al., 2013 (21). ProMuscle Study.</td>
<td>106 frail /prefrail participants. Netherlands ≥65 years</td>
<td>MMSE, Word Learning Test Wechsler digit span forward &amp; backward test, TMT A &amp; B, Stroop Colour-Word Test, Verbal Fluency test &amp; Reaction Time Task.</td>
<td>recall, recognition, attention, working memory, information-processing speed, executive functioning &amp; more.</td>
<td>Isotope dilution-online solid phase extraction LC-MS. Tertiles: T1: &lt;34 T2: 34-52 T3: 53-125 Categories: &lt;30 (17%) &lt;50 (53%) ≥75 (23%)</td>
<td>Age, gender, BMI, education, smoking, alcohol intake, PA &amp; season.</td>
<td>Linear regression: MMSE: no association (p = 0.77). Better executive function associated with higher s25OHD: β 0.007 ± 0.003 p = 0.01. No association with attention &amp; working memory, information processing speed &amp; episodic memory.</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td>-------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Buell et al., 2010 (22). Nutrition &amp; Memory in Elders study.</td>
<td>318 low income community dwellers with diminished ability to perform activities &amp; had an unmet need. US 2003-2007 65–99 years</td>
<td>Specialist assessment. Assessed: AD with ADRDA criteria. Vascular dementia with AIREN criteria. Other dementias with DSM-IV criteria.</td>
<td>RIA Categories: Def.: &lt;25 Insufficient: 25-50 Sufficient &gt;50.</td>
<td>Age, race, gender, BMI, education, chronic diseases, multivitamin use, season, PA, plasma homocysteine &amp; APOE ε4 allele status.</td>
<td>Logistic regression: s25OHD associated with a higher prevalence of dementia (n=70): vs &gt;50 (p=0.02) ≤50: OR 2.2 (1.1, 4.3). s25OHD borderline associated with a higher prevalence of AD (n=37): vs &gt;50 (p=0.05) ≤50: OR 2.7 (0.99, 7.2).</td>
<td></td>
</tr>
<tr>
<td>Oudshoorn et al., 2008 (303).</td>
<td>225 outpatients diagnosed with probable AD. 60-94 years</td>
<td>MMSE. Dementia diagnosed with DSM-IV &amp; AD with ADRDA. Orientation, registration, attention, recall, naming, repetition, comprehension writing &amp; construction.</td>
<td>RIA Categories: Def.: &lt; 50 (63%) Sufficient: ≥ 50</td>
<td>Age, gender, mobility score, action radius, education &amp; other vitamin levels.</td>
<td>Linear regression: Lower s25OHD associated with lower MMSE scores: β=0.05 p=0.01.</td>
<td></td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td>------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Buell et al., 2009 (23). Nutrition and Memory in Elders study.</td>
<td>1,080 (377 black &amp; 703 Caucasian) low income community dwellers with a diminished ability to perform activities &amp; had an unmet need (307). US 2006 + 65-99 years</td>
<td>MMSE &amp; North American Adult Reading Test (for eligibility) &amp; Neuropsychological test battery.</td>
<td>Language, memory, executive function, processing speed, visuospatial &amp; anxiety.</td>
<td>RIA Categories: Def. = &lt;25 (18%) Insufficient = 25-50 (47%) Sufficient ≥50 (35%)</td>
<td>Age, gender, race, BMI, education, site, kidney function, season, PA, alcohol use, homocysteine, APOE ε4 status, plasma B vitamins, multivitamin use, diet &amp; chronic conditions.</td>
<td>Linear regression (continuous s25OHD): Higher s25OHD <strong>associated</strong> with better cognition (TMT A &amp; B, digit symbol, matrix reasoning, block design &amp; digit span tests) (p &lt;0.05). <strong>No association</strong> with memory tests. Linear regression (categorical s25OHD): Higher s25OHD <strong>associated</strong> with better cognition: vs &lt;25 (p &lt;0.05) &gt;50: executive function, TMT A, TMT B, matrix reasoning &amp; digit span 25-50: executive function, TMT B, block design, and digit span. <strong>No association</strong> with memory tests.</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Lee et al., 2009 (88). European Male Ageing Study.</td>
<td>3,369 community-dwelling men. Italy, Belgium, Poland, Sweden, UK, Spain, Hungary, Estonia. 2005 40–79 years</td>
<td>Rey–Osterrieth Complex Figure test, the Camden Topographical Recognition Memory test &amp; Digit Symbol Substitution Test (DSST).</td>
<td>Visuospatial (constructional), memory, recognition &amp; processing speed.</td>
<td>RIA Categories: Def.: &lt;25 Insufficient: 25–49 Suboptimum: 50–75</td>
<td>Age, education, depression, BMI, PA, physical performance, smoking, alcohol use, centre &amp; season.</td>
<td>Linear regression: Lower s25OHD associated with worse DSST scores only: Continuous: $\beta/10$ nmol/L = 0.152 ($p&lt;0.01$). Categorical: vs ≥75 50-74: $\beta$=-0.76 ($p&lt;0.05$) 25-49: No association $\beta$=-0.77 ($p&gt;0.05$) &lt;25: $\beta$=-1.4 ($p&lt;0.05$). Stratified by age: DSST associated in &gt;60yrs not with 50-59yrs.</td>
</tr>
<tr>
<td>McGrath et al., 2007 (28). Third National Health &amp; Nutrition Examination Survey.</td>
<td>11,232 (12-19 years = 1,676, 20-59 years = 4,747, 60-90 years = 4,809) community-dwellers (oversampled non-Hispanic blacks &amp; Mexican-Americans). US 1988-1994 12-90 years</td>
<td>20-59 years: 5 measures from the Neurobehavioural Evaluation System psychometric measures. (average of several trials, with lower scores = superior performance). 60-90 years: Learning and memory (higher score = superior performance).</td>
<td>20-59 years: visual attention, psychomotor speed, coding speed, general attention, concentration, learning &amp; immediate memory. 60-90 years: Learning and memory.</td>
<td>RIA Quintiles: 20-59 years: Q1: &lt;40 Q2: 40-52 Q3: 53-66 Q4: 67-85 Q5: &gt;85 60-90 years: Q1: &lt;42 Q2: 42-55 Q3: 56-69 Q4: 70-85 Q5: &gt;85</td>
<td>Age, sex, race/ethnicity &amp; PA.</td>
<td>Linear regression: Negative association between s25OHD &amp; learning &amp; memory task. 60-90 year: lower scores found with Q2 &amp; Q5 ($p=0.02$) Q1: 6.5, Q2: 6.4 Q3: 6.6, Q4: 6.6 Q5: 6.4. Logistic regression: No association but negative association at Q2: vs Q5 ($p=0.11$) 20-59 years: Q1: 0.75 (0.52, 1.09) Q2: 0.67 (0.47, 0.96) Q3: 1.00 (0.72, 1.40) Q4: 0.80 (0.58, 1.11).</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>--------------------------</td>
<td>-------------------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Llewellyn et al., 2011 (26). Third National Health and Nutrition Examination Survey.</td>
<td>3,325 community-dwellers. US 1988-1994 ≥65 years</td>
<td>MMSE (6 items), East Boston Memory Test story recall task &amp; Weschler Adult Intelligence Scale digits. Impairment = worst 10% of the composite scores distribution.</td>
<td>Immediate &amp; delayed verbal memory, orientation &amp; attention.</td>
<td>RIA Categories: Severe def.: &lt;25 Def: 25-49 Insufficient: 50-74 Sufficient: ≥75</td>
<td>Age, gender, ethnicity, education, BMI, season, PA, smoking, alcohol use, serum vitamin E, combined family income, chronic diseases &amp; impaired mobility.</td>
<td>Logistic regression: Cognitive impairment is associated with lower s25OHD: vs ≥75 (p = 0.02) &lt;25: OR 3.9 (1.5, 10.4) 25-49: OR 1.4 (1.0, 2.1). No association but memory impairment is negatively &amp; positively associated with s25OHD: vs ≥75 (p = 0.18) &lt;25: OR 3.2 (1.2, 8.44) 50-74: OR 0.6 (0.4, 0.8).</td>
</tr>
<tr>
<td>Seamans et al., 2010 (29). ZENITH study.</td>
<td>387 community-dwellers. France, Northern Ireland &amp; Italy. 55-87 years</td>
<td>Selected Cambridge Neuropsychological Testing Automated Battery.</td>
<td>Visual memory, working memory &amp; attention.</td>
<td>Enzyme-linked immunosorbent assay. &lt;30(12%) &lt;50(36%) Tertiles: T1: &lt;48, T2: 48-86, T3: &gt;86</td>
<td>Excluded if took &gt;3 drugs / supplements. Centre, gender, age, season, erythrocyte zinc status &amp; BMI.</td>
<td>Linear regression: Less spatial working memory errors associated with higher s25OHD: All: T1 vs T3: ß = 5.36 (p=0.04). Female: T1 vs T3: ß = 10.84 (p=0.004). T1 vs T2 = no association.</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Slinin et al., 2012 (20). Study of Osteoporotic Fractures.</td>
<td>6,257 community-dwelling, healthy Caucasian women. US 1992-1994 ≥65 years</td>
<td>mMmMMSE &amp; TMT B. Cognitive impairment = mMmMMSE below &amp; TMT B. scores above 1.5 SD of sample mean.</td>
<td>Orientation, concentration, language, executive function &amp; memory.</td>
<td>Mass spectrometry. Categories: Severely def.: &lt;25 (7%) Def.: 25-49 (33%) Insufficient: 50-74 (39%) Sufficient: ≥75 (21%)</td>
<td>Site, season, age, chronic diseases, education, health, impairment of daily activities, PA, smoking, BMI, depression, vitamin D supplements.</td>
<td>Logistic regression: No association (study concluded association but not significant) mMmMMSE: vs ≥75 (p=0.29) &lt;25: OR 1.60 (1.05, 2.42). TMT B: no association (p=0.314).</td>
</tr>
<tr>
<td>Wilson et al., 2014 (19). Health, Aging &amp; Body Composition study.</td>
<td>2,777 Medicare-eligible, well-functioning, community-dwellers. US 1997-1998 70-79 years</td>
<td>Modified MMSE &amp; Digital Symbol Substitution Test Orientation, concentration, language, visuospatial function, memory processing speed &amp; executive function.</td>
<td>RIA (at 1 year follow-up). Categories: Def.: &lt;50 (33%) Insufficient: 50-74 (36%) Sufficient: ≥75 (31%)</td>
<td>Education, age, gender, race, site, season, BMI, alcohol use, smoking, PA, chronic diseases, depression &amp; calcium, vitamin D &amp; multivitamin supplements.</td>
<td>Linear regression: Higher s25OHD associated with better cognitive function scores: mMmMMSE (n=2708): p=0.02 &lt;50: ß = 89.9 (89.4, 90.4) 50-74: ß = 91 (90.5, 91.4) ≥ 75: ß = 90.6 (90.2, 91.1). DSST (correct answers) (n=2680): p=0.01 &lt;50: ß = 35.2 (34.5, 36) 50-74: ß = 35.9 (35.2, 36.6) ≥ 75: ß = 37.0 (36.3, 37.8).</td>
<td></td>
</tr>
</tbody>
</table>

* all values in this column are s25OHD levels measured in nanomoles/litre and % is the percentage of participants with those s25OHD levels

**this column has mainly the significant results, values are s25OHD (nanomoles/litre), odds ratio (OR) or ß coefficient (ß) and 95% confidence interval in brackets

‡ the % or number is the number of participants with MCI, cognitive impairment or dementia.

Abbreviations: AD = Alzheimer’s disease, ADRDA = AD & Related Disorders Association criteria, AIREN = Association Internationale pour la Recherche´ et l’Enseignement en Neurosciences, APOE ε4 = ε4 alleles in apolipoprotein E, ß = beta coefficient, BMI = body mass index, DDST = Digit Symbol Substitution Test, def. = deficient, DSM-IV = Diagnostic and Statistical Manual of Mental Disorders (4th Edition), LC-MS = liquid chromatography-tandem mass spectrometry, MCI = mild cognitive impairment, mMmMMSE = modified MMSE, MMSE = Mini Mental State Examination, MRI = magnetic resonance imaging, n= number, OR = odds ratio, PA = physical activity, PTH = parathyroid hormone, Q = quintiles or quartiles, RIA = radioimmunoassay, s25OHD = serum 25-hydroxyvitamin D, SD = standard deviations, TMT = Trial Making Test, T = tertiles, US = United States, vs = verses / compared to, yrs = years, ZENITH = Zinc Effects in Nutrient/Nutrient Interactions and Trends in Health and Ageing.
<table>
<thead>
<tr>
<th>Reference &amp; Study</th>
<th>Sample characteristics</th>
<th>Cognitive performance tests and measure ‡</th>
<th>Cognitive domains assessed</th>
<th>s25OHD assessment method &amp; categories *</th>
<th>Confounders considered &amp; exclusions</th>
<th>Analytical method &amp; adjusted results**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afzal et al., 2014 (33). Copenhagen City Heart Study.</td>
<td>10,186 adults with no dementia. Denmark 1981-1983 20-100 years ±21 year follow-up.</td>
<td>Incident diagnoses of AD &amp; vascular dementia from the national Danish Patient Registry and the national Danish Causes of Death Registry using International Classification of Diseases.</td>
<td>Chemiluminescent immunoassay (LIAISON® 25OH vitamin D TOTAL assay). Stored till measured in 2009-2010. Categories: Def. = &lt;25 Insufficient =25-49 Sufficient = ≥50</td>
<td>Age, gender, smoking, BMI, PA, alcohol use, income, education, chronic diseases, creatinine &amp; season.</td>
<td>Cox proportional hazards regression: Low s25OHD is association with risk of dementia &amp; AD. Seasonally adjusted percentile categories: Risk of dementia: vs &gt;50 (p=0.02): ≤ 25 = HR 1.27 (1.0, 1.6) 26-50 = HR 1.24 (1.0,1.5). Risk of AD: vs &gt;50 (p=0.03) ≤ 25 = HR 1.29 (1.01, 1.66). Vascular dementia = no association.</td>
<td></td>
</tr>
<tr>
<td>Knekt et al., 2010 (302). Mini-Finland Health Survey.</td>
<td>3,173 participants free of Parkinson disease. Finland 1978 to 1980 50-79 years 29 year follow-up.</td>
<td>Parkinson disease cases identified through linkage with the nationwide Drug Imbursement Register of the Social Insurance Institution.</td>
<td>RIA Frozen till 2002 when measured. Quartiles: men: Q1: 8-28 Q2: 29-41 Q3: 42-56 Q4: 57-159 women: Q1: 7-25 Q2: 26-36 Q3: 37-49 Q4: 50-151</td>
<td>Gender, age, marital status, education, alcohol use, leisure-time PA, smoking, BMI &amp; month of blood draw.</td>
<td>Higher s25OHD levels associated with a reduced risk of Parkinson disease: vs Q1 (p=0.005) Q4: RR 0.33 (0.14, 0.80).</td>
<td></td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>--------------------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Bartali et al., 2014 (18). Nurses’ Health Study.</td>
<td>1,185 well educated healthy Caucasian women. US 1989-2001 60-70 years 9 year &amp; 6 year (every 1.5-2 years) follow-up</td>
<td>Initial Telephone Interview of Cognitive Status, telephone adapted MMSE &amp; other tests. Combined global composite score &amp; verbal memory composite score.</td>
<td>Memory, language &amp; processing speed.</td>
<td>RIA (baseline measurement) Quintiles: Q1: &lt;44 Q2: 44-57 Q3: 58-68 Q4: 69-83 Q5: 84-184 Categories: &lt;37 37-75 &gt;75</td>
<td>Excluded if low baseline cognition scores. Age, education, assay batch, time between blood draw &amp; cognitive interview, season, husband’s education, smoking, aspirin use, menopause age, vitamin E supplements, depression, chronic diseases, alcohol use, hormone use, BMI, PA &amp; health status.</td>
<td>Lower s25OHD associated with worse cognitive performance nine years late. Mean difference highest in Q1: vs Q5 Global composite score: (p=0.009) Q1: -0.20, Q2: -0.07, Q3: -0.03, Q4: -0.10 Category fluency score: (p=0.01) Q1: -1.17, Q2: -0.36 Q3: -0.36, Q4: 0.18 A weak non-linear relationship was found. No association with verbal score, TICS score &amp; digit backward. Categories: vs &gt;75 Mean difference in global score was highest at: &lt;37: -0.18 (-0.31, -0.04) No associated with 3 other follow-ups (6yrs later).</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>----------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Breitling et al., 2012 (32). ESTHER study.</td>
<td>1,639 who visited general practitioners. Germany 2000–2002 ≥ 65 years 5 year follow-up.</td>
<td>At 5 year follow up: COGTEL version A, phone interview based on Wechsler Memory Scale-Revised &amp; the Wechsler Adult Intelligence Scale-Revised.</td>
<td>Prospective, verbal short-/long-term working memory, verbal fluency &amp; inductive reasoning.</td>
<td>Automated chemiluminescence assays: Women: LIAISON® competitive immuno-luminometric. Men: delayed competition chemiluminescent immunoassay by Immunodiagnostic Systems. Categories female: Q1: 17, Q2: 24 Q3: 32, Q4: 45 Categories male: Q1: 37, Q2: 51 Q3: 64, Q4: 79</td>
<td>Age, school education, BMI, current smoking, alcohol intake, season, cancer, cerebrovascular disease &amp; depression.</td>
<td>Linear regression: Adjusted for: education, age, BMI &amp; season: lower scores overtime association with lower s25OHD: Women: vs Q5 (p=0.018) Q1: -2.1, Q2: -0.43 Q3: -0.37, Q4: 0.04 Men: vs Q5 (p=0.045) Q1: -1.68, Q2: -1.75 Q3: -0.89, Q4: 0.91. Further adjustment = no association in men / women Cubic spline: showed non-linear association, scores decrease with lower s25OHD (p=0.015).</td>
</tr>
<tr>
<td>Slinin et al., 2010 (30). Osteoporotic Fractures in Men Study.</td>
<td>Approximately 1,138 community-dwelling men (multicentre). US 2000-2002 ≥65 years ± 4.6 follow-up years.</td>
<td>mMMS &amp; TMT B. Measured at baseline &amp; follow-up. Cognitive impairment = &lt;80 score or ≥ 5 pt decline on follow-up (± 1SD change) &amp; TMT B (shorter time better score) = ≥ 1 SD above mean change in time &gt;50.7 sec.</td>
<td>Orientation, concentration, language, executive function, praxis, attention, psychomotor speed, cognitive shifting, complex sequencing function &amp; memory.</td>
<td>LC-MS Quartiles: &lt;50 50-62 63-74 ≥ 75</td>
<td>Excluded those with baseline cognitive impairment. Age, season, site latitude, ethnicity, education, health status, functional status, smoking, alcohol, BMI &amp; PA.</td>
<td>Lower decline in MMSE scores were borderline associated with higher s25OHD when adjusted for only age, site &amp; season: Q1: 1.53 (0.99, 2.37). Further adjustment = no association. TMT B = no association.</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------</td>
<td>------------------------------------------</td>
<td>-------------------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Slinin et al., 2012 (20). Study of Osteoporotic Fractures.</td>
<td>5,336 community-dwelling, healthy Caucasian women. US 1992–1994 ≥65 years 4 year follow up.</td>
<td>mMMSE &amp; TMT B. Cognitive decline = decline &gt;1 SD from mean change in score.</td>
<td>Orientation, concentration, language, praxis, executive function &amp; memory.</td>
<td>Mass spectrometry. Categories: Severely def. = &lt;25 Def. = 25-49 Insufficient = 50-74 Sufficient = ≥ 75</td>
<td>Excluded those with baseline cognitive impairment / dementia. Site, season, age, education, health status, impairment in daily living activities, smoking, BMI, hypertension, diabetes, PA, depression, baseline cognitive function &amp; vitamin D supplementation.</td>
<td>Logistic regression: Lower s25OHD associated with higher odds of cognitive decline: mMMSE: vs 75 (p &lt; 0.003) &lt; 25: OR 1.58 (1.12, 2.22) 25-49: OR 1.31 (1.04, 1.64). TMT B: no association (p=0.931).</td>
</tr>
<tr>
<td>Reference &amp; Study</td>
<td>Sample characteristics</td>
<td>Cognitive performance tests and measure ‡</td>
<td>Cognitive domains assessed</td>
<td>s25OHD assessment method &amp; categories *</td>
<td>Confounders considered &amp; exclusions</td>
<td>Analytical method &amp; adjusted results**</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Llewellyn et al., 2010 (25). InCHIANTI Study (Invecchiare in Chianti).</td>
<td>858 participants Italy 1998-2006 6 year follow up (assessment every 3 years) ≥ 65 years</td>
<td>MMSE, TMT A &amp; B. Substantial cognitive decline (34%) = decline of ≥ 3 MMSE score points &amp; scoring in the worst 10% of cognitive decline/too many mistakes on TMT A &amp; B at follow-up.</td>
<td>Visuospatial scanning, sequential processing, motor speed, attention &amp; executive function.</td>
<td>RIA Categories: severely def. = &lt;25 (20%); def. = 25-49 (42%); insufficient = 50-74 (19%); Sufficient ≥ 75 (18%)</td>
<td>Age, gender, education, baseline cognitive score, season tested, alcohol use, smoking, BMI, depression, total energy intake, serum vitamin E level &amp; impaired mobility.</td>
<td>Logistic regression: Those with s25OHD &lt;25 declined by 0.3 MMSE points per year more than those with ≥ 75: MMSE: vs ≥75 (p=0.02) &lt;25 RR 1.60 (1.19, 2.00). TMT A: no association. TMT B: vs ≥ 75 (p=0.04) &lt;25 RR 1.31 (1.03-1.51).</td>
</tr>
<tr>
<td>Wilson et al, 2014 (19). Health, Aging &amp; Body Composition study.</td>
<td>2,234 Medicare-eligible, community-dwellers. US 1997-1998 70-79 years 4 year follow-up.</td>
<td>mMMSE &amp; Digital Symbol Substitution Test. Measured at baseline &amp; 4 year follow-up.</td>
<td>Orientation, concentration, recall, language, visuospatial, verbal fluency, processing speed, executive function &amp; working memory.</td>
<td>RIA Measured at 1 year follow-up. Categories: Def.: &lt;50 (33%); Insufficiency: 50-74 (36%); Sufficiency ≥ 75 (31%)</td>
<td>Education, age, gender, race, site, season, BMI, alcohol, smoking, PA, renal function, depression, use of calcium, multivitamins, vitamin D supplements, baseline cognitive scores, diabetes &amp; Cardio vascular disease.</td>
<td>Linear regression: Lower s25OHD is borderline associated with greater decline in cognitive function scores: mMMSE scores (n=2207): (p=0.05) &lt;50: -1(-1.5, -0.6) 50-74: -0.8(-1.2, -0.3) ≥ 75: -0.2(-0.7 to -0.2). No association with DSST (n=2175) (p=0.22).</td>
</tr>
</tbody>
</table>

Abbreviations: AD = Alzheimer’s disease, ADRDA = AD & Related Disorders Association criteria, ß = beta coefficient, BMI = body mass index, DDST = Digit Symbol Substitution Test, def. = deficient, DSM-IV = Diagnostic and Statistical Manual of Mental Disorders (4th Edition), HR = hazards ratio, LC-MS = liquid chromatography-tandem mass spectrometry, MCI = mild cognitive impairment, mMMSE = modified MMSE, MMSE = Mini Mental State Examination, OR = odds ratio, PA = physical activity, Q = quintiles or quartiles, RIA = radioimmunoassay, RR = relative risk, s25OHD = serum 25-hydroxyvitamin D, SD = standard deviations, SPMS = Pfeiffer’s Short Portable Mental Status, TMT = Trial Making Test, T = tertiles, US = United States, vs = verses / compared to.

*all values in this column are s25OHD levels measured in nanomoles/litre and % is the percentage of participants with those s25OHD levels.

**this column has mainly the significant results, values are s25OHD (nanomoles/litre), OR, HR, RR or ß coefficient and 95% confidence interval are in brackets.

‡ the % or number is the number of participants with MCI, cognitive impairment or dementia.
relationship (32, 88), for example, a similar trend was seen in the unadjusted results of a large study, where those diagnosed with dementia were more likely to have s25OHD levels <36nmol/L and >59 nmol/L (304), and those with the lowest prevalence of dementia had s25OHD levels between 49-59 nmol/L (304). Brouwer-Brolsma et al. used spline regression to explore the non-linear dose-response relationship between s25OHD levels and cognitive performance, and found a threshold level of approximately 60-80 nmol/L (305).

Out of the ten prospective studies summarised in Table 14 seven papers found an association with lower s25OHD and worse cognitive function (18-20, 25) or higher odds of dementia (16, 33, 302). Two other studies (30, 32) found the same association but only when minimally adjusted, when further adjustments were made there was no association found; and one study found only a non-linear association (31). Even though, a significant difference in scores over time was observed, the differences were modest, for example 0.2 (18) and 0.7-0.8 (19). This decline may be due to normal age-related decline and may not reflect MCI. Breitling et al. (32) suggested that a 0.3 point decrease in the COGTEL was normal per year.

2.3.2.1 Association according to gender

Studies with only female participants showed an association between lower s25OHD levels and worse cognitive function (16-18, 20), but studies with only male participants had inconsistent results, some found no association (24, 30) and others found an association (27, 88). When Breitling et al. (32) and Seaman et al. (29). stratified their analysis by gender they found the association was stronger in women and no longer significant for men. However, the opposite was found by Llewellyn at al. (27), where the association was stronger for men and no longer significant for women.
2.3.2.2 Middle aged sample

Most of the papers assessing the relationship between cognitive function and s25OHD levels are with participants \( \geq 60 \) years of age (17, 19-24, 30, 303). Only a few studies have assessed those between the ages of 40 and 60 years. Maddock et al. (31) assessed a sample of 50 year olds and observed only a non-linear relationship. Lee et al. (88) assessed both middle aged and older men (n=3,133, 40-79 years) and found an overall slower information processing speed with lower s25OHD levels, but when they stratified their analysis by age, no significant linear association was seen in those aged 40-59 years (88).

McGrath et al. (28) assessed 12-90 year olds and only found a possible negative association between s25OHD and various cognitive function tests in 20-59 year olds. Seaman et al. (29) assessed 55-87 year olds and found a positive association between less memory errors and higher s25OHD levels. Afzal et al. (33) assessed 20-100 year olds and Knekt et al. (302) assessed 50-79 year olds, and both found low s25OHD levels were associated with a higher risk of dementia and Parkinson disease, respectively. As only three studies were found at the time of this thesis that did an analysis with just a middle aged sample, it is difficult to conclude the effect of s25OHD on cognition in a middle aged sample.

2.3.3 Possible reasons for inconclusive results

It is difficult to compare studies as there are numerous factors that can influence the results, some of these are discussed below.

2.3.3.1 Sample Characteristics

Studies have different sample characteristics and thus results are limited to those populations. For example, some studies had a sample that was well-educated (18, 19, 24), Caucasians (15,
female (17, 18) or just male (24, 88). Some papers assessed only middle aged adults (31), adults over 60 years of age (16, 19, 23, 24) or a wide age range (28, 88). Some samples varied in size, from 25 to 17,099 participants (34, 35), with the smaller samples (15) having less power to detect small differences compared to larger samples (17, 27, 28). Certain studies exclude participants, for example those taking vitamin supplements (24, 303).

Some studies enrolled participants who may not get out as much as community-dwelling participant and thus their s25OHD levels may be affected. These participants included those with memory complaints (15), a diminished ability to perform certain activities (22, 23), frail (21) or those with probable AD (303). Study populations also had different s25OHD levels, in some studies more than 50% of the sample had levels <50 nmol/L (21, 23), but one study (24) only had 6% with levels <50 nmol/L, this may have affected their results, as they found no significant association between s25OHD and cognitive function.

2.3.3.2 Statistical Analysis

Studies used different methods to analyse the data, and because a non-linear relationship is becoming more evident, the method used may partly explain the final results (34). Some performed logistic regression (15, 27) (cognitive function scores as a categorical variable), linear regressions (17, 88) (cognitive function scores as a continuous variable) or Cox proportional hazards regression (33). Some found an association when using linear regression with continuous variables (23, 88) and others only found an association when s25OHD concentrations were analysed as a categorical variable (17).
2.3.3.3 Vitamin D definitions and assay methods

Across studies different definitions, cut-off points (19, 35) quartiles (30), tertiles (21) and quintiles (28) were used. Different assay methods were used to measure s25OHD levels with some being more accurate and precise than others (section 2.2.4.1), most studies used RIA (18, 23, 25, 26, 88), and only a few used LC-MC (21, 30), the current gold standard method (247, 252).

Some studies used baseline data collected and analysed many years ago (18), thus the assessment method may not have been as accurate or precise as methods today. Others analysed blood samples that had been frozen for a few years and may have expired (33, 302). Also all the studies in Table 13 and 14 are based on one measure of s25OHD and there is no guarantee that this single measure reflects a participant’s general vitamin D status throughout the year (195).

2.3.3.4 Cognitive function test

Most of the studies summarized in Table 13 and 14 used different neuropsychological test batteries, making it difficult to compare studies (35). The tests most commonly used were MMSE (15, 21, 23, 26, 35) and modified MMSE (19, 20, 30). Some tests are more sensitive than others at detecting cognitive function changes (section 2.1.3.1) (77). For example, two different studies (26, 28) analysed the same data, but used different cognitive tests and found different results.

Many tests do not have defined validated cut-off points to diagnose MCI or dementia and assess only a lower or higher score (19, 21, 23), which does not mean the participant is cognitively impaired. Some studies use 1.5 SD below or above the sample mean (20, 30), which is not an accurate measure of cognitive impairment. Other studies (15) used a comprehensive measure of
MCI, which included MMSE alongside a specialist assessment, blood tests and magnetic resonance imaging scans (15).

Tests can also have ceiling or floor effect when used on younger and well-educated participants (77, 78). Perhaps Chan et al. (24) found no association between cognitive function and s25OHD levels, as they may have used the wrong test for their well-educated sample (24). Likewise, Maddock et al. (31) may have used cognitive function tests that were not sensitive enough to detect early cognitive function decline and thus their results may be inaccurate.

Studies using a number of different cognitive function tests (18, 21, 88) are more likely to find an association than those only using one or two tests. Some studies measured a number of cognitive domains (15, 21, 28), other studies only measured a few (17, 24, 27, 88). There have been suggestions that s25OHD affects only certain cognitive domains, however, this was not confirmed in the review by van der Schaft et al. (34).

2.3.3.5 Confounders

Adjusting for confounders produces more accurate results and can influence findings. Confounders shown to be significant include general health, exercise, age, level of education, BMI and gender (34). The systematic review by van der Schaft et al. found a number of studies had not adjusted for education, BMI, and six did not adjust for any potential confounders (34). When studies were excluded from the review due to no adjustment for education, the number of studies that found a significant association with vitamin D deficiency and worse cognitive function increased from 72% to 87% (34).
A few studies found no significant association between s25OHD levels and cognitive function after adjusting for confounders (12, 21, 28, 30-32). McGrath et al. (28) was a large study but only adjusted for four confounders and did not adjust for education, which may partly explain their results.

2.3.3.6 Prospective studies

Two prospective studies (18, 32) had no measure of baseline cognitive function and only had one baseline s25OHD measure which was compared to a cognitive function assessment a few years later. Wilson et al. (19), on the other hand, measured cognitive function at baseline and at the 4 years follow-up, but only measured s25OHD levels one year after the baseline cognitive function tests had been done. Thus some studies were unable to adjust for baseline cognitive function or to exclude those that were already cognitively impaired.

If cognitive function decline is gradual, changes may not be detected over a short period of time, thus studies with short follow-up times may be inaccurate. The follow-up period of current prospective studies ranged from 4 (19, 20, 34) to 29 years (302).

2.3.4 Vitamin D supplementation random control trials

Good RCTs examining the effect of vitamin D supplementation on cognitive function, are scarce, Table 15 lists some of the RCT’s available. Results of the trials may be affected by a low vitamin D dose (308, 309), a young sample (310) or a short follow up period (308, 310).

2.3.5 Conclusion

Experiments in animals and in vitro studies give enough evidence that vitamin D plays a role in the brain, however, vitamin D’s effect on the human brain is unclear. The majority of the
Table 15: Random control trials investigating the effect of vitamin D supplementation on cognitive function.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age</th>
<th>No.*</th>
<th>Duration</th>
<th>Dose</th>
<th>Type</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dean et al., 2010</td>
<td>18-30</td>
<td>128</td>
<td>6 weeks</td>
<td>5000 IU/day</td>
<td>Double blind trial.</td>
<td>No association between group allocation &amp; cognitive outcomes.</td>
</tr>
<tr>
<td>(310).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosom et al., 2012</td>
<td>65-80</td>
<td>4,143</td>
<td>7.8 years</td>
<td>400 IU/day with calcium</td>
<td>Randomized double-blind placebo-controlled trial.</td>
<td>No association between treatment assignment &amp; incident cognitive impairment.</td>
</tr>
<tr>
<td>(309).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annweiler et al., 2012</td>
<td>Median age = 80.6</td>
<td>44</td>
<td>16 months</td>
<td>800 or 100,000 IU/day</td>
<td>Pre-post.</td>
<td>Found an improvement in cognitive function overtime.</td>
</tr>
<tr>
<td>(311).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manders et al., 2009</td>
<td>60+ years</td>
<td>176</td>
<td>24 weeks</td>
<td>Nutrient dense drink with a number of vitamins</td>
<td>Randomized, double-blind, placebo-controlled, parallel-group, intervention trial.</td>
<td>Treatment improved cognition but was not statistically significant, it was significant in a subgroup (n=29) of institutionalized elderly people with low BMI only.</td>
</tr>
<tr>
<td>(308).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI = body mass index, IU = international units

Available published literature is cross sectional (17, 21-23, 26, 27, 88), which does not prove causality but indicates an association. More prospective studies are now available (18, 19, 31, 32), which may possibly confirm the findings in cross-sectional studies, however, better designed prospective studies are required.

The heterogeneity between study characteristics, measurements and methods make it difficult to directly compare results (section 2.3.3). The majority of the studies show an association between lower s25OHD levels and worse cognitive function, especially at levels <25 nmol/L (6, 35). A non-linear association is also becoming more evident. As Sempos et al. found the reverse J-shaped association was stronger for persons aged 20 to 64 years (240), perhaps, in order to detect dementia early, more cross-sectional and prospective studies with middle aged adults (40-
60 years) are needed. These will require sensitive cognitive function tests that can detect early stages of cognitive function decline.

Whether low s25OHD levels are a consequence or a cause of cognitive function decline is still unknown. Good RCT’s are scarce and better designed RCT’s are required in order to make recommendations about vitamin D and cognitive functioning, and to determine whether vitamin D supplementation can prevent cognitive decline in those with s25OHD <25nmol/L.
3 Objective

There is no cure or prevention for dementia, but early detection may help prolong its onset. Available literature suggests a positive relationship between low s25OHD and worse cognitive function in those 60 years and older. Few papers have been published on the association between cognitive function and vitamin D status, with a younger (middle aged) study sample. Thus it is unclear whether an association exists between s25OHD and MCI in a middle aged population, as available studies found inconsistent results.

Hence the aim of this thesis is to investigate if a positive association exists between MCI and s25OHD in a middle aged population (50 years) living in the Canterbury DHB region in New Zealand. MCI is assessed using the MoCA, a highly sensitive test for detecting early cognitive decline. Some papers have reported a stronger association between cognitive function and vitamin D status in females compared to males therefore this study also investigated whether there was a gender difference.

3.1 Hypotheses

1. That a low serum 25-hydroxyvitamin D is associated with mild cognitive impairment.

2. That the association between serum 25-hydroxyvitamin D and mild cognitive impairment is stronger in women than in men.
4 Methods

4.1 Study design

This thesis uses baseline cross-sectional data from Canterbury Health, Ageing and Life Course (CHALICE) study participants. The observational prospective CHALICE study, randomly enrolled 403 community-dwelling middle aged adults (49-52 years) living in the Canterbury DHB region, between August 2010 and September 2013. Details concerning the CHALICE study have been described elsewhere (312). The participant selection and recruitment, as well as data collection and management procedures relating to this thesis are briefly outlined below.

The aim of the CHALICE study is to build-up a comprehensive database of established and novel factors that determine health and inform new models to financially sustain the delivery of health services for the aging population (312). The CHALICE study has two phases: a cross-sectional baseline survey plus follow-up assessments. The initial assessment comprises seven modules and involves self-completed questionnaires, a 4-6 hour face to face interview, physical measurements, diagnostic tests, 7 day physical activity log and a 4 day food and beverage diary (FBD). Annually a short postal, phone or email questionnaire is completed for each participant and the intention is to follow up with a full assessment every five years, for the rest of their lives.

The CHALICE study conformed to the ethical standards for human experimentation as established by the Helsinki Declaration 1964 (sixth revision 2008) (312) and ethical approval for the study was obtained from the Upper South A Regional Ethics Committee (URA/10/03/021) (Appendix 1)
4.1.1 Participant selection and recruitment

Between 2010 and 2012, extracts were made in June each year from the Canterbury electoral roll, for electors turning 50 years of age during that year. The New Zealand electoral roll, on which all New Zealanders must be registered to vote (18 years and older), is maintained regularly and was used as a sampling frame. In order to make people aware of the CHALICE study, it received widespread local media coverage in 2009 (312) and community networks were created to help encourage participation by Māori.

Stratified random sampling was used, where the electors were stratified by Māori and non-Māori ethnic groups. As Maori have a higher risk of disease (313), the aim was to oversample the Māori to obtain a ratio of 4:1 non-Māori to Māori. Selected participants were then randomly assigned to one of the available trained CHALICE interviewers. The CHALICE study selection criteria are: participants are around 50 years of age at enrolment living in the Canterbury DHB region, who are non-institutionalized and can complete the assessment.

In 2010, using the electoral roll addresses, selected participants were posted an invitation to participate in the CHALICE study. They were asked to contact the interviewer by return free-post or telephone. If there was no response, participants telephone numbers were obtained from the White Pages or the internet. Participants were called up to four times, over 10-20 days, on different days and at different times (including evenings and weekends). If contact was not made, a second invitation letter was posted approximately 4–6 weeks after the first letter. Another four telephone calls were attempted over another 10–20 days.

If no telephone number was found or if contact was still unsuccessful, two home visits were scheduled, normally on the weekend or in the evening. At this stage if participants were still not
contactable they were classified as ‘End of line (EOL) – protocol complete’. Alternatively, participants were classified as ‘EOL - no valid address or phone number’ if the house was abandoned due to, for example, earthquake damage or if no valid phone number or address was ever found. For these participants, from 2011, the electoral roll was rechecked in November to establish if a participant had another address in the Canterbury DHB region. If a new address was found the protocol was started again, aiming to complete interviews by January the following year.

When participants were contacted, the study was described in detail, if they were interested and met the criteria, an appointment was made to complete the assessment at the CHALICE study offices. Participants were reminded, the day before their assessment, of their appointment and to remember to fast overnight (312). All participants were well informed about the CHALICE study before they supplied informed written consent (Appendix 2). Participants were aware that they could withdraw from the study and request return of their biological samples at any stage (312).

4.1.2 Participant assessment and follow up

The assessment at the CHALICE offices took approximately 4-6 hours, and was completed on a single day for most participants. A few participants did it over two days, for example when services were interrupted by an earthquake, a blind participant needed extra time or a scheduled weekend assessment with diagnostic tests completed on a weekday. The typical structure of an assessment day is illustrated in the flow chart in Figure 1. The assessment consisted of seven modules which involved physical measures, health history, family and social questions, heart tests, mental health questions, cognitive health assessment, and lifestyle questionnaires. Table 16 gives a more detailed description of the modules.
**Figure 1: Typical CHALICE study assessment day flow chart.**
Two weeks after the assessment, a follow up phone call was made to the participant to assess the progress of their 4 day FBD and 7 day exercise log. Once the FBDs were returned, they were checked by trained nutritionists. If there were incomplete sections the interviewers contacted the participants to obtain the required missing information.

A feedback letter was sent to all participants detailing their results. Abnormal results, such a high BMI, abnormal blood test results, elevated blood pressure, impaired cognitive function, psychological health issues and markers of eye disease, were highlighted. If necessary, participants were advised to contact their general practitioner, who in most cases also received their feedback results. Due to the time taken to return, check, enter and analyse the FBDs, nutritional intake feedback was not included.

4.2 Data collection procedures and variable coding

Only the standardised CHALICE study protocols, questions and data relevant to this thesis are described below.

4.2.1 Physical measurements and blood samples

4.2.1.1 Blood samples

Blood samples were collected between 7am and 9am at the assessment, after an overnight fast (10-12 hours) without food or drink, except for water. The research nurse performed venepuncture after the participant rested for 10 minutes. Blood samples were sent on the same day to Canterbury Health Laboratories in Christchurch, which was accredited International Accreditation New Zealand (IANZ) (314) and registered with Vitamin D External Quality Assessment Scheme (315). Samples were stored at -20°C until they were analysed (316).
Table 16: A brief description of the CHALICE study assessment modules.

<table>
<thead>
<tr>
<th>No.</th>
<th>Module</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical measurements.</td>
<td>Blood samples; urine sample; blood pressure &amp; pulse; body measurements; bio-impedance assessment; &amp; fundus photograph.</td>
</tr>
<tr>
<td>2</td>
<td>Personal health history.</td>
<td>Date of birth; ethnicity; relationship status; education; income; employment; home ownership; economic living standards index; medical insurance; current medication; chronic conditions; infections &amp; immunization; digestive disease; sleep pattern; health service utilisation; screening programmes; environmental conditions; tobacco use; &amp; alcohol use.</td>
</tr>
<tr>
<td>3</td>
<td>Wellbeing, family and social history.</td>
<td>Family medical history &amp; its impact; earthquake questionnaire; &amp; attitude &amp; beliefs on: family, relationships, health, ageing, religion, discrimination, experiences, social capital &amp; social standing.</td>
</tr>
<tr>
<td>4</td>
<td>Heart health assessment.</td>
<td>Electrocardiograph (ECG) &amp; ultrasound examination of heart (echocardiograph); blood pressure; &amp; heart rate.</td>
</tr>
<tr>
<td>5</td>
<td>Mental health.</td>
<td>Depression; dysthymia; suicidality; anxiety; manic episode; panic disorder; agoraphobia; social phobia; obsessive-compulsive disorder &amp; hoarding; post-traumatic stress disorder; alcohol abuse &amp; dependence; &amp; substance use.</td>
</tr>
<tr>
<td>7</td>
<td>Lifestyle &amp; physical function.</td>
<td>Exercise history, diet history, explanation of 4 day food &amp; beverage diary, explanation of 7 day exercise log; &amp; physical functional assessment.</td>
</tr>
</tbody>
</table>

No. = number, CHALICE = Canterbury Health, Ageing and Life Course

4.2.1.1.1 Serum 25-hydroxyvitamin D

Blood samples were analysed for s25OHD, a reliable and commonly used marker of vitamin D status (241, 242) (section 2.2.4). Serum 25OHD levels were measured using isotope dilution
high-performance LC-MS (316, 317), which is currently considered the gold standard for measuring s25OHD levels (195, 251). The unit of measure was nmol/L. The inter-assay coefficient of variation (CV) for 25OHD₃ was 11.6% (n=1000), 14.0% (n=1000), 10.9% (n=800), 9.0% (n=1000) and 6.5% (n=1000), at 24.6, 53.3, 71.6, 102.5 and 196 nmol/L, respectively (316). The inter-assay CV for 25OHD₂ was 19.3% (n=800) at 47.8 nmol/L (316). The detection limit was <2 nmol/L for both metabolites (316).

Currently there is no consensus on the optimal s25OHD levels (section 2.2.4.2), the most commonly used cut-off is 50 nmol/L (35, 88, 135), and thus in this thesis s25OHD was dichotomised into insufficient vitamin D (≤50 nmol/L) and sufficient vitamin D (>50 nmol/L), as reported previously (22, 30). In order to test the sensitivity of the analysis, vitamin D was also categorised into: <50 nmol/L, 50-75 nmol/L and >75 nmol/L based on previously reported cut-offs (19). A <25 nmol/L category was not possible as there were only nine participants in this group. Due to the various cut-offs, used in the literature, analyses were also conducted on the extreme tails of s25OHD levels: <40 nmol/L and >80 nmol/L, the 20th and 80th percentiles, respectively.

To enable adjustment for seasonal differences in s25OHD levels (section 2.2.6), the date of assessment was used to determine the season. Season was dichotomized into summer/autumn (December to May months) and winter/spring (June to November months), as has been reported previously (21, 218).

4.2.1.1.2 Serum creatinine

Serum creatinine was measured by Classical Jaffe Reaction using the Abbott c8000 analyser with Abbott reagents (317). Creatinine concentration in the sample is directly proportional to the
degree by which absorbance increases at 500 nanometres due to a complex formed when the serum creatinine bonds with picrate at an alkaline pH (318). The unit of measure was micromoles per litre (µmol/L). The imprecision of the creatinine assay is ≤6% total CV (318).

Serum creatinine is often used as a marker of kidney function (319), where high serum creatinine levels indicate a reduced kidney function. Normal creatinine levels are between: 50-110 µmol/L for adult males and 45-90 µmol/L for adult females (317). As kidney function can have an effect on s25OHD concentrations (219), any participants with values over the normal level had their estimated glomerular filtration rate (eGFR) calculated.

The eGFR is a more accurate guide to kidney function (320) and was calculated using an online calculator (321), utilizing Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (Appendix 3). The CKD-EPI formula, an accurate formula for assessing adult kidney function (320), requires the age, gender, race (black or white) and serum creatinine values of a participant. An eGFR <60 mL/minute per 1.73 m² was used as an indication of renal impairment as reported previously (22, 23, 219, 321, 322).

4.2.1.1.3 Intact parathyroid hormone

Intact PTH was measured in picomoles per litre (pmol/L), using a chemiluminescent microparticle immunoassay (Architect Intact PTH assay) on an ARCHITECT i system (323). This two-step immunoassay involves intact PTH binding with anti-PTH coated paramagnetic microparticles (323). A chemiluminescent reaction is created after adding a conjugate and solutions (323). The reaction is measured as relative light units, which reflects the amount of intact PTH concentration in the sample (323). The reference range for intact PTH is between: 1.6-7.0 pmol/L (317). The measurement range is 0.3-330 pmol/L and the assay is designed to
have precision of \( \leq 9\% \) total CV for the low control and \( \leq 7\% \) CV for medium and high control (323).

### 4.2.1.2 Body mass index and body fat percentage

Body height was measured using a fixed stadiometer with a sliding horizontal headpiece, as done elsewhere (324). Participants stood straight against the wall, looking ahead, without shoes, while the headpiece was lowered and rested firmly on the top of their head. Measurements were recorded to the nearest 0.1 cm.

Bio-impedance analysis was used to measure body weight and body composition, using a calibrated Tanita Body Composition Analyser TBF-300 (325). The participants were asked to remove heavy items like belts and jackets. The information entered for each participant was: age (years), height (meters (m)), weight for clothes (set at 0.0 kilograms (kg)) and body type (kept as standard male or female). Once on the weighing platform their bare feet were positioned to touch boot metal footplates. BMI (weight (kg)/height (m\(^2\))) and body fat percentage were automatically calculated by the analyser.

The BMI was used as a continuous variable as well as a categorical variable. The WHO’s international classification of BMI was used (326), as used in other studies (150): underweight (BMI <18.5 kg/m\(^2\)); normal weight (BMI 18.5-24.99 kg/m\(^2\)); overweight (BMI \(\geq\) 25-29.99 kg/m\(^2\)) and obese (BMI \(\geq\) 30 kg/m\(^2\)). Only one participant in this study was underweight, therefore the underweight and normal weight categories were combined (underweight/normal weight: <24.99 kg/m\(^2\)). To test the sensitivity of the analysis to BMI, a severely obese category was also formed (\(\geq\) 40 kg/m\(^2\)).
4.2.2 Demographics

Demographics formed part of module 2, the face to face interview. The participants responded to questions from a list of answers on a show card and the interviewer recorded the answer on the questionnaire (Appendix 4). Most of the questions in module 2 were based on the 2006/07 NZ Health Survey adult questionnaire (327). Age, gender and date of birth were also recorded in this questionnaire.

4.2.2.1 Ethnicity

Participants were asked, which ethnic group or groups they belonged to (328). Participants were categorised into all the identified ethnic groups and thus a participant could belong to more than one ethnic group (328). To make comparisons between Māori and non-Māori participants, the Māori group consisted of all the participants that self-identified with being Māori, regardless of any other ethnic group they chose. All others were then classed as non-Māori, as done elsewhere (328).

The CHALICE study did not have a self-reported or objective measurement of skin pigmentation which could affect s25OHD levels (177) (section 2.2.6). Thus in this study ethnicity was used as a proxy measure of skin colour, as done previously (182), which has been found to be a better determinant of s25OHD levels compared to objective measures (266). Participants were classified as non-European if they identified with any of the ethnic groups defined as skin of colour or ‘ethnic skin’ (Talakoub and Wesley’s table) (279), regardless of what other ethnic groups they identified with. The rest of the participants were classified as European. Table 17 indicates which CHALICE study ethnic groups went into each category.
Table 17: Ethnic categories and the associated CHALICE study ethnic groups.

<table>
<thead>
<tr>
<th>Ethnic category</th>
<th>Ethnic group the CHALICE study participant identified with</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>British, Hungarian, NZ European, Dutch, Polish, South African, New Zealander, Australian, European English, European</td>
</tr>
<tr>
<td>Non-European</td>
<td>Māori, Cook Islands Māori, Samoan, Tongan, Niuean, Chinese, Iranian, Filipino, Indian, South Korean, Papua New Guinea, Sri Lankan, Malaysian, Malay Singapore, Persian, Indonesian</td>
</tr>
</tbody>
</table>

NZ = New Zealand, CHALICE = Canterbury Health, Aging and Life Course

4.2.2.2 Household income

Household income includes income from everyone who contributes towards living costs in a single private dwelling (329), and is before tax and housing costs, from all sources. The question on household income (1.17 of Appendix 4), comprised 17 options: 15 different income categories from 0 to over $150,001, a ‘don’t know’ category and a ‘refused’ category.

Annual household income was used as a basic indicator of household wealth, and was dichotomised due to the large amount of categories. The cut-off point between low and high household income was based on the average median annual household income results, over three years (June 2010 to June 2013), obtained from the Statistics New Zealand Household Economic Survey (from all sources) (329). The average national median household income was $66,983. Therefore, in this thesis all household income groups below $70,000 were categorised as low annual household income, those above $70,000 were categorised as high annual household income.
To test the sensitivity of the analysis to income, very low and very high household income categories were also used. At the time of this thesis no official measure of poverty had been defined in NZ (330), and others used 50% below the median household income as an indication of poverty (330, 331). In this thesis 50% below the $70,000 cut-off was $35,000, however, there was only a $30,001-$40,000 category, and thus the very low household income threshold group comprised all income categories below $40,000. For the very high household income threshold, the highest household income category ‘$150,001 or more’ was used as the cut-off, as 19% of the participants fell into this category (approximately the 80th percentile), as used by others (332).

4.2.2.3 Education

Participants were asked about their highest completed education qualification that was longer than 3 months of full-time study (1.10 of Appendix 4). The qualification options were ‘no qualification’, ‘secondary school qualification’, ‘post-secondary certificate, diploma or trade diploma’, ‘university degree’, ‘other’, ‘don’t know’ and ‘refuse’.

Education was also dichotomised into low and high education groups. The low education category included: ‘no qualification’ and ‘secondary school qualification’. The high education category included: ‘post-secondary certificate, diploma, or trade diploma’ and ‘University degree’. Where participants choose ‘other’: the ‘high school education’ was classed as low education and ‘apprenticeship’, ‘trade certificate’ and ‘a graduate of business & commerce’ was classed as high education.

4.2.3 Risk Factors
Both smoking and alcohol questions were the same as those in the NZ health survey questionnaire (328).

4.2.3.1 Current Smoking status

CHALICE participants were asked a number of questions on tobacco smoking (4.06 in Appendix 4), however, the question used to assess whether a participant was a current smoker or not was question 4.06c, ‘How often do you now smoke?’ If they responded with: 1. ‘You don’t smoke now’ they were coded ‘no’ to current smoker, and if they responded with: ‘less often than once a month’, ‘at least once a month’, ‘at least once a week’, or ‘at least once a day’ they were coded ‘yes’.

4.2.3.2 Typical alcohol consumption

Various questions relating to alcohol use were asked, however, in this thesis only the first three questions were used to assess typical alcohol use over the last 12 months (4.07 a-c in Appendix 4). All answers had answer options of ‘don’t know’ or ‘refused’.

The participants were first asked if they had a drink containing alcohol in the last year (‘yes’ or ‘no’). If they responded ‘yes’, they were further asked how often they drank (‘monthly or less’, ‘up to 4 times a month’, ‘up to 3 times a week’, ‘4 or more times a week’) and how many drinks they drank on a typical day (‘1 or 2’, ‘3 or 4’, ‘5 or 6’, ‘7 to 9’, ‘10 or more’). The interviewer used ‘the straight up guide to standard drinks’ (333) (Appendix 5) to help participants accurately describe the amount of standard drinks they consumed according to the type of alcohol they drank.
The categorical data on typical alcohol consumption was converted to a continuous variable (grams per day), as previously reported (334). If a participant answered ‘no’ to the first question ‘Have you had a drink containing alcohol in the last year’ they were allocated 0 g/day/year. If a participant said ‘yes’ to this question they were asked how often they had a drink containing alcohol, the answer was then multiplied by either 12 (the number of months in a year) or 52 (number of weeks in a year), whichever was appropriate (Appendix 6 shows the calculations in a table form). For example, if they said three times a week, three was multiplied by 52 weeks to indicate how often they drank in a year.

The answer was then multiplied by the answer to the third question (how many drinks they drank on a typical day). For example, seven to nine, was averaged to eight (the nearest whole number). The answer was then multiplied by 10g of alcohol which is equal to one standard drink (Appendix 5) and divided by 365 days to obtain the g/day.

### 4.2.4 Cognitive function

Cognitive function was assessed using the standardized and validated MoCA test, version 2004 (335). Appendix 7 shows a score sheet; information on the 16 tasks; and the points allocated for each task. The MoCA test has proven to be an effective, accurate and sensitive measure of MCI (45, 78) (section 2.1.3.1). The maximum score obtainable is 30 points, a validated score of 26 or above is considered NCF and a score below 26 indicates MCI (84).

If a participant had ≤12 years of study, they were traditionally given an extra point towards the final MoCA score. However, in the CHALICE study, it was not possible to stratify the education data in this way, so an alternative approach was use where the logistic regression models were adjusted for education. To test the sensitivity of the analysis to the omitted point, a
point was also added to the MoCA scores if the participant had low education and a separate analysis was run with this adjusted data.

### 4.2.5 Depression

In order to detect the presence of current depression, the CHALICE study used part of the Mini-International Neuropsychiatric Interview (MINI); a validated, structured, short (15 minutes) diagnostic interview (336). The MINI was created to be over-inclusive and not miss any true cases. It consists of close ended questions and can be administered by someone with minimal training (336). It is compatible with the International Classification of Diseases and the Diagnostic and Statistical Manual of Mental Disorders (336).

The first two questions of the MINI (A1 and A2) (Appendix 8, section A) are the screening questions. If the participant answered ‘no’ to both, they were asked no further questions (336) and were coded as ‘no’ to current depression. If the participant answered ‘yes’ to questions A1 or/and A2, they were asked a further 7 questions (A3). Participants were marked as ‘yes’ to current depression if they responded ‘yes’ to the A1 and A2 questions and 3 of the A3 questions or they answered ‘yes’ to either A1 or A2 questions and 4 of the A3 questions.

### 4.2.6 General health

Health status was assessed with a validated and well-established questionnaire (337, 338), the improved Medical Outcomes Study Short Form-36 version 2 (SF-36). The SF-36 questionnaire was completed by the participant before the CHALICE assessment day. The SF-36 consists of 36 questions (Appendix 9). Questions included: opinion on general health; assessing whether work or activities were affected by limited physical functioning, emotional problems or pain;
and feelings of vitality or fatigue. The scores were expressed on a 0 to 100 scale, with a higher score indicating better health status.

4.2.7 Chronic diseases

The question ‘Have you ever been told by a doctor that you have or have had…?’ on chronic diseases is part of module 2 (2.02 of Appendix 4): The two chronic diseases used in this thesis are heart disease (heart attack, angina, heart failure, inadequate pumping of the heart, build-up of fluid in the legs or lungs, problems with heart rhythm, heart valves, intermittent claudication and venous thrombosis) and diabetes (excluding gestational diabetes). Participants were coded as either ‘yes’ or ‘no’ for both questions.

4.2.8 Dietary assessment

The four day estimated FBD was used to establish the participant’s usual food and nutrient intake. This method has been shown to be accurate (169) and is commonly used to assess dietary intake in large studies (184, 185). Participants were instructed on how to complete the diary properly. They were asked to supply detailed information, such as type of food or drink, brand, quantity, cooking method and recipe (Appendix 10).

Data from the FBDs were entered, by trained nutritionists, into a food and nutrient analysis programme; Kai-culator (version v1.08d) developed in the Department of Human Nutrition, University of Otago. The food composition database included FOODfiles from Plant and Food Research Ltd as well as recipes calculated for the 2008/09 New Zealand Adult Nutrition Survey. Agreed upon assumptions and standardised protocols were used to ensure consistency and reduce bias. A second nutritionist rechecked all the FBD entries and corrected any errors.
Kai-culator automatically analysed a participant’s dietary protein (g), calcium (milligrams (mg)) and vitamin D (µg) intake for each of the four days. For each nutrient the average over the four days was used as the participant’s average nutrient intake per day.

4.2.8.1 Vitamin D supplementation

All non-prescription and prescription medication and supplements were self-reported by the participant and brought to the assessment for verification, where the CHALICE interviewer wrote down the name, the dose and frequency of use. Ingredients of all supplements and medication recorded were assessed for vitamin D. If vitamin D was one of the ingredients, the dose was recorded and coded ‘yes’ for vitamin D supplement use and if there was no vitamin D present, the data was coded ‘no’.

4.3 Data management

A Data Management Committee managed the CHALICE data (312). A study identification (ID) number was allocated to each participant at the assessment and was used on all the collected data related to them. Only the interviewers were aware of the participants’ names. The raw data are locked in cabinets at the University of Otago CHALICE office. Using only the ID numbers, apart from the FBD data, all data has been entered into the password-protected Progeny database (Progeny Software, Needham, South Norfolk, UK).

A general audit was conducted on all data and a standard protocol was used to clean the data, which involved: a random selection of 10% of the participants; their data were re-entered with different ID numbers; these were compared to the original entries; an error rate was determined; and errors, if any, were corrected. The Progeny database is stored in accordance with the
requirements of the New Zealand Privacy Act (1993) and the Health Information Privacy Code (1994) (312).

4.4 Sample size

Due to the exploratory nature of this thesis, no formal sample size calculation was performed. By Dec 2013, at the time of data analysis for this thesis, 400 participants had been interviewed, and their data had been entered and cleaned.

4.5 Statistical Data Analysis

All data used in this thesis were stored on an Excel spreadsheet. All statistical analyses were conducted using the statistical package R (version 3.0.2 (2013-09-25), R Foundation for Statistical Computing, Auckland, New Zealand). All tests were two-tailed and p-values < 0.05 were considered significant.

4.5.1 Descriptive statistics

Participants’ baseline characteristics were summarized for all participants and various groups using frequencies and percentages (%) or means and standard deviations (SD), which ever were appropriate. Differences in means and frequencies according to the different groups were assessed using the independent Welch’s t-test for continuous variables and the Pearson’s chi-squared ($\chi^2$) test for categorical variables, unless the sample size was small or the expected frequency was <5 then the Fisher’s exact tests was used instead (339). Differences found between the groups were adjusted for where possible in the models.
4.5.2 Exploratory data analysis

Exploratory data analyses using graphs were performed on all variables. Variable distributions were assessed and where appropriate, in order to satisfy statistical test assumptions, log transformations were performed if variables were not normally distributed. Relationships between s25OHD levels and cognitive function, as well as potential confounders were also evaluated using scatterplots.

4.5.3 Univariate preliminary models

Univariate logistic regression models established the relationship between cognitive function (NCF and MCI) and s25OHD levels, as well as all the potential confounders identified in the literature. The associations between vitamin D and factors identified that could potentially confound the relationship between cognitive function and s25OHD levels were also explored using univariate logistic regression models, with vitamin D as a binary outcome variable (≤50 nmol/L and >50 nmol/L). Sensitivity analyses were also conducted on various variable thresholds to determine threshold effect.

Identified potential confounders included: age, gender, education, annual household income, ethnicity, BMI, current smoking status, alcohol intake, creatinine, season of blood draw, PTH, general health status, current depression, vitamin D intake from food, calcium intake from food, dietary protein intake and vitamin D supplement use. Significant interactions in the preliminary univariate models were added to the final multivariate models. Some predictor variables were tested as a continuous variable and retested as a categorical variable. Some categorical variables were tested as two categories and then retested as numerous categories to test the analyses sensitivity to that variable.
4.5.4 Multivariate models

Multivariate logistic regression models were used to establish whether s25OHD levels were associated with the probability of being diagnosed with MCI or NCF (339), while adjusting for and at the same time determining the effects of various potential confounders. All logistic regression results are expressed as beta coefficients ($\beta$) (standard error (SE)), odds ratio (OR (95% confidence intervals (CI)) and Wald type p-value).

Multivariate models were adjusted for demographics first and then variables which were significantly associated with cognitive function in the univariate models. The significant interactions with s25OHD status were added to the final multivariate model individually, to test their effect on the model parameters, if they had no effect they were not included to avoid multicollinearity and over-adjustment. The final model was also adjusted for season of blood draw to account for the seasonal difference in sun exposure, as reported previously (27, 304) and vitamin D supplementation use. Effect change by variables that mutually were predictive of cognitive function and s25OHD were also determined by including interaction terms into the regression models.

A secondary analysis was performed to examine whether the association had the same pattern when analysing men and women separately; adding a point to the MoCA scores for low education; excluding participants who were prescribed the monthly 1.25mg vitamin D tablet or took vitamin D supplements over 1000IU per day; and excluding participants with possible kidney damage. Information on dietary calcium, dietary protein and vitamin D intake existed for a subgroup of 234 participants, and analyses were performed to determine if a similar association was evident when further adjustment was made for these variables.
4.5.5 Assessing the model

To assess which model was the best fit for the data and for predicting cognitive function, deviance (-2LL) using the likelihood ratio with its chi-squared statistic and p-value; Akaike information criterion (AIC) and three different $R^2$ statistics (Hosmer and Lemeshow, Cox and Snell and Nagelkerke), were computed (339).

Standardized residuals were checked for any outliers (any cases above 1.96) (339). To verify if any cases excessively influenced the model parameters the following residual statistics were checked: Cook’s distance >1; hat values 3 times the average leverage value\(^2\), and DFBeta >1 (339). Sensitivity analyses were performed on any outliers, influential cases or odd observations to assess if the conclusion was sensitive to these observations; this was achieved by removing the observations individually and as a group and refitting the model to assess if the conclusion changed substantially (340).

To assess the accuracy of the model the assumptions were checked (339). Collinearity was tested using the variance inflation factor (VIF) (values >10 were considered problematic); the mean VIF (values substantially >1 may indicate bias in the model) and the tolerance statistic (values <0.2 indicate potential problems) (339). To test whether there was a linear relationship between the continuous predictors in the model and the logit of the outcome variable, the interaction term between the predictors and its log transformation were added to each model. A significant value would indicate that the main effect had violated the assumption of linearity of the logit (339).

\(^2\) Number of predictors in a model plus 1 divided by the sample size (339).
5 Results

5.1 Participation rate and sample size

The CHALICE study baseline participation rate\(^3\) at the time analyses for this thesis were conducted was 62\% (n=403), 52\% for Māori and 64\% for non-Māori. At the time of this thesis three participants had not completed their assessment and thus data on only 400 participants was available. Participants’ data were excluded if they had incomplete data necessary for this thesis.

Three of the 400 participants did not complete the MoCA test: one was blind, one did not speak English and one had a reaction to the eye drops given during the assessment. Another 19 participants were unable to do the test properly and interviewers felt that their responses were impaired due to reasons other than natural cognitive function decline (Appendix 11 lists these reasons). A further 12 participants were excluded due to missing total annual household income data. Complete data was available for 366 participants as shown by the flow chart in Figure 2.

5.2 Sample characteristics

A comparison of baseline characteristics between participants included in the study and those that were excluded (n=34) revealed no significant differences in mean age, BMI, general health scores, s25OHD levels, education, gender, current depression and current smoking status (Appendix 12). There was a borderline difference in ethnicity, a possibility that excluded participants were more likely to be non-European than European (OR 2.22 (0.96, 4.95) \(p=0.046\)). The excluded participants also drank on average 0.4g less alcohol per day (\(p=0.011\)) compared to the included participants.

\(^3\) Participation rate = total participants assessed divided by (letters sent out minus no response minus ineligible).
Figure 2: Flow chart of the participants included in this thesis

n = number of participants, MoCA = Montreal Cognitive Assessment
Baseline characteristics of the participants in the study are listed in Table 1 and are also divided according to gender, Māori and non-Māori groups. Among the 366 participants (mean age: 50.8 years, range: 49.1-52.6) included in the analysis 53% were female, 80% were European and 15% self-identified with the Māori ethnic group. Only 29% of the total sample was in the normal or underweight BMI category (BMI <25), with an average BMI of 28.4±6.4. More than half of the participants had a post school education and reported having a household income over $70,000.00.

Mean MoCA test scores were 27±2.3 out of 30. Participants diagnosed with MCI (MoCA scores <26) made up 24% of the total sample. The mean s25OHD level was 63 nmol/L (range: 16-148 nmol/L). Only 124 participants (34%) had s25OHD levels ≤50 nmol/L, nine participants (3%) were deficient (<25nmol/L) and 251 (69%) had levels below 75nmol/L. More blood was drawn during winter/spring than in the summer/autumn period. Serum 25OHD levels were significantly higher (p <0.001) in summer/autumn (mean 74.4±22 nmol/L) than in spring/winter (mean 56.3±23). Supplements containing vitamin D were used by 18% (n=67) of the sample and 4% (n=16) of the sample were prescribed the monthly 1.25 mg (1250 µg) vitamin D\( _3 \) tablet.

5.2.1 Differences between males and females and Māori and non-Māori

There were few differences in baseline characteristics between males and females and the Māori and non-Māori. Non-Māori participants had on average a significantly lower BMI, and the odds of them having a post school qualification was higher (OR 2.02 (1.08, 3.82) p = 0.02) than if they were Māori. With regards to the genders, the significant standardized residuals (std res) showed (339) that significantly more males than expected (std res: 2.16) and less females than expected (std res: -2.04) were overweight and less males than expected used supplements that contained vitamin D (std res: -2.05) compared to females (OR 0.41 (0.22, 0.75) p = 0.002).
Table 18: Baseline characteristics for participants in the study and difference between gender and self-reported ethnicity (Māori and non-Māori).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total participants (n=366)</th>
<th>Males (n=172)</th>
<th>Females (n=194)</th>
<th>Māori (n=53)</th>
<th>Non- Māori (n=313)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.8 ± 0.7</td>
<td>50.7 ± 0.7</td>
<td>50.9 ± 0.7 ***</td>
<td>50.8 ± 0.7</td>
<td>50.8 ± 0.7</td>
</tr>
<tr>
<td>Education (post school qualification)</td>
<td>220 (60)</td>
<td>109 (63)</td>
<td>112 (58)</td>
<td>24 (45)</td>
<td>196 (63) *</td>
</tr>
<tr>
<td>Household annual income (≥ $70,001)</td>
<td>247 (68)</td>
<td>121 (70)</td>
<td>126 (65)</td>
<td>34 (64)</td>
<td>213 (68)</td>
</tr>
<tr>
<td>Female</td>
<td>194 (53)</td>
<td>-</td>
<td>-</td>
<td>30 (57)</td>
<td>164 (52)</td>
</tr>
<tr>
<td>Ethnicity (European)</td>
<td>294 (80)</td>
<td>139 (81)</td>
<td>155 (80)</td>
<td>0 (0)</td>
<td>294 (94) ***</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.4 ± 6.4</td>
<td>28.1 ± 4.6</td>
<td>28.7 ± 7.4</td>
<td>30.1 ± 6.6</td>
<td>28.1 ± 6.1 *</td>
</tr>
<tr>
<td>Normal &amp; underweight (BMI &lt;25 kg/m²)</td>
<td>105 (29)</td>
<td>37 (22)</td>
<td>68 (35) ***</td>
<td>9 (17)</td>
<td>96 (31)</td>
</tr>
<tr>
<td>Overweight (BMI: 25-29.99 kg/m²)</td>
<td>145 (40)</td>
<td>86 (50)</td>
<td>59 (30)</td>
<td>23 (43)</td>
<td>122 (39)</td>
</tr>
<tr>
<td>Obese (BMI ≥30 kg/m²)</td>
<td>116 (32)</td>
<td>49 (29)</td>
<td>67 (35)</td>
<td>21 (40)</td>
<td>95 (30)</td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD (nmol/L)</td>
<td>63.1 ± 24.3</td>
<td>64.0 ± 23.7</td>
<td>62.3 ± 24.5</td>
<td>61 ± 21.7</td>
<td>63.4 ± 24.5</td>
</tr>
<tr>
<td>s25OHD insufficient (≤50 nmol/L)</td>
<td>124 (34)</td>
<td>53 (31)</td>
<td>71 (37)</td>
<td>17 (32)</td>
<td>107 (34)</td>
</tr>
<tr>
<td>Season of blood draw (summer/autumn)</td>
<td>137 (37)</td>
<td>70 (41)</td>
<td>67 (35)</td>
<td>18 (34)</td>
<td>119 (38)</td>
</tr>
<tr>
<td>Vitamin D supplement use (yes)</td>
<td>67 (18)</td>
<td>20 (12)</td>
<td>47 (24) **</td>
<td>10 (19)</td>
<td>57 (18)</td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol intake (grams/day)</td>
<td>1.06 ± 1.10</td>
<td>1.08 ± 1.05</td>
<td>1.04 ± 1.18</td>
<td>1.13 ± 1.22</td>
<td>1.05 ± 1.1</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>55 (15)</td>
<td>24 (14)</td>
<td>31 (16)</td>
<td>13 (25)</td>
<td>42 (13)</td>
</tr>
<tr>
<td>General health (score out of 100)</td>
<td>71.4 ± 19.7</td>
<td>71.0 ± 17.5</td>
<td>71.8 ± 21.5</td>
<td>70.2 ± 17.9</td>
<td>71.6 ± 20.0</td>
</tr>
<tr>
<td>Current depression</td>
<td>31 (9)</td>
<td>11 (6)</td>
<td>20 (10)</td>
<td>6 (11)</td>
<td>25 (8)</td>
</tr>
<tr>
<td><strong>Cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoCA test score</td>
<td>27 ± 2.3</td>
<td>27 ± 2.5</td>
<td>27 ± 2.1</td>
<td>27 ± 2.6</td>
<td>27 ± 2.6</td>
</tr>
<tr>
<td>Mild cognitive impairment (MoCA &lt;26)</td>
<td>89 (24)</td>
<td>43 (25)</td>
<td>46 (24)</td>
<td>16 (30)</td>
<td>73 (23)</td>
</tr>
</tbody>
</table>

Significantly different: Independent t-test (continuous variables) & Fisher’s Exact Test (categorical variables), p-value:*<=0.05, ** <=0.01, *** <=0.001

BMI = body mass index, MoCA = Montreal Cognitive Assessment, n = number of participants, s25OHD = serum 25-hydroxyvitamin D
Table 19: Baseline characteristics of participants with mild cognitive impairment and normal cognitive function.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mild cognitive impairment b (n= 89)</th>
<th>Normal cognitive functiond (n=277)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.7 ± 0.7</td>
<td>50.8 ± 0.7</td>
<td>0.555</td>
</tr>
<tr>
<td>Education (post school qualification)</td>
<td>37 (42)</td>
<td>183 (66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annual household income (≥$70,001)</td>
<td>57 (64)</td>
<td>190 (69)</td>
<td>0.426</td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>46 (52)</td>
<td>148 (53)</td>
<td>0.774</td>
</tr>
<tr>
<td>Ethnicity (European)</td>
<td>68 (77)</td>
<td>226 (82)</td>
<td>0.284</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.4 ± 7.8</td>
<td>27.8 ± 5.5</td>
<td>0.005g</td>
</tr>
<tr>
<td>Normal &amp; underweight (BMI &lt;25 kg/m²)</td>
<td>20 (23)</td>
<td>85 (31)</td>
<td>0.149</td>
</tr>
<tr>
<td>Overweight (BMI: 25-29.99 kg/m²)</td>
<td>34 (38)</td>
<td>111 (40)</td>
<td></td>
</tr>
<tr>
<td>Obese (BMI ≥30 kg/m²)</td>
<td>35 (39)</td>
<td>81 (29)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD (nmol/L)</td>
<td>65.3 ± 22.5</td>
<td>62.3 ± 24.6</td>
<td>0.286</td>
</tr>
<tr>
<td>Insufficient s25OHD (≤50 nmol/L)</td>
<td>21 (24)</td>
<td>103 (37)</td>
<td>0.019</td>
</tr>
<tr>
<td>Season (summer/autumn)</td>
<td>35 (39)</td>
<td>102 (37)</td>
<td>0.671</td>
</tr>
<tr>
<td>Vitamin D supplement use</td>
<td>22 (25)</td>
<td>45 (16)</td>
<td>0.072</td>
</tr>
<tr>
<td>Lifestyle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol intake (grams/day)</td>
<td>1.06 ± 1.01</td>
<td>1.05 ± 1.15</td>
<td>0.954</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>11 (12)</td>
<td>44 (16)</td>
<td>0.418</td>
</tr>
<tr>
<td>General Health (score out of 100)</td>
<td>68.2 ± 21.0</td>
<td>72.3 ± 19.2</td>
<td>0.096</td>
</tr>
<tr>
<td>Currently depressed</td>
<td>16 (18)</td>
<td>15 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cognition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOCA test score</td>
<td>23.7 ± 1.7</td>
<td>27.9 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Independent t-test (continuous variables) & Pearsons chi-squared test (categorical variables).
BMI=body mass index, MoCA = Montreal Cognitive Assessment scores, s25OHD = 25-hydroxyvitamin D
bMoCA <26 and dMocA ≥26
Effect size: g r=0.26

5.2.2 According to cognitive function groups and vitamin D status groups

Table 19 shows characteristics of the participants according to the cognitive function categories: MCI and NCF. Participants with MCI had a significantly higher BMI; were less likely to have a post school education; and were more likely to have s25OHD levels >50nmol/L, however, there
was no significant difference between their mean s25OHD levels. The standardized residuals (std res: 3.08) also showed that significantly more participants with MCI had current depression.

Table 20 lists the participant characteristics according to s25OHD status: ≤50nmol/L and >50nmol/L. A number of differences were found between the two groups. Participants with s25OHD >50nmol/L had lower mean MoCA scores and were less likely to have NCF and be current smokers. Significantly more participants than expected with s25OHD ≤50nmol/L were obese (standardized residual = 2.185) and had a slightly higher mean BMI than those with s25OHD >50nmol/L. Participants with s25OHD levels ≤50nmol/L where also four times (OR 4 (1.88, 9.58)) as likely to not take vitamin D supplements than the >50nmol/L group. On average participants with s25OHD >50nmol/L had significantly higher serum creatinine levels, lower serum PTH levels and the season of blood draw was nearly five (OR 4.5 (2.59, 8.08)) times more likely to be in summer/autumn rather than winter/spring.

5.3 Preliminary Assessment

The MoCA scores, as a continuous variable, were not normally distributed but negatively skewed and transforming the data did not improve the distribution (Appendix 13). The initial assessment using a scatterplot to investigate the relationship between s25OHD levels and cognitive function, as continuous variables, found no non-linear or linear relationship (Figure 3). To overcome the problem of MoCA scores not being normally distributed and the lack of a linear relationship between s25OHD levels and MoCA scores (339), the MoCA scores were dichotomized and converted to a binary variable. The two categories formed were MCI and NCF.
Table 20: Baseline characteristics of study participants according to serum vitamin D status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S25OHD levels (n=124)</th>
<th>S25OHD levels (n=242)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤50 nmol/L</td>
<td>&gt;50 nmol/L</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.9 ± 0.7</td>
<td>50.8 ± 0.7</td>
<td>0.043&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Education (post school qualification)</td>
<td>78 (63)</td>
<td>142 (59)</td>
<td>0.435</td>
</tr>
<tr>
<td>Annual household income (≥$70,001)</td>
<td>85 (69)</td>
<td>162 (67)</td>
<td>0.756</td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>71 (57)</td>
<td>123 (51)</td>
<td>0.243</td>
</tr>
<tr>
<td>Ethnicity (European)</td>
<td>103 (83)</td>
<td>191 (79)</td>
<td>0.346</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.9 ± 6.7</td>
<td>27.7 ± 5.8</td>
<td>0.002&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal &amp; underweight (BMI &lt;25 kg/m²)</td>
<td>25 (20)</td>
<td>80 (33)</td>
<td>0.002</td>
</tr>
<tr>
<td>Overweight (BMI: 25-29.99 kg/m²)</td>
<td>46 (37)</td>
<td>99 (41)</td>
<td></td>
</tr>
<tr>
<td>Obese (BMI ≥30 kg/m²)</td>
<td>53 (43)</td>
<td>63 (26)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D &amp; other serum tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (nmol/L)</td>
<td>37.8 ± 8.7</td>
<td>76.0 ± 18.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>79.9 ± 10.8</td>
<td>82.7 ± 10.2</td>
<td>0.016&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>5.1 ± 2.2</td>
<td>4.1 ± 1.6</td>
<td>&lt;0.001&lt;sup&gt;m&lt;/sup&gt;</td>
</tr>
<tr>
<td>Season (summer/autumn)</td>
<td>21 (17)</td>
<td>116 (48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D supplement use</td>
<td>9 (7)</td>
<td>58 (24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lifestyle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol intake (grams/day)</td>
<td>1.05 ± 1.3</td>
<td>1.06 ± 1.0</td>
<td>0.916</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>27 (22)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>28 (12)</td>
<td>0.01</td>
</tr>
<tr>
<td>General health (score out of 100)</td>
<td>70.9 ± 19.0</td>
<td>71.6 ± 20.1</td>
<td>0.748</td>
</tr>
<tr>
<td>Currently depressed</td>
<td>9 (7)</td>
<td>22 (9)</td>
<td>0.551</td>
</tr>
<tr>
<td>Cognition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montreal Cognitive Assessment test score</td>
<td>27.2 ± 1.9</td>
<td>26.7 ± 2.5</td>
<td>0.044&lt;sup&gt;p&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mild cognitive impairment (MOCA score &lt;26)</td>
<td>21 (17)</td>
<td>68 (28)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

<sup>a</sup> Independent t-test (continuous variables) & Pearsons chi-squared test (categorical variables).

BMI=body mass index, MoCA = Montreal Cognitive Assessment, n=number.

Effect size: <sup>e</sup>r=0.13, <sup>k</sup>r=0.21, <sup>.cleanup_r</sup>r=0.16, <sup>m</sup>r=0.30, <sup>p</sup>r=0.12

In the preliminary univariate logistic regression with cognitive function as the outcome variable and vitamin D as a continuous predictor variable, the result did not reach statistical significance (OR 1 (0.99, 1.01) p=0.306) (Table 21). But when s25OHD was split into insufficient (≤50 nmol/L) and sufficient (>50 nmol/L) vitamin D status, an association, opposite to the
Figure 3: Scatterplots investigating the relationship between serum 25-hydroxyvitamin D and Montreal Cognitive Assessment test scores with an added linear regression line (left) and a smoother (right).

hypothesis, was seen (OR 0.52 (0.30, 0.89) p=0.020) (Table 21). This is illustrated by the boxplot in Figure 4, where participants with insufficient s25OHD levels had mostly higher MoCA scores, however, participants with sufficient s25OHD levels had more varied MoCA scores, both high and low scores.

The univariate logistic regression models identified which potential confounders were significantly associated with cognitive function and vitamin D status (Table 21). A significant association was observed between cognitive function and education (OR 2.74 (1.68, 4.49) p<0.001), BMI (kg/m²) (OR 0.94 (0.90, 0.98) p = 0.001) and current depression (OR 0.26 (0.12, 0.56) p <0.001). Gender, ethnicity (European verses non-European), current smoker, alcohol intake (g/day) and general health, among others, were not associated with cognitive function.
Figure 4: Boxplot of Montreal Cognitive Assessment (MoCA) test scores according to serum 25-hydroxyvitamin D insufficient (≤50 nmol/L) and sufficient levels (>50 nmol/L).

When annual household income was divided at $70,000, no significant association was found with cognitive function groups, however when the lower threshold (>40,000.00) was used to test the sensitivity of cognitive function to income, a significant association was found (OR 2.35 (1.21, 4.48) p < 0.010). Thus this income category was used in the multivariate logistic regression models.

Vitamin D status was significantly associated with: age, current smoker, season of blood draw (OR 4.52 (2.70, 7.86) p < 0.001); serum creatinine; PTH, vitamin D supplement use (OR 4.03, (2.01, 8.99) p < 0.001), BMI (OR 0.94 (0.91, 0.98) p = 0.002) and the obese BMI category compared to the normal/underweight BMI category (OR 0.37 (0.21, 0.66) p=0.001) (illustrated in the scatterplot in Appendix 14) (Table 21).
Table 21: Determinants of cognitive function and serum 25-hydroxyvitamin D levels: univariate logistic regression

<table>
<thead>
<tr>
<th>Predictor variable ‡</th>
<th>Normal cognitive function (n=366)*</th>
<th>Serum 25OHD levels &gt;50 nmol/L (n=366)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.11 (0.18)</td>
<td>0.90 (0.63, 1.27)</td>
</tr>
<tr>
<td>Gender Female (Male)</td>
<td>-0.07 (0.24)</td>
<td>1.07 (0.66, 1.73)</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>-0.31 (0.29)</td>
<td>0.73 (0.42, 1.32)</td>
</tr>
<tr>
<td>Education High (Low)*</td>
<td>1.01 (0.25)</td>
<td>2.74 (1.68, 4.49)</td>
</tr>
<tr>
<td>Annual household income &gt; $70,000 (≤ 70,000)</td>
<td>0.17 (0.28)</td>
<td>1.18 (0.68,2.02)</td>
</tr>
<tr>
<td>Annual household income &gt; $40,000 (≤ $40,000)</td>
<td>0.85 (0.33)</td>
<td>2.35 (1.21, 4.48)</td>
</tr>
<tr>
<td>Annual household income &gt; $150,000 (≤ $150,000)</td>
<td>0.10 (0.32)</td>
<td>1.11 (0.61, 2.10)</td>
</tr>
<tr>
<td>s25OHD levels (nmol/L) (continuous)</td>
<td>-0.01 (0.01)</td>
<td>1.00 (0.99, 1.01)</td>
</tr>
<tr>
<td>Sufficient s25OHD levels &gt; 50 (s25OHD ≤ 50)</td>
<td>-0.65 (0.28)</td>
<td>0.52 (0.30, 0.89)</td>
</tr>
<tr>
<td>s25OHD levels &gt; 75</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>s25OHD levels &lt; 50</td>
<td>0.51 (0.33)</td>
<td>1.66 (0.88, 3.18)</td>
</tr>
<tr>
<td>s25OHD levels between 50-75</td>
<td>-0.06 (0.29)</td>
<td>0.95 (0.53, 1.66)</td>
</tr>
<tr>
<td>MoCA score (continuous)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal cognitive function, MoCA ≥ 26 (MoCA &lt; 25)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>-0.06 (0.02)</td>
<td>0.94 (0.90, 0.98)</td>
</tr>
<tr>
<td>BMI - normal (&lt;25 kg/m²)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>BMI - overweight (25 - 29.99 kg/m²)</td>
<td>-0.26 (0.32)</td>
<td>0.77 (0.41, 1.42)</td>
</tr>
<tr>
<td>BMI - obese (≥ 30 kg/m²)</td>
<td>-0.61 (0.32)</td>
<td>0.54 (0.29, 1.01)</td>
</tr>
<tr>
<td>Current Smoker Yes (No)</td>
<td>0.29 (0.36)</td>
<td>1.34 (0.68, 2.84)</td>
</tr>
<tr>
<td>Alcohol intake (grams/day)</td>
<td>-0.01 (0.11)</td>
<td>0.99 (0.81, 1.24)</td>
</tr>
<tr>
<td>General Health (Score out of 100)</td>
<td>0.01 (0.01)</td>
<td>1.01 (1.00, 1.02)</td>
</tr>
<tr>
<td>Current Depression Yes (No)</td>
<td>-1.34 (0.38)</td>
<td>0.26 (0.12, 0.56)</td>
</tr>
<tr>
<td>Season: summer/autumn (winter/spring)</td>
<td>-0.11 (0.25)</td>
<td>0.90 (0.55, 1.48)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>-0.00 (0.01)</td>
<td>1.00 (0.97, 1.02)</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>-0.02 (0.06)</td>
<td>0.98 (0.87, 1.12)</td>
</tr>
<tr>
<td>Vitamin D supplements use: Yes (No)</td>
<td>-0.53 (0.30)</td>
<td>0.59 (0.33, 1.07)</td>
</tr>
<tr>
<td>Vitamin D intake from food (µg) †</td>
<td>0.05 (0.06)</td>
<td>1.05 (0.95, 1.20)</td>
</tr>
</tbody>
</table>

Each row is a univariate logistic regression with * cognitive function binary outcome variable (mild cognitive impairment & normal cognitive function) or ‡ vitamin D binary outcome variable (vitamin D ≤ 50, vitamin D > 50nmol), ‡ predictor variables placed into a univariate logistic regression model with either cognitive function or vitamin D status (the reference group is in brackets), † only 234 participants returned diet history. *High education = post school education & low education = no education and secondary school education. Abbreviations: β = β coefficient, BMI = body mass index, CI = confidence intervals, MoCA = Montreal Cognitive Assessment, n = number, OR = odds ratio, s25OHD = serum 25-hydroxyvitamin D, SE = standard error.
Gender, education, income, ethnicity, alcohol intake, general health, current depression and vitamin D dietary intake from food were not associated with vitamin D status. A preliminary scatterplot (Appendix 15) revealed a relationship between creatinine and gender, a slight positive relationship was observed with s25OHD levels and females. When an interactive term creatinine x gender was entered into the preliminary logistic regression with s25OHD, there was no significant association (OR 1.01 (0.93, 1.09), p=0.912)(data not shown).

### 5.4 Final Logistic Regression Models

Three different confounder logistic regression models were created to evaluate the relationship between cognitive function groups and s25OHD levels: model 1, gender-adjusted only; model 2, adjusted for gender, education, income (<$40,000/≥$40,000), ethnicity (European/non-European), BMI (kg/m²), depression (yes/no); model 3, adjusted for all the variables in model 2 and further adjusted for season (summer/autumn or winter/spring) and vitamin D supplement use. Table 22 shows the 3 different hierarchical multiple logistic regression models.

The unadjusted preliminary model suggested s25OHD >50 nmol/L was inversely associated with NCF (Table 21) (opposite to the hypothesis). This result remained significant in the gender adjusted model 1 (OR=0.53, p=0.02) and was more pronounced in the fully adjusted model 2 (OR=0.44, p=0.008) and model 3 (OR=0.48, p=0.022) when the effect of the other variables was held constant. Adjusting for season and supplement use (model 3) did not significantly change the results by very much, the effect changed by 3% and thus model 2 was accepted as the final model to avoid multicollinearity and over-adjustment. The adjusted odds of a participant having sufficient vitamin D with NCF were 56% less likely than if the participant had MCI, with a true population effect between 76% and 21% (model 2).
Table 22: Multivariate logistic regression showing the association between normal cognitive function (dependent variable) and serum 25-hydroxyvitamin D (independent variable)

Model 1 (n=366)

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>β (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.61 (0.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (s25OHD ≤50nmol/L)</td>
<td>-0.65 (0.28)</td>
<td>0.53 (0.3, 0.89)</td>
<td>0.020</td>
</tr>
<tr>
<td>Gender Male (Female)</td>
<td>-0.04 (0.25)</td>
<td>0.97 (0.6, 1.57)</td>
<td>0.885</td>
</tr>
</tbody>
</table>

Model 2 (final model) (n=366)

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>β (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.49 (0.80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (s25OHD ≤50nmol/L)</td>
<td>-0.82 (0.31)</td>
<td>0.44 (0.24, 0.79)</td>
<td>0.008</td>
</tr>
<tr>
<td>Gender Male (Female)</td>
<td>-0.21 (0.26)</td>
<td>0.81 (0.48, 1.36)</td>
<td>0.432</td>
</tr>
<tr>
<td>Education High (Low)</td>
<td>0.86 (0.26)</td>
<td>2.36 (1.41, 3.96)</td>
<td>0.001</td>
</tr>
<tr>
<td>Household Income ≥$40,001 (&lt;$40,001)</td>
<td>0.71 (0.37)</td>
<td>2.03 (0.98, 4.12)</td>
<td>0.054</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>-0.06 (0.32)</td>
<td>0.95 (0.51, 1.79)</td>
<td>0.861</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.06 (0.02)</td>
<td>0.95 (0.91, 0.99)</td>
<td>0.009</td>
</tr>
<tr>
<td>Depression Yes (No)</td>
<td>-0.95 (0.42)</td>
<td>0.39 (0.17, 0.89)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Model 3 (n=366)

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>β (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.73 (0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (s25OHD ≤50nmol/L)</td>
<td>-0.74 (0.32)</td>
<td>0.48 (0.25, 0.89)</td>
<td>0.022</td>
</tr>
<tr>
<td>Gender Male (Female)</td>
<td>-0.30 (0.27)</td>
<td>0.74 (0.43, 1.25)</td>
<td>0.263</td>
</tr>
<tr>
<td>Education High (Low)</td>
<td>0.90 (0.27)</td>
<td>2.46 (1.46, 4.16)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Household Income ≥$40,001 (&lt;$40,001)</td>
<td>0.67 (0.37)</td>
<td>1.95 (0.94, 3.99)</td>
<td>0.069</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>-0.03 (0.32)</td>
<td>0.97 (0.53, 1.84)</td>
<td>0.928</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.06 (0.02)</td>
<td>0.94 (0.90, 0.98)</td>
<td>0.007</td>
</tr>
<tr>
<td>Depression Yes (No)</td>
<td>-0.99 (0.42)</td>
<td>0.37 (0.16, 0.86)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

*the variable reference group is in brackets
Comparing the models:
Model 1: −2LL= 400; compared to model with just constant χ² = 5.82 (2) p =0.55, AIC: 406; R²: Hosmer & Lemeshow = 0.01, Cox & Snell = 0.02, Nagelkerke = 0.02.
Model 2: −2LL = 363; compared to model 1 χ² = 37.10 (5) p=<0.001; AIC = 379; R²: Hosmer & Lemeshow = 0.11, Cox & Snell = 0.11, Nagelkerke = 0.17.
Model 3: −2LL = 360; compared to model 2 χ² = 3.07 (2) p=0.22; AIC: 380; R²: Hosmer & Lemeshow = 0.11, Cox & Snell = 0.12, Nagelkerke = 0.18.
Model 1 adjusted for Gender
Model 2 adjusted for Gender, Education, very low household income (less than 40,000), ethnicity, BMI and depression
Model 3 adjusted for Gender, Education, very low household income (less than 40,000), ethnicity, BMI, depression, season blood was drawn & vitamin D supplement use
β = beta coefficient, BMI = body mass index, CI = confidence intervals, n = number, OR = odds ratio, s25OHD = serum 25-hydroxyvitamin D, SE = standard error
Each factor that showed a significant association with s25OHD levels was added to model 2 individually to assess the effect on model parameters (Appendix 16). There was no effect on the relationship between s25OHD and cognitive function when model 2 was adjusted for current smoking, age, creatinine, PTH and categorical variable BMI (Appendix 16). Thus they were left out to avoid multicollinearity. As vitamin D status was significantly predicted by BMI and so was cognitive function it was added as an interactive term in a separate analysis, however the interactive term was not significantly associated with cognitive function and just increased the standard error in the s25OHD level coefficients (Appendix 16).

In addition, high education (model 2 adjusted OR = 2.36 (1.41, 3.96) p=0.001), BMI (kg/m²) (model 2 adjusted OR = 0.95 (0.91, 0.99), p=0.009 (Appendix 17 illustrates this relationship)) and having current depression (model 2 adjusted OR = 0.39 (0.17, 0.89) p=0.023) were also associated with NCF. When a model was created using BMI per 10 units increase, the odds of having normal cognitive function were 43% less than a 10 unit lower BMI (OR 57 (0.37, 0.86) p=0.009) (Appendix 16). In the final model (model 2) the β coefficients did not reach statistical significance if the participant was male, non-European and had a household income >$40,000.00 per annum.

To test the sensitivity of the analysis to different s25OHD levels, s25OHD was categorised into s25OHD <50 nmol/L (n=117), between 50-75 nmol/L (n=144) and >75 nmol/L (n=105) and added to model 2. The only significant association was found between s25OHD <50 nmol/L compared to s25OHD between 50–75 nmol/L (model 2 adjusted OR 2.2 (1.1, 4.51) p=0.028) (Appendix 16). Indicating that the odds of a participant having normal cognitive function are twice as high if they have vitamin D levels <50nmol/L than if they have vitamin D levels between 50 and 75 nmols/L (opposite to hypothesis). A multivariate logistic regression was also performed using continuous
s25OHD in the final model instead of the categorical s25OHD, the association was close to significant but also opposite to the hypothesis (adjusted OR 0.99 (0.98, 1.00) p=0.084).

In a secondary analysis women and men were examined separately and a different pattern of association was observed (Table 23). In women cognitive function was significantly inversely associated with s25OHD levels and depression. In men the effect size remained similar but the association between cognitive function and s25OHD levels was no longer significant, and cognitive function was significantly associated with BMI and education. When model 2 was further adjusted for season and vitamin D supplement use (model 3 in Table 23) the effect size remained the same but was no longer significant in women, the results remained unchanged in men.

Additional models adjusted for self-reported diabetes (yes/no) and heart disease (yes/no) did not significantly change the results (Appendix 16). Similar results were also seen when participants were excluded due to: possible kidney damage (eGFR was over 60 mL/min/1.73 m²) (model 2 adjusted OR 0.45 (0.24, 0.81) p=0.009); and vitamin D supplements use (over 1000IU per day and the monthly prescribed 1.25mg tablet) (model 2 adjusted OR 0.44 (0.24, 0.79) p=0.008) (data not shown). When a point was added to the MoCA scores for all those that did not have a post school education the results did not change appreciably (Appendix 18). Finally when the final logistic regression model was run using all the data (excluded and included participants), cognitive function and vitamin D status association was still evident (OR 0.56 (0.33, 0.94) p=0.032).
Table 23: Logistic regression models illustrating the odds of normal cognitive function according to s25OHD status separated by gender.

### Women (n=194)

<table>
<thead>
<tr>
<th>Predictor variables *</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ß (SE)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Constant</td>
<td>2.26 (0.98)</td>
<td>2.59 (1.05)</td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (s25OHD ≤50nmol/L)</td>
<td>-0.85 (0.42)</td>
<td>0.43 (0.18, 0.94)</td>
</tr>
<tr>
<td>Education High (Low)</td>
<td>0.68 (0.37)</td>
<td>1.98 (0.96, 4.13)</td>
</tr>
<tr>
<td>Household Income ≥$40,001 (&lt;$40,001)</td>
<td>0.59 (0.47)</td>
<td>1.8 (0.69, 4.50)</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>0.34 (0.46)</td>
<td>1.4 (0.59, 3.66)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.04 (0.03)</td>
<td>0.96 (0.91, 1.01)</td>
</tr>
<tr>
<td>Depression Yes (No)</td>
<td>-1.12 (0.54)</td>
<td>0.33 (0.11, 0.96)</td>
</tr>
<tr>
<td>Season: winter/spring (summer/autumn)</td>
<td></td>
<td>-0.31 (0.41)</td>
</tr>
<tr>
<td>Vitamin D supplement use: yes (no)</td>
<td>-0.34 (0.42)</td>
<td>0.71 (0.31, 1.65)</td>
</tr>
</tbody>
</table>

### Men (n=172)

<table>
<thead>
<tr>
<th>Predictor variables *</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ß (SE)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Constant</td>
<td>3.03 (1.47)</td>
<td>3.25 (1.53)</td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (s25OHD ≤50nmol/L)</td>
<td>-0.73 (0.48)</td>
<td>0.48 (0.18, 1.19)</td>
</tr>
<tr>
<td>Education High (Low)</td>
<td>1.07 (0.38)</td>
<td>2.92 (1.38, 6.28)</td>
</tr>
<tr>
<td>Income ≥$40,001 (&lt;$40,001)</td>
<td>0.88 (0.59)</td>
<td>2.40 (0.74, 7.68)</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>-0.47(0.45)</td>
<td>0.62 (0.26, 1.55)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.09 (0.04)</td>
<td>0.91 (0.84, 0.99)</td>
</tr>
<tr>
<td>Depression Yes (No)</td>
<td>-0.88 (0.68)</td>
<td>0.41 (0.11, 1.64)</td>
</tr>
<tr>
<td>Season: winter/spring (summer/autumn)</td>
<td></td>
<td>0.09 (0.41)</td>
</tr>
<tr>
<td>Vitamin D supplement use: yes (no)</td>
<td>-0.92 (0.55)</td>
<td>0.40 (0.14, 1.20)</td>
</tr>
</tbody>
</table>

*variables model was adjusted for with the variable reference group in brackets

β = beta coefficient, SE = standard error, CI = confidence intervals, n = number, OR = odds ratio, BMI = body mass index
5.5 Assessing the models and assumptions

The deviance for model 1 (−2LL= 400) was not statistically better at predicting the outcome than if there were no predictors in the model ($\chi^2 = 5.82 (2) \ p = 0.55$). Model 2 produced a significant improvement in the fit of the model compared to a model 1 (−2LL = 363, $\chi^2 = 37.1 (5) \ p < 0.001$) and model 3 (−2LL = 360, $\chi^2 = 3.07 (2) \ 0.22$) had a lower deviance but was no better than model 2 (Table 22).

Less than 5% of the residuals had absolute values over 1.96 in model 2. Sensitivity analyses found none of these residuals caused any undue influence on the model parameters, and the conclusion did not change substantially when the model was rerun after individual outliers and influential cases were removed individually and all together.

When the VIF, the mean VIF and the tolerance statistic were examined no collinearity was evident within the data. The only continuous predictor in the models was BMI. When the interactive term between BMI and its log transformation was added to each model the interaction term was not significant (data not shown).
6 Discussion and conclusion

6.1 Main Findings

The main finding in this observational study of community dwelling middle aged Cantabrians, was that MCI, as assessed by the MoCA test (scores <26), was generally associated with sufficient s25OHD levels (>50 nmol/L). This cross-sectional inverse relationship remained significant after adjustments were made for potential confounders and participants with possible kidney damage (eGFR <60 mL/minute per1.73 m²) and high vitamin D supplement use were excluded. This association between s25OHD levels and MCI was stronger in women, which appears to be due to supplement use.

6.1.1 Association between serum 25-hydroxyvitamin D and cognitive function

The first hypothesis in this study: that a low serum 25-hydroxyvitamin D is associated with MCI, was not supported by this thesis, but surprisingly an association opposite to the hypothesis was found. The adjusted odds of having NCF was 56% less likely if participants had s25OHD levels >50 nmol/L than if they had s25OHD levels ≤50 nmol/L, with a true population effect between 76% and 21%, when the effect of gender, ethnicity, education, very low income, BMI and depression were held constant.

The results in this thesis are in agreement with some of the findings in a large nationally representative study in the US (28), which also assessed middle aged adults (Table 13). Even though the McGrath et al. study (28) found no association between lower s25OHD levels and impaired cognitive performance, they did, however, find selected negative associations. Those ≥60 years of age (n=4809) with s25OHD levels between 43-56 nmol/L (quintile 2) and >85.4 nmol/L (quintile 5), were found to have inferior performance compared to the rest of the
quintiles (p=0.02) (28). Also in 20-59 year olds (n=4,747), the odds of having poorer cognitive function was lower in those with s25OHD levels between 40-53 nmol/L compared to those with >90 nmol/L (OR 0.7 (0.57,0.96) p=0.11) (the overall comparison did not reach statistical significance) (28). McGrath et al. also found no linear, U- or J-shaped relationship (28).

McGrath et al. commented that interpreting these results required caution and was most likely a false-positive result because they had made no adjustment for multiple comparisons (28). Even though the McGrath et al. study had enough power to identify small effect sizes, they did not adjust for education, one of the most important confounders; and they only assessed learning and memory in the 60-90 year olds.

Llewellyn et al. (26) assessed the same data set as McGrath et al., but only for those ≥65 years of age (n=3,325) and with different cognitive function tests, which covered more cognitive function domains. This time they found a positive association with lower s25OHD and worse cognitive performance. However, they also found a negative association; those at s25OHD levels between 50-74 nmol/L were less likely to have impaired memory than those with levels ≥75 nmol/L (OR 0.6 (0.4,0.8) p = 0.18) (the overall comparison did not reach statistical significance) (26).

Similar findings were found by others. Wilson et al. (19) found a non-linear association, with better cognitive performance at s25OHD levels between 50-74 nmol/L and worse performance at levels <50 and ≥75 nmol/L. Bartali et al. (18) found a weak non-linear relationship and the highest difference in cognitive function over time were found in those with s25OHD levels <44 nmol/L and between 69-83 nmol/L, however the lowest difference found was at levels between 44-69 nmol/L (Table 14). Breitling et al. (32) also found a significant non-linear association and
in their age, BMI, education and season adjusted model, men with s25OHD levels in quartile 2 (51 nmol/L) had a higher cognitive decline than those in the other quartiles. All these study results are in agreement with the results in this thesis, however, because the sample characteristics, vitamin D groups and cognitive function tests differ to those used in this thesis, it is difficult to directly compare results.

A recent study with 50 year old adults by Maddock, et al. (31) (n= 6496) found no association between s25OHD levels and cognitive function when performing linear regression. However, they did find a non-linear association between s25OHD levels (measured 5 years previously) and immediate word recall, where those with levels between 50-75 nmol/L performed better than those with s25OHD levels <25 and ≥75 nmol/L (31). This non-linear association is becoming more evident (19, 21, 32, 88). Recently a reverse J-shape relationship was found between vitamin D status and mortality (240) and a U-shape relationship between vitamin D and fracture risk (304). A similar trend was seen in the unadjusted figures for dementia, participants with the lowest prevalence of dementia had s25OHD levels between 49 and 59 nmol/L (304) and a spline regression in another study indicated a threshold level of approximately 60-80 nmol/L (305).

In this thesis a non-linear relationship was suggested, as linear regression found no significant association between s25OHD and MCI and logistic regression found an association only when s25OHD was a categorical predictor variable, these results are supported by another study with similar findings (17). However, in this thesis when vitamin D was categorised into 3 groups to test sensitivity at different levels, a significant association was found only in the <50nmol group. This suggested that the odds of a participant having NCF compared to MCI, with
s25OHD levels <50 nmol/L were twice as likely (OR 2.2 (1.1,4.5) p=0.028) than those with s25OHD levels between 50-75nmols/L, when adjusted for potential confounders.

Due to the heterogeneity of various assessment methods and the sample characteristic, comparing studies is difficult and may partly explain the different results across studies (section 2.3.3). Maddock et al. used a non-comprehensive cognitive assessment, measuring only two verbal memory tasks, one verbal fluency task and one speed of processing task (31), which may not have been sensitive enough to detect early cognitive function decline in their middle aged sample. Also s25OHD was measured using automated Immunodiagnostic Systems Limited OCTEIA assay which is not as accurate as LC-MS (247). These different methods make it difficult to directly compare results from the Maddock et al. study to the findings in this thesis.

In this thesis the MoCA test was used which is a highly sensitive test, especially in detecting the first stages of cognitive decline (45) and is a better global assessment test than the MMSE (78). At the time of this thesis, no other study was found that used the MoCA test to assess the relationship between cognitive function and s25OHD levels, and therefore no direct comparisons could be made across studies. This thesis also measured s25OHD using the ‘gold standard’, LC-MS (247). These methods may have enabled this thesis to detect changes more accurately, that may have not been evident when using other tests.

The MoCA has a validated cut-off point that defines MCI and NCF, many tests used in other studies did not have well-defined cut-off points (20, 26, 30). Most studies used RIA (19, 25-27, 303) to measure s25OHD levels, and only a few used LC-MS (21, 30). The sample in this thesis, however, was smaller than other studies (28, 31, 88), was not nationally representative as it was based in the Canterbury DHB region and only assessed participants in their early 50’s. Only
Maddock et al. assessed middle aged participants (31), the rest of the studies either assessed a wide age range (28, 88) or older participants (19, 20, 30). This thesis also adjusted for most of the important confounders which a few studies failed to do (34).

Recent systematic reviews and meta-analyses concluded that lower s25OHD levels are associated with worse cognitive performance or higher odds of cognitive impairment or dementia (34, 35, 300). This association is especially evident with s25OHD levels <25 nmol/L (section 2.3.2). The association between s25OHD levels >25nmol/L produced inconsistent results (26, 27, 29, 88). In this thesis only 3% (n=9) of the sample had vitamin D levels below 25 nmol/L, thus this lower level was not assessed and perhaps is the reason why the results were not positively associated with MCI. The results in this thesis reflect more the results found by others at s25OHD levels >25nmol/L (section 2.3.2).

The results of this thesis suggest that high doses of vitamin D may not be beneficial with regards to cognitive functioning in this group, supporting suggestions made by others (341, 342). Even though participants with insufficient vitamin D levels had mainly NCF some in this group also had MCI (Figure 3 and 2). In the sufficient s25OHD group there was a large variation in MoCA scores, and even though the majority had NCF a substantial amount of participants had scores below 26. This shows that there is considerable individual variation in cognitive function within the study population. Therefore, though the odds of having NCF are a lot higher if a participant had s25OHD ≤50 nmol/L, it does not mean that all participants with high s25OHD levels had MCI. The variance explained by s25OHD levels is small ($R^2 = 0.11-0.17$) and other factors are probably influencing these results.
It can be speculated that participants with NCF have demanding jobs that do not allow them to go outdoors as often, affecting their s25OHD levels. On the other hand they may have higher s25OHD levels because they are well-educated and thus take more supplements and do more exercise outdoors. Others that are not as well-educated probably have lower MoCA scores and may have higher s25OHD levels because they have jobs based outdoors or they do not have a demanding job that keeps them from spending hours indoors. There is also a possibility that those who have noticed a cognitive decline may start taking vitamin supplements to improve their health and cognition, however these are only conjectural comments.

### 6.1.2 Gender Association

The findings in this study were opposite to the first hypothesis however this inverse relationship was stronger in women than in men as stated in the second hypothesis. Initially when comparing unadjusted characteristics, no significant difference was found in the mean s25OHD levels and in the s25OHD categories between males and females. Yet when a logistical regression was performed to assess the relationship between cognitive function and s25OHD levels in only the females in this thesis (adjusted for model 2 confounders), the association was significant and showed a similar effect as in the whole sample (OR 0.43 (0.18,0.94) p=0.041). But when the same analysis was performed with only the males in this thesis, the effect was similar but the result was no longer significant (OR 0.48 (0.17,1.19) p=0.127). This finding is supported by other studies (16-18, 20, 29, 32, 34) but not all (27) (section 0).

As there was a significant difference in supplement use between males and females, the models were further adjusted for season and vitamin D supplement use. The effect size was still the same for the female participants but it was now borderline significant (OR 0.43 (0.17, 0.99) p=0.053), suggesting that the association might be explained by supplement use. The results
remained the same for the male participants. Caution is warranted in interpreting these results as the sample size was reduced by nearly half which could have influenced the results. Also the supplement use data may not be an accurate reflection of actual use. To further investigate this relationship, a few interactive terms were added to the final model with all participants: gender x s25OHD levels, gender x education, and gender x vitamin D supplement use. However none of these interactive terms were significant and did not change the results substantially (data not shown).

6.2 Sample characteristics

The study sample was comparable to the 2013 Canterbury census gender figures (50-54 years) (53% compared to 51% Canterbury females) but had a higher Māori representation (15% compared to 6%), because the CHALICE study intended a 20% Māori participation (343). Compared to the 2012/2013 NZ national health survey (45-54 years) the study sample had a lower prevalence of obesity (32% compared to 36%) and smoked less (15% compared to 20%) (344).

At the time of this thesis, census figures for certain characteristics were not available for Canterbury 50-54 year olds, thus the study sample was compared to those in Canterbury ≥15 years of age instead. The study sample had a higher post school education (60% compared to 29%) and reported a higher household income ≥$70,000 (68% compared to 48%) compared to those ≥15 years living in Canterbury (343). The most likely explanation for these differences is probably age, 15 year olds generally would not have had an opportunity to obtained a post school education and earn a higher household annual income.
The interviewers felt that MoCA test scores for 19 participants (5%) should be excluded as they were unable to complete the test properly. In order to reduce bias, the conservative approach was taken, and these participants’ data were excluded. The main reason for exclusion, was that English was their second language, also reported by others (70) (Appendix 11). This could possibly explain why excluded participants were probably more likely to be non-European than European. Excluded participants also drank significantly less alcohol per day, however on average the difference was only 0.4g (Appendix 12), which is not much considering a standard drink contains approximately 10g of alcohol (Appendix 5) (333).

The prevalence of MCI in this study population was 24% (without adjustment for education) and 20% (when roughly adjusted for education). This prevalence is high for a middle aged population (71, 72) (section 2.1.2), and may be due to the ability of the MoCA test to effectively detect early cognitive decline (78). The prevalence does, however, fall within the range that was found for a middle aged sample that was assessed with different MCI criteria (73).

The mean s25OHD levels in this study were good (63 nmol/L), similar to findings in other studies (88, 304) and similar to the 2008/09 NZ national nutrition survey levels for 45-54 year olds (61.2 nmol/L) (193). Only 3% of the participants were deficient (<25 nmol/L) and 34% had insufficient levels (≤50 nmol/L), which does not support earlier findings of a high vitamin D deficiency prevalence in New Zealand (265) and in Christchurch (345) (which lies at a latitude of 44° South (55) making it a vulnerable region for vitamin D deficiency).

No difference was found in s25OHD levels between Māori and non-Māori in agreement with the NZ nutrition survey (193) but contradicting other reports (13, 266), this may be due to the variability of skin colour in those identifying with the Maori ethnic group. Participants with
sufficient vitamin D were 4.52 times more likely to have their blood drawn in summer/autumn than in winter/spring, an association found by others (32, 88). The average daily vitamin D intake from food was approximately 4.5 µg per day, which is within the range found by Thomson et al. (145), however, this data has limitations (section 2.2.2.1).

Supplements containing vitamin D were used by 18% of the sample; and 4% of the participants were prescribed the monthly 1.25 mg (1250 µg) vitamin D₃ tablet, which is similar to the previously reported 5% (135). Males and females differed significantly in their vitamin D supplement use, more women were likely to use supplements than men, consistent with other findings (23, 153). However, there was no difference between their average s25OHD levels.

The majority of the study sample was overweight or obese (71%), was well educated (60% had a post school education) and had a high annual household income (68% had a household income ≥$70,001). Significantly more males and less females than expected were in the overweight group, as found elsewhere (32). However, significantly more females (n=16) than males (n=3) were in the morbidly obese group (BMI ≥40) (OR 0.2 (0.04, 0.71) p = 0.005) (data not shown). Māori had a higher BMI than non-Māori, similar to previous reports (328), however the difference was small (2 kg/m²). Only 45% of the Māori had a post school education compared to 63% of the non-Māori, which was also found by others (346).

6.3 Other associations found

Education significantly predicted cognitive function and the odds of a participant having NCF and a higher education was 2.4 times higher than having a lower education, when all other variables were held constant, which is consistent with other studies (17, 32). This thesis did not find an association with education and vitamin D, contradicting some studies (19, 32, 34).
A one unit increase in BMI significantly reduced the odds of a participant having NCF by only 5%, which was not a very big effect, as found by others (17). But when the analysis was performed using a 10 unit increase in BMI, the odds of having NCF were 50% less likely than having MCI, when the other variables in the model were held constant, in line with other findings (32). A higher BMI was also found to be associated with lower s25OHD concentrations, possibly due to the sequestrating of vitamin D into fat cells in the body (281, 282), confirmed by many other studies (18, 19, 33, 34, 88, 180, 218, 271, 273).

Depression was also a potential confounder and the odds of a participant having current depression were 61% less likely if the participant had NCF than if they had MCI. These findings are also supported by others (24, 70) but not all (24, 32, 58, 90). No association was found between s25OHD levels and depression, as found by others (88).

Cognitive function and s25OHD levels were not associated with alcohol consumption, also found by others (19) but not all (18, 25, 32, 33, 88, 218, 347). The alcohol consumption figures in the thesis did not vary by much and thus may have been too small to detect an effect. Lower s25OHD levels were found to be associated with current smoking, which supports previous findings (32, 218, 235, 271, 273).

Higher serum PTH levels were associated with lower s25OHD levels, which is not surprising as PTH increases the production of 1,25-dihydroxyvitamin D₃ and thus decreases s25OHD levels (114). Also as expected, creatinine levels were higher with higher s25OHD levels, possibly indicating kidney damage and thus a lower conversion to 1,25-dihydroxyvitamin D₃ (219).
Low s25OHD levels as well as cognitive function have been found to have a relationship with cardiovascular disease (CVD) and diabetes (6, 19, 89). The final model was further adjusted for self-reported CVD and diabetes, however, the associations between s25OHD levels and MoCA scores remained unchanged (data not shown), in line with other findings (32). Only 4% of the participants had diabetes and 14% had heart disease thus this analysis may not have had enough power to detect an association.

Higher levels of s25OHD were significantly associated with current vitamin D supplementation, in line with other studies (19, 153, 180, 185). As non-prescribed supplements in New Zealand vary in the actual vitamin D content compared to the declared value, the supplement data in this study needs to be interpreted with caution and may not be accurate (187). When the final model was adjusted for Vitamin D supplement use, the results did not change significantly. When participants were excluded for using daily vitamin D supplements over 1000 IU or the monthly 1.25mg vitamin D tablet, the results remained unchanged.

6.4 Limitations of the study

This thesis had limitations that should be considered when interpreting the results. Potential weaknesses of the CHALICE study have been previously outlined (312). Regardless of earthquake disruptions (312), the CHALICE study baseline participation rate was 62% and 52% for Māori, however results should not be significantly affected as Māori were oversampled.

It should be noted though, that participants who agreed to participate in the CHALICE study may differ to those who did not. The CHALICE sample might be healthier than those not participating, as participants had to be able to complete the assessment at the CHALICE offices. As the analysis was established on an internal evaluation between participants who took part,
the results are unlikely to be significantly influenced by selection factors. Participants whose data were not included in this thesis may differ from those who’s data were included, however, when all participants’ data were included in a separate analysis, results were not substantially affected (data not shown) and there were few characteristic differences between the two groups. This thesis was based on 50 year olds living in the Canterbury DHB region in New Zealand and thus findings should not be generalised to other populations.

Another limitation of this thesis is that the CHALICE study participants with 12 years of education or less were not awarded a point in the MoCA test, instead analyses were adjusted for education in the final model. In a separate analysis a point was awarded to all the participants that did not have a post school education and the results remained unchanged (Appendix 18). The MoCA has been validated but further research is required to confirm test utility is maintained across individuals of different cultural backgrounds (348).

In this study the household income did not take the household composition into account, and thus the household wealth data may be inaccurate. Although this thesis adjusted for most of the important confounders, this is an observational study and there will most likely be possible residual confounding influencing the findings. These may be unknown confounders that were not included in the analysis or known confounders that were not adjusted for. For example, physical activity was not adjusted for in this thesis, as this data were not ready at the time of data analysis. This is an important confounder that has been found to be positively associated with vitamin D and cognitive function (18, 19).

Ethnicity was used as a proxy measure of skin colour, as participants with darker skin were found to be associated with lower s25OHD levels (220), however this finding was not supported
by this thesis. This may be due to the variation in skin pigmentation levels between the various ethnic groups that were categorised together in this thesis, possibly leading to bias (19, 220).

Only one s25OHD measure was obtained, because of the cross sectional nature of this thesis, and there is doubt as to whether a single measurement of s25OHD can accurately reveals a participant’s vitamin D status (195) and seasonal changes. However, all characteristics were assessed on the same day or within a few days (for a minimal few). Thus models were adjusted for seasonal variation, however the results remained similar, and it is unlikely the results were biased. In this thesis there was no objective measurement of sun exposure, which is associated with s25OHD levels (21) and possibly cognitive function (32, 94), therefore, even though s25OHD levels were taken in the summer it does not mean the participant was exposed to the sun.

This thesis also did not adjust for calcium and multivitamin supplement use, which were found to be associated with higher s25OHD levels (19). However, a strong association was found between higher s25OHD levels and the use of vitamin D supplements. This data may not be accurate as the participants brought their supplements into the interview, and it is possible that they might have forgotten to bring some in, or that they did not take the recommended or reported dosage. Vitamin D dietary intake from food was also assessed, however the composition tables in New Zealand do not reflect the true vitamin D levels in food (section 2.2.2.1) and thus the vitamin D food intake data is likely to be inaccurate. Also self-reported data can be less accurate and can be influenced by various factors.

The possibility of reverse causality due to the cross-sectional nature of this thesis may mean that due to MCI participants may have taken more vitamin D supplements. Those that have noticed a
cognitive decline may be more health conscious and may be getting outdoors more to try and reverse the age related changes occurring at middle age.

6.5 Strengths of this study

The main strengths of this thesis include a substantial sized random sample with an acceptable response rate; standardized methods and procedures to collect data; a ‘gold-standard’ measure used to determine s25OHD levels at a good quality controlled single laboratory; and a validated, effective and accurate MoCA test used to assess MCI, which has a validated cut-off score and is a highly sensitive screening tool.

The wide range of variables measured in the CHALICE study allowed for analyses to account for an extensive list of confounders. The variables that were chosen were based on the evidence for such a relationship. Only important confounders and those that were significantly associated with cognitive function were left in the final model. Other potential confounders were explored but left out if they did not significantly affect the final results to avoid multicollinearity. This thesis validated the models by comparing predicted values with actual measures and running several sensitivity analyses to test if any points had a major effect on the results.

6.6 Conclusion

Current reviews and most of the current studies have found an association between lower s25OHD levels and worse cognitive function, especially at levels <25 nmol/L. Even though some studies have found a linear association between s25OHD levels and cognitive function, many are discovering a non-linear relationship, with ideal levels at certain thresholds. Thresholds differ among studies, which may partly be explained by the heterogeneity of various
study characteristics and assessment methods. As this thesis did not have many participants with 
s25OHD levels <25 nmol/L, it could not confirm or reject this association.

This observational study of community dwelling middle aged Cantabrians, found that MCI, as 
assessed by a highly sensitive test, was generally associated with sufficient s25OHD levels 
(>50nmol/L). This cross-sectional inverse relationship remained significant after adjustments 
were made for potential confounders. This association between s25OHD levels and MCI was 
stronger in women, and appears to be explained by supplement use.

Findings on s25OHD levels >25 nmol/L are inconsistent, and thus the results from this thesis 
add to these inconsistent results. The findings in this thesis may play an important role in 
guiding future more detailed and better designed prospective studies. At the time of this thesis 
few studies acknowledged their findings that showed an inverse association between cognitive 
function and s25OHD levels, with some dismissing these findings as false-positive results.

The results also suggest that maintaining high s25OHD levels may not be necessary, as has been 
suggested by some (252). The clinical implications of the findings in this thesis are not clear, 
however it should at least make people cautious about taking large doses of vitamin D 
supplements, especially woman.

Caution is required when interpreting the results of this study as cognitive function variance is 
not predicted very well by the predictor variables found to be associated with it in this study. 
This is not unusual for an observational study, but means that there are other factors predicting 
the cognitive function in individuals that have not been accounted for. Also causality cannot be 
concluded due to the cross-sectional design of this study.
6.7 Future studies

Based on the results in this thesis, large prospective studies are required to assess the association between cognitive function and s25OHD levels >25 nmol/L, instead of s25OHD levels <25 nmol/L, which have already been found to have an adverse effect on cognitive function. Various s25OHD levels should be analysed and methods should be standardised to enable comparison among studies. Longer follow-ups are necessary with repeated measures of s25OHD levels and cognitive function.

More cross-sectional studies are required to assess the association between cognitive function and s25OHD levels with a middle aged population, using standardised sensitive cognitive function measures that can detect early cognitive changes. Different cognitive domains should be assessed to determine which cognitive domains are affected by vitamin D status and based on the results in this thesis, more potential confounders may need to be identified.

Randomized controlled trails are also required in order to assess causality, and investigate if vitamin D supplementation has any benefit for those with s25OHD levels <25 nmol/L. Randomized control trials do, however, need to be done with caution due to the number of studies that indicate supplementation and higher s25OHD levels are not always beneficial.

6.8 Suggestions

Even though this study was unable to deduce whether high vitamin D status was due to sun exposure or due to higher vitamin D supplementation, there was a correlation between higher supplement use and vitamin D status, especially among woman. Thus it is possibile that the higher vitamin D levels that are associated with lower cognitive function might be due to
supplement use and thus caution should be taken when using supplements with high amounts of vitamin D.

Even though a couple of dermatologists concluded that the preferred approach to maintaining optimal vitamin D levels are through dietary or supplemental intake (205). As toxicity cannot occur due to sun exposure, safe sun exposure combined with physical activity may be beneficial as both have been found to improve cognitive function as well as s25OHD levels.
7 References


116


315. Elder P. Vitamin D EQAssessment [online]. Email to Peter Elder (peter.elder@cdhb.health.nz) 2014 Jul 3 [cited 2014 Jul 3]

316. Elder P. Serum vitamin D test [online]. Email to Peter Elder (peter.elder@cdhb.health.nz) 2014 Mar 13 [cited 2014 Mar 14].


340. Pearson J. Quick few questions [online]. E-mail to John Pearson (john.pearson@otago.ac.nz) 2014 May 8 [cited 2014 May 8].


348. Gallant K. Different cultural backgrounds [online]. Email to Dr Nasreddine (info@mocatest.org) 2014 Dec 10 [cited 2014 Dec 11].
8 Appendices

8.1 Appendix 1: Ethical approval letter

14 June 2010

Professor Peter Joyce
Department of Psychological Medicine
Christchurch School of Medicine & Health Sciences
P O Box 4345
Christchurch

Attn: Janet Spittlehouse

Dear Professor Joyce,

URA/10/03/021 Canterbury Health, Ageing and Life Course Study
Investigators Prof P Joyce, Mr C Lacey, A/Prof V Cameron, Prof S Chambers,
Dr R Gearry, Dr H Jamieson, Prof M Kennedy

This study was given ethical approval by the Upper South A Regional Ethics Committee on 14 June 2010.

Approved Documents
- Protocol version 2.1 dated 18.05.10
- Information sheet and Consent form version 2.1 dated 12.05.10
- CHALICE Yearly health questionnaire version 1.0 dated 02.06.10

This approval is valid until 31 August 2016, provided that Annual Progress Reports are submitted (see below).

Access to ACC
For the purposes of section 32 of the Accident Compensation Act 2001, the Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out. Participants injured as a result of treatment received in this trial will therefore be eligible to be considered for compensation in respect of those injuries under the ACC scheme.

Amendments and Protocol Deviations
All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:
- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
• the design or duration of the study
• the method of recruitment
• information sheets and informed consent procedures.

Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

**Annual Progress Reports and Final Reports**

The first Annual Progress Report for this study is due to the Committee by 30 June 2011. The Annual Report Form that should be used is available at www.ethicscommittees.health.govt.nz. Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.

A Final Report is also required at the conclusion of the study. The Final Report Form is also available at www.ethicscommittees.health.govt.nz.

**Requirements for the Reporting of Serious Adverse Events (SAEs)**

For the purposes of the individual reporting of SAEs occurring in this study, the Committee is satisfied that the study’s monitoring arrangements are appropriate.

SAEs occurring in this study must be individually reported to the Committee within 7-15 days only where they:

• are *unexpected* because they are not outlined in the investigator's brochure, and
• are not defined study end-points (e.g. death or hospitalisation), and
• occur in patients located in New Zealand, and
• if the study involves blinding, result in a decision to break the study code.

There is no requirement for the individual reporting to ethics committees of SAEs that do not meet all of these criteria. However, if your study is overseen by a data monitoring committee, copies of its letters of recommendation to the Principal Investigator should be forwarded to the Committee as soon as possible.

Please see www.ethicscommittees.health.govt.nz for more information on the reporting of SAEs, and to download the SAE Report Form.

We wish you all the best with your study.

Yours sincerely

**Alieke Dierckx**

Administrator
Upper South A Regional Ethics Committee

Email: alieke_dierckx@moh.govt.nz
8.2 Appendix 2: Participant consent form

CONSENT FORM

[if known]

Full Name:_____________________________________
NHI Number: _____ _____

Participant Number [office use only]: _______________
Date of Birth:___/___/19____

- I have read and understand the information sheet about this study, and I understand what is involved.
- I have been given the opportunity to discuss this study and to ask questions about it. I am satisfied with the answers I have been given.
- I have had enough time to consider whether to take part, and to discuss my decision with a person of my choice.
- I know who to contact if I have questions about the study.

I understand that:

(Please tick)

☐ I will be asked to complete questionnaires about my medical history and lifestyle.
☐ I will be asked to provide blood and urine samples.
☐ I will have an electrocardiograph (ECG).
☐ I will have an ultrasound examination of heart (echocardiograph).
☐ I will have a fundus photograph taken of my retina, using eye drops to dilate my pupil.

(Please read)

- Taking part is voluntary and I am free to withdraw at any time and for any reason.
• I will be contacted by CHALICE staff after my assessment day to organise the return of the CHALICE food and activity diaries and to clarify further details, if necessary.
• I will be re-contacted by CHALICE staff each year and in 4 to 5 years time for another assessment.
• I will be asked to provide contact details for 2 family and/or friends, and I understand that they may be contacted in the event that CHALICE staff are unable to contact me.
• My participation in this study is confidential and no information that could identify me will be used in any reports on this study.
• This study has received ethical approval from the Upper South A Regional Ethics Committee.

Please Circle

I consent to have my General Practitioner notified of my participation in this study..................YES / NO
I wish to receive a summary of my results including any previously undiagnosed problems or abnormal laboratory results............................................................YES / NO
I wish for my GP to receive a summary of my results including any previously undiagnosed problems or abnormal laboratory results ............................................................YES / NO
I consent for my medical records to be accessed through the National Health Index (NHI) database ............................................................YES / NO
I consent to researchers storing my samples for later use;
  Blood and plasma..................................................................................YES / NO
  Urine.................................................................................................YES / NO
  DNA.................................................................................................YES / NO
I consent to being contacted in future to ask about participating in related studies ........YES / NO
I consent to the non-identifying use of my information in related studies ..................YES / NO
I am aware that the study will collect, store and examine my DNA (genetic make-up) in relation to medically relevant traits and I consent to such analysis being performed .........YES / NO
I understand that if I consent to such analysis, I am not giving up any rights and no rights will be created for the researcher to my genetic information..........................YES / NO
I consent to researchers using my samples and DNA for later use as part of research with other New Zealand research collaborators (subject to approval by a NZ Ethics Committee) ..................................................................YES / NO
I consent to researchers storing my samples and DNA for later use as a part of future research with international researcher collaborators ..................................................YES / NO
I consent to my samples and DNA being sent overseas ............................................YES / NO
I understand that I can request to have my samples and DNA destroyed at any time .........YES / NO
I elect to have all my samples disposed of with an appropriate karakia. ......................YES / NO
I wish to receive copies of newsletters which will contain general findings of this study. ..................................................YES / NO

I ________________________________ (print full name) hereby consent to take part in this study.

Signature: __________________________ Date: ______________

Consent obtained by:

CHALICE staff signature: __________________________ Date: ______________

CHALICE staff name: __________________________

8.3 Appendix 3: CKD-EPI formula

The CKD-EPI formula (321)

Black Female
If serum creatinine (Scr) <= 0.7
GFR = 166 × (Scr/0.7)\(^{0.329}\) × 0.993\(^{\text{Age}}\)
If serum creatinine (Scr) > 0.7
GFR = 166 × (Scr/0.7)\(^{1.209}\) × 0.993\(^{\text{Age}}\)

Black Male
If serum creatinine (Scr) <= 0.9
GFR = 163 × (Scr/0.9)\(^{0.411}\) × 0.993\(^{\text{Age}}\)
If serum creatinine (Scr) > 0.9
GFR = 163 × (Scr/0.9)\(^{1.209}\) × 0.993\(^{\text{Age}}\)

White or other race Female
If serum creatinine (Scr) <= 0.7
GFR = 144 × (Scr/0.7)\(^{0.329}\) × 0.993\(^{\text{Age}}\)
If serum creatinine (Scr) > 0.7
GFR = 144 × (Scr/0.7)\(^{1.209}\) × 0.993\(^{\text{Age}}\)

White or other race Male
If serum creatinine (Scr) <= 0.9
GFR = 141 × (Scr/0.9)\(^{0.411}\) × 0.993\(^{\text{Age}}\)
If serum creatinine (Scr) > 0.9
GFR = 141 × (Scr/0.9)\(^{1.209}\) × 0.993\(^{\text{Age}}\)
8.4 Appendix 4: CHALICE study module 2 questionnaire

Module 2 Questionnaire
Personal Health History

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Participant Study Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer’s Name</td>
<td>Interviewer’s Number</td>
</tr>
</tbody>
</table>

TABLE OF CONTENTS

1. DEMOGRAPHICS.........................................................................................................................
   Date of birth and ethnicity.................................................................................................... 2
   Relationship status................................................................................................................. 3
   Education, income and employment....................................................................................... 4
   ELSI-SF: Economic Living Standards Index Short Form....................................................... 6
   Home ownership and medical Insurance............................................................................... 8

2. CHRONIC CONDITIONS............................................................................................................ 9
   Current medication............................................................................................................... 9
   Long-term conditions......................................................................................................... 11
   Infection and immunisation history................................................................................... 22
   Digestive disease............................................................................................................... 23
   Sleep patterns..................................................................................................................... 24

3. HEALTH SERVICE UTILISATION.......................................................................................... 26
   GP use............................................................................................................................... 26
   Medical Specialists.......................................................................................................... 26
   Complementary or alternative health care workers............................................................ 28
   Secondary Health Care Services (Hospital Use)................................................................. 29

4. RISK AND PROTECTIVE FACTORS...................................................................................... 31
   Screening programmes....................................................................................................... 31
   Environmental conditions................................................................................................. 32
   Tobacco.............................................................................................................................. 33
   Alcohol.............................................................................................................................. 33
1. DEMOGRAPHICS

First, I am going to ask you some general questions about you and your household. Then we will go on to talk about your health.

1.01 You are male/female...? [Circle one]
1 Male
2 Female

Date of birth

1.02 Firstly, what is your date of birth? [Record]

Enter eight digit date (e.g. 4 March 1946 = 04031946).

______/_____/_____________
.R Refused

Ethnicity

[Showcard 1.03a]

1.03a Which ethnic group or groups do you belong to? Call the number or numbers of the ones that apply to you from Card 1.03a. [record all mentioned]

1 New Zealand European
2 Māori
3 Samoan
4 Cook Island Māori
5 Tongan
6 Niuean
7 Chinese
8 Indian
9 Other, such as Dutch, Japanese, Tokelauan
.K Don’t know

GO TO THE QUESTIONNAIRE FOR MAORI PARTICIPANTS

1.03b What other ethnicity or ethnicities do you belong to? [Record]

____________________________________

GO TO 1.03b

1.04a Are you descended from Māori? That is did you have a Māori ancestor? [Circle one]

1 Yes
5 No
.K Don’t remember
.R Refused

GO TO 1.05a

1.04b What are your iwi affiliations? [Record all]

____________________________________

.K Don’t remember
.R Refused
1.05a Which country were you born in? [Circle one]

1 New Zealand  
2 Australia  
3 England  
4 Scotland  
5 China (People’s Republic of)  
6 South Africa  
7 Samoa  
8 Cook Islands  
9 Other [specify the present name of the country] ____________________________  
   .K Don’t know .R Refused

1.05b In what year did you arrive to live in New Zealand? [Record 4 digit year]

__________________  
   .K Don’t remember  
   .R Refused

1.06 How long have you lived in Canterbury? [Record years and months]

Years ____________ Months ____________  
   .K Don’t remember  
   .R Refused

Marital/Relationship Status

[Showcard 1.07a]
1.07a Looking at Card 1.07a, which one of these statements is true about your CURRENT relationship status?

1 I am married (or living together for 1 year or more)  
2 Separated  
3 Divorced  
4 Widowed  
5 Never married  
   .K Don’t know .R Refused

1.07b Are you currently in a relationship? How long (in years) have you been in your current relationship?

______________  

1.08 How long (in years) is/was the longest intimate relationship you’ve had in your life?

______________

Sexuality

[Showcard 1.09]
1.09 Looking at Card 1.09, which of the following best describes yourself?

1 Heterosexual (“straight”)  
2 Gay  
3 Lesbian  
4 Bisexual  
5 Transsexual  
6 Can’t choose  
   .K Don’t know .R Refused

Education
[Showcard 1.10]
1.10 What is your highest qualification? Please do not count incomplete qualifications or qualifications that take less than 3 months of full-time study to get. Please tell us your highest qualification, shown on Card 1.10. [Record one]

1 No qualification
2 Secondary school qualifications
3 Post secondary certificate, diploma, or trade diploma
4 University degree
5 Other [specify] ____________________________________________
.K Don’t know
.R Refused

Income support and employment

[Showcard 1.11]
1.11 Looking at Card 1.11, are you currently receiving any of these types of income support? [Circle yes or no and, if yes, circle all mentioned]

1 Yes
5 No
.K Don’t know/unsure
.R Refused

1 NZ Superannuation
2 Working for Families (Family Support, In Work Payment, Family Tax Credit)
3 Unemployment benefit
4 Domestic purposes benefit
5 Sickness benefit
6 Invalid’s benefit
7 Student allowance
8 Disability allowance
9 ACC (as income support, not reimbursement for health services)
10 Other government benefits (independent youth benefit, war pension, etc)
.K Don’t know
.R Refused

1.12 In the past 12 months, have you been out of paid work at any time for more than one month? Please do not include time out of paid work which was from your own choice, such as being a homemaker, caregiver, or full-time student.

1 Yes
5 No
.K Don’t know/unsure
.R Refused

1.13 What is your trained trade or profession? [Record]
[Showcard 1.14a]
1.14a Which of the statements on Card 1.14a best describes your current work situation. Please also say if you are self employed. [Circle one]

☐ Self employed are to be coded as 1 (working in paid employment). Please also tick the box “self employed”.

☐ Working in paid employment (1) includes students (full time or part time) if they have any paid employment.

1 Working in paid employment. [Tick if self employed ]

2 Not in paid work, and looking for a job

3 Not in paid work, and not looking for a job (for any reason, such as being retired, a homemaker, caregiver, or full-time student).

Specify___________

4 Other Specify___________________

.K Don’t know .R Refused

1.14b How many hours a week do you usually work? [Record hours]

_________________________

.K Don’t know .R Refused

1.14c What is your current occupation? (What is your job called? What kind of work do you do?) [Record]

_____________________________________________

[Showcard 1.15]
1.15 Looking at Card 1.15, in the last 4 weeks, which of these have you done, without pay? [Circle yes or no and, if yes, circle all mentioned]

1 Yes

5 No

.K Don’t know/unsure

.R Refused

1 Household work, cooking, repairs, gardening, etc, for my own household

2 Looked after a child who is a member of my household

3 Looked after a member of my household who is ill or has a disability

4 Looked after a child (who does NOT live in my household)

5 Helped someone who is ill or has a disability (who does NOT live in my household)

6 Other voluntary work for or through any organisation, group or marae

7 Studied for 20 hours or more per week at school or any other place

8 Studied for less than 20 hours per week at school or any other place

.K Don’t know

.R Refused

143
Income

[Showcard 1.16]
1.16 Looking at Card 1.16, what is the total income that you yourself got from all sources, before tax or anything was taken out of it, in the last 12 months? [Record one]

1 Less than $5,000
2 $5,001 - $10,000
3 $10,001 - $15,000
4 $15,001 - $20,000
5 $20,001 - $25,000
6 $25,001 - $30,000
7 $30,001 - $40,000
8 $40,001 - $50,000
9 $50,001 - $60,000
10 $60,001 - $70,000
11 $70,001 - $80,000
12 $80,001 - $100,000
13 $100,001 - $120,000
14 $120,001 - $150,000
15 $150,001 or more
.K Don’t know .R Refused

Household income

[Showcard 1.16]
1.17 Still looking at Card 1.16, what is the total income that your household got from all sources, before tax or anything was taken out of it, in the last 12 months? [Record one]

1 Less than $5,000
2 $5,001 - $10,000
3 $10,001 - $15,000
4 $15,001 - $20,000
5 $20,001 - $25,000
6 $25,001 - $30,000
7 $30,001 - $40,000
8 $40,001 - $50,000
9 $50,001 - $60,000
10 $60,001 - $70,000
11 $70,001 - $80,000
12 $80,001 - $100,000
13 $100,001 - $120,000
14 $120,001 - $150,000
15 $150,001 or more
.K Don’t know .R Refused

ELSI (Economic Living Standard Index)

[Showcard 1.18]
1.18 I’m now going to ask you some questions about things you may or may not have access to in your household. Looking at card 1.18 for the answer, do you have......

minated: “Does this include a cellphone?”: Access to a telephone in the household is the key concept, for example, if there is a cellphone and no landline then ‘Yes’, but only if cellphone is in the house whenever the respondent is home and they can make a phone call on it.
<table>
<thead>
<tr>
<th>Activity</th>
<th>1 Yes</th>
<th>2 No (don’t want it)</th>
<th>3 No (due to the cost)</th>
<th>4 No (other reason)</th>
<th>Refused (R) Don’t know (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Telephone (see note above)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Washing machine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Heating available in all main rooms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) A good pair of shoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) A best outfit for special occasions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) Personal computer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Home contents insurance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(h) Enough room for family to stay the night</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**[Showcard 1.18]**

1.19 Still looking at Card 1.18 for the answer, do you do the following activities?

<table>
<thead>
<tr>
<th>Activity</th>
<th>1 Not at all</th>
<th>2 A little</th>
<th>3 A lot</th>
<th>Refused (R) Don’t know (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Give presents to family and friends on birthdays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Visit the hairdresser at least once every 3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Have holidays away from home every year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Have a holiday overseas at least once every 3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) Have a night out at least once a fortnight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) Have family or friends over for a meal at least once a month</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**[Showcard 1.20]**

1.20 Now I’m going to ask you about some things some people do to help keep costs down. Looking at Card 1.20, in the last 12 months, have you done any of these things not at all, a little, or a lot?

<table>
<thead>
<tr>
<th>Activity</th>
<th>1 Not at all</th>
<th>2 A little</th>
<th>3 A lot</th>
<th>Refused (R) Don’t know (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Gone without fresh fruit and vegetables to keep costs down</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Continued wearing clothing that was worn out because you couldn’t afford a replacement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Put off buying clothes for as long as possible to help keep down costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Stayed in bed longer to save on heating costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) Postponed or put off visits to the doctor to help keep down costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) NOT picked up a prescription to help keep down costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Spent less on hobbies than you would like to help keep down costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(h) Gone without or cut back on trips to the shops or other local places to help keep down costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The next questions are about your material standard of living – the things that money can buy. Your material standard of living does NOT include your capacity to enjoy life. You should NOT take your health into account for these questions.

[Showcard 1.21]
1.21 Looking at Card 1.21, generally, how would you rate your material standard of living? Would you say that it is high, fairly high, medium, fairly low or low? [Circle one]

1 High
2 Fairly high
3 Medium
4 Fairly low
5 Low
.K Don’t know .R Refused

[Showcard 1.22]
1.22 Looking at Card 1.22, generally, how satisfied are you with your material standard of living? Would you say you were very satisfied, satisfied, neither satisfied nor dissatisfied, dissatisfied or very dissatisfied? [Circle one]

1 Very satisfied
2 Satisfied
3 Neither satisfied nor dissatisfied
4 Dissatisfied
5 Very dissatisfied
.K Don’t know
.R Refused

[Showcard 1.23]
1.23 Looking at Card 1.23, how well does your (and your partner's combined) total income meet your everyday needs for such things as accommodation, food, clothing and other necessities? Would you say you have not enough money, just enough money, enough money, or more than enough money? [Circle one]

1 Not enough
2 Just enough
3 Enough
4 More than enough
.K Don’t know
.R Refused

Home Ownership

[Showcard 1.24]
1.24 Who owns your home? [Circle one]

1 You own or partly own your house or flat (with or without a mortgage)
2 Family members
3 A family trust
4 A private landlord
5 A local authority or city council
6 Housing New Zealand
Medical Insurance

1.25 Are you covered by any health or medical insurance scheme? [Circle one]

1 Yes
5 No
.K Don't know .R Refused
2. CHRONIC CONDITIONS

The next section of this survey is about chronic health conditions you may have. A chronic condition is a physical or mental illness that has lasted, or is expected to last, for more than six months. The symptoms may come and go or be present all the time.

First, I would like to know about any medications you are taking.

**Current Medication**

2.01 **What are your current medications/prescriptions for yourself?** Please include all medications such as Inhalers, aerosol, injections, tablets and ‘over the counter medication’ etc.

Please copy from the packet or bottle the drug name, dose and number taken per day.

<table>
<thead>
<tr>
<th><strong>2.01a Drug Name - Prescription drugs from a Dr</strong></th>
<th><strong>2.01b Dose</strong></th>
<th><strong>2.01c Number/day</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>2.01d Drug Name – Non-prescription drugs</strong></th>
<th><strong>2.01e Dose</strong></th>
<th><strong>2.01f Number/day</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.01g **Have you had any adverse reactions to drugs?** [Circle one]

1. [specify] ____________________________________________

5. No

K. Don’t know

R. Refused
2.02 Have you ever been told by a doctor that you have or have had [Circle all mentioned]

1 Heart disease including:
   (a) Heart attack
   (b) Angina
   (c) Heart failure
   (d) Inadequate pumping of the heart
   (e) Build-up of fluid in the legs or lungs
   (f) Problems with heart rhythm (atrial fibrillation, supraventricular tachycardia (SVT), ventricular tachycardia (VT), ectopic beat)
   (g) Problems with heart valves (leaky or blocked)
   (h) Intermittent claudication (vascular spasm in the legs)
   (i) Clot in the leg (venous thrombosis)

2 Stroke

3 Diabetes

4 Allergies

5 Asthma

6 Chronic bronchitis or emphysema (COPD)

7 Arthritis (including gout, lupus and psoriatic arthritis)

8 High blood pressure

9 High cholesterol

10 Cancer

If none of the above GO TO 2.19a Page 21

Go to each relevant section in turn.

Remember to complete the “ACCESS TO SERVICES” questionnaire for participants who have diagnosed heart disease, diabetes, COPD, high blood pressure or high cholesterol and they have seen their GP about this condition in the last 12 months. If a participant has more than one of these conditions, ask them which has been most significant in the last 12 months and
use this condition to answer the access to services questionnaire. You only need to complete one per participant.

**Heart disease**

The first few questions are about heart disease. Please do not include high blood pressure or high blood cholesterol here, as I will ask you about those later.

2.03a **Have you ever been told by a doctor that you have had a heart attack?** [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 2.04a

2.03b **Have you ever been admitted to hospital with a heart attack?** [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 2.04a

2.03c **How old were you when you were first admitted to hospital with a heart attack?** [Record age]

.K Don’t know
.R Refused

2.03d **In the past 12 months, have you been admitted to hospital with a heart attack?** [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

2.04a **Have you ever been told by a doctor that you have angina?** *(interviewer probe – angina is typically chest pain when you walk or do exercise)* [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 2.05a

2.04b **How old were you when you were told by a doctor that you had angina?** [Record age]

.K Don’t know
.R Refused

2.05a **Have you ever been told by a doctor that you have heart failure?** That is inadequate heart pumping, or a build-up of fluid in the lungs or legs. [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 206
2.05b How old were you when you were told by a doctor that you had heart failure? [Record age]
  ☐ If from birth record 0
  ☐ K Don’t know
  ☐ R Refused

2.06 Have you ever been told by a doctor that you have any other heart disease? Please include problems with heart rhythm (atrial fibrillation, supraventricular tachycardia (SVT), ventricular tachycardia (VT), ectopic beat), heart valves (eg leaky or blocked valve), intermittent claudication (cramping and/or pain in the legs, usually when walking. Sometimes called vascular spasm in the legs) and clot in the leg (venous thrombosis) but not high blood pressure or high cholesterol. [Circle one]
  ☐ If the respondent has a leaking or blocked heart valve please ask “which valve” and record below.

  1. Yes [specify] ____________________________________________
  5. No
  ☐ K Don’t know
  ☐ R Refused

2.07 Have you been to a GP about your heart disease in the past 12 months?

  1. Yes
  5. No
  ☐ K Don’t know
  ☐ R Refused

2.08 Looking at Card 2.08, what treatments do you now have for your heart condition(s)? [Circle yes or no and, if yes, circle all mentioned]
  ☐ Probe “Any others?” until no other treatment mentioned

  1. Medicines, tablets or pills (including spray under the tongue or patches on the skin)
  2. Diet
  3. Exercise
  4. Other [specify] ____________________________________________

2.09a Have you ever had bypass surgery or angioplasty (sometimes called a stent) for your heart condition(s)? [Circle one]

  1. Yes
  5. No
  ☐ K Don’t know
  ☐ R Refused

2.09b How old were you when you had bypass surgery or angioplasty? [Record age]

  ☐ K Don’t know
  ☐ R Refused
Stroke

2.10a Have you ever been told by a doctor that you have had a stroke? Please do not include “mini-stroke” or transient ischaemic attack. [Circle one]
   - A stroke is a definite event that has left permanent neurological damage (eg lost vision or feeling etc.)
   1 Yes
   5 No
   .K Don’t know
   .R Refused

   GO TO 2.10b

2.10b How old were you when you were first told by a doctor that you had had a stroke? [Record age or circle appropriate answer]

   .K Don’t know
   .R Refused

2.10c Have you had a stroke during the past 12 months? [Circle one]
   1 Yes
   5 No
   .K Don’t know
   .R Refused
   [Showcard 2.10d]

2.10d What treatments do you now have for your stroke? [Circle yes or no and, if yes, circle all mentioned]
   1 Yes
   5 No
   .K Don’t know
   .R Refused

   1 Medicines, tablets or pills
   2 Diet
   3 Exercise or rehabilitation (include speech therapy, occupational therapy, physiotherapy)
   4 Other [specify]

Diabetes

2.11a Have you ever been told by a doctor that you have diabetes? <IF RESPONDENT IS FEMALE, ADD…> Please do not include diabetes during pregnancy. [Circle one]

   1 Yes
   5 No
   .K Don’t know
   .R Refused

   GO TO 2.11b

2.11b Is that type I or type II?

   1 Type I
   2 Type II
   .K Don’t know
   .R Refused

2.11c How old were you when you were first told by a doctor that you had diabetes? [Record age or circle appropriate answer]
2.11d Have you been to a GP about your diabetes in the past 12 months?

1 Yes  
5 No  
.K Don’t know  
.R Refused

[Showcard 2.11e]

2.11e What treatments do you now have for your diabetes? [Circle yes or no and, if yes, circle all mentioned]

1 Yes  
5 No  
.K Don’t know  
.R Refused

1 Medicines, injections, tablets or pills  
2 Diet  
3 Exercise  
4 Other [specify]  
5 Other

2.11f In the past 12 months have you had a “Get Checked” free annual diabetes check with your GP or nurse? [Circle one]

1 Yes  
5 No  
.K Don’t know  
.R Refused

Allergies

2.12a Have you ever been told by a doctor that you have allergies? [Circle one]

1 Yes  
5 No  
.K Don’t know  
.R Refused

[Showcard 2.12b]

2.12b Looking at Card 2.12b, what substances are you allergic to? [Multiple answers allowed]

1 Pollen  
2 Mould  
3 Dust mites  
4 Animals  
5 Chemicals  
6 Shellfish  
7 Peanuts  
8 Gluten  
9 Fish  
10 Eggs  
11 Not identified  
12 Other [specify]  
.K Don’t know  
.R Refused

2.12c In the last 12 months, have you had problems with allergies? [Circle one]

1 Yes
2.12d Looking again at card 1.12b, which allergies have affected you in the last 12 months? (If respondent answers more than one kind, say: Which affects you most?) [Circle all allergies mentioned and underline the ‘most’]

1 Pollen
2 Mould
3 Dust mites
4 Animals
5 Chemicals
6 Shellfish
7 Peanuts
8 Gluten
9 Fish
10 Eggs
11 Not identified
12 Other [specify] ________________________________

.K Don’t know
.R Refused

[Showcard 2.12e]
2.12e What treatments do you now have for allergies? [Circle yes or no and, if yes, circle all mentioned]
Probe “Any others?” until no other treatment mentioned

1 Yes
5 No
.K Don’t know
.R Refused

1 Medicines, tablets or pills
2 Avoidance
3 Nasal steroids
4 Immunotherapy
5 Other [specify] ________________________________

[Asthma]
2.13a Have you ever been told by a doctor that you have asthma? [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 2.14a

2.13b How old were you when you were first told by a doctor that you had asthma? [Record age]

If from birth record 0

.K Don’t know
.R Refused

[Showcard 2.13c]
2.13c In the last 12 months, how many asthma attacks have you had? [Circle one]

1 None
2 1-5

GO TO 2.12e

GO TO 2.14a
2.13d In the last 12 months, have you been woken by an attack or shortness of breath at any time? [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

[Showcard 2.13e]
2.13e What treatments do you now have for asthma?
[Circle yes or no and, if yes, circle all mentioned]
Probe “Any others?” until no other treatment mentioned

1 Yes
5 No
.K Don’t know
.R Refused

1 Inhalers, aerosol, or tablets
2 Other [specify]____________________________

COPD (Chronic obstructive pulmonary disease)

2.14a Have you ever been told by a doctor that you have chronic bronchitis or emphysema? [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

2.14b How old were you when you were told by a doctor that you had this condition? [Record age]

.K Don’t know
.R Refused

2.14c Have you been to a GP about your chronic bronchitis or emphysema in the past 12 months?

1 Yes
5 No
.K Don’t know
.R Refused

[Showcard 2.14d]
2.14d What treatments do you now have for this condition?
[Circle yes or no and, if yes, circle all mentioned]
Probe “Any others?” until no other treatment mentioned

GO TO 2.14a
GO TO THE QUESTIONNAIRE ABOUT ACCESS TO SERVICES
Arthritis

2.15a Have you ever been told by a doctor you have arthritis? Please include gout, lupus and psoriatic arthritis. [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

1 Inhalers, aerosol, or tablets
2 Physiotherapy
3 Oxygen
4 Other [specify]____________________________

2.15b What kind of arthritis was that? One answer only (If respondent answers more than one kind, say: Which affects you most?) [Circle one only]

1 Rheumatoid
2 Osteoarthritis
3 Other [specify]____________________________
.K Don’t know
.R Refused

[Showcard 2.15c]

2.15c Looking at Card 2.15c, which joints were affected first? [Circle one]

1 Small joints like fingers or hands
2 Large joints like knees or hips
.K Don’t know
.R Refused

2.15d How old were you when you were first told by a doctor that you had arthritis? [Record age]  If from birth record 0

.K Don’t know .R Refused

[Showcard 2.15e]

2.15e What treatments do you now have for arthritis?
[Circle yes or no and, if yes, circle all mentioned]

 probes “Any others?” until no other treatment mentioned

1 Yes
5 No
.K Don’t know
.R Refused

1 Medicines, tablets, or pills
2 Exercise or physiotherapy
3 Injections
4 Other [specify]____________________________
2.15f Have you ever had an operation or surgery because of your arthritis. Please don’t include joint replacement surgery? [Circle one]

1 Yes  
5 No  
.K Don’t know  .R Refused

2.15g Have you ever had joint replacement surgery because of your arthritis? [Circle one]

1 Hip  
2 Knee  
3 No  
4 Other [specify] ________________________________  
.K Don’t know  
.R Refused

High Blood Pressure

2.16a Have you ever been told by a doctor that you have high blood pressure?  [Circle one]  
READ OUT IF FEMALE - Please do not include high blood pressure you may have had during pregnancy.)

1 Yes  
5 No  
.K Don’t know  
.R Refused

2.16b How old were you when you were told that you have high blood pressure? [Record in years]

____________________________

.K Don’t know  
.R Refused

2.16c Have you been to a GP about your high blood pressure in the last past 12 months?  
1 Yes  
5 No  
.K Don’t know  
.R Refused

[Showcard 2.16d]

2.16d What treatments do you now have for your high blood pressure?  
[Circle yes or no and, if yes, circle all mentioned]  
≥ Probe “Any others?” until no other treatment mentioned

1 Yes  
5 No  
.K Don’t know  .R Refused

1 Medicines, tablets or pills  
2 Diet  
3 Exercise  
4 Other ________________________________

Cholesterol

2.17a Have you ever been told by a doctor that you have high cholesterol levels in your blood?  
[Circle one]
2.17b How old were you when you were told that you have high cholesterol levels in your blood? [Record in years]

.K Don’t know
.R Refused

2.17c Have you been to a GP about your high cholesterol in the last past 12 months?

1 Yes
5 No
.K Don’t know
.R Refused

[Showcard 2.16d]

2.17d What treatments do you now have for high cholesterol?
[Circle yes or no and, if yes, circle all mentioned]

Probe “Any others?” until no other treatment mentioned

1 Yes
5 No
.K Don’t know
.R Refused

1 Medicines, tablets or pills
2 Diet
3 Exercise
4 Other

GO TO 2.18a
Cancer

2.18a Have you ever been told by a doctor that you have cancer? [Circle one]

1 Yes  2 No
.K Don’t know  .R Refused

[Showcard 2.18b]
2.18b Now looking at Showcard 2.18b what kind or kinds of cancer were you diagnosed with? [Tick all mentioned in 2.18b column below]  ☐ For each Cancer mentioned, ask 2.18c.  
2.18c How old were you when you were first told by a doctor that you had this kind of cancer? [Record age in 2.18c column below]

<table>
<thead>
<tr>
<th>Kind of cancer</th>
<th>2.18b Tick if yes</th>
<th>2.18c Age diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Bowel/rectal/colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Cervical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Prostate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Melanoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Skin cancer (not melanoma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Bladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Gallbladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Hodgkin’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Leukaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Lip, mouth, pharynx, throat (oesophageal, laryngeal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Ovarian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Non-Hodgkin’s lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 Stomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Testicular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Pancreatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 Thyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23a Other [specify] [Record up to two ‘Other’]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23b Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

.K Don’t know  .R Refused

[Showcard 2.18d]
2.18d What treatments do you now have for cancer?
[Circle yes or no and, if yes, circle all mentioned]  ☐ 
☐ Probe “Any others?” until no other treatment mentioned

1 Yes  5 No
.K Don’t know  .R Refused
1 Chemotherapy
Other long-term conditions

[Showcard 2.19a]
2.19a Have you ever been told by a doctor that you have any other long term condition that we have not discussed already, such as those listed on Card 2.19a? Please include any condition that has lasted, or is expected to last, *six months or more*, and remember, a long-term condition may come and go or be present all the time. [Multiple answers allowed]

For each long-term condition mentioned in 2.19a ask 2.19b.

2.19b How old were you when you were first told by a doctor that you had [insert condition]? [Record age in 2.19b column below]

### Other physical conditions

<table>
<thead>
<tr>
<th>Other physical conditions</th>
<th>2.19a Tick if yes</th>
<th>2.19b Age diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Migraine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Stomach ulcer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Irritable bowel syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Gall bladder problems/gall stones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Endometriosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Prostate problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Thyroid conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Eczema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Inflammatory Bowel Disease (Ulcerative Colitis, Crohn’s etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Chronic Kidney Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12a Other long term physical health conditions [specify] [Record up to six]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12b ‘Other’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12f</td>
<td>.K Don’t know</td>
<td></td>
</tr>
<tr>
<td></td>
<td>.R Refused</td>
<td></td>
</tr>
</tbody>
</table>

2.20a Have you ever been knocked out or knocked unconscious?

1 Yes  
5 No  
.K Don’t know  
.R Refused  

If none GO TO 2.20a  

GO TO 2.21a
2.20b Did you have to stay overnight or longer for observation in hospital because of being knocked out?

1 Yes
5 No
.K Don’t know
.R Refused

Infections and immunisations

[Showcard 2.21a]
2.21a Have you ever been told by a doctor that you have any of the following conditions. These conditions may come and go or be present all the time. [Multiple answers allowed]

For each condition mentioned in 2.21a ask 2.21b.

2.21b How old were you when you were first told by a doctor that you had [insert condition]? [Record age in 2.21b column below]

<table>
<thead>
<tr>
<th>Infection condition</th>
<th>2.21a Tick if yes</th>
<th>2.21b Age diagnosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 None</td>
<td>GO TO 2.22a</td>
<td></td>
</tr>
<tr>
<td>1 Chicken Pox</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Shingles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Rheumatic fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Treatment for urinary tract infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Hepatitis B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Hepatitis C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Septicaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Cellulitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.K Don’t know</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.R Refused</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Showcard 2.22a]
2.22a Have you ever had any of the following immunisations. [Multiple answers allowed]

For each condition mentioned in 2.22a ask 2.22b.

2.22b How old were you when you had [insert immunisation]? [Record age of most recent immunisation in 2.22b column below]

<table>
<thead>
<tr>
<th>Immunisation</th>
<th>2.22a Tick if yes</th>
<th>2.22b Age of most recent immunisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 None</td>
<td>GO TO 2.23</td>
<td></td>
</tr>
<tr>
<td>1 Influenza (flu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Hepatitis B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Pneumococcal vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.K Don’t know</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.R Refused</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
[Showcard 2.23]
2.23 Looking at show card 2.23, in the last 12 months have you had either of these conditions? [Circle one]

1 Acute gastroenteritis (vomiting/diarrhoea)
2 Influenza (flu)
3 Both
4 Neither
5 Don’t know
6 Refused

Digestive Disease

[Showcard 2.24]
2.24 In the last 3 months, how often have you noticed blood in your stools?

1 Never or rarely
2 Sometimes (about 25% of the time)
3 Often (about 50% of the time)
4 Most of the time (about 75% of the time)
5 Always (100% of the time)
6 Don’t know
7 Refused

[Showcard 2.24]
2.25 In the last 3 months, how often have you noticed black stools (not due to medication such as Iron supplements or charcoal tablets)?

1 Never or rarely
2 Sometimes (about 25% of the time)
3 Often (about 50% of the time)
4 Most of the time (about 75% of the time)
5 Always (100% of the time)
6 Don’t know
7 Refused

[Showcard 2.24]
2.26 In the last 3 months, how often have you vomited blood?

1 Never or rarely
2 Sometimes (about 25% of the time)
3 Often (about 50% of the time)
4 Most of the time (about 75% of the time)
5 Always (100% of the time)
6 Don’t know
7 Refused

[Showcard 2.24]
2.27 In the last 5 years, have you been told by your doctor that you are anaemic (a low blood count or low iron)? (If female, not due to your menstrual period.)

1 Yes
2 No
3 Don’t know
2.28 In the last 3 months, have you unintentionally lost over 4.5 kilograms (10 pounds)?

1 Yes
5 No
.K Don’t know .R Refused

2.29 Have you had a recent major change in bowel movements (change in frequency or consistency)?

1 Yes
5 No
.K Don’t know .R Refused

Sleep Patterns

[Showcard 2.30]
2.30 To what degree do you feel you are a “morning person” or a “night person”?

1 Definitely a morning person (energetic in the morning and tired at night)
2 To some degree a morning person
3 To some degree a night person
4 Definitely a night person (tired in the morning and energetic at night)
.K Don’t know .R Refused

The next three questions ask about events outside of your control that may lead to an interruption of your sleep.

[Showcard 2.31a]
2.31a Looking at card 2.31a in the last 12 months has your sleep been regularly interrupted by any of these events, so that you were awake for at least 20 minutes? [Circle all mentioned]

0 None
1 Night shift work
2 Traffic noise from nearby roadways
3 Crying babies
4 Barking dogs
5 Snoring partner
6 An undiagnosed health problem [specify]______________________________
7 Job requirements, e.g., being “on call”
8 Noisy neighbours
9 Other [specify]______________________________
.K Don’t know .R Refused
2.31b *For how long IN TOTAL has your sleep been interrupted by these events? [record months and years]*

Years ___________ Months __________

.K Don’t know
.R Refused

[Showcard 2.32]
2.32 *In the last 6 months, have you had any problems falling asleep?*

1 Never
2 Seldom
3 Sometimes
4 Usually
5 Always
.K Don’t know
.R Refused

[Showcard 2.32]
2.33 *In the last 6 months, have you felt sleepy during work or freetime?*

1 Never
2 Seldom
3 Sometimes
4 Usually
5 Always
.K Don’t know
.R Refused

[Showcard 2.34]
2.34 *In the last 6 months, how do you think you have slept on the whole?*

1 Very good
2 Pretty good
3 Neither good nor bad
4 Pretty bad
5 Very bad
.K Don’t know
.R Refused

[Showcard 2.35]
2.35 *In the last 6 months, have you been waking up too early and not being able to sleep again?*

1 Never
2 Seldom
3 Sometimes
4 Usually
5 Always
.K Don’t know
.R Refused

[Showcard 2.35]
2.36 *In the last 6 months, have you had a feeling of not having had enough sleep on wakening?*

1 Never
2 Seldom
3 Sometimes
4 Usually
5 Always
In the last 6 months, have you had disturbed or uneasy sleep (not due to environmental factors)?

1. Never
2. Seldom
3. Sometimes
4. Usually
5. Always

For how long in the last 6 months have you had disturbed sleep? [Record time in months]

GO TO 3.01

.S. Don't know .R. Refused

.S. Don't know .R. Refused
3. HEALTH SERVICE UTILISATION

3.01 In the last 12 months, have you seen your GP about your own health?
If parents consult doctor about own health issue at the end of a consultation for their children, that is included. Visits at home or elsewhere are included.

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 3.03

3.02 How many times have you seen your GP about your own health in the past 12 months?
[Record number of times]

.K Don’t know
.R Refused

[Showcard 3.03]

3.03 Over the last 12 months have you had carried out any of the following shown on Card 3.03? [Circle all mentioned]

0 None of the below
1 Weight measurement
2 Blood sample
3 Urine sample
4 Blood pressure measurement
5 Cholesterol test
6 Diabetes test
.K Don’t know .R Refused

Medical Specialists

The next few questions are about medical specialists, such as those listed on Card 3.04. By medical specialist I mean the kind of doctor that people go to for a particular health condition, problem or service, not a GP. You may have seen the medical specialist as an outpatient in a hospital or at their private rooms or clinic. Please do not include medical specialists you may have seen as an inpatient at a public hospital.

The definition of an inpatient is ‘An inpatient is someone who is admitted to hospital at least overnight’

[Showcard 3.04]

3.04 In the last twelve months, have you seen any medical specialists listed on Card 3.04 about your own health? [Circle and/or record all mentioned]

Please note, a General Physician is not a General Practitioner.

1 Yes
5 No
.K Don’t know
.R Refused

If none GO TO 3.07

3.05 How many times have you seen each of those specialists in the past 12 months? [Record number of times in 3.05 column below]
### 3.04 Medical specialists

<table>
<thead>
<tr>
<th>3.04 Medical specialists</th>
<th>3.04 Tick if yes</th>
<th>3.05 Number of times seen in past 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 General physician (not a General Practitioner)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Dermatologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Neurologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Cardiologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Haematologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Endocrinologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Respiratory physician</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Gastroenterologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Oncologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 General surgeon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Orthopaedic surgeon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Ophthalmologist (Eye specialist)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Ear, nose and throat specialist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Urologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 Obstetrician or Gynaecologist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 Geriatrician</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Psychiatrist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Infectious disease physician</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 Immunologist (Allergy specialist)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Other [specify]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Other [specify]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Showcard 3.06]

3.06 **Looking at Card 3.06, the last time you saw a medical specialist about your own health, where was this?** Please do not include inpatient visits to a public hospital.  
[Circle one only]

1 Public hospital as an outpatient
2 Private hospital
3 Specialist’s private rooms or clinic
4 Other [please specify]_________________________________________
.K Don’t know
.R Refused

[Showcard 3.07]

3.07 **Thinking about health care generally do any of these things stop you from getting health care?**

0 None
1 Costs of Doctor visits
2 Costs of prescriptions
3 Transport to Health Services
4 Not being able to get to an appointment when I need to
5 Family commitments
6 The service provided by Health Services
7 I have had a bad experience(s) and do not wish to go back
8 Other [please specify] ____________________________________________
   .K Don’t know
   .R Refused

[Showcard 3.08]
3.08 What helps you to get health care/services?

0 None
1 Whanau/Family
2 Relationship with Doctor
3 Relationship with Pharmacist
4 Close proximity of services
5 Maori Health Providers
6 Getting good service
7 Health promotion material
8 Other [please specify] ____________________________________________
   .K Don’t know
   .R Refused

Complementary or alternative health care workers

The next set of questions are about complementary or alternative health care workers. This includes Māori or Pacific traditional healers, and traditional healers from other cultures. Please do not include any health care worker that we have already talked about.

[Showcard 3.09]
3.09 In the last twelve months, have you seen any of the complementary or alternative health care workers on Card 3.09 about your own health? Please mention those you have seen.

1 Yes
5 No
   .K Don’t know
   .R Refused

   If none GO TO 3.13a

3.10 How many times have you seen each of those health care workers in the past 12 months? [Record in 3.10 column below]
[Showcard 3.11]

Now please look at Card 3.11, the last time you saw a complementary or alternative health care worker about your own health, what was it for? [Circle all mentioned]

1. A long-term illness, chronic condition or disability
2. A short-term illness or temporary condition
3. An injury or poisoning
4. Mental or emotional health
5. Physical well-being / to feel good
6. Contraception or family planning
7. Something else [please specify]
8. Don’t know
9. Refused

3.12 The last time you saw an alternative or complementary health care worker, did you also see a GP about the same condition? [Circle one]

1. Yes
2. No
3. Don’t know
4. Refused

Secondary Health Care Services (Hospital Use)

The last few questions in this section are about your use of hospitals over the past 12 months. I’ll begin by asking you about public hospitals, that’s where you don’t have to pay, and then move on to private hospitals, where you, your insurance, or a government agency like ACC would pay.

3.13a In the last 12 months, have you yourself used a service at, or been admitted to, a public hospital as a patient? This could have been for a physical or a mental health condition. [Circle one]

1. Yes
2. No
3. Don’t know
4. Refused

GO TO 3.14a
[Showcard 3.13b]

3.13b Looking at Card 3.13b, in the last 12 months, at a public hospital, which of the following happened? [Circle all mentioned]

1. You yourself used Emergency Department
2. You yourself used an outpatients department, that is, a ward or clinic or specialist where you went as an outpatient
3. You were admitted for day treatment, that is, day surgery or medical care for which you had to stay in hospital for more than 3 hours but not overnight
4. You were admitted as an inpatient, that is, stayed as a patient overnight
5. Other
   .K Don’t know
   .R Refused

3.14a In the last 12 months, have you yourself used a service at, or been admitted to, a private hospital? [Circle one]

1. Yes
2. No
   .K Don’t know
   .R Refused

   GO TO 4.01

[Showcard 3.14b]

3.14b In the last 12 months, at a private hospital, which of the following happened? [Read out and circle all mentioned]

1. You were admitted as an inpatient, that is, stayed as a patient overnight
2. You were admitted for day treatment, that is, day surgery or medical care for which you had to stay in hospital for more than 3 hours but not overnight
3. Other
   .K Don’t know
   .R Refused
4. RISK AND PROTECTIVE FACTORS

The next section is about medical, biological and lifestyle factors that can influence your health.

Screening programmes

Female respondents to be asked following questions

The next few questions are about your periods and two cancer screening programmes run by the Ministry of Health: the National Cervical Screening programme and BreastScreen Aotearoa.

4.01a In the last 12 months, have your periods been. [Circle one]
1 Regular
2 Irregular
3 None
.K Don’t know
.R Refused

GO TO 4.02

4.01b How old were you when your periods stopped? [Record in years]

.K Don’t know
.R Refused

4.01c Why did your periods stop?
1 Natural menopause
2 Surgical (hysterectomy - your uterus or womb is removed)
3 Other [specify] ____________________________________________________
.K Don’t know
.R Refused

4.02 In the last 2 years, have you had a mammogram? A mammogram is a breast x-ray that helps to check for early signs of breast cancer. [Circle one]
1 Yes
5 No
.K Don’t know
.R Refused

4.03a In the last 3 years, have you had a cervical smear to check for cervical cancer? A cervical smear is a screening test where cells are taken from the cervix. It is not a swab or check for sexually transmitted infections. [Circle one]
1 Yes
5 No
.K Don’t know
.R Refused

GO TO 4.05a

4.03b How about in the last 5 years, have you had a cervical smear? [Circle one]
1 Yes
5 No
.K Don’t know
.R Refused

GO TO 4.05a

Prostate cancer testing

Male respondents only

FEMALE RESPONDENTS GO TO 4.05a
The next question is about testing for prostate cancer.

4.04 **In the past 12 months, have you had a PSA (prostate-specific antigen) blood test for prostate cancer?**

1 Yes  
2 No  
.K Don’t know  
.R Refused

**Environmental Conditions**

The next few questions are about environmental conditions. By environmental conditions we mean factors in your surroundings that may influence your health and well-being.

[Showcard 4.05a]

4.05a **Looking at Card 4.05a, have you ever worked or lived in an area in which you were exposed to environmental conditions such as these mentioned?**

1 Yes  
2 No  
.K Don’t know  
.R Refused  

[Showcard 4.05a]

4.05b **Looking at Card 4.05a, which environmental condition or conditions did you experience for 6 months or more and how many years were you exposed for? [circle all mentioned]**

<table>
<thead>
<tr>
<th>Environmental Condition</th>
<th>4.05a Tick if yes</th>
<th>4.05b How many years exposed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Outdoor air pollution (e.g., exhaust, pollutants, particulate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Indoor air pollution (e.g., tobacco, mould, dust)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Water pollution (e.g., contaminated drinking water, PCBs, dioxin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Hazardous waste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Heavy metals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Pesticides, insecticides, herbicides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Odours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Noise pollution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Radiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Pollution of rivers and ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Other [specify]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.K Don’t know</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.R Refused</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tobacco

Now, some questions on smoking tobacco.

4.06a Have you ever smoked a total of more than 100 cigarettes in your whole life? [Circle one]

1 Yes
5 No
.K Don’t know
.R Refused

GO TO 4.07

4.06b How old were you when you started smoking regularly? [Record in years]

.K Don’t know
.R Refused

4.06c How often do you now smoke? [Circle one only]

Read answer options. If more than one frequency given, code the highest one.

1 You don’t smoke now
2 Less often than once a month
3 At least once a month
4 At least once a week
5 At least once a day
.K Don’t know
.R Refused

GO TO 4.06e

4.06d How old were you when you stopped smoking regularly (daily)? [Record in years]

.K Don’t know
.R Refused

4.06e From when you started smoking regularly to now/when you stopped, did you ever give up smoking for 6 months or more?

1 Yes, once
2 Yes, twice
3 Yes, three times or more
4 No
.K Don’t know
.R Refused

GO TO 4.06g

4.06f In total, taking into consideration all the times you stopped, how long did you give up smoking for? [Record in years]

Round up (or down) to the nearest year.

.K Don’t know
.R Refused

4.06g Which of these products do you/have you smoke/d the most? [Circle one]

1 Tailor-made cigarettes (that is, manufactured cigarettes in a packet)
2 Roll your owns using loose tobacco
3 Both tailor-mades and roll your owns
4 Pipes
5 Cigars

GO TO 4.07
On average, over all your years of smoking, how many cigarettes do/did you smoke a day? [Circle one]

If respondent is unable to suggest an average, ask for the typical number of cigarettes smoked in a week and divide by 7.

1 Less than 1 per day
2 1-5 per day
3 6-10 per day
4 11-15 per day
5 16-20 per day
6 21-25 per day
7 26-30 per day
8 31 or more a day
.K Don’t know/unsure
.R Refused

Are you seriously considering quitting within the next 6 months? Please answer from Card 4.06i. [Circle one only] IF not applicable circle here: N/A

1 No, I have no intention of quitting
2 Yes, I am thinking of quitting
3 Yes, I am thinking of quitting within the next 30 days
4 Yes, I have managed to stop smoking for at least a day now
.K Don’t know/unsure
.R Refused

Alcohol

I will now ask you some questions about your use of alcoholic drinks. Many New Zealanders enjoy alcohol. However, sometimes it can affect our health.

Have you had a drink containing alcohol in the last year? [Circle one]

1 Yes
2 No
.K Don’t know
.R Refused

How often do you have a drink containing alcohol? [Circle one]

Don’t prompt answer. Wait and code
1 Monthly or less
2 Up to 4 times a month
3 Up to 3 times a week
4 4 or more times a week
.K Don’t know
.R Refused

How many drinks containing alcohol do you have on a typical day when you are drinking? [Circle one]

Take average and round to nearest whole number if necessary e.g. if respondent says 4 or 5, average is 4.5, round to nearest whole number = 5, that is code 5

1 1 or 2
2 3 or 4
3 5 or 6
4 7 to 9
5 10 or more
.K Don’t know
.R Refused

Please refer to the leaflet “The straight up guide to standard drinks” and record the number of STANDARD drinks.
For the next series of questions please refer to Card 4.07d.

4.07d Looking at Card 4.07d, how often do you have six or more drinks on one occasion? [Circle one]

1 Never  
2 Less than monthly  
3 Monthly  
4 Weekly  
5 Daily or almost daily  
.K Don’t know  
.R Refused

4.07e How often during the last year have you found that you were not able to stop drinking once you had started? [Circle one]

1 Never  
2 Less than monthly  
3 Monthly  
4 Weekly  
5 Daily or almost daily  
.K Don’t know  
.R Refused

4.07f How often during the last year have you failed to do what was normally expected from you because of drinking? [Circle one]

1 Never  
2 Less than monthly  
3 Monthly  
4 Weekly  
5 Daily or almost daily  
.K Don’t know  
.R Refused

4.07g How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session? [Circle one]

1 Never  
2 Less than monthly  
3 Monthly  
4 Weekly  
5 Daily or almost daily  
.K Don’t know  
.R Refused

4.07h How often during the last year have you had a feeling of guilt or remorse after drinking? [Circle one]

1 Never  
2 Less than monthly  
3 Monthly  
4 Weekly  
5 Daily or almost daily  
.K Don’t know  
.R Refused
4.07i How often during the last year have you been unable to remember what happened the night before because you had been drinking? [Circle one]

1 Never
2 Less than monthly
3 Monthly
4 Weekly
5 Daily or almost daily
.K Don’t know
.R Refused

[Showcard 4.07j]
4.07] Now please look at card 4.07j, have you or someone else been injured as a result of your drinking? [Circle one]

1 Yes, but not in the last year
2 Yes, during the last year
3 No
.K Don’t know
.R Refused

[Showcard 4.07j]
4.07k Again referring to card 4.07j, has a relative or friend, or a doctor or other health worker, been concerned about your drinking or suggested you cut down? [Circle one]

1 Yes, but not in the last year
2 Yes, during the last year
3 No
.K Don’t know
.R Refused

**THIS CONCLUDES MODULE 2**

**Interviewer observations**

please comment here if, for example, the respondent had language or cognitive difficulties or if they had assistance from a friend or family member.
8.5 Appendix 5: The Alac straight up guide to standard drink
A quick guide to how much alcohol you're drinking

178
### Appendix 6: Calculating typical alcohol consumption per day in grams

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Working out</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you had a drink containing alcohol in the last year</td>
<td>No</td>
<td></td>
<td>0g/day/year</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Go to Q2 and multiply the answer by Q3’s answer and by 10g</td>
<td></td>
</tr>
<tr>
<td>2. How often do you have a drink containing alcohol?</td>
<td>Monthly or less</td>
<td>1 x 12 =</td>
<td>12 days/year</td>
</tr>
<tr>
<td></td>
<td>Up to 4 times a month</td>
<td>4 x 12 =</td>
<td>48 days/year</td>
</tr>
<tr>
<td></td>
<td>Up to 3 times a week</td>
<td>3 x 52 =</td>
<td>156 days/year</td>
</tr>
<tr>
<td></td>
<td>4 or more times a week</td>
<td>4 x 52 =</td>
<td>208 days/year</td>
</tr>
<tr>
<td>3. How many drinks containing alcohol do you have on a typical day when</td>
<td>1 or 2</td>
<td>= 2</td>
<td>2 x 10g alcohol</td>
</tr>
<tr>
<td>you are drinking?</td>
<td>3 or 4</td>
<td>= 4</td>
<td>4 x 10g alcohol</td>
</tr>
<tr>
<td></td>
<td>5 or 6</td>
<td>= 6</td>
<td>6 x 10g alcohol</td>
</tr>
<tr>
<td></td>
<td>7 to 9</td>
<td>= 8</td>
<td>8 x 10g alcohol</td>
</tr>
<tr>
<td></td>
<td>10 or more</td>
<td>= 10</td>
<td>10 x 10g alcohol</td>
</tr>
</tbody>
</table>

Once Q2 is multiplied by Q3 and by 10g, the answer is divided by 365 days = g/day

Q = Question, g = grams
8.7 Appendix 7: MoCA test score sheet, tasks and point allocation

**Montreal Cognitive Assessment (MoCA)**

**Date of Assessment** | **Participant Study Number**
---|---

**Interviewer’s Name** | **Interviewer’s Number**
---|---

**Montreal Cognitive Assessment (MOCA)**

- **Executive Function**: Copy cube and Draw CLOCK (10 past eleven) (3 points)
- **Naming**: Contour Numbers Hands (3 points)
- **Memory**: Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.
- **Attention**: Read list of digits (1 digit/sec.). Subject has to repeat them in the forward order and in the backward order.
- **Language**: Fluency - Name maximum number of words in one minute that begin with the letter F (N ≥ 11 words)
- **Abstraction**: Similarity between e.g. banana - orange = fruit, train - bicycle = watch - ruler
- **Delayed Recall**: Has to recall words with no cue
- **Orientation**: Date, Month, Year, Day, Place, City

**Scores**

- **Face**: 1st trial 2, 2nd trial 3
- **Velvet**: 1
- **Church**: 1
- **Daisy**: 1
- **Red**: 1
- **Contour Numbers Hands**: 1
- **Total**: 10/30

*Add 1 point if ≤ 12 yr old*
A summary of the Montreal Cognitive Assessment fields, tasks and point allocation

<table>
<thead>
<tr>
<th>Category and activity</th>
<th>Instructions</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visuospatial/executive:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternating trail making</td>
<td>Draw a line, from a number to a letter in ascending order.</td>
<td>1</td>
</tr>
<tr>
<td>Visuo-constructional skills</td>
<td>Copy and draw the cube as accurately as possible.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Draw a clock with the numbers and set the time to 10 past 11.</td>
<td>3</td>
</tr>
<tr>
<td>Naming:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naming</td>
<td>Name the 3 animals in the picture: lion, rhino and camel.</td>
<td>3</td>
</tr>
<tr>
<td>Memory:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td>5 words are read out twice, participant repeat them after each time they hear them and then they need to remember them for later.</td>
<td>0</td>
</tr>
<tr>
<td>Attention:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward digit span</td>
<td>Read out numbers, which need to be repeated in same order.</td>
<td>1</td>
</tr>
<tr>
<td>Backward digit span</td>
<td>Read out 3 numbers that have to be repeated backwards.</td>
<td>1</td>
</tr>
<tr>
<td>Vigilance</td>
<td>List of letters read out, participant taps hand only when they hear the letter A.</td>
<td>1</td>
</tr>
<tr>
<td>Serial 7s</td>
<td>Count by subtracting seven from 100, and then, keep subtracting seven from the answer until participant is told to stop.</td>
<td>3</td>
</tr>
<tr>
<td>Language:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentence repetition</td>
<td>Two sentences are read out (one at a time) and have to be repeated word for word.</td>
<td>2</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Need to think of as many words as can in 60 sec starting with F.</td>
<td>1</td>
</tr>
<tr>
<td>Abstraction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstraction</td>
<td>Two pairs of words are given; participant says what each item has in common with the other.</td>
<td>2</td>
</tr>
<tr>
<td>Delayed recall:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed recall</td>
<td>Recall the words that were given earlier.</td>
<td>5</td>
</tr>
<tr>
<td>Orientation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orientation</td>
<td>Participant has to answer some questions: date today (year, month, day), day of the week, name of the place and city they are in.</td>
<td>6</td>
</tr>
<tr>
<td>Education years:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education years</td>
<td>If participant has had 12 or less years of education they get an extra point.</td>
<td>1</td>
</tr>
<tr>
<td>Total possible score</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>
8.8 Appendix 8: Mini-International Neuropsychiatric Interview

A. MAJOR DEPRESSIVE EPISODE (CURRENT)

(\ means: go to the diagnostic boxes, circle NO in all diagnostic boxes, and move to the next module)

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.1</td>
<td>Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?</td>
<td>0</td>
</tr>
<tr>
<td>A.2</td>
<td>In the past two weeks, have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time?</td>
<td>0</td>
</tr>
</tbody>
</table>

IS A1 OR A2 CODED YES?

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

A.3 Over the past two weeks, when you felt depressed or uninterested:

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td>Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by ≤5% of body weight or ±8 lbs. or ±3.5 kgs., for a 160lb./70kg. Person in a month)? IF YES TO EITHER, CODE YES</td>
<td>0</td>
</tr>
<tr>
<td>b)</td>
<td>Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning waking or sleeping excessively)?</td>
<td>0</td>
</tr>
<tr>
<td>c)</td>
<td>Did you talk or move more slowly than normal or were your fidgety, restless or having trouble sitting still almost every day?</td>
<td>0</td>
</tr>
<tr>
<td>d)</td>
<td>Did you feel tired or without energy almost every day?</td>
<td>0</td>
</tr>
<tr>
<td>e)</td>
<td>Did you feel worthless or guilty almost every day?</td>
<td>0</td>
</tr>
<tr>
<td>f)</td>
<td>Did you have difficulty concentrating or making decisions almost every day?</td>
<td>0</td>
</tr>
<tr>
<td>g)</td>
<td>Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?</td>
<td>0</td>
</tr>
</tbody>
</table>

ARE 3 OR MORE A3 ANSWERS CODED 1? (OR 4 A3 ANSWERS IF A1 OR A2 ARE CODED 0)?

<table>
<thead>
<tr>
<th></th>
<th>MAJOR DEPRESSIVE EPISODE CURRENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

IF PATIENT HAS CURRENT MAJOR DEPRESSIVE EPISODE THEN LIFETIME MAJOR DEPRESSIVE EPISODE (ON PAGE 6) MUST BE CODED 1.
8.9 Appendix 9: Medical Outcomes Study Short Form-36 version 2

Thank you for agreeing to take part in the Chalice study. We really appreciate you giving up your time to help complete this important research project. Please will you take a few minutes to read over and answer the following questions?

**HEALTH STATUS (SF-36v2)**

For each of the following questions, please select the one response that best describes your answer. Please enter the date that you are completing this questionnaire: ____/____/____

1. In general, would you say that your health is:
   1. Excellent
   2. Very good
   3. Good
   4. Fair
   5. Poor

2. Compared to one year ago, how would you rate your health in general now?
   1. Much better now than one year ago
   2. Somewhat better now than one year ago
   3. About the same as one year ago
   4. Somewhat worse now than one year ago
   5. Much worse now than one year ago

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

<table>
<thead>
<tr>
<th>Activity Description</th>
<th>1 Yes, Limited a lot</th>
<th>2 Yes, Limited a little</th>
<th>3 No, Not Limited at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Lifting or carrying groceries.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Climbing several flights of stairs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) Climbing one flight of stairs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) Bending, kneeling or stooping.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


(g) Walking more than a kilometre.
(h) Walking half a kilometre.
(i) Walking 100 metres.
(j) Bathing, showering or dressing yourself

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities, as a result of your physical health?

<table>
<thead>
<tr>
<th></th>
<th>1 All of the time</th>
<th>2 Most of the time</th>
<th>3 Some of the time</th>
<th>4 A little of the time</th>
<th>5 None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cut down on the amount of time you spent on work or other activities.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Accomplished less than you would like.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Were limited in the kind of work or other activities.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Had difficulty performing the work or other activities (for example, it took extra effort).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

<table>
<thead>
<tr>
<th></th>
<th>1 All of the time</th>
<th>2 Most of the time</th>
<th>3 Some of the time</th>
<th>4 A little of the time</th>
<th>5 None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cut down on the amount of time you spent on work or other activities.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Accomplished less than you would like.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Did work or activities less carefully than usual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

   1. Not at all
   2. A little bit
   3. Moderately
   4. Quite a bit
   5. Extremely

7. How much bodily pain have you had during the past 4 weeks?
1. No bodily pain
2. Very mild
3. Mild
4. Moderate
5. Severe
6. Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

1. Not at all
2. A little bit
3. Moderately
4. Quite a bit
5. Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.........

<table>
<thead>
<tr>
<th>Question</th>
<th>1 All of the time</th>
<th>2 Most of the time</th>
<th>3 Some of the time</th>
<th>4 A little of the time</th>
<th>5 None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Did you feel full of life?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Have you been very nervous?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Have you felt so down in the dumps that nothing could cheer you up?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Have you felt calm and peaceful?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) Did you have a lot of energy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(f) Have you felt downhearted and depressed?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Did you feel worn out?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(h) Have you been happy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Did you feel tired?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc)?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. None of the time
11. How TRUE or FALSE is each of the following statements for you?

<table>
<thead>
<tr>
<th></th>
<th>1 Definitely true</th>
<th>2 Mostly true</th>
<th>3 Don’t know</th>
<th>4 Mostly false</th>
<th>5 Definitely false</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) I seem to get sick a little easier than other people</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) I am as healthy as anybody I know</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) I expect my health to get worse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) My health is excellent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
If you have any questions about this diary please contact the CHALICE team.
Part 3: Food Diary

How to fill in your diary
Below is a step-by-step guide on how to fill in your food diary. It is very important that you do not change what you normally eat or drink just because you are keeping a diary so that we get a true picture of what you eat and drink. Try to fill in the diary each time you have something to eat or drink rather than leaving it until the end of the day so that you don't forget anything.

Step 1: When
Write down the exact time you ate or drank something. So, for example, if you had breakfast at 7.30am, write in "7.30am".

Step 2: Where
Please record where you were when you ate something. The next column along in the food diary is for you to write in where you were when you ate or drank something. This could be:
- At home – e.g. in the kitchen, in bed
- Away – e.g. in the street, in the car/on a bus, at a friend's or relative's house,
- In a cafe/restaurant (please specify McDonalds, Pizza Hut, etc.),
- At work – e.g. in canteen, in lunchroom, at your desk.

Step 3: Who with
In the next column in the food diary, please write down who you were with when you ate or drank something. For example, you might have been alone, with family or with friends. Experts have shown that by thinking who you were with during the day can help you to remember what you have eaten. We do not use this data in our research, it is just there to aid your memory.
Step 4: Food and drink
The next step in the food diary is to describe what you ate or drank. The more details you are able to give about the food and drink you have consumed, the better we will be able to estimate your nutrient intake. Include any extras like sugar and milk in your tea or cereal, butter or other spreads on your bread and sauces such as tomato sauce and mayonnaise. Do not forget to include drinking water.

Step 5: Brand and details
It would also help us if you can write down the brand name of any foods or drinks if you know it (e.g. Watties, Parm, Arnott's). If convenient, staple the wrapper to the back page of this book.
For breakfast cereals, as well as the brand name, please write down the name of the cereal (e.g. Coco Pops, Cornflakes, Sanitarium toasted museli: golden oats and fruit).
For sandwiches, please describe the type of bread used, how many slices of bread were used and give details of the filling.
For salad or mixed vegetables, please describe what is in it (e.g. 1 lettuce leaf, half a tomato, 8 slices of cucumber).
For pizza, please describe the topping (e.g. cheese and tomato, ham and pineapple).

Step 6: Preparation and cooking
If you know the cooking method used (e.g. roast, baked, boiled, fried) please write it down in this section.

Step 7: Quantity
In the next column, please write in the size of the portion of food or drink you had. For drinks, you can specify glass, cup, or mug or bottle/can size. Other descriptions include: packet (e.g. for crisps), number (e.g. for biscuits), slice (e.g. for cake, pizza), teaspoon (e.g. for sugar), tablespoon (e.g. for tomato sauce, pasta), cupful (e.g. for cooked pasta or rice), handful (e.g. for nuts, grapes, berries), package weights (e.g. 150g Fresh and Fruity yoghurt). On the next page you will find some more information on how to describe the food and drink that you consume.

If you have kitchen scales it is helpful to weigh foods and record these amounts.
For **mixed food dishes and recipes** it may be easier to list the total ingredients, then describe the proportion of this recipe that you consumed.

* e.g. 1/3 of recipe 1

**Recipe example**  Creamy tuna pasta (recipe 1)

- 250g Diamond spiral pasta
- ½ cup Oxo chicken stock, pre-mixed with water
- ¼ cup Chopped parsley
- 2 cups Sliced button mushrooms
- 220g John West tuna canned in oil, liquid drained
- 1 cup Carnation evaporated skim milk
- 1 tablespoon Parmesan cheese, dried
- ¼ teaspoon Freshly ground black pepper

I had one third of this recipe

If you make your food from separate ingredients then you can write the recipes down in the recipe list at the back of this diary.

Please write down all the ingredients for each recipe (including brand names, amounts and preparation or cooking details). Indicate the proportion of the recipe you consumed.

Don’t forget about any drinks that you have between meals e.g. tea, coffee, wine, beer, orange juice.
How to describe your food and drink using household measures

Below are some suggestions on how to describe certain food and drink items together with their household measures.

<table>
<thead>
<tr>
<th>Food</th>
<th>Description of food or drink and brand</th>
<th>Household measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon</td>
<td>Shoulder or streaky; fried or grilled rashers, smoked or unsmoked</td>
<td>Number</td>
</tr>
<tr>
<td>Bread</td>
<td>Type of bread, eg. white, brown, wholemeal, granary, French stick, ciabatta, currant.</td>
<td>Number of slices</td>
</tr>
<tr>
<td>Canned drinks</td>
<td>Type, brand name For example: 335ml can Diet Coca Cola</td>
<td>Number or full or half can</td>
</tr>
<tr>
<td>Crisps</td>
<td>Type, brand name e.g. 30g Rashuns</td>
<td>Packet weight</td>
</tr>
<tr>
<td>Fruit</td>
<td>Type and size of fruit e.g. large Granny Smith apple For tinned fruit; slices/ halves etc in juice or syr</td>
<td>Number of pieces or tablespoons</td>
</tr>
<tr>
<td>Jams</td>
<td>Type, brand name e.g. Pam's strawberry jam</td>
<td>Teaspoons, heaped or flat</td>
</tr>
<tr>
<td>Milk</td>
<td>Type; full cream, trim, semi-trim</td>
<td>Pints, glasses or cups</td>
</tr>
<tr>
<td>Oil</td>
<td>Type e.g canola oil, sunflower oil, corn oil, olive oil</td>
<td>Tablespoons</td>
</tr>
<tr>
<td>Prepacked foods eg.</td>
<td>Brand name e.g. Pam’s olive oil</td>
<td>Number</td>
</tr>
<tr>
<td>beefburgers, pies,</td>
<td>Full name of product including brand name For example: Bird’s Eye fish fingers.</td>
<td></td>
</tr>
<tr>
<td>biscuits, confectionery</td>
<td>Keep the package.</td>
<td></td>
</tr>
<tr>
<td>Sandwiches</td>
<td>Describe fully if homemade or if bought; Full name, place of purchase and price, describe bread as above and note loaf size.</td>
<td>Number of slices of bread or number of rolls</td>
</tr>
<tr>
<td>Spreads on bread or</td>
<td>Type e.g. butter, low fat spread, rice bran oil spread, canola spread, reduced fat canola spread,</td>
<td>Number of teaspoons or thinly, average or thickly spread</td>
</tr>
<tr>
<td>toast</td>
<td>Weight Watchers spread. Full description, and brand name. Keep the package.</td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>Type e.g. caster, rich brown, white</td>
<td>Teaspoons, heaped or flat</td>
</tr>
<tr>
<td>Sweets, chocolate and</td>
<td>Name, size (weight) and price (if known) For example: king size Mars bar 89c</td>
<td>Weight of bar or number of sweets</td>
</tr>
<tr>
<td>snack bars</td>
<td>Keep the wrapper.</td>
<td></td>
</tr>
<tr>
<td>Takeaways</td>
<td>Describe in full, give name of restaurant For example: One scoop chips, The High Street chip shop.</td>
<td>Portion size and price</td>
</tr>
<tr>
<td>Vegtableas</td>
<td>Type; fresh, frozen, tinned or dried Brand name</td>
<td>Tablespoons, full or heaped</td>
</tr>
</tbody>
</table>

Adapted from NUGENOB study (www.nugenob.com)
<table>
<thead>
<tr>
<th>Date</th>
<th>When</th>
<th>Where</th>
<th>Who with</th>
<th>Food or Drink</th>
<th>Brand and details</th>
<th>Preparation/ Cooking</th>
<th>Quantity</th>
</tr>
</thead>
</table>
Recipes

Please write down the ingredients of your recipes in this section.

<table>
<thead>
<tr>
<th>Recipe Number</th>
<th>Food or Drink</th>
<th>Brand and details</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Are there any special reasons why this week may differ from ‘normal’ in terms of household food (for example a child’s birthday party or other family celebration)?

Please circle either Yes or No

If yes please state reason:

__________________________________________

Please check that you have answered all the questions in parts 1, 2 and 3 and please make sure that you have filled in your diary for all four days.

Don’t forget to include any:

- Drinks e.g. tea, coffee, wine, beer, orange juice, soft drinks, water
- Snacks between meals e.g. biscuits, crisps, peanuts, slices, muffins
- Lollies or sweets

THANK YOU!
8.11 Appendix 11: Reasons for missing MoCA test scores

<table>
<thead>
<tr>
<th>Interviewer reasons for missing or invalid Montreal Cognitive Assessment scores</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did not speak English or English was the second language</td>
<td>9</td>
</tr>
<tr>
<td>Partially deaf, lip reader or hearing problems</td>
<td>2</td>
</tr>
<tr>
<td>Carbon monoxide poisoning</td>
<td>1</td>
</tr>
<tr>
<td>Blind</td>
<td>1</td>
</tr>
<tr>
<td>Tired due to a recent (3 months ago) mastectomy.</td>
<td>1</td>
</tr>
<tr>
<td>Learning difficulty</td>
<td>4</td>
</tr>
<tr>
<td>On medication and has depression</td>
<td>1</td>
</tr>
<tr>
<td>Memory impairment</td>
<td>1</td>
</tr>
<tr>
<td>Organic brain disease</td>
<td>1</td>
</tr>
<tr>
<td>Eye drop problems</td>
<td>1</td>
</tr>
</tbody>
</table>

**Total test scores missing:** 22
### 8.12 Appendix 12: Baseline characteristics of participants included and excluded from this thesis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants excluded (n=34)</th>
<th>Participants included (n=366)</th>
<th>P-value †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (standard deviation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong> (years)</td>
<td>50.9 (0.7)</td>
<td>50.8 (0.7)</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>BMI</strong> (kg/m²)</td>
<td>30.9 (7.6)</td>
<td>28.4 (6.2)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Serum 25OHD</strong> (nmol/L)</td>
<td>55.4 (25.9)</td>
<td>63.1 (24.1)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Alcohol</strong> (g/day)</td>
<td>0.66 (0.79)</td>
<td>1.06 (1.12)</td>
<td>0.01*a</td>
</tr>
<tr>
<td><strong>General Health</strong> (score out of 100)</td>
<td>71.1 (20.1)</td>
<td>71.4 (19.8)</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Creatinine</strong> (µmol/L)</td>
<td>81.0 (11.6)</td>
<td>81.7 (10.5)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Parathyroid Hormone</strong> (pmol/L)</td>
<td>4.4 (1.7)</td>
<td>4.4 (1.9)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Number of participants (percentage)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender Female (Male)</td>
<td>19 (56)</td>
<td>194 (53)</td>
<td>0.86</td>
</tr>
<tr>
<td>Ethnicity European (non-European)</td>
<td>22 (65)</td>
<td>294 (80)</td>
<td>0.05*</td>
</tr>
<tr>
<td>Education High (Low education) b</td>
<td>17 (50)</td>
<td>219 (60)</td>
<td>0.28</td>
</tr>
<tr>
<td>Current smoker (current non-smoker)</td>
<td>5 (15)</td>
<td>55 (15)</td>
<td>1.00</td>
</tr>
<tr>
<td>Current depression (no depression)</td>
<td>2 (6)</td>
<td>31 (9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Serum 25OHD d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient vitamin D</td>
<td>12 (35)</td>
<td>124 (34)</td>
<td>0.85</td>
</tr>
<tr>
<td>Sufficient vitamin D</td>
<td>22 (64)</td>
<td>242 (66)</td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index</strong> e</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/Underweight</td>
<td>7 (21)</td>
<td>105 (29)</td>
<td>0.21</td>
</tr>
<tr>
<td>Overweight</td>
<td>11 (32)</td>
<td>145 (40)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>16 (47)</td>
<td>116 (32)</td>
<td></td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter/Autumn</td>
<td>23 (68)</td>
<td>229 (63)</td>
<td>0.80</td>
</tr>
<tr>
<td>Summer/Spring</td>
<td>11 (32)</td>
<td>137 (37)</td>
<td></td>
</tr>
</tbody>
</table>

† Independent Welch’s t-test (continuous variables) & Fisher’s Exact Test (categorical variables)

Effect size: *r = 0.09, *OR 2.2 (0.96, 4.95)

bHigh education = post school education, low education = no education or secondary school education

cInsufficient vitamin D = ≤ 50 nmol/L, sufficient vitamin D = > 50 nmol/L

*normal/underweight = BMI ≤ 24.99 kg/m²; overweight = BMI ≥ 25 – 29.99 kg/m² & obese =BMI ≥ 30kg/m²

Abbreviations: BMI = body mass index, 25OHD = 25-hydroxyvitamin D, n = number, OR = odds ratio
8.13 Appendix 13: Distribution of MoCA scores and the transformed data

Transformations performed on MOCA scores

A histogram (left) of MoCA scores with a normal curve and a normal Q-Q plot (right) of the MoCA scores against the values of a normal distribution

1. Reciprocal transformation (-1/MOCA scores)

The Shapiro-Wilk normality test: W = 0.8338, p-value < 0.001
2. **Square Root transformation**

The Shapiro-Wilk normality test: \( W = 0.9045, \) p-value < 0.001

3. **Square Root transformation on reverse score transformation (highest score (30) subtracted from all the MOCA scores)**

The Shapiro-Wilk normality test: \( W = 0.9448, \) p-value < 0.001
Box Cox transformation (MOCA scores\(^3\))

The Shapiro-Wilk normality test: \(W = 0.956, p\)-value < 0.001

8.14 Appendix 14: Scatterplot with BMI and s25OHD levels according to BMI categories.

Abbreviations: BMI = body mass index, s25OHD = serum 25-hydroxyvitamin D.
Appendix 15: Scatterplot with creatinine and s25OHD according to gender.
### Appendix 16: Other variables added to model 2 to see if there was a significant effect on the model parameters

<table>
<thead>
<tr>
<th>Model 2 with continuous s25OHD instead:</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD levels (nmol/L)</td>
<td>-0.01 (0.01)</td>
<td>0.99 (0.98, 1.00)</td>
<td>0.089</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for current smoking:</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.79 (0.31)</td>
<td>0.45 (0.24, 0.82)</td>
<td>0.011</td>
</tr>
<tr>
<td>Current Smoker (non-smoker)</td>
<td>0.42 (0.40)</td>
<td>1.53 (0.72, 3.46)</td>
<td>0.284</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for creatinine:</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.82 (0.31)</td>
<td>0.44 (0.23, 0.79)</td>
<td>0.008</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>0.00 (0.02)</td>
<td>1.00 (0.97, 1.04)</td>
<td>0.827</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for parathyroid hormone:</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.81 (0.316)</td>
<td>0.45 (0.24, 0.82)</td>
<td>0.011</td>
</tr>
<tr>
<td>Parathyroid Hormone (pmol/L)</td>
<td>0.01 (0.08)</td>
<td>1.01 (0.87, 1.17)</td>
<td>0.937</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for BMI category</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.74 (0.30)</td>
<td>0.48 (0.26, 0.86)</td>
<td>0.015</td>
</tr>
<tr>
<td>Normal &amp; underweight (BMI &lt;25 kg/m²)</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Overweight (BMI: 25 – 29.99 kg/m²)</td>
<td>-0.14 (0.34)</td>
<td>0.87 (0.44, 1.68)</td>
<td>0.676</td>
</tr>
<tr>
<td>Obese (BMI ≥ 30 kg/m²)</td>
<td>-0.48 (0.35)</td>
<td>0.65 (0.32, 1.30)</td>
<td>0.226</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for age</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.82 (0.31)</td>
<td>0.44 (0.24, 0.79)</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.09 (0.19)</td>
<td>0.92 (0.63, 1.34)</td>
<td>0.648</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 with an interactive term (s25OHD x BMI)</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.56 (1.39)</td>
<td>0.57 (0.04, 8.98)</td>
<td>0.688</td>
</tr>
<tr>
<td>s25OHD &gt; 50 nmol/L x BMI</td>
<td>-0.01 (0.04)</td>
<td>0.99 (0.91, 1.08)</td>
<td>0.849</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 using BMI/10 rather than BMI</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.82 (0.31)</td>
<td>0.44 (0.24, 0.79)</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI per 10 kg/m²</td>
<td>-0.57 (0.22)</td>
<td>0.57 (0.37, 0.86)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 with 3 vitamin D categories instead of two</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD = 50-75nmol/L</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>s25OHD ≤50 nmol/L</td>
<td>0.79 (0.36)</td>
<td>2.2 (1.10, 4.51)</td>
<td>0.028</td>
</tr>
<tr>
<td>s25OHD &gt;75</td>
<td>0.10 (0.31)</td>
<td>1.1 (0.60, 2.02)</td>
<td>0.748</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for Diabetes</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.82 (0.31)</td>
<td>0.44 (0.24, 0.79)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes: yes (no)</td>
<td>0.00 (0.65)</td>
<td>1.00 (0.29, 3.88)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 further adjusted for cardiovascular disease</th>
<th>$\beta$ (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s25OHD &gt; 50 nmol/L (s25OHD ≤ 50 nmol/L)</td>
<td>-0.84 (0.31)</td>
<td>0.43 (0.23, 0.78)</td>
<td>0.007</td>
</tr>
<tr>
<td>Cardiovascular disease: yes (no)</td>
<td>0.19 (0.37)</td>
<td>1.21 (0.59, 2.59)</td>
<td>0.608</td>
</tr>
</tbody>
</table>

$\beta$ = beta coefficient, BMI = body mass index, CI = confidence intervals, OR = odds ratio, s25OHD = serum 25-hydroxyvitamin D, SE = standard error
Appendix 17: Scatterplot of BMI (kg/m²) and MoCA test scores

Appendix 18: Model 2 logistic regression rerun with data amended by adding additional point to MoCA scores for low education

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>β (SE)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.30 (0.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s25OHD &gt;50nmol/L (≤50nmol/L)</td>
<td>-0.71 (0.32)</td>
<td>0.49 (0.26, 0.90)</td>
<td>0.024</td>
</tr>
<tr>
<td>Gender male (female)</td>
<td>-0.30 (0.28)</td>
<td>0.74 (0.43, 1.27)</td>
<td>0.273</td>
</tr>
<tr>
<td>Education High (Low)</td>
<td>0.31 (0.28)</td>
<td>1.37 (0.80, 2.34)</td>
<td>0.259</td>
</tr>
<tr>
<td>Household Income ≥ $40,001 (&lt;$40,001)</td>
<td>0.62 (0.38)</td>
<td>1.86 (0.86, 3.83)</td>
<td>0.102</td>
</tr>
<tr>
<td>Ethnicity non-European (European)</td>
<td>0.01 (0.33)</td>
<td>1.01 (0.54, 2.00)</td>
<td>0.971</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.07 (0.02)</td>
<td>0.94 (0.90, 0.98)</td>
<td>0.002</td>
</tr>
<tr>
<td>Depression Yes (No)</td>
<td>-0.46 (0.44)</td>
<td>0.63 (0.27, 1.54)</td>
<td>0.290</td>
</tr>
</tbody>
</table>

β = beta coefficient, SE = standard error, CI = confidence intervals, OR = odds ratio