VITAMIN D DEFICIENCY:
COGNITION, MORTALITY AND RESOURCE UTILISATION –
PROSPECTIVE ASSOCIATIONS

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

UNIVERSITY OF DUBLIN
TRINITY COLLEGE

July 2019
Declaration

I declare that the work in this thesis is entirely my own except where credit is given in the acknowledgements.

I have contributed to the study upon which this thesis is based by recruiting over one hundred and thirty participants for subsequent study participation.

All subjects participating in the studies gave full and informed consent and ethical approval was granted by the `Joint Research and Ethics Committee of St. James’s Hospital.

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

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish

I agree to deposit this thesis in the University’s open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

____________________
Avril Beirne

July 2019
Summary

Introduction
The association between Vitamin D and bone health has long been known. In recent times there has been increasing research into the effects of Vitamin D beyond this well-established link. Although there has been extensive work completed, the results have been variable to date.

Aims
The aim of this body of work was to prospectively assess the extra-osseous associations of Vitamin D in an older Irish population who are at significant risk of Vitamin D deficiency. The areas of interest include all-case mortality, resource utilisation, including hospital admission, Emergency Department (ED) attendance and cognition.

Methods
These studies come from prospectively determining outcomes for participants of the TUDA study some five years later. The TUDA study was a large cross sectional study from Northern and Southern Ireland that compared cognition and diet in a community population from three disease-defined cohorts (mild cognitive impairment (MCI), hypertension and osteoporosis). Four individual studies were conducted to evaluate the relationship between Vitamin D and mortality, hospital admission and Emergency Department (ED) attendances and cognition. The 3,093 participants included were the TUDA cohorts recruited between 2008 and 2012 through Saint James’s Hospital (from the MCI and osteoporosis cohort) and outcomes were assessed between 2013 and 2015.

Results
During the follow-up period of four years, 40% of participants were admitted, 51% attended the Emergency Department and 15% died. In the three studies looking at mortality or hospital attendance, Vitamin D was inversely related to all the outcomes of interest. Vitamin D deficiency was not associated with cognitive change over time in four of the five RBANS Indices. Performance on Index IV improved significantly in cases.
Summary (continued)

All statistical models were adjusted for multiple covariates including potential confounders of Vitamin D, frailty and chronic medical conditions.

Conclusion
These studies add to a growing literature that shows prospective associations between Vitamin D deficiency and poor outcome measures in relation to mortality and resource utilisation. The improvement in RBANS Index IV in patients who were initially Vitamin D deficient may have been a chance effect or may reflect a benefit in cognitive function with repletion of deficient Vitamin D levels over time. All associations were independent of markers of cognitive and physical frailty. These results support an effect of Vitamin D beyond falls and bone health. The need for further evaluation of Vitamin D deficiency and supplementation, as a modifiable factor in processes beyond the current recommendations for bone health, is warranted and should focus on randomised controlled trials.
Publications and Presentations Related to Thesis

Presentations
1. Vitamin D Deficiency and Cognition Function- A Case Control Study
Irish Gerontological Society Annual Meeting, September 2017
Avril M Beirne, Kevin G McCarroll, J Bernard Walsh, Miriam C Casey, Eamon Laird, Helene McNulty, Mary Ward, Leanne Hoey, Anne Molloy, Martin Healy, Conal J Cunningham

Abstracts
1. A Prospective Study of Mortality in the Trinity University of Ulster and Department of Agriculture (TUDA) Cohort
A M Beirne, K McCarroll, M C Casey, H McNulty, E Laird, C Walsh, J B Walsh, M Walsh, L Hoey, A Molloy, M Healy, JJ Strain, C J Cunningham
Irish Journal of Medical Science (2014) 183 (Suppl 7): S320

2. Vitamin D Deficiency and Cognition Function- A Case Control Study
Avril M Beirne, Kevin G McCarroll, J Bernard Walsh, Miriam C Casey, Eamon Laird, Helene McNulty, Mary Ward, Leanne Hoey, Anne Molloy, Martin Healy, Conal J Cunningham
Age and Ageing (2017) 46 (Suppl 3) Siii12

3. Vitamin D Deficiency and Resource Utilisation – A Prospective Association
Avril M Beirne, Kevin G McCarroll, J Bernard Walsh, Miriam C Casey, Eamon Laird, Helene McNulty, Mary Ward, Leanne Hoey, Anne Molloy, Martin Healy, Conal J Cunningham
Age and Ageing (2017) 46 (Suppl 3) Siii55
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Finally, to my husband Seán, my parents and family, I am eternally grateful for your encouragement, understanding and unfaltering belief and support as always.
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<td>Addenbrooks Cognitive Examination</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute Coronary Syndrome</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>ADAS-Cog</td>
<td>Alzheimer’s Disease Assessment Scale-Cognitive Subset</td>
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<td>ADL</td>
<td>Activity of Daily Living</td>
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<td>aMM</td>
<td>Appendicular Muscle Mass</td>
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<td>AMTS</td>
<td>Abbreviated Mental Test Score</td>
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<td>APACHE</td>
<td>Acute Physiology and Chronic Health Evaluation</td>
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<td>ApoE</td>
<td>Apolipoprotein E</td>
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<tr>
<td>ARF</td>
<td>Acute Renal Failure</td>
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<td>ARI</td>
<td>Acute Respiratory Infection</td>
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<td>ASA</td>
<td>American Society of Anaesthesiologists Physical Status Classification</td>
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<td>Auditory Verbal Learning Test</td>
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<td>BMD</td>
<td>Bone Mineral Density</td>
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<td>Body Mass Index</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>CAB</td>
<td>Cognitive Assessment Battery</td>
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<tr>
<td>CABG</td>
<td>Coronary Artery Bypass Graft</td>
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<td>CAM</td>
<td>Confusion Assessment Measure</td>
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<td>CANTAB</td>
<td>Cambridge Neuropsychological Test Automated Battery (CANTAB)</td>
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<td>CAP</td>
<td>Community Acquired Pneumonia</td>
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<td>CCF</td>
<td>Congestive Cardiac Failure</td>
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<td>CDR</td>
<td>Clinical Dementia Rating</td>
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<tr>
<td>CDT</td>
<td>Clock Drawing Task</td>
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<td>CES-D</td>
<td>Centre of Epidemiologic Studies Depression Scale</td>
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<td>CERAD</td>
<td>Consortium to Establish a Registry for Alzheimer’s Disease</td>
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<td>CGA</td>
<td>Comprehensive Geriatric Assessment</td>
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CHD  Coronary Heart Disease
CI   Confidence Interval
CKD  Chronic Kidney Disease
CNS  Central Nervous System
COGTEL  Cognitive Telephone Screening Instrument (COGTEL)
COPD Chronic Obstructive Pulmonary Disease
CRF  Chronic Renal Failure
CRP  C-Reactive Protein
CRF  Cardiorespiratory Fitness
CRT  Choice Reaction Time
CT   Computerised Tomography
CTRM Camden Topographical Recognition Memory
CURB (65) Confusion/Urea/Respiratory Rate/Blood Pressure/Aged >65years
CV   Coefficient of variation
CVA  Cerebrovascular Accident (Stroke)
CVD  Cardiovascular Disease
CVLT (II) California Verbal Learning Test (Second Edition)
DAD  Disability Assessment in Dementia
DBP  Vitamin D Binding Protein
DEQAS Vitamin D External Quality Assessment Scheme
DNA  Deoxyribonucleic Acid
DRG  Diagnostic Related Group
DSM IV Diagnostic and Statistical Manual IV
DSST Digit Symbol Substitution Test
D(E)XA Dual Energy X-Ray Absorptiometry
DVT  Digital Vigilance Task
DWMI Deep White Matter Ischaemia
DWRT Delayed Word Recall Test
ECG  Electrocardiogram
ED   Emergency Departme
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<td>EF</td>
<td>Ejection Fraction</td>
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<td>EFS</td>
<td>Edmonton Frailty Scale</td>
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<tr>
<td>EMS</td>
<td>Elderly Mobility Score</td>
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<td>eGFR</td>
<td>Glomerular Filtration Rate (estimated)</td>
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<td>FAB</td>
<td>Frontal Assessment Battery</td>
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<td>FEV1</td>
<td>Forced Expiratory Volume</td>
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<td>FI</td>
<td>Frailty Index</td>
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<td>FIM</td>
<td>Functional Independence Measure</td>
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<td>Forced Vital Capacity</td>
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<td>GDNF</td>
<td>Glial Cell Line Derived Neurotrophin Factor</td>
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<td>GDS</td>
<td>Geriatric Depression Scale</td>
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<td>GSR</td>
<td>Global Solar Radiation</td>
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<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
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<td>HD</td>
<td>Huntington’s disease</td>
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<td>HF</td>
<td>Heart Failure</td>
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<td>HGS</td>
<td>Hand Grip Strength</td>
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<td>HR</td>
<td>Hazard Ratio</td>
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<td>Health Service Executive</td>
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<td>HTN</td>
<td>Hypertension</td>
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<td>Instrumental Activities of Daily Living</td>
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<td>ICD</td>
<td>International Classification of Diseases</td>
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<td>ICU</td>
<td>Intensive Care Unit</td>
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<td>IHD</td>
<td>Ischaemic Heart Disease</td>
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<td>ITT</td>
<td>Intention to Treat</td>
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<td>ITU</td>
<td>Intensive Therapy Unit</td>
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<td>IU</td>
<td>International Units</td>
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<td>KFI</td>
<td>Kaplan-Feinstein Index</td>
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<td>LCMS</td>
<td>Liquid chromatography mass spectroscopy</td>
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<td>LOS</td>
<td>Length of Stay</td>
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<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<td>aMCI</td>
<td>Amnestic Mild Cognitive Impairment</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<td>MICE</td>
<td>Multivariate Imputation by Chained Equations</td>
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<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<td>MMMSE (3MS)</td>
<td>Modified Mini Mental State Examination</td>
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<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
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<td>Meals on Wheels</td>
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<td>MRI</td>
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<td>North American Adult Reading Test</td>
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<td>NGF</td>
<td>Nerve Growth Factor</td>
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<td>NH</td>
<td>Nursing Home</td>
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<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association</td>
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<td>NIST</td>
<td>National Institute of Standards and Technology</td>
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<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
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<td>Neuropsychological Testing</td>
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<td>Neurotrophin-3</td>
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<td>OR</td>
<td>Odds Ratio</td>
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<td>PASE</td>
<td>Physical Activity Scale for the Elderly</td>
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<td>PCI</td>
<td>Percutaneous Coronary Intervention</td>
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<td>Parkinson’s disease</td>
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<td>PEFR</td>
<td>Peak Expiratory Flow Rate</td>
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<td>Positron Emission Tomography</td>
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<td>Physical Performance Test</td>
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<td>Prostate Specific Antigen</td>
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<td>Physical Self Maintenance Scale</td>
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<td>Parathyroid Hormone</td>
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<td>PVD</td>
<td>Peripheral Vascular Disease</td>
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<tr>
<td>PVWMI</td>
<td>Periventricular White Matter Ischaemia</td>
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<td>QALY</td>
<td>Quality Adjusted Life Year</td>
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<td>QoL</td>
<td>Quality of Life</td>
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<td>RAA</td>
<td>Renin Angiotensin-Aldosterone</td>
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<td>RBANS</td>
<td>Repeatable Battery for the Assessment of Neuropsychological Status</td>
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<td>Raven’s Coloured progressive Matrices</td>
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<td>RCT</td>
<td>Randomised Control Trial</td>
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<tr>
<td>(m)RNA</td>
<td>(messenger) Ribonucleic Acid</td>
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<td>ROC</td>
<td>Receiver Operating Characteristic</td>
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<td>Roy-Osterrieth Complex Figure (ROCF)</td>
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<td>Relative Risk</td>
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<td>SBT</td>
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<td>Standard Deviation</td>
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<td>SJH</td>
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<td>Systemic Inflammatory Response Syndrome</td>
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<td>SNP</td>
<td>Single Nucleotide Polymorphisms</td>
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<td>SOFA</td>
<td>Sequential Organ Failure Assessment</td>
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<td>SOL</td>
<td>Space Occupying Lesion</td>
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<td>SPMSQ</td>
<td>Short Portable Mental Status Questionnaire</td>
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<td>Self Rated Health</td>
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<td>SRT</td>
<td>Simple Reaction Time</td>
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<td>SUVR</td>
<td>Regional standard uptake value ratio</td>
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<td>SVD</td>
<td>Small Vessel Disease</td>
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<td>TIA</td>
<td>Transient Ischaemic Attack</td>
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<td>TUDA</td>
<td>Trinity, University of Ulster, Department of Agriculture</td>
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<td>TUG</td>
<td>Timed Up and Go</td>
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<td>UV(B)</td>
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<tr>
<td>VC</td>
<td>Vital Capacity</td>
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<td>VCI</td>
<td>Vascular Cognitive Impairment</td>
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<td>VD</td>
<td>Vascular Dementia</td>
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<td>VF</td>
<td>Verbal Fluency</td>
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<td>VDBP</td>
<td>Vitamin D Binding Protein</td>
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<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
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<td>Vitamin D Response Element</td>
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<td>VDRP</td>
<td>Vitamin D Receptor Protein</td>
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<td>Word Fluency Test</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>White Matter Ischaemia</td>
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<td>1,25(OH)(_2)D(_3)</td>
<td>1,25-dihydroxyvitamin D</td>
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<td>6MWT</td>
<td>6 Minute Walk Test</td>
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**Appendix**
Section 2: Vitamin D – An Introduction
Chapter 1: A Brief History of Vitamin D

1.1 Introduction

Rickets, a disease of the bone, although observed as early as the first and second century AD in the Greek-Roman era (Hess, 1928), was first identified as a distinct clinical disorder, in the 1600s by Whistler and Glisson (Clarke, 1962). It is a disease of childhood caused by lack of sufficient amounts of calcium, phosphate and Vitamin D to allow the normal calcification of growing bones (Weick, 1967). During the late 1700s it became rampant in Europe as people began to live in large, over-populated, polluted cities with reduced exposure to sunlight. It was estimated that towards the beginning of the 20th century, 80-90% of children in Europe and certain parts of the United States had evidence of rickets (Holick, 2006).

1.2 Vitamin D and Cod Liver Oil

In the 1890s, the search began for specific foods and remedies that could potentially prevent rickets. This is based on two major theories: the effects of either sunlight and or cod liver oil in the development of rickets. This search would eventually lead to the discovery of Vitamin D. Scientists including Palm, Kassowitz and Schmorl in the late 1800s and early 1900s proposed that a lack of sunlight exposure, latitude and seasonality were factors and causes in the development of rickets.

In 1914, McCollum and his colleagues isolated a fat-soluble factor from butterfat, which they named “fat-soluble factor A,” which later became “vitamin A” that was found to be necessary for normal growth and prevention of specific eye diseases in young rats. Based on this work, Sir Edward Mellanby described the deficiency of a fat-soluble nutrient in cod-liver oil as the cause for rickets in dogs. He wrote:

“Rickets is a deficiency disease which develops in consequence of the absence of some accessory food factor or factors. It therefore seems probable that the cause of rickets is a diminished intake of an anti-rachitic factor, which is either [McCollum’s] fat-soluble factor A, or has a similar distribution to it” (Mellanby, 1989).
In 1922, McCollum observed that oxidation destroys “fat-soluble A” without destroying another substance which plays an important role in bone growth and concluded that fat-soluble factor A consisted of two entities, one later called “vitamin A” and the other the newly discovered antiachitic factor which he called Vitamin D (McCollum et al., 2002).

![Elmer Verner McCollum (early 1900s)](image)

Subsequently, Goldblatt and Soames demonstrated that skin exposed to sunlight or ultraviolet (UV) light produced a substance with similar properties to this nutrient (Goldblatt and Soames, 1923). This ultimately led to the discovery of the chemical structure of vitamin D by Adolf Windaus who was awarded the Nobel Prize in Chemistry in 1928;

“For his studies on the constitution of the sterols and their connection with vitamins” (Wolf, 2004).

As a result, by the 1930s the use of cod liver oil in the treatment of prevention of rickets had become common practice (Weick, 1967).

1.3 Vitamin D and Heliotherapy

The effect of sunlight and in particular Ultraviolet (UV) radiation in the prevention of rickets was also under evaluation. As far back as 1822, Sniadecki had recognised the medical benefits of sunlight, when he found that there were higher incidences of rickets in urban dwelling Polish children compared with their rural counterparts (W., 1939). In the
early 1900s, a number of scientists across Europe noted that exposure to sunlight and artificially produced UV light could cure children with rickets (Chick, 1976, Hess, 1922). Hess and Gutman went on to establish the chemical basis of heliotherapy by demonstrating that sunlight therapy improved the status of rickets clinically, radiologically and biochemically and claimed their results;

“furnish the first definitive evidence of metabolic change in the animal body brought about by the solar rays” (Hess AF, 1922).

Steenbock later found that irradiation of not only the rat, but also their food, could prevent or cure rickets and concluded that an inactive lipid in the diet and skin could be converted by UV light into an active anthracitic substance (Steenbock, 1924). This discovery was a major breakthrough. It became possible to enhance the Vitamin D content of common infant foods such as milk and cereal in an inexpensive and palatable way. Consumption of such Vitamin D–enhanced foods led to the eradication of “epidemic” rickets and within two decades, a wide variety of foods and beverages were fortified with Vitamin D.
Chapter 2: Basic Science and Biochemistry of Vitamin D

2.1 Vitamin D Structure

Vitamin D refers to a family of lipid soluble compounds with a four-ringed cholesterol backbone and exists in two major physiological forms: Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). The difference between the two forms is based on the formations of their side chains.

**Figure 1: Chemical Structure of Vitamin D**

Vitamin D indicates a molecule with the general structure of rings A, B, C, and D with differing side chain structures. The A, B, C, and D ring structure is derived from the cyclopentanoperhydrophenanthrene ring structure for steroids. Technically, vitamin D is classified as a seco-steroid, i.e. those in which one of the rings has been broken. In Vitamin D, the 9,10 carbon-carbon bond of ring B is broken.

2.2 Vitamin D Metabolism

Humans obtain Vitamin D from three main sources: exposure to sunlight, diet and dietary supplements. Vitamin D is a pro-hormone that must be metabolised in the body to its biologically active form 1,25-dihydroxcholecalciferol or 1,25-dihydroxyvitamin D (1,25(OH)_{2}D_{3}). Vitamin D2 is produced in plants, yeasts and fungi when ergosterol is exposed to UVB irradiation. Vitamin D3 can be produced photo-chemically in the skin of...
vertebrates on exposure of 7-dehydroxycholesterol to UVB radiation (wavelength, 290 to 315 nm) (Holick, 2006).

Dietary Vitamin D is ingested in the form of Vitamin D2 (ergocalciferol) from plant sources, Vitamin D3 (cholecalciferol) from animal sources and in fortified foods such as milk, fatty fish and cod liver oil. It is incorporated into micelles in the intestine, absorbed by enterocytes and incorporated into chylomicrons and transported bound to Vitamin D Binding Protein (VDBP) to the liver via the portal circulation. In the liver Vitamin D is hydroxylated by 25-hydroxylase to form 25-hydroxyvitamin D (25(OH)D or calcidol), which is the major circulating form of Vitamin D and is used in practice as a measure of a person’s Vitamin D level as it has stable levels and a long half-life of approximately two to three weeks (Holick, 2006). CYP2R1 is now considered the key enzyme responsible for the conversion of vitamin D to 25(OH)D3 (Cheng et al., 2003).

In these forms, Vitamin D is biologically inert, and so after its synthesis in the liver, 25(OH)D3 is transported by DBP to the kidney where it is internalised by megalin, a transmembrane protein that acts as a surface receptor for DBP (Nykjaer et al., 1999), where further hydroxylation of 25(OH)D by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase) (CYP27B1) occurs. This takes place in the mitochondria of the proximal tubules of the kidney to 1,25(OH)2D3, the bioactive form of Vitamin D. The renal production of 1,25(OH)2D3 is tightly regulated by plasma parathyroid hormone (PTH) levels and serum calcium and phosphorus levels (Holick, 2007).
Figure 2: Metabolism of Vitamin D

- UVB (290-315nm) 
  - Skin
    - 7 - Dehydrocholesterol
  - Solar radiation 
    - Inactive photoprotectors

- Heat
  - Heat 
    - Vitamin D$_3$
  - Fat Cell

- Circulation
  - Circulation
  - 25-Vitamin D-hydroxylase Liver

- Phosphorus, calcium, FGF and other factors +/- 
  - 25 (OH) D
    - Main circulating metabolite
    - 1 Hydroxylase Kidney
      - 1,25 (OH)$_2$D
      - Osteoblast Bone Osteoblast
        - Ca$^{2+}$ and HPO$_4^{2-}$
        - Calcification
      - Parathyroid Hormone
    - Parathyroid
      - Intestinal Calcium Absorption
      - Bile
      - Excretion

- 1,25 (OH)$_2$D
  - Calcium Absorption
2.3 Vitamin D Receptor

The Vitamin D hormone functions through the nuclear Vitamin D Receptor (VDR), a 427 amino acid peptide, which has been identified in many tissues throughout the body which likely accounts for the diverse biological actions of the most active metabolite of Vitamin D, 1,25(OH)_{2}D_{3} (Jones et al., 1998). VDR belongs to the nuclear hormone receptor superfamily and acts as a ligand inducible transcription regulation factor (Mangelsdorf et al., 1995).

Figure 3: Vitamin D Receptor
The actions of Vitamin D have been thought of as “classical” describing those involved in calcium, phosphate and bone homeostasis (Bouillon et al., 2008) and “non classical “being those functions related to cellular growth, proliferation and immune function (DeLuca, 2004). These actions are initiated when there is direct interaction between 1,25(OH)2D3, which acts as a ligand, and the intracellular VDR, which results in changes in gene expression.

The VDR bind to DNA as VDR/VDR homodimers (Freedman et al., 1994) or VDR/RXR (retinoid x receptor) heterodimers in order to regulate gene expression (Issa et al., 1998). Activation of the VDR, the liganded VDR, prompts rapid binding of the VDR to regulatory regions of target genes, forming large protein complexes, which in turn direct changes in transcription (Haussler et al., 1998, Darwish and DeLuca, 1993).

**Figure 4:** Actions of Vitamin D
2.4 Vitamin D and Bone

The benefits of Vitamin D on bone health have previously been mentioned in terms of the eradication of rickets in children, and prevention of osteomalacia and osteoporosis in adults. These actions are mediated by maintaining calcium homeostasis. In severely deficient Vitamin D deficient states, the osteoid (new bone) is not mineralised. In less severe deficiency, there is an increase in production of PTH, which in turn causes bone resorption and can lead to osteoporosis and fractures.

In hypocalacemic states, 1,25(OH)₂D₃ and PTH act to maintain calcium homeostasis. 1,25(OH)₂D₃ acts to increase calcium absorption from the intestine. If normal calcium is unable to be maintained by intestinal calcium absorption, then 1,25(OH)₂D₃ and PTH, together acting via their receptors, release calcium from the bone stores and increase reabsorption of calcium from the distal tubule of the kidney (Veldurthy et al., 2016). In bone, both PTH and 1,25(OH)₂D₃ stimulate osteoclastogenesis (Christakos et al., 2016). Osteoclasts cause bone resorption, resulting in the release of calcium from bone to maintain calcium levels.
Chapter 3: Vitamin D Deficiency

It is estimated that a billion people in the world are either deficient or have insufficient levels of Vitamin D (Holick, 2007). Prevalence rates vary depending on definitions used, populations sampled, locations and time of year samples are taken. An Irish study from the TUDA population, reported the prevalence of vitamin D deficiency (<50nmol/l) in non-supplemented subjects as 43.4%, 66.0% and 75.0% in the respective bone, hypertensive and cognitive cohorts. Severe deficiency (<25nmol/L) was most prevalent (32.8%) in non-supplemented cognitive cohort participants. The mean difference in 25(OH)D due to supplementation was between 21.4 and 35.4nmol/L (McCarroll et al., 2015).

The effects of Vitamin D deficiency on bone are well established, causing rickets and osteomalacia, osteoporosis, falls (Bischoff-Ferrari et al., 2004b) and fractures (Bischoff-Ferrari et al., 2005). In more recent years extra-osseous effects of Vitamin D have been proposed, including cancer, diabetes (Zella and DeLuca, 2003) cardiovascular disease (Grandi et al., 2010) and inflammatory conditions such as Multiple Sclerosis (Cantorna et al., 1996). The biologic basis for these associations of Vitamin D and extra skeletal effects are presumed related to the expression of VDR and 1\(\alpha\) hydroxylase in multiple tissues throughout the body as previously outlined.

Vitamin D status is most commonly measured using serum 25-hydroxyvitamin D (25(OH)D) levels, as this provides a relatively accurate marker of bioavailability (Rosen, 2011). The most common cut-off for Vitamin D deficiency is <50nmol/L (Holick, 2007), however there are many varying definitions from the Institute of Medicine (IoM) and the Endocrine Society of America. Vitamin D deficiency is prevalent in Northern Europe affecting up to 50% or more of older adults (Hirani et al., 2010, Klenk et al., 2013). In locations at high latitude such as Ireland (52°N), little or no cutaneous synthesis occurs during the winter months (Webb et al., 1988).

Deficiency of Vitamin D is more common in older adults and is likely related to a combination of factors. Despite similar sun exposure, Vitamin D production is
approximately 75% less when compared to young adults, which may result from reduced epidermal levels of 7-dehydrocholesterol (MacLaughlin and Holick, 1985, Holick et al., 1989). In older adults, lifestyle and physiological factors including, reduced exposure to sunlight, increased adiposity, poor dietary intake and decreased capacity of skin to produce Vitamin D by photoisomerization, impaired hepatic or renal hydroxylation or end-organ resistance to Vitamin D may also negatively affect 25-hydroxyvitamin D [25(OH)D] status (Holick, 2007).

In an Irish study evaluating the determinants of Vitamin D deficiency, in a large cohort of community dwelling older adults, Vitamin D supplement use and Global Solar Radiation (GSR) were positive predictors of 25(OH)D, whereas the only universal negative predictor was Body Mass Index (BMI). Although supplement use was the most important determinant of Vitamin D status, Vitamin D fortified milk and spending time in the sun, even in the oldest old, were found to be useful strategies in improving 25(OH)D levels (McCarroll et al., 2015).

Identifying and understanding factors associated with Vitamin D deficiency are important as this can help identify populations at risk. Optimisation of 25(OH)D status in these people may reduce falls and fracture risk (Bischoff-Ferrari et al., 2012). In addition, as increasing evidence purports a role for vitamin D in cognition, cardiovascular, neurological and autoimmune disease, as well as cancer and depression, there may also be a role for Vitamin D in modifying risk in these conditions (Rejnmark et al., 2017).
Chapter 4: Vitamin D and Frailty

4.1 Frailty
Frailty is a term increasingly used in the description of older adults. Frailty has been considered as a clinical syndrome characterised by age associated decline in physiological and functional ability that carries increased risk of poorer health outcomes such as disability, falls, co-morbidity, and mortality along with other characteristics (Fried et al., 2001, Graham et al., 2009, Ensrud et al., 2009, Romero-Ortuno and Kenny, 2012). However a standardised definition has yet to be established, although many definitions and descriptions have been purported either based on a physical phenotype (Fried et al., 2004) or summation of co-morbidities and physical and psychological factors (Rockwood and Mitnitski, 2007).

Some of the dimensions of frailty, such as muscle weakness, poorer performance and slowness can be as a result of Vitamin D deficiency (Janssen et al., 2002). As previously outlined, Vitamin D mediates its effects on muscle weakness, sarcopenia and changing muscle morphology through a number of mechanisms. Also with low serum 25(OH)D concentrations, 1,25(OH)2D3 absorption decreases causing a rise in PTH, which results in increased inflammatory cytokine production and also reduced muscle fibre levels (De Martinis et al., 2002).

The importance of considering frailty when evaluating potential factors in resource utilisation has been demonstrated in previous studies (Kawryshanker et al., 2014, Hubbard and Theou, 2012). Interventions that can improve outcomes in frail older adults attending acute hospital environments are increasingly important and improving on factors that can impact on these outcomes are of significant concern. Previous studies demonstrate that frailty is associated with increased ED attendance and increased rates of hospitalisation (Chamberlain et al., 2016, Hastings et al., 2008). An Australian study previously showed that intermediate and high frailty groups had 22 and 43% higher healthcare costs in the six months following a hospital admission compared with those with low levels of frailty (Comans et al., 2016).
4.2 Vitamin D and Frailty

To date interventions such as Comprehensive Geriatric Assessment (CGA), multidisciplinary approach to care, elder-friendly environments and focused exercise programmes have been shown to be beneficial in managing frail older patients (Ellis et al., 2011, Van Craen et al., 2010, Cohen et al., 2002).

There is increasing evidence to support the role of Vitamin D deficiency in frailty states and functional outcomes in older adults. There are many ways in which Vitamin D could contribute to frailty including reduced muscle protein synthesis, sarcopenia, falls and increased muscle weakness (Lips, 2006). These effects are thought to be mediated through increased levels of inflammatory cytokines such as Interleukin 12 and Interleukin 2 as well as through secondary hyperparathyroidism (Lips, 2006, Halfon et al., 2015). Identification of predictors of frailty and reduced functional status may offer mechanisms to target those at increased risk of resource utilisation.

4.3 Cross Sectional Studies

Positive Studies

Kiebzak et al performed a pilot study looking at the relationship between Vitamin D and short-term rehabilitation progress and functional status in a US study (Kiebzak et al., 2007). A convenience sample of 100 patients admitted to a rehab unit was recruited. 25(OH)D was measured as well as grip strength, bone status using ultrasound of the heel and the Functional Independence Measure (FIM). The mean age of the participants was 70 years with mean Vitamin D levels of 44nmol/L. Serum 25(OH)D concentration was found to be positively associated with FIM scores (r=0.25 p<0.011) and grip strength (r=0.23 p<0.022). Serum 25(OH)D was not significantly correlated with either FIM efficiency or change in total FIM score during admission. An inverse relationship was also found between Vitamin D and hospital LOS, (Spearman r =0.235, p<0018).

Boxer et al evaluated the relationship between Vitamin D and frailty in patients with heart failure with an Ejection Fraction (EF) <40% aged >60 years (Boxer et al., 2008). Aerobic capacity was tested using the six-minute walk test (6MWT). Frailty was defined using the
Freid et al criteria. Sixty participants attending an outpatient Heart Failure (HF) clinic participated, with a mean age of 77 years, mean 25(OH)D level was 57.9nmol/L and a mean 6MWT of 309 meters. According to frailty criteria, 28% were not frail and 25% had 3-5 frailty characteristics. Significant correlations were found between Vitamin D levels and the 6MWT with lower Vitamin D levels associated with poorer performance, β 0.42, P<0.05. Vitamin D was also associated with frailty scores, β -0.30, p<0.05 and subsets of physical activity and grip strength were also associated with Vitamin D levels.

As part of a longitudinal study looking at the relationship between Vitamin D, frailty and mortality, Smit et al reported cross-sectional results from NHANES III (Smit et al., 2012). Frailty was defined as meeting three of the following five criteria; unintentional weight loss, slowness, muscle weakness, exhaustion and low physical activity. Of the participants included, the mean age was 71.7 years and a mean Vitamin D of 65.9nmol/L, 9.6% were frail and 40.5% were pre-frail. Those who were frail had lower Vitamin D levels at 60.4nmol/L compared with not frail at 71.9nmol/L. Participants within the lowest quartile of Vitamin D (49.5nmol/L) were more likely to be frail, OR 1.67 (1.00-2.82) p=0.02. The longitudinal element of this study investigated the relationship between Vitamin D, frailty and mortality.

In 2018, a further cross-sectional study found that 25(OH)D concentrations were associated with frailty status and physical performance (Vaes et al., 2018). In 756 participants, physical performance was measured using handgrip strength (HGS) of the dominant hand, and gait speed was measured an average of two attempts to walk fifteen feet and also using the Timed Up and Go (TUG) in a subgroup of 494 participants and knee extensions. Frailty status was assessed using the Fried criteria as previously outlined. The mean age of participants was 73.8 years and mean Vitamin D level was 54nmol/L. In multivariate regression analysis (covariates included age, gender. Vitamin D levels (<50nmol/L) were associated with TUG OR 0.73, (0.14-1.32, 95% CI) and gait speed, OR -0.04 (-0.08 - -0.01, 95% CI), but not with HGS, OR (-0.92 (-2.25- 0.40, 95% CI), or knee extensions, OR 9.89 (-12.8 – 32.6, 95% CI). Also Vitamin D was associated with Frailty compared with non-frail, OR 2.30 (1.11 – 4.76, 95% CI).
Negative Studies

Pellicane et al evaluated the effect of Vitamin D deficiency and recovery of function measured by the Functional Independence Measure (FIM) (Pellicane et al., 2011). A convenience sample of 65 patients admitted to a rehabilitation unit in Chicago was recruited. A sample for 25(OH)D analysis was drawn within 24 hours of admission. The mean age of participants was 70.8 years and mean Vitamin D level was 71.4nmol/L. In adjusted models no difference between Vitamin D groups and FIM efficiency was noted. No statistically significant difference between Vitamin D groups and Length of Stay (LOS) was noted.

Martinez et al reported a potential association between serum 25(OH)D concentration and functional impairment and frailty in institutionalised octogenarian women (Martinez et al., 2014). Participants must be institutionalised for over 6 months, aged over 65 years and be able to rise from a chair and walk six meters. Frailty was measured using the Fried criteria. This study included 104 women with a mean age of 84 years, mean MMSE of 24, mean Barthel of 65 and mean Lawton Index of 4. According to criteria, 21% of the population were robust, 21% pre-frail and 58% were frail. The serum Vitamin D concentrations for each of these groups was 113.4nmol/L, 72.4nmol/L and 69.9nmol/L respectively which was a statistically significant difference, p<0.01. No statistically significant difference was found between Vitamin D concentration in pre-frail and frail women.
### Table 1: D and Frailty: Cross Sectional Studies

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<th>Confounders</th>
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<td>Kiebzak et al 2007</td>
<td>100</td>
<td>70</td>
<td>Mobile</td>
<td>Liver and kidney disease, calcium disorders, Psychiatric disorders and physical disability preventing assessment</td>
<td>44nmol/L</td>
<td>Age, Grip strength, Body weight</td>
<td>Vitamin D levels positively correlated with FIM scores and grip strength. Vitamin D inversely related to hospital LOS</td>
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<td>60</td>
<td>77</td>
<td>Heart Failure (EF &lt;40%) &gt;60 years</td>
<td>Metastatic/active cancer, ESKD, Connective tissue disorders, Androgen, DHEAS, liver disease, PD, Immobile, MI &lt;3months</td>
<td>57.9nmol/L</td>
<td>Age, Gender, hsCRP</td>
<td>Vitamin D levels associated with frailty scores and subsets of physical activity and grip strength. Also associated with increased distance on 6MWT</td>
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<td>71.7</td>
<td>&gt;60 years Complete data on frailty</td>
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<td>65.9nmol/L</td>
<td>Age, Gender, BMI, Ethnicity; smoking, Education, Chronic disease index, Latitude</td>
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<td>Vaes et al 2018</td>
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<td>73.8</td>
<td>&gt;65 years</td>
<td>Not available</td>
<td>54nmol/L</td>
<td>Age, Gender, Ethnicity, Physical activity, Vitamin D supplement, BMI, Smoking, Season, Chronic diseases</td>
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<td>Martinez et al 2014</td>
<td>104</td>
<td>84</td>
<td>Women &gt;60 years</td>
<td>Able to rise from chair and mobilise 6 meters</td>
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<td>No significant difference between frailty status and Vitamin D levels</td>
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4.4 Prospective Studies

Positive Studies

A study from the LASA population evaluated the relationship between Vitamin D and PTH on loss of muscle strength and sarcopenia (Visser et al., 2003). Sarcopenia was measured using grip-strength and was defined as 40% loss of grip strength over a 3 year follow up period and body mass was measured using appendicular skeletal mass, measured by DXA. Of the 1,008 participants with complete data included in this analysis, the mean age was 75.5 years and 9.2% of the population had serum Vitamin D level <25nmol/L. Those who lost grip strength were older, had lower weight, and had more chronic diseases and weaker grip strength at baseline. Regression models revealed a significant association between low serum 25(OH)D levels (<25nmol/L) and loss of grip strength, OR 2.57 (1.40-4.70, 95% CI) compared with Vitamin D levels >50nmol/L. 25(OH)D levels <50nmol/L were found to be associated with loss of appendicular skeletal muscle strength over time, OR 2.25 (1.11-2.56, 95% CI).

The association between Vitamin D and lower limb extremity strength (assessed using the eight foot (2.4meters) walking speed test and five repetitions of the sit-to-stand test) in 4,100 ambulatory older US adults from NHANES III was investigated (Bischoff-Ferrari et al., 2004c). The mean age of participants was 71.4 years with mean 25(OH)D levels of 57.9nmol/L in the inactive participants versus 68.4nmol/L in those who are active. There was an association between 25(OH)D levels and lower limb extremity function in adjusted models, performance speed was lowest at lower 25(OH)D levels and speed increased with increasing Vitamin D levels.

Puts et al found increased risk of frailty associated with Vitamin D in the LASA study population (Puts et al., 2005). Cross-sectional analysis was performed on 1,271 participants. Prospective data was available for 885, which excluded those lost to follow-up, those who died, those who were frail at baseline (n=242), and those who were not contactable at 3-year follow-up. Nine frailty indicators were measured including: BMI, MMSE, PEFR, vision and hearing impairment, incontinence, low sense of mastery and depressive symptoms. At baseline those who were frail were older (79.2 years compared
with 74.5 years in the non-frail) and were more likely to have a low Vitamin D levels (23.2% of frail versus 8.3% of non-frail had 25(OH)D levels <25nmol/L). At 3-year follow-up, 14.1% of participants had transitioned to frail from non-frail status at baseline. Logistic regression for cross-sectional data revealed that low 25(OH)D levels had greater odds of frailty, OR 2.55 (1.56-617, 95% CI) in fully adjusted models. In longitudinal regression models, there was increased odds of frailty in those participants with 25(OH)D levels <25nmol/L, OR 1.90 (0.92-3.95, 95% CI).

In 2006, Carpintero et al assessed the relationship between 1,2-dihydroxycholecalciferol (1,25(OH)2D3) and older adults with hip fracture following low trauma injury (Carpintero et al., 2006). Participants were seen at three and twelve months post hip fracture. This study included 109 participants with a mean age 81.4 years and with mean 25(OH)D levels of 26.9nmol/L and mean 1,25(OH)2D3 of 23.9mcg/ml. Multivariate logistic regression showed an increased odds of dependence at year one, OR 6.97 (1.7-28.4, 95% CI) with 1,25(OH)2D3 levels but report no association based on 25(OH)D and functional outcome, although results were not reported.

Wicherts et al report a relationship between Vitamin D and decline in physical performance over a three-year period in an older Dutch population (n=1,234) from the LASA study (Wicherts et al., 2007). After three years, 979 participants completed a repeat assessment. The mean age of participants at baseline was 75.3 years, with a mean 25(OH)D level of 53.9nmol/L, 48% of the study population had Vitamin D levels <50nmol/L at baseline. In fully adjusted models, physical performance was found to be poorer at lowest Vitamin D levels and appeared to increase with increasing serum 25(OH)D levels up to approximately 50nmol/L. Multivariate regression models showed Vitamin D levels <50nmol/L were associated with poorer physical performance compared with the reference group, OR -1.65 (-2.24 - -1.07, 95% CI) p <0.001. Regression models showed greater risk of decline in physical performance after three years in those with lower 25(OH)D levels (<25nmol/L) at baseline, OR 2.21 (1.00-4.87, 95% CI).
A study from the Hawaiian Osteoporosis Study (HOS) found an association between serum 25(OH)D and quadriceps strength (Pramyothin et al., 2009). Participants were female, n=495, with a mean age of 74.5 years with a mean Vitamin D level of 79.9nmol/L. Among the performance based tests and three strength tests carried out, quadriceps strength was associated with Vitamin D levels, β 0.114, p=0.006. Vitamin D was not correlated with any Activates of Daily Living (ADLs). Longitudinal data over the follow up period (mean 2.7 years) showed there was increased risk of vertebral fractures with increasing 25-hydroxyvitamin D3 levels, OR 1.55, p=0.040. There was no significant association between Vitamin D and falls, although there were small event numbers for multivariate analysis models.

Shardell et al evaluated the relationship between frailty and Vitamin D status in a longitudinal Italian study, the InChianti study (Shardell et al., 2009). Data was collected from residents randomly selected from a population registry and further data collected at three and six years. Frailty was defined using the five criteria proposed by Fried et al. 904 participants aged >65 years were included. The median 25(OH)D level was 40nmol/L and mean age was 74.4 years. At baseline, those with lower Vitamin D levels (<50nmol/L) tended to be older, lower MMSE scores and higher frailty prevalence. 295 participants died over the 6-year follow-up period. Standardised frailty state transition models based on 25(OH)D levels (<50nmol/L) were provided. Pre-frail participants with low 25(OH)D levels were 8.9% more likely to die than those with levels >50nmol/L (2.5-152%, 95% CI). Pre-frail participants with low Vitamin D levels were more likely to become frail at three years, 7.7% (-3.5-18.7%, 95% CI) and were less likely to become robust after three years than those with high 25(OH)D levels. Using 25(OH)D as a continuous variable, each 7.5nmol/l decrease in 25(OH)D was associated with increased odds of death in those without exhaustion at baseline and increased odds of dying rather than recovery in those with exhaustion. It was also associated with greater odds of developing weakness (as a frailty category) in those without weakness, OR 1.11 (1.10-2.72, 95% CI) or dying, OR 2.63 (1.18-10.4, 95% CI).
A study from NHANES III was reported in 2010 (Wilhelm-Leen et al., 2010). Participants were included if they had; full frailty data available (defined using modified Fried criteria), 25(OH)D measured, aged >60 years and 5,048 met inclusion criteria. The mean 25(OH)D in whites was 70.4nmol/L and in non-whites was 54.9nmol, which was a statistically significant difference (p<0.0001). 25(OH)D levels were associated with frailty in both whites, OR 3.7 (2.1-6.8, 95% CI) and non-whites, OR 4.0 (1.7-9.2, 95% CI).

Chang et al explored the relationship between Vitamin D and frailty in an older community dwelling Taiwanese population (Chang et al., 2010). Subjects had participated in a prior study regarding integrated care for frail older adults, those (n=548) who scored 3-6 on the Chinese Canadian Study of Health and Ageing Clinical Frailty Scale Telephone Version (CCSHA_CFS_TV) were invited to participate in the current study. Their mean age was 71.1 years, mean MMSE of 25.1 and mean 25(OH)D of 99.6nmol/L. Frailty was assessed using the Fried Frailty Index (FFI) and the Edmonton Frail Scale (EFS). Analysis found increased odds of frailty associated with low levels of Vitamin D (<50nmol/L), using the FFI, OR 2.67 (1.29-5.52, 95% CI) for pre-frail versus robust and OR 8.26 (2.82-24.24, 95% CI) for frail versus robust on basic regression models and findings remained significant on fully adjusted models. Using the EFS results are similar but the model lost significance with multivariate analysis.

An Italian study including 904 participants from the InChianti study investigated the relationship between 25(OH)D and frailty states (Shardell et al., 2012). Frailty was again defined using the Fried criteria. The mean age of participants was 74.4 years and the median Vitamin D level was 39.9nmol/L. Participants were followed at 3 and 6 years after recruitment. Robust participants with low Vitamin D levels (<50nmol/L) were 4.9% less likely to remain robust, 6.4% more likely to become pre-frail and 1.4% more likely to become frail than those with high Vitamin D levels. Pre-frail participants were 7.7% less likely to become robust, 4.3% less likely to stay pre-frail and 3% more likely to become frail and 8.9% more likely to die than those with high Vitamin D levels. Findings for frail participants were less consistent, as those with low Vitamin D levels were 0.5% more
likely to die and 13.5% more likely to become frail than those with high Vitamin D levels over the follow-up period.

A population study of European men from the EMAS study reported an association between Vitamin D, Parathyroid Hormone (PTH) and frailty (Tajar et al., 2013). Frailty in this study was defined using the Fried criteria to calculate their own frailty measures including the EMAS FP (Frailty Phenotype) and EMAS FI (Frailty Index). This study included 1,504 men with a mean age of 69.5 years and mean Vitamin D of 62.9nmol/L. Using the FP with lower Vitamin D levels there was increased risk of being pre-frail, OR 1.45 (1.26-1.67, 95% CI) or frail, OR 1.89 (1.30-2.76, 95% CI) compared with being robust in fully adjusted models. Analysis for individual frailty factors showed that lower levels of Vitamin D were associated with increased risk of weakness, slower walking speed, lower activity levels and exhaustion.

A prospective study from the HIMS population was performed assessing the link between Vitamin D and frailty (measured using the FRAIL scale which has five domains; fatigue, resistance, ambulation, illness and weight loss (Wong et al., 2013). The mean age of the 4,203 men was 76.6 years with a mean 25(OH)D of 68.3nmol/L. At baseline, 16.1% were frail. After a mean follow-up period of 5.3 years, 1,817 men responded and of these, 25.3% were frail. On cross-sectional analysis, low Vitamin D levels were associated with increased odds of frailty, OR 1.96 (1.52-2.52, 95% CI). In longitudinal analysis again lower Vitamin D levels were associated with increased odds of frailty, OR 1.56, 1.07-2.27, 95% CI).

An Australian study (the CHAMP Study) found an association between Vitamin D and frailty in older community dwelling men (Hirani et al., 2013). Vitamin D was measured using 25(OH)D and also 1,25(OH)2D3 and frailty was defined as having three out of the five following markers; weight loss, weakness/reduced muscle strength, slow walking speed, exhaustion and low activity level. The mean age of the 1,659 participants was 77 years, mean Vitamin D was not provided. The prevalence of frailty was 9.2% in those with 25(OH)D measured and 8.6% for those with 1,25(OH)2D3 measured. Logistic regression
analysis showed increased odds of frailty in those with 25(OH)D levels <40nmol/L, OR 2.66 (1.32-5.26, 95% CI) and increased odds in those with 1,25(OH)2D3 levels <62mmol/L, OR 1.86 (1.04-3.59, 95% CI).

A cross sectional and longitudinal study from the LASA study population reported an association between Vitamin D deficiency and functional limitations (Sohl et al., 2013). The study population was divided into two cohorts based on age at time of study recruitment. Functional limitations were assessed using a questionnaire. The mean age of participants in the older cohort was 75.3 years versus 60.0 years in the younger cohort and the mean 25(OH)D levels were 53.9nmol/L versus 56.7nmol/L. Regression analysis showed an increased odds of functional limitation in those with Vitamin D levels <50nmol/L compared with higher levels, in the older cohort, OR 1.7 (1.2-2.5, 95% CI) and younger cohort, OR 2.1 (1.2-3.5, 95% CI). In longitudinal analysis, Vitamin D deficiency (50nmol/L) was associated with increased risk of functional limitation after three years in the older participants, OR 2.9 (1.1-3.5, 95% CI) but not at six years, the association was present in younger participants after six-years, OR 3.3 (1.1-10.1, 95% CI).

In 2014 Wang et al found an association between 25(OH)D levels, Vitamin D Binding Protein (VDBP) and frailty (Wang et al., 2014). Participants (n=418) had a mean age of 76.4 years, and mean Vitamin D level of 41.6nmol/L. Frail participants (defined using Fried criteria) were older, less educated, higher DBP and lower Vitamin D levels than their non frail counterparts. The odds of being frail versus non-frail was assessed across Vitamin D quartiles and showed increased odds of frailty in those with in the lowest quartile of Vitamin D and highest quartile of DBP, OR 3.18 (1.46-4.56, 95% CI) and also increased odds of frailty in those with lowest quartile of Vitamin D and lowest quartile of DBP, OR 2.63 (1.31-3.68, 95% CI) compared with those in the reference group (lowest quartile of DBP and highest quartile Vitamin D).

Wang et al also evaluated the relationship between Vitamin D deficiency and outcomes after hip fractures in a case control study (Wang et al., 2015). Participants were admitted with a first acute hip fracture (n=265) over an eighteen-month period. A similar number of
age and sex matched cases were also recruited. The median age of patients was 69 years. Cases were more likely to have lower serum calcium and 25(OH)D levels than controls, 77% of cases were Vitamin D deficient versus 57.4% of controls. Those with hip fractures had significantly lower 25(OH)D levels at 40.6nmol/L than the control group, 46nmol/L. Functional outcome was based on the Barthel Index (BI), with a favourable outcome score defined as 50-100. At discharge 24% were defined as having poor functional outcome. In multivariate analysis, 25(OH)D deficiency was found to be associated with poorer functional outcome, OR 5.25 (3.12-8.16, 95% CI).

A further prospective study reported an association between 25(OH)D and frailty and all-cause mortality (Vogt et al., 2015). The KORA Age Study was a follow-up study of four German cross-sectional studies of community dwelling adults. Of the original participants who took part in the baseline assessment, 822 participants were re-examined after a median of 2.9 years. Participants unable to attend the study centre were re-examined at home. Frailty was assessed using the Fried criteria. The mean age of participants was 75.5 years and the mean 25(OH)D level was 46.7nmol/L. The incidence of frailty at follow up was 29% and the incidence of pre-frailty was 21.1%. Those with Vitamin D levels <30nmol/L at baseline had higher odds of becoming pre-frail, OR 2.43 (1.17-5.03, 95%CI) and becoming frail, OR 2.53 (1.23-5.22, 95% CI). For individual markers of frailty, 25(OH)D levels <30nmol/L were found to be significantly associated with slow walking speed, OR 3.8 (1.53-9.25, 95% CI) and physical inactivity, OR 5.1 (1.63-16.17, 95% CI).

A Chinese post hoc analysis study evaluated the effects of Vitamin D levels and functional outcome in women with hip fractures (Liu et al., 2015). The population included 261 postmenopausal women admitted with their first hip fracture to an Orthopaedic Unit, not taking Calcium/Vitamin D supplementation. Median 25(OH)D was 37.9nmol/L and median age was 68 years. The median Barthel Index (BI) on discharge was 70 and 29.1% of participants were classified as having a poor functional outcome. Authors found a correlation between Vitamin D and BI (r=0.384, p<0.0001). In those with unfavourable functional outcome Vitamin D levels were lower compared with those with favourable
outcomes. In multivariate analysis Vitamin D was found to be an independent predictor of an unfavourable outcome, OR 0.83 (0.79-0.90, 95% CI).

A further study from the KORA Age study population was published reporting an association between Vitamin D and frailty. Frailty was defined using the Fried criteria (Pabst et al., 2015). The mean age of the 940 participants was 75.6 years with a mean serum 25(OH)D level of 46.8nmol/L. With the lowest levels of Vitamin D as the reference range, 25(OH)D levels >75nmol/L were less frequently frail or pre-frail compared with those with Vitamin D levels 30 - <50nmol/L, OR 0.38 (0.23-0.60, 95% CI) versus OR 0.51 (0.33-0.78, 95% CI) respectively. Vitamin D was also found to be associated with the individual frailty criteria of exhaustion, physical inactivity and slowness.

In a study evaluating the prevalence of muscle weakness and its association with Vitamin D in people living in Ecuador an association was noted between 25(OH)D concentrations and muscle strength (Orces, 2016). Muscle weakness was measured via Grip strength. The study comprised 2,205 participants with a mean age of 71.7 years with mean Vitamin D concentrations of 67nmol/L. Multivariate analysis showed a significant mean difference in Vitamin D levels based on grip strength category; intermediate -3.2 and weak -3.2, p=0.05.

In a cohort of older women residing in nursing homes, Vitamin D deficiency at baseline was found to be associated with functional decline (Kotlarczyk et al., 2017). Participants (n=137) were nursing home residents aged 65 years and older and were followed for two years. Functional assessment was based on Katz Activities of Daily Living (ADLs) and Instrumental ADLs and the nursing home Physical Performance Test (PPT). Cognition was measured with SPSMQ. Participants had a mean Vitamin D level of 66.1nmol/L and mean age of 85.4 years. Those deficient in Vitamin D at baseline were more likely to have slower gait speed and also lower IADL scores. Those deficient in Vitamin D were supplemented with 800IU of Vitamin D daily and serum levels increased from 34.4 to 88.6nmol/L after two years, no information was available on compliance. However, at follow up, those initially deficient in Vitamin D showed greater functional decline than those sufficient at baseline; decreased IADL score -2.5 compared with -1.2 (p=0.0153), decline in PPT score -
3.1 compared with -0.5 and greater decline in cognitive score on SPSMQ. Those deficient in Vitamin D experienced a greater number of falls, 88.5% compared with sufficient participants, 66.2 with AOR 4.01 p=0.037.

Buta et al evaluated the relationship between Vitamin D and frailty in a female population from The Women’s Health and Ageing Study (WHAS II) (Buta et al., 2017). Frailty was defined as having three out of the following five criteria; weight loss, weakness, exhaustion, slowness, and less physical activity. Participants, n=369, were non-frail at baseline and were followed for a mean of 8.5 years and 23.8% developed frailty over that time. The incidence of frailty in women with 25(OH)D levels <25nmol/L was 32.2/1,000 person years compared with 12.9/1,000 person years in those with Vitamin D levels >75nmol/L. In Cox regression analysis lower Vitamin D levels (<25nmol/L) were associated with increased risk of frailty in fully adjusted models, HR 2.55 (1.04-6.29, 95% CI) p<0.05. When the model was adjusted for cardiovascular risk factors, this association remained, but lost statistical significance, HR 2.29 (0.92-5.69, 95% CI) p=0.07.

In a study involving 3,369 men, authors found an association between Vitamin D status and frailty, with higher levels of serum Vitamin D associated with reduced risk of progression of frailty (Swiecicka et al., 2017b). Participants were part of the EMAS study and were followed for a median of 4.3 years. The mean age was 59 years and mean 25(OH)(D level was 64.6nmol/L. Frailty was measured using the EMAS Frailty Index (FI) and Frailty Phenotype (FP). Fully adjusted multivariate models showed reduced risk of frailty with baseline Vitamin D levels, OR 0.84 (0.75-0.95, 95% CI) p=0.007.

Negative Studies
A US study evaluated the association of Vitamin D and frailty (using Fried criteria) in a cohort of 4,551 community dwelling women classed as robust/non-frail at baseline (Ensrud et al., 2010). These women were followed after approximately 4.5 years and their frailty status was reassessed. Those with one or two components were considered intermediate frailty and those with greater than three components were considered to be frail. The mean age of participants was 76.7 years with mean Vitamin D levels of 57.8nmol/L. In cross-
sectional analysis, 25(OH)D levels <37.5nmol/L were associated with greater odds of frailty (frail versus robust/intermediate) in basic and multivariate models, OR 1.47 (1.19-1.82, 95% CI). Of the 4,119 participants with repeat frailty assessment at year ten, 432 (9.2%) had died prior to follow-up examination. There was no association between baseline 25(OH)D levels and odds of becoming intermediate/frail/dead (versus robust) at follow-up.

Ensrud et al also evaluated the relationship between 25(OH)D and frailty in 1,606 older men in the MrOS study population (Ensrud et al., 2011). Of these, 1,476 were defined as non-frail at baseline. Frailty status was defined using the Fried criteria. The mean age at baseline was 73.8 years. Those with lower Vitamin D levels at baseline were more likely to be frail or intermediately frail compared with those with higher Vitamin D levels and they had higher prevalence of each individual component of frailty. In cross sectional, fully adjusted, analysis those with 25(OH)D levels <50nmol/L had greater odds of frailty than those with levels >75nmol/L, OR 1.60 (1.19-2.16, 95% CI). In longitudinal analysis, men defined as non-frail (robust or intermediate frailty), were followed for a mean of 4.6 years. In multivariate models 25(OH)D levels were not associated with increased odds of frailty, OR 1.01 (0.71-1.45, 95% CI).

A Belgian study found no association between Vitamin D and physical performance in older adults (Mathei et al., 2013). This prospective study included 367 participants aged greater than 80 years with physical performance measured by static balance, gait speed and grip strength. Mean age was 84.7 years and the prevalence of Vitamin D insufficiency (defined as <47nmol/L) was 32.9% and severe insufficiency (<25nmol/L) was 32.7%. In basic and adjusted models there was no association between balance, gait speed and grip strength.

A French prospective study including 321 participants assessed at the local Geriatric Frailty Unit reported no significant association between Vitamin D and frailty (Krams et al., 2016). Frailty was defined using an adapted version of the Fried criteria. The mean age of participants was 82.9 years and the mean Vitamin D level was 40.4nmol/L. Those who were deemed frail were older, had lower weights and had higher co-morbidities based on
Charlson co-morbidity index. Analysis showed a non-significant association between Vitamin D and frailty, OR 0.97 (0.95-0.99, 95%CI).
### Table 2: Vitamin D and Frailty: Longitudinal Studies

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Mean f/u</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Confounders</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visser et al 2003 Netherlands LASA</strong></td>
<td>1,008</td>
<td>75.5</td>
<td>3 years</td>
<td>55-85 years</td>
<td>Inability to participate, Missing data</td>
<td>Not available</td>
<td>9.6% levels &lt;25nmol/L</td>
<td>Age, Gender, Height, BMI, Creatinine, Season, Smoking, Chronic diseases, Physical activity</td>
</tr>
<tr>
<td><strong>Bischoff-Ferrari et al 2004 USA NHANES III</strong></td>
<td>4,100</td>
<td>71.4</td>
<td>-</td>
<td>&gt;60 years</td>
<td>Full data available</td>
<td>Not available</td>
<td>68.4nmol/L (Active), 57.9nmol/L (Inactive)</td>
<td>Age, Gender, Ethnicity, BMI, Number of medical co-morbidities, Calcium intake, poverty-income ration</td>
</tr>
<tr>
<td><strong>Puts et al 2005 Netherlands LASA</strong></td>
<td>885</td>
<td>76.9</td>
<td>3 years</td>
<td>&gt;60 years</td>
<td>Frailty at baseline</td>
<td>Not available</td>
<td>Age, Gender, IGF-1, CRP, PVD, Education, DM, Season, Smoking and Alcohol consumption, PTH, COPD, Stroke, Cancer, Number of Chronic diseases, Cardiac Disease</td>
<td>Increased risk of frailty in longitudinal analysis in those with Vitamin D levels &lt;25nmol/L</td>
</tr>
<tr>
<td><strong>Carpintero et al 2006 Spain</strong></td>
<td>109</td>
<td>81.4</td>
<td>1 year</td>
<td>&gt;65 years</td>
<td>Hip fracture due to high energy trauma, Pathological fracture, Liver or kidney disease</td>
<td>Not available</td>
<td>26.9nmol/L</td>
<td>1,25(OH)D levels were associated with increased dependency levels at one year 25(OH)D levels not associated with functional outcome</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Wicherts et al 2007 Netherlands LASA</td>
<td>979</td>
<td>75.3</td>
<td>3 years</td>
<td>&gt;65 years</td>
<td>Missing data, No blood sample</td>
<td>53.9nmol/L</td>
<td>Age, Gender, Number of chronic diseases, BMI, Alcohol consumption, Physical activity</td>
<td>Vitamin D levels &lt;50nmol/L associated with poorer physical performance and lower Vitamin D levels were associated with greater risk of physical decline</td>
</tr>
<tr>
<td>Pramyothin et al 2009 Hawaii Osteoporosis Study (HOS)</td>
<td>495</td>
<td>74</td>
<td>2.7 years</td>
<td>Post menopausal Japanese women</td>
<td>Not available</td>
<td>79.9nmol/L</td>
<td>Age, Height, Weight, Quadriceps strength</td>
<td>Longitudinal analysis: No association with falls, but increased rates of vertebral fractures with lower Vitamin D levels</td>
</tr>
<tr>
<td>Shardell et al 2009 USA</td>
<td>904</td>
<td>74.4</td>
<td>6 years</td>
<td>&gt;65 years</td>
<td>Community Dwelling</td>
<td>40nmol/L (Median)</td>
<td>Age, Gender, Education, Season, Smoking, Alcohol consumption, cognition, Co-morbidities, BMI</td>
<td>Participants with lower Vitamin D levels: more likely to die. Pre-frail participants with low Vitamin D levels: more likely to become frail. Vitamin D levels associated with increased risk of weakness over time</td>
</tr>
<tr>
<td>Wilhelm-Leen et al 2010 USA NHANES III</td>
<td>5,048</td>
<td>-</td>
<td>&gt;60 years</td>
<td>Full frailty criteria</td>
<td>Missing data for frailty</td>
<td>70.4nmol/L (Whites) 54.9nmol/L (Non-whites)</td>
<td>Season and latitude adjusted 25(OH)D</td>
<td>25(OH)D associated with frailty in both white and non-white study population</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Chang et al 2010</td>
<td>548</td>
<td>71.1</td>
<td>-</td>
<td>65-79 years Community dwelling</td>
<td>Not available</td>
<td>99.6nmol/L</td>
<td>Increased risk of frailty based on the FFI with Vitamin D levels &lt;50nmol/L</td>
<td></td>
</tr>
<tr>
<td>Shardell et al 2012</td>
<td>904</td>
<td>74.4</td>
<td>3 &amp; 6 years</td>
<td>&gt;65 years</td>
<td>Not available</td>
<td>39.9nmol/L</td>
<td>Robust participants with lower Vitamin D levels were less likely to remain robust and those who were pre-frail; were more likely to remain pre-frail or become frail</td>
<td></td>
</tr>
<tr>
<td>Sohl et al 2013 LASA</td>
<td>762</td>
<td>75.3 (older cohort)</td>
<td>6 years</td>
<td>&gt;55 years (older cohort, recruited in 1992) 55-65 years (younger cohort recruited in 2002)</td>
<td>Missing values, Missing Vitamin D levels</td>
<td>53.9nmol/L, 56.7nmol/L</td>
<td>Age, Gender, Number of Chronic diseases, Season, BMI, Education, Creatinine, Multivitamin use, Physical activity, Smoking, Alcohol, Urbanisation</td>
<td>Participants with lower Vitamin D levels were more likely to have functional limitations. At 3 years older participants: relationship between Vitamin D (&lt;50nmol/L) and functional limitation. At 6 years the younger cohort: association between Vitamin D and functional limitations</td>
</tr>
<tr>
<td>Tajjar et al 2013 EMAS</td>
<td>1,504</td>
<td>69.5</td>
<td>-</td>
<td>Men 40-79 years</td>
<td>Not available</td>
<td>62.9nmol/L</td>
<td>Lower Vitamin D associated with increased risk of being pre-frail and with weakness, slower walking speed, lower activity levels and exhaustion</td>
<td></td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Hirani et al 2013</td>
<td>1,659</td>
<td>77</td>
<td>5.3 years</td>
<td>Men &gt;70 years</td>
<td>Living in residential care facility</td>
<td>Not available</td>
<td>Age, Season, GFR Vitamin D supplement, Self-reported health status, Health conditions, PTH, Income, Dementia, 1,25(OH)<em>{2}D</em>{3}</td>
<td>Increased risk of frailty in participants with 25(OH)D levels &lt;40nmol/L and with 1,25(OH)D levels &lt;62nmol/L</td>
</tr>
<tr>
<td>Australia CHAMP</td>
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<tr>
<td>Wong et al 2013</td>
<td>1.817</td>
<td>76.6</td>
<td>5.3 years</td>
<td>Men &gt;65 years</td>
<td>Not available</td>
<td>68.3nmol/L</td>
<td>Age, Education, Living status, DM Smoking, Physical activity, Vitamin supplements, CVD disease, HTN, CCI Dyslipidaemia</td>
<td>Lower Vitamin D levels associate with increased risk of frailty</td>
</tr>
<tr>
<td>HIMS</td>
<td></td>
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<tr>
<td>Wong et al 2014</td>
<td>518</td>
<td>76.4</td>
<td>-</td>
<td>Men &gt;70 years</td>
<td>Unable to mobilise without assistance Abnormal hepatic or renal function</td>
<td>41.6nmol/L</td>
<td>Age, Education, Smoking, BMI</td>
<td>Increased odds of frailty in those in the lowest quartile of Vitamin D and also lowest levels of Vitamin D binding protein</td>
</tr>
<tr>
<td>China</td>
<td></td>
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<tr>
<td>Wang et al 2015</td>
<td>530</td>
<td>69</td>
<td>1.5 years</td>
<td>&gt;60 years First hip fracture Matched case control study</td>
<td>Fractures due to RTA, malignancy, primary hyperparathyroidism</td>
<td>40. 7nmol/L (cases) 45.9nmol/L (controls)</td>
<td>Age, Gender, Hip fracture type, Sun exposure, Activity level, MMSE &lt;24 (cognitive impairment), Neurological impairment, Time to blood collection from fracture</td>
<td>Vitamin D deficiency associated with poor functional outcome at discharge post acute hip fracture, based on Barthel Index scores</td>
</tr>
<tr>
<td>China</td>
<td></td>
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<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
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<tr>
<td>Vogt et al 2015 KORA-Age</td>
<td>822</td>
<td>75.5 (Median)</td>
<td>2.9 years</td>
<td>&gt;65 years</td>
<td>Not available</td>
<td>46.7nmol/L</td>
<td>Age, Gender, BMI, Season, Education, PTH, Alcohol, Smoking, CVD, DM, Multi-morbidity,</td>
<td>Vitamin D levels &lt;30nmol/L associated with increased incidence of frailty and pre-frailty over follow-up</td>
</tr>
<tr>
<td>Liu et al 2015 China</td>
<td>261</td>
<td>68 (Median)</td>
<td>-</td>
<td>Post menopausal women First fracture</td>
<td>Fractures due to RTA, Malignancy, Primary hyperparathyroidism</td>
<td>37.9nmol/L</td>
<td>Age, Gender, Fracture type, Sun exposure, Activity level, MMSE &lt;24, Neurological impairment, Season</td>
<td>Vitamin D associated with unfavourable outcomes</td>
</tr>
<tr>
<td>Pabst et al 2015 Germany KORA-Age</td>
<td>940</td>
<td>75.6</td>
<td>-</td>
<td>&gt;65 years</td>
<td>Missing variables</td>
<td>46.8nmol/L</td>
<td>Age, Gender, Education, Alcohol, Smoking, BMI, Season, CVD, DM, PTH, Comorbidities</td>
<td>Participants with 25(OH)D levels &gt;75nmol/L were less likely to be frail/pre-frail than those who were deficient. Also associated with exhaustion, slowness &amp; inactivity</td>
</tr>
<tr>
<td>Orces et al 2016 Ecuador</td>
<td>2,205</td>
<td>71.7</td>
<td>-</td>
<td>&gt;60 years</td>
<td>Not available</td>
<td>67nmol/L</td>
<td>Age, Literacy, BMI, Area of residence, Dairy consumption, Smoking, Physical Activity, Comorbidities</td>
<td>Vitamin D associated with grip strength</td>
</tr>
<tr>
<td>Kotlarczyk et al 2017</td>
<td>137</td>
<td>85.4</td>
<td>2 years</td>
<td>Female NH residents &gt;65 years</td>
<td>Not available</td>
<td>66.1nmol/L</td>
<td>Not available</td>
<td>Participants deficient in Vitamin D at baseline were more likely to fall and more likely to have a functional decline over follow-up</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<td><strong>Positive</strong></td>
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</tr>
<tr>
<td>Buta et al 2017</td>
<td>369</td>
<td>73.8</td>
<td>8.5 years</td>
<td>Women 70-79 years                                                                   Frailty free at baseline Missing follow-up visit and Vitamin D data Non-African-American, Non-Caucasian</td>
<td>Not available</td>
<td>64.6nmol/L</td>
<td>Age, Ethnicity, Education, Smoking, Season, BMI, CVD, DM, HTN, Hyperlipidaemia</td>
<td>Vitamin D deficiency (25(OH)D &lt;25nmol/L) was associated with increased risk of frailty but not significantly when model included cardiovascular risk factors</td>
</tr>
<tr>
<td>WHAS II USA</td>
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</tr>
<tr>
<td>Swiecicka et al. 2017</td>
<td>3,369</td>
<td>59</td>
<td>4.3 years</td>
<td>Men Adrenal or pituitary disease, Medications affecting PTH, DHEAS</td>
<td>64.6nmol/L</td>
<td>Baseline Frailty, Age, Centre, BMI, PTH</td>
<td>Higher baseline Vitamin D levels associated with reduced risk of worsening frailty status</td>
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<tr>
<td><strong>Negative</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>57.8nmol/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ensrud et al 2010</td>
<td>4,551</td>
<td>76.7</td>
<td>4.5 years</td>
<td>Women &gt;65 years Black women Bilateral hip replacement Unable to walk unaided</td>
<td>57.8nmol/</td>
<td>Age, Season, BMI, Baseline frailty, Self-reported health status, Smoking, Education, Alcohol, Co-morbidity, Short MMSE score</td>
<td>Vitamin D levels at baseline were not associated with increased odds of death, intermediate frailty or frailty at follow-up</td>
<td></td>
</tr>
<tr>
<td>MrOS</td>
<td>1,476</td>
<td>73.8</td>
<td>4.6 years</td>
<td>Men Non-frail at baseline Bilateral hip replacement Unable to mobilise independently</td>
<td>Not available</td>
<td>Age, Gender, Site, Season, BMI, 3MS, Education, Living alone, smoking, alcohol, frailty, Co-morbidity score, self reported health,</td>
<td>Lower Vitamin D levels at baseline were associated with increased risk of intermediate frailty/frailty in cross-sectional analysis but not in longitudinal analysis</td>
<td></td>
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<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Mathei et al 2013 Belgium BFC80+</td>
<td>367</td>
<td>84.7</td>
<td>-</td>
<td>&gt;80 years</td>
<td>Dementia Palliative Medical emergency</td>
<td>Not available 32.7% &lt;25nmol/L</td>
<td>Age, Gender, Education, Institutionalisation, BMI, hsCRP, GFR, Smoking, Number of chronic diseases, Calcium, Vitamin D supplement, Season, Diuretics</td>
<td>No association between with balance, gait speed and grip strength</td>
</tr>
<tr>
<td>Krams et al 2016 France</td>
<td>321</td>
<td>82.9</td>
<td>-</td>
<td>GP referrals to Geriatric Frailty Clinic</td>
<td>Vitamin D supplementation 6 months prior to study</td>
<td>40.4nmol/L</td>
<td>Age, Gender, Season, CCI</td>
<td>No association between Vitamin D and frailty in this older population</td>
</tr>
</tbody>
</table>

ADLS: Activities of Daily Living, BI: Barthel Index, BMI: Body Mass Index, CCI: Charlson Co-morbidity Index, CES-D: Center for Epidemiologic Studies Depression Scale, COPD: Chronic Obstructive Pulmonary Disease, CRP: C-Reactive Protein, CVD: Cardiovascular Disease, DBP: (Vitamin) D Binding Protein, DM: Diabetes Mellitus, EFS: Edmonton Frailty Scale, FFI: Fried Frailty Index, FIM: Functional Independence Measure, GFR: Glomerular Filtration Rate, GP: General Practitioners, HTN: Hypertension, IGF1: Insulin-like Growth Factor, LOS: Length of Stay, NH: Nursing Home, PTH: Parathyroid Hormone, PVD: Peripheral Vascular Disease, RTA: Road Traffic Accident, 3MS: Modified Mini Mental State Examination, 6MWT: 6 Minute Walk Test
4.5 Randomised Control Trials
Positive Studies
The PROVIDE study is an RCT investigating if baseline Vitamin D concentrations and dietary intake influence muscle mass and function in older community dwelling adults (Verlaan, 2017). Participants were recruited across eighteen centres in Europe, and were eligible for participation if they had mild to moderate limitations in function based on Short Physical Performance Battery (SPPB) and low skeletal muscle mass using biometric impedance analysis. Participants were randomised to receive the intervention over thirteen weeks; a whey protein and Vitamin D (800IU) and leucine enriched nutritional drink taken twice daily, the control group received an iso-caloric control product. In this post-hoc analysis, 380 participants were included and the muscle related outcomes were Appendicular Muscle Mass (aMM) and the chair stand test. The mean age of participants was 77.8 years with mean Vitamin D level of 50.5nmol/L. Those with lower Vitamin D at baseline had lower MMSE scores and had higher depression scores and were less likely to be living alone. In those with higher Vitamin D concentrations at baseline (>50nmol/L) there was a greater change in aMM compared with those with levels <50nmol/L at baseline, β 0.35 versus -0.01 respectively (p=0.034) in response to the intervention. There was no difference in the chair to stand time between the 25(OH)D subgroups in response to the intervention.

4.6 Meta Analyses
Positive Studies
A meta-analysis from 2016, included seven prospective studies, examined the association between Vitamin D insufficiency and frailty in a total of 17,815 participants (Zhou et al., 2016). The prevalence of frailty ranged from 3.9-31.9% in individual studies. Frailty criterion used included Fried, modified Fried, Frailty Index or nine individual frailty indicators. The pooled OR of frailty for the lowest versus highest Vitamin D level was 1.27 (1.17-1.38, 95% CI). Subgroup results showed low level Vitamin D was associated with frailty in women, OR 1.27 (1.15-1.40, 95% CI) but not in men. This association was not present when the non-Fried derived frailty scores were used.
A further meta-analysis investigated the effects of Vitamin D on muscle strength and mobility and found no clear benefit in supplementation (Rosendahl-Riise et al., 2017). Studies included those with participants aged >65 years, community dwelling, Vitamin D as the intervention in all forms, with or without calcium, and with Timed Up and Go (TUG) and Hand Grip Strength (HGS) as measures of muscle strength. A total of 15 studies were included with 2,866 participants with a ratio of men to women of 1:9. In the majority of studies, Vitamin D was measured using 25(OH)D concentrations, and there were differing metabolites of Vitamin D used with varying doses and administration routes, in the intervention groups of individual studies. For the outcome of HGS, seven studies were included; there was a non-significant improvement in HGS, after Vitamin D supplementation, of 0.2kg (0.25-0.7, 95% CI). For TUG, five studies were included in the analysis and using a random effects model, showed an increase in TUG of 0.3 seconds, after Vitamin D supplementation (0.1-0.5, 95% CI) indicating deterioration in function. Season of blood draw as an important covariate of Vitamin D was stated in 9 out of 15 studies, while other covariates of Vitamin D status were usually not considered. Significant heterogeneity was noted.

Annweiler et al performed a meta-analysis assessing the effect of Vitamin D on walking speed in older adults (Annweiler et al., 2017). A total of 15 studies were included in the meta-analyses; with four separate analyses performed. The first compared the difference between usual pace walking speed and Vitamin D status, the second looked at fast pace walking speed and Vitamin D status, the third assessed speed of TUG performance and the fourth examined the proportion of participants with slow walking speed according to Vitamin D status. Each meta-analysis confirmed a positive association between Vitamin D deficiency (either severe deficiency <25nmol/L, deficiency <50nmol/L, or insufficiency <75nmol/L) and gait speed.

\[ 4.6 \] Discussion

There have been increasing numbers of studies evaluating the effect of Vitamin D on frailty. These studies have shown varying results based on the study population used and the definition of frailty applied. For example in some studies with older populations
(Krams et al., 2016, Mathei et al., 2013), there does not appear to be the same association as is seen in other studies with slightly younger populations (Pabst et al., 2015, Swiecicka et al., 2017b).

Overall the results of large prospective studies and suggest an association between deficient levels of Vitamin D and frailty, although it remains difficult to exclude reverse causality as a factor. There have been two positive meta-analyses to date but limited data from RCT trials to support the benefit of Vitamin D in frailty.

Results to date suggest an association between Vitamin D and frailty and there appears to be a clear pathophysiological basis for this link. However, further RCTs, with large population samples, with adequate levels of Vitamin D supplementation and standardised methods of frailty measurement are needed to evaluate the potential relationship between Vitamin D and frailty. In addition prospective studies that adjust for potential confounding factors and more objective outcomes such as resource utilisation would have value.
Chapter 5: Vitamin D and Resource Utilisation

5.1 Introduction
Increased use of healthcare resources by the older population continues to be a significant and complex issue in modern healthcare service provision. Aside from the economic consequences, particularly in the elderly, this is also associated with negative outcomes in function, independent living and quality of life.

In Ireland in 2014 approximately 22% of all Emergency Department (ED) attendances were aged 65 years or more, and almost 12.5% were aged over 75 years with the proportion of over 65 years admitted on an emergency basis increasing from 32% in January to 38% in December 2014. This trend will have a significant impact on access to services (HSE, 2015).

The hospital environment traditionally focuses on the management of acute illnesses and while this is a necessary service and an important function, the older population is at risk of decline from a cognitive and functional perspective following hospital admission. There is increased risk of falls, delirium, hospital acquired infection, adverse drug events and also pressure sore development. These are due to a combination of many factors including acute illness and associated deconditioning during their hospital stay. While effective treatment and efficient delivery of care are important elements of management in the acute hospital setting, maintenance of the functional status of the elderly person is equally important and consideration of the most appropriate environment and any factors that may improve patient care and outcomes following admission are important considerations.

Many studies have been published evaluating and identifying those at increased risk of hospitalisation, frequent attendances to EDs and also those at increased risk of nursing home admission.

Although the effects of Vitamin D on bone and muscle function have long been known, the effects and implications of Vitamin D beyond bone are increasingly studied, such as the
effects of Vitamin D on frailty and associated outcome measures as previously outlined. In more recent times, studies have been published looking at the association between Vitamin D and resource utilisation through surrogate measures such as infections, exacerbations of chronic illnesses, admissions to critical care and intensive care facilities and functional outcomes following falls, fractures and acute illnesses.

5.2 Vitamin D: Infections and Sepsis

5.2.1 Cross Sectional Studies

Positive Studies

A Finnish study from 2007 investigated the link between Vitamin D and acute respiratory tract infections in a young population of military men (Laaksi et al., 2007). A retrospective review of military men serving in a base in southwest Finland was performed documenting any respiratory tracts infections over a six-month period and 756 soldiers were included in the analysis. The mean 25(OH)D level was 80.2nmol/L. 25(OH)D levels were associated with increased rates of Respiratory Tract Infections and increased leave of absence from duties (median 4 days) versus those with concentrations >40nmol/L (median 2 days).

Youssef et al reported the relationship between Vitamin D and inpatient and outpatient costs in veterans infected with Clostridium Difficile, Methicillin Sensitive Staphylococcus Areus, (MSSA) Methicillin Resistant Staphylococcus Areus (MRSA) or Pseudomonas Auerginosa (Youssef et al., 2010, Youssef et al., 2012). Vitamin D deficiency appeared to be associated with higher inpatients costs, but not consistently associated with longer Length of Stay (LOS).

Leow et al prospectively examined the relationship between 25(OH)D levels and antimicrobial peptide levels in the setting of Community Acquired Pneumonia (CAP) and the outcomes of these conditions (Leow et al., 2011). Participants were recruited during admission to an acute care setting in New Zeeland and the admitting physician, diagnosed CAP. CURB-65 score and the Charlson Index scores were recorded. Blood samples were collected within 24 hours of admission for 25(OH)D and C-Reactive Protein (CRP) analysis along with antimicrobial peptides analysis. The primary outcome of interest was
30-day mortality. A total of 128 eligible patients were assessed during the study period and of these 112 were included in the analysis. The mean age of participants was 76 years with a mean Vitamin D level of 54nmol/L. 25(OH)D levels were not correlated with either antimicrobial peptide level. Participants with severe 25(OH)D deficiency had higher 30-day motility rates, OR 13.5 (2.6-69.1, 95% CI). Of the potential covariates, CURB-65 was significantly associated with mortality, OR 2.23 (1.13-4.40, 95% CI).

A further cross-sectional study involving 300 randomly selected patients from the larger CAPNETZ study investigated the association of 25(OH)D and 1,25(OH)2D3 with community acquired pneumonia (CAP) outcomes and disease severity (Pletz et al., 2014). The inclusion criteria for this study were; age >18 years, infiltrate on CXR, focal signs on chest auscultation, fever, cough, purulent sputum. The mean age of participants was 59.9 years with a mean 25(OH)D level was 34.1nmol/L. Those participants requiring hospitalisation had lower Vitamin D levels, 31.9nmol/L versus 40.4nmol/L. All further analysis was performed using age and season adjusted Vitamin D levels. There was no significant association between 25(OH)D and CURB score (used as a marker of severity of pneumonia). However there was an association between 1,25(OH)2D3 and CURB on simple linear models, p =0.011, lower levels of Vitamin D were also associated with longer hospitalisation, F=17.7 versus F=46.2, P<0.001.

Jovanivich et al looked at the association between Vitamin D and the risk of hospital admission due to CAP and sepsis in a retrospective case control study (Jovanovich et al., 2014). Data was gathered from healthcare data storage for a healthcare organisation with 22 hospitals and 150 outpatient clinics. There were 187,132 admissions with CAP based on ICD-9 criteria and 4,352 cases of sepsis. Controls were randomly selected from a pool of 62,757 without sepsis or CAP diagnosis. Those included were required to have serum 25(OH)D level measured in the 3-15 month period prior to admission. 132 participants were included in the CAP and 422 in the sepsis analysis. The mean age of participants in the CAP sub-analysis was 60 years. There was no significant difference in 25(OH)D levels between cases (70.1nmol/L) and controls (79.3nmol/L). There was no association between 25(OH)D as a continuous variable and CAP but in logistic regression analysis there was an
association between 25(OH)D and CAP in those with levels <37.5nmol/L versus >37.5nmol/L, OR 2.57 (1.08-6.08, 95% CI) p=0.03. In logistic regression models with 25(OH)D levels categorized as >/<50nmol/L and >/<37.5nmol/L there was an increased odds of sepsis, OR 1.75 (1.11-2.77, 95% CI) and OR 1.89 (1.09-3.31, 95% CI) P=0.02 respectively.

5.2.2 Prospective Studies
Positive Studies
Ginde et al performed a small prospective observational pilot study to evaluate the association of Vitamin D deficiency (<75nmol/L) and higher sepsis severity in Emergency Department (ED) patients (Ginde et al., 2011). A total of 81 patients were recruited, aged over 18 years with suspected infection as identified by the admitting physician. Patients were classified as having sepsis if they had suspected infection with two or more Systemic Inflammatory Response Syndrome (SIRS) markers. Severe sepsis was presence of suspected infection with one or more organs acutely dysfunctional. Other markers of illness severity used in the study were the Acute Physiology and Chronic Health Evaluation (APACHE) and Sequential Organ Failure Assessment (SOFA) scores. The median age of participants was 62 years and median Vitamin D level was 52nmol/L. Participants were assessed at baseline and at 24 hours. Those with lower 25(OH)D levels at baseline were more likely to be older, male and white in ethnicity. Lower Vitamin D levels were also associated with higher SOFA and APACHE scores on admission and higher prevalence of severe sepsis. Lower Vitamin D levels were associated with increased SOFA scores at 24hours from baseline. Patients with lower Vitamin D levels had lower lactate levels and higher Interleukin-6 (IL-6) and IL-1 levels on admission.

A study from Finland investigated the relationship between 25(OH)D and incident hospitalisation with pneumonia in men from the KIHD study population (Aregbesola et al., 2013). Two cohorts were recruited, 1,166 men aged 54 years and 1,516 men aged 42, 48, 54 or 60 years. Approximately ten years later all men in the second cohort were invited to participate in a repeat assessment. At this time a sample of 920 postmenopausal women aged 53-73 years entered the study and 723 men and 698 women were included in this
analysis. Mean age of participants was 62.5 years with mean 25(OH)D levels of 43.5 nmol/L. Mean follow-up time was 9.8 years. During this time 78 participants had at least one hospitalisation due to pneumonia. In multivariate models, there was a 2.4 higher risk of developing pneumonia in those with the lowest tertile of Vitamin D over follow-up period compared with subjects in the highest tertile, OR 2.4 (1.2-4.9, 95% CI) p=0.02.

5.2.3 Randomised Control Trials
Positive Studies
A recent RCT investigated the effects of high dose Vitamin D supplementation compared with low dose supplementation in Long Term Care (LTC) residents in relation to rates of Acute Respiratory Infections (ARIs) (Ginde et al., 2017). Residents aged >60 years from 25 different facilities were eligible for inclusion. Those with a history of sarcoidosis, hypercalcaemia, renal failure, renal stones, active cancer, Vitamin D supplementation >1,000 IU/day and immunosuppression use were excluded. A total of 107 residents were randomised, 55 to receive high dose Vitamin D (3,00-4,000IU/day) and 52 to receive standard dose Vitamin D (400-1,000IU/day). The mean age of participants in the high dose group was 80 years compared with 82 years in the standard group was with a mean serum 25(OOH)D level of 57.4 nmol/L in both groups initially. Over the twelve-month follow-up period, there was a lower incidence of overall ARI rates in the high dose group compared with the low dose group, RR 0.60 (0.38-0.94, 95% CI). In subgroup analysis, the high dose group had lower rates of upper ARI, but there was no difference between groups in relation to lower ARI rates. Also, there was no difference between mortality and all cause hospitalisation between the groups. There was of note higher incidence of falls in the higher dose Vitamin D group, the reasons for which are unclear.

5.3 Vitamin D and Hospital Admissions/Re-admissions
5.3.1 Cross Sectional Studies
Positive Studies
A retrospective study from 2016 showed that untreated Vitamin D deficiency appeared to be associated with increased rates of hospital re-admission over a twelve-month period (Zaidi et al., 2016). Records of 209 patients admitted to medical wards, selected at random,
were reviewed. Over the ten-month study period, details of patients treated for Vitamin D deficiency were recorded as well as re-admission rates. The mean age of patients was 76 years and 32.5% of the population had serum 25(OH)D levels <30nmol/L. Of those not treated for Vitamin D deficiency, there was a re-admission rate of 75% over the following twelve months compared with 48% in those treated for Vitamin D deficiency. Falls were the most frequent cause of admission in the Vitamin D deficient group at 25.5%.

5.3.2 Prospective Studies

Negative Studies
Konstari et al investigated the relationship between Vitamin D and development of hip/knee OA and hospitalisation rates (Konstari et al., 2014). The study population was from a larger Finnish population study with participants recruited using a two-stage cluster sampling method. A total sample of 2,454 men and 2,820 women were included. Incidence and information regarding Osteoarthritis (OA) was obtained from national healthcare records. Follow-up commenced from initial recruitment until first hospitalisation for knee or hip OA, death or until study completion. During follow-up, 127 participants developed incident OA of the knee and 45 of the hip. Higher Vitamin D levels were found to be associated with increased incidence of hospitalisation due to OA over the follow-up period, knee OA and hospitalisation with Vitamin D 55-134nmol/L RR 1.45 (0.85-2.47, 95% CI), hip OA (same quartile) RR 2.81 (1.01-7.65, 95% CI). It is unclear if these participants were on higher levels of Vitamin D supplementation or had higher rates of osteoporosis or falls.

5.54 Vitamin D and Nursing Home Admissions

5.4.1 Cross Sectional Studies

Positive Studies
In 2006, Visser et al examined the association of Vitamin D and Nursing Home (NH) admission in the LASA population (Visser et al., 2006). 11% of the study population was admitted to a NH over the 6-year follow-up period. There was an inverse relationship between Vitamin D deficiency and NH admission in fully adjusted models, HR 3.48 (1.39-8.75, 95%CI). However, those admitted to NH had a higher rate of dementia and the population was a frail group.
5.5 Vitamin D and Hospital Length of Stay (LOS)

5.5.1 Cross Sectional Studies

Positive Studies

Amrein et al looked at the relationship between changes in pre-hospital Vitamin D status and hospital LOS after hospital admission in a retrospective cohort study (Amrein et al., 2016). Patients were included if they had at least two samples measuring 25(OH)D levels between 7-365 days prior to admission. Of the 1.6 million patient admissions between 1993 and 2011, 982 were eligible for inclusion. At admission the mean age was 61 years and mean 25(OH)D was 80.4nmol/L. In patients with initial 25(OH)D levels <50nmol/L, decreased LOS was found to be associated with increasing 25(OH)D levels. For an increase in 25(OH)D of 25nmol/L in adjusted models, LOS reduced by 0.4 days (0.29-0.49, 95% CI) compared with the estimated LOS for the Diagnostic Related Group (DRG) estimates.

An observational study from 2016 aimed to evaluate the relationship between Vitamin D concentrations and 30-day mortality, functional impairment, LOS, Quality of Life (QoL) and falls and fractures in medical patients presenting for in-hospital care (Graedel et al., 2016). Of the total 4,257 participants, 35.4% had 25(OH)D concentrations between 25-50nmol/L (insufficient) and 18.7% had levels <25nmol/L (deficient). The mean age of participants was 63 years. Hospital LOS was associated with a stepwise increase based on Vitamin D status, 3, 4 and 5 days (p<0.001) for sufficient, insufficient and deficient groups respectively. In fully adjusted models, Vitamin D deficiency was associated with longer LOS, OR 1.8 (1.4-2.8, 95% CI) p<0.001. There was no association between Vitamin D levels and 30-day re-admission rates, falls and fractures. There was an association between Vitamin D deficiency and patient QoL reports and subcategories of mobility and self-care.

In a study from 2016, the effects of Vitamin D levels on hospital LOS in a patient population post hip arthroplasty surgery was investigated (Maier et al., 2016). 1,083 participants were consecutively recruited with a mean age of 76 years and a mean Vitamin D level of 42.7nmol/L. The mean LOS was 13.2 days and there was a significant difference in LOS between groups based on serum 25(OH)D levels; 15.6 days (levels <50nmol/L)
versus 11.3 days (levels >50nmol/L). In multivariate analysis, this association remained with a mean difference in LOS of 4.3 (0.5-6.8, 95% CI) p=0.002.

**Negative Studies**
A further study assessed the association between Vitamin D and Length of Stay (LOS) and number of acute illnesses suffered as secondary outcomes in 399 acute geriatric inpatients over a ten-month period (Annweiler et al., 2010a). The mean age of participants was 84.5 years with a mean Vitamin D level of 34.8nmol/L. There was no statistically significant difference between the number of hospital days and the number of acute illnesses between sufficient and insufficient (<50nmol/L) 25(OH)D groups in fully adjusted regression models.

An Italian study investigated the relationship between Vitamin D, LOS and common causes of hospital admission (Marra et al., 2014). Participants (n=115) were patients consecutively admitted to two medical wards in a major hospital. 25(OH)D levels were measured at baseline, demographic information was recorded and also common medical conditions were documented. The mean age of participants was 78.4 years with a mean 25(OH)D level of 15nmol/L and 12.2% were bed bound. The mean LOS was 8 days and 4 (3.5%) died during the incident admission. In logistic regression models, infection was the only condition associated with 25(OH)D levels <20nmol/L, OR 2.66, (1.03-6.88, 95% CI). In fully adjusted models including age and PTH levels, Vitamin D was not found to be associated with LOS.

**5.5.2 Prospective Studies**
**Positive Studies**
A prospective cohort study of NH residents admitted to an acute hospital facility found an association between admission Vitamin D levels and Length of Stay (LOS) (Mc Williams et al., 2011). Seventy-one participants were included in the analysis with a mean age of 82.2 years and mean Vitamin D levels of 56.7nmol/L and median level of 57.4nmol/L. Of the 71 participants, 72% had 25(OH)D levels <75nmol/L. These participants had a longer
LOS than those with normal Vitamin D concentrations (>75nmol/L), 13.7 versus 7.7 days, p=0.002.

Helard et al assessed if Vitamin D levels were a marker of longer LOS in an acute French geriatric unit (Helard et al., 2013). Participants included a convenience sample of patients aged over 75 years, acutely admitted to a geriatric unit over a six-month period and 253 patients met inclusion criteria. The mean age of the study population was 86.2 years with a mean Vitamin D level of 33.9nmol/L. The mean LOS was 14.7 days, which was longer in those with lower Vitamin D (>/>=50nmol/L), 15 versus 12 days, p=0.017. Multivariate linear regression showed an inverse relationship between 25(OH)D levels and LOS, β=-0.07, (-0.14 - -0.02, 95% CI) p=0.043.

A French study evaluated the effect of Vitamin D on the LOS in 531 patients who were admitted (unplanned) to an acute geriatric care unit (Beauchet et al., 2013). Information relating to age, medications, home help, cognition measured using the Confusion Assessment Methods (CAM), the Kaplan Feinstein Index (KFI) score was recorded to explore the number and severity of chronic diseases, along with the reason for admission. The mean age of participants was 85 years. The mean LOS was 11.5 days. Those with longer LOS had higher rates of 25(OH)D levels <25nmol/L. Those with intermediate LOS had a higher prevalence of mobility disorders as the reason for admission. Multivariate analysis showed that LOS was predicted by male sex, delirium and 25(OH)D levels, β=2.14 (0.96-3.31, 95% CI) and on backwards reduced model β=2.20 (1.02 -3.37, 95% CI).

5.6 Vitamin D: Intensive and Critical Care Unit Admissions and Outcomes

5.6.1 Cross sectional Studies

Positive Studies

A study from Iran with 70 participants showed that Vitamin D levels in critically ill surgical patients can potentially predict intensive care unit LOS (Alizadeh et al., 2015). The study population consisted of surgical patients admitted to the ICU. Participants had a mean age of 54.8 years and 74.3% were defined as having deficient levels of Vitamin D.
(defined <75nmol/L in this study). Cox regression analysis showed a statistically significant relationship between lower Vitamin D levels and ICU LOS.

A retrospective study from the USA investigated the effect of Vitamin D levels in ICU patients and their discharge destination (Brook et al., 2015). Adults, aged >18 years, who were deemed likely to require >48 hours ICU treatment were included in the analysis. A total of 300 participants was included with a mean age of 66 years and mean 25(OH)D concentration of 47.4nmol/L and mean LOS of 9 days. The overall mortality rate in this group was 16 and among those who survived there was a non-home discharge destination in 30%. Analysis revealed a near linear relationship between Vitamin D levels and the risk of non-home discharge destination up to levels of 25nmol/L. Regression analysis revealed an inverse relationship between 25(OH)D concentrations and non home discharge, OR per 2.49nmol/L, 0.88 (0.82-0.95, 95% CI). In logistic regression models those participants with Vitamin D levels <50nmol/L were more likely to have a non-home discharge destination, OR 2.7 (1.23-6.14, 95% CI).

5.6.2 Prospective Studies

Positive Studies

A study from 2012 reported on the effects of Vitamin D deficiency in patients admitted to a surgical ICU in the USA (Flynn et al., 2012). Sixty-six patients were recruited consecutively, with a mean age of 56 years and mean APACHE score of 17. A mean Vitamin D level was not provided but 74% of the study population had levels <50nmol/L. Patients with Vitamin D levels <50nmol/L versus those with levels >50nmol/L had a longer length of hospital stay (29 versus 17, p=0.03). The ICU LOS was also longer but was not statistically significant (19 versus 13, p=0.30).

Borgermann et al investigated the relationship between Vitamin D and outcomes in cardiac surgery patients (Borgermann et al., 2012). This was a parallel two-arm study and 1,25(OH)_2D_3 was measured. Participants included those adults who underwent cardiac surgery. The sample size was small with 29 younger (mean age of 58.8 years) and 30 older adults with a mean age of 77 years included in the analysis. The primary end-point in this
study was the difference in the post-operative time course between the two groups and secondary end-points included differences until discharge in the composite of MI, stroke, and low cardiac output syndrome and in-hospital mortality. In odds adjusted models there was increased risk of adverse outcome with lower Vitamin D status OR 0.26 (0.86-0.99, 95% CI) p=0.037.

Matthews et al looked at the association between Vitamin D and adverse outcomes in a surgical Intensive Care Unit and mortality (Matthews et al., 2012). A prospective observational study of 258 patients admitted over a 16 month period showed that 91% had 25(OH)D levels <65nmol/L and 54% had levels <33nmol/L on admission to the ICU. All participants were given 50,000IU Vitamin D per week once they could tolerate nutritional support. They found that the mean length of stay (LOS) was 13 days in the severe Vitamin D deficient group versus 5.7 days in mildly deficient group, with mean treatment cost for ICU stay of $51,413 in severely deficient group versus $20,414 in the mild group.

Negative Studies
A study from China investigated the changes in the Vitamin D-Calcium and PTH axis in a critically ill patient population (Hu et al., 2013). This prospective study included 216 patients admitted to a medical ICU. All patients were >18 years and spent >48hrs in the ICU. Median age was 64 years, median APACHE II score was 21 and 44% of the population were Vitamin D deficient (defined as serum 25(OH)D level <75nmol/L). The 90-day mortality was 28.7%. Those deficient in 25(OD)D were more likely to die, had higher PTH levels, higher incidence of positive blood cultures, and multi-organ failure syndrome. There was no difference between ICU LOS or ventilation days between Vitamin D groups.

A study from 2016, showed no significant association between Vitamin D levels and a number of outcomes including, ICU LOS, mortality and duration of ventilation in 185 ICU patients (Vosoughi et al., 2016). The mean age of participants was 55.6 years with a mean serum 25(OH)D level of 39.4nmol/L. in multivariate analysis there was no significant
association between 25(OH)D levels and all cause mortality, OR 0.56 (0.11-2.92, 95% CI), ICU LOS; Beta -0.08, p=0.58 and Ventilation time, β= -0.02, p=0.75.

5.6.3 Randomised Control Trials
Negative Studies
An Austrian RCT aimed to investigate the potential beneficial effects of restoring and maintaining Vitamin D levels over six months in ICU patients. (Amrein et al., 2014b) The primary outcome was hospital LOS. Secondary outcomes included, ICU LOS, the percentage of patients with 25 hydroxyvitamin D levels higher than 30 ng/mL at day 7, hospital mortality, and 6-month mortality. The final study population included medical and surgical ICU patients, all Caucasian (n=475) with vitamin D deficiency (≤50nmol/L) assigned to receive either vitamin D3 (n=237) given orally or via nasogastric tube once at a dose of 540,000IU followed by monthly maintenance doses of 90,000 IU for 5 months or placebo (n=238). The mean age of participants was 64.6 years with mean Vitamin D levels of 32.1nmol/L. The median hospital LOS was not significantly different between groups, 20.1 days for vitamin D3 compared with 19.3 days for placebo, p = 0.98. There was no significant difference between the two study groups: 20.1 days for vitamin D3 versus 19.0 days for placebo.

5.6.4 Meta-Analysis
Negative Studies
Putzu et al performed a meta-analysis to evaluate the effects of Vitamin D on outcomes in critically ill adults. (Putzu et al., 2017) A total of seven Randomised Control Trials (RCTs) were included. Six studies administered Vitamin D3 (cholecalciferol) and one study administered Vitamin D2 (ergocalciferol). All had placebo as comparative. Five studies reported on ICU and hospital LOS and three studies reported on length of mechanical ventilation. This analysis found no association between groups. Authors report quality of evidence as low to moderate.

A recent meta-analysis looked at the relationship between Vitamin D supplementation and outcomes in a critically ill population admitted to medical, surgical or neurological ICU
facilities (Langlois et al., 2017). In this study 6 RCTs were included with a total study population of 677 patients. The authors found no difference in mortality rates, ICU LOS and hospital LOS in those treated with Vitamin D supplementation compared with those receiving placebo or usual Vitamin D treatment.

5.7 Vitamin D: Chronic Conditions
5.7.1 Prospective Studies
Positive Studies
A Scottish study looked at how Vitamin D levels affected hospital admission rates in patients with Multiple Sclerosis (MS) (Disanto et al., 2011). Information was obtained on admissions for a Scottish population of 712 individuals along with vitamin D levels during the month of admission, and the month, two months and three months prior to admission. Months 2 and 3 were inversely associated with risk of admission (Spearman's r =-0.587, p=0.045). The strongest correlation was found for the average Vitamin D levels in the 32 months prior to admission (Spearman's r =-0.57, p=0.02).

Malinoschi et al evaluated if low serum Vitamin D levels in patients with Chronic Obstructive Pulmonary Disease (COPD) affected exacerbation rates, hospitalisation and decline in Forced Expiratory Volume (FEV1) over the course of one year (Malinovschi et al., 2014). Participants were recruited from a respiratory outpatient clinic. Inclusion criteria: age >40yrs, FEV1/VC <0.7, and at least one year follow up at the clinic. In total 132 participants were included, (n=97 excluded) with median age of 67.5 years and mean Vitamin D levels of 30nmol/L. The majority of participants had GOLD stage II COPD and 51.6% had been admitted to hospital in the previous twelve months with an exacerbation of COPD. No significant relationship was found between Vitamin D levels and FEV1 predicted, Vital Capacity (VC) predicted, FEV1/VC ratio or decline. Analysis of stratified by number of exacerbations of COPD showed the higher the number exacerbations in the year preceding the study the lower the 25(OH)D level p for trend <0.001. Vitamin D deficiency was associated with frequent attenders, OR 18.1, (4.98-65.8, 95% CI). Severe deficiency was independent of frequent exacerbations in multiple regression analysis, OR 34.9 (4.89-249.0, 95% CI) p<0.001. Severe Vitamin D deficiency was independently
associated with hospitalisation, adjusted OR 8.45 (1.82-39.2, 95% CI) p=0.006 and with further adjustments for co-morbidities this remained significant p=0.02. ROC yielded an AUC 0.74 (0.65-0.84).

Negative Studies
A further study from Norway evaluated the longitudinal effects of Vitamin D deficiency on outcomes in COPD (Persson et al., 2015). The population included patients attending a hospital with a diagnosis of COPD who had Vitamin D sampled as part of the Bergen COPD Cohort Study. The mean age of participants was 63.4 years and results were otherwise provided in terms of Vitamin D status (</>50nmol/L). Those who were deficient in Vitamin D at baseline were more likely to be cachexic, current smokers, and had more frequent exacerbations of COPD. In terms of mortality at 5 years, 25(OH)D levels did not appear to be predictive, HR 1.04 (0.36-2.94, 95% CI) for those with levels <25nmol/L at baseline. Also Vitamin D levels at baseline did not show association with increased exacerbation rates during the follow-up period.

5.7.2 Randomised Control Trials
Negative Studies
Lehouck et al explored the effects of Vitamin D supplementation on COPD exacerbation rates in those with moderate to very severe COPD (Lehouck et al., 2012). In this Belgian single centre, double-blind placebo controlled trial participants were recruited over a 1.5 year period. Participants were randomised in two strata, one Vitamin D naive and the other receiving 400IU-800IU Vitamin D for osteoporosis at baseline recruitment (20% of the study population were receiving Vitamin D supplementation prior to recruitment). Participants were randomly assigned to blocks of twenty. In each consecutive block participants were randomised to receive monthly oral dose of Vitamin D 100,00IU or placebo. 340 were eligible for recruitment of whom 182 were randomly assigned and 150 completed the study. The mean age of participants was 68 years with a mean Vitamin D level of 50nmol/L. There were a total of 468 exacerbations over the study period with an annual rate of 2.8 exacerbations per patient per year. There was no significant difference between time to first exacerbation and time to first hospitalisation for an exacerbation.
between the two groups. There were 15 deaths in the year after randomisation with no significant difference in survival between the groups on Kaplan Meier analysis.

5.8 Vitamin D: Falls
5.8.1 Introduction
Falls frequently occur in the elderly and are often a source of morbidity and mortality including fractures. Although there are many factors, which contribute to falls, loss of muscle strength results in functional impairment and increased risk of falls and fractures (Wolfson et al., 1995). An estimated 95% of hip fractures are due to falls (Mitchell et al., 2016).

Vitamin D is related to falls due to its effects on proximal muscle strength and balance (Bischoff et al., 1999, Mowe et al., 1999). Myopathy has long since been recognised to co-exist with decreased bone mineralisation in Vitamin D deficiency states such as osteomalacia (Schott and Wills, 1976). Vitamin D supplementation has a protective effect on fracture reduction and its effects appear to be mediated through bone health benefits (Chapuy et al., 1992, Dawson-Hughes et al., 1997). Vitamin D deficiency is also associated with lower muscle strength and mass (Bischoff et al., 1999, Zamboni et al., 2002).

Vitamin D is also believed to affect muscle function and morphology. 1,25(OH)2D3 receptors have also been identified in human muscle tissue (Simpson et al., 1985) and the expression of VDR in muscle has been found to decline with age (Bischoff-Ferrari et al., 2004a). Vitamin D also regulates muscular calcium uptake and influx via voltage-gated calcium channels which affects muscle function (Boland, 1986, Ebashi, 1985).

Falls are a frequent cause of presentation to the acute hospital setting and are estimated to account for 10% of emergency hospital visits and 6% of hospital admission with increasing frequency with increasing age. Fall related injuries among older adults, especially among older women, are associated with substantial economic costs (Stevens et al., 2006). Developing an effective intervention or modifying a potentially reversible risk factor, such
as Vitamin D deficiency, which could decrease the incidence and healthcare costs of these injuries is an important consideration.

5.8.2 Prospective Studies

Positive Studies
A Dutch study in 2006, involving the Longitudinal Ageing Study Amsterdam (LASA) population, found that lower Vitamin D levels (defined as <25nmol/L) were in dependently associated with increased risk of falls (Snijder et al., 2006). Participants had a mean age of approximately 76 years and mean 25(OH)D level of approximately 50nmol/L. They were followed for reported falls for a one-year period. Multivariate logistic regression analysis showed an increased risk of recurrent falls OR 2.23 (95% CI, 1.17–4.25) in Vitamin D deficient participants in fully adjusted models.

Negative Studies
An Iranian study examining the association between serum, 25(OH)D levels and falls in an older population did not show an association (Ghafouri et al., 2016). Participants (n=82) included those who presented to the local ED following a fall. These participants were followed after a six-month period. The mean age of participants was 75 years with a mean Vitamin D level of 94.8nmol/L. There was no association between Vitamin D levels and falls or recurrent falls noted.

5.8.3 Randomised Control Trials

Positive Studies
A Swiss RCT investigated the effects of Vitamin D and Calcium supplementation in the elderly (Bischoff et al., 2003). Participants included a convenience sample awaiting transfer to a long stay facility, aged >60 years and mobile for distances greater than three meters. The study involved a six-week pre-treatment and twelve-week treatment period. Falls were recorded during the inpatient stay. Subjects were randomised to the treatment group (Vitamin D 800IU and Calcium 1,200mg per day) and control group (1,200mg Calcium per day). The mean age of the 122 participants was 84.9 years in the treatment group and 85.4 years in controls. Median 25(OH)D levels in the treatment group were
30.7nmol/L and in controls was 29.0nmol/L. In the follow-up period there were 80 falls in total, 25 falls in the treatment group and 55 falls in the control group. In models looking at percentage change in musculoskeletal strength measured by numerous parameters such as grip strength and TUG, there was an overall percentage increase in the treatment group compared with controls, p 0.0094. Multivariate regression models showed reduced rate of falls in the treatment group at 49%, estimate -0.68 (95% CI 14-71%) p=0.01 compared with controls. A student t test suggested reduced risk of recurrent falls in the treatment group with p=0.045.

Results from a prior double blind placebo controlled RCT in the US were used to evaluate a possible association between Vitamin D supplementation and falls in an elderly nursing home population (Broe et al., 2007). Participants (n=124) were followed over a five-month period and were randomly assigned at recruitment to five groups receiving differing doses of Vitamin D (200IU, 400IU, 600IU, 800IU) or placebo. Participants already on multivitamins (63% of the study population) were continued on these. Mean age of the population was 89 years with mean Vitamin D level of 48.7nmol/L at baseline. Those randomised to placebo were significantly younger than those randomised to receive Vitamin D. Those receiving higher doses of Vitamin D (800IU per day) had reduced rates of falls compared with all other groups, RR 0.28 (0.10-0.75, 95% CI).

In 2015, Scragg et al published the methodology for the “ViDA Study”, a large RCT investigating the effects of Vitamin D supplementation on cardiovascular disease, respiratory tract infections falls and non-vertebral fractures (Scragg et al., 2015). This double-blinded placebo controlled RCT aims to evaluate the efficacy of monthly Vitamin D supplementation in reducing CVD morbidity and mortality and incidence of acute respiratory infections, falls and non-vertebral fractures. The study population consists of 5,110 adults aged between 50-84 years recruited in New Zealand and the results are awaited.
Negative Studies

Dhesi et al performed an RCT to investigate the effects of Vitamin D on neuromuscular function in older adults who fall (Dhesi et al., 2004). Participants (n=139) were recruited thorough a falls clinic and baseline assessments functional performance, psychomotor function using the Choice Reaction Time (CRT), postural stability and quadriceps strength. After initial assessment, patients were randomised, with the treatment group receiving a single dose of 600,000IU intramuscular ergocalciferol and placebo group receiving equivalent volumes of normal saline. The mean age of patients was 76.6 years in the placebo versus 77 years in the intervention group. At baseline 25(OH)D levels were similar in both groups as was baseline screening cognitive assessment with AMTS. At baseline CRT was notably slower in the intervention group at 2.69 seconds versus 2.29 seconds in the placebo group. Six months after the intervention, there was little change in the measure of psychomotor function in the placebo group (2.32 seconds), however in the intervention an improvement in time of 0.41 seconds was noted, suggesting a correlation but this was not statistically significant.

Burleigh et al performed a RCT to evaluate the relationship between Vitamin D and falls in an inpatient population (Burleigh et al., 2007). Participants were new admissions to a general geriatric assessment/rehabilitation unit. Serum 25(OH)D level was measured along with any falls or fractures during the study period. MMSE, Barthel scores, elderly mobility scores (EMS) and falls risk assessment were measured. Participants were randomised to either placebo (calcium carbonate alone) or treatment arm (800IU Vitamin D and 1200mg calcium carbonate). The mean age of participants was 83 years, and Vitamin D levels for the total cohort were low with a median level of 22nmol/L with a median LOS of 30 days (IQR 14.6-71). No statistically significant difference between number of falls, time to first fall or fracture, and BI or Elderly Mobility Scale (EMS) scores between groups was reported.

A further RCT showed that supplementation with Ergocalciferol (Vitamin D2) along with Calcium benefited women known to be at increased risk of falling (Prince et al., 2008). This study population consisted of older community dwelling females with a mean age of
77.2 years and mean Vitamin D level of 44.7nmol/L. Participants were excluded if they had serum 25(OH)D levels >60nmol/L. All participants received 1g of calcium citrate per day for the twelve-month study period and were randomised to treatment arm (1,000IU ergocalciferol) or placebo equivalent. There were more falls in the control group (62.9%) compared with the placebo group (53.0%). The treatment group had lower risk of falling compared with controls, OR 0.61 (0.37-0.99, 95% CI). There was no difference between groups in those who suffered multiple falls.

5.8.4 Meta Analysis
Positive Studies
A meta-analysis from 2004 showed a 22% reduction in falls with Vitamin D treatment compared with calcium or placebo in a population of older adults living in the community or in long stay geriatric units (Bischoff-Ferrari et al., 2004b). This study evaluated the relationship between persons receiving Vitamin D and the risk of having at least one fall. Five RCTs with 1,237 participants were included. Treatment in the individual studies varied between two months to three years and Vitamin D doses varied from 400 to 800IU daily. Primary analysis revealed Vitamin D prevented falls compared with control group, OR 0.78 (0.64 – 0.92, 95% CI). Further sensitivity analysis with a further five RCTs included again showed a preventative effect of Vitamin D treatment in the reduction of falls, OR 0.87 (0.80 – 0.96, 95% CI).

Cranney et al performed a systematic review and meta-analysis evaluating the effects of Vitamin D supplementation on bone health (Cranney et al., 2008). The review included 112 RCTs, 19 prospective studies 30 case-control studies and six before/after studies. 72 studies looked at 25(OH)D concentrations and bone health outcomes, 41 focused on post-menopausal women and older men. Fifteen RCTs evaluated the effects of Vitamin D supplementation on fractures in post-menopausal women and older men; most used oral Vitamin D as the supplement ranging from 300-800IU. Combined results from twelve of these RCTs showed a modest reduction in falls with Vitamin D supplementation, OR 0.89 (0.80-0.99, 95% CI). In the combined results from eight RCTs where Vitamin D 700-
800IU or 1,000IU combined with calcium (500-1,200mg) was given, there was a significant reduction in falls, OR 0.84 (0.76-0.93, 95% CI).

Negative Studies
A Cochrane review in 2012 evaluated potential interventions aimed at preventing falls in older adults living in the community (Gillespie et al., 2012). In trials specifically looking at Vitamin D and falls, Vitamin D supplementation did not reduce the rate of falls, RR 1.00 (0.90-1.11, 95% CI) in 7 trials and 9,324 participants, or the risk of falling, RaR 0.96 (0.89-1.03, 95% CI) in 13 trials with 26,747 participants.

A meta-analysis reported that Vitamin D had no beneficial effect on falls (Bolland et al., 2018). This study included 81 RCTs with participants (n=513,537) aged >18 years, using Vitamin D as a monotherapy compared with placebo or RCTs that compared high with low dose Vitamin D treatment. 70% of treatment groups were on doses of 1,000 units/day or less. The co-primary endpoints were participants with at least one fracture, at least one hip fracture or at least one fall. 37 studies with falls outcome data were included. RCTs with Vitamin D supplementation of any dose were pooled and analysis was performed using trial sequential analysis for each outcome. 57% of trials were in population with mean 25(OH)D <50nmol/L but only 6% had levels <25nmol/L. The majority of studies had a follow-up period of one year or less. In the falls analysis no significant association was found, RR 0.97 (0.93-1.02, 95% CI). Variable doses of Vitamin D were used in many studies with a short follow-up period in many of the studies. Although half the population had serum 25(OH)D levels below 50nmol/L, only 6% were truly deficient (<25nmol/L).

5.8.5 Economic Studies
Poole et al reported a cost saving analysis with empiric treatment of all adults aged over 65 years with 800IU daily of cholecalciferol in relation to falls (Poole et al., 2015). A Markov health state transition model was created and used a 5-year horizon and annual cycles to assess costs and benefits of treatment. Health states were defined as well: living in the community, minor fall: ED attendance but no admission, major fall: necessitating hospital admission and death. The authors report the model predicts over five years treatment with
Vitamin D in adults aged >60 years would prevent 430,000 minor falls, 190,000 major falls, 1,579 acute deaths, and avoid 84,000 person-years in residential care facilities. The greatest benefit was noted in those aged >75 years. In relation to cost-effectiveness treatment with a ratio of 19,759 per Quality Adjusted Life Year (QALY) gained based on a reduction in falls alone.

5.9 Vitamin D: Osteoporosis and Fractures

As outlined in Section 2, Vitamin D has been well established in the maintenance of calcium homeostasis and bone metabolism and Vitamin D deficiency is known to be related to abnormal bone turnover and associated increased risk of osteopenia and osteoporosis.

Boonen et al performed a comparative meta-analysis of RCTs to evaluate the effect of Vitamin D and calcium supplementation on fracture reduction (Boonen et al., 2007). Authors performed an adjusted indirect comparison of two pooled risk estimates. A total of 9 RCTs were included with 53,260 participants in the first meta-analysis of Vitamin D alone compared with placebo versus no treatment. All used cholecalciferol, at a dose of 700-800IU in 6 trials and 400IU in 3 trials. Calcium/Vitamin D was given in 6 RCTs. In 4 RCTs comparing Vitamin D supplementation alone with placebo, including 9,083 participants, the pooled RR of hip fracture was 1.10 (0.89-1.36, 95% CI). In the meta-analysis of 6 RCTs with 45,509 participants, comparing combined Vitamin D and calcium supplementation with placebo/no treatment, the pooled RR 0.82 (0.71-0.94, 95% CI). The pooled risk difference was 0.4% giving a NNT of 2,376 to prevent one hip fracture (165-843, 95% CI) over the treatment period of 24-84 months. The indirect comparison of the pooled estimates for hip fracture from the two meta-analyses gave an adjusted RR 0.75 (0.58-0.96, 95% CI) in favour of combining Vitamin D and calcium, suggesting a 25% reduction in hip fracture using combination treatment compared with Vitamin D alone.

Hip fractures are a common consequence of falls in older people and are particularly devastating in terms of their impact on an individual’s health and ability. In older adults who have survived a hip fracture, 29% will not have returned to their baseline level of
function twelve months on (Bertram et al., 2011). It is reported that 20% of adults with a neck of femur fracture will return to the ED within twelve months due to a fall or another complication associated with their fracture and three quarters will require admission (Bryson et al., 2011).

A 2014 Cochrane review evaluated the use of Vitamin D in fracture prevention in older men and post-menopausal women (Avenell et al., 2014). Hip fracture prevention was the primary outcome of interest. RCTs and quasi-RCTs were included in the review with Vitamin D alone or combined with calcium compared with placebo. Fifty-three trials were included with 91,791 participants (31 trials had a median age of greater than 80 years). Vitamin D alone was found to be unlikely to prevent hip fractures, RR 1.12 (0.98-1.29, 95% CI) or any new fracture, RR 1.03 (0.96-1.11, 95% CI). Overall the combination of Vitamin D and calcium reduces the risk of any type of fracture and there is high quality of evidence to support this, RR 0.97 (0.90-0.99, 95% CI). In terms of adverse outcomes there were increased risks of gastrointestinal symptoms and renal disease.

Hiligsmann et al developed an economic model to assess the cost effectiveness of Vitamin D supplementation in the treatment of osteoporosis in the elderly using a Markov micro simulation model in the Belgian population (Hiligsmann et al., 2015). The study population included men and women aged >60 years and osteoporosis was defined as BMD T score >-2.5 and the first hip fracture incidence was taken from the national database of hospital bills and other fracture risk was estimated from Swedish incidence rates. The authors report a cost per QALY gained with Vitamin D and calcium supplementation was estimated at €40,578 in women and €23,477 in men. Treatment with Vitamin D and calcium remained cost-effective in those aged 80 years compared with the cost of osteoporotic fracture in the no treatment group.

In 2015 a study was published examining the relationship between 25(OH)D status, Vitamin D Binding Protein (DBP) Single Nucleotide Polymorphisms (SNPs) and race with incident fracture hospitalisations (Chamberlain et al., 2016). Participants were from an on-going prospective US study, the ARIC study. Data on fracture related hospital admissions
was obtained through annual phone calls to participants. The study consists of 12,781 participants with a mean age 57.1 years and median 25(OH)D levels of 59.2nmol/L (63.9nmol/L for whites and 45.5nmol/L for blacks). Over a median of 19.6 years follow-up there were 1,122 incident hospitalisations. Fracture incident rate was 5.9 per 1,000 person years. Hip fracture incidence was 1.2 per 1,000 person years. In fully adjusted logistic regression models, there was increased odds of all fracture types based on Vitamin D levels <50nmol/L, although not statistically significant, OR 1.19 (1.03-1.37, 95% CI) p=0.20. There was no difference in fracture risk based on Vitamin D levels in blacks but there was a trend towards significance in lower Vitamin D levels and fracture incidence in whites, OR 1.19 (1.02-1.39, 95% CI).

A recent meta-analysis reported that Vitamin D had no beneficial effect on outcomes of fracture, falls and bone mineral density (BMD) (Bolland et al., 2018). This study included 81 RCTs with participants (n=513,537) aged >18 years, using Vitamin D as a monotherapy compared with placebo or RCTs that compared high with low dose Vitamin D treatment. 70% of treatment groups were on doses of 1,000IU per day or less. The co-primary endpoints were participants with at least one fracture, at least one hip fracture or at least one fall. The secondary end point was percentage change in BMD measured with DEXA. 42 studies with fracture outcome data and 41 with BMD data. RCTs with Vitamin D supplementation of any dose were pooled and analysis was performed using trial sequential analysis for each outcome. 57% of trials were in population with mean 25(OH)D <50nmol/L but only 6% had levels <25nmol/L. The majority of studies had a follow-up period of one year or less. Of the 36 trials included in the meta-analysis for fracture, there was no significant association found with Vitamin D treatment, RR 1.00 (0.99-1.07, 95% CI). For the 34 studies included in the analysis of increasing BMD, there appeared to be some potential benefit with Vitamin D at the lumbar spine, RR 0.25 (0.00-0.49, 95% CI). The between group differences for BMD at the neck of femur was 0.76% (0.43-1.09, 95% CI) and 0.25% (0.00-0.49%, 95% CI). In subgroup analysis, those with 25(OH)D levels <25nmol/L appeared to have increased spine and hip BMD with Vitamin D supplementation. Although a well-conducted analysis, as with all meta-analyses it is dependent on the studies included and their associated variability in design and study
populations. Variable doses of Vitamin D were used in many studies with a short follow-up period in many of the studies. Although half the population had serum 25(OH)D levels below 50nmol/L, only 6% were truly deficient (<25nmol/L).

5.10 Discussion
There have been a number of studies evaluating how Vitamin D affects outcomes in patients relating to specific conditions and disease processes. These outcomes can vary depending on the population being evaluated in the relevant study. Many have very specific conditions or meet specific defined inclusion criterion, such as ICU patients, those with COPD or hip arthroplasty patients. Therefore the results of these studies are difficult to translate to the general older population. However, these are common presentations to acute services and frequently result in admissions and therefore are important to consider.

The effects of Vitamin D on bone heath are well established and the majority of studies outlined above show an overall benefit in Vitamin D in fracture prevention. The role of Vitamin D in falls prevention is less clear but there appears to be a relationship and certainly some economic studies suggest a benefit financially in supplementation of Vitamin D in falls prevention.

In addition, there have been many studies evaluating the effects of Vitamin D on frailty, which of course has been shown to impact on functional outcomes in older adults. Overall, these studies appear to show beneficial effects of higher serum Vitamin D levels in outcomes in frail older adults (see Chapter 4).

Evidence suggests Vitamin D has the potential to impact on multifactorial disease processes, which are associated with frequent use of resources such as COPD and Multiple sclerosis.

However, there are few studies evaluating the effects of Vitamin D on resource utilisation directly. Given the feasibility of supplementation as a potential strategy for prevention, further studies to explore the effects of Vitamin D on these disease processes and
specifically on resource utilisation, such as ED attendance and hospital admissions would be both informative and beneficial in older adults.
Chapter 6: Vitamin D and Mortality

6.1 Introduction
There have been a significant number of studies in recent years assessing the relationship between Vitamin D and all-cause and cause-specific mortality, particularly cancer and cardiovascular related deaths. The underlying mechanism of action of Vitamin D on mortality is most likely mediated by a number of potential mechanisms including anti-inflammatory, immune modulating effects and its effects on cell proliferation and apoptosis.

As outlined in earlier sections, Vitamin D deficiency is also linked to bone health and risk of fractures as well as frailty syndrome, both of which are associated with increased mortality rates.

6.2 Vitamin D and Cancer
Biological findings have reinforced the likelihood of the vitamin D hypothesis. Firstly, vitamin D receptors have been found in various organs, as previously outlined and activation of these by calcitriol, induces cell differentiation and inhibits proliferation, invasiveness, angiogenesis, and metastatic potential (Ordonez-Moran et al., 2005). These biological activates are typical of cancer genesis and some, such as differentiation and proliferation, are also involved in cardiovascular ischaemic diseases.

Vitamin D appears to affect the incidence of many types of cancer through a number of different mechanisms including inhibiting tumour angiogenesis and strengthening of the inhibition of proliferation of adjacent cells within a tissue. Vitamin D metabolites also help to maintain a normal calcium gradient in the colon epithelial crypts (Lipkin and Newmark, 1985). 1,25(OH)2D inhibits mitosis of breast epithelial cells (Campbell et al., 1997). Pulsatile release of ionised calcium, which is enhanced by 1,25(OH)2D, also reduces differentiation and apoptosis (Brenner et al., 1998).
6.3 Vitamin D and Cardiovascular Disease and Associated Risk Factors
Hypovitaminosis D may affect cardiovascular related mortality through its purported associations with the renin-angiotensin aldosterone system (RAA), hypertension, diabetes, metabolic syndrome and coronary artery disease. Studies have indicated Vitamin D deficiency as a marker of cardiovascular risk (Pilz et al., 2013), promoting atherosclerosis (Wang et al., 2008) and subsequent cardiovascular events (Kunadian et al., 2014). Low levels of Vitamin D have also been linked to inflammation, higher coronary artery calcium scores, increased platelet volume and vascular stiffness (Kunadian et al., 2014, Cumhur Cure et al., 2014).

Vitamin D suppresses inflammation via several pathways, such as inhibition of prostaglandin pathways, up-regulation of anti-inflammatory cytokines, decrease of cytokine-induced expression of adhesion molecules, and down-regulation of the RAA (Ferder et al., 2013, Kunadian et al., 2014). Vitamin D deficiency stimulates systemic and vascular inflammation, enabling atherogenesis (Lee et al., 2008). Hypertension is also associated with lack of vitamin D, due to activation of the RAA system, causing endothelial dysfunction, which is involved in plaque formation.

Chronic Vitamin D deficiency causes secondary hyperparathyroidism, increasing insulin resistance, impairing beta-pancreatic cell function, and enabling the development of metabolic syndrome and diabetes mellitus (Lee et al., 2008). Many studies, including cellular, experimental, and observational support the role of vitamin D in the pathogenesis of both type 1 and 2 diabetes (Takiishi et al., 2010).

6.4 Vitamin D and Mortality: Cross Sectional Studies
Positive Studies
In 2010 Annweiler et al assessed the association between Vitamin D and in-hospital mortality in 399 acute geriatric inpatients over a ten-month period, with a mean age of 84.5 years and mean Vitamin D level of 34.8nmol/L (Annweiler et al., 2010a). There were seventeen deaths in this study group. Higher serum levels of vitamin D were found to be associated with fewer in-hospital deaths in this acute care unit, OR 0.87 p=0.029.
A US study looked at the retrospective association of Vitamin D and mortality in a medical ICU over a four-month period (Venkatram et al., 2011). Actual mortality was compared with predicted mortality, which was calculated using the APACHE IV score. 437 patients participated in the study with a mean age of 59.9 years. 340 (77.8%) patients had baseline serum Vitamin D levels <49.9nmol/L. Hospital mortality rates were higher in the Vitamin D deficient groups and also observed mortality was higher than predicted mortality in Vitamin D deficient group (24.1% versus 8%). Logistic regression analysis for mortality and Vitamin D deficiency showed an association, OR 8.7 (1.03-72.8, 95% CI) p=0.047.

Leow et al examined the relationship between 25(OH)D levels and antimicrobial peptide levels in the setting of Community Acquired Pneumonia (CAP) and the outcomes of 30-day mortality in these conditions (Leow et al., 2011). The primary outcome of interest was 30-day mortality. A total of 128 eligible patients were assessed during the study period and of these 112 were included in the analysis. The mean age of participants was 76 years with a mean Vitamin D level of 54nmol/L. 25(OH)D levels were not correlated with either antimicrobial peptide level. Participants with severe 25(OH)D deficiency had higher 30-day mortality rates, OR 13.5 (2.6-69.1, 95% CI). Of the potential covariates, CURB-65 was significantly associated with mortality, OR 2.23 (1.13-4.40, 95% CI).

A Dutch study looked at the association between Vitamin D and outcomes in CAP including ICU admission and also 30-day mortality (Remmelts et al., 2012). The primary clinical outcome of interest was an adverse clinical outcome and defined as the need for ICU admission or death within 30 days of hospital admission. There were 304 participants included, with a mean age of 63.5 years and a median 25(OH)D level of 47.4nmol/L. Those in the Vitamin D deficient group were more likely to be older and had higher incidence of co-morbidities at baseline. Over the study period, 5.3% of patients were admitted to the ICU (4 of these died) and 5.9% (16 patients) died at 30 days. Vitamin D deficiency was found to be associated with the combined outcome of ICU admission and mortality in adjusted models, OR 2.95 (0.06-13.59, 95% CI). In analysis when Vitamin D was considered as a continuous variable, it was found to be a significant predictor of mortality.
Padhi et al. evaluated the relationship between Vitamin D and outcomes in critically ill patients of the Medical ICU of a teaching hospital in Eastern India (Padhi et al., 2014). All patients admitted to the MICU in a twelve-month period with available Vitamin D levels were included. Of the 300 patients admitted to the MICU, 152 had available Vitamin D levels. The mean age and mean Vitamin D levels were 60 years and 60.6nmol/L respectively. Those with lower Vitamin D levels were more likely to die, OR 0.39 (0.29-0.67, 95% CI), have a longer ICU stay and longer mechanical ventilation time, OR 0.53 (0.62-1.87, 95% CI).

A US Study looked at the relationship between Vitamin D and mortality in a hospitalised population (Lange et al., 2013). The study population of 23,603 comprised patients aged >18 years admitted to two hospitals who had Vitamin D levels taken within 7-365 days prior to admission. The primary end point was 30-day mortality after hospital admission and secondary end points included all-cause mortality and blood-stream infection. Mean age of participants was 61.2 years and 30-day mortality rate was 3.6%. 73.4% of Vitamin D levels were taken within 6 months of admission and 20.6% within one month of admission. In fully adjusted modules, pre-hospital 25(OH)D levels of <37.44nmol/L were associated with increased odds of mortality 30 days after hospital admission, OR 1.45 (1.21-1.74, 95% CI) and in-hospital mortality, OR 1.78 (1.42-1.72, 95% CI).

Amrein et al. hypothesised that 25(OH)D levels prior to hospital admission had a U shaped association with mortality (Amrein et al., 2014a). In this US, two-centre retrospective study, hospitalised adults with a Vitamin D sample taken within the year prior to admission were recruited. The sample size was 24,094 participants. The primary end point was all-cause 90-day mortality. Participants were followed for a total of 365 days. The mean age at hospital admission was 61 years and the mean Vitamin D level was 69.6nmol/L. Inpatient mortality rate was 2%, 30-day mortality was 4%, 90-day was 7%, and 365-day mortality was 13%. Pre-hospital Vitamin D levels were found to be a strong predictor of 90-day mortality. The adjusted OR for mortality with a Vitamin D level <25nmol/L was 2.0 (1.68-2.40, 95% CI) compared with those with Vitamin D levels between 75-125nmol/L. Vitamin D was also found to be a predictor of 365-day mortality in adjusted models, OR
2.6 (2.26-2.96, 95% CI). Models adjusted for age, gender, race, medical versus surgical patient, season and Deyo-Charlson Index.

Moraes et al investigated the relationship between Vitamin D and mortality and other health outcomes in 135 patients admitted to an ICU in Brazil over an eight-month period (Moraes et al., 2015). The mean age of participants was 57.9 years and the median Vitamin D level was 33.2nmol/L was a 28-day mortality rate of 21.5%. 74.8% of participants were insufficient of 25(OH)D (<50nmol/L). In ROC analysis the AUC was 0.61 (0.489-0.73, 95%CI) at admission for 25(OH)D levels in relation to mortality. Mortality was found to be associated with Vitamin D, adjusted RR 2.20 (1.07-4.54, 95% CI). Vitamin D was not found to be associated with ICU LOS, mechanical ventilation and infection rates.

A study from 2016 aimed to evaluate the relationship between Vitamin D concentrations and 30-day mortality, functional impairment, LOS, Quality of Life (QoL), falls and fractures in medical patients presenting for in-hospital care (Graedel et al., 2016). Of the total 4,257 participants, 35.4% had 25(OH)D concentrations of 25-50nmol/L (insufficient) and 18.7% had levels <25nmol/L (deficient). The mean age of participants was 63 years. With lower Vitamin D levels there was a stepwise increase in mortality of 3.4%, 5.6% and 7.8% in patients with sufficient, insufficient and deficient levels of Vitamin D. In fully adjusted regression models, Vitamin D deficiency (<25nmol/L) was associated with increased risk of 30-day mortality, OR 1.93 (1.29-2.14, 95% CI) p=0.001.

Amrein et al also looked at the relationship between pre-hospital Vitamin D status and 30-day mortality after hospital admission in a retrospective cohort study (Amrein et al., 2016). 982 admissions were eligible for inclusion on the current analysis. At admission mean age was 61 years and the mean final 25(OH)D level was 80.4nmol/L. In hospital mortality rate was 2.4%, 30-day mortality was 3.1% and 90 day was 6%. Mortality at 30 days after admission was lower in those with an increase in Vitamin D levels prior to admission. Each 25nmol/L increase in Vitamin D levels throughout the year leading up to admission was associated with a relative decrease in 30-day mortality, OR 0.92 (0.85-0.99, 95% CI). For those with levels >75nmol/L, analysis did not show a change in mortality rate with levels
increasing by 25nmol/L in the year prior to admission. In hospital mortality rates also decreased for every 25nmol/L increase in pre-hospital serum 25(OH)D levels, OR 0.74 (0.62-0.88, 95%CI). There was a non-statistically significant reduction in 30 and 90-day post discharge mortality rates.

**Negative Studies**
A study from Turan et al investigated the effects of 25(OH)D deficiency on cardiac surgery outcomes including 30-day mortality and cardiac morbidities (Turan et al., 2013). Of 18,064 patients screened for inclusion in this prospective study only 426 were eligible for inclusion. The median Vitamin D level was 47.4nmol/L. Vitamin D levels were not associated with mortality, the incidence of 30-day mortality was 1.4%, OR 0.89 (0.55-1.46, 95% CI).

A further study of 70 surgical ICU patients, again did not find a significant association between Vitamin D levels at admission to ICU and mortality rates over a twelve-month study period (Alizadeh et al., 2015). Mean Vitamin D levels were not provided but approximately 25% of the study population had levels >75nmol/L.

A further study assessing the relationship between Vitamin D and mortality (90-day) in ICU patients was reported on 2016. This analysis included participants from the FINNAKI study with a diagnosis of severe sepsis or septic shock within 24 hours of ICU admission (Ala-Kokko et al., 2016). Severe Vitamin D deficiency was defined as <25nmol/L. A total of 610 participants were included with a mean age 62.4 years and median 25(OH)D levels of 48.1nmol/L. Fully adjusted Cox regression models revealed no association between Vitamin D deficiency and 90-day mortality, HR 0.49 (0.22-0.87, 95% CI).

In a small retrospective study from 2017, authors investigated the relationship between Vitamin D and mortality in an ICU population (Atalan and Gucyetmez, 2017). Over a twelve-month period, 491 patients were admitted. The mortality rate was 21.6% and the mean age of survivors was 63 years compared with 62.5 years in non-survivors with mean Vitamin D levels of 20.5nmol/L and 18.7nmol/L respectively. There was no significant
difference in Vitamin D levels at admission to ICU between groups. Multivariate analysis found no significant association between Vitamin D levels and mortality, OR 0.5 (0.2 -1.4, 95% CI) p =0.206. The small sample size makes it difficult to draw conclusions.
Table 3: Vitamin D and Mortality: Cross Sectional Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Confounders</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annweiler et al</td>
<td>2010</td>
<td>399</td>
<td>17 deaths (4.3%)</td>
<td>84.5</td>
<td>Geriatric inpatients</td>
<td>Not available</td>
<td>34.8nmol/L</td>
<td>Age, Gender, BP, Number of acute and chronic diseases, GFR, BMI, Season of blood draw and hospital admission</td>
<td>Higher Vitamin D concentrations are associated with lower hospital mortality rates</td>
</tr>
<tr>
<td>Venkatram et al</td>
<td>2011 USA</td>
<td>437</td>
<td>92 deaths (21%)</td>
<td>Not available</td>
<td>All admissions to MICU with Vitamin D level</td>
<td>Re-admission to MICU</td>
<td>Not available</td>
<td>Age, APACHE IV, Ventilator Days, ICU LOS, Creatinine, Albumin, Glucose</td>
<td>Better survival rates in those with higher Vitamin D level</td>
</tr>
<tr>
<td>Leow et al</td>
<td>2011 New Zealand</td>
<td>112</td>
<td>9 deaths (8%)</td>
<td>76 (Median)</td>
<td>Adults admitted with CAP</td>
<td>Not available</td>
<td>54nmol/L (Median)</td>
<td>Age, Gender, CURB score, Charlson Index (rates co-morbidities), CRP, residential living</td>
<td>Association between Vitamin D deficiency and 30 day mortality from CAP</td>
</tr>
<tr>
<td>Remmelts et al</td>
<td>2012 Netherlands</td>
<td>272</td>
<td>16 deaths</td>
<td>63.5</td>
<td>CAP</td>
<td>Receiving calcitrol or 1alpha-hydroxyvitamin D3</td>
<td>47.4nmol/L (Median)</td>
<td>Age, Race, Gender, Season, NH resident, Liver &amp; Renal disease, DM, Malignancy, COPD, Albumin</td>
<td>Vitamin D deficiency is a predictor of 30-day mortality in patients with CAP</td>
</tr>
<tr>
<td>Lange et al</td>
<td>2013</td>
<td>23,063</td>
<td>3.6%</td>
<td>61.2</td>
<td>Missing Data</td>
<td>High dose Vitamin D supplement</td>
<td>69.3nmol/L</td>
<td>Age, Gender, Race, Deyo-Charlson Index, Season, Urea, Creatinine, Time from Vitamin D to admission</td>
<td>Pre-admission Vitamin D levels were predictors of in-hospital and 30-day mortality</td>
</tr>
<tr>
<td>Padhi et al</td>
<td>2014</td>
<td>152</td>
<td>18.4%</td>
<td>60.0</td>
<td>All ICU admissions with Vitamin D levels</td>
<td>Not available</td>
<td>60.6nmol/L</td>
<td>Not available</td>
<td>Vitamin D deficiency associated with increased mortality, LOS and longer ventilation times</td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>No.</td>
<td>Mortality Rate</td>
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<td>Inclusion Criteria</td>
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<td>Mean Vitamin D</td>
<td>Confounders</td>
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<td><strong>Positive</strong></td>
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<tr>
<td>Amrein et al</td>
<td>2014</td>
<td>24,094</td>
<td>13% 365-day</td>
<td>61.2</td>
<td>&gt;18 years, Hospitalised, 25(OH)D level within 12 months of admission</td>
<td>Foreign patients</td>
<td>69.6nmol/L</td>
<td>Age, Gender, Race, Season, Dayo-Clarkson Index</td>
<td>Increased risk of mortality with deficient Vitamin D levels and also levels &gt;12nmol/L (U shaped association)</td>
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<tr>
<td>USA</td>
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<td>7% 90-day</td>
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<tr>
<td>Amrein et al</td>
<td>2016</td>
<td>4,344</td>
<td>134 deaths</td>
<td>61 years</td>
<td>Two Vitamin D level within 7-365 days prior to admission, &gt;18 years</td>
<td></td>
<td>80.4nmol/L</td>
<td>Age, Gender, Race, Season, Dayo-Clarkson Index, Creatinine, Haematocrit</td>
<td>Change in Vitamin D status prior to admission associated with mortality risk</td>
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<td>USA</td>
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<tr>
<td>Moraes et al</td>
<td>2015</td>
<td>135</td>
<td>21.5%</td>
<td>57.9</td>
<td>Medical ICU admissions</td>
<td>ESKD on dialysis, Pregnancy, Granulomatous diseases, Parathyroid dysfunction</td>
<td>33.2nmol/L</td>
<td>Lactate, Albumin, APACHE, SOFA, Sepsis, BMI</td>
<td>Inverse association between Vitamin D and 28-day ICU mortality</td>
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<tr>
<td>Brazil</td>
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<tr>
<td>Graedel et al</td>
<td>2016</td>
<td>4,257</td>
<td>115 deaths</td>
<td>63.0</td>
<td>Adult patients requiring hospitalisation</td>
<td>Surgical and Paediatric patients</td>
<td>Not available</td>
<td>Age, Gender, Co-morbidities, Main diagnosis</td>
<td>Vitamin D deficiency (&lt;25nmol/L) associated with mortality</td>
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<tr>
<td>USA</td>
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<td>(2.7%)</td>
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<td><strong>Negative</strong></td>
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<tr>
<td>Turan et al</td>
<td>2013</td>
<td>426</td>
<td>1.4%</td>
<td>Not available</td>
<td>Vitamin D level within three and one month prior to surgery</td>
<td>&lt;18 years, No General Anaesthetic</td>
<td>47.5nmol/L</td>
<td>Age, Gender, BMI, Smoking, Race, Alcohol, Smoking, Dialysis, CCF, HTN, Arrhythmia, Prior vascular/cardiac surgery</td>
<td>No association between peri-operative Vitamin D levels and 30-day post-operative mortality</td>
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<td>USA</td>
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<td>Ala-Kokko et al 2016</td>
<td>610</td>
<td>29.5%</td>
<td>62.4</td>
<td>&gt;18 years</td>
<td>Dialysis, Elective admission, Organ donors, Non-resident in Finland</td>
<td>48.1nmol/L (Median)</td>
<td>APACHE II, Corticosteroid use, Hospital LOS, DM, Multi-organ failure</td>
<td>Vitamin D levels not associated with 90-day mortality in ICU patients with septic shock</td>
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<td>Finland FINNAKI</td>
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<tr>
<td>Alizadeh et al 2017</td>
<td>70</td>
<td>25%</td>
<td>54.5 years</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
<td>No association between Vitamin D and ICU mortality in surgical patients</td>
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<td>Iran</td>
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<tr>
<td>Atalan at al 2017</td>
<td>491</td>
<td>21.6%</td>
<td>63 years</td>
<td>&gt;18 years</td>
<td>Elective surgery</td>
<td>19.6nmol/L</td>
<td>Age, Sepsis diagnosis, APACHE II score, Number of organ dysfunction</td>
<td>No significant association between baseline admission Vitamin D levels and ICU mortality</td>
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<td>Turkey</td>
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</tbody>
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APACHE IV: Acute Physiology and Chromic Health Evaluation, BMI: Body Mass Index, BP: Blood Pressure, CAP: Community Acquired Pneumonia, CCF: Congestive Cardiac Failure, CRP: C-Relative Protein, CURB: Confusion Urea Respiratory Rate Blood Pressure, GFR: Glomerular Filtration Rate, HTN: Hypertension, LOS: Length of Stay, (M)ICU: (Medical) Intensive Care Unit
6.5 Prospective Studies

Positive Studies

The effects of Vitamin D and mortality were studied in 3,258 participants from the LURIC study (Dobnig et al., 2008). Participants were recruited through referrals for coronary angiogram. Mean age was 63.7 years and mean Vitamin D at baseline was 42nmol/L. Participants were followed for a median of 7.7 years and there were 737 (22.6%) deaths over this time. Results showed an association between Vitamin D and mortality in the lower quartiles of 25(OH)D, HR 2.08, (1.60-2.70, 95% CI).

A study from the NHANES III project looked at the association of deficiency and mortality (Melamed et al., 2008). Participants (n=13,331) were aged 20 years or more, had physical examination and laboratory tests completed at baseline (between 1988 to 1994) with follow complete follow-up (through to 2000). Mortality was recorded the National Death Index. The cause of death was recorded using the relevant ICD codes at the time of death. The mean age of participants at baseline was 44.8 years. The median time to follow-up was 9.7 years. There were 1,806 deaths with the most common cause documented as CVD (43%). On multivariate analysis, the lowest quartile of Vitamin D was found to be associated with increased all cause mortality risk, MRR 1.26 (1.06-1.46, 95% CI). A non-statistically significant association was found between the lowest Vitamin D quartile and CVD mortality, MRR 1.20 (0.87-1.64, 95% CI) on fully adjusted models. No association was found with cancer related mortality in this study.

A study from Germany found that lower levels of Calcitrol levels were associated with mortality in a population of 510 participants (Zittermann et al., 2009), 228 had end-stage heart failure awaiting cardiac transplantation. The remainder had been part of two Vitamin D RCTs, complete at the time if this study, of these, 178 were heart failure patients with obesity participating in a weight reduction programme and 104 were heart failure (HF) patients not awaiting transplantation. None were taking Vitamin D supplementation at enrollment for this study. The mean age of participants with end-stage HF was 56.5 years and the main age of the remaining participants was 51.2 years, the mean serum 25(OH)D level was 36.2 and 57.9nmol/L respectively, thus those participants who had been involved
in the Vitamin D supplementation trials had higher serum levels of Vitamin D. The mean Calci
trol level for the study was 29.0ng/L. During the one year follow-up, 82 participants
died, 95% of these had end-stage HF. In fully adjusted models, low serum calcitrol levels
were associated with increased mortality, HR 3.93 (1.34-11.55, 95% CI).

Semba et al report a positive association between Vitamin D and all cause mortality in a
population of older community dwelling women in Baltimore in the US (Semba et al.,
2009). Participants were recruited as part of the Women’s Health and Ageing Studies
(WHAS) with mean age of 74 years and mean Vitamin D level of 50.9nmol/L. Median
time to follow-up for mortality was 72 months. There were 100 deaths, 19.2% in the lowest
Vitamin D quartile versus 8.12% in the highest quartile of Vitamin D. In fully adjusted
models women in the lowest quartile of Vitamin D had higher mortality rates compared
with those in the highest quartile, HR 2.45 (1.12-5.36, 95% CI). Models were adjusted for
age, race, education, season, BMI, smoking supplement use, physical activity, cholesterol
and chronic diseases.

Semba et al went on to look at Vitamin D and all-cause and cancer mortality in a
community dwelling Italian population aged over 65 years from the InCHIANTI Study
(Semba et al., 2010). 1,006 participants were eligible for inclusion. The mean age of
participants was 74 years. Participants were followed for 6.5 years. Participants were
divided into Vitamin D quartiles. During the follow-up period there were 228 deaths
(22.7%). In fully adjusted models, there were higher rates of all-cause mortality in those in
the lowest Vitamin D quartile compared with the highest, HR 2.11 (1.22-3.64, 95% CI). In
a sub-analysis for cardiovascular mortality, again on fully adjusted models, lower Vitamin
D levels were associated with increased mortality, HR 2.64 (1.68-2.19, 95% CI). A further
analysis of 729 participants who had 25(OH)D levels taken at baseline and at 3 year
follow-up, who died of all causes in the 6.5 year follow-up period was performed. Those
who had Vitamin D level below the median at baseline and at follow-up had a higher
mortality risk (17.3%) than those who maintained a Vitamin D level above the median at
baseline and at follow-up (8.3%). Those participants who increased their Vitamin D levels
from below to above the median over the 3 years also had a lower mortality risk (10.8%).
From the NHANES III study (Ginde et al., 2009) the association between Vitamin D and all cause mortality was assessed in 6,408 participants with a mean age of 73 years, mean Vitamin D level of 66nmol/L and median follow-up period of 7.3 years. There were 1,493 deaths over this time. Lower baseline Vitamin D was associated with higher rates of mortality with adjustments for a number of factors including common IHD risk factors.

In a prospective study of postmenopausal Japanese women recruited through clinic visits were followed for a period of 6.9 years (Kuroda et al., 2009). The mean age of participants was 63.9 years and 47% of the cohort had 25(OH)D levels <50nmol/L at baseline. The death rate was reported as 12.6 deaths/1,000 person years. In multivariate regression analysis 25(OH)D was associated with mortality, HR 2.17 (1.27-3.27, 95% CI).

In a French study aimed at identifying predictors for bone loss and fractures in older men, the MINOS study, 782 men with serum Vitamin D levels were followed for a ten-year period (Szulc et al., 2009). Participants were recruited through an insurance register in France. Over this time 182 men died (23.3%). Mean age of the survivors was 64 years and in the deceased group was 70 years. Survivors had a higher baseline mean 25(OH)D level at 70nmol/L versus 57.5nmol/L in those who died. In fully adjusted models, there was an association between Vitamin D and mortality, HR 1.22 (1.01-1.48, 95% CI).

In the USLAM study from Sweden 1,194 men with a mean age of 71 years with serum 25(OH)D levels measured were followed for a median of 12.7 years (Michaelsson et al., 2010). Of these, 584 (49%) died over the study period. 119 men (9.9-%) had a serum 25(OH)D level <50nmol/L. Lower Vitamin D levels were associated with higher overall mortality rates, HR 1.43, 95% CI 1.11-1.84, and with higher rates of cancer mortality, HR 1.99, 95% CI 1.29-3.08. Higher Vitamin D levels (>98nmol/L) also appeared to be associated with increased mortality rates, HR 1.67 (1.19-2.35, 95% CI).

In a US study (Anderson et al., 2010), the prevalence of Vitamin D deficiency and its association with Cardiovascular (CV) outcomes including mortality was investigated. Participants were selected through a database search identifying 41,497 subjects with
Vitamin D measured between 2000-2009. Participants mean age was 55 years and mean follow-up time was one year, 17% of study population had Vitamin D levels <37.5nmol/L. Those participants had higher rates of hypertension, hyperlipidaemia, diabetes and Peripheral Vascular (PVD). Over the follow-up period, 1,193 (4.3%) had died. Vitamin D levels <37.5nmol/L were found to be associated with mortality, HR 1.77 (1.5-2.1, 95% CI) and incident development of CAD, HF, Stroke and PVD in fully adjusted models.

A Norwegian study reported an association between Vitamin D and mortality in 7,161 participants from the Tromso study (Hutchinson et al., 2010). Participants had a mean age 58.9 years, non-smokers had a mean Vitamin D of 52.9nmol/L and smokers had a mean Vitamin D of 71.7nmol/L. Mean follow-up time was 11.6 years during which 1,359 (18.9%) died. In the fully adjusted models, in the lowest Vitamin D quartile in the non-smokers, there was slightly increased risk of all cause mortality, HR 1.32 (1.07-1.62, 95% CI). No association was found in the smoking group HR 1.06 (0.83-1.35, 95% CI).

A Finnish group reported an association between Vitamin D deficiency and all cause mortality in 2011 (Virtanen et al., 2011). The study population consisted of men and women recruited as part of a population based study to investigate the risk factors for CVD and other chronic diseases. Men aged between 42-60 years of age were recruited between 1986-1989. Postmenopausal women aged between 53-73 years were later recruited between 1998-2001. A total of 1,136 were included. There was an average of 9.1 years follow-up where all causes of mortality were recorded using ICD codes. The mean age of the population was 61.8 years with a mean Vitamin D of 43.7nmol/L. There were 87 deaths in the follow-up period. The mean serum 25(OH)D concentration was 39.5nmol/L in those who died versus 44.1nmol/L in those who survived. In fully adjusted models, low serum 25(OH)D levels were associated with increased risk of death, HR 2.06 (1.12-3.8, 95% CI).

A further study from the US looked at the association of low Vitamin D levels and mortality in 2,399 critically ill ICU patients (Braun et al., 2011). Participants had a pre-admission 25(OH)D level, with a mean level of 64.9nmol/L. The mean age was 64.9 years and participants were followed for 11 years. Mortality risk was 1.7 fold higher in Vitamin
D deficient patients compared to those with sufficient levels at baseline at 30 days and 1.6 fold higher at 90 and 365-day mortality. Multivariate analysis for 30-day mortality showed increased risk for participants with Vitamin D levels < 37nmol/L, OR 1.69 (1.26-2.26, 95% CI).

Welsh et al reported an association between Vitamin D deficiency and all cause mortality in a Scottish population but found no association with CVD related events (Welsh et al., 2012). Participants were involved in the MIDSPAN Family Study, and were offspring of people who were involved in a previous study. The total number of participants included was 2,081 with a mean age of 45 years and a median Vitamin D level of 46.4nmol/L. In fully adjusted models there was no association found between CVD deaths and Vitamin D deficiency, HR 1.07 (0.94-1.23 95% CI). However low serum 25(OH)D levels were found to be associated with all cause mortality in fully adjusted models, HR 2.02 (1.17-3.51, 95%CI).

In a study of 182,152 Israeli participants recruited through a national healthcare/insurance company an inverse relationship between Vitamin D and all cause mortality was found (Saliba et al., 2012). The mean Vitamin D level in those that died was 44.8nmol/L versus 51nmol/L in those who were still alive. Median follow up period was 1.4 years during which time there were 7,247 deaths. The increase in mortality was only noted in the groups with Vitamin D (25(OH)D) levels <50nmol/L with no mortality disadvantage seen in those with levels greater than this.

A subset of 2,878 men from the MrOS study was followed for mean of 6 years to assess for an association between Vitamin D and mortality (Johansson et al., 2012). They ranged in age from 70-81 years with a baseline mean Vitamin D level of 66.9nmol/L. At three-year follow-up, baseline Vitamin D levels below 60nmol/L were strong predictors of mortality but weakened this association significantly at 6 years.

Matthews et al looked at the association between Vitamin D and adverse outcomes in a surgical Intensive Care Unit and mortality (Matthews et al., 2012). A prospective
observational study of 258 patients admitted over a 16 month period, 91% had Vitamin D levels <65nmol/L and 54% had levels <33nmol/L on admission to the ICU. All participants were given 50,000IU Vitamin D per week once they could tolerate nutritional support. In terms of mortality an inverse relationship was noted between Vitamin D levels and mortality, with levels <65nmol/L associated with increased mortality rates.

Michos et al reported on Vitamin D deficiency’s association with fatal stoke in whites and blacks in the NHANES III study (Michos et al., 2012). 7,981 participants were followed over a median of 14.1 years. The mean age of participants was 48.4 years with a mean Vitamin D level of 75.8nmol/L in whites and 47.7nmol/L in blacks. There were 176 cases of fatal strokes, 116 whites and 60 blacks. In those deficient in Vitamin D (<37.5nmol/L) there was increased risk of fatal stroke in whites HR 2.13 (1.01-4.50, 95% CI) but not in blacks, HR 0.93 (0.49-1.80, 95% CI).

Tromson et al reported on a study of 65,409 participants of the Whitehall study and also reported on two meta-analyses. The first included twelve prospective studies looking at vascular deaths and the second included eighteen prospective studies relating to all cause mortality (Tomson et al., 2012). In the Whitehall study, the mean age of participants at baseline was 76.9 years with a mean Vitamin D of 56nmol/L. Participants were followed for a mean of 13 years, over which time there were 3,215 deaths (59.5%). Vitamin D was found to be associated with cause specific and all-cause mortality, HR 0.79 (0.72-0.85, 95% CI). The meta-analyses showed consistent trends for association between all cause and vascular related mortality, HR 0.79 (0.72-0.87, 95% CI).

A longitudinal study from the NHANES data looked at the relationship between Vitamin D, frailty and mortality (Smit et al., 2012). Of the participants included, the mean age was 71.7 years and mean Vitamin D level of 65.9nmol/L. In longitudinal analysis with a median follow up time of 12.6 years, those participants in the lowest quartile for Vitamin D (<49.5nmol/L) had increased risk of mortality, HR 1.27 (1.09-1.47, 95%CI). In the lowest Vitamin D quartile there was increased mortality risk, HR 2.98 (2.01-4.42, 95% CI) compared with those who were not frail HR 1.25 (0.97-1.60, 95% CI) in those with lowest
levels of Vitamin D and HR 1.11 (0.88-1.40, 95% CI) for those with Vitamin D levels ranging between 66.5-84.1nmol/L.

Pilz et al reported an association between Vitamin D and mortality in a population of female NH residents aged over 70 years in Austria (Pilz et al., 2012). 963 participants were included in this analysis with mean age of 83.7 years with a median Vitamin D level of 17.5nmol/L. 284 deaths were recorded over the mean follow-up time of 27 months. In multivariate analysis, Vitamin D deficiency (<14nmol/L) was associated with mortality when compared with higher levels, HR 1.56 (1.01-2.40, 95% CI).

Durup et al report a reverse J shaped association between 25(OH)D levels and all cause mortality in a Danish population (Durup et al., 2012). Subjects were recruited from the Copenhagen General Practitioner Laboratory Database. 247,574 subjects with 25(OH)D levels taken between 2004 and 2010 were included in the analysis. These included children from birth, 54.4% of the study population had levels <50nmol/L. Mortality data was retrieved from the Civil Registration Database. Results found that both low (<10nmol/L) with a HR 2.13 and high levels (>140nmol/L) with a HR 1.42 of Vitamin D were associated with increased risk of all cause mortality. The lowest morbidity rates were found in those with Vitamin D levels between 50-60nmol/L.

An Argentinean study investigated the relationship between 25(OH)D and all cause and cardiac related mortality in a female chest pain/suspected Acute Coronary Syndrome (ACS) population with 5-year follow-up (Naesgaard et al., 2012). A total of 982 patients were included, mean age was 62.2 years (males 59.7 years, females 65.9 years). As well as being older, women were more deficient in Vitamin D. At 5-year follow-up period 173 participants (17.6%) had died. Statistical analysis in the female population with multivariate regression models revealed HR 0.16 (95% CI 0.06-0.42), compared with HR 0.70 (95% CI 0.36-1.34) in males. In ROC analysis, the AUC was 0.29 for mortality in females compared with 0.41 for males.
A further study from the LURIC population looked at the effects of optimal levels of Vitamin D on CVD mortality and all cause mortality in a population of people referred for coronary angiograms with metabolic syndrome, in a centre in southwest Germany (Thomas et al., 2012). 1,801 subjects were included in the analysis. The mean age was 63.4 years with 22.2% having severe Vitamin D deficiency (<25nmol/L). The median follow up time was 7.7 years and there were 462 deaths recorded from local registries. In fully adjusted models there was a dose dependant reduction in all cause mortality, HR 0.28 (0.14-0.55, 95% CI) in those with optimal Vitamin D status (>75nmol/L). For CVD specific mortality those with higher Vitamin D levels again had lower risk of death in fully adjusted model, HR 0.36 (0.17-0.76, 95 CI).

Skaaby et al assessed the prospective association between vitamin D and all cause mortality, cardiovascular disease (CVD) related (including ischaemic heart (IHD) and stroke disease) mortality (Skaaby et al., 2012a). The mean age of participants was 49.8 years and mean Vitamin D in the baseline groups varied from 48-61nmol/L. There were 633 all cause deaths and 478 deaths secondary to CVD. Authors found no association between Vitamin D and IHD but did note an inverse relationship with all cause mortality.

In a further study from this population, cause specific mortality in a subset of 9,146 participants was reported and found an association between Vitamin D and mortality of respiratory, endocrine, nutritional and digestive causes but not secondary to neoplasm and cardiovascular disease (Skaaby et al., 2012b).

Signorello et al assessed the association between Vitamin D and mortality in African Americans and Non-African Americans (Signorello et al., 2013). A study population of approximately 85,000 was followed prospectively for 8 years. 1,852 participant deaths occurred over one year after study enrolment and it was these deaths that were examined. Participants were included aged between 40 and 79 years and were followed for 8 years with a mean baseline Vitamin D level of 40.4nmol/L. Higher quartiles of Vitamin D were associated with decreasing risk of all cause mortality. This effect was somewhat stronger in the African American group.
An inverse relationship between 25(OH)D and all cause mortality was reported from a Danish study (Blicher et al., 2013). The population consisted of 5,147 patients admitted to a university hospital in Denmark. At baseline the mean age for men was 74.2 years and women was 78.5 years. Median Vitamin D levels for the population were 47nmol/L for females and 40nmol/L for males. Median follow-up time was 2.7 years during which time there were 1,689 deaths. Cox regression analysis showed decreased 25(OH)D concentrations were associated with increased mortality risk, HR 1.07 (1.05-1.10, 95% CI).

Sempos et al investigate the possibility of a reverse association between Vitamin D and all-cause mortality in the NHANES dataset (Sempos et al., 2013). Participants who were aged >17 years and underwent baseline assessment between 1988 and 1994, were followed up until 2006 for mortality data obtained from the National Death Index. The sample included 11,315 individuals who were alive and 3,784 presumed dead. Those who died were older at baseline, had lower 25(OH)D levels (mean 60nmol/L), were less educated and had higher rates of medication usage and higher rates of self reported medical problems. Analysis showed a J shaped association between 25(OH)D levels and mortality in the NHANES III participants. The J shaped association remained after deaths within 3 years of baseline assessments were excluded. The upswing on the J shaped curve on fully adjusted models appeared evident at 25(OH)D levels <40nmol/L and >120nmol/L.

Results of both cross-sectional and longitudinal analysis from The Health in Men Study (HIMS) were reported looking at the effects of 25(OH)D deficiency on all cause mortality in men aged 70-88 years (Wong et al., 2013). A total of 4,203 men had complete data available including 25(OH)D levels. Frailty was scored using the FRAIL score and parameters were obtained from self-reported questionnaires. Mortality data was obtained from the Western Australian Data Linkage System (WADLS). Mean follow-up time for mortality data was 6.7 years, during which 1,144 men died, 322 who were frail and 822 were non-frail. Those who died had lower concentrations of 25(OH)D, 66.2 versus 69.1nmol/L, p<0.001. In adjusted Cox regression models there was increased risk of all cause mortality with lower Vitamin D levels, HR 1.20 (1.02-1.42, 95% CI).
Rohrmann et al reported a twenty-year follow-up study from the Swiss MONICA study assessing the relationship between Vitamin D and mortality in 3,181 participants (Rohrmann et al., 2013). At baseline participants had a mean age of 47.1 years and mean follow-up time was 18 years and 459 (14.4%) participants died. Results showed a reverse association between Vitamin D levels and all cause mortality, comparing those with 25(OH)D levels <25nmol/L versus those with levels >100nmol/L, HR 0.46 (0.24-0.88, 95% CI). When all-cause mortality was evaluated with 25nmol/L increase in 25(OH)D, there was only a statistically significant association in men, HR 0.79 (0.68-.091, 95% CI). In women a reverse association was noted in CVD mortality HR 0.68 (0.46-1.00, 95% CI) and in men it was associated with cancer mortality HR 0.72 (0.57-0.91, 95% CI).

A German cohort study from the ESTHER population also looked at 25(OH)D concentrations and all cause and cause specific mortality (Schottker et al., 2013b). 25(OH)D levels were taken at baseline and at 5 years. 9,578 participants were included in the current analysis. The mean age of participants was 62 years, mean Vitamin D level was 43.8nmol/L and median follow-up time was 9.5 years, during which time there were 1,083 deaths. At 5 year follow-up 511 study participants had died. There was an inverse relationship between all cause mortality and Vitamin D deficiency, HR 1.68 (1.41-2.01, 95% CI) in fully adjusted models in those with Vitamin D concentrations <30nmol/L. In analysis for cause specific mortality, there was also an association between Vitamin D deficiency and CVD, HR 1.29 (0.94-1.76, 95% CI) and cancer mortality HR 1.42 (1.08-1.87, 95% CI).

A study from NHANES looked at Vitamin D and all cause and cardiovascular mortality (Amer and Qayyum, 2013). The study sample included 10,170 participants with a mean age of 46.6 years and median Vitamin D of 52nmol/L. There were higher rates of mortality in those with lower Vitamin D levels, 5.7% versus 4.8% (p<0.05). Those with lower levels had higher rates of obesity, HTN, and elevated glucose levels. There were 509 cases of all cause mortality in the follow-up period. Multivariate analysis showed an inverse association between Vitamin D and mortality up to levels of 52nmol/L but not above this
level, HR 0.54 (0.35-0.84, 95% CI) versus HR 0.83 (0.63-1.11, 95% CI) respectively. A non-linear association was found between Vitamin D and all cause mortality.

Skaaby et al investigated the effect liver damage had on 25(OH)D and mortality (Skaaby et al., 2013). Participants were from the Monica 1 study, a general population based study in Denmark. 2,649 participants were included with 736 deaths in the median follow-up time of 17.0 years. In adjusted models those with the lower 25(OH)D levels had higher risk of mortality, HR 0.80 (0.64-0.99, 95% CI), compared with those in the higher Vitamin D quartile, HR 0.73 (0.57-0.93, 95% CI). When those with deranged liver function were excluded from the analysis, there was no attenuation in the association between Vitamin D and mortality. Thus this study showed no relationship between liver dysfunction (marked by deranged LFTs) and the association between 25(OH)D and all-cause mortality.

A further prospective study looking at the association of Vitamin D and critical care outcomes was reported (Aygenel et al., 2013). Of the 334 patients admitted to the Medical ICU in Turkey during the 16-month study period, 201 were included in the study. Participants were divided based on Vitamin D status, >50nmol/L v <50nmol/L. The mean age of participants was 66 years with a mean Vitamin D level of 37.2nmol/L. Patients were classified into two groups; insufficient (<50nmol/L) and sufficient (>50nmol/L). Baseline characteristics were similar in the two groups aside from higher APACHE II and SOFA scores in the Vitamin D insufficient group and higher albumin and calcium levels in the sufficient, lower rates of sepsis/septic shock, mortality, and fewer rates of mechanical ventilation. There were higher numbers of non-survivors in the Vitamin D insufficient group (p=0.027).

Schottker et al report a cross sectional study and longitudinal study looking at the possible association between Vitamin D deficiency and frailty and mortality in the ESTHER study (Schottker et al., 2014b). The study population included 9,949 men and women recruited by GPs from 2000-2002, were followed at 2, 5 and 8 years. Serum 25(OH)D levels were taken at baseline and at year eight. Participants were asked to rate their general health status, which was reported as Self Reported Health (SRH). Also Frailty indexes (FI) were
calculated based on self-reported elements of diseases, disease markers and disability and cut off points for continuous FI were calculated based on a subpopulation at 8 year follow up, where a home visit was carried out and frailty was calculated using a modified Fried criterion. The mean age of the 9,579 participants at baseline was 62.2 years, 15.1% of participants had a 25(OH)D level of <30nmol/L (deficient). In cross-sectional analysis, Vitamin D deficiency was found to be associated with poorer SRH, HR 1.7 (1.2-2.5, 95% CI) and also frailty status, HR 1.9 (1.3-2.78, 95% CI). In longitudinal analysis, Vitamin D deficiency was found to be associated with increased risk of all cause mortality, HR 1.5 (1.3-10.8, 95% CI).

An Australian study from 2014 looked at the relationship between 25(OH)D levels, in 1,659 men, and all cause mortality and disability (Hirani et al., 2014). Mortality data was obtained from registers of birth and deaths and marriages. The mean age of participants was 77 years and the mean 25(OH)D level was 55.9nmol/L. In the follow up period there were 355 deaths. Mean Grip Strength and Dynamic Balance scores were lower in 25(OH)D levels <50nmol/L. Low serum levels of Vitamin D were associated with all cause mortality on fully adjusted models, HR 1.40 (1.04-1.89, 95%CI), and no association with Vitamin D levels > 50nmol/L, HR 0.89 (0.65-1.22, 95% CI).

Lee et al looked at the relationship between Vitamin D and mortality in 2,816 older community dwelling men (Lee et al., 2014). Participants were followed for a median of 4.3 years and 187 died. Those who died were older (69.3 versus 59.2 years), had lower 25(OH)D levels (48.3 versus 64.3nmol/L) and had lower scores in terms of physical function and activity. In fully adjusted regression models, lower Vitamin D levels were found to be associated with all cause mortality, HR 2.37 (1.33-4.24, 95% CI). No association was found between 25(OH)D and cancer and cardiovascular related mortality.

Joshi et al investigated the link between Vitamin D deficiency and mortality in critically ill ICU patients in India (Joshi et al., 2014). Eighty five consecutive patients were admitted, who were able to provide consent were recruited. The mean age of participants was 42.5 years with a mean Vitamin D level of 103.6nmol/L, 52% of participants died. Vitamin D
deficiency/insufficiency (n=20/27) was found to be associated with higher rates of mortality than those with sufficient levels (n=24/58) p=0.004. Logistic regression revealed a significant association between Vitamin D and morality, OR 4.13 (1.6-11.1, 95% CI).

A study from Israel evaluated the effect of body weight on the association between mortality and Vitamin D status (Saliba et al., 2014). Information was derived from the Clalit Heath Service database, which derives information from a number of sources including primary care, hospitalisation, communities and pharmacies. 175,781 participants were included, mean age was 60.6 years and mean Vitamin D levels was 54.6mmol/L in those with a BMI <25 and 45nmol/L in those with a BMI of >30kg/m2. The median follow-up time was 48 months during which time 12,337 (7%) died. Vitamin D was associated with all cause mortality, HR 0.91 (0.90-0.92, 95% CI) for every 10nmol/L increase in Vitamin D, a non-linear relationship.

Khaw et al evaluated the relationship between Vitamin D and mortality in a UK population (Khaw et al., 2014). 25,639 participants were recruited from GP registers including both men and women aged between 40-79 years between 1993-1997 and 15,000 were followed for approximately 13 years. Of these, 14,641 with 25(OH)D samples available were included. The mean age of participants was approximately 62 years and the Vitamin D level was 56.6nmol/L. In fully adjusted models, those with higher Vitamin D levels had lower risk of all cause mortality compared with those with levels <30nmol/L, HR 0.73 (0.59-0.90, 95% CI). Also with a 20nmol/L increase in serum Vitamin D level there was an 8% reduction in mortality rate, in fully adjusted models, HR 0.92 (0.88-0.96, 95% CI).

Samefors et al performed a study assessing the association between Vitamin D and mortality in Swedish nursing home residents, as part of the SHADES study (Samefors et al., 2014), which included 333 subjects. Vitamin D levels were categorized into quartiles. The majority of participants were women (68%), mean age of participants was 84.5 years. Participants were followed for 3 years, during the study period 44% of the participants died. The Vitamin D levels in those who died were lower at inclusion than those who
survived 37.6nmol/L versus 42.2nmol/L p<0.001. After the 3 years, 59% of participants in the lowest Vitamin D quartile had died compared with 28% of those in the highest quartile.

Mursu et al evaluated the link between Vitamin D and mortality rates in Finnish men (Mursu et al., 2015). Participants were recruited as part of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), which was originally designed to evaluate risk factors for cardiovascular disease; the current study included 1892 men. Deaths were recorded using the Finnish Death Register. The mean age of participants was 52.5 years and the mean Vitamin D level was 43.5nmol/L. The mean follow-up time was 22.2 years and 670 men had died related to disease (accidents and suicides were excluded). Those in the lowest versus higher Vitamin D tertiles had increased mortality risk on fully adjusted models, HR 1.23 (1.01-1.51, 95% CI) p=0.048.

In Germany a study looking at 25(OH)D and frailty and all-cause mortality in the KORA Age Study was completed (Vogt et al., 2015). Of the original participants who took part in the baseline assessment in 2009, 822 participants were re-examined in 2012. Mortality data was obtained using death certificate from local health authorities. The mean age of participants was 75.5 years and the mean 25(OH)D level was 46.7nmol/L. The mean follow-up time was 2.9 years. In fully adjusted models, Vitamin D levels <30nmol/L were found to be associated with increased mortality, OR 3.86 (1.22-12.29, 95% CI).

Holter et al investigated the relationship between 25(OH)D and all-cause mortality of patients admitted to an acute hospital in Norway with Community Acquired Pneumonia (CAP). (Holter et al., 2016) Patients treated with alfacalcidiol and calcitrol were excluded from this analysis. There were 241 adults included with a mean age of 66 years and mean Vitamin D level of 37.4nmol/L. Over the median follow-up time of 5 years, 72 participants (29.9%) died. In basic models Vitamin D deficiency (<30nmol/L) and inadequacy (30-49nmol/L) were not statistically significantly associated with increased risk of all-cause mortality. However when the model included season, Vitamin D supplementation, COPD history and immunocompromise, Vitamin D deficiency was associated with all cause
mortality, HR 1.91 (1.06-3.45, 95% CI) p=0.031, there was no association with Vitamin D inadequacy.

A longitudinal study from a young Norwegian population investigated the association between Vitamin D and all cause and cause specific mortality in men with prostate cancer, without prostate cancer and for the total population (Meyer et al., 2016). 2,259 participants had prostate cancer with a matched control group of 2,120 without. Mean follow-up from time of cancer diagnosis was 4.7 years. Mean age of participants was 47.4 years and mean Vitamin D level was 61.6nmol/L. Over the study period, 27% of the men with prostate cancer died and 12.6% of the controls died. There was increased risk of all cause mortality in those with prostate cancer, HR 1.43 (0.93-2.22, 95% CI) but no association in those without prostate cancer, HR 0.90 (0.421, 90, 95% CI). For the total population there was an inverse relationship between Vitamin D and all cause mortality, HR 1.19 (1.03-1.38, 95% CI).

A further prospective study from 2016 showed an inverse association between Vitamin D concentrations and all cause mortality in a cohort of elderly women from the OPRA Study (Buchebner et al., 2016). 715 attended for five-year follow-up (aged 80 years) and 382 attended for 10 year follow up (aged 85 years). Vitamin D was measured at each time point and defined as low: <50nmol/L, intermediate: 50-75nmol/L and high: >75nmol/L. The mean age of participants at baseline was 75.2 years with mean 25(OH)D level of 63nmol/L. Based on 25(OH)D concentration at baseline, Vitamin D was not associated with mortality risk over the five year follow-up. Over the ten-year period, low Vitamin D levels were associated with increased risk of mortality in fully adjusted models, HR 1.4 (1.0-1.9,95% CI). Based on Vitamin D levels taken at second assessment (aged 80years) there was increased risk of five-year mortality with lower Vitamin D levels, HR 2.1 (1.2-3.6, 95% CI). Ten-year mortality risk based on this measurement was also associated inversely associated with Vitamin D level, HR 1.9 (1.2-2.6, 95% CI). At final measurement of Vitamin D, aged 85 years, the association remained, with increased risk of mortality associated with lower 25(OH)D concentration, HR 2.0 (1.1-3.7, 95% CI).
A further analysis from the NHANES III population confirms an inverse relationship between Vitamin D and all cause mortality (Daraghmeh et al., 2016). A total of 10,577 participants were included with a mean age of 54 years, and 25% had Vitamin D levels <44nmol/L. Information on deaths was obtained from the National Death Index. Results were presented based on 25(OH)D quartiles. For all cause mortality there was decreased mortality risk with higher levels of Vitamin D, HR 0.74 (0.57-0.96, 95%CI) p=0.015. For cause specific mortality, there was an inverse association with decreased Coronary Heart Disease (CHD) mortality risk and higher Vitamin D levels in unadjusted models, HR 0.71 (0.57-0.88, 95% CI) p=0.012 but lost statistical significance in fully adjusted models.

A recent study from Norway evaluated the association between Vitamin D quartiles and all-cause mortality in a community dwelling population (n=6,277) after a median of 18.5 years follow-up (Sun et al., 2017). This study showed that over the follow-up period, 24.1% of participants died. The median level of Vitamin D in the total population was 47.3nmol/L. Cox regression analysis revealed a non-linear association between Vitamin D levels and all-cause mortality, with an increasing death rate in levels <35nmol/L. With Vitamin D considered as quartiles, those in the first quartile (34.5nmol/) had increased risk of mortality compared with those in the fourth quartile (>58.2nmol/L), HR 1.24 (1.12-1.72, 95% CI).

A small study from India in 2017 looked at the relationship between Vitamin D and outcomes in ICU patients with sepsis (Vipul et al., 2017). There were a total of 88 participants included, who were admitted in the Emergency Department or Intensive Care Unit with sepsis. The mean age of participants was 45 years, with a mean serum 25(OH)D level of 40.8nmol/L. Vitamin D levels were higher in those who did well compared with those who died in the study, t=2.1, p=0.04. Vitamin D deficiency was associated with increased length of stay, lower discharge rates and higher mortality compared with those participants with sufficient levels.
Negative Studies
In 2006, Visser et al examined the association of Vitamin D and Nursing Home (NH) admission and mortality in the LASA population (Visser et al., 2006). 30.2% of the 1,260 study participants died over the 6-year follow-up period. 46.8% of the population had Vitamin D levels <50nmol/L. There was an inverse relationship between Vitamin D deficiency and NH admission in fully adjusted models, HR 3.48 (1.39-8.75, 95%CI). Vitamin D deficiency was associated with higher mortality risk in unadjusted models but after adjustment for frailty measures of mobility performance and serum albumin levels, the association lost statistical significance.

From the MrOS study 1,490 community dwelling US men with a mean age of 73.7 years and 25.2% of participants with Vitamin D levels <50nmol/L were followed (Cawthon et al., 2010). Over the mean 7.3 years of follow-up, 330 participants (22.2%) died. This study found no association between Vitamin D deficiency and mortality, HR 0.95 (0.68-1.34, 95% CI) for Vitamin D levels <50nmol/L and all cause mortality.

A further study from NHANES assessed the association of Vitamin D and all cause mortality (Ford et al., 2011). The study consisted of 7,531 participants with 347 deaths. The mean age of participants was 45.8 years and the mean Vitamin D was 60.6nmol/L and participants were followed for a mean period of 3.8 years. The mean Vitamin D level was 54.1nmol/L in those who died versus 60.7nmol/L who survived. In survivors, Vitamin D was not significantly associated with mortality, HR 1.28, (0.86-1.90, 95% CI).

In a US study in 2011, a post hoc analysis of data from three nested case control studies from the WHI study was reported (Eaton et al., 2011). This cohort consisted of 2,429 post-menopausal women with a mean age of 65.8 years. Mean follow up time was 10.5 years and the mortality rate was 9.2% (224 deaths). Vitamin D was reported as quartiles. Multivariate analysis revealed that Vitamin D deficiency (<50nmol/L) was not associated with mortality in this group, HR 1.25 (0.8-1.95, 95% CI).
In a study of 130 severely ill ICU patients requiring mechanical ventilation the role of Vitamin D in mortality rates was evaluated (Arnson et al., 2012). Those with Vitamin D supplementation prior to admission were excluded. 57 deaths occurred over a 60-day follow-up period. Mean age was 70.2 years and mean Vitamin D was 37.2nmol/L. A longer average survival was noted in Vitamin D sufficient versus deficient groups (16 vs 9.8 days) but no significant difference between groups in terms of mortality.

A prospective study in China reported no association between Vitamin D concentration and mortality or cause specific mortality (Lin et al., 2012). This study included 1,101 participants, recruited as part of the General Population Trial of Linxium, with a mean age of 56.5 years and they were followed over a 24-year period. 73% of participants had a Vitamin D level of <50nmol/L. 793 participants (72%) died in the follow up period. Adjusted HR for the association between continuous Vitamin D levels and death showed no significant differences, in the overall group of 793 deaths, HR 1.01, (0.97-1.05, 95% CI). Models were adjusted for age, gender, hypertension, smoking, BMI and alcohol consumption.

Flynn et al reported on the effects of Vitamin D deficiency in patients admitted to a surgical ICU in the USA. 66 patients were recruited consecutively, inclusion criteria included ICU stay >48 hours and having a Vitamin D level checked on admission and every 7 days during their hospital stay (Flynn et al., 2012). Mean age of participants was 56 years with a mean APACHE score of 17, 74% of the study population had levels <50nmol/L. The overall in hospital mortality rate was 7% (5/66) and this was not associated with Vitamin D levels.

Participants of the CaMos study were followed over a 10-year period and authors report an association between Vitamin D and mortality in this population (Langsetmo et al., 2013). Data was taken at baseline and at 5 years relating to supplement use and dietary Vitamin D and Calcium intake. The mean age of men was 60.3 years and women 63.5 years. Information was presented in terms of Vitamin D intake and divided into low (<800IU), moderate (800-1,200IU) and high (>1,200IU). Vitamin D levels were not presented and
therefore compliance with stated intake cannot be assured. Overall results were not impressive, although in women supplementation was associated with lower risk of mortality with HR 0.84 (0.71-0.99, 95% CI) but was not maintained at higher doses, HR 0.91 (0.62-1.32, 95% CI).

A Spanish study looked at the relationship between Vitamin D and CVD and all cause mortality in 312 community dwelling adults aged 85 years at the time of initial recruitment (Formiga et al., 2014). Participants were followed for a median of 2.8 years. The mean serum 25(OH)D level was 30.5nmol/L. Over the follow-up period 58 (18.5%) of the participants died with 8% of deaths attributed to CVD. No relationship was found between Vitamin D and mortality or CVD related mortality.

Puhan et al investigated the relationship between Vitamin D deficiency and exacerbations of COPD and all cause mortality in 356 patients (Puhan et al., 2014). Participants were recruited from primary care settings in Switzerland. Those aged >40 years with a confirmed diagnosis of COPD and free from exacerbations within the last 4 weeks and with a life expectancy of >12 months were included. Participants had a mean age of 67.2 years and a mean Vitamin D level was 38.7nmol/L and 9.6% of participants died within the 2 year follow-up. Although Vitamin D levels were lower in those who died (39.4nmol/L versus 32.5nmol/L) there was no statistically significant association between Vitamin D and mortality in regression models.

Granic et al reported no association between Vitamin D and older adults from the Newcastle 85+ study (Granic et al., 2015a). Data was available for 845 individuals. Follow up period was approximately six years and in that time, 47.7% of participants died. The mean survival time was 4.3 years. In unadjusted models an association between Vitamin D status and survival was noted ($\chi^2 = 7.9, \ p=0.02$). In models adjusted for sociodemographic variables a U shaped relationship was noted between 25(OH)D and survival, with increased mortality in lowest quartile of Vitamin D (5-17nmol/L), HR 1.31 (1.01-1.69, 95% CI) and in the highest season specific quartile of Vitamin D, HR 1.44 (1.12-1.85, 95% CI). In further models adjusted for lifestyle factors, only those in the highest quartile of Vitamin D
 (>69nmol/L) had increased mortality rate HR 1.37 (1.06-1.77, 95% CI). When models were fully adjusted to include mood and cognition, there was no significant relationship noted, HR 1.25 (0.97-1.63, 95% CI).

Lee et al did not show a relationship between Vitamin D and mortality in patients with hip fracture (Lee et al., 2015). Participants (n=489) aged >50 years with hip fracture were recruited and followed for at least twelve months with a mean age of 78.9 years and mean Vitamin D level of 39.2nmol/L. During the minimum follow-up period of twelve months, 23.3% had died. In multivariate models, no association between Vitamin D and 90-day mortality was found.

Guo et al report a negative longitudinal association between Vitamin D intake and all-cause mortality in 452 men over a 20-year follow-up period (Guo et al., 2017). This population was originally recruited as part of the CAOS study investigating cardiovascular (CVD) risk factors in men. There were 281 deaths over the 20 years follow-up but no association between Vitamin D intake and all cause mortality was found though the confidence intervals were quite wide due to small sample size, OR 0.89 (0.49-1.62, 95% CI).
<table>
<thead>
<tr>
<th>Author Year</th>
<th>Country</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age (years)</th>
<th>Mean f/u</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Confounders</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Kuroda et al 2008 Japan</td>
<td></td>
<td>12,321</td>
<td>107 Deaths (8.7%)</td>
<td>63.9</td>
<td>6.9 years</td>
<td>Post-menopausal</td>
<td>Critical illness, bed bound</td>
<td>45% levels &lt;50nmol/L</td>
<td>Age, Smoking, Alcohol, BMD, CVD, Dementia, Malignancy</td>
<td>Vitamin D deficiency appeared to be associated with mortality</td>
</tr>
<tr>
<td>Dohling et al 2008 Germany LURIC</td>
<td></td>
<td>3,258</td>
<td>737 deaths (22.6%)</td>
<td>64.3</td>
<td>7.7 years (Median)</td>
<td>White race Referred for coronary angiography</td>
<td>Acute &amp; Chronic non-cardiac illness, Malignant neoplasm in past 3 years</td>
<td>50.2% levels &lt;42nmol/L</td>
<td>Age, Gender, BMI, Physical activity, DM, Smokers, Albumin, pro-BNP, BP, Cholesterol, ACE-I, B-Blockers, Statins, Aspirin</td>
<td>Inverse relationship between Vitamin D and all cause mortality</td>
</tr>
<tr>
<td>Melamed et al 2008 USA NHANES III</td>
<td></td>
<td>13,331</td>
<td>1,806</td>
<td>44.8</td>
<td>9.7 years</td>
<td>&gt;20 years</td>
<td>Incomplete data</td>
<td>Not available (quartiles provided)</td>
<td>Age, Gender, Race, Season, CVD, DM, BMI, Cholesterol, Smoking, Physical activity, Vitamin D supplementation</td>
<td>Association between Vitamin D and all-cause mortality, non-significant association with cancer &amp; CVD mortality</td>
</tr>
<tr>
<td>Ginde et al 2009 USA NHANES III</td>
<td></td>
<td>3,408</td>
<td>1,493 deaths (43.8%)</td>
<td>73</td>
<td>7.3 years (Median)</td>
<td>Community Dwelling</td>
<td>&lt;65 years</td>
<td>66nmol/L (Median)</td>
<td>Age, Gender, BMI, Ethnicity, Income, Season, Physical activity, Smoking, Alcohol, Asthma, COPD, Renal, MI, HTN, function, DM,</td>
<td>Inverse association between Vitamin D deficiency and CVD and mortality</td>
</tr>
<tr>
<td>Zitterman et al 2009 Germany</td>
<td></td>
<td>510</td>
<td>82 deaths (16%)</td>
<td>53.5</td>
<td>1 year</td>
<td>End Stage Heart Failure waiting for cardiac transplant</td>
<td>&lt;18yrs, Cardiac transplant during follow-up</td>
<td>Calcitrol 29.0ng/L</td>
<td>Age, BMI, Aspirin, Smoking, Renal function, CRP, TNF-a, HTN, DM, CVD, End stage HF,</td>
<td>Calcitrol in lowest quintiles had 1 year mortality risk 3.9 times higher than those in the highest quintile</td>
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<td>Author Year</td>
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<tr>
<td>Semba et al 2009 USA WHAS</td>
<td>714</td>
<td>100 deaths (14%)</td>
<td>74</td>
<td>72 months</td>
<td>Community dwelling 70-79 years Post-menopausal women</td>
<td>Not available</td>
<td>50.9nmol/L</td>
<td>Age, Race, Education, Season, BMI, Smoking, Supplement use, Physical Activity, Cholesterol, Chronic Disease</td>
<td>Lowest quartile of Vitamin D associated with highest mortality risk</td>
<td></td>
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<tr>
<td>Szulc et al 2009 France MINOS</td>
<td>782</td>
<td>182 deaths (23.3%)</td>
<td>67</td>
<td>10 years</td>
<td>50-85 years</td>
<td>Not available</td>
<td>Survivors: 70.6nmol/L Deceased: 57.5nmol/L</td>
<td>Age, Leisure and Physical activity, DM, HTN, IHD, Parkinson’s, BMI, Smoking, Alcohol,</td>
<td>Higher mortality in lowest quintile of Vitamin D</td>
<td></td>
</tr>
<tr>
<td>Semba et al 2010 Italy InChianti</td>
<td>1,006</td>
<td>228 deaths (22.7%)</td>
<td>74</td>
<td>6.5 years</td>
<td>&gt;65 years</td>
<td>Not available</td>
<td>Not available (Quartiles)</td>
<td>Age, Gender, Education, Season, BMI, Smoking, Aspirin, Physical Activity, Cholesterol, MMSE, Chronic Disease</td>
<td>Higher rates of all cause mortality in those with lower Vitamin D levels &amp; lower quartiles associated with higher CVD mortality risk</td>
<td></td>
</tr>
<tr>
<td>Michaelsson et al 2010 Sweden ULSAM</td>
<td>1,194</td>
<td>584 deaths (49%)</td>
<td>71</td>
<td>12.7 years (Median)</td>
<td>&gt;50 years</td>
<td>Cancer and/or CVD at baseline</td>
<td>Not available (Percentiles)</td>
<td>Age, Weight Height, Calcium intake, DM, Alcohol, Smoking, Season, Social class, Physical activity, PTH, Calcium, PO4, BP, CRP, Cholesterol,</td>
<td>Low concentrations of Vitamin D associated with higher risks of death due to cancer and CVD. High concentrations (&gt;95th percentile) are associated with higher cancer mortality risk</td>
<td></td>
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<tr>
<td>Anderson et al 2010 USA</td>
<td>41,504</td>
<td>1,193 deaths (4.3%)</td>
<td>66.6</td>
<td>1.3 years</td>
<td>Healthcare database with one Vitamin D test</td>
<td>Not available</td>
<td>Not available 17% had Vitamin D &lt;37.5nmol/L</td>
<td>Age, Gender, DM, HTN, PVD, Hyperlipidaemia</td>
<td>Inverse relationship with Vitamin D and outcomes including MI, HF, stroke, PVD and mortality</td>
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<tr>
<td>Hutchinson et al 2010 Norway Tromso</td>
<td>7,161</td>
<td>1,359 deaths (18.9%)</td>
<td>58.9</td>
<td>11.6</td>
<td>&gt;25 years</td>
<td>Not available</td>
<td>52.3nmol/L</td>
<td>Age, Gender, Physical activity, BMI, DM, HTN, Prior CVD and Cancer</td>
<td>Lower Vitamin D levels associated with all cause mortality in non-smokers and lesser degree in smokers</td>
<td></td>
</tr>
<tr>
<td>Braun et al 2011 USA</td>
<td>2,399</td>
<td>6.7% 30day 23.2% 90day 32.3% 365day</td>
<td>64.9</td>
<td>11</td>
<td>&gt;18 years ICU admission</td>
<td>&lt;18yrs No Vitamin D level Vitamin D supplements post blood test</td>
<td>65.9nmol/L</td>
<td>Age, Gender, Race, Deyo-Clarkson index, Season, Sepsis</td>
<td>Vitamin D deficiency associated with mortality at 30, 90 &amp; 365 days</td>
<td></td>
</tr>
<tr>
<td>Virtanen et al 2011</td>
<td>1,136</td>
<td>87 deaths</td>
<td>61.8</td>
<td>9.1</td>
<td>Men: 42-60 years, Women: 53-73 years</td>
<td>IHD, Stroke, Cancer at baseline</td>
<td>43.7nmol/L</td>
<td>Age, Gender, DM, Month and year, HTN, BMI, Education, Lipids</td>
<td>Low Vitamin D levels associated with increased mortality risk</td>
<td></td>
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<tr>
<td>Skaaby et al 2012 Denmark Monica10 Inter99</td>
<td>8,329</td>
<td>Total: 8,131 IHD: 478 All cause: 633</td>
<td>49.8</td>
<td></td>
<td>All cause mortality 10.2 years, CVD: 10.2 years</td>
<td>Community dwelling</td>
<td>61nmol/L &amp; 48nmolL (Median)</td>
<td>Age, Gender, Education, Season, Study group, Fish intake, Physical activity, Smoking, Alcohol, BMI</td>
<td>Inverse relationship between Vitamin D and all cause mortality No association with IHD mortality</td>
<td></td>
</tr>
<tr>
<td>Skaaby et al 2012 Denmark Monica10 Inter99</td>
<td>9,146</td>
<td>830 deaths (9.7%) 49.8</td>
<td></td>
<td></td>
<td>All cause mortality 10.2 years, CVD: 10 years</td>
<td>Community dwelling</td>
<td>61nmol/L &amp; 48nmolL (Median)</td>
<td>As above</td>
<td>Association between Vitamin D and mortality due to respiratory, endocrine, nutritional and digestive causes No association with neoplastic and CVD mortality</td>
<td></td>
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<tr>
<td>Author Year</td>
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<tr>
<td>Saliba et al 2012</td>
<td>182,152</td>
<td>7,247 deaths (4%)</td>
<td>60.4</td>
<td>2.4 years &gt; 20 years</td>
<td>Vitamin D level available</td>
<td>&lt;20yrs</td>
<td>Age, Gender, CVD, Ethnicity, Season, Vitamin D supplement, Socio-economic status</td>
<td>Inverse relationship between Vitamin D and all cause mortality</td>
<td></td>
<td></td>
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<tr>
<td>Johansson et al 2012</td>
<td>2,878</td>
<td>577 deaths (20%)</td>
<td>75.4</td>
<td>6 years 70-81 years</td>
<td>Unable to give informed consent or mobilise unaided</td>
<td>66.9nmol/L</td>
<td>Age, hip BMD, Cancer, Angina, Diabetes, Physical activity, Medications, General health</td>
<td>3 years: low Vitamin D was a strong predictor of mortality 6 years: low Vitamin D weaker at predicting mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matthews et al 2012</td>
<td>258</td>
<td>12.3% &lt;34.9nmol/L 11.5% in &lt;64.9nmol/L</td>
<td>45.9</td>
<td>15 months Consecutive admissions to a surgical ICU</td>
<td>None</td>
<td>53.5% &lt;32.5nmol/L 91.9% &lt;64.9nmol/L</td>
<td>Age Gender, MI, ARF, Pneumonia</td>
<td>Levels &lt;64.9nmol/L associated with increased mortality (No deaths in levels &gt;65nmol/L)</td>
<td></td>
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<tr>
<td>Michos et al 2012</td>
<td>7,981</td>
<td>176 fatal strokes (2%)</td>
<td>48.4</td>
<td>14.1 years &gt;30 years</td>
<td>Non-white &amp; non-black No Vitamin D Unable to provide information</td>
<td>Blacks 48.4nmol/L Whites 76.8nmol/L</td>
<td>Race, Age, Gender, Income, BMI, Education, Smoking, Alcohol, Physical activity, Season, CRP, Diabetes, HTN, Cholesterol</td>
<td>Increased risk of fatal stroke in Vitamin D deficient whites but not in blacks</td>
<td></td>
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</tr>
<tr>
<td>Tomson et al 2012</td>
<td>5,409</td>
<td>Whitehall 3,215 (59.4%) Whitehall 113 years</td>
<td>Whitehall 76.9 years</td>
<td>White males</td>
<td></td>
<td>56nmol/L (Median)</td>
<td>Age, Season, Prior disease, CVD risk factors, Self-reported daily activities</td>
<td>Whitehall: Vitamin D inversely associated with risk of vascular deaths and at levels of 40-90nmol/L with all cause mortality</td>
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<tr>
<td>Author Year</td>
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<td>Mean Age (years)</td>
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<td>Positive</td>
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<tr>
<td>Welsh et al 2012</td>
<td>2,081</td>
<td>100 deaths</td>
<td>45.1</td>
<td>14.4 years</td>
<td>30-59 years Offspring of a prior study cohort</td>
<td>Not within the local region, Died before commencement</td>
<td>46.4nmol/L (Median)</td>
<td>Age, Gender, DM, Season, Glucose, Smoking, BP, Cholesterol, CRP, Alcohol, Waist circumference, Creatinine, Meds, Education</td>
<td>Vitamin D associated with all case mortality, No association with CVD related mortality</td>
<td></td>
</tr>
<tr>
<td>Durup et al 2012</td>
<td>247,574</td>
<td>15,198 deaths</td>
<td>51.0</td>
<td>3.1 years</td>
<td>GP patients with serum 25(OH)D levels measured</td>
<td>Not available</td>
<td>54.4% had 25(OH)D levels &lt;50nmol/L</td>
<td>Age, Gender, PTH, Albumin, Calcium</td>
<td>J shaped association between Vitamin D and mortality</td>
<td></td>
</tr>
<tr>
<td>Næsgaard et al 2012</td>
<td>982</td>
<td>173</td>
<td>62.2</td>
<td>5 years</td>
<td>Hospitalised with chest pain/ACS</td>
<td>&lt;18 years</td>
<td>46.2nM</td>
<td>Age, Gender, Smoking, Smoking, HTN, DM, CHF, Angina, IHD, CABG, PCI</td>
<td>25(OH)D associated with increased mortality risk in females, but not in males</td>
<td></td>
</tr>
<tr>
<td>Smit et al 2012</td>
<td>4,731</td>
<td>Not available</td>
<td>71.7</td>
<td>12.6 years</td>
<td>&gt;60 years Complete frailty and Vitamin D data</td>
<td>Not available</td>
<td>65.9nmol/L</td>
<td>Age, Gender, Race, Education, BMI, Smoking, Latitude, Chronic diseases</td>
<td>Lowest quartile of Vitamin D levels was associated with increased mortality risk</td>
<td></td>
</tr>
<tr>
<td>Pilz et al 2012</td>
<td>961</td>
<td>284 deaths</td>
<td>83.7</td>
<td>2.3 years</td>
<td>&gt;70 years Female NH Residents Independently Mobile</td>
<td>Malignancy, Hypercalcaemia, CKD, Bilateral Hip Replacement Osteomalacia, Steroid use, CCF,</td>
<td>17.5nmol/L (Median)</td>
<td>Age, BMI, Albumin, CAD, Creatinine clearance, HTN, PTH, Calcium, Phosphate, Knee extensor strength, Mobility status</td>
<td>Association between Vitamin D and mortality in female NH residents</td>
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</tr>
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<td>Author</td>
<td>Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age</td>
<td>Mean f/u</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Thomas et al</td>
<td>2012</td>
<td>1,801</td>
<td>462 deaths</td>
<td>63.3</td>
<td>7.7 years (Median)</td>
<td>Caucasian</td>
<td>Acute illness (not ACS), Non-Cardiac</td>
<td>Not available</td>
<td>Age, Gender, Smoking Alcohol, Physical Activity, VMI, Waist, BP, DM, Cholesterol, CRP, CVD, Education</td>
<td>Association between Vitamin D and mortality</td>
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<tr>
<td>UK LURIC</td>
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<td></td>
<td>Chronic Disease, Malignant Neoplasm</td>
<td>65% levels &lt;50nmol/L</td>
<td></td>
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<tr>
<td>Schottker et al</td>
<td>2013</td>
<td>9,578</td>
<td>1,083 deaths (11.3%)</td>
<td>62</td>
<td>9.5 years (Median)</td>
<td>Men and women</td>
<td>Missing Data</td>
<td>43.8nmol/L</td>
<td>Age, Gender, Month, BMI, Multivitamin supplements, Education, BP, CKD, Physical activity, Smoking, CRP, Cholesterol, Diabetes, CVD, Hypertension, Cancer</td>
<td>Inverse association between Vitamin D and all-cause mortality</td>
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<tr>
<td>Germany ESTHER</td>
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<td>511 had died at 5 years</td>
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<td></td>
<td>Aged 50-74 years</td>
<td>Unable to follow-up mortality data</td>
<td></td>
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<tr>
<td>Wong et al</td>
<td>2013</td>
<td>4,203</td>
<td>1,144 deaths</td>
<td>76.7</td>
<td>6.7 years</td>
<td>None Stated</td>
<td>70-88 years Men</td>
<td>59.3nmol/L</td>
<td>Age, Education, Living Status, Smoking, Physical Activity, Vitamin Supplement, eGFR, Season</td>
<td>Increased risk of all-cause mortality with lower Vitamin D levels</td>
</tr>
<tr>
<td>Australia</td>
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<tr>
<td>Rohrmann et al</td>
<td>2013</td>
<td>3,191</td>
<td>459 deaths</td>
<td>47.1</td>
<td>18.4 years</td>
<td>Not available</td>
<td>No Vitamin D level</td>
<td>Not available (quartiles)</td>
<td>Age, Season, Nationality, Diet, BP, Smoking Status</td>
<td>Inverse relationship between Vitamin D and all-cause mortality.</td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
<td>Exclusion Criteria</td>
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<tr>
<td>Sempos et al</td>
<td>2013 USA</td>
<td>15,099</td>
<td>3,784 deaths</td>
<td>45</td>
<td>13.8 years</td>
<td>&gt;20 years</td>
<td>Missing data</td>
<td>Pregnant at baseline</td>
<td>Association between low Vitamin D levels and all-cause mortality</td>
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<tr>
<td>NHANES III</td>
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<tr>
<td>Signorello et al</td>
<td>2013 USA</td>
<td>85,000</td>
<td>1,852 deaths (2%)</td>
<td>Not available</td>
<td>8 years</td>
<td>40-79 years</td>
<td>Not treated for cancer in past 12mths Non English speaking</td>
<td>40.4nmol/L</td>
<td>BMI, Smoking, Income, Physical activity</td>
<td>Increasing risk of all cause mortality with decreasing quartiles of Vitamin D Association between Vitamin D and CVD mortality but not cancer mortality</td>
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<tr>
<td>SCCS Study</td>
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<tr>
<td>Blicher et al</td>
<td>2013 Denmark</td>
<td>5,147</td>
<td>1,689 deaths</td>
<td>76.7</td>
<td>2.7 years</td>
<td>20-90 years</td>
<td>Not available</td>
<td></td>
<td>43.5nmol/L</td>
<td>Not available</td>
</tr>
<tr>
<td>Aygencel et al</td>
<td>2013 Turkey</td>
<td>201</td>
<td>76 deaths (Median)</td>
<td>66</td>
<td>2 years</td>
<td>Medical ICU admission</td>
<td>&lt;18 years &lt;48hours in ICU Terminal cancer, Bone metabolism medications</td>
<td>37.2nmol/L</td>
<td>Not available</td>
<td>Vitamin D deficiency associated with mortality in ICU patients</td>
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<tr>
<td>Skaaby et al</td>
<td>2013 Denmark</td>
<td>2,649</td>
<td>736</td>
<td>55.4</td>
<td>17.0 years</td>
<td>Not available</td>
<td>Liver Disease, Alcohol Related Physical or Mental Illness</td>
<td>61.0nmol/L</td>
<td>Gender, Education, Season, Diet, BMI, Physical Activity, Smoking, Alcohol</td>
<td>Relationship between Vitamin D and mortality independent of liver enzyme levels</td>
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<tr>
<td>Monica 1</td>
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103
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age (years)</th>
<th>Mean f/u</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Confounders</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Amer &amp; Qayyum</td>
<td>2013</td>
<td>USA NHANES</td>
<td>10,170</td>
<td>509</td>
<td>46.6</td>
<td>3.8 years (Median)</td>
<td>&gt;18 years</td>
<td>51.8nmol/L</td>
<td>Age, Gender, Race, HTN, Smoking, CRP, Obesity, Cholesterol, Serum Glucose, Renal function</td>
<td>Inverse relationship between all cause and CVD related mortality</td>
</tr>
<tr>
<td>Khaw et al</td>
<td>2014</td>
<td>UK</td>
<td>14,641</td>
<td>2,776 deaths</td>
<td>62.1</td>
<td>13 years</td>
<td>42-82 years</td>
<td>56.6nmol/L</td>
<td>Age, Gender, Month, BMI, Social class, Physical activity, Alcohol, CVD, Cancer, DM</td>
<td>Inverse association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Joshi et al</td>
<td>2014</td>
<td>India</td>
<td>85</td>
<td>44 deaths (52%)</td>
<td>42.4</td>
<td>Not available</td>
<td>Patients admitted to critical care unit</td>
<td>103.6nmol/L</td>
<td>Age, Gender, Chronic disease, GCS, Blood sugar, Creatinine, Albumin, SAPS II score, Cortisol</td>
<td>Vitamin D deficiency associated with increased mortality rates</td>
</tr>
<tr>
<td>Schottker et al</td>
<td>2014</td>
<td>Germany ESTHER</td>
<td>9,579</td>
<td>1,450 deaths (15.1%)</td>
<td>62.2</td>
<td>8 years</td>
<td>50-74 years</td>
<td>Not available</td>
<td>Age, Gender, BMI, Smoking, Season, Fr, SRH, Light Physical Activity</td>
<td>Increased risk of all-cause mortality with Vitamin D deficiency</td>
</tr>
<tr>
<td>Samefors et al</td>
<td>2014</td>
<td>Sweden SHADES</td>
<td>333</td>
<td>147 deaths (44%)</td>
<td>85.0</td>
<td>3 years</td>
<td>&gt;65 years</td>
<td>40.5nmol/L</td>
<td>Age, Gender, BMI, BP, GFR, Season, Duration in NH, Physical activity, Dementia, CVD, Cancer</td>
<td>Association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Hirani et al</td>
<td>2014</td>
<td>Australia CHAMP</td>
<td>1,659</td>
<td>355 deaths (21.4%)</td>
<td>77.0</td>
<td>5 years</td>
<td>Men Community Dwelling &gt;70 years</td>
<td>55.9nmol/L</td>
<td>Age, Season, BMI, Smoking, Physical activity, Vitamin D supplements, Psychotropic Meds</td>
<td>Lower levels of Vitamin D were associated with all cause mortality</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
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<tr>
<td>Lee et al 2014 Belgium EMAS</td>
<td>2,816</td>
<td>187 deaths (6.6%)</td>
<td>62.8</td>
<td>4.3 years (Median) Community dwelling Men</td>
<td>Not available</td>
<td>56.3nmol/L</td>
<td>Age, Centre, Smoking, Alcohol, Co-morbidities, PASE score, Creatinine, PPT rating</td>
<td>Lower levels of Vitamin D associated with increased risk of all-cause mortality</td>
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<tr>
<td>Saliba et al 2014  Israel</td>
<td>175,781</td>
<td>12,337 deaths (7%)</td>
<td>60.6</td>
<td>2 years (Median) &gt;20 years BMI information available</td>
<td>Not available</td>
<td>50.9nmol/L</td>
<td>Age, Gender, Ethnicity, Season, Vitamin D supplements, Statin, Smoking, HTN, CVD, DM, Cancer</td>
<td>Relationship between Vitamin D and mortality (presented by BMI category, inversely related to BMI)</td>
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<tr>
<td>Vogt et al 2015 Germany KORA Age</td>
<td>727</td>
<td>98 deaths</td>
<td>75.5</td>
<td>2.9 years (Median) Community dwelling</td>
<td>Not available</td>
<td>46.7nmol/L</td>
<td>Age, Education, BMI, Smoking, Alcohol, Season, CVD, DM, Multi-morbidity, Frailty</td>
<td>Vitamin D associated with all-cause mortality, frailty status also contributed to relationship</td>
<td></td>
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<tr>
<td>Mursu et al 2015 Finland KIHID</td>
<td>1,892</td>
<td>670 deaths</td>
<td>52.4</td>
<td>22.2 years Men No Cancer or CVD history</td>
<td>Missing Data CVD or cancer at baseline</td>
<td>43.5nmol/L</td>
<td>Age, Month, Year, BMI, Smoking, Physical activity, Education, Income, Alcohol, BP, DM Cholesterol, CRP</td>
<td>Lower Vitamin D levels associated with increased mortality risk</td>
<td></td>
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<tr>
<td>Holter et al 2016 Norway</td>
<td>241</td>
<td>72 deaths</td>
<td>66 (Median)</td>
<td>5 years (Median) Hospitalised with CAP &gt;18 years</td>
<td>No CXR evidence of pneumonia, Hospitalisation in prior 2weeks, Calcitrol/alfacalcidol supplements</td>
<td>37.4nmol/L</td>
<td>Age, COPD, Immuno-compromise, Season</td>
<td>Association between Vitamin D and mortality in patients admitted with CAP</td>
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<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
<td>Exclusion Criteria</td>
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<tr>
<td>Meyer et al 2016 Norway</td>
<td>4,379</td>
<td>27% cases 12.6% controls</td>
<td>62.9</td>
<td>21.1 years</td>
<td>Men diagnosed with prostate cancer &amp; Matched controls</td>
<td>Not available</td>
<td>62.2nmol/L</td>
<td>Age, Month, Education, BMI, Smoking, Physical activity</td>
<td>Inverse association between Vitamin D and all-cause mortality</td>
<td></td>
</tr>
<tr>
<td>Buchebner et al 2016 OPRA</td>
<td>1,044</td>
<td>96 deaths (91.2%)</td>
<td>75.2</td>
<td>715: 5 year follow-up 382: 10 year follow-up</td>
<td>Women &gt;75 years</td>
<td>None applied</td>
<td>63nmol/L</td>
<td>CVD, CKD, Diabetes, Osteoporosis, Respiratory disease</td>
<td>Inverse association between Vitamin D and mortality</td>
<td></td>
</tr>
<tr>
<td>Daraghmeh et al 2016 NHANES III</td>
<td>10,517</td>
<td>23 deaths</td>
<td>54</td>
<td>20 years</td>
<td>&gt;35 years</td>
<td>Pregnant, eGFR &lt;15ml/min, Missing Vitamin D</td>
<td>Not available</td>
<td>Age, Ethnicity, Income, Diabetes, CVD, BP, Vitamin D supplements, Smoking, Alcohol, Family history Cancer</td>
<td>Decreased mortality risk with higher levels of Vitamin D</td>
<td></td>
</tr>
<tr>
<td>Vipul et al 2017 India</td>
<td>88</td>
<td>14 deaths (15.4%)</td>
<td>45</td>
<td>1 year</td>
<td>ICU/Medical ED with sepsis</td>
<td>Pregnant or lactating mothers, Resuscitated patients, Vitamin D supplements, Malabsorption</td>
<td>40.9nmol/L</td>
<td>Not Available</td>
<td>Vitamin D associated with mortality and longer length of hospital stay in small patient population with sepsis</td>
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<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
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<tr>
<td>Sun et al 2017</td>
<td>6,377</td>
<td>1,539 (24.1%)</td>
<td>Not available</td>
<td>18.5 years</td>
<td>&gt;20 years</td>
<td>Not available</td>
<td>47.3nmol/L (Median)</td>
<td>Age, Gender, Season, Alcohol, Smoking, Falls, Physical activity, Education, BMI, Economic difficulties, Chronic Illness</td>
<td>Lowest quartile of Vitamin D was associated with increased risk of all-cause mortality</td>
<td></td>
</tr>
<tr>
<td>Visser et al 2006</td>
<td>1,260</td>
<td>138 admitted to NH (11%)</td>
<td>Not available</td>
<td>6 years</td>
<td>Community dwelling 55-85yrs</td>
<td>Not living independently at baseline</td>
<td>46.8% levels &lt;50nmol/L</td>
<td>Sex, Gender, BMI, Education, Chronic illness, creatinine, Albumin, Cognition, Depression, C2H5, Smoking, Physical activity, Mobility performance, Cholesterol</td>
<td>Vitamin D deficiency was associated with higher mortality risk but after adjustment for frailty measures, the association lost statistical significance</td>
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<tr>
<td><strong>Negative</strong></td>
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<tr>
<td>Cawthon et al 2010</td>
<td>1,490</td>
<td>330 deaths (22.2%)</td>
<td>73.5</td>
<td>7.3 years</td>
<td>Community dwelling</td>
<td>Walk with assistance</td>
<td>25.2% levels &lt;50nmol/L</td>
<td>Age, Clinic, Season, Calcium and PO4, GFR, Percentage body fat, Weight, Race, Health status, Alcohol, Education, Activity level, Marital status</td>
<td>No association between Vitamin D and mortality</td>
<td></td>
</tr>
<tr>
<td>Eaton et al 2011</td>
<td>2,429</td>
<td>224 deaths (9.2%)</td>
<td>65.7</td>
<td>10.5 years</td>
<td>Post-menopausal women 50-79 years</td>
<td>Not available</td>
<td>Not available</td>
<td>Age, Ethnicity, HTN Smoking, Diabetes, CVD, Fracture, BMI, Physical activity, Waist circumference</td>
<td>No association between Vitamin D and mortality</td>
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<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
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<tr>
<td><strong>Negative</strong></td>
<td></td>
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<tr>
<td><strong>Ford et al 2011</strong> USA NHANES</td>
<td>7,531</td>
<td>347 deaths (4.6%)</td>
<td>45.5 (Median)</td>
<td>&gt;20 years</td>
<td>Incomplete data</td>
<td>60.6nmol/L</td>
<td>Age, Gender, Race, Education, HbA1C, Calcium, Smoking, Alcohol, Physical activity, HDL, CRP</td>
<td>Vitamin D not significantly associated with reduced all cause mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Flynn et al 2012 USA</strong></td>
<td>66</td>
<td>5 deaths (7%)</td>
<td>56.0 None Stated</td>
<td>ICU stay &gt;48 hours</td>
<td>Not available</td>
<td>Not available (76% levels &lt;50nmol/L)</td>
<td>Not available</td>
<td>No association with Vitamin D and surgical ICU mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Arnson et al 2012 Israel</strong></td>
<td>130</td>
<td>57 deaths (44%)</td>
<td>70.2 None Stated</td>
<td>&gt; 18 years Requiring Mechanical Ventilation</td>
<td>Vitamin D supplementation before admission</td>
<td>37.2nmol/L</td>
<td>Age APACHE score, MI, CVA, CHF</td>
<td>No difference between Vitamin D sufficient &amp; deficient groups for mortality Longer survival with Vitamin D sufficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lin et al 2012 China</strong></td>
<td>1,101</td>
<td>793 deaths (72%)</td>
<td>56.5 24 years</td>
<td>Healthy community dwellers 40-69 years</td>
<td>Not available</td>
<td>73% levels &lt;50nmol/L</td>
<td>Age, Gender, HTN, Smoking, BMI, Alcohol</td>
<td>No association between Vitamin D and all cause mortality and cause specific mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Langsetmo et al 2013 Canada CaMos</strong></td>
<td>9,033</td>
<td>1,160</td>
<td>61.9 10 years</td>
<td>&gt;25 years Community Dwelling French English Chinese speaking</td>
<td>Not available</td>
<td>Not available</td>
<td>Age, Education, Centre, BMI, Health status, Smoking, Physical activity, Sun exposure, Co-morbidity, Medication</td>
<td>No association between Vitamin D intake and mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Formiga et al 2013 Spain Octabaix</strong></td>
<td>312</td>
<td>58 deaths (18.5%)</td>
<td>Not available</td>
<td>85 years Missing blood tests</td>
<td>Not available</td>
<td>69.0nmol/L</td>
<td>Gender, Marital status, Education, Physical activity, Charlson Co-morbidity Index</td>
<td>No association between Vitamin D and all-cause or CVD related mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean f/u Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounders</td>
<td>Results</td>
<td></td>
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<tr>
<td>Puhan et al</td>
<td>2014</td>
<td>356</td>
<td>34 deaths (9.6%)</td>
<td>67.2</td>
<td>2 years</td>
<td>&gt;40 years, Confirmed COPD, Exacerbation free &gt;4 weeks</td>
<td>Patients taking Vitamin D supplements</td>
<td>38.7nmol/L</td>
<td>Age, Gender, FEV1, Country, Season, Smoking Status</td>
<td>No significant association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Hak Lee et al</td>
<td>2015</td>
<td>S Korea 489</td>
<td>114 deaths (23.3%)</td>
<td>76.5</td>
<td>2 years</td>
<td>&gt;50 years, Post hip fracture</td>
<td>Not available</td>
<td>39.2nmol/L</td>
<td>Age, Gender, BMI, ASA Score, DXA T Score, Pre-ambulatory status</td>
<td>No association noted</td>
</tr>
<tr>
<td>Granic et al</td>
<td>2016</td>
<td>UK Newcastle 85+ 775</td>
<td>332 deaths (57.6%)</td>
<td>85 at baseline</td>
<td>6 years</td>
<td>Community and Institutionalised adults</td>
<td>Not available</td>
<td>39nmol/L (Median)</td>
<td>Gender, Education, Marital status, Income, Smoking, Alcohol, Physical activity, Mental health, MMSE &lt;23, CKD, waist-hip</td>
<td>No association in fully adjusted models</td>
</tr>
<tr>
<td>Guo et al</td>
<td>2017</td>
<td>UK CAPS 452</td>
<td>218 (62.2%)</td>
<td>52 years</td>
<td>20 years</td>
<td>Men</td>
<td>Missing Data Prior MI</td>
<td>Not Measured (Vitamin D intake)</td>
<td>Age, BMI, Social class, Alcohol intake, Smoker, Leisure activity, Food energy intake, Calcium intake</td>
<td>No significant association between Vitamin D intake and all-cause mortality</td>
</tr>
</tbody>
</table>
6.6 Economic Studies

A Canadian study looked at the economic burden of Vitamin D deficiency and estimated the risk of premature death (Grant et al., 2010). The authors performed a meta-analysis of observational data and RCTs to calculate dose-response relationships for Vitamin D and a number of diseases and mortality. Mortality data was collected from the Statistics Bureau Canada and economic burden data was collected from Economic Burden of Illness Canada 1998. It was assumed that cancer incidence and mortality rates have similar dose response relationships. From the meta-analysis it was estimated that increasing Vitamin D levels from 75 to 105nmol/L would decrease cancer incidence rates by 2%. For cardiovascular disease, increasing serum Vitamin D levels from 62.56nmol/L to 105nmol/L would reduce incidence by 25%. A summary of deaths in Canada from 2004 was provided with an estimate of prevented deaths with an increased Vitamin D level to 100nmol/L. It was estimated that 37,000 (16.1%) of annual deaths could be considered premature and the economic burden could be reduced by 7.3%.

In 2011, Grant produced a paper estimating the global reduction in mortality rates with doubling of serum Vitamin D levels (Grant, 2011). He identified a number of diseases in which Vitamin D deficiency is thought to be associated with increased incidence and mortality rates and examined the evidence available to date, determined dose mortality rate relations for each type of disease, calculated Vitamin D levels for each region and also obtained mortality figures. He then calculated mortality rate reduction figures for each region. The fraction of Vitamin D related disease that could be postponed was assumed to be 20% based on the dose-response relations to date. The HR for CVD death dropped by 18% when Vitamin D levels were increased from 54 to 110nmol/L. For the same change in Vitamin D level, the OR for certain cancers dropped by 33%.

6.7 Genetic Studies

Positive Studies

The Leiden Longevity Study reported on the possible association between 25(OH)D and familial longevity (Noordam et al., 2012). The study was initially designed to look at the genetic and phenotypic markers related to familial longevity. 421 families of nonagenarians
were recruited consisting of long-lived white siblings, offspring and offspring’s partners. Families had to have at least two long-loved siblings (>89 years). Genotypes of offspring and controls were determined at three Single Nucleotide Polymorphisms (SNPs). Following exclusion of those with missing data (36%), 1,038 offspring were included in the analysis (from 380 families) and 461 controls. Mean age of offspring was 59.5 years versus 58.9 years for controls, p=0.09. In adjusted models controls were found to have higher Vitamin D levels (68.4nmol/L) than the offspring (64.3nmol/L), Offspring were found to have lower frequency of common genetic variants in the CYP2R1 gene, n = 0.371 versus controls n= 0.418, p= 0.04, which is associated with increased levels of Vitamin D.

Afzal et al performed a Mendelian randomisation analysis from three cohorts to investigate if low Vitamin D levels were associated with increased mortality (Afzal et al., 2014). The three cohorts were from Danish population studies with no crossover between studies. Participants were followed in each study for a mean of 19.8, 5.8 and 7.9 years respectively. Mortality data was obtained from the Danish Civil Registration System. Genotyping was conducted blinded to 25(OH)D concentrations and mortality data. Genetic variants considered were DHC87 and CYP2R1. Vitamin D Receptor (VDR) polymorphisms were not considered as authors state they are not related to 25(OH)D biological activity directly. Results showed a 20nmol/L lower 25(OH)D level was associated with all cause mortality. In multivariate analysis the adjusted HR was 1.19 (1.14-1.25, 95% CI). 25(OH)D levels were 4.6nmol/L lower for four versus no variant alleles with DHCR7 and 6.1nmol/L for CYP2R1. For all cause mortality the HR for combined DHCR7 and CYP2R1 allele score increase was 1.02 (1.00-1.03, 95%CI). For genetically determined 20nmol/L lower 25(OH)D concentration from all cause mortality, OR 1.30 (1.05-1.61, 95% CI).

A more recent study investigated the influence of genetic variants, SNPs in the Vitamin D pathway, and mortality (Ordonez-Mena et al., 2017). Participants were part of the ESTHER study cohort and this included 8,417 participants with a mean age of 63.6 years and mean 25(OH)D level of 49.9nmol/L. Four SNPs around the Vitamin D pathway were measured. Over the twelve year follow-up period, 1,338 (15.9%) died. Two SNPs, the first on the GC gene which codes for the VDP and the second on the DHCR7 gene which codes for 7-
dehydroxycholesterol reductase were associated with low 25(OH)D concentrations and increased odds of lower Vitamin D status, OR 1.27 (1.18-1.36, 95% CI) and OR 1.16 (1.08-1.25, 95% CI) respectively. The genotype score was associated with all cause mortality, HR 0.93 (0.88-0.99, 95% CI) but not for CVD mortality. The SNPs were not associated with the mortality endpoints in this study.

Negative Studies
Jorde et al report on the polymorphisms of Vitamin D associated with risk of MI, diabetes and mortality in the Tromso study (Jorde et al., 2012). This longitudinal population based study looked at lifestyle related diseases. Mortality data was retrieved by linkage with the National Causes of Death Registry and death certificates were obtained for an underlying or contributing diagnosis of Cardiovascular Disease (CVD) or sudden unexpected death. Seventeen SNPs were selected for this analysis based on previous genome wide association studies. A total of 9,528 subjects were included in this analysis and at least one SNP was available for 9,471 participants, 3,828 had died in the follow-up period. No relationship was found between the selected SNPs, their associated genes and mortality.

A Mendelian randomisation study assessing the association between genetic SNPs for Vitamin D deficiency and mortality was reported in 2013 (Trummer et al., 2013). This study aimed to analyse the genotype-risk factor association and the genotype-outcome association to estimate a risk factor-outcome association. The investigated cohorts from the LURIC study consisted of 3,316 participants with a mean age of 62.6 years and mean Vitamin D of 43.4nmol/L and were followed for a median of 9.9 years, over which time 995 people died (30%). Samples were taken for genotyping for genetic loci including GC, DHCR7 and CYP2RI. On multivariate Cox regression analysis, the SNPs were not found to be associated with mortality.

6.8 Randomised Control Trials
Negative Studies
In 2003 a UK study looking at the effects of Vitamin D supplementation four monthly on fractures and mortality was reported (Trivedi et al., 2003). This trial was a feasibility trail
for a planned study that subsequently did not materialise. Participants were randomised to receive one capsule containing 100,000IU Vitamin D3 every four months or placebo. The study was conducted by post with one capsule posted every four months to participants for five years. Mean baseline Vitamin D levels were not provided. 2,686 male and female participants were recruited through mailed letters. If they were advised to commence Vitamin D >2,00IU/day during the trial, they were excluded from follow up. Results showed no significant difference in mortality rates between participant groups. Age adjusted relative risk for all cause mortality HR 0.88 (0.74-1.06, 95% CI). Although, it did show lower rates of fracture at any site in those allocated to treatment with Vitamin D.

A study published from the WHI population looked at the association between Vitamin D supplementation and cardiovascular events including coronary heart deaths and myocardial infarctions (Hsia et al., 2007). The population group and randomisation and treatment arms are as outlined above. No effects were noted with Calcium and Vitamin D supplementation in any of the stated outcomes.

LaCroix et al report the effects of Vitamin D and calcium (CaD) supplementation on all cause mortality and cause specific mortality in the Women’s Health Initiative Calcium and Vitamin D RCT (WHI CaD Trial) (LaCroix et al., 2009) including 36,282 community dwelling US postmenopausal women. Participants were initially enrolled to assess HRT and dietary modification. One year later they were recruited to a further study of Calcium and Vitamin D RCT with a primary outcome to assess the effects on fracture prevention and on other fractures or colorectal cancer as secondary outcomes. All participants from WHI CaD were followed until they were lost to follow-up or until death. The mean age of study participants was 62.4 years, with a mean baseline Vitamin D of 42nmol/L. The mean follow up time was 7 years. Participants were randomised to receive 400iu Vitamin D and 1,000mg calcium or placebo. Cause of death was determined using medical records, death certificate and autopsy reports. There were 1,551 (4.2%) deaths in this study group, 744 in CaD intervention group and 807 in placebo. There was a non-significant trend for intervention with CaD on total mortality, HR 0.91 (0.83–1.01, 95% CI). CaD HRs were non-significant for stroke and cancer mortality, whereas HRs were close to unity for CHD.
and other causes of death. Intention-to-treat (ITT) HRs by age at baseline suggested lower HRs among younger women for total, stroke, and other causes of death. The HR for total mortality was 0.89 (0.79–1.01, 95% CI) in women <70 years compared with HR 0.95 (0.80–1.12, 95% CI) in women >70 years (p for age interaction = 0.10). HRs for cancer death were similar across age categories.

A small pilot RCT including 25 patients in a medical ICU was performed looking at the effects of high dose Vitamin D on serum levels and also on some clinical outcomes, including mortality and length of ICU and hospital stay (Amrein et al., 2011). The primary end-point was the percentage that reached serum levels of 75nmol/L on Day 7. Treatment Arm: 540,000IU Vitamin D3 once off enteral dose, Placebo: Herbal Oil Only. The mean age of participants was 62.5 years. The mean Vitamin D level was 36.7nmol/L in the Treatment group versus 35.2nmol/L in the Placebo Group at baseline. There was a significant rise in serum 25(OH)D in the treatment group on Day 7 to 95.3nmol/L. There was no change in the placebo group. There was no difference in mortality, LOS in ICU or in hospital stay.

Avenel et al reported long-term follow up results for mortality and cancer in a RCT of Vitamin D and/or calcium (RECORD Trial) (Avenell et al., 2012). A total of 5,292 participants were recruited through 21 centres through fracture clinics and orthopaedic wards in Scotland and England with a mean age of 77 years. The majority of participants were female, (approximately 84%). Vitamin D at baseline was 38nmol/L in a subgroup of 60 trial participants. The median follow up time was 6.2 years. All cause mortality, mortality due to vascular disease and cancer were included as pre-specified outcomes in the main trial protocol. This paper reports on mortality at three years. Participants were randomised to four groups to receive: 800IU Vitamin D3/day, 1000mg Calcium/day, 800IU Vitamin D3 and 1,000mg Calcium or placebo. There were 1,717 deaths (32.4%), 836 deaths of the 2,649 participants allocated to Vitamin D compared with 881 of 2,643 participants not allocated to receive Vitamin D. The main cause of death was recorded as vascular (42.3% of deaths) and cancer in 19.2% of deaths. In those allocated to Vitamin D versus no Vitamin D, there was no difference between the number of deaths and ITT.
analysis, HR 0.93 (0.85-1.02, 95% CI). There was no difference in the groups allocated to calcium or placebo. Post-hoc analysis adjusted for compliance did not affect the results.

An Austrian RCT investigated the potential beneficial effects of restoring and maintaining Vitamin D levels over six months in ICU patients (Amrein et al., 2014b). The primary outcome was hospital length of stay (LOS). Secondary outcomes included, length of ICU stay, percentage of patients with 25(OH)D levels higher than 75nmol/L at day seven, hospital mortality, and six-month mortality. The final study population included medical and surgical ICU patients (n=475) with vitamin D deficiency (≤50nmol/L) assigned to receive either Vitamin D3 (n=237) given orally or via nasogastric tube once at a dose of 540,000IU followed by five monthly maintenance doses of 90,000 IU for 5 months or placebo (n=238). The mean age of participants was 64.6 years with mean Vitamin D levels of 32.1nmol/L. Hospital mortality was not significantly different, 28.3% (22.6-34.5, 95% CI) for Vitamin D3 versus 35.3% (29.2-41.7, 95% CI) for placebo, HR 0.81 (0.58-1.11, 95% CI), p = 0.18. Six-month mortality rate was 35.0% (29.0-41.5, 95% CI) for Vitamin D3 compared with 42.9% for placebo (36.5%-49.4%, 95% CI), HR, 0.78 (95% CI, 0.58-1.04), p= 0.09. In subgroup analysis (n=200) with severe Vitamin D deficiency (<30nmol/L), hospital mortality was significantly lower with 28.6% patients deaths for Vitamin D3 compared with 46.1% for placebo, HR 0.56 [0.35-0.90, 95% CI], p =0.04, but not six-month mortality 34.7% [25.4-45.0, 95% CI] for Vitamin D3 versus 50.0% [39.9%-60.1%, 95% CI] for placebo; HR 0.60 (0.39-0.93, 95% CI.), p = 0.12.

Neale et al have recently outlined their double blind placebo controlled trial, The D Health Trial which has at present recruited and randomised 21,315 participants, to receive either 60,000IU Cholecalciferol orally once monthly versus placebo (Neale et al., 2016). The primary end-point is all-cause mortality and results of this study are awaited.

A recent RCT looked at the effect of Vitamin D supplementation on all-cause mortality in patients with heart failure (Zittermann et al., 2017). 400 participants with heart failure (either on heart transplant programme or on waiting list for transplantation) with Vitamin D levels <75nmol/L were randomised to receive 4,000IU Vitamin D or placebo for three
years. 129 participants stopped taking the study medication while another 40 were lost to follow-up. The mean age of participants was 55 years and the mean Vitamin D level was 35.2nmol/L in the placebo group and 31.3nmol/L in controls. Mortality rates were 19.6% versus 17.9% in Vitamin D and placebo groups respectively. Survival analysis revealed no significant difference, HR 1.09 (0.69-1.71, 95% CI).

A more recent RCT including 25,871 US men aged >50 years and women aged >55 years investigated the effects of Vitamin D and Omega-3 fatty acid supplementation on cardiovascular and cancer outcomes (Manson et al., 2018). Participants had a mean age of 67.1 years and mean 25(OH)D level of 77nmol/L at baseline recruitment. All were free of cancer (except non-melanoma skin cancer) and cardiovascular disease at recruitment. Participants were limited in taking Vitamin D supplementation from sources beyond the trial (to maximum 800 units per day. Participants were randomised in a two-by-two factorial design to receive Vitamin D 2,000 IU per day or placebo along with active Omega-3 fatty acids or placebo. Over the 5.3-year follow-up period, 1,033 people had died. The primary end-point was invasive cancer, which developed in 1,617 participants, with no significant difference between Vitamin D and placebo groups, HR 0.96 (0.88-1.06, 95% CI) or in deaths between the groups. In analysis where deaths in the first two years of the study were excluded, there appeared to be fewer deaths in those participants in the Vitamin D group compared with placebo, HR 0.79 (0.63-0.99, 95% CI). There were 805 cardiovascular events over the study follow-up period, (including MI, stroke and cardiovascular death), 396 in Vitamin D group and 409 in the placebo group, HR 0.97 (0.85-1.12, 95% CI). Vitamin D did not appear to be beneficial in terms of cancer, cardiovascular disease and mortality in this study. There is a potential association with Vitamin D supplementation and cancer deaths beyond the initial study, and further post-intervention follow-up is ongoing. Participants in this study were younger adults, who overall were not deficient in Vitamin D and thus results do not answer the question of the beneficial effects of Vitamin D in older adults who are deficient in Vitamin D.
Table 5: Vitamin D and Mortality: Intervention/Randomised Control Trials

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age (years)</th>
<th>Mean follow-up</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Confounder</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trivedi et al</td>
<td>2003</td>
<td>UK</td>
<td>2,686</td>
<td>Fracture and mortality</td>
<td>74.8</td>
<td>4 years</td>
<td>65-85 years</td>
<td>Vitamin D supplements, Renal Calculi Sarcoidosis Malignancy</td>
<td>Not available</td>
<td>Age</td>
<td>100.00IU Vitamin D3 every 4/12 Vitamin D placebo</td>
</tr>
<tr>
<td>LaCroix et al</td>
<td>2009</td>
<td>USA</td>
<td>36,282</td>
<td>1,551 deaths: Intervention: 744 Placebo: 807</td>
<td>62.4</td>
<td>7 years</td>
<td>Community Dwelling Post-menopausal women</td>
<td>Predicted survival &lt;3yrs, Renal Calculi, Hypercalcaemia Steroids, Calcitrol or &gt;600IU Vitamin D/day</td>
<td>42nmol/L (Median)</td>
<td>Age, Ethnicity, Latitude</td>
<td>Calcium 1,000mg &amp; VitD3 400IU/day Placebo</td>
</tr>
<tr>
<td>Hsia et al</td>
<td>2007</td>
<td>USA</td>
<td>36,282</td>
<td>Intervention: 499 Placebo: 475</td>
<td>62.4</td>
<td>7 years</td>
<td>Community Dwelling Post-menopausal women</td>
<td>Predicted survival &lt;3yrs, Renal Calculi, Hypercalcaemia Steroids, Calcitrol or &gt;600IU Vitamin D/day</td>
<td>42nmol/L (Median)</td>
<td>Not available</td>
<td>Calcium 1,000mg &amp; VitD3 400IU/day Placebo</td>
</tr>
<tr>
<td>Avenil et al</td>
<td>2012</td>
<td>UK</td>
<td>5,292</td>
<td>1,717 deaths (32.4%)</td>
<td>77</td>
<td>6.2 years (Median)</td>
<td>&gt;70 years</td>
<td>AMTS &lt;7, Immobile, Cancer likely to metastasise to bone in preceding 10 years, Renal calculi, Hypercalcaemia Life expectancy &lt;6/12</td>
<td>38nmol/L (recorded in 60 participant s)</td>
<td>Age, Gender, Type of fracture, Time since fracture</td>
<td>4 groups: 1. VitD3 2. Calcium and Vitamin D3 3. Calcium 4. Placebo Vitamin D3: 800 IU/day Calcium: 1000mg/day</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age (years)</td>
<td>Mean follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Confounder</td>
<td>Intervention</td>
<td>Results</td>
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<tr>
<td>Amrein et al 2011 Austria Pilot Trial</td>
<td>25</td>
<td>12 deaths</td>
<td>62</td>
<td>15 days</td>
<td>Medical ICU patients &gt;18 years, ICU LOS &gt;48 hours 25(OH)D &lt;50nmol/l</td>
<td>Life expectancy &lt;24hours, Hypercalcaemia, Ileus, Sarcoid, Pregnancy, TB, Renal calculi</td>
<td>33.9nmol/L</td>
<td>Not available</td>
<td>540,000IU Vitamin D3 Herbal Oil with 200IU Vitamin D3 (Placebo)</td>
<td>No difference in hospital mortality between groups</td>
<td></td>
</tr>
<tr>
<td>Amrein et al 2014 Austria</td>
<td>475</td>
<td>Intervention: 34.7% Placebo: 50%</td>
<td>64.6</td>
<td>6 months</td>
<td>Medical &amp; Surgical ICU patients As above</td>
<td>GI impairment, Participation in pilot trial, Pregnant, lactating women, Sarcoid, TB, Renal calculi, Hypercalcaemia</td>
<td>32.1nmol/L</td>
<td>Age, Gender, SAPS II, Co-morbidity, Calcium, Albumin, Procalcitonin, PTH</td>
<td>540,000IU Vitamin D3 initially then five monthly maintenance of 90,000IU (650IU/day equivalent) Placebo</td>
<td>No significant difference between mortality rates in placebo versus intervention group</td>
<td></td>
</tr>
<tr>
<td>Zitterman et al 2017 Germany EVITA</td>
<td>400</td>
<td>Intervention: 19.6% Placebo: 17.9%</td>
<td>55</td>
<td>3 years</td>
<td>Heart Failure Patients on transplant programme or awaiting transplant 18-79 years</td>
<td>Urgent transplant listing, Hypercalcaemia, Vitamin D intake &gt;800IU/day, Vitamin D level &gt;75nmol/L</td>
<td>33.3nmol/L</td>
<td>Not available</td>
<td>4,000IU daily or placebo</td>
<td>No significant reduction in mortality heart failure patients</td>
<td></td>
</tr>
<tr>
<td>Manson et al 2018</td>
<td>25,871</td>
<td>1,033 deaths Intervention: 485 Placebo: 493</td>
<td>67.1</td>
<td>5.3 years</td>
<td>No history of cancer or CVD, Vitamin D supplementation &lt;800IU/day</td>
<td>Renal failure, Cirrhosis, Hypercalcaemia “Other serious medical condition”</td>
<td>77nmol/L</td>
<td>Age, Gender, n-3 fatty acid randomisation</td>
<td>2,000U/day or placebo with Omega-3 fatty acid or placebo</td>
<td>No difference between groups in cancer or cardiovascular incidence or mortality</td>
<td></td>
</tr>
</tbody>
</table>

AMTS: Abbreviated Mental Test Score, CVD: Cardiovascular Disease, ICU: Intensive Care Unit, IU: International Units, PTH: Parathyroid Hormone, SAPS II: Simplified Acute Physiology Score, TB: Tuberculosis
6.9 Meta-Analyses

Positive Studies

Autier et al performed a meta-analysis of 18 RCTs with a total of 57,311 male and female participants in 2007 (Autier and Gandini, 2007). The primary outcome of the majority of these RCTs was to assess the effects of Calcium and Vitamin D on fractures, bone mineral density and osteoporosis. The mean length of follow up was 5.8 years and 4,777 deaths (8.3%) of any cause occurred. Baseline mean Vitamin D was not documented but mean Vitamin D supplementation was 528IU/day. Analysis showed significant risk reduction in all cause mortality in those supplemented with Vitamin D, SRR 0.92 (0.86-0.99, 95% CI), reporting a 7% decrease in mortality in this group. There appeared to be no significant difference between lower and higher doses of Vitamin D supplementation in subgroup analysis 300-799IU Vitamin D, SRR 0.93, (0.85-1.03, 95% CI) versus >800IU Vitamin D, SRR 0.92, (0.82-1.03, 95% CI).

A Cochrane review published in 2011 (Bjelakovic et al., 2011) reviewed RCTs that compared Vitamin D supplementation with placebo or no intervention. Vitamin D supplementation included Vitamin D2, D3, alfacalcidol and calcitrol. Analysis included 50 RCTS with 94,148 participants. All trials came from high-income countries and the main primary outcomes included bone mineral density, falls, fractures and mortality. The mean age of participants was 74 years. The median duration of Vitamin D supplementation was two years. Participants in 18 trials had levels >50nmol/L and the remaining 22 trials had baseline levels <50nmol/L. The mean daily dose of Vitamin D3 (administered in 32trials) was 804IU/day. 32 trials used calcium combined with Vitamin D at a mean dose of 929mg/day. Overall mortality was lower in Vitamin D groups (11.2%) compared with placebo or no intervention groups (11.4%), RR 0.97 (0.94 to 1.00, 95% CI) p=0.03. When participants were deficient in Vitamin D, supplementation with Vitamin D3 reduced mortality, RR 0.94 0.90 to 0.99, 95% CI) p=0.02 but there was no benefit in Vitamin D3 supplementation when patients were not deficient at baseline, RR 0.92 (0.79 to 1.07, 95% CI) p=0.27. Trials using Vitamin D2 supplementation showed no significant effects on mortality overall, RR 1.02 (0.97 to 1.09, 95% CI) p=0.42. Of note, when adverse events were assessed, no significant effect on risk of hypercalcaemia was noted with Vitamin D2
or D3 RR 1.26, (0.78 to 2.05, 95% CI) p=0.34 but Vitamin D3 combined with calcium significantly increased the risk of nephrolithiasis, RR 1.17 (1.00 to 1.34, 95% CI) p=0.02. Alfacalcidol and calcitrol were associated with increased the risk of hypercalcaemia, RR 3.18 (1.17-8.68, 95% CI).

Schottker et al published a systematic review and meta-analysis looking at the relationship between Vitamin D and mortality in 2012 (Schottker et al., 2013a). This analysis involved original longitudinal cohort studies conducted in the general population reporting an association of measured 25(OH)D levels and all cause mortality, only 12 studies were included, with 31,528 participants and 6,921 deaths over the follow-up time ranging from 5.9 to 14 years. The meta-analysis summarises the results of 12 cohort studies. The pooled HR demonstrated an inverse association between 25(OH)D and mortality, HR 0.92 (0.89-0.95, 95% CI). For a 25nmol/L increase in 25(OH)D the HR for mortality was 0.90 (0.86-0.94, 95% CI) and for a 50nmol/L increase, HR 0.81 (0.74-0.88, 95% CI).

A further meta-analysis was conducted in 2012 (Zittermann et al., 2012) and included observational studies that reported Relative Risks (RR) or crude data on Vitamin D and mortality. Ultimately 14 studies were included in non-parametric and 11 in parametric analysis. In non-parametric analysis the summary estimate for the highest versus lowest Vitamin D categories showed reduced mortality RR 0.71 (0.50-0.91, 95% CI). On parametric analysis the median reference category of Vitamin D was 27.5nmol/L, the best fitting model presented the following estimates, β1 0.085, (-0.120, -0.050, 95% CI) and β2 0.0018, (0.0008, 0.0030, 95% CI). An increase in Vitamin D concentration was associated with decreased mortality.

Rejnmark et al published a review in 2012 assessing the effects of Vitamin D supplementation on mortality using both Individual Participant Data (IPD) level and study level meta-analyses (Rejnmark et al., 2012). Inclusion criteria specified only RCTs with administration of Vitamin D2 or Vitamin D3 in one intervention arm and one arm without Vitamin D. Their search revealed 11 eligible trials for inclusion. IPD analysis included 70,528 participants, 86.8% female and median age 70 years. A total of 27,345 were
randomised to Calcium and Vitamin D, 7,771 to Vitamin D alone, and 35,412 received placebo or no Vitamin D. The mean follow up time was 3 years and 3,832 participants died (5.4%). In those randomised to receive Vitamin D (either alone or with calcium) 1,870 deaths occurred (5.3%) whereas 1,962 deaths (5.5%) occurred in those not receiving Vitamin D, HR 0.94 (0.88-1.00, 95% CI). When adjusted, risk of death was reduced by 7%, HR 0.93 (0.88-0.99, 95% CI). When analysis of studies using stratified Cox regression models was performed, risk of death was reduced among these participants receiving Calcium and Vitamin D, HR 0.91 (0.84-0.98, 95% CI), but assessing risk of death with Vitamin D alone there was no significant difference noted HR 0.96 (0.87-1.06, 95% CI).

In 2013 Zhang et al reported a meta-analysis of the effects of long-term Vitamin D and overall mortality (Zheng et al., 2013). After exclusion criteria were applied 42 RCTS with a total of 85,466 participants were included. The mean age ranged form 37-89 years. Vitamin D levels were reported in 37 studies. Participants in 22 studies had 25(OH)D levels <50nmol/L. Prolonged treatment with Vitamin D, for longer than 3 years (13 studies, 3693 participants randomised to Vitamin D and 3,880 randomised to placebo), was found to significantly reduce mortality with a RR 0.94 (0.90-0.98, 95%CI) whereas shorter treatment duration (<3years, 29 trials, 1,175 randomised to Vitamin D and 1,118 randomised to placebo) was not found to significantly affect all-cause mortality, RR 1.04 (0.97-1.12, 95% CI). In subgroup analyses, participants with 25(OH)D levels <50nmol/L were found to have significant reduction in mortality when treated with Vitamin D, RR 0.93 (0.89-0.98, 95% CI) compared with those with levels >50nmol/L, RR 0.96 (0.89-1.03, 95% CI). Cholecalciferol was found to be more effective in reduction of mortality (RR 0.93, 0.89-.97, 95% CI) compared with ergocalciferol RR 0.98, (0.90-1.06, 95% CI).

Rush et al performed a systematic review and meta-analysis to evaluate the relationship between Vitamin D and premature mortality in a Scottish population (Rush et al., 2013). Nine studies were suitable for inclusion in the meta-analysis. In synthesis of results the overall effect size using adjusted HR for all cause mortality for lowest quartile of 25(OH)D was HR 1.19 (1.12-1.27, 95% CI). In age stratified analysis the pooled effect size was HR 1.12 (1.01-1.24, 95% CI) for those with a mean age <65 years versus HR 1.25 (1.14-1.36,
95% CI) for those >65 years. Overall there was a statistically significant association between all cause mortality and 25(OH)D level, HR 1.42 (1.30-1.55, 95% CI). In fully adjusted models, this remained significant, HR 1.19 (1.12-1.27, 95% CI).

A meta-analysis of eight observational studies was reported in 2014 (Schottker et al., 2014a) and looked at the relationship between Vitamin D levels and all-cause, CVD and cancer related mortality. Participant information for those aged between 50-79 years was included from each study. Results showed a non-significant level of heterogeneity between studies. The median Vitamin D levels varied between 24-62nmol/L with a median follow-up time between 4.2-15.8 years during which time 6,695 participants died. The meta-analysis revealed a pooled estimate effect for all cause mortality at 1.6 fold higher in the bottom quartile of Vitamin D compared with the top quartile.

A systematic review and meta-analysis from 2014 evaluated the effect of Vitamin D deficiency on infection rates, sepsis and mortality in critically ill patients (de Haan et al., 2014). A total of 14 studies including 9,715 participants were included. The mean 25(OH)D level was 45nmol/L, mean age 62 years and mean 30-day mortality rate was 17.5% and mean in-hospital mortality rate was 18.4%. In pooled analysis for 30-day mortality associated with Vitamin D deficiency in a subgroup of 2,572 participants was RR 1.76 (1.37-2.26, 95% CI) and the pooled in-hospital mortality, RR 1.79 (1.49-2.16, 95% CI) for 3,606 participants.

A meta-analysis reported by Garland et al analysed the strength of the inverse association between Vitamin D and age-adjusted mortality (Garland et al., 2014). Studies reporting a measure of association according to two or more categories of 25(OH)D were included with 32 studies considered eligible. Of these, 25 showed a significant inverse relationship between 25(OH)D concentration and age adjusted mortality rates. In a further two studies an inverse relationship existed but was not statistically significant. The overall age-adjusted mortality HR was 1.9 (1.6-2.2, 95% CI) p<0.001 when comparing lowest 25(OH)D levels (<22.5nmol/L) to highest levels (>125nmol/L). When 25(OH)D levels >75nmol/L were compared with levels <75nmol/L, lower levels were once again found to be associated with
higher all cause mortality, p<0.01. A pooled dose response curve showed a plateau at 25(OH)D concentrations beyond 125nmol/L.

Bjelakovic et al completed a Cochrane review in 2014 looking at Vitamin D supplementation for the prevention of mortality in adults (Bjelakovic et al., 2014). 56 RCTs with 95,286 participants, with Vitamin D compared to placebo or no intervention were included in this analysis. The age ranged from 18 to 107 years. Vitamin D was administered for a weighted mean of 4.5 years. 80% of trials reported Vitamin D levels at baseline and of these, participants in 19 trials had levels >50nmol/L and those participants in the remaining 26 trials had insufficient levels of Vitamin D (<50nmol/L). More than 8% of participants dropped out. When all 56 trials were analysed together it was found that Vitamin D appeared to be associated with reduced mortality, RR 0.97 (0.94-0.99, 95% CI). Only Vitamin D3 was found to decrease mortality when different forms of Vitamin D were analysed, RR 0.94 (0.91-0.98, 95% CI). There was no mortality benefit found with Vitamin D2, or alfacalcidol or calcitrol. Also combining Vitamin D3 with calcium increased the risk of nephrolithiasis.

Putzu et al performed a meta-analysis to evaluate the effects of Vitamin D on outcomes in critically ill adults including mortality (Putzu et al., 2017). A total of seven RCTs were included. Six studies administered Vitamin D3 (cholecalciferol) and one study administered Vitamin D2 (ergocalciferol). All studies used placebo as control. Analysis based on the trials with low risk of bias (n=5) showed that administering Vitamin D in critically ill patients was associated with reduced mortality, (31.6% in the Vitamin D group compared with 40.1% in the control group), OR 0.70 (0.50-0.98, 95% CI) p=0.04. The effect on mortality remained when all seven studies were included in the analysis.

A recent independent participant data (IPD) meta-analysis of eight prospective European studies with a total of 26,916 participants found an association with lower Vitamin D levels and all-cause, and cardiovascular mortality but no association with cancer related mortality (Gaksch et al., 2017). The mean age of participants was 61.6 years with a mean 25(OH)D concentration of 53.8nmol/L. There were 6,802 deaths in the mean follow-up period of
10.5 years. Participants were grouped based on their Vitamin D level with deficiency defined as <30nmol/L, inadequacy; 30-39.9nmol/L and 40-49.9nmol/L and sufficiency; 75-99.9nmol/L. All cause mortality was significantly increased in those with serum 25(OH)D levels from 30-39.9nmol/L, HR 1.24 (1.07-1.42, 95% CI) and also in those with levels below 30nmol/L, HR 1.50 (1.28-1.71, 95%CI). Heterogeneity was reported as low.

Negative Studies
A meta-analysis was published by Chowdhury et al looking at the relationship between Vitamin D and mortality in observational cohort studies, along with quantifying the effects of Vitamin D supplementation on mortality when given alone and compared with placebo/no treatment in RCTs (Chowdhury et al., 2014). 95 studies were included comprising a total of 880,128 participants and 71,625 mortality outcomes. In 73 observational cohort studies, the median age of participants was 63 years and the follow-up period ranged from 0-29 years and the median baseline Vitamin D level was 51.7nmol/L. For primary prevention cohorts the pooled RR for all cause mortality for those in the lowest versus highest Vitamin D concentrations were 1.35 (1.22-1.49, 95%CI). In further analysis, for each 25nmol/L decline in Vitamin D concentration there was a 16% increase in all cause mortality. 22 RCTs were included in the analysis of Vitamin D supplementation and all-cause mortality with a follow-up period ranging from 0.38 to 6.8 years. Fourteen studies reported the effects of Vitamin D3 and the remaining eight reported the effects of Vitamin D2. The mean age of participants ranged from 56-85 years. There were a total of 2,527 all cause mortality events in intervention group versus 2,587 in the control group. The total RR was 0.8 (0.94-1.02, 95% CI), a RR of 0.89 (0.80-0.99, 95%CI) for Vitamin D3 supplementation and a RR of 1.04 (0.97-1.11, 95% CI) for Vitamin D2 supplementation.

Zheng et al performed a further meta-analysis in 2015 looking at the effect of high dose intermittent Vitamin D on fracture and overall mortality prevention in adults (Zheng et al., 2015). RCTs referring to high dose intermittent Vitamin D supplementation were included, daily dosing was excluded, the dose of Vitamin D was >100,000IU (with or without calcium supplementation) and treatment times must be greater than one month. Controls
received no treatment or calcium alone. Of the nine trials included, mortality data was available in seven, hip fracture data in four, falls data in eight and vertebral fracture data in five. Follow-up ranged from 6 months to 5 years. Mean age ranged from 77-85 years. This study showed no significant benefit from intermittent high dose Vitamin D in the risk of all-cause mortality falls and fracture risk.

6.10 Discussion

In recent years there have been many studies assessing the effects of Vitamin D on mortality. Cross-sectional and prospective studies suggest an association between Vitamin D and mortality risk, although there are inconsistencies in study findings.

Overall RCTs to date do not show a benefit in Vitamin D supplementation in mortality, although these studies were often carried out in specific populations such as ICU or post-operative patients, with the exception of the recent 2018 trial, and so results are not applicable to a more general population. Better management of patients in ICU may compensate for Vitamin D associated risk though this is obviously speculative. Meta-analyses also show mixed results without conclusive evidence to support the benefit of Vitamin D in all-cause mortality.

The variability in study results are likely due to multiple factors including limitations in individual study design; such as young study populations, participants who were not deficient in Vitamin D at baseline, short follow-up study periods for mortality data and incomplete or inadequate adjustment for confounding.

Further studies, including large prospective studies with prolonged follow-up periods, but ideally larger RCT of older adults who are deficient in Vitamin D are needed to fully evaluate the association between Vitamin D deficiency and mortality in older adults.
Chapter 7: Vitamin D and Cognition

7.1 Introduction
There have been a number of studies published in regarding Vitamin D status and its relationship to cognitive impairment, ranging from small case control studies to cross-sectional and longitudinal studies, with some conflicting results and findings. The effects of Vitamin D on the brain and on cognition are potentially mediated through a number of mechanisms, including effects on neurotransmitters, neuro-inflammation, the Vitamin D Receptor and genetic effects.

7.2 Vitamin D and the Brain
In the past it was thought that cerebral 1,25-dihydroxyvitamin D3 was derived from serum levels but the presence of Vitamin D Receptors (VDR) and 1α-hydroxylase in neuronal and glial cells of the human brain (Eyles et al., 2005), as well as 1,25(OH)2D3 in cerebrospinal fluid (CSF) suggest a biosynthetic pathway for Vitamin D within the central nervous system (CNS) (Balabanova et al., 1984).

Previous animal studies in the 1980s and 1990s confirmed the presence of VDR in animal models in both developing and adult brains of rats and hamsters (Balabanova et al., 1984, Musiol et al., 1992, Prufer et al., 1999, Stumpf and O’Brien, 1987). The earliest evidence to support the presence of VDR in the human brain came in 1992 via insitu hybridisation. Sutherland et al showed the presence of VDR mRNA in the brains of participants with AD and Huntington’s Disease (HD) post mortem (Sutherland et al., 1992).

Subsequently the distribution of VDR along with 1α-hydroxylase (CYP27B1) was mapped in the human brain using immunohistochemisrty and immunofluorescence (Eyles et al., 2005). The authors found both the enzyme and receptor present in neurons and glial cells throughout the brain with highest concentrations in the hypothalamus and within the large neurons of the substantia nigra.
There is also evidence of down regulation of VDR in the hippocampi of those with AD (Brewer et al., 2001) and long-term treatment with 1,25-OHD in rats has been shown to slow hippocampal aging and protect neuron density (Brewer et al., 2006). Also VDR polymorphisms are associated with development of AD and PD (Kim et al., 2005, Lefebvre d'Hellencourt et al., 2003).

### 7.3 Vitamin D: Neuroinflammation and Cytokines

Neuroinflammation is another postulated mechanism for cognitive impairment through an association between systemic inflammation and age-related cognitive decline (Alley et al., 2008, Bermejo et al., 2008) and also in the development of amyloid plaques (Cunningham, 2011).

Vitamin D is thought to be an anti-inflammatory agent with effects on T Cell function (Hayes et al., 2003) potentially reducing the production of TNF-α in the brain (van Etten and Mathieu, 2005) and also appears to inhibit the production of pro-inflammatory cytokines in the microglial cells (Lefebvre d'Hellencourt et al., 2003).

Animal studies have investigated the effects of Vitamin D supplementation on inflammatory markers in the brain. Oral Vitamin D has been shown to reduce production of pro-inflammatory cytokine IL-1β in the hippocampi of rats (Moore et al., 2005) and subcutaneous Vitamin D supplementation in older rats modulated age-related pro-inflammatory state, through IL-1β and IL-10 (van Etten and Mathieu, 2005).

### 7.4 Vitamin D and Neuronal Protection

Vitamin D is also thought to protect the structure and function of neurons by inhibiting nitric oxide synthase, (Garcion et al., 1997) up-regulating neurotrophins, (Neveu et al., 1994) and neuronal calcium homeostasis (Brewer et al., 2001).

Neurotrophins are proteins involved in neuronal survival in ageing and also in pathological processes affecting neurons (Siegel and Chauhan, 2000). Vitamin D up-regulates neurotrophins such as neurotrophin-3 (NT-3), which is found in the hippocampus and has
also been shown to up-regulate glial cell line derived neurotrophic factor (GDNF) (Neveu et al., 1994) which is involved in the survival and differentiation of dopaminergic cells in the brain and evidence supports its involvement in synaptogenesis (Ledda et al., 2007). Studies involving Vitamin D deficient new-born Sprague Dawley rats showed changes in their brains, including enlarged cortex and ventricles, reduced expression of GDNP and NGF with increased mitotic cells and reduced apoptotic cells compared with controls (Ko et al., 2004, McGrath et al., 2004).

Lastly, Nerve Growth Factor (NGF) is involved in nerve cell growth and survival, particularly in the cholinergic neurons of the forebrain and is also found in the hippocampal regions where it is involved in neurotransmission and synaptic plasticity (Korsching et al., 1985). Vitamin D has been shown to regulate a particular receptor to which NGF binds, which is involved in apoptosis (Naveilhan et al., 1996).

### 7.5 Vitamin D and Calcium Regulation

Vitamin D modulates calcium levels in the peripheral tissues of the body as well as L-type voltage calcium channels. Neurodegeneration, neuronal ageing and hippocampal volume loss have been attributed to L-type voltage sensitive calcium channel (L-VSCC) density and glucocorticoid toxicity (Kimura et al., 1998). Vitamin D appears to regulate neuronal calcium homeostasis and calcium entry into the brain, protecting neurons from excess calcium (Brewer et al., 2001).

Excessive calcium levels have been shown to have a negative effect on memory and cognitive functioning (Veng et al., 2003, Thibault et al., 2001) and elevated calcium levels intracellularly as well as L-type calcium channels have been observed in AD (Coon et al., 1999). β-Amyloid is also thought to cause neurodegeneration by inducing L-VSCC and by suppressing VDR expression (Dursun et al., 2011). Studies examining the effects of treatment with Vitamin D showed; in older rats there was restoration of L-type calcium channel activity similar to levels seen younger animals (Brewer et al., 2006) and down-regulation of L-VSCC activity and up-regulation of VDR levels (Dursun et al., 2011).
Vitamin D also modulates three calcium-binding proteins within the brain; carbindon, paralvumin and calretinin. These are uniquely distributed throughout the adult brain and act as calcium buffers and thus are believed to have neuro-protective effects (Fierro and Llano, 1996).

Calcitrol also appears to have a role in regulating abnormal intracellular levels of calcium in AD, as evidenced by finding in animal models; through decreasing L-VSCC activity in vulnerable neurons and reducing age-related changes due to calcium dysregulation (Brewer et al., 2001, Brewer et al., 2006).

7.6 Vitamin D and Vascular Disease
Vascular disease is a risk factor for cerebral dysfunction and is also a risk factor for AD. Langa et al estimate the prevalence of mixed dementia, with both AD and VD pathology, in approximately 45% of cases (Langa et al., 2004). Disturbance of glucose metabolism is also a feature of brains of patient’s with AD and diabetes itself is a risk factor for AD (Ott et al., 1999, Kroner, 2009).

Vitamin D deficiency has also been linked to vascular risk factors, such as hypertension (Vaidya and Forman, 2010), diabetes (Pittas et al., 2007) and incident cardiovascular disease (Wang et al., 2008). Reduced Vitamin D levels have been found in patients with obesity (Liel et al., 1988) and serum 25(OH)D levels have been found to be inversely associated with body weight (Cheng et al., 2010). Insufficient levels of Vitamin D have been found to related to large vessel infarcts, white matter hyperintensity volume (Buell et al., 2010) and atrial fibrillation (Demir et al., 2014). Therapeutic intervention with Vitamin D has been found to modulate blood pressure control and plasma rennin activity (Burgess et al., 1990, Lind et al., 1989). Pre-treatment with 1,25(OH)2D3 for eight days in adult rats with ligated middle cerebral artery ligate reduced the volume cerebral infarction (Wang et al., 2000).

Vitamin D may help modify the damage mediated by vascular disease by ameliorating the effects of inflammation, calcium regulation and oxidative stress. During ischaemic events,
transforming growth factor (TGF) and GDNF are unregulated in the hippocampus to promote survival. Vitamin D helps antioxidant defences by increasing glutathione and GDNF concentrations (Wion et al., 1991). Vitamin D has been shown to inhibit antigen presenting cell maturation and stimulate anti-inflammatory cytokine production in both in-vitro and animal models (Timms et al., 2002, Carthy et al., 1989). Also Vitamin D has been shown to have an inverse relationship with inflammatory markers such as CRP in prior epidemiological studies (Timms et al., 2002).

7.7 Vitamin D and Alzheimer’s disease (AD)
Pathological characteristics of AD include hippocampal volume and neuronal loss with the presence of amyloid plaques and tangles. Concentration of the 42 amino acid form of amyloid B, total-tau and hyper-phosphorylated-tau in the CSF are considered to be the core biomarkers for AD (Blennow et al., 2010).

Early in the AD process, the hippocampus is affected. Sutherland et al found there was a reduction in the VDR mRNA levels in the hippocampal CA1 and CA2 pyramidal cells of humans with AD compared with controls (Sutherland et al., 1992). Studies have shown that treatment with Vitamin D attenuated hippocampal atrophy and protected neuronal density in ageing rats (Landfield and Cadwallader-Neal, 1998). In 2014, a study showed that higher plasma 25(OH)D levels were associated with increased CSF β-amyloid levels, increased cerebral volumes of the medial temporal lobe (Hooshmand et al., 2014).

Treatment with 1,25(OH)2D3 has also been shown to reduce β-amyloid (Aβ) peptide concentrations by increasing amyloid efflux from the brain and possibly improved permeability across the blood-brain barrier (Ito et al., 2011b, Ito et al., 2011a). Another possible mechanism of Vitamin D in AD relates to macrophage function. A study in 2011 showed treatment with 1,25(OH)2D3 improved macrophage ability to phagocytose soluble Aβ protein (Mizwicki et al., 2012).

Also higher concentrations of VDR polymorphisms have been found in brains with AD compared with controls (Gezen-Ak et al., 2007). There are higher levels of Vitamin D
Binding Protein (DBP) in the CSF of those with AD. DBP was found to prevent deaths mediated by Aβ in mouse cultured hippocampal cells and also reduced synaptic loss due to Aβ in mice post injection of Aβ into the lateral ventricle (Moon et al., 2013).

7.8 Vitamin D and Cognition: Cross Sectional Studies

Positive Studies

A Japanese study showed high prevalence of Vitamin D deficiency in elderly women with Alzheimer’s disease (AD) (Sato et al., 1998). Participants (n=46) were recruited from a nursing home, including ambulatory women aged over seventy years who met the Diagnostic and Statistical Manual of Mental Disorders Third Edition (DSM III) and National Institute of Neurological and Communicative Disorders/Alzheimer’s Disease and Related Disorders Association (NINCDS ADRDA) criteria for dementia and probable AD. Control group (n=140) included healthy aged matched women without fractures recruited from the community. Controls were younger, had higher BMI, BMD and serum 25(OH)D (53.9nmol/L versus 17.7nmol/L, P<0.0001) and 1,25(OH)2D3 levels than patient group.

A retrospective chart review of patients attending a community-based clinic was performed to assess the association between Vitamin D and B12 deficiency and cognitive impairment (Przybelski and Binkley, 2007). Full data including Mini Mental State Examination (MMSE), Vitamin D and B12 levels were available for 32 participants. Mean MMSE was 19.2 and 78% of participants had 25(OH)D level <75nmol/L (defined as suboptimal in this study). A correlation between 25(OH)D concentration and MMSE score was noted, however this was a small study with no potential confounders considered.

Wilkins et al performed a small cross sectional study in 2006 comparing 40 persons with AD and 40 non-demented persons (Wilkins et al., 2006). The mean Vitamin D level in this group was 46.5nmol/L suboptimal levels, defined as <44nmol/L were found in 58% of the sample. Vitamin D deficiency was associated with worse performance on the Short Blessed Test (SBT), which is a 6-item cognitive assessment tool, and poorer scores on the Clinical Dementia Rating (CDR) sum of boxes.
In 2008 a cross-sectional study of 962 patients seen in a geriatric outpatient clinic with a mean age of 77.6 years was reported (Oudshoorn et al., 2008). Vitamin D status was compared with cognitive function measured by MMSE in a subset of 225 patients given a consensus diagnosis of probable AD. Dementia was diagnosed using DSM-IV criteria and probable AD was diagnosed based on NINCDS-ADRDA criteria. Mean MMSE was 45.4nmol/L and 63% of the group had 25(OH)D levels <50nmol/L. This study showed a likely association between Vitamin D and cognitive function with a lower Vitamin D level associated with lower MMSE score. The mean MMSE score in those with 25(OH)D <50nmol/L was 18.5 versus 21.5 in the Vitamin D replete group.

A study of 60 patients, comparing African Americans to European Americans revealed a lower Vitamin D level in the African American subgroup (Wilkins et al., 2009). Cognitive assessment included the Short Blessed Test (SBT) and Clinical Dementia Rating (CDR). Subjects were divided into two groups: normal 25(OH)D levels (>50nmol/L) (n=31) and deficient (<50nmol/L) (n=29). Mean 25(OH)D level was 48nmol/L. At baseline, the African Americans had a lower mean 25(OH)D level at 44.8nmol/L than their European counterparts with a mean of 55.5nmol/L. Those in the Vitamin D deficient group performed worse on SBT and Physical Performance Test (PPT) than those in the "normal" group. No differences were noted on MMSE.

A further cross-sectional study, as part of the Nutrition And Memory in Elders (NAME) study, of 1,080 urban dwellers aged >60 years (mean age 75.7 years) in the Boston area was performed (Buell et al., 2009). Participants with MMSE <10 or verbal IQ <75, measured using the North American Adult Reading Test (NAART), were excluded. The study group consisted of 703 non-black and 377 black participants, and were 75% female. The black population tended to be younger, less educated, have higher BMI, higher prevalence of diabetes and hypertension, but had lower 25(OH)D levels at 40.8nmol/L versus the non-black group at 47nmol/L. The full cognitive test battery included the Wechsler Memory Scale (WMS III), NAART, Trails A and B, matrix reasoning and digit symbol coding. Vitamin D levels were defined as sufficient (>50nmol/L), insufficient (25-50nmol/L) and deficient (25nmol/L). Results showed that lower Vitamin D levels were
associated with lower scores on tests of executive function (including Trails A and B, matrix reasoning and digit symbol coding) and with poorer scores on tests of attention and processing speed. No significant association was noted on tests of memory (including WMS III recall and logical memory recognition).

From the European Male Aging Study (EMAS), in 3,133 non-institutionalised males with a mean age of 59.9 years, the association between vitamin D (deficiency <25nmol/L, insufficient 25-49nmol/L, suboptimal 50-75nmol/L) and cognition was assessed (Lee et al., 2009). Cognitive tests included the Rey-Osterrieth Complex Figure (ROCF), Camden Topographical Recognition Memory (CTRM) and Digital Symbol Substitution Test (DSST), which is a measure of speed of information processing. A positive association between Vitamin D and DSST was noted in fully adjusted models, particularly at levels <35nmol/L. No association was noted between Vitamin D and ROCF or CTRM tests.

A study from 2009 examined if low serum 25(OH)D was associated with increased odds of cognitive impairment in 1,766 adults residing in care homes recruited from the Health Survey for England (Llewellyn et al., 2009). Cognitive function was measured with the Abbreviated Mental Test Score (AMTS). 1,554 were deemed as cognitively normal and 212 as cognitively impaired. In unadjusted models, those in the lowest 25(OH)D quartile were more likely to be cognitively impaired with a linear relationship noted, OR 4.17 (2.56-6.78, 95% CI) p <0.001. In fully adjusted models the association was attenuated but remained present, OR 2.28 (1.36-3.83, 95% CI). In analysis by sex, this association was only noted in males.

A multicentre study, Epidemiology of Osteoporosis (EPIDOS), with 5,596 community dwelling females with mean age 80.9 years, reported an association between dietary Vitamin D intake and global cognitive function. Cognition was measured using the Short Portable Mental State Questionnaire (SPMSQ) with impairment defined as a score <8 (Annweiler et al., 2010c). 14.2% of these women had inadequate dietary intake of Vitamin D, defined as <35mcg/week and 11.2% were deemed cognitively impaired based on study
criteria. Those with lower weekly Vitamin D intake, which was estimated using food frequency questionnaire, were more likely to have poorer scores on SPMSQ.

A further study, from EPI DOS, of 752 French women, with a mean age of 80.7 years, investigated the relationship between Vitamin D levels and cognition (Annweiler et al., 2010b). Cognition was assessed and defined as outlined above. Patient groups were divided based on Vitamin D concentrations of >25nmol/L and <25nmol/L and 17.2% of participants were found to have Vitamin D level <25nmol/L. An association between Vitamin D deficiency and cognitive impairment was found, with those in the <25nmol/L group more had a lower score on SPMSQ. Vitamin D deficiency was associated with cognitive impairment in logistic regression models, with OR 2.03 (1.17-3.53, 95% CI) after confounders were considered.

Buell et al reported an association between Vitamin D and dementia and cerebrovascular pathology in nursing home care receivers from the NAME study (Buell et al., 2010). The mean age of the 318 participants was 73.5 years and mean Vitamin D level of 47.9nmol/L. Seventy six participants were classified as having AD, 21 as Vascular Dementia (VD), 31 with stroke and no dementia and 14 were classified as having dementia of other aetiology. Vitamin D deficiency (<50nmol/L) was associated with increased risk of all cause dementia, OR 2.21 (1.13-4.32, 95% CI) and was associated with increased risk of AD, in fully adjusted models, OR 2.65 (0.99-7.16, 95% CI). It was also found to be associated with increased risk of stroke (with or without dementia) OR 2.04 (1.03-4.04, 95%CI).

Seamans et al also showed an association between Vitamin D and cognition in a study involving 387 community dwelling Europeans aged between 55-87 years (Seamans et al., 2010). The Cambridge Neuropsychological Testing Automated Battery (CANTAB) was used to assess cognition in areas of visual, working memory and attention. 48% of this study population had Vitamin D level <50nmol/L. Serum Vitamin D concentrations were found to be inversely related to scores on subsets of Spatial Working Memory (SWM), with higher error rates in those with lower Vitamin D levels.
A study from NHANES reported an association between Vitamin D deficiency and cognitive impairment in a subset of 3,396 community dwelling adults with a mean age 73.7 years (Llewellyn et al., 2011). A composite score of different aspects of the neuropsychological battery used in the original NHANES III study was used and cognitive impairment was defined as the lowest 10% of distribution of cognitive performance. Vitamin D levels were classified as follows: sufficient >75nmol/L, insufficient 50-75nmol/L, deficient 25-50nmol/L and severely deficient <25nmol/L. Those deficient or severely deficient in Vitamin D were more likely to cognitively impaired, OR 3.68 with 25(OH)D levels <25nmol/L in fully adjusted models.

In a study of 288 patients, with a mean age of 86 years, admitted to an acute geriatric unit the association between Vitamin D deficiency and dementia was assessed. Dementia was diagnosed based on the DSM-IV. Of this sample, 33% were classed as moderate to severe dementia (MMSE <15) (Annweiler et al., 2011a). Mean serum 25(OH)D was 35.3nmol/L and severe deficiency (<25nmol/L) was associated with moderate to severe dementia, OR 2.57 in the fully adjusted model.

A Polish study in 2012 assessed the link between Vitamin D and cognition and also grip strength and balance in 140 patients from a geriatric ward or long term care facility (Skalska et al., 2012). Cognition was assessed using the AMTS and muscle strength measured using the Hand Grip strength and mobility was assessed with TUG. The mean age of participants was 79.6 years and mean 25(OH)D level was 44.7nmol/L. Analysis revealed that Vitamin D in the tertile of 23.3-47.8nmol/L was associated with increased risk of poorer performance on AMTS, OR 3.2 (1.0 – 9.7, 95% CI) p=0.04. This is a small study with a very crude cognitive test used.

Results from the Chinese Longitudinal Healthy Longevity Survey (CLHLS) showed a relationship between Vitamin D and cognitive function (Chei et al., 2014). Cognitive function was measured with the MMSE, and cognitive impairment was defined as an MMSE of <18. Of the total 2,378 participants, 2,004 were included in the analysis and those without Vitamin D levels and MMSE scores were excluded. Mean age of the study...
population was 84.9 years with a mean Vitamin D of 43.1nmol/L. Multivariate analysis of quartiles of Vitamin D and cognitive impairment showed there was an association with low Vitamin D levels and cognitive impairment in fully adjusted models, OR 2.15 (1.05-4.41, 95%CI) p=0.05.

Peterson et al performed a cohort study evaluating the relationship between serum Vitamin D and motor and cognitive change in 159 community dwelling older adults (Peterson et al., 2012). The mean age was 85 years with a mean Vitamin D level of 94.1nmol/L. Cognition was assessed across a number of domains including Memory, Attention, Processing speeds, Executive function and visuospatial construction. There was an association between Vitamin D and global cognitive scores but not on individual tests of cognitive domains. Those with mild dementia (measured using the Clinical Dementia Rating (CDR) scale) had lower levels of Vitamin D compared with cognitively intact subjects p=0.02.

A study including 253 women with a mean age of 77.5 years and mean 25-hydroxyvitamin D level of 58.9nmol/L reported an association between the volume of white matter hyperintensity (WMH) and atrophy on Magnetic Resonance Imaging (MRI) of the brain in older women with a diagnosis of either amnestic Mild Cognitive Impairment (aMCI) or AD (Sakurai et al., 2014). Adjusted multivariate regression models revealed an inverse association between 25(OH)D and WMH volume, β= -0.163, p= 0.008. There was no association between Vitamin D and parenchymal volume demonstrated.

Pre-hospital Vitamin D concentrations were found to be associated with hospital acquired new onset delirium (HANDO) in a recent study (Quraishi et al., 2015). The population was abstracted from databases relating to patients admitted to two hospitals in Boston. The final study cohort consisted 198 (4%) of the participants with HANDO. Association between Vitamin D and HANOD was statistically significant, OR 2.15 (1.32-3.5, 95% CI) for 25(OH)D levels <25nmol/L.

In 2015, Ahn et al investigated the cross-sectional relationship between physical fitness, Vitamin D and cognition in the elderly (Ahn and Kang, 2015). A total of 467 participants
aged >65 years were recruited from eight community healthcare facilities in South Korea over three separate visits. The assessments included cognitive screening with the MMSE, physical fitness assessment with the Senior Fitness Test (SFT) and demographic data and 25(OH)D measurement. For the 412 participants included, the mean age of participants was 73.4 years, mean MMSE was 25.8 and the mean Vitamin D level was 50nmol/L. In fully adjusted regression models, Vitamin D was found to be a predictor of cognitive function assessed with the MMSE, R² 0.210, p=0.012.

Darwish et al examined the role of Vitamin D in adults aged 30-60 years and those aged >60 years in Beirut (Darwish et al., 2015). 254 adults aged >30 years were recruited through a University affiliated medical centre outpatient clinics and nursing homes (NH) in the greater Beirut area. 22/97 older adults (>60 years) were recruited from NH. Cognitive assessments included the Montreal Cognitive Assessment (MoCA), ROCF trial and recognition tests, Symbol Digit Modalities Test (SDMT), a screening questionnaire that inquires about the participant’s general health (The Brief Risk Factor Surveillance System). Of the 254 included, 61.8% of adults were aged 30-60 years and 38.2% were aged >60 years. 67% of participants had received >12 years education and the mean 25(OH)D level was 67.7nmol/L. On bivariate correlation analysis, 25(OH)D level was positively correlated with ROCF immediate and delayed recall, r=0.19 and r=0.183 respectively. In older adults as well as the ROCF, 25(OH)D was correlated with SDMT r=0.260. 25(OH)D was not found to correlate with MoCA.

A study from Asia in 2015 looked at the relationship between Vitamin D and vascular dementia secondary to small vessel disease (Prabhakar et al., 2015). Participants were >60 years who presented to the Neurological services at the National Institute of Neurosciences in Bangalore India. Inclusion criterion consisted of the presence of vascular cognitive impairment and the presence of lacunes or Periventricular White Matter Ischaemia (PVWMI) (graded using Feazekas rating scale) on MRI Brain scan. A control group was also recruited and consisted of age and sex matched "clinically normal" subjects without history of CVD. The total study population consisted of 140 cases and 132 controls. There was no significant difference between the groups in terms of 25(OH)D levels, cases:
39.7nmol/L and controls 41.4nmol/L. In fully adjusted regression models for 25(OH)D levels <30nmol/L and vascular dementia revealed, OR 21.9 (1.03-6.09, 95% CI). However in the same model for those with insufficient Vitamin D levels (30-50nmol/L), a non-significant association was found.

Vedak et al investigated the relationship between Vitamin D and cognition in an elderly Indian population (Vedak et al., 2015). 25(OH)D levels were measured in those patients with dementia and MCI and were compared to an age matched elderly population of controls. 86 participants aged >50 years were enrolled and subdivided into three groups: AD, VD (Vascular Dementia) and VCI (Vascular Cognitive Impairment) (not demented), n=32, n=24 in MCI group and n=30 in the control group. Cognitive assessments included the MMSE and Addenbrooks Cognitive Examination (ACE). MMSE scores of <24 and ACE scores of <90 were considered as indicating cognitive impairment. The mean age of those with dementia was 68.5 years versus 65.4 years in the MCI group 66.8 years in the controls. Those with dementia had a lower mean MMSE at 20.3 versus 27.5 in the MCI group. The mean serum 25(OH)D level was also significantly lower in those with dementia at 18.9nmol/L compared with MCI group at 45.5nmol/L and controls at 50.1nmol/L. Spearman’s coefficient revealed a positive correlation between 25(OH)D levels and cognitive decline at r=0.5 for MMSE, p<0.001 and r=0.53 for ACE, p<0.001.

Pettersen evaluated if supra-therapeutic levels of Vitamin D affected executive functioning in a Canadian population (Pettersen, 2015). 142 healthy adults were included. Inclusion criteria was age >20 years and no physical or visual impairment that would impact on ability to complete cognitive assessment. Cognition was assessed using a broad number of tests including phonemic fluency, forward and backward digit span tests of working memory and also verbal recognition memory recall. Demographic information was obtained using a self-reported questionnaire. The mean age of participants was 56.3 years with a mean Vitamin D level of 80nmol/L. 62% of the population were taking Vitamin D supplementation. Participants were subdivided into groups based on their Vitamin D concentration, insufficient (<50nmol/L), sufficient (50-75nmol/L), high sufficient (75-100nmol/L) and supra-therapeutic (>100nmol/L). Those in the insufficient group were
younger than those in the supra-therapeutic group, 47.3 v 64.0 years respectively. When Vitamin D levels were compared with performance across a number of different cognitive domains, the means scores increased for verbal fluency (VF) subsets and this was found to be statistically significant (insufficient group mean VF score 12.0 versus supra-therapeutic group M=13.1, (p=0.048). In multiple regression models, only the analysis predicting VF scores was found to be significant, $R^2 =0.14$, $F=3.05$, p<0.005.

A study involving a population from Singapore reported an association between Vitamin D deficiency and cognitive impairment, assessed with the AMT (Annweiler et al., 2016). Participants (n=2,273) were involved in the Singapore Kidney Eye Study with a mean age of 70.4 years, mean AMT of 8.2 and mean 25(OH)D level of 58.2nmol/L. In univariate and multivariate analysis, increased Vitamin D levels were associated with increased AMT scores, $\beta= 0.08$, p=0.029.

A recent cross-sectional study looked at the relationship between serum Vitamin D and Alzheimer’s disease (AD) and subcortical dementia (sVAD) (Moretti et al., 2017). Participants with AD (n=87) were compared with those with sVAD (n=456) and controls (n=567). Those with AD or sVAD were more likely to be deficient in Vitamin D compared with controls. Univariate regression analysis revealed that Vitamin D levels were associated with AD, OR 5.6 (8.9-11.6, 95% CI) and sVAD, OR 6.7 (7.3-9.6, 95% CI). There appeared to be an inverse relationship between Vitamin D levels and AD and sVAD in this study.

A recent cross-sectional study assessed the association between 25(OH)D and 1,25(OH)\textsubscript{2}D\textsubscript{3} levels in patients with Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (AD) (Ouma et al., 2018). This study included 230 participants subdivided in to three groups, healthy subjects (HS, n=61, mean age 74.5), MCI (n=61, mean age 75.5) and AD subdivided into mild (n=41, mean age 74.8) moderate (n=35, mean age 82.2) and severe (n=32, mean age 77.7). MCI was diagnosed using Peterson’s criteria and severity of AD was classified using the MMSE (mild 20-27, moderate 10-20, severe <10). The mean 25(OH)D levels in HS was 65.3nmol/L in women and 68.4nmol/L in men, while in the
MCI participants these levels were significantly lower; 45.5nmol/L in women and 52.5nmol/L in men p=0.003. There was no difference in Vitamin D levels between MCI and AD participants. No significant difference in 1,25(OH)_{2}D_{3} levels between MCI and AD groups was detected. In univariate analysis, only gender was found to be a predictor of MMSE in HS, while 25(OH)D was a predictor of MMSE variability in MCI and AD groups. 1,25(OH)_{2}D_{3} was not found to be significant predictor of MMSE. In ROC analysis, 25(OH)D appeared to show differential diagnosis power in MCI; AUC 0.77 (0.85-0.96, 95% CI), sensitivity 90%, specificity 54% and differential diagnosis power in AD; AUC 0.84 (0.78-0.90, 95% CI) sensitivity 97%, specificity 79%.

A further study from the US showed an inverse relationship between Vitamin D and cognitive impairment (Pavlovic et al., 2018). Participants (n=4,358) were recruited from a preventative medicine clinic in Texas. 27% were men and all were aged >55 years. Cognitive function was assessed with the MoCA with a score of <25 defined as indicating cognitive impairment. The mean age of participants was 60.8 years, with a mean 25(OH)D level of 90.4nmol/L and mean MoCA score of 26.9. In those with lower Vitamin D concentrations (<75nmol/L), there was an increased odds of having cognitive impairment on MoCA, OR 1.24 (1.01-1.51, 95% CI) compared with those participants with levels >74nmol/L.

**Negative Studies**

McGrath et al assessed the relationship between 25(OH)D levels and cognition in a population, from NHANES III, of greater than 11,000, divided into three age groups, adolescents (12-17 years), adults (20-60 years) and elderly (60-90 years) (McGrath et al., 2007). They did not find an association between Vitamin D deficiency and cognitive impairment, but those with higher levels of 25(OH)D were more impaired on learning and memory tasks.

In a study of delirium in ICU patients, the relationship between serum Vitamin D and delirium in the VALID study population was investigated (Morandi et al., 2013). All patients who were screened for delirium by two assessors were included. Patients were
assessed daily for delirium using the Confusion Assessment Measurement for ICU (CAM ICU) and 25(OH)D was taken within 24 hours of ICU admission. 120 patients were enrolled. Median age was 52 years, mean Vitamin D was 38nmol/L, mean LOS in ICU was 9 days and prevalence of delirium on day one was 41%. In multivariate analysis, there was no association found between Vitamin D and APACHE II score (marker of severity of illness). There was no association between 25(OH)D and delirium on the day of measurement or day after, OR 1.01 (0.99-1.03, 95% CI).

A cross sectional study from a memory clinic in France, reports an association between Vitamin D and executive dysfunction in a population of 110 adults with subjective memory complaints aged >60 years (Annweiler et al., 2014). Executive function was measured using the Trails B, the N-Back Test, Stroop Test and the Go No Go Test. The mean age of participants was 71.0 years, mean MMSE of 28.2 and mean Vitamin D levels of 60.4nmol/L. There was a trend for association between Vitamin D deficiency and Trails B on multivariate analysis, β= 1.48 (0.36 - 2.61, 95% CI) p=0.01. Otherwise this small study showed no relationship between Vitamin D and executive function tests.

Lam et al looked at the association of Vitamin D and verbal episodic memory in an Australian study (Lam et al., 2015). A total of 250 adults aged 40 years and older were recruited between December 2011 and February 2012. 40 participants were excluded and of the remaining, 23 did not complete the cognitive testing (RAVLT) and 2 did not complete the NART, a total of 179 were included in the final analysis. The mean age of participants was 65.7 years and the mean Vitamin D level was 84.7nmol/L. On subsets of the RAVLT there was some suggestion of an association between Vitamin D and episodic memory, however these subsets are difficult to interpret individually and thus of unlikely significance.

A study from 2018 found no cross-sectional association between Vitamin D levels and Beta-amyloid (Aβ) levels an Apolipoprotein E (ApoE) genotype (Nourhashemi et al., 2018). Participants and data were derived from the Multidomain Alzheimer’s Prevention Trial (MAPT) which included 178 people with data available for 25(OH)D levels and
cerebral amyloid using Positron Emission Tomography (PET). Cortical regional standard uptake value ratios (SUVRs) were generated using the whole cerebellum as the reference region and obtaining mean signals from pre-defined cortical regions. Participants were excluded from the analysis if they had a CDR >1 as this is suggestive of dementia. Participants had a mean age of 76.2 years and mean 25(OH)D level of 55.9nmol/L and 60% of participants were female. Vitamin D level was not associated with cortical Aβ load or with ApoE ε4 status.
<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Cognitive Assessment</th>
<th>Confounders</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
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<tr>
<td>Sato et al 1998</td>
<td>46</td>
<td>Not available</td>
<td>Cases: NH Residents with AD Controls: Community Dwellers, No AD</td>
<td>Not available</td>
<td>53.9nmol/L v 17.7nmol/L</td>
<td>Not available</td>
<td>Not available</td>
<td>Cases more likely to be deficient in Vitamin D than controls</td>
</tr>
<tr>
<td>Wilkins et al 2006</td>
<td>80</td>
<td>74.8</td>
<td>40 with AD and 40 non-demented</td>
<td>Not available</td>
<td>47.3nmol/L</td>
<td>SBT MMSE CDR</td>
<td>Age, Race, Gender, Season</td>
<td>Association between Vitamin D deficiency and impairment on SBT &amp; CDR None with MMSE</td>
</tr>
<tr>
<td>Przybelski et al 2007</td>
<td>32</td>
<td>79.5</td>
<td>MMSE, Vitamin D and B12 measurements on same day</td>
<td>Not available</td>
<td>54nmol/L</td>
<td>MMSE</td>
<td>Not available</td>
<td>Association between MMSE and Vitamin D deficiency</td>
</tr>
<tr>
<td>Oudshoorn et al 2008</td>
<td>225</td>
<td>77.6</td>
<td>Consensus Diagnosis AD</td>
<td>Vitamin supplements</td>
<td>45.4nmol/L</td>
<td>MMSE</td>
<td>Age, Gender, Mobility, Education, Sunlight Exposure, B1, B6 and B12</td>
<td>Association with Vitamin D deficiency &amp; lower MMSE</td>
</tr>
<tr>
<td>Wilkins et al 2009</td>
<td>60</td>
<td>79.5</td>
<td>&gt;55years Independently Mobile Mild CI</td>
<td>Stroke, Cognitive Impairment, CKD, PD, Osteoporosis meds or Vitamin D</td>
<td>48nmol/L</td>
<td>MMSE SBT</td>
<td>Age, Gender, Race, Education, Weight</td>
<td>Association with SBT and Vitamin D deficiency No association with MMSE</td>
</tr>
</tbody>
</table>

Table 6: Vitamin D and Cognition: Cross Sectional Studies
<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Cognitive Assessment</th>
<th>Confounders</th>
<th>Results</th>
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</thead>
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<tr>
<td><strong>Positive</strong></td>
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<tr>
<td>Llewellyn et al 2009 UK HSE</td>
<td>1,766</td>
<td>78.2</td>
<td>Household Dwellers (adults and children) NH Residents (&gt; 65 years)</td>
<td>None Stated</td>
<td>Not available (Quintiles)</td>
<td>AMTS</td>
<td>Age, Gender, Ethnicity, Season, Stroke, Smoking, DM, Alcohol, HTN Mobility</td>
<td>Association between Vitamin D and lower AMTS. Only in males when analysed by gender</td>
</tr>
<tr>
<td>Lee et al 2009 UK/Europe EMAS</td>
<td>3,133</td>
<td>59.9</td>
<td>Non-institutionalised men 40-79 years</td>
<td>None Stated</td>
<td>62.5nmol/L</td>
<td>ROCF, DSST, CTRM</td>
<td>Age, Education, Depression, BMI, Smoking, Alcohol, Season Physical activity</td>
<td>Association with DSST with Vitamin D &lt;35nmol/L No association on CTRM or ROCF</td>
</tr>
<tr>
<td>Buell et al 2009 USA</td>
<td>1,080</td>
<td>75.7</td>
<td>English speaking &gt;60 years No hearing or visual impairment</td>
<td>HIV, Epilepsy, BPAD, Schizophrenia, Brain tumour, MMSE &lt;10 NAART &lt;75</td>
<td>47nmol/L</td>
<td>MMSE, WMS III, NAART, Trails A/B, Matrix Reasoning, DSC</td>
<td>Age, Gender, Race, Education, BMI, Kidney function, Season, Alcohol</td>
<td>Association on tests of executive function and attention/processing speed No association on memory tests</td>
</tr>
<tr>
<td>Annweiler et al 2010 France EPIDOS</td>
<td>752</td>
<td>80.4</td>
<td>&gt;75 years Healthy French females Community Dwelling</td>
<td>Inability to mobilise independently, Hip fracture, Bilateral hip replacement, Inability to understand or answer question</td>
<td>18nmol/L</td>
<td>SPMSQ</td>
<td>Age, Physical activity, BMI, Depression, Chronic diseases, DM, Education, PTH, Psychoactive medication</td>
<td>Association between Vitamin D deficiency (&lt;25nmol/L) and impairment on SPMSQ</td>
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<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Seamans et al 2010</td>
<td>380</td>
<td>68</td>
<td>Healthy men</td>
<td>Dementia, Major physical or mental health problems, Taking &gt;3 medications</td>
<td>76.2nmol/L</td>
<td>CANTAB</td>
<td>Visual &amp; Working Memory Attention</td>
<td>Association with Spatial Working Memory (Only in females)</td>
</tr>
<tr>
<td>ZENTH</td>
<td></td>
<td></td>
<td>55-70 years &amp; 70-87 years</td>
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<tr>
<td>Annweiler et al 2010</td>
<td>5,596</td>
<td>80.7</td>
<td>&gt;75 years</td>
<td>Inability to mobilise independently, hip fracture, Bilateral hip replacement, Inability to understand</td>
<td>Assessed dietary intake. No measures of serum Vitamin D</td>
<td>SPMSQ</td>
<td>Age, ADLs, Chronic disease, BMI, Education, Depression, Season, Sunlight exposure, Psychoactive medication</td>
<td>Association between Vitamin D intake and cognitive impairment on SPMSQ</td>
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<tr>
<td>France</td>
<td></td>
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<td>Community Dwelling Females</td>
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<td>EPIDOS</td>
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<td>No Vitamin D supplements</td>
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<tr>
<td>Buell et al 2010</td>
<td>318</td>
<td>73.5</td>
<td>English speaking</td>
<td>HIV, Epilepsy, BPAD, Schizophrenia, Brain tumour, MMSE &lt;10 NAART &lt;75</td>
<td>47.9nmol/L</td>
<td>DSM IV</td>
<td>NINDS-AIREN Criteria</td>
<td>Vitamin D deficiency associated with all cause dementia and stroke</td>
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<tr>
<td>NAME</td>
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<td></td>
<td>&gt;60 years</td>
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<td>No hearing or visual impairment</td>
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<tr>
<td>Llewellyn et al 2011</td>
<td>3,396</td>
<td>73.7</td>
<td>60-90 years</td>
<td>None Stated</td>
<td>29%:&lt;50nmol/L</td>
<td>Composite of MMSE, WAIS and East Boston Memory Test</td>
<td>Age, Gender, Race, Education, BMI, Kidney function, Season, Alcohol</td>
<td>Vitamin D deficiency associated with cognitive impairment on composite score</td>
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<tr>
<td>USA</td>
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<td>Household US population</td>
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<td>2.7%:&lt;25nmol/L</td>
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<tr>
<td>NHANES III</td>
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<td>Author</td>
<td>No.</td>
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<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Annweiler et al 2011 France</td>
<td>288</td>
<td>86</td>
<td>Moderate to Severe Dementia MMSE &lt;15</td>
<td>“Confused patients” excluded based on CAM</td>
<td>Mean 35.2nmol/L</td>
<td>MMSE 33% had mod-severe dementia</td>
<td>Age, Gender, Co-Morbid Illnesses, Psychoactive meds, Albumin, Morphine, Steroids, PTH</td>
<td>Association between Vitamin D deficiency and moderate to severe cog impairment</td>
</tr>
<tr>
<td>Peterson et al 2012 USA</td>
<td>159</td>
<td>85</td>
<td>Independent living &gt;80 years Not demented CDR &lt;0.5 MMSE &gt;24</td>
<td>Not stated</td>
<td>94.1nmol/L</td>
<td>MMSE CDR, Trails, Digit Symbol Coding, Letter Fluency, Stroop Test</td>
<td>Age, Gender, Education</td>
<td>Association between Vitamin D and global cognitive scores, and mild dementia (CDR)</td>
</tr>
<tr>
<td>Skalska et al 2012 Poland</td>
<td>140</td>
<td>79.6</td>
<td>Age &gt;60 years Independent mobility (with or without aid)</td>
<td>Acute illness</td>
<td>44.7nmol/L</td>
<td>AMTS</td>
<td>Age, Gender, BP, Falls in last year, Number of medications, Use of walking aid</td>
<td>Vitamin D associated with poor cognition based on AMTS score</td>
</tr>
<tr>
<td>Chei et al 2014 China CLHLS</td>
<td>2,004</td>
<td>84.9</td>
<td>Community Dwelling</td>
<td>Not available</td>
<td>43.1nmol/L</td>
<td>MMSE</td>
<td>Age, Gender, BMI, BP, Education, GFR, ADLs, Smoking, Chronic Conditions</td>
<td>Association between lower Vitamin D quartiles and CI (MMSE &lt;18)</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Annweiler et al 2014</td>
<td>253</td>
<td>77.5</td>
<td>Women &gt;65 years</td>
<td>Severe Cardiac Failure, Renal or Liver Disorders, Musculoskeletal Disease, Cortical Lesion on MRI</td>
<td>58.6nM</td>
<td>MMSE</td>
<td>Age, HTN, DM, CKD, Lipid Abnormality</td>
<td>Inverse association between Vitamin D and White Matter Intensities on MRI Brain</td>
</tr>
<tr>
<td>Sakur et al 2014</td>
<td>253</td>
<td>77.5</td>
<td>Women &gt;65 years</td>
<td>Severe cardiac failure, renal, liver or musculoskeletal disorders, cortical lesion on MRI</td>
<td>58.9nmol/L</td>
<td>MMSE</td>
<td>HTN, Diabetes, Lipid abnormalities, CKD</td>
<td>An inverse association between 25(OH)D and WMH volume. No association with parenchymal volume demonstrated</td>
</tr>
<tr>
<td>Quraishi et al 2015</td>
<td>198</td>
<td>59</td>
<td>&gt;18 years Hospital Acquired New onset Delirium</td>
<td>No social security details Existing Dementia/ Delirium</td>
<td>54.9</td>
<td>Not available</td>
<td>Age, Gender, Race, Medical versus Surgical, Co-morbidity Index</td>
<td>Significant association between Vitamin D levels and new onset delirium</td>
</tr>
<tr>
<td>Darwish et al 2015</td>
<td>254</td>
<td>38.2% &gt;60 years</td>
<td>&gt;30 years</td>
<td>Neurological or Psychiatric Disorders, TBI, CI, C2H5OH or drug abuse</td>
<td>67.7nmol/L</td>
<td>MoCA ROCF Recognition Tests</td>
<td>SDMT</td>
<td>Not available</td>
</tr>
<tr>
<td>Pettersen et al 2015</td>
<td>142</td>
<td>56.3</td>
<td>&gt;20 years</td>
<td>Dementia, Brain Tumour, Stroke, Brain Trauma</td>
<td>80nmol/L</td>
<td>Phonemic Fluency, Forward/ Backward DST, Verbal Recognition &amp; Recall</td>
<td>Age, Gender, Education, Physical activity, BMI, BDI II, Vitamin D</td>
<td>Supra-therapeutic levels of Vitamin D (&gt;100nmol/L) were associated with Verbal Fluency only.</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Ahn et al 2015 Korea</td>
<td>412</td>
<td>73.4</td>
<td>&gt;65 years</td>
<td>Did not complete assessment</td>
<td>50</td>
<td>MMSE SFT</td>
<td>Age, Gender, GDS, Education, Co-Morbidity Index</td>
<td>Association between Vitamin D and MMSE scores</td>
</tr>
<tr>
<td>Prabhakar et al 2015 India</td>
<td>140</td>
<td>59.9</td>
<td>&gt;60 years</td>
<td>Large Vessel Stroke, Delirium, SOL, CNS Infection, Head Injury, Cerebral Irradiation</td>
<td>39.7nmol/L cases</td>
<td>41.4nmol/L controls</td>
<td>None Stated</td>
<td>Increased risk of VD with deficient level (&lt;30nmol/L) but not for levels of 30-50nmol/L.</td>
</tr>
<tr>
<td>Annweiler et al 2016 Singapore SEED</td>
<td>2,273</td>
<td>70.4</td>
<td>&gt;60 years</td>
<td>Not available</td>
<td>58.2nmol/L</td>
<td>AMT</td>
<td>Not available</td>
<td>Higher Vitamin D levels associated with higher AMT scores</td>
</tr>
<tr>
<td>Moretti et al 2017 Italy</td>
<td>86</td>
<td>77.9</td>
<td>AD: NINDCS-ADRDA &amp; DSM sVAD: NINDS-AIREN</td>
<td>Absence of informant Unavailable neurological examination Psychotropic meds in 2month prior to study</td>
<td>27nmol/L</td>
<td>Not available</td>
<td>Not available</td>
<td>Inverse relationship between Vitamin D and AD and sVAD on univariate analysis</td>
</tr>
<tr>
<td>Pavlovic et al 2018 USA</td>
<td>4,358</td>
<td>60.8</td>
<td>All who completed baseline assessment with MoCA CRF and 25(OH)D levels included</td>
<td>&lt;55 years, BMI &lt;18.5</td>
<td>90.4nmol/L</td>
<td>MoCA</td>
<td>Age, Gender, Education, BMI, Season, Ethnicity, BP, CRF, CVD, Smoking, Glucose,</td>
<td>Association between lower Vitamin D concentrations and cognitive impairment on MoCA (&lt;25/30</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
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<tr>
<td>Ouma et al 2018 Japan</td>
<td>230</td>
<td>74.5</td>
<td>HS: 74.5 MCI: 75.5</td>
<td>AD Mild: 74.8 Moderate: 82.2 Severe: 77.7</td>
<td>&gt;74 years</td>
<td>Free of hepatic and renal disorders</td>
<td>HS: 65.3nmol/L (women) 68.4nmol/L (Men) MCI: 45.5nmol/L (women) 52.5 (men)</td>
<td>MMSE</td>
</tr>
<tr>
<td>McGrath et al 2007 USA HANES III</td>
<td>4,809</td>
<td>60-90</td>
<td>Household US population</td>
<td>Not available (Quintiles)</td>
<td>Adults: DDST SDLT Elderly: Short Story Recall</td>
<td>38nmol/L</td>
<td>CAM ICU</td>
<td>Age, APACE II score</td>
</tr>
<tr>
<td>Morandi et al 2013 USA VALID</td>
<td>120</td>
<td>52</td>
<td>ICU Screened for delirium</td>
<td>&lt;18 years, ICU &gt;3days prior to enrolling, Post cardiac arrest, Cardiothoracic surgery, Severe Lung Disease</td>
<td>60.4nmol/L</td>
<td>Trails B, N Back Test, Go No Go Test</td>
<td>Age, Gender, Season, BMI, Co-Morbidities, Calcaemia, MMSE, GFR, Depressive Symptoms</td>
<td>No association between Vitamin D and delirium in ICU population</td>
</tr>
<tr>
<td>Anweiler et al 2014 France AIT</td>
<td>110</td>
<td>71.0</td>
<td>Subjective Memory Complaints</td>
<td>&lt;60 years, MMSE &lt;10, Dependant Mobility, Prior Stroke, Delirium, Depression, Medical Illness within 3 months</td>
<td></td>
<td></td>
<td></td>
<td>Overall no association Trend for association on Trails B</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders</td>
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<tr>
<td>Lam et al 2015</td>
<td>179</td>
<td>65.7</td>
<td>&gt;40 years</td>
<td>MMSE &gt;24</td>
<td>84.7nmol/L</td>
<td>RAVLT</td>
<td>Ionised Calcium, PTH, Age, Gender, Depression, Anxiety, Stress</td>
<td>No association between 25(OH)D and verbal episodic memory</td>
</tr>
<tr>
<td>Nourhashemi et al 2018 France MAPT Study</td>
<td>178</td>
<td>76.2</td>
<td>&gt;70 years</td>
<td>One of these: Spontaneous Memory Complaint, Slow gait speed Limitation in &gt;1 ADLs</td>
<td>55.9nmol/L</td>
<td>Cortical SUVRs measured using PET</td>
<td>Age, Gender, BMI, Season, education, Cognition assessed with MMSE, Time to PET, ApoE ε4 status or cortical β amyloid levels</td>
<td>No association between Vitamin D and ApoE ε4 status or cortical β amyloid levels</td>
</tr>
</tbody>
</table>
7.9 Prospective Studies

Positive Studies

In the INCHIANTI study, 858 adults aged 65 and older were assessed and followed for a median 6 years and were then assessed three yearly (Llewellyn et al., 2010). Cognition was assessed using MMSE, Trails A and B. Participants with dementia at baseline were excluded. Cognitive decline was defined as MMSE score decline of 3 or more points or scoring in the lowest 10% or Trails A and B being discontinued secondary to multiple mistakes. Vitamin D was measured in quartile values, severely deficient <25nmol/L, deficient >25 <50nmol/L, insufficient >50 <75nmol/L and sufficient >75nmol/L. In linear regression models, those with Vitamin D levels <25nmol/L were more likely to have cognitive decline. They also reported with a random effects model that those with levels <25nmol/L had a 0.3 point decline in their MMSE per year compared with 0.1 point decline in those with levels of 50-75nmol/L. No association between Vitamin D and Trails A and B were noted.

In a subgroup of the EPIDOS study 40 women (mean age 78.4 years) with a baseline serum Vitamin D were assessed after seven years for onset of dementia, classified as AD, non-AD (NAD) and not demented (Annweiler et al., 2011b). No participant was diagnosed with dementia at baseline based on DSM IV criteria. At follow up assessment, 30 (75%) of the participants were not demented, with 26 of these having Vitamin D level >25nmol/L, 15% at follow up were diagnosed NAD and 10% AD. Vitamin D deficiency at baseline, defined as <25nmol/L, was found to be associated with onset of NAD within 7 years but not with onset of AD.

Annweiler et al reported the relationship between dietary Vitamin D intake and likelihood of developing dementia in a subgroup of the EPIDOS cohort after seven year follow up (Annweiler et al., 2012b). 498 females were assessed with MMSE and were classified as non-demented (72.5%), AD (14.1%), and other dementias (OD) (13.4%). Dementia was diagnosed using the DSM-IV criteria and AD was diagnosed using the NINCDS/ADRDA criteria. Those with highest Vitamin D intake were less likely to develop AD but Vitamin D intake was not associated with developing OD.
In the ESTHER cohort 1,639 Germans were assessed after five year follow up for an association between cognitive function, assessed using the Cognitive Telephone Screening Instrument (COGTEL), and Vitamin D (Breitling et al., 2012). Linear regression models showed an association between Vitamin D levels in the lowest quintile and lower COGTEL scores in women but not in men. Women in the lowest quintile of Vitamin D scored approximately two points less than the women in the highest quintile group.

In a cohort of 6,257 healthy, community dwelling Caucasian females with mean age 76.6 years, the association between lower 25(OH)D levels and cognitive impairment and the risk of cognitive decline over 5 years was evaluated (Slinin et al., 2012). Cognitive testing performed at baseline included modified MMSE (mMMSE) and Trails B. Cognitive impairment was defined as >1.5 standard deviations (SD) below mean on MMSE or >1.5 SD above mean on Trails B and cognitive decline was defined as >2.9 score change on MMSE or >1 SD above sample mean on completion time of Trails B. Those who reported a diagnosis of dementia at baseline were excluded. Vitamin D levels were defined as severely deficient (<25nmol/L), deficient (25-49nmol/L), insufficient (50-74nmol/L) and sufficient (75nmol/L). 7.3% of this cohort was severely deficient in Vitamin D and 32.7% were deficient. In multivariate analyses, cognitive impairment at baseline and cognitive decline on mMMSE were associated with severe Vitamin D deficiency, but no association was found on Trails B.

A further prospective study assessed the risk of developing VD and/or AD in a Danish population (Afzal et al., 2013). The diagnosis rates were recorded from Danish Patient Registry and Danish Causes of Death Registry over a mean follow up period of 21 years. Mean age was 58 years. Results were based on 25(OH)D levels subdivided into deficient (<25nmol/L), insufficient (25-50nmol/L) and sufficient (>50nmol/L). Regression analysis showed an association between Vitamin D levels <25nmol/L and subsequent diagnosis of AD, HR of 1.29 (1.01-1.66, 95% CI), for Vitamin D levels 26-50nmol/L, HR 1.23. For VD HR 1.22 (0.77-1.91, 95% CI) and HR 1.22 (0.79-1.87, 95% CI) for the respective Vitamin D categories. Analysis also showed an association with development of both VD and AD.
combined with a HR of 1.27 for those with a Vitamin D level <25nmol/L. Overall this study suggested an increase in the risk of AD with decreasing serum 25(OH)D levels.

In a Finnish longitudinal study an inverse dementia risk was associated with Vitamin D concentrations in women but not in men (Knekt et al., 2014). The study population was enrolled from a national register of Finnish adults aged 30 years and over, from 1978 to 1980. 5,010 participants were included in this study (aged between 40-79 years) and were longitudinally followed until the date of dementia occurrence, which was the primary outcome measure. This was defined using ICD 8 criteria. The mean age of participants at baseline was 56 years in non-dementia cases and 69 years in those who developed dementia. The mean 25(OH)D levels was 45nmol/L in men and 40nmol/l in women. During the 17 year follow-up period there were 151 incident cases of dementia. An inverse association between age and sex adjusted Vitamin D status with onset of dementia was found, HR 0.31 (0.14-0.68, 95% CI) in fully adjusted models in women with higher Vitamin D being associated with lower rates of dementia.

Perna et al reported a longitudinal relationship between Vitamin D and cognition in a German population in the ESTHER study (Perna et al., 2014). At 5 year follow up participants aged >70 years were invited to participate in a telephone cognitive assessment called the COGTEL. Only 527 participants were included in the longitudinal study. Mean age at baseline was 73.7 years with mean COGTEL score of 29.6 and at follow-up mean age was 78.3 years with a mean COGTEL of 32.4. Mean Vitamin D levels were not given. Participants within the lower Vitamin D quintiles had lower COGTEL scores and regression analysis showed Vitamin D to be a predictor of lower COGTEL scores at follow up. Participants in lower quintiles for Vitamin D had lower COGTEL scores than those in the higher quintiles which was statistically significant with p=0.0348.

Another prospective study evaluated the association of Vitamin D with cognitive function in a population of 1,927 community dwelling adults who were cognitively intact at baseline (Toffanello et al., 2014). These participants were followed after 4.5 years and had a repeat cognitive testing with a MMSE, performed. Mean age of participants was 73.9 years with a
mean MMSE of 24.8 at baseline and mean Vitamin D level of 84.1nmol/L. Severe Vitamin D deficiency (levels <25nmol/L) were identified in only 6.8% of the overall sample. An MMSE of <24/30 was defined as cognitively impaired and a decline of three points or more over the study follow up period was defined as cognitive decline. In logistic regression models, 25(OH)D levels <50nmol/L were associated with cognitive decline in fully adjusted models in those who were cognitive intact at baseline, RR 1.40 (1.11-1.74, 95% CI) p=0.04. Further logistic regression analysis also showed that Vitamin D insufficiency and deficiency were associated with a higher risk of cognitive decline in the 4.4 year follow-up period, RR 1.36 (1.04-1.80, 95% CI) p=0.04.

A Canadian prospective study suggests an association between Vitamin D and cognition (Pettersen et al., 2014). Participants were divided into groups based on Vitamin D status, insufficient <75nmol/L, sufficient >75nmol/L. Cognition was assessed using a number of tools to assess verbal learning, non-verbal learning, executive function and working memory along with the CANTAB. Data was also compared for both summer and winter months of 25(OH)D collection. In cross-sectional analysis, in the summer period there were 32 participants with a mean age of 52 years and with 44% having insufficient Vitamin D levels and in the winter period there were only 19 participants eligible for inclusion with 63% having insufficient Vitamin D levels. Those with insufficient Vitamin D levels in the summer group had lower scores on tests of working memory (DSB), p=0.018. In the winter group, insufficient Vitamin D levels were associated with poorer scores in tests of working memory on the CANTAB, p=0.05. In longitudinal analysis, those participants with larger drops in Vitamin D levels from summer to winter samples, were associated with greater drops in tests scores on a test of working memory and executive function called the One Touch Stockings of Cambridge (OTS) p<0.01.

A prospective study from the US reported an association between Vitamin D and dementia in 1,658 participants (Littlejohns et al., 2014). Participants were recruited as part of the CHS (Cardiovascular Health Study) from 1989-1993. Mean follow-up time was 5.6 years. Mean age of participants at baseline was 73.6 years. Those with 25(OH)D deficiency were older, less educated, more likely to be black. 171 participants developed all-cause dementia
and 102 developed AD. Fully adjusted models showed those who were severely deficient in Vitamin D were more likely to develop either all-cause dementia, HR 2.25 (1.23-4.13, 95% CI) or AD dementia, HR 2.22 (1.02-4.83, 95% CI).

Bartali et al used the Nurse Health Study (NHS) to assess the prospective relationship between Vitamin D and cognition in 1,185 females (Bartali et al., 2014). Blood samples were taken in 1989 and then in 1995 NHS participants aged >70 were selected for a cognition sub-study. Mean age of participants at this time 74 years. Cognition was assessed using a composite score of a six cognitive tests including; the Telephone Interview of Cognitive Status (TICS), telephone adapted MMSE, immediate and delayed recall from East Boston Memory test, category fluency, delayed recall of TICS and backward digit span. Participants were followed over 6 years. Mean Vitamin D levels were not provided. At baseline, lower Vitamin D levels were associated with poorer scores on the global composite cognitive score and also on category fluency but not on other subsets. After 6 year follow-up there was no association between Vitamin D and decline in cognitive scores.

Jorde et al reported a cross-sectional and prospective association between Vitamin D and cognition from the Tromso study (Jorde et al., 2015). In Tromso 4 (1994-1995) there were 3,435 participants included who had at least one cognitive test in Tromso 5 (2001-2002) and Tromso 6 (2007-2008). Linear regression revealed an association between Vitamin D and cognitive function in domains of digit symbol coding, word recall and finger tapping. In fully adjusted models, 25(OH)D was only associated with finger tapping.

Assmann et al report the association of midlife Vitamin D and cognition in a subpopulation of the SU.VI.MAX study (Assmann et al., 2015). Baseline 25(OH)D measurements were available for a subpopulation who were involved in a nested case control study looking at the effects of Vitamin D on cancer risk. From this study, 1,009 participants from the control group with full data available were included in the current study. Mean age of participants was 66.6 years and there was a mean study follow up time of 13.4 years. Participants with lower education and Vitamin D deficiency at baseline were found to have
poorer scores 13 years later on Backward Tracking, $\beta=0.17$, (0.06-0.29, 95% CI), p=0.004. There was no association found between cognitive domains and Vitamin D deficiency in those with either second or higher level education.

A positive association between Vitamin D and cognition was reported in a study population consisting of diverse ethnic groups aged $>65$ years (Miller et al., 2015). The sample consisted of 382 participants with 318 of these having two separate assessments allowing for longitudinal analysis. Cognition was assessed using the Spanish and English Neuropsychological Assessment Scales. Mean age of participants was 75.5 years with mean Vitamin D level of 47.9nmol/L, 17.3% had dementia, and 49.5% were cognitively normal. Vitamin D deficiency was defined as $<30$nmol/L and 26.2% were Vitamin D deficient. Baseline Vitamin D levels were also lower in those with dementia (40.4nmol/L) compared with cognitively normal individuals (48.6nmol/L). In cross-sectional analysis, those with Vitamin D deficiency had lower semantic memory, visuospatial and executive function scores. Longitudinal analysis with mean follow-up of 4.8 years, reported higher levels of Vitamin D were associated with slower decline in cognitive scores, in fully adjusted models with Vitamin D as both continuous and categorical variable.

The Korean Longitudinal Study on Health and Ageing (KLoSHA) investigated the association of Vitamin D status with the future risk of MCI and dementia (Moon et al., 2015). 412 participants were analysed in this study. The mean age of participants was 72.6 years. 66% of the study population had levels $<25$nmol/L, overall mean not available. Mild Cognitive Impairment (MCI) was diagnosed according to the revised International Working Group on MCI and dementia was diagnosed using DSM IV criteria. At baseline 304 (74%) were cognitively normal, 25% had MCI and the remaining 1.7% had dementia at baseline. At follow-up, 295 (72%) were normal. 23% had MCI and 5.6% had dementia. Over the 5 year follow up period, 67 (16%) participants showed progression to either MCI or dementia. Overall the association of Vitamin D deficiency and development of cognitive impairment appeared to be associated but was not statistically significant. In subgroup analysis, in those with an MMSE of $<27$ and not demented at baseline, there was an
association with the development of cognitive impairment, HR 4.66 (1.46-14.88, 95% CI) and MCI in those with Vitamin D <25nmol/L, HR 7.13 (1.54-32.92, 95% CI).

In the prospective Rotterdam study, the relationship between Vitamin D levels and incident dementia was assessed in 6,220 participants (Licher et al., 2017). At baseline, 127 participants had dementia and analysis revealed a non-significant association between Vitamin D and dementia, OR 1.2 (0.95-1.52, 95% CI) but not AD. Of note, mean Vitamin D levels were not provided. Over the study period, 795 developed dementia (641 had AD). Lower serum 25(OH)D levels were associated with higher risk of incident dementia, OR 1.11 (1.02-1.20, 95% CI) and also higher rates of AD, OR 1.13 (1.03-1.24, 95% CI).

Feart et al suggested higher Vitamin D levels may slow cognitive decline in older adults in a prospective study (Feart et al., 2017). The study population consisted of 916 people who were followed for 12 years, with a mean age of 73.3 years and a mean serum 25(OH)D level of 35.8nmol/L. Over the follow-up period, there were 177 incident cases of dementia and 124 of these were AD. In longitudinal analysis Vitamin D deficiency was associated with faster decline in cognition, based on cognitive Z scores for episodic memory using the FCSRT. Those with Vitamin D deficiency were also associated with increased risk of all-cause dementia at follow-up, HR 1.98 (1.17-3.36, 95% CI).

Goodwill et al sought to investigate the relationship between midlife Vitamin D and cognition in later life in 252 women from the Women’s Healthy Ageing (WHA) study (Goodwill and Szoeke, 2017). The mean age at recruitment was 59.8 years with a mean Vitamin D level of 49.4nmol/L. Cognition was assessed at baseline (n=252) and at 10 year follow-up (n=176) using; Trail Making Test (TMT), Consortium to Establish a Registry for Alzheimer’s Disease (CREAD) and California Verbal Learning Test, Second Edition (CVLT II). 71 participants were lost to follow-up. At baseline those with Vitamin D levels <25nmol/L were more impaired on tests of executive function, Trails B and verbal fluency, than those with levels >25nmol/L. At 10 year follow-up, those with 25(OH)D levels >25nmol/L at baseline, scored better on tests of overall executive function and Trails B. There was no association between Vitamin D and cognitive decline (decline >1SD of the mean sample change from 2002-2012).
Goodwill et al published further results showing the association between midlife Vitamin D concentrations and executive function ten years later from the Women’s Healthy Ageing Project (Goodwill et al., 2018). 252 participants were included in this analysis, with a mean age of 59.8 years and mean serum 25(OH)D concentration of 49.4nmol/L. Cognition was assessed using the cognitive tests as outlined above. At baseline, higher Vitamin D levels (>25nmol/L) were associated with better scores on TMT-B, verbal fluency and on a composite score for executive function. On longitudinal analysis there was an association between Vitamin D and composite score of executive function, but not on individual cognitive test scores.

A recent US study assessed if Vitamin D status and intake were associated with domain specific cognition (Beydoun et al., 2018). The study sample included 2,574 participants from the HANDLS study with a mean age of 46.9 years and mean Vitamin D level of 50nmol/L. There was a mean follow up time of 4.6 years. Cognition was measured using CVLT list learning and delayed free recall, Trails A and B, verbal fluency and MMSE. Results were stratified by age, gender and race. Higher baseline Vitamin D levels were associated with slower decline in verbal fluency but no association was noted across other domains.

**Negative Studies**
As part of the MrOS (Osteoporotic Fractures in Men Study) 1,604 men underwent cognitive testing with 3MS (Modified Mini Mental State Exam) and Trails B and were included in analyses for link between 25(OH)D and cognition. Cognitive impairment was defined as 3MS score <80 or Trails B time >226.5secs (>1SD above the mean) (Slinin et al., 2010). At baseline, the mean 3MS score was 93.2 and mean Trails time was 136.1 seconds, with 179 men were classified as impaired cognitively. Vitamin D levels were divided into quintiles. There was no cross-sectional association between Vitamin D and baseline cognitive impairment with either 3MS or Trails B. 1,138 men underwent a second assessment 4.6 years later and had 3MS, Trails B and 25(OH)D levels available. Those with impairment at baseline were excluded from the longitudinal analysis. In 8% of this subgroup, incident cognitive impairment was noted at follow up. Vitamin D deficiency
(<49.9 nmol/L) showed some association with cognitive decline at follow-up on 3MS testing but there was no significant association noted on Trails B.

A study involving middle-aged black and white participants did not show an association between Vitamin D and cognitive function and dementia risk (Schneider et al., 2014). Participants from the ARIC Brain MRI Ancillary Study, with a mean age of 62 years and mean 25(OH)D concentration of 43.2 nmol/L in blacks and 63.6 nmol/L in whites, were followed for a median of 10.6 years. Cognitive function was assessed using the Delayed Word Recall Test (DWRT), Digital Symbol Substitution Test (DSST) and Word Fluency Test (WFT). In cross-sectional analysis Vitamin D was not associated with cognitive test scores by race specific 25(OH)D tertiles. There was no significant association in longitudinal analysis between Vitamin D concentrations and decline across the three cognitive tests. In terms of incident hospitalisation due to dementia, there were 145 events for the 1,652 included in this analysis, again no significant association was found.

Data from the Newcastle 65+ study suggests a U shaped association between Vitamin D and cognitive decline. Participants were initially recruited from GP practices and were followed over a three-year period (Granic et al., 2015b). Cognition was assessed using the SMMSE and CRT, Simple Reaction Time (SRT) and Digital Vigilance Task (DVT). A total of 845 participants were included in the analysis. 25(OH)D was compared across three season specific quartiles, to account for the seasonal variation of serum Vitamin D levels. Mean Vitamin D levels were not reported. Those in lowest and highest season specific groups had greater rates of cognitive impairment. A total of 773 participants with full data on cognition and serum Vitamin D levels were included in logistic regression models, with 27.4% classified as impaired on SMMSE (score <25). Regression models predicted those in the lowest and highest season specific quartiles of Vitamin D had higher risk of cognitive impairment compared with the middle group, OR 1.66 (1.06-2.60, 95% CI) (for the lowest Vitamin D group). Longitudinal analysis included 470 participants, the remaining participants either died (n=360) or did not complete assessment (n=15). Of those with Vitamin D status at 3-year follow up (n=299), 33.3% were cognitively impaired.
There was no association between season specific Vitamin D and odds of incident cognitive impairment three years later, OR 1.03 (0.57-1.89, 95% CI).

In a cohort from the Framingham study, Vitamin D was found to be associated with impairment in certain cognitive domains, and also reduced hippocampal volumes on MRI Brain (Karakis et al., 2016). Participants from the study were divided into two cohorts for analysis of incident dementia (n=1,663) and the cognitive and MRI cohort (n=1291) who underwent detailed Neuropsychological Testing (NPT) including Trails A and B, Delayed Logical Memory, the Hooper Visual Reproductions and Visual Reproductions Delayed Test. MRI of the brain measured total brain volume, hippocampal volume, hyper-intensities and infarcts. Both imaging and NPT were performed approximately 1.8 years after Vitamin D measurement. Dementia was defined using DSM IV criteria and AD diagnosed based on NINCDS-ADRDA. Participants in the incident dementia cohort were older (72.4 versus 59.5 years) and had higher serum 25(OH)D levels than the cognitive MRI cohort (62.6 versus 49.4nmol/L). In the incident dementia group, 16.1% developed dementia over the 9-year surveillance period. There was no association between serum 25(OH)D and dementia in this study cohort. Vitamin D levels were associated with poorer performance on cognitive scores, including Trails A and B; β=-0.05, p= 0.023, and the Hooper Visual Reproducive Test β=-0.10 p=0.036, however both models lost statistical significance when adjusted for BMI and supplementation use. Finally, Vitamin D (as a categorical variable) was associated with hippocampal volumes, β=-0.01 p=0.034, but again the model lost significance with adjustment for BMI and Vitamin D supplementation.

Van Schoor et al report a cross sectional association between Vitamin D deficiency across numerous cognitive domains but did not remain significant on longitudinal analysis (van Schoor et al., 2016). Participants were part of the LASA study. Cognitive tests were across a number of domains using the following: MMSE, RCPM, information processing speed, Auditory Verbal Learning Test (AVLRT). For the total population the mean age was 75.6 years with mean Vitamin D concentration of 53.7nmol/L, 48.4% had levels <50nmol/L. On adjusted cross sectional models, Vitamin D deficiency was associated with speed of
information processing and also MMSE. Longitudinal analysis revealed no association between Vitamin D and the cognitive domains tested.

A Swedish study evaluated the longitudinal association between Vitamin D and all cause dementia, incident AD, VD and performance on MMSE over a median follow-up of 12.1 years (Olsson et al., 2017). Participants were men recruited as part of the Uppsala Longitudinal Study with a mean age of 71 years at baseline and 82 years at follow-up. Of the original 2,322 men recruited, 1,182 were included in this study, who had survived and had serum 25(OH)D measured at baseline. AD was defined based on NINCDS ADRDA and DSM IV criteria. A secondary measure included cognitive decline on MMSE defined as >3 point decline on scores over follow-up period or MMSE <25. Mean 25(OH)D level at baseline for the total cohort was 68.7nmol/L. There was no significant association between Vitamin D, either as a categorical or continuous variable and incident dementia, AD, VD or all cause dementia.

A study from 2018 found Vitamin D levels were not associated with baseline or incident cognitive impairment in the Health ABC Study (Kilpatrick et al., 2018). This study comprised 2,786 community dwelling adults aged 70-79 years. Cognition was assessed using the Modified Mini Mental State Test (3MS) and the Digital Symbol Substitution Tests (DSST). Cognitive impairment was defined as >1.5 SD below sample derived race and educational mean. Covariates included in the analysis were: age, gender, educational attainment, smoking and alcohol status, season and minutes walked per week, BMI, medical co-morbidities and supplementation use. The mean age of participants was 73.5 years and mean Vitamin D was not provided. At baseline in both black and white populations, those with Vitamin D levels >75nmol/L had lower BMIs and were significantly more active (in terms of minutes walked per weeks) than those with levels <50nmol/L. At baseline, 11.8% of whites and 13.1% of blacks met the study definition for cognitive impairment, no significant association between 25(OH)D concentrations and baseline cognitive impairment was found. At 5 year follow-up, 13.8% whites and 13.3% blacks went on to develop incident cognitive impairment. In adjusted models, (Vitamin D
<50nmol/L) was not associated with increased odds of developing cognitive impairment, in blacks, OR 1.06 (0.55-2.06, 95% CI), or in whites, OR 1.02 (0.6401.67, 95%CI).
Table 7: Vitamin D and Cognition: Longitudinal Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Mean Follow-up</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Cognitive Assessment</th>
<th>Confounders Considered</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llewellyn et al 2010</td>
<td>858</td>
<td>73.8</td>
<td>5.2 years</td>
<td>&gt;65 years</td>
<td>Community Dwelling Cognitive Impairment</td>
<td>Dementia (DSM IV)</td>
<td>MMSE Trails A and B</td>
<td>Age, Gender, Education, season, BMI, Alcohol, Smoking, Depression, impaired mobility and cognition</td>
<td>Association with MMSE</td>
</tr>
<tr>
<td>Italy InCHIANTI</td>
<td></td>
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<td></td>
<td>62.3% &lt;50nmol/L 20.3% &lt;25nmol/L</td>
<td></td>
<td></td>
<td>No association on Trails A or B</td>
</tr>
<tr>
<td>Annweiler et al 2011</td>
<td>40</td>
<td>78.4 median</td>
<td>7 years</td>
<td>&gt;75 years</td>
<td>Healthy French females Ambulatory</td>
<td>Vitamin D supplement, Institutionalisation Prior hip fracture, Bilateral hip replacement, Inability to walk independently</td>
<td>SPMSQ (7.55% had &quot;subtle cog impairment at baseline)</td>
<td>Age, HTN, DM, BMI, Physical activity, Smoking</td>
<td>Vitamin D deficiency at baseline associated with developing Non-AD at 7 year follow up</td>
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<tr>
<td>EPIDOS</td>
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<td></td>
<td>36.25nmol/L (Median)</td>
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<tr>
<td>Annweiler et al 2012</td>
<td>498</td>
<td>80.6</td>
<td>7 years</td>
<td>&gt;75 years</td>
<td>Community Dwelling French females Ambulatory</td>
<td>Vitamin D supplement, Institutionalisation Prior hip fracture, Bilateral hip replacement, Inability to walk independently</td>
<td>MMSE Grober &amp; Buschke</td>
<td>Age, BMI, Education, Disability, Physical activity, Sun exposure, Depression, HTN, Number of chronic diseases</td>
<td>Higher Vitamin D intake associated with lower risk of AD</td>
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<td>France EPIDOS</td>
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<td>Author</td>
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<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
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<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
<td>Results</td>
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<tr>
<td>Breitling et al 2012</td>
<td>1,639</td>
<td>74</td>
<td>5 years</td>
<td>&gt;65 years</td>
<td>Insufficient knowledge of German, Unwilling or unable to participate</td>
<td>Not available</td>
<td>COGTEL</td>
<td>Age, Education, Gender, BMI, Smoking, Alcohol, CVD, Depression</td>
<td>Association between COGTEL and Vitamin D deficiency in women, not in men</td>
</tr>
<tr>
<td>Slinin et al 2012 USA</td>
<td>6,257</td>
<td>76.6</td>
<td>4 years</td>
<td>Women &gt;65 years Ambulatory Caucasians</td>
<td>Black women, Previous hip fracture, Bilateral hip replacement, Dementia</td>
<td>Not available</td>
<td>mMMSSE</td>
<td>Age, Gender, Season, Clinic site, Education, Self-reported health, ADLs, Smoking, BMI, Depression</td>
<td>Association between Vitamin D and mMMSSE but not with Trails B</td>
</tr>
<tr>
<td>Afzal et al 2013 Denmark</td>
<td>10,186</td>
<td>58</td>
<td>21 years</td>
<td>20-100 years</td>
<td>Not available</td>
<td>41nmol/L (median)</td>
<td>ICD 8 ICD 10</td>
<td>Age, Gender, Month, BMI, Smoking, DM, Alcohol, HTN, Education, Income, Activity, Cholesterol</td>
<td>Increased risk of developing AD in those with Vitamin D &lt;25nmol/L at baseline</td>
</tr>
<tr>
<td>Pettersen et al 2014 Canada</td>
<td>19</td>
<td>52</td>
<td>4 months</td>
<td>&gt;20 years, Literate in English, No Visual or Hearing Impairment</td>
<td>Brian Tumour Brain Injury Symptomatic Stroke</td>
<td>Not available</td>
<td>CANTAB</td>
<td>Not available</td>
<td>Seasonal decline in Vitamin D was associated with decline in tests of executive/working memory</td>
</tr>
<tr>
<td>Knekt et al 2014 Finland Mini-Finland Health Survey</td>
<td>5,010</td>
<td>62.5</td>
<td>17 years</td>
<td>40-79 years Free from Dementia</td>
<td>Not available</td>
<td>42.5nmol/L</td>
<td>ICD-8</td>
<td>Age, Gender, Month, BMI, Education, BP, Marital Status, Activity, Smoking, Alcohol, Glucose</td>
<td>Inverse association between Vitamin D and incident dementia in women</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
<td>Results</td>
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<tr>
<td>Toffanello et al 2014</td>
<td>1,927</td>
<td>73.9</td>
<td>4.5 years</td>
<td>Community Dwelling</td>
<td>Caucasian</td>
<td>84.1nmol/L</td>
<td>MMSE</td>
<td>Age, Gender, Baseline MMSE, BMI, smoking, mobility, physical activity, ADLs, Depression, DM, GFR, COPD</td>
<td>Association between Vitamin D and cognitive decline on MMSE</td>
</tr>
<tr>
<td>Perna et al 2014</td>
<td>527</td>
<td>73.7</td>
<td>5 years</td>
<td>Baseline data and 25(OH)D</td>
<td>&gt;70 years baseline impairment</td>
<td>Not available</td>
<td>COGTEL</td>
<td>Age, Education, Season, MI, DM, Stroke, Depression, CKD, Smoking, Alcohol, Physical Activity</td>
<td>Lower Vitamin D quartiles associated with lower COGTEL scores</td>
</tr>
<tr>
<td>Littlejohns et al 2015</td>
<td>1,658</td>
<td>73.6</td>
<td>5.6 years</td>
<td>Community Dwelling</td>
<td>Missing Vitamin D or Dementia information, Prevalent dementia at recruitment</td>
<td>Not available (quartiles)</td>
<td>NINCDS-ADRDA</td>
<td>Age, Gender, Season, Education, Smoking, Alcohol, Depressive Symptoms</td>
<td>Severe Vitamin D deficiency associated with incident all-cause dementia</td>
</tr>
<tr>
<td>Assmann et al 2015</td>
<td>1,009</td>
<td>66.6</td>
<td>13.4 years</td>
<td>Women: 35-60 years Men: 45-60 years Free from disease that impacted participation</td>
<td>Not available</td>
<td>52nmol/L</td>
<td>Phonemic &amp; Semantic Fluency RI-48 Cued Recall DST Forward &amp; Backward</td>
<td>Age, Gender, Education, Physical Activity, Smoking, Alcohol, BMI, Season</td>
<td>Deficient Vitamin D associated with poorer scores on Backward Tracking</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
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<tr>
<td>Miller et al 2015 USA</td>
<td>318</td>
<td>75.5</td>
<td>4.8 years</td>
<td>&gt;60 years English or Spanish Speaking Community Dwelling</td>
<td>Not Institutionalised No Psychiatric Disorder No Substance Abuse</td>
<td>47.9nmol/L</td>
<td>Spanish and English Neuropsychological Scales</td>
<td>Age, Gender, Race, Education, BMI, Season, Vascular Risk Score, ApoE4</td>
<td>Higher Vitamin D was associated with slower rate of cognitive decline</td>
</tr>
<tr>
<td>Jorde et al 2015 Norway Tromso</td>
<td>3,435</td>
<td>57.7</td>
<td>6 years</td>
<td>None Stated</td>
<td>None Stated</td>
<td>Quartiles</td>
<td>MMSE Finger Tapping DSCT</td>
<td>Age, Gender, BMI, Education, Systolic BP, Exercise level</td>
<td>Vitamin D only associated with finger tapping in longitudinal analysis</td>
</tr>
<tr>
<td>Goodwill et al 2017 WHAP USA</td>
<td>252</td>
<td>59.8</td>
<td>10 years</td>
<td>45-55 years at baseline Women</td>
<td>Not stated</td>
<td>49.4nmol/L</td>
<td>CERAD, CVLT II, Trails B, Animal Fluency, MMSE</td>
<td>Age, Gender, Education, BMI, Physical activity, Smoking status, CESD, HTN, Co-morbidities: Diabetes, CHD</td>
<td>Midlife Vitamin D levels associated with executive function at baseline and 10 year follow-up</td>
</tr>
<tr>
<td>Licher et al 2017 Rotterdam Study</td>
<td>6,220</td>
<td>Not available (aged &gt;55 years)</td>
<td>68,884 person-year follow-up</td>
<td>&gt;55 years Not available</td>
<td>Not available</td>
<td>Not available</td>
<td>Age, Gender, Season, Ethnicity, Education, CV risk factors, Calcium, Renal function, Depression, outdoor activity, APOE status</td>
<td>Longitudinal association between Vitamin D and incident dementia and AD</td>
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<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
<td>Results</td>
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<tr>
<td>Feart et al 2017</td>
<td>916</td>
<td>73.3</td>
<td>&gt;12 years</td>
<td>Community dwelling &gt;65 years</td>
<td>Dementia at baseline</td>
<td>35.8nmol/L</td>
<td>MMSE, TMT, FCSRT</td>
<td>Age, Gender, Education, Income, DM, Depression, Number of meds, APOE status, BMI, Physical exercise, CVD, HTN, Stroke, Smoking, Hypercholesterol</td>
<td>Vitamin D deficiency association with faster decline in FCSRT and also associated with increased risk of incident dementia at follow up</td>
</tr>
<tr>
<td>Three City (3C) Study</td>
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<tr>
<td>Goodwill et al 2018</td>
<td>252</td>
<td>59.8</td>
<td>10 years</td>
<td>Women Aged 45-55 years at baseline</td>
<td>Not available</td>
<td>49.4nmol/L</td>
<td>CERAD, CVLT II, Trails B, Animal Fluency, MMSE</td>
<td>Age, Gender, Education, BMI, Physical activity, Smoking status, CESD, HTN, Co-morbidities: Diabetes, CHD</td>
<td>Baseline Vitamin D &gt;25nmol/L associated with better scores on TMT-B, verbal fluency and composite score of executive function. Longitudinally Vitamin D association with composite score of executive function</td>
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<tr>
<td>WHAP Australia</td>
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<tr>
<td>Beyboun et al 2018</td>
<td>2,574</td>
<td>46.9</td>
<td>4.6 years</td>
<td>Not available</td>
<td>Not available</td>
<td>50nmol/L</td>
<td>CVLT list learning and delayed recall, Trails A &amp; B, MMSE, animal fluency</td>
<td>Age, Gender, Race, Marital status, Education, BMI, Poverty Income ratio, Illicit drug use, Smoking status, CESD, Healthy Index Eating Score</td>
<td>Higher baseline Vitamin D levels associated with slower decline in verbal fluency but no association across other domains</td>
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<tr>
<td>HANDLS USA</td>
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<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
<td>Results</td>
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<tr>
<td>Slinin et al 2010 USA</td>
<td>1,138</td>
<td>73.7</td>
<td>4.6 years</td>
<td>&gt;65 years Community dwelling</td>
<td>Mobility with assistance, Bilateral hip replacements, Severe medical condition</td>
<td>Not available</td>
<td>3MS Trails B</td>
<td>Age, Gender, Season, Race, BMI, Smoking, Alcohol, education, chronic illness</td>
<td>No association on Trails B or 3MS</td>
</tr>
<tr>
<td>Schneider et al 2014 USA</td>
<td>1,652</td>
<td>62</td>
<td>16.6 years</td>
<td>&gt;55 years Community based</td>
<td>Not available</td>
<td>43.2nmol/L</td>
<td>DWRT DSST WFT</td>
<td>Age, Gender, Education, Income, Physical Activity, Smoking, Alcohol, BMI, CKD, calcium, phosphate, PTH, DM, HTN</td>
<td>No longitudinal association with cognitive decline or incident dementia risk</td>
</tr>
<tr>
<td>Granic et al 2015 UK</td>
<td>845</td>
<td>Not available</td>
<td>3 years</td>
<td>Single birth cohort</td>
<td>Not available</td>
<td>Not available</td>
<td>SMMSE CRT SRT DVT</td>
<td>Age, Gender, Education, Chronic diseases, Osteoporosis, Smoking, Alcohol</td>
<td>No association between Vitamin D and cognitive impairment. U shaped (low and high season specific levels) association with Vitamin D and cognitive function</td>
</tr>
<tr>
<td>Karakis et al 2016 Framingham Study (Original and Offspring)</td>
<td>1,663 Demen</td>
<td>tia cohort</td>
<td>72.4</td>
<td>9 years</td>
<td>&gt;60 years</td>
<td>Not available</td>
<td>62.6nmol/L</td>
<td>Trails A &amp; B, Delayed Logical Memory, Hooper Visual Reproductions Test (HVRT)</td>
<td>Age, Gender, Smoking, HTN, DM, CVD, BMI, Vitamin D supplement</td>
</tr>
<tr>
<td></td>
<td>tia cohort</td>
<td>1,291 MRI Cohort</td>
<td>59.5</td>
<td></td>
<td></td>
<td>49.4nmol/L</td>
<td></td>
<td></td>
<td>Associated with poorer scores on Trails and HVRT</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean Follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Confounders Considered</td>
<td>Results</td>
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<tr>
<td>Van Schoor et al 2016</td>
<td>3,107</td>
<td>75.6</td>
<td>3 years</td>
<td>&gt;55 years</td>
<td>Not available</td>
<td>53.7nmol/L</td>
<td>MMSE RCPM</td>
<td>Age, Gender, Season, Education, DM, Depressive symptoms, IHD, HTN, Alcohol, Smoking, Physical Activity</td>
<td>No significant association between Vitamin D and cognition longitudinally</td>
</tr>
<tr>
<td>Olsson et al 2017 Sweden ULSAM</td>
<td>1,182</td>
<td>71</td>
<td>18 years (total)</td>
<td>Community Dwelling</td>
<td>Not available</td>
<td>69nmol/L</td>
<td>MMSE NINDS-ADRDA</td>
<td>Age, Gender, Education, Season, BMI, HTN, DM, Smoking, Cholesterol</td>
<td>No association between Vitamin D and incident dementia, AD, VD, or cognitive decline</td>
</tr>
<tr>
<td>Kilpatrick et al 2018 Health ABC Study USA</td>
<td>2,786</td>
<td>73.5</td>
<td>5 years</td>
<td>70-79 years</td>
<td>Life-threatening illness</td>
<td>Not available</td>
<td>3MS DSST</td>
<td>Age, Gender, Educational attainment, Season, Smoking and Alcohol status, Co-morbidities (DM, medication use, CKD, CVD) BMI, Supplement use</td>
<td>No association between Vitamin D and baseline or incident cognitive impairment</td>
</tr>
</tbody>
</table>

7.10 Intervention Trials

Positive Studies

In 2012 Annweiler et al reported a retrospective pre and post intervention study assessing the effects of supplementing Vitamin D3 on cognition of patients attending their memory clinic (Annweiler et al., 2012a). 44 subjects were included, with median age of 80.6 years. Cognition was assessed at baseline and follow up visits with MMSE, Cognitive Assessment Battery (CAB) and Frontal Assessment Battery (FAB). Baseline median MMSE was 27.0, FAB was 15.5 and CAB was 88.0. Participants were not on previous Vitamin D supplementation or anti-dementia medications. The 24 participants in the control group were similar in baseline characteristics aside from higher 25(OH)D levels at baseline, median 25(OH)D 63nmol/L versus controls at 42nmol/L. Twenty patients received Vitamin D3 supplementation on their first visit, noted from prescription records, with doses of 800IU per day or 100,000IU per month. Participants were followed for 16 months and following this the treatment group were noted to have higher 25(OH)D levels, with median 75.0nmo/L versus control of 48nmol/L. Overall scores on cognitive tests were higher in the treatment group but tests scores remained stable in control group.

Negative Studies

A small non-blinded intervention trial of 63 nursing homes residents, with a mean age of 87 years, was reported assessing the affects of supplementing ergocalciferol in patients deficient of Vitamin D (defined as <62.5nmol/L) (Przybelski et al., 2008). The comparison group consisted of patients replete in Vitamin D (>62.5nmol/L) who continued with their normal treatment. Sixty-three patients (47 women, 16 men) were included and 95% (n= 61) of these completed the study. Higher numbers were in the comparison group at 38 participants versus 25 in the intervention group. No limitation on calcium or Vitamin D intake at baseline was specified in this study. In fact 43% of participants were receiving calcium and 46% were receiving Vitamin D at baseline, with the majority in the comparison group (85%) also taking supplements. Baseline calcium levels were equal in both groups but baseline Vitamin D levels were significantly higher in the comparison group than in the intervention group, at 86.9nmol/L versus 43.2nmol/l respectively. Patients in the treatment group received 50,000 IU ergocalciferol three times weekly for a
four-week period. The comparison group received no increase in Vitamin D supplementation nor did they receive placebo. After 4 weeks an increase in serum 25(OH)D was noted in the intervention group. Cognitive assessment included the Clock Drawing Task (CDT) and Semantic Fluency Test (SFT) with use of the neuropsychiatric inventory to assess for behavioural disturbances. No significant differences were noted across any of these parameters in either group after the 4 week treatment period.

7.11 Randomised Control Trials (RCTs)

Positive Studies
A recent RCT from Canada compared high dose Vitamin D supplementation with low dose supplementation and how this affects cognitive scores (Pettersen, 2017). Participants had a serum Vitamin D level <100nmol/L at baseline, supplementation at baseline was not restricted but participants were advised not to alter their dose during the 18-week study period. The treatment group received Vitamin D3 (cholecalciferol) 4,000IU/day and controls received 400IU/day. Cognition was assessed with tests of verbal learning memory, non-verbal learning memory, working memory and executive function. A total of 82 participants completed the study with a mean age of 54.7 years and mean Vitamin D of 63.9nmol/L. Vitamin D deficiency at baseline (<50nmol/L) was present in 55% of the treatment group and in 67.5% of the control group. For the whole population there was a significant improvement in cognitive sub-scores for recognition portion of the Verbal Memory Task in the low dose group but not in the high dose group, Δ=0.49, p=0.021. The low dose group also improved on verbal fluency, whereas the high dose did not have a significant improvement, Δ=0.39, p=0.016 and Δ=0.38, p=0.077 respectively. In subgroup analysis of those with levels <75nmol/L at baseline, compared with those in the high dose group showed an improvement in non-verbal memory, Δ=0.65, p=0.005.

Negative Studies
Stein et al reported a two-phase intervention study, including a pilot phase and an RCT (Stein et al., 2011). Participants were living in the community, aged over sixty years, English speaking and had mild to moderate AD (defined as MMSE score of 12 to 24). In the eight-week pilot study, 13 participants, with AD and a median MMSE of 21.5, median
ADAS-Cog of 25 and median 25(OH)D of 66nmol/L were assessed. At baseline serologic blood tests and the Alzheimer’s disease assessment Scale-Cognitive (ADAS-Cog) and the Disability Assessment in Dementia Questionnaire (DAD) were performed. Patients were treated with 3,000 IU Vitamin D2 weekly and the ADAS-Cog and DAD were repeated at eight-weeks. Results showed a 6-point increase in the ADAS-Cog following treatment with Vitamin D2 and also the DAD score increased by two, indicating less disability. In the RCT, 31 participants with AD and a median age 77.5 years participated (median MMSE 19.5 and 25(OH)D 49nmol/L). All participants received an eight-week run in period treatment of 1,000IU Vitamin D2 once daily and all continued on this through the subsequent 8-week RCT. At baseline and eight weeks, cognitive assessment was repeated with ADAS-Cog, Wechsler Memory Scale- Revised (WMS-R) Logical Memory subsets of immediate and delayed memory, GDS and DAD. Then participants were randomised to treatment group receiving high dose Vitamin D2, 6,000IU capsules or placebo for eight-weeks. Treatment group received two capsules three times daily initially and then dose was altered depending on serum 25(OH)D levels at 2, 4, and 6 weeks. Results showed no change in ADAS-Cog scores or WMS subsets of immediate and delayed recall in those on the high dose Vitamin D2 compared with the placebo controls receiving physiological doses of D2. Of note, following the 8-week RCT phase there was a further treatment arm with intranasal insulin.

In 2012 Rossom et al performed a post hoc analysis of a RCT to assess the effects of Vitamin D on cognitive function in 4,143 women aged 65 years and older without baseline cognitive impairment (mean age of 71 years at screening) who participated in the WHICaD Trial (Rossom et al., 2012). Initial outcomes were related to bone health and fracture but this study reports on cognitive outcomes. All were enrolled in the WHI Memory Study (WHIMS) and also participated in the WHI Hormone Therapy Trails. Cognitive assessments were performed annually until the end, and then participants were followed for post trial clinic assessments. All participants were not cognitively impaired at baseline. Participants in the WHI Calcium and Vitamin D trial were randomised to receive total calcium carbonate 2,000mg and Vitamin D 800IU daily, or placebo. The use of personal vitamin D supplements up to 600IU/day and calcium supplements of 1,000mg/day was
permitted. Primary outcomes included probable dementia, MCI or normal cognition defined as per study protocol. Dementia diagnosis was based on DSM IV criteria. MCI was defined as performance <10th percentile on at least one Consortium, to establish a Registry for AD (CREAD) test. Global cognition was assessed with 3MSE and domain specific tests were performed in a subset of 1,420 participants including: digit span, card Rotations Test, Primary Mental Abilities Vocabulary and letter and semantic fluency tests. Results showed that 4.8% of treatment group and 5.1% of placebo group went on to develop cognitive impairment in the 7.8 year follow up period. 39 treatment participants and 37 placebo participants developed incident dementia in the follow up period. No differences in 3MS scores or in the subsets of domain specific tests were noted.
Table 8: Vitamin D and Cognition: Intervention/Randomised Control Trials

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Mean follow-up</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Cognitive Assessment</th>
<th>Treatment</th>
<th>Confounders</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Annweiler et al 2012</td>
<td>44</td>
<td>80.6</td>
<td>16 months</td>
<td>No recent Vitamin D supplement</td>
<td>No anti-dementia medication</td>
<td>Recent Vitamin D supplement</td>
<td>MMSE CAB FAB</td>
<td>Intervention: Vitamin D3 800IU/day or 100,000IU per month</td>
<td>Age, Gender, cognitive score at baseline</td>
<td>Some improvement in scores on MMSE and FAB</td>
</tr>
<tr>
<td>Dhesi et al UK 2004</td>
<td>123</td>
<td>76.6 (placebo) 77.0 (Intervention)</td>
<td>6 months</td>
<td>&gt;65 years</td>
<td>Lived in their own homes, Fall in prior 8/52, Vitamin D &lt;25nmol/L</td>
<td>Normal bone biochemistry</td>
<td>25nmol/L CRT</td>
<td>Placebo: Normal saline IM</td>
<td>Not available</td>
<td>Intervention group: faster CRT than control after six months</td>
</tr>
<tr>
<td>Peterson et al 2017</td>
<td>82</td>
<td>56.7 (High Dose) 52.6 (Low Dose)</td>
<td>18 weeks</td>
<td>&gt;20 years</td>
<td>English literacy</td>
<td>Baseline 25(OH)D &lt;100nmol/L</td>
<td>High Dose: 67.2nmol/L Low Dose: 60.5nmol/L</td>
<td>SDMT VF DS-F DS-B CANTAB</td>
<td>Not available</td>
<td>Some improvement in test scores in verbal memory in the high dose treatment group</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age (years)</th>
<th>Mean follow-up</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Mean Vitamin D</th>
<th>Cognitive Assessment</th>
<th>Treatment</th>
<th>Confounders</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
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<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
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<td>Inclusion Criteria</td>
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<td>Mean Vitamin D</td>
<td>Cognitive Assessment</td>
<td>Treatment</td>
<td>Confounders</td>
<td>Results</td>
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<tr>
<td>Przybelski et al, 2008, USA</td>
<td>63</td>
<td>87</td>
<td>4 weeks</td>
<td>Nursing Home Residents, Independently mobile or with assistance, No limitation on Vitamin D intake</td>
<td>Hepatic or renal failure, PTH, Calcium or Phosphate abnormality, Granulomatous disorders, Vitamin D intoxication</td>
<td>Treatment group: 43.3nmol/L Comparison Group: 87nmol/L</td>
<td>CDT SFT</td>
<td>Intervention: Vitamin D2 50,000IU PO three times weekly for 4 weeks Controls: Usual treatment</td>
<td>Not available</td>
<td>No significant difference in either group</td>
</tr>
<tr>
<td>Rossom et al, 2012, USA WHIMS WHI</td>
<td>4,143</td>
<td>71</td>
<td>7.8 years</td>
<td>&gt;65 years No cognitive impairment at baseline Calcium supplements up to 1,000mg/day Vitamin D supplements up to 600IU/day</td>
<td>Cancer, stroke, TIA, heart disease, MI, hypertension, liver or kidney disease</td>
<td>Intervention: 50nmol/L Placebo: 48nmol/L</td>
<td>Probable dementia: DSM IV MCI (&lt;10%ile on CREAD test) 3MSE 1,420 participants received domain specific cognitive tests</td>
<td>Intervention: 2 tablets daily containing Calcium Carbonate 1,000mg and Vitamin D 400IU Placebo equivalent</td>
<td>Smoking, Baseline 3MSE, Study year, Age, Assignment to other WHI Trials.</td>
<td>No significant differences in 3MSE or domain specific cognitive scores No difference in incidence of cognitive impairment between groups</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mean Age (years)</td>
<td>Mean follow-up</td>
<td>Inclusion Criteria</td>
<td>Exclusion Criteria</td>
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<td>Cognitive Assessment</td>
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<td>Confounders</td>
<td>Results</td>
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</tr>
<tr>
<td><strong>Stein et al 2011 Australia</strong></td>
<td>Pilot: 13 RCT: 32</td>
<td>Pilot: 77.5 weeks</td>
<td>Pilot: 8 weeks</td>
<td>Mild to Moderate AD Community Dwelling</td>
<td>&gt;60 years</td>
<td>Cerebro-vascular disease, MS, epilepsy, T1DM, renal calculi, Malabsorb, Poorly controlled psychiatric disorder, Excessive alcohol intake</td>
<td>Pilot: 66nmol/L RCT: 49nmol/L</td>
<td>MMSE ADAS Cog WMS: RLM &amp; delayed scores</td>
<td>Pilot: 3,000IU D2 for 8weeks</td>
<td>CVD, MS, T1DM, Renal calculi, Malabsorb, Excessive C2H5 intake</td>
</tr>
</tbody>
</table>

7.12 Meta-Analyses

Positive Studies

Etgen et al evaluated the relationship between Vitamin D and cognitive impairment and dementia through a meta-analysis of case control, cross-sectional, longitudinal, prospective and interventional trials (Etgen et al., 2012). Five cross-sectional studies including 5,686 participants were included. Lower Vitamin D concentrations had significantly increased risk of cognitive impairment, OR 2.37 (1.77-3.17, 95% CI) p= <0.0001. There was significant heterogeneity. Only two studies were included in the longitudinal meta-analysis. Vitamin D deficiency was associated increased risk of cognitive impairment at follow up, OR 2.49 (1.74-3.56, 95% CI) p= <0.001.

A further meta-analysis from 2012 suggests lower concentrations of Vitamin D are associated with poor cognition and dementia (Balion et al., 2012). A total of 37 studies were included in the analysis, from cross-sectional to RCTs, with sample sizes varying from 27 to 17,099. MMSE was the most frequently used cognitive test. In the meta-analysis of Vitamin D and AD versus control groups, six studies with 888 participants were included. The results showed those with AD had lower concentrations of serum 25(OH)D compared with controls, OR -6.2 (-10.6 - -1.8, 95% CI). In further analysis comparing concentrations of 25(OH)D >50nmol/L and <50nmol/L with MMSE scores from eight studies with 2,749 participants, analysis showed higher Vitamin D levels associated with higher MMSE scores, OR 1.2 (0.5 – 1.9, 95% CI).

In 2013, Zhao et al published a meta-analysis looking at the association of Vitamin D deficiency, AD and PD (Zhao et al., 2013). Six AD studies met the eligibility criteria with included studies comparing people with AD or PD with healthy controls. Results showed that in the overall analysis, those with AD were more likely to have lower 25(OH)D levels. However there was significant heterogeneity between all the studies included in the analysis. Five studies on PD met criteria and again analysis showed that those with PD were more likely to have lower Vitamin D levels. Once again there was significant heterogeneity between the studies included.
A further meta-analysis of five studies reported an association between Vitamin D and dementia and Alzheimer’s Disease (Shen and Ji, 2015). Of the five studies, two were prospective cohort studies and three were cross-sectional. In terms of Vitamin D deficiency and AD, three studies were included in the analysis, two prospective and one cross-sectional. There was increased risk of AD in those subjects with Vitamin D levels <50nmol/L, OR 1.21 (1.01-1.40, 95% CI). There was also an association between Vitamin D deficiency (<50nmol/L) and development of dementia, OR 1.63 (1.09-2.16, 95% CI). No heterogeneity was noted.

Annweiler et al performed a meta-analysis of studies evaluating the relationship between Vitamin D and cognitive impairment in Asians and included seven studies with 1,179 participants (Annweiler et al., 2016). The mean difference between 25(OH)D between cognitively impaired and cognitively normal individuals was -17nmol/L (-28.4 - -5.7, 95%CI). A subgroup analysis based on AD as the cognitive outcome showed a mean 25(OH)D difference of -37.2nmol/L (-39.3 - -34.9, 95% CI).

A more recent meta-analysis reports an association between Vitamin D deficiency and increased dementia risk in longitudinal, prospective, and RCT studies (Sommer et al., 2017). The authors included five articles in the analysis with a total of 18,933 participants and found that Vitamin D deficiency defined as <25nmol/L was associated with higher risk of dementia, OR 1.54 (1.19-1.99, 95% CI) with low heterogeneity reported.

Goodwill et al report from their meta-analysis that observational data points to an association between Vitamin D and cognition but interventional studies are as yet to show convincing evidence of a benefit from supplementation with Vitamin D (Goodwill and Szoeke, 2017). This meta-analysis was performed using 26 observational studies and three interventional studies. Low Vitamin D levels were associated with poorer cognitive scores, OR 1.24 (1.14-1.35, 95% CI) and cognitive decline, OR 1.26 (1.09-1.22, 95% CI). In intervention studies, Vitamin D supplementation yielded no benefit compared with controls.
A recent meta-analysis from 2018 found an association between Vitamin D concentrations and dementia (Jayedi et al., 2018). This meta-analysis included retrospective and prospective cohort studies and excluded case control studies, studies with only two categories of Vitamin D concentrations, and studies conducted in populations with specific diseases. A total of eight studies were included with 28,354 participants with 1,953 cases of dementia and 1,607 cases of dementia with a median follow-up time ranging from 5 to 21 years. In the seven studies analysing relationship between Vitamin D and dementia, Vitamin D concentrations of 25-50nmol/L did not appear to be associated with risk of dementia, pooled HR 1.09 (0.95-1.24, 95% CI). Vitamin D concentration <25nmol/L was associated with risk of dementia, pooled HR 1.33 (1.08-1.58, 95% CI). There appeared to be a decreased risk of dementia with higher concentrations of 25(OH)D, with a pooled HR 0.83 (0.70-0.96, 95% CI) with 25nmol/L increment in serum 25(OH)D concentrations, but in the context of marked heterogeneity of individual studies. Six studies evaluated the relationship between Vitamin D and risk of AD. In those with Vitamin D concentrations of 25-50nmol/L were not associated with risk of AD, pooled HR 1.19 (0.96-1.4, 95% CI) or with concentrations <25nmol/L, HR 1.31 (0.98-1.675, 95% CI). There appeared to be a dose response relationship with a U shaped curve for Vitamin D and risk of dementia, and a more linear relationship for risk of AD.

7.13 Discussion
There have been many studies investigating the relationship between Vitamin D and cognitive function. A number of studies have shown evidence of a link between Vitamin D deficiency and poor cognition but this link appears to be more consistent in overall test scores and domains related to executive function and attention rather than memory subsets.

Difficulties in comparing these studies include the heterogeneity of the study populations, different definitions of MCI and dementia applied, different assays for Vitamin D and definitions of deficiency and sufficiency used. Many studies also used very crude measures for cognitive assessment.
Given the effects of Vitamin D may be mediated by neuro-inflammation, VDR and vascular effects, it is possible its effects are multifactorial.

Ultimately a large placebo controlled RCT is required to determine the effects of Vitamin D on cognition and cognitive decline, using a comprehensive and detailed neuropsychological assessment battery with sufficient follow up period and adequate dosing of Vitamin D to assess the effects of Vitamin D repletion in cognition.

Observational studies still have value in identifying possible confounders and effect modifiers.
Section 3: Study Population
Chapter 8: Study Population of Trinity University of Ulster Department of Agriculture (TUDA)

These observational investigations were conducted as part of the Trinity, University of Ulster, Department of Agriculture (TUDA) Study, which is a large study of older Irish adults designed to investigate nutritional factors, related gene-nutrient interactions and a range of health and lifestyle factors in the development of chronic diseases of ageing in non-institutionalised adults. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and ethical approval was granted by the relevant authorities in each jurisdiction: the Research Ethics Committee of St. James’s Hospital and The Adelaide and Meath Hospital, Dublin, and the Office for Research Ethics Committees Northern Ireland (ORECNI; reference 08/NI/RO3113) with corresponding approvals from The Northern and Western Health and Social Care Trusts, Northern Ireland. All participants provided written informed consent at the time of enrolment.

Participants for the TUDA Study were identified and recruited between September 2008 and December 2012, from either hospital clinics or the community, in sub-cohorts to focus on three common diseases of ageing: cognitive dysfunction, bone disease including osteopenia and osteoporosis, and hypertension, i.e. the Cognitive, the Bone and the Hypertensive sub-cohorts, respectively.

Two of the cohorts, cognitive and bone, were recruited from outpatient services at the Department of Medicine for the Elderly at St. James’s Hospital, Dublin. Subjects in the cognitive cohort were recruited from general geriatric clinics and a day hospital service and had cognitive impairment based on testing with the RBANS (Repeatable Battery for the Assessment of Neuropsychological Status). Subjects in the bone cohort had a diagnosis of osteoporosis or osteopenia (within three years of recruitment) based on DXA results as defined by standard WHO criteria and were recruited from a specialist bone health service.

The Hypertension sub-cohort was recruited from General Practitioner practices in the Western and Northern Health and Social Care Trusts, Northern Ireland. Recruitment was
on the basis of having a diagnosis of hypertension at the time of recruitment. Apart from raised blood pressure, the Hypertensive sub-cohort was considered generally healthy; all participants were ‘free-living’ and were recruited in the community, although relevant medical details and drug usage were recorded as for all TUDA study participants.

For all TUDA study sub-cohorts, participants were deemed eligible for recruitment if they were aged over 60 years, were able to provide consent, scored 16 or more on Mini Mental State Examination (MMSE) and were without a diagnosis of dementia, and they and their parents were born in the North or South of Ireland. A much smaller number of participants identified at recruitment were also included (i.e. just 3% of total sample) who did not fulfill the requirement of being born in Ireland, but considered themselves Irish on the basis of one or both parents being born in Ireland.
Recruitment of the Cognition sub-cohort was from the Geriatric unit at St. James’s Hospital Dublin
Recruitment of the Bone sub-cohort was from the Geriatric unit at St. James’s Hospital Dublin
Recruitment of the Hypertensive sub-cohort was from General Practitioner clinics in Northern Ireland
Cognitive impairment defined as a RBANS < 80
Osteoporosis defined as a Bone mineral Density T-Score ≤ 2.5 at any measurement site
Hypertensive defined as a systolic blood pressure of 140 or a diastolic pressure as 90 or blood pressure medication

The Trinity Ulster Department of Agriculture (TUDA) Study
n=5,186

Cognition Cohort1
n =1,699
Cognitive impairment:62.3%
Hypertensive: 79.3%
Osteoporosis: Not measured

Bone Cohort2
n =1,394
Cognitive impairment:26.0%
Hypertensive: 56.1%
Osteoporosis: 81.2%

Hypertensive Cohort3
n =2,093
Cognitive impairment:28.2%
Hypertensive: 95.1%
Osteoporosis: 15.6%
### Table 9: Baseline Characteristics of Cognitive and Bone Cohorts

<table>
<thead>
<tr>
<th></th>
<th>Cognitive Cohort (n=1,699)</th>
<th>Bone Cohort (n=1,394)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean)</td>
<td>80.7</td>
<td>71.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Gender (female %)</td>
<td>67.0</td>
<td>85.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total Education (years)</td>
<td>11.5</td>
<td>12.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Living Alone (%)</td>
<td>61.7</td>
<td>33.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>11.3</td>
<td>14.7</td>
<td>0.005*</td>
</tr>
<tr>
<td>Previous Smoker (%)</td>
<td>43.5</td>
<td>37.5</td>
<td>0.001*</td>
</tr>
<tr>
<td>Current Alcohol (%)</td>
<td>47.6</td>
<td>68.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Previous Alcohol (%)</td>
<td>24.7</td>
<td>11.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>55.4</td>
<td>76.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Median 25(OH)D (nmol/L)</td>
<td>50.7</td>
<td>76.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Vitamin D supplementation (%)</td>
<td>61.8</td>
<td>69.0</td>
<td>&lt;0.001*</td>
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<tr>
<td>Sun Holiday Prior Six Months (%)</td>
<td>12.3</td>
<td>19.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GSR J/cm/sq (mean)</td>
<td>31,245.2</td>
<td>30,527.6</td>
<td>0.256*</td>
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<tr>
<td>MMSE (mean)</td>
<td>26.3</td>
<td>26.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FAB (mean)</td>
<td>14.5</td>
<td>15.2</td>
<td>&lt;0.001*</td>
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<td>RBANS Total (mean)</td>
<td>80.6</td>
<td>85.8</td>
<td>&lt;0.001*</td>
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<td>RBANS Index I (mean)</td>
<td>87.7</td>
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<td>&lt;0.001*</td>
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<td>RBANS Index II (mean)</td>
<td>82.0</td>
<td>86.0</td>
<td>&lt;0.001*</td>
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<tr>
<td>RBANS Index III (mean)</td>
<td>87.0</td>
<td>89.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>RBANS Index IV (mean)</td>
<td>82.6</td>
<td>87.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>RBANS Index V (mean)</td>
<td>83.2</td>
<td>87.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HADS (mean)</td>
<td>3.0</td>
<td>3.2</td>
<td>0.242c</td>
</tr>
<tr>
<td>CES-D (mean)</td>
<td>6.7</td>
<td>5.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TUG seconds (mean)</td>
<td>18.8</td>
<td>14.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMI kg/m2 (mean)</td>
<td>27.0</td>
<td>26.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Waist Hip Ratio (mean)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.003*</td>
</tr>
<tr>
<td>IADLS (mean)</td>
<td>22.1</td>
<td>23.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PSMS (mean)</td>
<td>22.6</td>
<td>22.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hypertension (%)**</td>
<td>84.0</td>
<td>73.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Vascular Disease (%)^</td>
<td>32.4</td>
<td>23.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.9</td>
<td>7.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Atrial Fibrillation (%)</td>
<td>17.6</td>
<td>12.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cancer (%)</td>
<td>18.7</td>
<td>16.2</td>
<td>0.075b</td>
</tr>
<tr>
<td>Chronic Kidney Disease (%)^^</td>
<td>64.5</td>
<td>34.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fall in the Last Year (%)</td>
<td>44.8</td>
<td>42.4</td>
<td>0.180b</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>7.2</td>
<td>6.5</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

a Independent t test  b Chi Square Test  c Mann Whitney U Test  *Statistically significant result  **Hypertension: self reported history, medication or BP >140/90mmHg  ^^Chronic Kidney Disease: eGFR <60mL/min  ^Vascular Disease: Self reported history of Stroke, Transient Ischaemic Attack, Peripheral Vascular Disease and/or Myocardial Infarction
Chapter 9: Study Design

9.1 Introduction
A previous study from TUDA investigated the prevalence and determinants of Vitamin D deficiency in the three cohorts (Bone, Hypertensive and Cognitive), from the study population and documents the full study methodology (McCarroll et al., 2015). Positive predictors of 25(OH)D in all cohorts were vitamin D supplement use and GSR whilst the only universal negative determinant was BMI. The relationship with diet and other factors including physical frailty, smoking, gender and sun exposure habits was inconsistent.

For the purpose of the following studies only those participants in the cognitive and bone cohorts, recruited through St James’s Hospital, Dublin, were included to allow for complete and accurate data collection relating to hospital admission, Emergency Department (ED) attendances and mortality to see the associations with Vitamin D and outcome measures including mortality, resource utilisation and mortality in an older Irish population with a high prevalence rate of Vitamin D deficiency.

9.2 Clinical, lifestyle and anthropometric information
A detailed health and lifestyle questionnaire was administered for each participant by fully trained researchers during a 90-minute study interview. Lifestyle details included smoking and alcohol status and vitamin supplement usage. Full details on dietary supplement usage and drugs, including dose, frequency and duration of medication was confirmed from products or prescriptions, which participants were asked to bring along to the appointment. Where dietary supplement or drug information was unknown, the details were collected via telephone shortly after the appointment. Limited dietary information was collected to obtain details on typical intakes and frequency of consumption of certain foods, including fortified products, identified as providing a rich source of specific micronutrients of interest; the information collected did not however permit detailed dietary analysis for nutrient intake values.
Anthropometric measurements included height to the nearest 1mm (using a wall mounted stadiometer, Seca Ltd), weight to the nearest 0.01kg (using electronic scales, Brosch Direct Ltd., Peterborough, UK) Body Mass Index (BMI) (kg/m$^2$) was calculated as weight (kg) divided by height (m$^2$). Waist and hip measurements were recorded to the nearest 0.1 centimetre (using a flexible tape measure, Seca Ltd). All participants were required to remove shoes and heavy clothing for all measurements.

9.3 Medical History
All medications a participant was taking were recorded along with a detailed medical history including whether a person had a diagnosis of hypertension, diabetes mellitus, myocardial infarction, transient ischaemic attack, stroke and peripheral vascular disease. Also recorded was a history of falls within the twelve months prior to study recruitment. A fall was defined as an event resulting in a person inadvertently coming to rest on the ground, floor or lower level.

Blood pressure was measured in a seated position using a clinically validated automated device (Omron 705CP-II). Readings were taken until both systolic and diastolic were within 5mmHg, otherwise a total of five recordings were made and an average of the last two readings was used.

9.4 Vitamin D Measurements
Non-fasting blood samples for 25(OH)D were taken on the day of assessment or within the following week. Bloods were processed on the same day and centrifuged within three hours. 25(OH)D samples were stored at –70°C and batched for later analysis at the biochemistry laboratory at St James’s Hospital, Dublin, a participant of the Vitamin D External Quality Assessment Scheme (DEQAS). 25(OH)D levels were measured by liquid chromatography mass spectroscopy (LCMS) using a standardised assay (Mass Chrom$^\text{®}$) and National Institute of Standards and Technology (NIST) vitamin D standard reference material. Inter and intra-assay coefficient of variation (CV) were 5.7% and 4.5%.
9.5 Neuropsychological Assessments

Global cognitive functioning was measured using a number of different neuropsychological measures including the MMSE (Folstein et al., 1975) and Frontal Assessment Battery (FAB), which specifically measures executive function (Dubois et al., 2000). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) provided a more comprehensive measure of a number of cognitive domains including; immediate (Index I) and delayed memory (Index V), visuospatial (Index II), language (Index III) and attention (Index IV) function (Randolph et al., 1998).

9.6 Biophysical and Psychological Measurements

Two assessments were completed evaluating a person’s function both in terms of Activities of Daily Living (ADL) and Instrumental ADL (IADL), these included Lawton’s IADLS and observer-rated Physical Self-Maintenance Scale (PSMS) (Lawton and Brody, 1969).

The PSMS was developed to gauge disability in elderly people currently in a community or institution. It is a six-item evaluation tool based on ADLs with a five-point scale for responses ranging from complete independence to total dependence and is recognised as a reliable and valid ADL scale for clinical and survey research. The Lawton Instrumental Activities of Daily Living Scale (IADL) is an appropriate instrument to assess independent living skills that are considered more complex than the basic activities of daily living as measured by PSMS. It is useful in identifying how a person is functioning at the present time and in identifying improvement or deterioration over time. There are eight domains of function measured.

Gait speed was measured using the Timed Up and Go (TUG) which is used to assess a person’s mobility and requires both static and dynamic balance (Podsiadlo and Richardson, 1991). It is also frequently used as a screening tool in those suspected to be at increased risk of falls. Participants were timed while they rose from a chair, walked at a comfortable and safe pace to a point three metres away, turned and walked back to the chair and sat down again.
Symptoms of depression were assessed using the short self-report scale Centre for Epidemiologic Studies Depression Scale (CES-D), which is a screening test designed to measure depressive symptomatology in the general population (Radloff, 1977). Scores range from 0 to 60, with high scores indicating greater depressive symptoms. Symptoms of anxiety were assessed using the seven questions for anxiety from the Hospital Anxiety and Depression Scale (HADS), which is a widely used and validated tool in general medical and community populations (Zigmond and Snaith, 1983).
Section 4: Vitamin D and Hospital Admissions
Chapter 10: Introduction

Increased use of healthcare resources by the older population continues to be a significant and complex issue in modern healthcare service provision, which can be associated with negative outcomes in relation to function, independent living and quality of life for older adults. Hospital admission rates and Length of stay (LOS) are measures frequently used to quantify resource utilisation. Reduction in LOS is often considered as a potential strategy to optimise resource consumption and reduce health care costs.

To date, many studies have tried to identify those at increased risk of hospitalization and nursing home admission. Given the postulated multisystem effects of Vitamin D, studies have recently been published looking at the association between Vitamin D and specific conditions such as infections, sepsis (Ginde et al., 2011, Jovanovich et al., 2014, Leow et al., 2011, Pletz et al., 2014), exacerbations of chronic illnesses, admissions to critical care and intensive care facilities (Langlois et al., 2017), functional outcomes following falls (Dhesi et al., 2004, Ghafouri et al., 2016, Scragg et al., 2015, Snijder et al., 2006) and fractures.

Frailty is another factor to consider as studies have demonstrated that it is relevant when considering outcomes. Previous studies demonstrate that frailty is associated with increased ED attendance and increased rates of hospitalisation (Chamberlain et al., 2016, Hastings et al., 2008) and with higher healthcare costs (Comans et al., 2016). A number of studies have evaluated the relationship between Vitamin D and frailty with associated outcome measures (Rosendahl-Riise et al., 2017, Zhou et al., 2016).

To date there is limited research looking at the association between Vitamin D levels and hospital admissions rates and length of stay in older adults. A small number of studies have specifically investigated the effect of Vitamin D on hospital admissions and results are variable (Table 10).
### Table 10: Vitamin D and Hospital Admission/Re-admission

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Sectional:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaidi et al 2016</td>
<td>209</td>
<td>76</td>
<td>32.5%</td>
<td>Higher hospital re-admission rates over 12 months in those not treated for Vitamin D deficiency</td>
</tr>
<tr>
<td>Prospective:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konstari et al 2014</td>
<td>2,454</td>
<td>Not available</td>
<td>Approximately 45nmol/L</td>
<td>Higher Vitamin D levels associated with increased hospitalisation rates due to OA</td>
</tr>
</tbody>
</table>

### Table 11: Vitamin D and Hospital Length of Stay (LOS)

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Sectional:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amrien et al 2006</td>
<td>982</td>
<td>61</td>
<td>80.4nmol/L</td>
<td>Shorter hospital LOS associated with higher Vitamin D levels</td>
</tr>
<tr>
<td>Graedel et al 2016</td>
<td>4,257</td>
<td>63</td>
<td>18.7% &lt;25nmol/L</td>
<td>Vitamin D deficiency associated with longer LOS</td>
</tr>
<tr>
<td>Maier et al 2016</td>
<td>1,083</td>
<td>76</td>
<td>42.7nmol/L</td>
<td>Lower LOS in those with higher Vitamin D levels</td>
</tr>
<tr>
<td>Cross Sectional:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annweiler et al. 2010</td>
<td>399</td>
<td>84.5</td>
<td>34.8nmol/L</td>
<td>No difference between Hospital LOS</td>
</tr>
<tr>
<td>Marra et al 2013</td>
<td>115</td>
<td>78.4</td>
<td>15nmol/L</td>
<td>Vitamin D not associated with hospital LOS</td>
</tr>
<tr>
<td>Prospective:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McWilliams et al 2011</td>
<td>71</td>
<td>82.2</td>
<td>56.7nmol/L</td>
<td>Those with lower Vitamin D levels (&lt;75nmol/L) had longer LOS</td>
</tr>
<tr>
<td>Helard et al 2013</td>
<td>253</td>
<td>86.2</td>
<td>33.9nmol/L</td>
<td>Lower Vitamin D levels associated with longer hospital LOS</td>
</tr>
<tr>
<td>Beauchet et al 2013</td>
<td>531</td>
<td>85</td>
<td>Not available</td>
<td>LOS was predicted by male sex, delirium and 25(OH)D levels</td>
</tr>
</tbody>
</table>
Chapter 11: Methodology

11.1 Study Population
All TUDA participants recruited through St James Hospital Dublin were included in this study. The TUDA population, as previously outlined in Section 3, is a large community dwelling population of Irish adults aged 60 years and older. It is a cross-sectional study designed to create a genotype/phenotype database for three population cohorts based on three disease states: cognition, bone health and hypertension.

Ethical approval was granted for this study from the local Research Ethics Committee (REC), (REC Reference 2013/05/05 Chairman’s Action).

11.2 Methodology
All TUDA study participants recruited through St James Hospital (SJH), Dublin (n=3,093) were included in this study, which included participants from the cognitive and bone cohorts. Full methodology is published elsewhere (McCarroll et al., 2015) and discussed in detail in Chapters 8 and 9.

Details relating to hospitalisation were accessed through the St James’s Hospital Electronic Patient Record (EPR) system, which includes clinical information, cardiac, radiological and laboratory investigations, as well as discharge summaries. Information was gathered from date of TUDA participation until completion of this study in June 2013. Information collected included Length of Stay (LOS), reason for admission and discharge destination. No exclusion criteria applied. Reason for admission was categorized, as was discharge destination.

Two researchers, RL and AB, independently investigated a review of records for reason for hospital admission. Overall thirty cases were reviewed for standardisation of key word analysis and reason for admission, which was achieved with a kappa score of greater than 0.8 for inter-rater reliability (Viera and Garrett, 2005).
All hospital admission records were reviewed using the EPR system and reason for admission was established by extracting relevant information and diagnosis through predefined keywords; “falls, fracture, cardiopulmonary, stroke/Transient Ischaemic Attack (TIA), gastrointestinal bleed, other/unclear”.

Admissions to other institutions were not captured in this study. However, <1% of the TUDA population live outside the catchment area of St. James’s Hospital, therefore our data collection captured events for 99% of the study population.

The aim of our study was to prospectively evaluate the relationship between serum 25-hydroxyvitamin D (25(OH)D) levels and hospital admissions in older Irish community dwelling adults participating in the Trinity, University of Ulster, Department of Agriculture (TUDA) Study.

11.3 Statistical Analysis
All parameters were inspected for normality and if significantly skewed were appropriately transformed. Normal assumptions for linear regression analysis were observed. Descriptive and comparative analyses were performed for all included participants and for a priori determined subgroups, admitted versus non-admitted patients.

Continuous variables are expressed as mean and standard deviations for normally distributed and median and quartiles for non-normally distributed data. Categorical variables were expressed as number of cases and percentages. Nominal or dichotomous variables were compared using Chi-squared test, non-normally distributed variables were compared with Mann-Whitney U test and normally distributed continuous variables were compared using the Student t test. These analyses were run using SPSS version 22.0 (SPSS, Inc., Chicago IL). Statistical significance was accepted when p <0.05.

Survival analysis was performed using R (R Development Core Team, 2013). Cox proportional Hazard Models were generated both for Vitamin D as a continuous variable and also as a categorical variable based on defined Vitamin D levels and were used to
evaluate the proposed relationship between Vitamin D and hospitalisation. The Vitamin D cut-offs were based on previously defined levels in the literature (Holick et al., 2011) with <50nmol/L defined as deficient and <25nmol/L as severely deficient. Missing data was dealt with using Multiple Imputation Modelling in Multivariate Imputation by Chained Equations (MICE) in R (R Development Core Team, 2013).

Multivariate linear regression models were generated to interrogate the potential relationship between total hospital length of stay (LOS) and Vitamin D levels. In regression models, these variables were log transformed as they were positively skewed on descriptive statistics. Logistic regression analysis was used in subgroup analysis to explore the relationship between Vitamin D and specific reason for admission.

Interaction terms for study cohort and admission status were looked for but were not found to be significant in regression analysis.
11.4 Covariates

The covariates in the basic Model 1 of this study were those known to effect serum 25-hydroxyvitamin D levels or hospital admission. These were; age, gender and total number of years in education, BMI, Vitamin D supplementation and Global Solar Radiation (GSR). The GSR in the month and preceding two months of subject recruitment was used as a surrogate marker of UVB exposure, which is known to effect serum Vitamin D concentrations. GSR represents the total amount of solar radiation (direct beam plus the diffuse component on a horizontal surface) received per unit area per month (MJm$^{-2}$), (http://www.met.ie/about/valentiaobservatory/solarradiation.asp). GSR data was obtained by request from the Irish Meteorological Service.
Model 2 consisted of Model 1 along with frailty markers covariates which are known to affect admission rates; Timed Up and Go (TUG) which is a frequently used screening tool to measure patients gait speed and is associated with a potential risk of falling, Mini Mental State Examination (MMSE) which is a screening tool for cognition and can be used as a marker of cognitive frailty and finally IADLS, a score reflecting a patient’s ability to function independently in their activities if daily living.

Lastly, Model 3 included Model 1 and 2 covariates and 3 pre-specified diseases (cancer, chronic kidney disease and falls) we investigated as possible effect modifiers of hospital admission.
Chapter 12: Results

12.1 Baseline Characteristics
Of the 3,093 participants included in this study, 1,269 participants (41%) were admitted over the mean follow-up period of 3.6 years (Table 12). Those admitted were older (80.0 versus 74.1 years, p<0.001), less educated and more likely to be female. Admissions were more frequent in the cognitive cohort group than the bone cohort, which is likely related to the fact that these participants were older and frailer at baseline recruitment. They were also more likely to have conditions such as hypertension, chronic kidney disease (CKD) and cancer than those who were not admitted.

Those participants admitted to hospital had lower mean Vitamin D levels at baseline, 58.4nmol/L versus 69.3nmol/L, than those not admitted (p<0.001). Those participants who were admitted were less independent in terms of function, with lower IADLS scores and also had slower TUG scores indicating slower gait speeds (16.0 versus 12.0 seconds).

Of the total study population with 25(OH)D levels available, 394 (12.7%) had levels <25nmol/L and 1,119 (36.2%) had levels <50nmol/L.

Of those participants admitted over the study follow-up period, 231 (18.2%) had 25(OH)D levels <25nmol/L and 584 (46%) had levels <50nmol/L.
Table 12: Baseline Characteristics By Admission Status

<table>
<thead>
<tr>
<th></th>
<th>Admitted (n=1,269)</th>
<th>Not Admitted (n=1,824)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Cohort (%)</td>
<td>76.6</td>
<td>39.9</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Bone Cohort (%)</td>
<td>23.4</td>
<td>60.1</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Age in years (mean)</td>
<td>80.0</td>
<td>74.1</td>
<td>&lt;0.001*a</td>
</tr>
<tr>
<td>Gender (female %)</td>
<td>68.5</td>
<td>79.8</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Total Education (median, years)</td>
<td>10.0</td>
<td>11.0</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Living Alone (%)</td>
<td>45.0</td>
<td>36.4</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>12.9</td>
<td>12.8</td>
<td>0.912a</td>
</tr>
<tr>
<td>Previous Smoker (%)</td>
<td>44.5</td>
<td>38.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Current Alcohol (%)</td>
<td>51.0</td>
<td>61.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Previous Alcohol (%)</td>
<td>24.2</td>
<td>15.0</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>25(OH)D nmol/L (mean)</td>
<td>58.4</td>
<td>69.3</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>25(OH)D nmol/L (median)</td>
<td>53.9</td>
<td>70.4</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>Vitamin D supplementation (%)</td>
<td>39.7</td>
<td>60.3</td>
<td>0.011*b</td>
</tr>
<tr>
<td>Sun Holiday Previous Six Months (%)</td>
<td>37.3</td>
<td>62.7</td>
<td>0.69b</td>
</tr>
<tr>
<td>MMSE (median)</td>
<td>27.0</td>
<td>27.0</td>
<td>0.033c</td>
</tr>
<tr>
<td>HADS (median)</td>
<td>2.0</td>
<td>2.0</td>
<td>0.309c</td>
</tr>
<tr>
<td>CES-D (median)</td>
<td>6.5</td>
<td>6.0</td>
<td>0.056a</td>
</tr>
<tr>
<td>TUG seconds (median)</td>
<td>16.0</td>
<td>12.0</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>BMI kg/m2 (mean)</td>
<td>26.9</td>
<td>26.6</td>
<td>0.875a</td>
</tr>
<tr>
<td>Waist Hip Ratio (mean)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.68a</td>
</tr>
<tr>
<td>IADLS (mean)</td>
<td>22.4</td>
<td>23.0</td>
<td>&lt;0.001*a</td>
</tr>
<tr>
<td>PSMS (median)</td>
<td>24.0</td>
<td>24.0</td>
<td>0.203c</td>
</tr>
<tr>
<td>Hypertension (%)**</td>
<td>83.2</td>
<td>76.6</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Vascular Disease (^)</td>
<td>29.6</td>
<td>27.8</td>
<td>0.267b</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.4</td>
<td>9.0</td>
<td>0.026*b</td>
</tr>
<tr>
<td>Atrial Fibrillation (%)</td>
<td>15.7</td>
<td>15.0</td>
<td>0.602b</td>
</tr>
<tr>
<td>Cancer (%)</td>
<td>20.9</td>
<td>15.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Chronic Kidney Disease (%)^^</td>
<td>65.3</td>
<td>41.5</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Fall in the Last Year (%)</td>
<td>4.3</td>
<td>4.0</td>
<td>0.716b</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>7.0</td>
<td>6.8</td>
<td>0.171a</td>
</tr>
</tbody>
</table>

a Independent t test  
b Chi Square Test  
c Mann Whitney U Test  
^^Chronic Kidney Disease: eGFR <60mL/min  
^Vascular Disease: Self reported history of Stroke, Transient Ischaemic Attack, Peripheral Vascular Disease and/or Myocardial Infarction  
**Hypertension: self reported history, medication or BP >140/90mmHg  
*Statistically significant result
Of the 41% of the TUDA participants admitted during the follow-up period, the median number of admissions was 2.0 (range 1-18), median hospital LOS per admission was 31.0 days (range 1-1,384) and median time to first admission of 10.0 months (range 0-51). The reasons for hospital admission are shown in Table 13 and Figure 7.

**Table 13: Reason for First Admission**

<table>
<thead>
<tr>
<th>Reason for First Admission</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>154 (12.1)</td>
</tr>
<tr>
<td>Fracture</td>
<td>83 (6.5)</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>375 (29.6)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>60 (4.7)</td>
</tr>
<tr>
<td>GI Bleed</td>
<td>25 (2.0)</td>
</tr>
<tr>
<td>Other</td>
<td>572 (45.1)</td>
</tr>
</tbody>
</table>

**Figure 7: Reason for First Hospital Admission**
12.2 Survival Analysis

In multivariate survival analysis, with Cox proportional hazard modelling, Vitamin D, considered as a continuous variable (Table 14), was found to be associated with hospital admission, HR 0.997 (0.995-0.998, 95% CI) p<0.001. This association remained in fully adjusted models, HR 0.996, (0.994-0.998, 95% CI) p<0.001. Thus for every 1nmol/L increase in 25(OH)D levels, the relative risk of hospital admission was reduced by 0.4%.

Table 14: Cox Proportional Hazard Model: 25 Hydroxyvitamin D and Admission
(Vitamin D as continuous time dependant variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>β Coefficient</th>
<th>HR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-3.485</td>
<td>0.997</td>
<td>0.995-0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-3.768</td>
<td>0.996</td>
<td>0.994-0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-4.035</td>
<td>0.996</td>
<td>0.994-0.998</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Statistically significant result

Further analysis considering Vitamin D as a categorical variable (Tables 15 and 16) revealed an association between hospital admission in participants with deficient (<50nmol/L) and severely deficient (<25nmol/L) Vitamin D levels compared with participants with normal Vitamin D levels. Once again this association was present in fully adjusted models with covariates for both physical and cognitive frailty considered. Thus for an individual with 25(OH)D levels above 50nmol/L the relative risk of hospital admission was decreased, i.e. 0.77 times that of those with levels <50nmol/L (Figures 8 to 11).
Table 15: Cox Proportional Hazard Model: 25 Hydroxyvitamin D >/<50nmol/L and Admission
(Vitamin D as a categorical variable)

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>HR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.2521</td>
<td>0.77</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2574</td>
<td>0.77</td>
<td>0.0004*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.2638</td>
<td>0.77</td>
<td>0.0003*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI, Time to Assessment
Model 2: Model 1 and TUG, MMSE, IADL
Model 3: Model 2 and History of Cancer, CKD, Fallen in the past year
*Statistically significant result

Figure 8: Survival Curve 25 Hydroxyvitamin D Levels >/<50nmol/L and Admission
Figure 9: Cumulative Hazard Curve 25 Hydroxyvitamin D Levels >/50nmol/L and Admission
**Table 16:** Cox Proportional Hazard Model: 25 Hydroxyvitamin D >/<25nmol/L and Admission  
(Vitamin D as categorical dependant variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>β Coefficient</th>
<th>RR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.2439</td>
<td>0.78</td>
<td>0.0010*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2060</td>
<td>0.81</td>
<td>0.0105*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.2195</td>
<td>0.80</td>
<td>0.0063*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI  
Model 2: Model 1 and TUG, MMSE, IADL  
Model 3: Model 2 and History of Cancer, CKD, Fallen in the past year

**Figure 10:** Survival Curve 25 Hydroxyvitamin D Levels >/<25nmol/L and Admission
Figure 11: Cumulative Hazard Curve: 25 Hydroxyvitamin D Levels >/<25nmol/L and Admission
12.3 Subgroup Analysis

In subgroup analysis using logistic regression models, Vitamin D concentrations were not associated with individual reasons for hospital admission Table 17).

Table 17: Association between 25(OH)D Concentration and Reason for First Hospital Admission

<table>
<thead>
<tr>
<th>Reason</th>
<th>Model 1</th>
<th>HR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>-0.001</td>
<td>0.99</td>
<td>0.99 – 1.01</td>
<td>0.678</td>
</tr>
<tr>
<td>Fracture</td>
<td>-0.004</td>
<td>0.99</td>
<td>0.99 – 1.00</td>
<td>0.349</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>-0.001</td>
<td>0.99</td>
<td>0.99 – 0.99</td>
<td>0.632</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>-0.004</td>
<td>0.99</td>
<td>0.99 – 1.005</td>
<td>0.350</td>
</tr>
<tr>
<td>GI Bleed</td>
<td>0.010</td>
<td>1.01</td>
<td>0.99 – 1.02</td>
<td>0.128</td>
</tr>
<tr>
<td>Other</td>
<td>0.002</td>
<td>1.00</td>
<td>0.99 – 1.01</td>
<td>0.238</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone (Y/N), Education, GSR, Vitamin D Supplementation, BMI
Model 2: Model 1 and TUG, MMSE, IADL

Vitamin D concentration (log transformed) was inversely associated with overall (accumulated over all hospital admissions) hospital LOS (log transformed) throughout the study period in multivariate linear regression models (Table 18).

Table 18: Association Between Total Hospital LOS (Log Transformed) and 25(OH)D Concentration (Log Transformed)

<table>
<thead>
<tr>
<th>Reason</th>
<th>Model 1</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>-0.74</td>
<td>-0.27 – -0.03</td>
<td>0.015*</td>
</tr>
<tr>
<td>Fracture</td>
<td>-0.95</td>
<td>-0.33 – -0.56</td>
<td>0.006*</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI Bleed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gender, Living Alone (Y/N), Education, GSR, Vitamin D Supplementation, BMI, Time to Assessment
Model 2: Model 1 and TUG, MMSE, IADL
*Statistically significant value
Figure 12: Scatterplot of the Association Between Total Hospital LOS and 25(OH)D Concentration
Chapter 13: Conclusion

13.1 Discussion
This study demonstrated a strong inverse association between Vitamin D levels and prospective hospital admission rates in a large population of older adults. Participants in our study whose serum 25-hydroxyvitamin D levels were deficient (<50nmol/L) or severely deficient (<25nmol/L) were more likely to be admitted to hospital than those participants with higher levels. This prospective relationship remained robust after full adjustment for multiple factors predictive of Vitamin D status and physical and cognitive frailty. Those participants with lower Vitamin D levels who were admitted to hospital were also more likely to have longer lengths of hospital stay (LOS) compared with Vitamin D replete participants.

This is the first study to examine the prospective association between Vitamin D and hospital admission in a large population of older adults. The findings are similar to prior studies which demonstrate that Vitamin D deficiency was associated with increased risk of hospital re-admission, nursing home admission and with increased hospital LOS compared with those with replete Vitamin D levels (Amrein et al., 2016, Beauchet et al., 2013, Graedel et al., 2016, Visser et al., 2006).

While certain studies have shown an association between Vitamin D and conditions leading to admission such as Multiple Sclerosis and COPD (Dhesi et al., 2004, Malinovschi et al., 2014), ICU admissions (Borgermann et al., 2012, Matthews et al., 2012) and infections (Aregbesola et al., 2013, Jovanovich et al., 2014, Pletz et al., 2014), this is the only study to date evaluating the relationship between Vitamin D status and hospital admission independent of physical and cognitive frailty measures rather than specific diseases or illnesses.

The prospective association in this study strengthens the need to further consider Vitamin D as an independent modifiable factor in hospital admission rates and the potential need for supplementation, in older adults deficient in Vitamin D beyond current bone health
recommendations. This association needs to be further investigated with a large randomised control trial (RCT), including older adults who are deficient in Vitamin D, with appropriate supplementation doses of Vitamin D before such recommendations can be made.

13.2: Strengths and Limitations

Although a large sample population of older adults was included in this analysis with a 3.6 year follow up, there are some potential limitations to our study. Participants were originally recruited to cohorts with underlying “diseases” of cognition and bone health and therefore the results may not be fully representative of a general older population.

Also, data on hospital admission was only available for our own institution so it is possible that participants could have been admitted to another facility and this data is not available for this study. However, as only approximately 1% of participants live outside our catchment area this is unlikely to be significant.

Finally, frailty is an important factor potentially contributing to the reason for ED attendance. While we did not use a specific frailty score or tool in this study, as there is no consensus on the best frailty index at present, frailty was considered and measured using markers of physical and cognitive frailty as previously outlined, which were included as confounders in this study’s analysis.
Section 5: Vitamin D and Emergency Department Attendances
Chapter 14: Introduction

Older people are an increasing user group of emergency departments (ED). ED attendance for older adults can herald potential significant decline and is associated with increased hospital admission rates and increased length of stay. Those who are not admitted are likely to re-attend with unplanned admissions, which in turn may be due to poor recognition of underlying disease processes and lack of awareness of cognitive or social difficulties or insufficient follow-up. Presentation to the ED for the older adult has been shown to be associated with poorer outcomes, including functional decline, morbidity, institutionalization and mortality.

A number of studies suggest an association between Vitamin D and frailty (Swiecicka et al., 2017a, Tajar et al., 2013, Verlaan et al., 2017, Vogt et al., 2015, Zhou et al., 2016), falls (Bischoff-Ferrari et al., 2004b, Broe et al., 2007, Cranney et al., 2008, Scragg et al., 2015) and resource utilization such as hospital admissions (Zaidi et al., 2016) and nursing home admission (Visser et al., 2006) as outlined previously in Chapters 4 and 5.

Attendance at ED may be influenced by many factors; thus we must seek to modify any factor, such as Vitamin D, that may be associated with increased risk of ED attendances. The relationship between Vitamin D and ED attendance is not well documented in the literature to date.

Given the paucity of evidence in this area, the aim of this study was to evaluate the prospective relationship between serum Vitamin D and Emergency Department (ED) attendances in older Irish community dwelling adults.
Chapter 15: Methodology

15.1 Study Population
All 3,093 TUDA participants recruited through St James’s Hospital were included in this study. The TUDA population is a large community dwelling population of Irish adults aged 60 years and older. It is a cross-sectional study designed to create a genotype/phenotype database for three population cohorts based on three disease states: cognition, bone health and hypertension. Full methodology published elsewhere (McCarroll et al., 2015) and discussed in detail in Chapters 8 and 9.

15.2 Methodology
Details relating to ED attendances were accessed through the SJH “Therefore system”, which is an electronic record of all patients ED attendances. Information was gathered from date of TUDA participation until June 2013, including total number of ED attendances, reason for attendance and discharge destination.

All ED attendance records, which are handwritten records scanned onto the hospitals ED computer programme “Therefore”, were reviewed. These records include medical history, examination details, electrocardiogram (ECG), and basic blood results, diagnosis and treatment plans. Reason for ED attendance was established from reading individual records, by AB and RL, and by extracting relevant information and diagnosis through predefined keywords; “falls, fracture, cardiopulmonary, stroke/Transient Ischaemic Attack (TIA), gastrointestinal bleed, other/unclear, planned”.

Two researchers, RL and AB, independently investigated a review of records for reason for ED attendance. Overall thirty cases were initially reviewed for standardisation of keyword analysis and reason for ED attendance. This was achieved with a kappa score of greater than 0.8 for inter-rater reliability (Viera and Garrett, 2005).
Attendance at Emergency Departments in other institutions was not captured in this study. However, only 1% of the TUDA population live outside the catchment area of St. James’s Hospital, therefore our data collection captured events for 99% of the study population.

Ethical approval was granted for this study from the local Research Ethics Committee (REC Reference 2013/05/05 Chairman’s Action).

15.3 Statistical Analysis

All parameters were inspected for normality and if significantly skewed were appropriately transformed. Normal assumptions for linear regression analysis were observed. Descriptive and comparative analyses were performed for all included participants and for a priori determined subgroups, ED attendance versus ED non-attendance.

Continuous variables are expressed as mean and standard deviations for normally distributed and median and inter-quartiles for non-normally distributed data. Categorical variables were expressed as number of cases and percentages. Nominal or dichotomous variables were compared using Chi-squared test, non-normally distributed variables were compared with Mann-Whitney U test and normally distributed continuous variables were compared using the Student t test. These analyses were run using SPSS version 22.0 (SPSS, Inc., Chicago IL). Statistical significance was accepted when p <0.05.

Survival analysis was performed using R (R Development Core Team, 2013). Cox proportional Hazard Models were generated both for Vitamin D as a continuous variable and also as a categorical variable based on defined Vitamin D levels and were used to evaluate the proposed relationship between Vitamin D and hospitalisation. The Vitamin D cut-offs were based on previously defined levels in the literature with <50nmol/L defined as deficient and <25nmol/L as severely deficient (Holick et al., 2011). Missing data was dealt with using Multiple Imputation Modelling in Multivariate Imputation by Chained Equations (MICE) using R (R Development Core Team, 2013).
Logistic regression analysis was used in subgroup analysis to explore the relationship between Vitamin D concentrations and the specific reason for ED attendance. In regression models, non-normally distributed variables, were log transformed for analysis.

Interaction terms for cohort and ED attendance status were looked for but were not found to be significant in regression analysis.

**Figure 13:** Absence of Interaction between ED Attendance Status and Study Cohort
15.4 Covariates

The basic model (Model 1) of this study considered those covariates known to effect serum 25-hydroxyvitamin D levels or ED attendance. These were age, gender and total number of years in education, BMI, Vitamin D supplementation and Global Solar Radiation (GSR). The GSR in the month and preceding two months of subject recruitment was used as a surrogate marker of UVB exposure, which is known to effect serum Vitamin D concentrations. GSR represents the total amount of solar radiation (direct beam plus the diffuse component on a horizontal surface) received per unit area per month (MJm$^{-2}$), (http://www.met.ie/about/valentiaobservatory/solarradiation.asp). GSR data was obtained by request from the Irish Meteorological Service.

Model 2 included all variables in Model 1 and also variables that were confounders for frailty (both physical and cognitive) which are known to affect admission rates; Timed Up and Go (TUG) which is a frequently used screening tool to measure patients gait speed and is associated with a potential risk of falling, Mini Mental State Examination (MMSE) which is a screening tool for cognition and can be used as a marker of cognitive frailty and finally IADLS, a score reflecting a patient’s ability to function independently in their activities of daily living.

Model 3 included all variables from Models 1 and 2 and pre-specified diseases/conditions found to be individual predictors of ED attendance.
Chapter 16: Results

16.1 Baseline Characteristics

Over the mean follow up period of 3.6 years, 1,577 (50.9%) TUDA participants attended the ED. The median time to first ED attendance was 10.0 months (range 0-51 months) and the median number of attendances was 2.0 (range 1-39 visits).

Those participants who attended the ED were older (79.2 versus 73.7 years); less educated (10.0 versus 12.0 years) and more likely to be living alone, were more depressed based on CES-D scores and were less independent in activities of daily living (Table 19).

Attenders were also frailer based on Timed Up and Go (TUG) at 16.0 versus 12.0 seconds. ED attenders had lower serum 25(OH)D levels than those who did not attend, 59.1 nmol/L compared with 70.6 nmol/L and were less likely to be taking Vitamin D supplements. Those who attended the ED were also more likely to have a history of Hypertension, Diabetes, Cancer and Chronic kidney disease (CKD) than those who did not attend the ED over the study period.

Of those who attended the ED over the study follow-up period, 698 (44.9%) had serum 25(OH)D levels <50 nmol/L and 272 (17.5%) had levels <25 nmol/L.
Table 19: Baseline Characteristics By Emergency Department (ED) Attendance Status

<table>
<thead>
<tr>
<th></th>
<th>ED Attenders (n=1557)</th>
<th>ED Non Attenders (n=1536)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Cohort (%)</td>
<td>72.7</td>
<td>37.0</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bone Cohort (%)</td>
<td>27.3</td>
<td>63.0</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age in years (mean)</td>
<td>79.2</td>
<td>73.7</td>
<td>&lt;0.001*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender (female %)</td>
<td>70.0</td>
<td>79.9</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Education (years, median)</td>
<td>10.0</td>
<td>12.0</td>
<td>&lt;0.001*&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Living Alone (%)</td>
<td>43.7</td>
<td>36.1</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>13.0</td>
<td>12.6</td>
<td>0.747&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Previous Smoker (%)</td>
<td>43.9</td>
<td>37.7</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Current Alcohol (%)</td>
<td>52.7</td>
<td>61.4</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Previous Alcohol (%)</td>
<td>22.9</td>
<td>14.6</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>59.1</td>
<td>70.6</td>
<td>&lt;0.001*&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median 25(OH)D (nmol/L)</td>
<td>55.0</td>
<td>71.9</td>
<td>&lt;0.001*&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin D supplementation (%)</td>
<td>62.8</td>
<td>67.3</td>
<td>0.012&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sun Holiday Previous Six Months (%)</td>
<td>13.7</td>
<td>17.2</td>
<td>0.008*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMSE (median)</td>
<td>27.0</td>
<td>27.0</td>
<td>0.074&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HADS (median)</td>
<td>2.0</td>
<td>2.0</td>
<td>0.574&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CES-D (median)</td>
<td>6.6</td>
<td>5.9</td>
<td>0.014*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TUG seconds (median)</td>
<td>16.0</td>
<td>12.0</td>
<td>&lt;0.001*&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI kg/m2 (mean)</td>
<td>26.9</td>
<td>26.6</td>
<td>0.109&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waist Hip Ratio (mean)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.022&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IADLS (mean)</td>
<td>22.3</td>
<td>23.1</td>
<td>&lt;0.001*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PSMS (median)</td>
<td>24.0</td>
<td>24.0</td>
<td>0.292&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypertension (%)**</td>
<td>83.1</td>
<td>75.5</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vascular Disease (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.4</td>
<td>26.6</td>
<td>0.019*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.5</td>
<td>8.4</td>
<td>0.003*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atrial Fibrillation (%)</td>
<td>16.0</td>
<td>14.6</td>
<td>0.270&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cancer (%)</td>
<td>19.5</td>
<td>15.6</td>
<td>0.005*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic Kidney Disease (%)&lt;sup&gt; ^^&lt;/sup&gt;</td>
<td>60.0</td>
<td>37.5</td>
<td>&lt;0.001*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fall in the Last Year (%)</td>
<td>44.2</td>
<td>43.3</td>
<td>0.610&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>7.0</td>
<td>6.8</td>
<td>0.084&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Independent t Test  
<sup>b</sup> Chi Square Test  
<sup>c</sup> Mann Whiney U Test  
*Statistically significant result  
<sup>a</sup>Vascular Disease: Self reported history of Stroke, Transient Ischaemic Attack, Peripheral Vascular Disease and/or Myocardial Infarction  
<sup>^</sup>Vascular Disease: Self reported history of Stroke, Transient Ischaemic Attack, Peripheral Vascular Disease and/or Myocardial Infarction  
<sup>^^</sup>Chronic Kidney Disease: eGFR <60mL/min  
**Hypertension: self reported history, medication or BP >140/90mmHg
Of the 50.3% of TUDA participants who attended the ED during the follow-up period, the median number of attendances was 2.0 (range 1-39) and median time to first ED attendance of 10.0 months (range 0-51). Reason for first ED attendance is outlined in Table 20 and Figure 14.

**Figure 14:** Reason For First ED attendance

**Table 20:** Reason for First Emergency Department Attendance

<table>
<thead>
<tr>
<th>Reason for ED Attendance</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>233 (15.0)</td>
</tr>
<tr>
<td>Fracture</td>
<td>130 (8.4)</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>342 (22.0)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>54 (3.5)</td>
</tr>
<tr>
<td>GI Bleed</td>
<td>26 (1.7)</td>
</tr>
<tr>
<td>Other</td>
<td>771 (49.6)</td>
</tr>
</tbody>
</table>
16.2 Survival Analysis

Fully adjusted Cox proportional hazard models with Vitamin D as continuous variable showed an association between Vitamin D and ED Attendances, HR 0.996 (0.995-0.998, 95% CI) p <0.001, which remained present in the fully adjusted model. For every 1nmol/L increase in serum 25(OH)D concentrations, there was a 0.4% reduction in risk of ED attendance.

Table 21: Cox Proportional Hazard 25 Hydroxyvitamin D and ED Attendance
(Vitamin D as a continuous time dependent variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>β Coefficient</th>
<th>HR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-3.570</td>
<td>0.996</td>
<td>0.995-0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-3.644</td>
<td>0.996</td>
<td>0.994-0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-3.868</td>
<td>0.996</td>
<td>0.994-0.998</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI
Model 2: Model 1 and TUG, MMSE, IADL
Model 3: Model 2 and Fall in the Last Year, History of Cancer
*Statistically significant value

Further modelling with Vitamin D levels presented as a dependant categorical variable, again showed an association between Vitamin D and ED attendance. Vitamin D deficiency (defined as <50nmol/L) and severe deficiency (defined as <25noml/L) were both associated with increased risk of ED attendance over the follow up period, HR 0.78, p=0.001. These models were adjusted for variables known to be associated with Vitamin D deficiency and frailty markers and certain clinical conditions found to be associated on subgroup analysis.
For an individual with 25(OH)D level above 50nmol/L the relative risk of ED attendance is decreased, i.e. 0.76 times that of those with levels <50nmol/L (Table 22 and Figures 15 and 16)

Table 22: Cox Proportional Hazards 25 Hydroxyvitamin D >/<50nmol/L and ED Attendance
(Vitamin D as a categorical variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>β Coefficient</th>
<th>RR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.2519</td>
<td>0.77</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2667</td>
<td>0.77</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.2740</td>
<td>0.76</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI
Model 2: Model 1 and TUG, MMSE, IADL
Model 3: Model 2 and Fall in the Last Year, History of Cancer
*Statistically significant value

Figure 15: Survival Curve 25 Hydroxyvitamin D Levels >/<50nmol/L and ED Attendance
**Figure 16:** Cumulative Hazard Curve 25 Hydroxyvitamin D Levels >/=50nmol/L and ED Attendance
In further models considering Vitamin D levels \(\geq\!<25\text{nmol/L}\), the association once again remained evident on both unadjusted and fully adjusted models (Table 23 and Figures 17 and 18).

**Table 23:** Cox Proportional Hazards 25 Hydroxyvitamin D \(\geq\!<25\text{nmol/L}\) and ED Attendance Status Total Cohort  
(Vitamin D as categorical variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>(\beta) Coefficient</th>
<th>HR</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.2707</td>
<td>0.76</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2409</td>
<td>0.79</td>
<td>0.0012*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.2445</td>
<td>0.78</td>
<td>0.0010*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI  
Model 2: Model1 and TUG, MMSE, IADL  
Model 3: Model 2 and Fall in the Last Year, History of Cancer  
*Statistically significant value

**Figure 17:** Survival Curve 25 Hydroxyvitamin D Levels \(\geq\!<25\text{nmol/L}\) and ED Attendance
Figure 18: Cumulative Hazard Curve 25 Hydroxyvitamin D Levels >/<25nmol/L and ED Attendance
16.3 Subgroup Analysis

In Logistic regression models (Table 24), there was no association noted between Vitamin D concentrations and specific reason for ED attendance, either in basic unadjusted models (Model 1) or fully adjusted models (Model 2).

Table 24: Association between 25(OH)D Concentrations (Log Transformed) and Reason for ED Attendance (First Attendance)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β Coefficient</td>
<td>HR</td>
<td>95% CI</td>
<td>p Value</td>
<td></td>
<td>β Coefficient</td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Fall</td>
<td>0.96</td>
<td>1.10</td>
<td>0.87 – 1.39</td>
<td>0.420</td>
<td>Fall</td>
<td>0.05</td>
<td>1.05</td>
<td>0.78 – 1.40</td>
</tr>
<tr>
<td>Fracture</td>
<td>-0.07</td>
<td>0.94</td>
<td>0.70 – 1.26</td>
<td>0.661</td>
<td>Fracture</td>
<td>0.71</td>
<td>1.07</td>
<td>0.75 – 1.54</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>-0.12</td>
<td>0.99</td>
<td>0.73 – 1.08</td>
<td>0.226</td>
<td>Cardiopulmonary</td>
<td>-0.07</td>
<td>0.93</td>
<td>0.73 – 1.81</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>-0.27</td>
<td>0.77</td>
<td>0.47 – 1.21</td>
<td>0.250</td>
<td>Stroke/TIA</td>
<td>-0.09</td>
<td>0.91</td>
<td>0.52 – 1.62</td>
</tr>
<tr>
<td>GI Bleed</td>
<td>0.44</td>
<td>1.56</td>
<td>0.78 – 3.10</td>
<td>0.208</td>
<td>GI Bleed</td>
<td>0.34</td>
<td>1.41</td>
<td>0.58 – 3.39</td>
</tr>
<tr>
<td>Other/Unclear</td>
<td>0.63</td>
<td>1.07</td>
<td>0.90 – 1.26</td>
<td>0.455</td>
<td>Other/Unclear</td>
<td>-0.01</td>
<td>0.10</td>
<td>0.81 – 1.22</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone (Y/N), Education (Log), GSR, Vitamin D Supplementation, BMI
Model 2: Model 1 and TUG(Log), MMSE(Log), IADL
Chapter 17: Conclusion

17.1 Discussion

This study demonstrates an inverse relationship between Vitamin D deficiency and Emergency Department attendance in an older population of community dwelling adults. Those participants with deficient serum levels of 25-hyrdoxyvitamin D (<50nmol/L) or severely deficient levels (<25nmol/L) were more likely to attend the ED compared with participants with higher Vitamin D levels. This relationship remained robust in fully adjusted models accounting for multiple cofounders for Vitamin D status, measures of physical and cognitive frailty and for a number of chronic conditions.

To my knowledge, this is the only study to date to examine the relationship between serum Vitamin D concentrations and ED attendance. Earlier studies suggest an association between Vitamin D and certain conditions associated with increased resource utilisation, such as chronic conditions, falls (Bischoff-Ferrari et al., 2004b), frailty (Rosendahl-Riise et al., 2017, Zhou et al., 2016) and fractures (Avenell et al., 2014, Boonen et al., 2007).

Our study’s findings suggest that Vitamin D deficiency is associated with increased risk of resource utilisation in older adults with Vitamin D deficiency independent of markers of physical and cognitive frailty. These results remained after adjusting for history of falls in the year prior to recruitment and a history of cancer (Model 3), suggesting these admissions were independent of these variables and support the extra-osseous effects of Vitamin D.

These results support the need to further consider Vitamin D as an independent modifiable factor in resource utilisation and the potential role for supplementation in older adults deficient in Vitamin D. This association needs to be investigated with a randomised control trial (RCT) with a large population of older adults, deficient in Vitamin D, with adequate doses of Vitamin D supplementation before such recommendations can be made.
17.2 Strengths and Limitations

In this large dataset, with a long follow-up period of 3.6 years, the inverse relationship between Vitamin D deficiency and ED attendance remained robust after adjustment for multiple potential confounds. However, our study has some potential limitations. The results of this study may not be applicable to all older populations, as the participants included are recruited with underlying disease states of cognition and bone health.

As ED attendance data was only available for our institution, it is possible participants may have attended other EDs and these events were not captured in this study. However, only 1% of the study population are estimated to live outside our institution’s catchment area, thus this is unlikely to be a significant factor.

Finally, frailty is an important factor potentially contributing to the reason for ED attendance. While we did not use a specific frailty score or tool in this study, as there is no consensus on the best frailty index at present, frailty was considered and measured using markers of physical and cognitive frailty as previously outlined, which were included as confounders in this study’s analysis.
Section 6: Vitamin D and Mortality
Chapter 18: Introduction

Vitamin D deficiency is common in the elderly and is likely related to lifestyle and physiological factors including, reduced exposure to sunlight, poor dietary intake and decreased capacity of skin to produce vitamin D by photoisomerization, impaired hepatic or renal hydroxylation or end-organ resistance to Vitamin D, as in Hereditary Vitamin D Resistant Rickets.

The effects of Vitamin D deficiency on bone are well established, causing rickets and osteomalacia, osteoporosis, falls (Bischoff-Ferrari et al., 2004b) and fractures (Bischoff-Ferrari et al., 2005). In more recent years extra-osseous effects of Vitamin D have been observed, including cancer, diabetes (Zella and DeLuca, 2003) cardiovascular disease (Grandi et al., 2010) and inflammatory conditions such as Multiple Sclerosis (Cantorn a et al., 1996). With the biologic basis for the association of Vitamin D and extra skeletal effects presumed to be related to the expression of VDR and 1α hydroxylase in multiple tissues throughout the body.

Mortality rates in Ireland have improved and this includes a greater life expectancy in both men and women (CSO, 2015). There are potential modifiable factors that may be causal in mortality and it is postulated Vitamin D may be one such factor. With its broad range of effects beyond bone health, it is reasonable to consider the effects of Vitamin D on mortality.

There have been a number of studies published assessing the potential relationship between serum Vitamin D levels and all cause mortality, including; cross-sectional (Alizadeh et al., 2015, Padhi et al., 2014), prospective studies (Ginde et al., 2009, Guo et al., 2017, Melamed et al., 2008, Sun et al., 2017, Vipul et al., 2017, Visser et al., 2006) the majority of which have been positive. Randomised Control Trials (RCTs) have been limited in number (Amrein et al., 2014b, Avenell et al., 2012, Zittermann et al., 2017) and the results of these have been disappointing to date, perhaps as individual studies include younger participants, or those who are not deficient in Vitamin D at baseline recruitment.
Table 25: Vitamin D and Mortality: Cross Sectional and Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Sectional Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annweiler et al 2010</td>
<td>France</td>
<td>399</td>
<td>17 deaths (4.3%)</td>
<td>84.5</td>
<td>34.8nmol/L</td>
<td>Higher Vitamin D concentrations associated with lower hospital mortality rates</td>
</tr>
<tr>
<td>Leow et al 2011</td>
<td>New Zealand</td>
<td>112</td>
<td>9 deaths (8%)</td>
<td>76 (Median)</td>
<td>54nmol/L (Median)</td>
<td>Association between Vitamin D deficiency and 30 day mortality from CAP</td>
</tr>
<tr>
<td>Venkatram et al 2011</td>
<td>USA</td>
<td>437</td>
<td>92 deaths (21%)</td>
<td>Not stated</td>
<td>Not available (77% &lt;50nmol/L)</td>
<td>Better survival rates in those with higher Vitamin D level</td>
</tr>
<tr>
<td>Remmelts et al 2012</td>
<td>Netherlands</td>
<td>272</td>
<td>16 deaths</td>
<td>63.5</td>
<td>47.4nmol/L (Median)</td>
<td>Vitamin D deficiency is a predictor of 30-day mortality in patients with CAP</td>
</tr>
<tr>
<td>Lange et al 2013</td>
<td></td>
<td>23,063</td>
<td>3.6%</td>
<td>61.2</td>
<td>69.3nmol/L</td>
<td>Pre-admission Vitamin D levels were predictors of in-hospital and 30-day mortality</td>
</tr>
<tr>
<td>Padhi et al 2014</td>
<td>India</td>
<td>152</td>
<td>18.4%</td>
<td>60.0</td>
<td>60.6nmol/L</td>
<td>Vitamin D deficiency associated with increased mortality, LOS and longer ventilation times</td>
</tr>
<tr>
<td>Amrein et al 2014</td>
<td>USA</td>
<td>24,094</td>
<td>13% 365-day</td>
<td>61.2</td>
<td>69.6nmol/L</td>
<td>Increased risk of mortality with deficient Vitamin D levels and also levels &gt;120nmol/L</td>
</tr>
<tr>
<td>Moraes et al 2015</td>
<td>Brazil</td>
<td>135</td>
<td>21.5%</td>
<td>57.9</td>
<td>33.2nmol/L</td>
<td>Inverse association between Vitamin D and 28-day ICU mortality</td>
</tr>
<tr>
<td>Graedel et al 2016</td>
<td></td>
<td>4,257</td>
<td>115 deaths (2.7%)</td>
<td>63.0</td>
<td>Not available</td>
<td>Vitamin D deficiency (&lt;25nmol/L) associated with mortality</td>
</tr>
<tr>
<td>Amrein et al 2016</td>
<td>USA</td>
<td>4,344</td>
<td>134 deaths</td>
<td>61</td>
<td>80.4nmol/L</td>
<td>Change in Vitamin D status prior to admission associated with mortality risk</td>
</tr>
</tbody>
</table>

Cross Sectional Negative

<p>| Turan et al 2013        | USA    | 426  | 1.4%          | Not stated | 47.5nmol/L     | No association between peri-operative Vitamin D levels and 30-day post-operative mortality |
| Ala-Kokko et al 2016    | Finland | 610  | 29.5%         | 62.4       | 48.1nmol/L (Median) | Vitamin D levels not associated with 90-day mortality in ICU patients with septic shock |</p>
<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Sectional Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alizadeh et al 2017 Iran</td>
<td>70</td>
<td>25% deficient 22% sufficient</td>
<td>54.5</td>
<td>Not available &lt;75nmol/L</td>
<td>No association between Vitamin D and ICU mortality in surgical patients</td>
</tr>
<tr>
<td>Prospective Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atalan at al 2017 Turkey</td>
<td>491</td>
<td>21.6%</td>
<td>63</td>
<td>19.6nmol/L</td>
<td>No significant association between baseline Vitamin D and ICU mortality</td>
</tr>
<tr>
<td>Kuroda et al 2008 Japan</td>
<td>12,321</td>
<td>107 Deaths (8.7%)</td>
<td>63.9</td>
<td>45% &lt;50nmol/L</td>
<td>Vitamin D deficiency associated with mortality</td>
</tr>
<tr>
<td>Dohling et al 2008 Germany</td>
<td>3,258</td>
<td>737 deaths (22.6%)</td>
<td>64.3</td>
<td>50.2% &lt;42nmol/L</td>
<td>Inverse relationship between Vitamin D and all cause mortality</td>
</tr>
<tr>
<td>Melamed et al 2008 USA</td>
<td>13,331</td>
<td>1,806</td>
<td>44.8</td>
<td>Not available (quartiles provided)</td>
<td>Association between Vitamin D and all-cause mortality, non-significant with cancer/CVD mortality</td>
</tr>
<tr>
<td>Ginde et al 2009 USA</td>
<td>3,408</td>
<td>1,493 deaths (43.8%)</td>
<td>73</td>
<td>66nmol/L (Median)</td>
<td>Inverse association between Vitamin D deficiency and CVD and mortality</td>
</tr>
<tr>
<td>Zitterman et al 2009 Germany</td>
<td>510</td>
<td>82 deaths (16%)</td>
<td>53.5</td>
<td>Calcitrol 29.0ng/L</td>
<td>Calcitrol in lowest quintiles had 1 year mortality risk 3.9 times higher than those in the highest quintile</td>
</tr>
<tr>
<td>Semba et al 2009 USA</td>
<td>714</td>
<td>100 deaths (14%)</td>
<td>74</td>
<td>50.9nmol/L</td>
<td>Lowest quartile of Vitamin D associated with highest mortality risk</td>
</tr>
<tr>
<td>Szulc et al 2009 France</td>
<td>782</td>
<td>182 deaths (23.3%)</td>
<td>67</td>
<td>Survivors: 70.6nmol/L Deceased: 57.5nmol/L</td>
<td>Higher mortality in lowest quintile of Vitamin D. Fully adjusted models: no association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Semba et al 2010 Italy</td>
<td>1,006</td>
<td>228 deaths (22.7%)</td>
<td>74</td>
<td>Not available (Quartiles)</td>
<td>Higher rates of all cause mortality in those with lower Vitamin D levels</td>
</tr>
<tr>
<td>Michaelsson et al 2010 Sweden</td>
<td>1,194</td>
<td>584 deaths (49%)</td>
<td>71</td>
<td>Not available (Percentiles)</td>
<td>Low Vitamin D associated with higher risks of death due to cancer and CVD High concentrations associated with higher cancer mortality risk</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>-------------</td>
<td>-------</td>
<td>----------------</td>
<td>---------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Anderson et al 2010 USA</td>
<td>41,504</td>
<td>1.193 deaths (4.3%)</td>
<td>66.6</td>
<td>Not available 17% &lt;37.5nmol/L</td>
<td>Inverse relationship with Vitamin D and outcomes including MI, HF, stroke, PVD and mortality</td>
</tr>
<tr>
<td>Hutchinson et al 2010 Norway</td>
<td>7,161</td>
<td>1.359 deaths (18.9%)</td>
<td>58.9</td>
<td>Smokers: 52.3nmol/L Non-Smokers: 72.0nmol/L</td>
<td>Lower Vitamin D levels associated with all cause mortality in non-smokers and to a lesser degree in smokers</td>
</tr>
<tr>
<td>Braun et al 2011 USA</td>
<td>2,399</td>
<td>6.7% 30day 23.2% 90day 32.3% 365day</td>
<td>64.9</td>
<td>65.9nmol/L</td>
<td>Vitamin D deficiency associated with mortality at 30, 90 &amp; 365 days</td>
</tr>
<tr>
<td>Virtanen et al 2011</td>
<td>1,136</td>
<td>87 deaths</td>
<td>61.8</td>
<td>43.7nmol/L</td>
<td>Low Vitamin D levels associated with increased mortality risk</td>
</tr>
<tr>
<td>Durup et al 2012 Denmark</td>
<td>247,574</td>
<td>15,198 deaths</td>
<td>51.0</td>
<td>54.4% levels &lt;50nmol/L</td>
<td>J shaped association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Skaaby et al 2012 Denmark</td>
<td>9,146</td>
<td>830 deaths (9.7%)</td>
<td>49.8</td>
<td>61nmol/L &amp; 48nmolL (Median)</td>
<td>Association between Vitamin D and mortality due to respiratory, endocrine, nutritional and digestive causes</td>
</tr>
<tr>
<td>Skaaby et al 2012 Denmark</td>
<td>8,329</td>
<td>IHD &amp; All cause deaths: 8,131</td>
<td>49.8</td>
<td>61nmol/L &amp; 48nmolL (Median)</td>
<td>Inverse relationship between Vitamin D and all cause mortality No association with IHD mortality</td>
</tr>
<tr>
<td>Saliba et al 2012 Israel</td>
<td>182,152</td>
<td>7,247 deaths (4%)</td>
<td>60.4</td>
<td>Deceased: 44.8nmol/L Living: 51nmol/L</td>
<td>Inverse relationship between Vitamin D and all cause mortality</td>
</tr>
<tr>
<td>Johansson et al 2012 Sweden</td>
<td>2,878</td>
<td>577 deaths (20%)</td>
<td>75.4</td>
<td>66.9nmol/L</td>
<td>3 year follow-up low Vitamin D was a strong predictor of mortality 6 year follow-up low Vitamin D was weaker at predicting mortality</td>
</tr>
<tr>
<td>Matthews et al 2012 USA</td>
<td>258</td>
<td>12.3% &lt;34.9nmol/L 11.5% in &lt;64.9nmol/L</td>
<td>45.9</td>
<td>53.5% &lt;32.5nmol/L 91.9% &lt;64.9nmol/L</td>
<td>Levels &lt;64.9nmol/L associated with increased mortality rates (No deaths in those with levels &gt;65nmol/L)</td>
</tr>
<tr>
<td>Michos et al 2012 USA</td>
<td>7,981</td>
<td>176 fatal strokes (2%)</td>
<td>48.4</td>
<td>Blacks 48.4nmol/L Whites 76.8nmol/L</td>
<td>Increased risk of fatal stroke in Vitamin D deficient whites but not in blacks</td>
</tr>
<tr>
<td>Welsh et al 2012 UK</td>
<td>2,081</td>
<td>100 deaths</td>
<td>45.1</td>
<td>46.4nmol/L (Median)</td>
<td>Vitamin D associated with all case mortality</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>-------------</td>
<td>-----</td>
<td>----------------</td>
<td>----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Prospective Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomson et al 2012 UK Whitehall</td>
<td>5,409</td>
<td>3,215 deaths</td>
<td>76.9 years</td>
<td>56nmol/L (Median)</td>
<td>Vitamin D inversely associated with risk of vascular deaths and at levels of 40-90nmol/L with all cause mortality</td>
</tr>
<tr>
<td>Naesgaard et al 2012 Denmark</td>
<td>982</td>
<td>173</td>
<td>62.2</td>
<td>46.2nM</td>
<td>25(OH)D associated with increased mortality risk in females</td>
</tr>
<tr>
<td>Smit et al 2012 USA</td>
<td>4,731</td>
<td>Not available</td>
<td>71.7</td>
<td>65.9nmol/L</td>
<td>Lowest quartile of Vitamin D levels was associated with increased mortality risk</td>
</tr>
<tr>
<td>Pilz et al 2012 Australia</td>
<td>961</td>
<td>284 deaths</td>
<td>83.7</td>
<td>17.5nmol/L (Median)</td>
<td>Association between Vitamin D and mortality in female NH residents</td>
</tr>
<tr>
<td>Thomas et al 2012 UK</td>
<td>1,801</td>
<td>462 deaths</td>
<td>63.3</td>
<td>Not available 65% levels &lt;50nmol/L</td>
<td>Association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Schottker et al 2013 Germany</td>
<td>9,578</td>
<td>1,083 deaths (11.3%)</td>
<td>62</td>
<td>43.8nmol/L</td>
<td>Inverse association between Vitamin D and all-cause mortality</td>
</tr>
<tr>
<td>Signorello et al 2013 USA</td>
<td>85,000</td>
<td>1,852 deaths (2%)</td>
<td>Not available</td>
<td>40.4nmol/L</td>
<td>Increasing risk of all cause mortality with decreasing quartiles of Vitamin D Association between Vitamin D and CVD mortality but not cancer mortality</td>
</tr>
<tr>
<td>Wong et al 2013 Australia</td>
<td>4,203</td>
<td>1,144 deaths</td>
<td>76.7</td>
<td>59.3nmol/L</td>
<td>Increased risk of all-cause mortality with lower Vitamin D levels</td>
</tr>
<tr>
<td>Rohrmann et al 2013 Switzerland</td>
<td>3,191</td>
<td>459 deaths</td>
<td>47.1</td>
<td>Not available (quartiles)</td>
<td>Inverse relationship between Vitamin D and all-cause mortality.</td>
</tr>
<tr>
<td>Blicher et al 2013 Denmark</td>
<td>5,147</td>
<td>1,689 deaths</td>
<td>76.7</td>
<td>43.5nmol/L</td>
<td>Inverse association between 25(OH)D and all-cause mortality</td>
</tr>
<tr>
<td>Sempos et al 2013 USA</td>
<td>15,099</td>
<td>3,784 deaths</td>
<td>45</td>
<td>64nmol/L</td>
<td>Association between low Vitamin D levels and all-cause mortality</td>
</tr>
<tr>
<td>Skaaby et al 2013 Denmark</td>
<td>2,649</td>
<td>736</td>
<td>55.4</td>
<td>61.0nmol/L</td>
<td>Relationship between Vitamin D and mortality independent of liver enzyme levels</td>
</tr>
<tr>
<td>Aygencel et al 2013 Turkey</td>
<td>201</td>
<td>76 deaths</td>
<td>66 (Median)</td>
<td>37.2nmol/L</td>
<td>Vitamin D deficiency associated with mortality in ICU patients</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>-------------</td>
<td>-----</td>
<td>----------------</td>
<td>----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Prospective Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amer &amp; Qayyum 2013 USA</td>
<td>10,170</td>
<td>509</td>
<td>46.6</td>
<td>51.8nmol/L</td>
<td>Inverse relationship between all cause and CVD related mortality</td>
</tr>
<tr>
<td>Khaw et al 2014 UK</td>
<td>14,641</td>
<td>2,776 deaths</td>
<td>62.1</td>
<td>56.6nmol/L</td>
<td>Inverse association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Joshi et al 2014 India</td>
<td>85</td>
<td>44 deaths (52%)</td>
<td>42.4</td>
<td>103.6nmol/L</td>
<td>Vitamin D deficiency associated with increased mortality rates</td>
</tr>
<tr>
<td>Samefors et al 2014 Sweden</td>
<td>333</td>
<td>147 deaths (44%)</td>
<td>85.0</td>
<td>40.5nmol/L</td>
<td>Association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Hirani et al 2014 Australia</td>
<td>1,659</td>
<td>355 deaths (21.4%)</td>
<td>77.0</td>
<td>55.9nmol/L</td>
<td>Lower levels of Vitamin D were associated with all cause mortality</td>
</tr>
<tr>
<td>Saliba et al 2014 Israel</td>
<td>175,781</td>
<td>12,337 deaths (7%)</td>
<td>60.6</td>
<td>50.9nmol/L</td>
<td>Relationship between Vitamin D and mortality (presented by BMI category, inversely related to BMI)</td>
</tr>
<tr>
<td>Lee et al 2014 Belgium</td>
<td>2,816</td>
<td>187 deaths (6.6%)</td>
<td>62.8</td>
<td>56.3nmol/L</td>
<td>Lower levels of Vitamin D associated with increased risk of all-cause mortality</td>
</tr>
<tr>
<td>Vogt et al 2015 Germany</td>
<td>727</td>
<td>98 deaths</td>
<td>75.5</td>
<td>46.7nmol/L</td>
<td>Vitamin D associated with all-cause mortality, frailty status also contributed to relationship</td>
</tr>
<tr>
<td>Mursu et al 2015 Finland</td>
<td>1,892</td>
<td>670 deaths</td>
<td>52.4</td>
<td>43.5nmol/L</td>
<td>Lower Vitamin D levels associated with increased mortality risk</td>
</tr>
<tr>
<td>Holter et al 2016 Norway</td>
<td>241</td>
<td>72 deaths (Median)</td>
<td>37.4nmol/L</td>
<td></td>
<td>Association between Vitamin D and mortality in patients admitted with CAP</td>
</tr>
<tr>
<td>Meyer et al 2016 Norway</td>
<td>4,379</td>
<td>27% cases 12.6% controls</td>
<td>62.9</td>
<td>62.2nmol/L</td>
<td>Inverse association between Vitamin D and all-cause mortality</td>
</tr>
<tr>
<td>Buchebner et al 2016</td>
<td>1,044</td>
<td>96 deaths (91.2%)</td>
<td>75.2</td>
<td>63nmol/L</td>
<td>Inverse association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Daraghmeh et al 2016 USA</td>
<td>10,517</td>
<td>23 deaths (25% levels &lt;44nmol)</td>
<td>54 years</td>
<td></td>
<td>Decreased mortality risk with higher levels of Vitamin D</td>
</tr>
<tr>
<td>Vipul et al 2017 India</td>
<td>88</td>
<td>14 deaths (15.4%)</td>
<td>45 years</td>
<td>40.9nmol/L</td>
<td>Vitamin D associated with mortality and longer length of stay in small population with sepsis</td>
</tr>
<tr>
<td>Author Year</td>
<td>No.</td>
<td>Mortality Rate</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>-------------</td>
<td>-----</td>
<td>----------------</td>
<td>----------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Sun et al 2017 Norway</td>
<td>6,377</td>
<td>1,539 (24.1%)</td>
<td>Not available</td>
<td>47.3nmol/L (Median)</td>
<td>Lowest quartile of Vitamin D was associated with increased risk of all-cause mortality</td>
</tr>
<tr>
<td>Visser et al 2006 Netherlands</td>
<td>1,260</td>
<td>380 deaths (30.2%)</td>
<td>Not available</td>
<td>46.8% levels &lt;50nmol/L</td>
<td>Vitamin D deficiency associated with higher mortality risk with adjustment for frailty, lost statistical significance</td>
</tr>
<tr>
<td>Prospective Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cawthon et al 2010 USA</td>
<td>1,490</td>
<td>330 deaths (22.2%)</td>
<td>73.5</td>
<td>25.2% levels &lt;50nmol/L 66% levels &lt;75nmol/L</td>
<td>No association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Eaton et al 2011 USA</td>
<td>2,429</td>
<td>224 deaths (9.2%)</td>
<td>65.7</td>
<td>Not available</td>
<td>No association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Ford et al 2011 USA</td>
<td>7,531</td>
<td>347 deaths (4.6%)</td>
<td>45.5</td>
<td>60.6nmol/L</td>
<td>Vitamin D not significantly associated with reduced mortality</td>
</tr>
<tr>
<td>Flynn et al 2012 USA</td>
<td>66</td>
<td>5 deaths (7%)</td>
<td>56.0</td>
<td>Not available (76% levels &lt;50nmol/L)</td>
<td>No association with Vitamin D and surgical ICU mortality</td>
</tr>
<tr>
<td>Arnason et al 2012 Israel</td>
<td>130</td>
<td>57 deaths (44%)</td>
<td>70.2</td>
<td>37.2nmol/L</td>
<td>No difference in mortality between Vitamin D sufficient and deficient groups.</td>
</tr>
<tr>
<td>Lin et al 2012 China</td>
<td>1,101</td>
<td>793 deaths (72%)</td>
<td>56.5</td>
<td>73% levels &lt;50nmol/L</td>
<td>No association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Langsetmo et al 2013 Canada</td>
<td>9,033</td>
<td>1,160</td>
<td>61.9</td>
<td>Not available</td>
<td>No association between Vitamin D intake and mortality</td>
</tr>
<tr>
<td>Formiga et al 2013 Spain</td>
<td>312</td>
<td>58 deaths (18.5%)</td>
<td>Not available</td>
<td>69.0nmol/L</td>
<td>No association between Vitamin D and all-cause or CVD related mortality</td>
</tr>
<tr>
<td>Puhan et al 2014</td>
<td>356</td>
<td>34 deaths (9.6%)</td>
<td>67.2</td>
<td>38.7nmol/L</td>
<td>No significant association between Vitamin D and mortality</td>
</tr>
<tr>
<td>Hak Lee et al 2015 S Korea</td>
<td>489</td>
<td>114 deaths (23.3%)</td>
<td>76.5</td>
<td>39.2nmol/L</td>
<td>No association noted</td>
</tr>
<tr>
<td>Granic et al 2016 UK</td>
<td>775</td>
<td>332 deaths (57.6%)</td>
<td>85 at baseline</td>
<td>39nmol/L (Median)</td>
<td>No association in fully adjusted models</td>
</tr>
<tr>
<td>Guo et al 2017 UK</td>
<td>452</td>
<td>218 (62.2%)</td>
<td>52 years</td>
<td>Not Measured (Vitamin D intake)</td>
<td>No significant association between Vitamin D intake and all-cause mortality</td>
</tr>
</tbody>
</table>
Table 26: Vitamin D and Mortality: Intervention and Randomised Control Trials

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age</th>
<th>Mean follow-up</th>
<th>Mean Vitamin D</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trivedi et al 2003 UK</td>
<td>2,686</td>
<td>Fracture and mortality</td>
<td>74.8</td>
<td>4 years</td>
<td>Not available</td>
<td>100,000IU Vitamin D3 every 4/12 vs Placebo</td>
<td>No association with Vitamin D and mortality</td>
</tr>
<tr>
<td>Hsia et al 2007 USA</td>
<td>36,282</td>
<td>Intervention: 499 Placebo: 475</td>
<td>62.4</td>
<td>7 years</td>
<td>42nmol/L (Median)</td>
<td>Calcium 1,000mg &amp; VitD3 400IU/day vs Placebo</td>
<td>Vitamin D had no significant effect on CHD deaths</td>
</tr>
<tr>
<td>LaCroix et al 2009 USA</td>
<td>36,282</td>
<td>1,551 deaths: Intervention: 744 Placebo: 807</td>
<td>62.4</td>
<td>7 years</td>
<td>42nmol/L (Median)</td>
<td>Calcium 1,000mg &amp; VitD3 400IU/day vs Placebo</td>
<td>Non statistically significant reduction in risk of death in CaD group</td>
</tr>
<tr>
<td>Amrein et al 2011 Austria</td>
<td>25</td>
<td>12 deaths</td>
<td>62</td>
<td>15 days</td>
<td>33.9nmol/L</td>
<td>540,000IU Vitamin D3 versus Herbal Oil with 200IU cholecalciferol (Placebo)</td>
<td>No difference in hospital mortality between groups</td>
</tr>
<tr>
<td>Avenil et al 2012 UK</td>
<td>5,292</td>
<td>1,717 deaths (32.4%)</td>
<td>77</td>
<td>6.2 years (Median)</td>
<td>38nmol/L (recorded in 60 participants)</td>
<td>4 groups: 1. VitD3 2. Calcium and Vitamin D3 3. Calcium 4. Placebo Vitamin D3: 800 IU/day Calcium: 1000mg/day</td>
<td>Vitamin D not found to significantly reduce mortality</td>
</tr>
<tr>
<td>Amrein et al 2014 Austria</td>
<td>475</td>
<td>Intervention: 34.7% Placebo: 50%</td>
<td>64.6</td>
<td>6 months</td>
<td>32.1nmol/L</td>
<td>540,000IU Vitamin D3 initially, Five monthly maintenance dose of 90,000IU (650IU/day equivalent) vs Placebo</td>
<td>No significant difference between mortality rates in placebo versus intervention group</td>
</tr>
<tr>
<td>Zitterman et al 2017 Germany</td>
<td>400</td>
<td>Intervention: 19.6% Placebo: 17.9%</td>
<td>55</td>
<td>3 years</td>
<td>33.3nmol/L</td>
<td>4,000IU daily or placebo</td>
<td>No significant reduction in mortality heart failure patients</td>
</tr>
</tbody>
</table>
The results of studies to date have been variable and seem to depend on the population selected, their Vitamin D levels, if the population was deficient in Vitamin D and the length of follow-up. The aim of this study is to evaluate the relationship between Vitamin D status and all cause mortality in a large community-dwelling population of older Irish adults.

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mortality Rate</th>
<th>Mean Age</th>
<th>Mean follow-up</th>
<th>Mean Vitamin D</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manson et al 2018 USA</td>
<td>25,871</td>
<td>1,033 deaths</td>
<td>67.1</td>
<td>5.3 years</td>
<td>77 nmol/L</td>
<td>Intervention: 485 Placebo: 493 2,000U/day or placebo with Omega-3 fatty acid or placebo</td>
<td>No difference between groups in cancer or cardiovascular incidence or mortality</td>
</tr>
</tbody>
</table>
Chapter 19: Methodology

19.1 Study Population
All 3,093 TUDA participants recruited through St James’s Hospital Dublin were included in this study. The TUDA population is a large community dwelling population of Irish adults aged 60 years and older. It is a cross-sectional study designed to create a genotype/phenotype database for three population cohorts based on three disease states: cognition, bone health and hypertension. Full methodology published elsewhere (McCarroll et al., 2015) and discussed in detail in Chapters 8 and 9.

19.2 Methodology
Information relating to mortality was collected from date of recruitment until June 2013 and was sourced through the Register of Births, Deaths and Marriages, which is a matter of public information. Permission was sought and obtained to conduct a search of the Register from the General Register Office (Government Offices). The database was reviewed using each study participant’s name, date of birth and address and each subject was checked against the register.

Data collected included date and place of death, age at death, cause of death, as well as antecedent causes and other significant conditions documented on the death certificate. Cause of death was categorised using predefined keywords from Section A on the individual death certificates. Section A is defined as the “Disease or condition directly leading to death” and the keywords included: “infection/pneumonia, cardiopulmonary, cancer, stroke/TIA, dementia, other”.

Ethical approval was obtained for this study from our local Research and Ethics Committee (REC Reference 2013/05/05 Chairman’s Action).

19.3 Statistical Analysis
All parameters were inspected for normality and if significantly skewed were appropriately transformed. Normal assumptions for linear regression analysis were observed. Descriptive
and comparative analyses were performed for all included participants and for a priori-determined subgroups, alive versus not alive.

Continuous variables are expressed as mean and standard deviations for normally distributed and median and inter-quartiles for non-normally distributed data. Categorical variables were expressed as number of cases and percentages. Nominal or dichotomous variables were compared using Chi-squared test, non-normally distributed variables were compared with Mann-Whitney U test and normally distributed continuous variables were compared using the Student t test. These analyses were run using IBM SPSS version 22.0 (SPSS, Inc., Chicago IL). Statistical significance was accepted when p <0.05.

Survival analysis was performed using R (R Development Core Team, 2013). Cox proportional Hazard Models were generated both for Vitamin D as a continuous variable and also as a categorical variable based on defined Vitamin D levels and were used to evaluate the proposed relationship between Vitamin D and mortality. The Vitamin D cut-offs were based on previously defined levels in the literature with <50nmol/L defined as deficient and <25nmol/L as severely deficient (Holick et al., 2011). Missing data was dealt with using Multiple Imputation Modelling in Multivariate Imputation by Chained Equations (MICE) using R (R Development Core Team, 2013).

Logistic regression analysis was used in subgroup analysis to explore the relationship between Vitamin D and specific cause of death. In regression models, non-normally distributed variables, were log transformed for analysis.

Interaction terms for cohort and mortality status were looked for but were not found to be significant in regression analysis.
19.4 Covariates

The basic model (Model 1) of this study included those covariates known to affect serum 25-hydroxyvitamin D levels. These include age, gender and total number of years in education, BMI, Vitamin D supplementation and Global Solar Radiation (GSR). The GSR in the month and preceding two months of subject recruitment was used as a surrogate marker of UVB exposure, which is known to effect serum Vitamin D concentrations. GSR represents the total amount of solar radiation (direct beam plus the diffuse component on a horizontal surface) received per unit area per month (MJm$^2$), (http://www.met.ie/about/valentiaobservatory/solarradiation.asp). GSR data was obtained by request from the Irish Meteorological Service.
Model 2 includes all covariates from Model 1 and also potential confounders for frailty (both physical and cognitive); Timed Up and Go (TUG) which is a frequently used screening tool to measure patients gait speed and is associated with a potential risk of falling, Mini Mental State Examination (MMSE) which is a screening tool for cognition and can be used as a marker of cognitive frailty and finally IADLS, a score reflecting a patient’s ability to function independently in their activities of daily living.

Lastly, model three covariates were included based on subgroup analysis of significant clinical conditions found to be predictors of mortality in the model.
Chapter 20: Results

20.1 Baseline Characteristics
There were 471 (15.2%) deaths in the mean follow up period of 3.9 years (Table 27). For those people who died, the mean time to death from assessment was 22.4 months (12.9 SD).

Those who died were older (82.7 versus 75.4 years), were more likely to be female and less educated (10.0 versus 11.0 years). Those who died had lower Vitamin D levels 52.4 nmol/L versus 66.7 nmol/L p<0.001. Participants who died were frailer based on Timed Up and Go Score (TUG), 18.0 versus 14.0 seconds p<0.001, had a lower BMI (25.8 versus 26.9, p<0.001).

Those who died were also less independent in their ADLS and more likely to have co-morbid conditions such as Chronic Kidney Disease (CKD), cancer and hypertension compared with those who survived.

Of those participants who died during the follow-up period, 109 (23.1%) had serum 25(OH)D levels < 25 nmol/L and 246 (52.5%) had levels < 50 nmol/L.
Table 27: Baseline Characteristics By Mortality Status

<table>
<thead>
<tr>
<th></th>
<th>Died (n=471)</th>
<th>Alive (n=2,622)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Cohort (%)</td>
<td>24.8</td>
<td>75.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Bone Cohort (%)</td>
<td>3.6</td>
<td>96.4</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Age in years (mean)</td>
<td>82.7</td>
<td>75.4</td>
<td>&lt;0.001*a</td>
</tr>
<tr>
<td>Gender (female %)</td>
<td>58.0</td>
<td>78.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Total Education (years)</td>
<td>10.0</td>
<td>11.0</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>Living Alone (%)</td>
<td>45.0</td>
<td>39.0</td>
<td>0.014*b</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>12.5</td>
<td>12.9</td>
<td>0.823b</td>
</tr>
<tr>
<td>Previous Smoker (%)</td>
<td>46.3</td>
<td>39.8</td>
<td>0.008*b</td>
</tr>
<tr>
<td>Current Alcohol (%)</td>
<td>46.9</td>
<td>58.9</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Previous Alcohol (%)</td>
<td>26.3</td>
<td>17.4</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>54.2</td>
<td>66.7</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>Median 25(OH)D (nmol/L)</td>
<td>47.0</td>
<td>66.7</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>Vitamin D supplementation (%)</td>
<td>65.7</td>
<td>61.5</td>
<td>0.091b</td>
</tr>
<tr>
<td>Sun Holiday Previous Six Months (N,%)</td>
<td>14.7</td>
<td>15.5</td>
<td>0.660b</td>
</tr>
<tr>
<td>MMSE (median)</td>
<td>27.0</td>
<td>27.0</td>
<td>0.015*c</td>
</tr>
<tr>
<td>HADS (median)</td>
<td>2.0</td>
<td>2.0</td>
<td>0.880c</td>
</tr>
<tr>
<td>CES-D (mean)</td>
<td>6.7</td>
<td>6.2</td>
<td>0.133c</td>
</tr>
<tr>
<td>TUG seconds (median)</td>
<td>18.0</td>
<td>14.0</td>
<td>&lt;0.001*c</td>
</tr>
<tr>
<td>BMI kg/m2 (mean)</td>
<td>25.8</td>
<td>26.9</td>
<td>&lt;0.001*a</td>
</tr>
<tr>
<td>Waist Hip Ratio (mean)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.178a</td>
</tr>
<tr>
<td>IADLS (mean)</td>
<td>22.2</td>
<td>22.8</td>
<td>0.007*a</td>
</tr>
<tr>
<td>PSMS (median)</td>
<td>24.0</td>
<td>24.0</td>
<td>0.579c</td>
</tr>
<tr>
<td>Hypertension (n,%)**</td>
<td>83.0</td>
<td>78.7</td>
<td>0.032*b</td>
</tr>
<tr>
<td>Vascular Disease (%)^</td>
<td>32.5</td>
<td>27.8</td>
<td>0.040*b</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>11.9</td>
<td>9.6</td>
<td>0.128b</td>
</tr>
<tr>
<td>Atrial Fibrillation (%)</td>
<td>14.8</td>
<td>15.4</td>
<td>0.746b</td>
</tr>
<tr>
<td>Cancer (%)</td>
<td>24.8</td>
<td>16.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Chronic Kidney Disease (%)^^</td>
<td>74.2</td>
<td>47.2</td>
<td>&lt;0.001*b</td>
</tr>
<tr>
<td>Fall in the Last Year (%)</td>
<td>42.2</td>
<td>44.0</td>
<td>0.469b</td>
</tr>
<tr>
<td>Number of Medications</td>
<td>7.2</td>
<td>6.8</td>
<td>0.052a</td>
</tr>
</tbody>
</table>

a Independent t Test  
b Chi Square Test  
c Mann Whitney U Test  
^Vascular Disease: Self reported history of Stroke, Transient Ischaemic Attack, Peripheral Vascular Disease and/or Myocardial Infarction  
^^Chronic Kidney Disease: eGFR <60mL/min  
**Hypertension: self reported history, medication or BP >140/90mmHg  
*Statistically significant result
Over the follow-up period, 471 (15.2%) participants died. The mean age at death was 84.1 years and mean time to death was 21.0 (0.1-51) months.

The most frequent cause of death documented on the death certificates was Infection/Pneumonia (38.9%) followed by cardiopulmonary (29.7%). The majority of participants died in hospital (69.4%), nursing home (16.1%) or hospice (6.4%). Only 8.1% of those who died were at home at the time of death (Table 28 and Figures 20 and 21).

**Table 28:** Cause of Death

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection/Pneumonia</td>
<td>183 (38.9)</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>140 (29.7)</td>
</tr>
<tr>
<td>Cancer</td>
<td>59 (12.5)</td>
</tr>
<tr>
<td>Stroke</td>
<td>27 (5.7)</td>
</tr>
<tr>
<td>Dementia</td>
<td>8 (1.7)</td>
</tr>
<tr>
<td>Other</td>
<td>54 (11.5)</td>
</tr>
</tbody>
</table>
**Figure 20:** Cause of Death

![Cause of Death Pie Chart]

**Figure 21:** Place of Death

![Place of Death Pie Chart]
20.2 Survival Analysis

Survival analysis with Cox Proportional Hazards revealed an association between serum 25(OH)D levels and mortality with a $\beta$ Coefficient of -5.1, HR 0.995 (0.992-0.998, 95% CI) $p=0.001$ on fully adjusted models, which indicates with every 1nmol/L increase in serum 25(OH)D concentrations there is a 0.005 (0.5%) increased mortality risk (Table 29).

Table 29: Cox Proportional Hazard Model: 25 Hydroxyvitamin D and Mortality
(Vitamin D as continuous and time dependent variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$ Coefficient</th>
<th>HR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-5.191</td>
<td>0.995</td>
<td>0.992-0.998</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-4.800</td>
<td>0.995</td>
<td>0.992-0.999</td>
<td>0.001*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-5.121</td>
<td>0.995</td>
<td>0.992-0.998</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Further models investigating the association between Vitamin D concentrations and mortality showed an association (Tables 30 and 31). In these models, Vitamin D was considered as a categorical variable with cut-offs based on those defined in the literature <50nmol/L: deficient (Figure 22 and 23) and <25nmol/L: severely deficient (Figure 24 and 25). Vitamin D was again found to be associated with mortality on both unadjusted and fully adjusted models, $\beta$ -0.304, HR 0.76, $p=0.011$. Fully adjusted models included confounders associated with Vitamin D, frailty and chronic medical conditions.

Table 30: Cox Proportional Hazard Model: 25 Hydroxyvitamin D >/<50nmol/L and Mortality
(Vitamin D as categorical dependant variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$ Coefficient</th>
<th>HR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.3053</td>
<td>0.74</td>
<td>0.0015*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2749</td>
<td>0.76</td>
<td>0.0163*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.3211</td>
<td>0.73</td>
<td>0.0008*</td>
</tr>
</tbody>
</table>
**Figure 22:** Cumulative Hazards 25 Hydroxyvitamin D Levels >/<50nmol/L and Mortality

![Cumulative Hazards Graph]

**Figure 23:** Survival Curve 25 Hydroxyvitamin D Levels >/<50nmol/L and Mortality

![Survival Curve Graph]
Table 31: Cox Proportional Hazard Model: 25 Hydroxyvitamin D >/=25nmol/L and Mortality
(Vitamin D as categorical dependant variable)

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>HR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.3426</td>
<td>0.71</td>
<td>0.0022*</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.2922</td>
<td>0.75</td>
<td>0.0151*</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.3042</td>
<td>0.74</td>
<td>0.0116*</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone, Total Education, Vitamin D supplementation, GSR, BMI
Model 2: Model 1 and TUG, MMSE, IADL
Model 3: Model 2 HTN, Diabetes, Atrial Fibrillation, History of Cancer, CKD, Fall in the Last Year
*Statistically significant result

Figure 24: Cumulative Hazards 25 Hydroxyvitamin D Levels >/=25nmol/L and Mortality
Figure 25: Survival Curve 25 Hydroxyvitamin D Levels >/<25nmol/L and Mortality
20.3 Subgroup Analysis

Logistic regression models were used to assess for potential links between Vitamin D concentrations and specific cause of death. In model 1, Vitamin D was associated with cancer related deaths, HR 1.01 (1.00 – 1.02, 95% CI), p=0.018, and this association remained after adjustment for TUG, MMSE and IADL, HR 2.13 (1.22 – 3.75, 95% CI) p =0.008.

Table 32: Association Between 25(OH)D Concentration (Log) and Cause of Death

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β Coefficient</td>
<td>HR</td>
</tr>
<tr>
<td>Infection/Pneumonia</td>
<td>-0.17</td>
<td>0.85</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>0.04</td>
<td>1.05</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.57</td>
<td>1.77</td>
</tr>
<tr>
<td>Stroke</td>
<td>0.23</td>
<td>1.26</td>
</tr>
<tr>
<td>Dementia</td>
<td>-0.38</td>
<td>0.68</td>
</tr>
<tr>
<td>Other</td>
<td>-0.34</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Model 1: Age, Gender, Living Alone (Y/N), Education (Log), GSR, Vitamin D Supplementation, BMI  
Model 2: Model 1 and TUG (Log), MMSE (Log), IADL  
*Statistically significant result
Chapter 21: Conclusion

21.1 Discussion

This large prospective study of older Irish community dwelling adults, shows an inverse relationship between serum Vitamin D levels and all-cause mortality and mortality due to cancer. Those participants with serum 25 hydroxyvitamin D levels either <50nmol/L or <25nmol/L were more likely to die over the 3.6year study follow-up period than those with higher Vitamin D levels. The significant relationship between Vitamin D and mortality remained robust after multiple confounding factors were considered, including those potentially associated with Vitamin D and frailty status.

This is the first Irish study to investigate the relationship between serum Vitamin D and all cause mortality. Our study’s findings are similar to those previously reported in the literature, including cross—sectional (Amrein et al., 2014b, Padhi et al., 2014, Venkatram et al., 2011), prospective (Amrein et al., 2014a, Durup et al., 2012, Saliba et al., 2012, Schottker et al., 2013b, Semba et al., 2009), which have all shown an association between lower levels of Vitamin D and mortality. There have also been a number of systematic reviews and meta-analyses reported, which have also found an association between Vitamin D and all-cause mortality (Autier and Gandini, 2007, Bjelakovic et al., 2011, Garland et al., 2014, Putzu et al., 2017, Schottker et al., 2014a, Bjelakovic et al., 2014) and cancer specific mortality.

Some positive studies to date suggest a plateau level of benefit in terms of Vitamin D supplementation beyond certain serum 25(OH)D levels (Bjelakovic et al., 2011), which may explain the U and J shaped results reported in some studies (Amrein et al., 2014a, Durup et al., 2012).

However, there have been a number of negative studies also reported in the literature. In fact the RCTs to date have shown no association between Vitamin D supplementation and mortality rates (Amrein et al., 2014b, Avenell et al., 2012, LaCroix et al., 2009, Zittermann et al., 2017, Manson et al., 2018). Often these are populations recruited for alternative
primary end point analysis, frequently are not deficient in Vitamin D and small doses of Vitamin D are used in the intervention arms of these studies.

The findings of this study supports a strong association between Vitamin D deficiency and mortality which warrants further evaluation in the form of an RCT, in an older population who are deficient in Vitamin D at recruitment, with prolonged follow-up and adequate Vitamin D supplementation, to clearly establish if Vitamin D deficiency is a modifiable factor that can affect mortality.

21.2 Strengths and Limitations

There are some limitations to our study. Although the study consisted of a large sample size, participants included were recruited with underlying disease states relating to cognition and bone health and so the results may not be applicable to the general population.

As this is a prospective study, we cannot draw conclusions regarding causality. However this is a large population sample, with multiple covariates considered in the analysis to account for potential confounds and our findings remained robust.

Another strength of this study is that we were able to adjust for a number of chronic conditions. Chronic diseases could either serve as a mediator or confounder in the pathway between Vitamin D and all-cause mortality. In our analysis, adjustment for multiple chronic diseases did not alter the association between Vitamin D concentrations and all-cause mortality.

Complete and accurate data was available on mortality for all study participants from a national registry. However, classification of cause of death is not standardised in the registry and thus information on cause-specific mortality was not available for analysis in this study population.
Section 7: Vitamin D and Cognition
Chapter 22: Introduction

It is estimated that a significant proportion of the population throughout the world is deficient in Vitamin D and the effects of Vitamin D on bone health and homeostasis are well documented. The effects of Vitamin D beyond bone are increasingly recognised, including cognition.

In recent times evidence supports a role for 25-hydroxyvitamin D (25(OH)D) in cognitive function, though results from studies are inconsistent. To date there have been a number of studies assessing the association between cognition and Vitamin D (Feart et al., 2017, Licher et al., 2017, Olsson et al., 2017).

A number of these studies use crude cognitive screening tools, such as the MMSE, as a measure of cognitive function (Llewellyn et al., 2010, Slinin et al., 2012, Toffanello et al., 2014). Also variability in the results of these studies is likely related to the heterogeneity of populations studied, small sample sizes, different definitions of Mild Cognitive Impairment (MCI), dementia, and of deficiency and sufficiency of Vitamin D levels.

The association of Vitamin D and cognition has previously been evaluated in the Trinity University of Ulster and Department of Agriculture (TUDA) Study, which revealed a cross-sectional relationship between Vitamin D and cognitive impairment. This was noted specifically in the RBANS cognitive domain of visuoconstruction (McCarroll, 2014).
### Table 33: Vitamin D and Cognition: Cross Sectional and Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cross Sectional Positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sato et al 1998 Japan</td>
<td>46</td>
<td>Not available</td>
<td>53.9nmol/L v 17.7nmol/L</td>
<td>Cases (nursing home residents with AD) more likely to be deficient in Vitamin D than controls (healthy adults)</td>
</tr>
<tr>
<td>Wilkins et al 2006 USA</td>
<td>80</td>
<td>74.8</td>
<td>47.3nmol/L</td>
<td>Association between Vitamin D deficiency and impairment on SBT &amp; CDR. None with MMSE</td>
</tr>
<tr>
<td>Przybelski et al 2007 USA</td>
<td>32</td>
<td>79.5</td>
<td>54nmol/L</td>
<td>Association between MMSE and Vitamin D deficiency</td>
</tr>
<tr>
<td>Oudshoorn et al 2008 Netherlands</td>
<td>225</td>
<td>77.6</td>
<td>45.4nmol/L</td>
<td>Association with Vitamin D deficiency &amp; lower MMSE</td>
</tr>
<tr>
<td>Wilkins et al 2009 USA</td>
<td>60</td>
<td>79.5</td>
<td>48nmol/L</td>
<td>Association with SBT and Vitamin D deficiency. No association with MMSE</td>
</tr>
<tr>
<td>Lee et al 2009 UK/Europe</td>
<td>3,133</td>
<td>59.9</td>
<td>62.5nmol/L</td>
<td>Association with DSST with Vitamin D &lt;35nmol/L. No association on CTRM or ROCF</td>
</tr>
<tr>
<td>Buel et al 2009 USA</td>
<td>1,080</td>
<td>75.7</td>
<td>47nmol/L</td>
<td>Association on tests of executive function and attention/processing speed. No association on memory</td>
</tr>
<tr>
<td>Llewellyn et al 2009 UK</td>
<td>1,766</td>
<td>78.2</td>
<td>Not available (Quintiles)</td>
<td>Association between Vitamin D and lower AMTS. Only in males when analysed by gender</td>
</tr>
<tr>
<td>Buell et al 2010</td>
<td>318</td>
<td>73.5</td>
<td>47.9nmol/L</td>
<td>Vitamin D deficiency associated with all cause dementia and stroke</td>
</tr>
<tr>
<td>Seamans et al 2010</td>
<td>380</td>
<td>68</td>
<td>76.2nmol/L</td>
<td>Association with Spatial Working Memory. (Only in females)</td>
</tr>
<tr>
<td>Annweiler et al 2010 France</td>
<td>5,596</td>
<td>80.7</td>
<td>Assessed dietary intake. No measures of serum Vitamin D</td>
<td>Association between Vitamin D intake and cognitive impairment on SPMSQ</td>
</tr>
<tr>
<td>Annweiler et al 2010 France</td>
<td>752</td>
<td>80.4</td>
<td>18nmol/L</td>
<td>Association between Vitamin D deficiency (&lt;25nmol/L) and impairment on SPMSQ</td>
</tr>
<tr>
<td>Llewellyn et al 2011 USA</td>
<td>3,396</td>
<td>73.7</td>
<td>29%: &lt;50nmol/L. 2.7%: &lt;25nmol/L</td>
<td>Vitamin D deficiency associated with cognitive impairment on composite score</td>
</tr>
<tr>
<td>Annweiler et al 2011 France</td>
<td>288</td>
<td>86</td>
<td>Mean 35.2nmol/L</td>
<td>Association between Vitamin D deficiency and moderate to severe cognitive impairment</td>
</tr>
<tr>
<td>Chei et al 2014 China</td>
<td>2,004</td>
<td>84.9</td>
<td>43.1nmol/L</td>
<td>Association between lower Vitamin D quartiles and Cognitive Impairment (MMSE &lt;18)</td>
</tr>
<tr>
<td>Annweiler et al 2014 France</td>
<td>253</td>
<td>77.5</td>
<td>58.6nM</td>
<td>Inverse association between Vitamin D and White Matter Intensities on MRI Brain</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cross Sectional Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sakur et al 2014</td>
<td>253</td>
<td>77.5</td>
<td>58.9nmol/L</td>
<td>An inverse association between 25(OH)D and WMH volume. No association with parenchymal volume.</td>
</tr>
<tr>
<td>Quraishi et al 2015 USA</td>
<td>198</td>
<td>59</td>
<td>54.9</td>
<td>Significant association between Vitamin D levels and new onset delirium.</td>
</tr>
<tr>
<td>Ahn et al 2015 Korea</td>
<td>412</td>
<td>73.4</td>
<td>50</td>
<td>Association between Vitamin D and MMSE scores.</td>
</tr>
<tr>
<td>Darwish et al 2015 Beirut</td>
<td>254</td>
<td>38.2% &gt;60years</td>
<td>67.7nmol/L</td>
<td>Association between ROCF and SDMT. No association with MOCA.</td>
</tr>
<tr>
<td>Pettersen et al 2015 Canada</td>
<td>142</td>
<td>56.3</td>
<td>80nmol/L</td>
<td>Supra-therapeutic levels of Vitamin D (&gt;100nmol/L) were associated with Verbal Fluency only.</td>
</tr>
<tr>
<td>Prabhakar et al 2015 India</td>
<td>140</td>
<td>59.9</td>
<td>39.7nmol/L</td>
<td>Increased risk of VD with deficient level (&lt;30nmol/L) but not for levels of 30-50nmol/L.</td>
</tr>
<tr>
<td>Vedak et al 2015 India</td>
<td>132</td>
<td>57.9</td>
<td>41.4nmol/L</td>
<td>Vitamin D associated with lower MMSE and ACE in dementia group compared with MCI.</td>
</tr>
<tr>
<td>Annweiler et al 2016 Singapore</td>
<td>2,273</td>
<td>70.4</td>
<td>58.2nmol/L</td>
<td>Higher Vitamin D levels associated with higher AMT scores.</td>
</tr>
<tr>
<td>Moretti et al 2017 Italy</td>
<td>86</td>
<td>77.9</td>
<td>27nmol/L</td>
<td>Inverse relationship between Vitamin D and AD and sVAD on univariate analysis.</td>
</tr>
<tr>
<td></td>
<td>AD 449</td>
<td>75.7</td>
<td>22.7nmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sVAD 567</td>
<td>76.4</td>
<td>47.2nmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouma et al 2018 Japan</td>
<td>230</td>
<td>HS: 74.5</td>
<td>HS: 65.3nmol/L</td>
<td>Association between 25(OH)D and variability in MMSE in MCI and AD but not in healthy subjects (HS). No association with 1,25(OH)2D3</td>
</tr>
<tr>
<td></td>
<td>MCI: 75.5</td>
<td>MCI: 45.5nmol/L</td>
<td>(women)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD Mild: 74.8</td>
<td>AD: 68.4nmol/L</td>
<td>(Men)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate: 82.2</td>
<td>MCI: 52.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe: 77.7</td>
<td>(women)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pavlovic et al 2018 USA</td>
<td>4,358</td>
<td>60.8</td>
<td>90.4nmol/L</td>
<td>Lower concentrations of Vitamin D associated with cognitive impairment on MoCA (scores &lt;25)</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>----------</td>
<td>----------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cross Sectional Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGrath et al 2007 USA</td>
<td>4,809</td>
<td>20-59 60-90</td>
<td>Not available (Quintiles)</td>
<td>No association between Vitamin D and cognitive impairment on tests: DDST SDLT, Short Story Recall</td>
</tr>
<tr>
<td>Morandi et al 2013 USA</td>
<td>120</td>
<td>52</td>
<td>38nmol/L</td>
<td>No association between Vitamin D and delirium in ICU population</td>
</tr>
<tr>
<td>Annweiler et al 2014 France</td>
<td>110</td>
<td>71.0</td>
<td>60.4nmol/L</td>
<td>Overall no association Trend for association on Trails B</td>
</tr>
<tr>
<td>Lam et al 2015</td>
<td>179</td>
<td>65.7</td>
<td>84.7nmol/L</td>
<td>No association between 25(OH)D and verbal episodic memory</td>
</tr>
<tr>
<td>Llewellyn et al 2010 Italy</td>
<td>858</td>
<td>73.8</td>
<td>62.3% &lt;50nmol/L 20.3% &lt;25nmol/L</td>
<td>Association with MMSE No association on Trails A or B</td>
</tr>
<tr>
<td>Prospective Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annweiler et al 2011 France</td>
<td>40</td>
<td>78.4 (Median)</td>
<td>36.25nmol/L (Median)</td>
<td>Vitamin D deficiency at baseline associated with developing Non-AD at 7 year follow-up</td>
</tr>
<tr>
<td>Annweiler et al 2012 France</td>
<td>498</td>
<td>80.6</td>
<td>Assessed Vitamin D intake and cognition</td>
<td>Higher Vitamin D intake associated with lower risk of AD</td>
</tr>
<tr>
<td>Slinin et al 2012 USA</td>
<td>6,257</td>
<td>76.6</td>
<td>Not available</td>
<td>Association between Vitamin D and mMMSM but not with Trails B</td>
</tr>
<tr>
<td>Breitling et al 2012 Germany</td>
<td>1,639</td>
<td>74</td>
<td>Not available</td>
<td>Association between COGTEL and Vitamin D deficiency in women, not in men</td>
</tr>
<tr>
<td>Afzal et al 2013 Denmark</td>
<td>10,186</td>
<td>58</td>
<td>41nmol/L (median)</td>
<td>Increased risk of developing AD in those with Vitamin D &lt;25nmol/L at baseline</td>
</tr>
<tr>
<td>Pettersen et al 2014 Canada</td>
<td>19</td>
<td>52</td>
<td>Not available</td>
<td>Seasonal decline in Vitamin D was associated with decline in tests of executive/ working memory</td>
</tr>
<tr>
<td>Perna et al 2014 ESTHER</td>
<td>527</td>
<td>73.7</td>
<td>Not available</td>
<td>Lower Vitamin D quartiles associated with lower COGTEL scores</td>
</tr>
<tr>
<td>Knekt et al 2014 Finland</td>
<td>5,010</td>
<td>62.5</td>
<td>42.5nmol/L</td>
<td>Inverse association between Vitamin D and incident dementia in women</td>
</tr>
<tr>
<td>Toffanello et al 2014 Italy</td>
<td>1,927</td>
<td>73.9</td>
<td>84.1nmol/L</td>
<td>Association between Vitamin D and cognitive decline on MMSE</td>
</tr>
<tr>
<td>Littlejohns et al 2015 USA</td>
<td>1,658</td>
<td>73.6</td>
<td>Not available (Quartiles)</td>
<td>Severe Vitamin D deficiency associated with incident all-cause dementia</td>
</tr>
<tr>
<td>Assmann et al 2015 France</td>
<td>1,009</td>
<td>66.6</td>
<td>52nmol/L</td>
<td>Deficient Vitamin D associated with poorer scores on Backward Tracking</td>
</tr>
<tr>
<td>Author</td>
<td>No.</td>
<td>Mean Age</td>
<td>Mean Vitamin D</td>
<td>Results</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>----------</td>
<td>----------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Prospective Positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller et al 2015 USA</td>
<td>318</td>
<td>75.5</td>
<td>47.9nmol/L</td>
<td>Higher Vitamin D was associated with slower rate of cognitive decline</td>
</tr>
<tr>
<td>Jorde et al 2015 Norway</td>
<td>3,435</td>
<td>57.7</td>
<td>Quartiles</td>
<td>Vitamin D associated with finger tapping in longitudinal analysis but not other domains</td>
</tr>
</tbody>
</table>
| Moon et al 2015 Korea                                    | 412   | 72.6     | Not available 60% <25nmol/L | Overall no association with Vitamin D and development of cognitive impairment
Subgroup association Vitamin D and MMSE <27 with no dementia at baseline and development of cognitive Impairment
Development of MCI in those with Vitamin D <25nmol/L. |
<p>| Feart et al 2017 France                                  | 916   | 73.3     | 35.8nmol/L      | Vitamin D deficiency association with faster decline in FCSRT. Associated with increased risk of incident dementia |
| Licher et al 2017                                        | 6,220 | Not available (&gt;55 years) | Not available | Longitudinal association between Vitamin D and incident dementia and AD                                                                 |
| Goodwill et al 2017                                      | 252   | 59.8     | 49.4nmol/L      | Midlife Vitamin D levels associated with executive function at baseline and 10 year follow-up                                               |
| Goodwill et al 2018                                      | 252   | 59.8     | 49.4nmol/L      | Longitudinally Vitamin D association with composite score of executive function, but not on individual cognitive test scores.           |
| <strong>Prospective Negative</strong>                                 |
| Slinin et al 2010 USA                                    | 1,138 | 73.7     | Not available   | No association on Trails B or 3MS                                                                                                        |
| Schneider et al 2014 USA                                 | 1,652 | 62       | 43.2nmol/L      | No longitudinal association with cognitive decline or incident dementia risk                                                              |
| Granic et al 2015 UK                                     | 845   |          | Not available   | No association between Vitamin D and cognitive impairment. U shaped association with Vitamin D and cognitive function |  |
| Karakis et al 2016                                       | 1,663 | 72.4     | 62.6nmol/L      | No association between Vitamin D and dementia or hippocampal volumes on MRI                                                            |
|                  | 1,291 | 59.5     | 49.4nmol/L      | Associated with poorer scores on Trails and HVRT                                                                                          |
| Van Schoor et al 2016                                    | 3,107 | 75.6     | 53.7nmol/L      | No significant association between Vitamin D and cognition longitudinally                                                             |</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olsson et al 2017</td>
<td>1,182</td>
<td>71</td>
<td>69nmol/L</td>
<td>No association between Vitamin D and incident dementia, AD, VD, or cognitive decline</td>
</tr>
<tr>
<td>Kilpatrick et al 2018</td>
<td>2,786</td>
<td>73.8</td>
<td>Not available</td>
<td>No association between Vitamin D and baseline or incident cognitive impairment</td>
</tr>
</tbody>
</table>
### Table 34: Vitamin D and Cognition: Intervention and Randomised Control Trials

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annweiler et al 2012 France</td>
<td>44</td>
<td>80.6</td>
<td>Treatment: 42.0nmol/L</td>
<td>Intervention: Vitamin D3 800IU/day or 100,000IU per month</td>
<td>Some improvement in scores on MMSE and FAB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 63.0nmol/L</td>
<td>Control: Usual treatment</td>
<td></td>
</tr>
<tr>
<td>Dhesi et al 2004 UK</td>
<td>123</td>
<td>Placebo: 76.6</td>
<td>Intervention: 77.0</td>
<td>Placebo: Normal saline IM</td>
<td>Intervention group: faster CRT than control after 6/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25nmol/L</td>
<td>Intervention: Single dose 600,000iu IM Vitamin D2</td>
<td></td>
</tr>
<tr>
<td>Peterson et al 2017 Canada</td>
<td>Total: 82 High Dose 42 Low Dose 40</td>
<td>High Dose: 56.7 Low Dose: 52.6</td>
<td>High Dose: 67.2nmol/L Low Dose: 60.5nmol/L</td>
<td>High Dose: 4,000IU per day Low Dose: 400IU per day</td>
<td>Some improvement in test scores in verbal memory in the high dose treatment group</td>
</tr>
<tr>
<td>Rossom et al 2012 USA</td>
<td>4,143</td>
<td>71</td>
<td>Intervention: 50nmol/L Placebo: 48nmol/L</td>
<td>Intervention: 2 tablets daily containing Calcium Carbonate 1,000mg and Vitamin D 400IU Placebo equivalent</td>
<td>No significant differences in 3MSE or domain specific cognitive scores after 7.8 years. No difference in incidence of cognitive impairment</td>
</tr>
<tr>
<td>Stein et al 2011 Australia</td>
<td>Pilot: 13 RCT: 32</td>
<td>Pilot: 77.5 RCT: Intervention: 75 Placebo: 79</td>
<td>Pilot: 66nmol/L RCT: 49nmol/L</td>
<td>Pilot: 3,000IU D2 x 8weeks RCT: Intervention: 6,000IU D2: initially 2tablets TDS then dose adjusted based on serum 25(OH)D levels Placebo: capsules with same capsule dose change as intervention “buddy”</td>
<td>Pilot: 6 point increase in ADAS Cog RCT: No difference between treatment and intervention groups in ADASCog or WMS Subsets</td>
</tr>
</tbody>
</table>

**Negative**

<table>
<thead>
<tr>
<th>Author Year</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Vitamin D</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Przybelski et al 2008 USA</td>
<td>63</td>
<td>87</td>
<td>Treatment Group: 43.3nmol/L Comparison Group: 87nmol/L</td>
<td>Intervention: 50,000IU PO three times weekly for 4weeks Vitamin D2 Controls: Usual treatment</td>
<td>No significant difference in either group</td>
</tr>
<tr>
<td>Rosom et al 2012 USA</td>
<td>4,143</td>
<td>71</td>
<td>Intervention: 50nmol/L Placebo: 48nmol/L</td>
<td>Intervention: 2 tablets daily containing Calcium Carbonate 1,000mg and Vitamin D 400IU Placebo equivalent</td>
<td>No significant differences in 3MSE or domain specific cognitive scores after 7.8 years. No difference in incidence of cognitive impairment</td>
</tr>
</tbody>
</table>
The aim of this study was to prospectively evaluate if association between serum 25-hydroxyvitamin D 25(OH)D and cognitive function persist, thus supporting a stronger relationship between Vitamin D deficiency and cognitive function.

The primary outcome of interest was the association between Vitamin D and cognitive decline in Index II of the RBANS, as this was found to be a significant association on prior cross sectional analysis (McCarroll, 2014). Secondary outcomes of interest include; the other four RBANS indices, TUG, mood, BMI and waist/hip ratio.
Chapter 23: Methodology

23.1 Study Population
All 3,093 TUDA participants recruited through St James Hospital Dublin were included in this study. The TUDA population is a large community dwelling population of Irish adults aged 60 years and older. It is a cross-sectional study designed to create a genotype/phenotype database for three population cohorts based on three disease states: cognition, bone health and hypertension. Full methodology is discussed in detail in Chapters 8 and 9.

This nested case control study was conducted using two sub-populations of participants from the TUDA cognitive and bone cohorts initially recruited through St James’s Hospital, Dublin. These sub-populations of interest include “cases” defined as those participants with serum Vitamin D levels <25nmol/L compared with age and sex matched “controls” with Vitamin D levels >75nmol/L. Inclusion criteria: those willing and able to consent to participate, with a MMSE score of >26/30 at initial TUDA recruitment. A total of 134 participants were included in this analysis, 67 cases and 67 controls (Figure 26). The mean time to follow up was five years.

23.2 Methodology
Those who met inclusion criteria as outlined were contacted by phone and those who were agreeable were forwarded information regarding the study and then were assessed at a later date. Those who consented to participate attended St James's Hospital for a repeat assessment, which included a structured interview and information collected included: cognitive, mood and psychosocial assessments, biophysical measurements, functional status and measurement of 25-hydroxyvitamin.

Cognitive assessments included: MMSE and the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Other assessments undertaken included biophysical, functional and psychological measurements similar to those form baseline TUDA
assessment, including BMI, Waist Hip Ratio, TUG, PSMS, and IADLS as well as a repeat serum 25(OH)D level.

Ethical approval for this study was obtained from the local Research Ethics Committee (REC Reference 2015-01 Chairman’s Action).

**Figure 26:** Nested Case Control Study Population Flow Chart

Those who did not attend for a full repeat assessment (n=75) were invited to participate in a mini telephone interview with completion of both IADLS and PSMS, this was completed in 59 participants. The remaining 16 participants were lost to follow-up.
23.3 Vitamin D Measurements
Non-fasting blood samples for 25(OH)D were taken on the day of assessment. Bloods were processed on the same day and centrifuged within three hours. 25(OH)D samples were stored at −70°C and batched for later analysis at the biochemistry laboratory at St James’s Hospital, Dublin, a participant of the Vitamin D External Quality Assessment Scheme (DEQAS). 25(OH)D were measured by liquid chromatography mass spectroscopy (LCMS) using a standardised assay (Mass Chrom®) and National Institute of Standards and Technology (NIST) vitamin D standard reference material. Inter- and intra -assay coefficient of variation (CV) were 5.7% and 4.5%.

23.4 Statistical Analysis
All parameters were inspected for normality and if significantly skewed were appropriately transformed. Normal assumptions for linear regression analysis were observed. Descriptive and comparative analyses were performed for all included participants.

Continuous variables are expressed as mean and standard deviations for normally distributed and median and inter-quartiles for non-normally distributed data. Categorical variables were expressed as number of cases and percentages. Nominal or dichotomous variables were compared using Chi-squared test, non-normally distributed variables were compared with Mann-Whitney U test and normally distributed continuous variables were compared using the Student t test. These analyses were run using IBM SPSS version 22.0 (SPSS, Inc., Chicago IL). Statistical significance was accepted when p <0.05.

Comparison between participants at baseline and follow up was performed using the paired t test and results were expressed as change in mean, standard deviation (SD) and associated confidence intervals (CI).

Multivariate linear regression models were created to explore the relationship between Vitamin D (>75nmol/L versus <25nmol/L) and cognitive function assessed using the individual RBANS Indices. Covariates in the model were; age, gender, education, BMI,
season of blood draw, GSR, supplement use, TUG and equivalent RBANS Index at baseline assessment.

Power Calculation: Assuming a standard deviation of ±17.8 based on prior research in similar populations and an alpha of 5%, a sample size of 126 (63 per group) would have an 80% power to show a significance of 10 on the RBANS scale, which is a clinically significant change.
Chapter 24: Results

24.1 Baseline Characteristics

At baseline there was no difference between ages, as all participants were both age and gender matched (Table 35).

Cases had mean 25(OH)D levels of 18.5nmol/L compared with the control group mean of 98.1nmol/L. At baseline cases had a slower gait speed, as measured by TUG, compared with controls (19.1 versus 12.5 seconds, p<0.001). They had an increased BMI compared with controls and were more likely to be depressed in the preceding week, as measured by CES-D and took more medications at baseline.

In terms of cognitive scores, there was no significant difference between the groups on MMSE scores (27.9 for cases versus 28.1 for controls), though performance on RBANS total was 6 points lower in cases. However there was a trend towards lower RBANS II (visuospatial index) in the cases groups compared with controls, p=0.055 and there was a significant difference in RBANS IV Index (attention index) at baseline between the groups.
Table 35: Characteristics of Cases and Controls at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Case n=67 (Mean, SD)</th>
<th>Control n=67 (Mean, SD)</th>
<th>Mean Difference</th>
<th>t test</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female n,%)</td>
<td>41 (61.2%)</td>
<td>41 (61.2%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.570 a</td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.6 ±7.3</td>
<td>76.3 ±7.6</td>
<td>-0.7</td>
<td>-0.5</td>
<td>-3.2 – 1.9</td>
<td>0.600 a</td>
</tr>
<tr>
<td>Total Education (years)</td>
<td>11.9 ±3.1</td>
<td>12.8 ±3.4</td>
<td>-0.8</td>
<td>-1.5</td>
<td>-1.9 – 0.3</td>
<td>0.140 a</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>18.5 ±4.9</td>
<td>98.1 ±18.0</td>
<td>-79.6</td>
<td>-34.8</td>
<td>-84.1 – -75.1</td>
<td>&lt;0.001* a</td>
</tr>
<tr>
<td>TUG (seconds)</td>
<td>19.1 ±11.8</td>
<td>12.5 ±6.3</td>
<td>6.6</td>
<td>3.9</td>
<td>3.2 – 10.0</td>
<td>&lt;0.001* a</td>
</tr>
<tr>
<td>Waist/Hip</td>
<td>0.9 ±0.1</td>
<td>0.9 ±0.1</td>
<td>0.0</td>
<td>0.5</td>
<td>-0.0 – 0.0</td>
<td>0.595 a</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.9 ±6.9</td>
<td>26.3 ±5.0</td>
<td>2.5</td>
<td>2.4</td>
<td>0.5 – 4.6</td>
<td>0.017* a</td>
</tr>
<tr>
<td>IADL</td>
<td>22.0 ±4.4</td>
<td>23.4 ±4.1</td>
<td>-1.3</td>
<td>-1.8</td>
<td>-2.8 – 0.2</td>
<td>0.079 a</td>
</tr>
<tr>
<td>PSMS</td>
<td>22.7 ± 1.9</td>
<td>22.9 ±2.1</td>
<td>-0.2</td>
<td>-0.7</td>
<td>-0.9 – 0.5</td>
<td>0.498 a</td>
</tr>
<tr>
<td>CES-D</td>
<td>7.1 ±8.5</td>
<td>4.1 ±5.3</td>
<td>3.0</td>
<td>2.5</td>
<td>0.6 – 5.5</td>
<td>0.014* a</td>
</tr>
<tr>
<td>HADS</td>
<td>3.7 ±4.3</td>
<td>2.8 ±3.0</td>
<td>0.9</td>
<td>1.3</td>
<td>-0.4 – 2.1</td>
<td>0.181 a</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.9 ±1.2</td>
<td>28.1 ±2.2</td>
<td>-0.2</td>
<td>-1.0</td>
<td>-0.6 – 0.2</td>
<td>0.316 a</td>
</tr>
<tr>
<td>RBANS Total Index</td>
<td>85.9 ±17.6</td>
<td>92.1 ±11.9</td>
<td>-6.2</td>
<td>-2.4</td>
<td>-11.5 – -1.0</td>
<td>0.020* a</td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>93.2 ±18.9</td>
<td>98.0 ±13.2</td>
<td>-4.8</td>
<td>-1.7</td>
<td>-10.4 – 0.8</td>
<td>0.093 a</td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>86.4 ±19.3</td>
<td>92.5 ±16.8</td>
<td>-6.1</td>
<td>-1.9</td>
<td>-12.3 – 0.1</td>
<td>0.055 a</td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>90.4 ±12.6</td>
<td>93.4 ±9.8</td>
<td>-3.0</td>
<td>-1.6</td>
<td>-6.9 – 0.8</td>
<td>0.122 a</td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>84.5 ±16.6</td>
<td>93.4 ±16.0</td>
<td>-8.9</td>
<td>-3.1</td>
<td>-14.6 – -3.2</td>
<td>0.002* a</td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>89.7 ±18.3</td>
<td>94.5 ±13.5</td>
<td>-4.7</td>
<td>-1.7</td>
<td>-10.2 – 0.8</td>
<td>0.093 a</td>
</tr>
</tbody>
</table>

| No. Medications          | 8.0 ±3.4             | 5.7 ±3.5                | 2.3             | 3.9    | 1.1 – 3.5     | <0.001* b |

a chi-squared test    b Student t Test    *Statistically significant result
Of the 134 participants selected for inclusion, follow-up data was available on 86% of participants, 50% of who attended in person for follow-up. Of the participants who did not complete the full follow-up (n=75), ten cases and six controls (n=16) were completely lost to follow-up and the remainder (n=59) declined to attend for full assessment but completed the IADLS and PSMS tools over the telephone.

Table 36: Baseline Characteristics Participants Complete Follow-Up v Not Complete

<table>
<thead>
<tr>
<th>n=134</th>
<th>Complete Follow Up (n=59)</th>
<th>Not Complete Follow Up (n=75)</th>
<th>Mean Difference</th>
<th>t test</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>73.7 ±7.8</td>
<td>77.8 ±6.6</td>
<td>-4.2</td>
<td>-3.2</td>
<td>-6.5 - -1.6</td>
<td>0.001*</td>
</tr>
<tr>
<td>Total Education (years)</td>
<td>13.1 ±3.5</td>
<td>11.8 ±2.9</td>
<td>1.3</td>
<td>2.3</td>
<td>0.2 - 2.4</td>
<td>0.026*</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>64.6 ±42.1</td>
<td>53.3 ±41.7</td>
<td>11.4</td>
<td>1.6</td>
<td>-3.0 - 25.8</td>
<td>0.121</td>
</tr>
<tr>
<td>TUG (seconds)</td>
<td>12.8 ±6.3</td>
<td>18.3 ±11.6</td>
<td>-5.5</td>
<td>-3.2</td>
<td>-9.0 - -2.1</td>
<td>0.002*</td>
</tr>
<tr>
<td>Waist/Hip</td>
<td>0.9 ±0.1</td>
<td>0.9 ±0.1</td>
<td>-0.0</td>
<td>-0.6</td>
<td>-0.0 - 0.0</td>
<td>0.537</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.6 ±6.2</td>
<td>27.6 ±6.3</td>
<td>-0.0</td>
<td>-0.0</td>
<td>-2.2 - 2.1</td>
<td>0.977</td>
</tr>
<tr>
<td>IADL</td>
<td>24.0 ±3.6</td>
<td>21.6 ±4.5</td>
<td>2.4</td>
<td>3.3</td>
<td>0.9 – 3.8</td>
<td>0.001*</td>
</tr>
<tr>
<td>PSMS</td>
<td>23.1 ±1.8</td>
<td>22.5 ±2.2</td>
<td>0.6</td>
<td>1.7</td>
<td>-0.1 - 1.3</td>
<td>0.096</td>
</tr>
<tr>
<td>CES-D</td>
<td>4.4 ±6.6</td>
<td>6.6 ±7.6</td>
<td>-2.2</td>
<td>-1.8</td>
<td>-4.7 - 0.2</td>
<td>0.077</td>
</tr>
<tr>
<td>HADS</td>
<td>2.6 ±2.9</td>
<td>3.8 ±4.2</td>
<td>-1.2</td>
<td>1.8</td>
<td>-2.5 - 0.9</td>
<td>0.069</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.2 ±1.1</td>
<td>27.7 ±1.2</td>
<td>0.5</td>
<td>2.3</td>
<td>0.1 – 0.9</td>
<td>0.023*</td>
</tr>
<tr>
<td>RBANS Total Index</td>
<td>92.9 ±14.8</td>
<td>85.8 ±15.1</td>
<td>7.2</td>
<td>2.7</td>
<td>1.9 – 12.4</td>
<td>0.008*</td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>98.2 ±14.9</td>
<td>85.8 ±17.4</td>
<td>4.4</td>
<td>1.5</td>
<td>-1.2 – 10.0</td>
<td>0.125</td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>94.6 ±18.6</td>
<td>85.5 ±17.1</td>
<td>9.1</td>
<td>2.9</td>
<td>2.9 – 15.3</td>
<td>0.004*</td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>94.5 ±10.0</td>
<td>89.8 ±11.9</td>
<td>4.7</td>
<td>2.4</td>
<td>0.9 – 8.5</td>
<td>0.016*</td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>92.8 ±18.5</td>
<td>85.8 ±14.9</td>
<td>6.9</td>
<td>2.4</td>
<td>1.2 – 12.8</td>
<td>0.019*</td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>93.7 ±14.8</td>
<td>90.8 ±17.1</td>
<td>2.9</td>
<td>1.0</td>
<td>-2.7 – 8.5</td>
<td>0.305</td>
</tr>
<tr>
<td>No. Medications</td>
<td>6.8 ±3.6</td>
<td>6.9 ±3.7</td>
<td>-0.2</td>
<td>-0.3</td>
<td>-1.4 – 1.1</td>
<td>0.789</td>
</tr>
</tbody>
</table>

*Statistically significant result
Those participants (n=75) who did not attend for follow-up, were older (77.8 versus 73.7 years), less educated (11.8 versus 13.1 years), physically slower (TUG 18.3 versus 12.8 seconds), less independent in ADLS (IADLS 21.6 versus 24.0). They were more likely to have lower cognitive scores at baseline compared with those who did attend (MMSE 27.7 versus 28.2) and had significantly poorer scores on RBANS Indices, including Total, Index II, III and IV at baseline (Table 36).

Overall those who did not attend follow-up were a frailer group in terms of physical and cognitive performance compared with those who attended for follow-up (Table 37).
**Table 37: Complete Follow-Up versus Telephone Follow-Up**

<table>
<thead>
<tr>
<th>n=118</th>
<th>Complete Assessment (n=59)</th>
<th>Phone Assessment (n=59)</th>
<th>t test</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female n,%)</td>
<td>37 (62.7)</td>
<td>34 (57.7)</td>
<td>-</td>
<td>-</td>
<td>0.879</td>
</tr>
<tr>
<td>Age (years) (mean, SD)</td>
<td>73.1 ±7.6</td>
<td>77.0 ±6.5</td>
<td>-2.8</td>
<td>-6.2 - -1.0</td>
<td>0.007*</td>
</tr>
<tr>
<td>Total Education (years)</td>
<td>13.1±3.5</td>
<td>11.6 ±3.0</td>
<td>2.5</td>
<td>0.3 – 2.7</td>
<td>0.015*</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>64.2 ±42.6</td>
<td>55.2 ±41.9</td>
<td>1.2</td>
<td>-6.4 -24.5</td>
<td>0.247</td>
</tr>
<tr>
<td>TUG (seconds)</td>
<td>12.9 ±6.6</td>
<td>16.5 ±10.0</td>
<td>-2.3</td>
<td>-6.9 - -0.5</td>
<td>0.026*</td>
</tr>
<tr>
<td>Waist/Hip</td>
<td>0.9 ±0.1</td>
<td>0.9 ±0.1</td>
<td>-0.4</td>
<td>-0.03 - 0.02</td>
<td>0.698</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.6 ±6.3</td>
<td>27.5 ±6.4</td>
<td>0.1</td>
<td>-2.2 – 2.4</td>
<td>0.935</td>
</tr>
<tr>
<td>IADLS</td>
<td>24.1 ±3.7</td>
<td>21.9 ±4.6</td>
<td>2.7</td>
<td>0.6 – 3.6</td>
<td>0.008*</td>
</tr>
<tr>
<td>PSMS</td>
<td>23.1 ±1.8</td>
<td>22.6 ±2.3</td>
<td>1.5</td>
<td>-0.2 – 1.3</td>
<td>0.147</td>
</tr>
<tr>
<td>CES-D</td>
<td>4.3 ±6.6</td>
<td>7.2 ±8.2</td>
<td>-2.1</td>
<td>-5.6 - -0.1</td>
<td>0.040*</td>
</tr>
<tr>
<td>HADS</td>
<td>2.5 ±2.9</td>
<td>4.1 ±4.4</td>
<td>-2.2</td>
<td>-2.9 - -0.2</td>
<td>0.027*</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.2 ±1.2</td>
<td>27.7 ±1.2</td>
<td>2.2</td>
<td>0.04 – 0.9</td>
<td>0.033*</td>
</tr>
<tr>
<td>RBANS Total Index</td>
<td>93.1±14.8</td>
<td>86.1±15.9</td>
<td>2.4</td>
<td>1.2 – 12.8</td>
<td>0.018*</td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>98.3 ±15.0</td>
<td>93.0 ±17.5</td>
<td>1.7</td>
<td>-0.7 0 11.2</td>
<td>0.084</td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>94.9 ±18.6</td>
<td>86.2 ±18.1</td>
<td>2.6</td>
<td>2.0 – 15.5</td>
<td>0.012*</td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>94.5 ±10.1</td>
<td>90.5 ±11.7</td>
<td>2.0</td>
<td>-0.0 - 8.0</td>
<td>0.053</td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>93.0 ±18.6</td>
<td>85.4 ±16.4</td>
<td>2.3</td>
<td>1.0 – 14.2</td>
<td>0.025*</td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>93.7 ±14.9</td>
<td>91.3 ±18.7</td>
<td>0.8</td>
<td>-3.5 – 8.2</td>
<td>0.430</td>
</tr>
<tr>
<td>IADLS 2</td>
<td>21.9 ±4.6</td>
<td>17.2 ±6.9</td>
<td>4.2</td>
<td>2.4 - 6.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PSMS 2</td>
<td>22.4 ±2.3</td>
<td>18.7 ±5.8</td>
<td>4.5</td>
<td>2.1 – 5.3</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Statistically significant result
24.2 Follow-Up Results

Participants were re-assessed after a mean follow up period of 5.3 years (5.6 years for cases and 4.7 years for controls, P<0.001). Of the 134 participants selected, 59 attended in person for a complete follow-up repeat assessment (24 cases and 35 controls).

Cases still had a significantly lower serum 25(OH)D level compared with controls (51.9 versus 83.3nmol/L, P<0.001). However, there was a significant increase in Vitamin D levels in cases between baseline and follow-up assessments (18.5 to 51.9nmol/L). This was likely due to the initiation of Vitamin D supplementation following their initial assessment. Cases had higher BMIs than their control counterparts, 29.9 versus 25.3kg/m2 p=0.006 (Table 38).

At repeat assessment participants were more frail, with mean Timed Up and Go (TUG) of 12.4 seconds (s) at baseline (Time 1) and 21.2s at follow up (Time 2). They were also less independent with lower Physical Self Maintenance Scores (PSMS) of 22.4 (Time 2) compared with 23.1 (Time 1) and IADLS score of 21.6 (Time 2) compared with 24.0 at Time 1.

There was also evidence of cognitive decline with lower MMSE scores (26.2 compared with 28.5 at Time 1, p<0.001). Of note there was significant decline in all RBANS indices in the follow up period, except in RBANS Index II (Visuospatial Index) and in RBANS Index IV (Attention Index) in cases.
### Table 38: Completed Group Characteristics: Comparison Baseline and Follow Up

<table>
<thead>
<tr>
<th>n=59</th>
<th>Mean</th>
<th>Change in</th>
<th>SD</th>
<th>t test</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 1</td>
<td>Time 2</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cohort (n=59)</td>
<td>73.7</td>
<td>78.7</td>
<td>±0.8</td>
<td>48.9</td>
<td>4.8 - 5.3</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>72.6</td>
<td>78.2</td>
<td>±0.9</td>
<td>30.1</td>
<td>5.2 – 5.9</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>74.4</td>
<td>79.1</td>
<td>±0.6</td>
<td>48.9</td>
<td>4.5 - 4.9</td>
</tr>
<tr>
<td><strong>25(OH)D (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cohort (n=57)</td>
<td>63.9</td>
<td>70.7</td>
<td>±36.9</td>
<td>1.4</td>
<td>-3.1 - 16.5</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>17.1</td>
<td>52.0</td>
<td>±34.3</td>
<td>5.0</td>
<td>20.5 – 49.4</td>
</tr>
<tr>
<td>Controls (n=33)</td>
<td>98.1</td>
<td>84.3</td>
<td>±22.6</td>
<td>3.5</td>
<td>-21.8 - -5.8</td>
</tr>
<tr>
<td><strong>TUG (seconds)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Total Cohort (n=53)</td>
<td>12.4</td>
<td>21.2</td>
<td>±10.1</td>
<td>6.3</td>
<td>5.9 – 11.6</td>
</tr>
<tr>
<td>Cases (n=21)</td>
<td>16.1</td>
<td>23.1</td>
<td>±9.6</td>
<td>3.4</td>
<td>2.7 – 11.5</td>
</tr>
<tr>
<td>Controls (n=32)</td>
<td>10.0</td>
<td>19.9</td>
<td>±10.4</td>
<td>5.4</td>
<td>6.1 – 13.7</td>
</tr>
<tr>
<td><strong>BMI (kg/m2)</strong></td>
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<tr>
<td>Total Cohort (n=57)</td>
<td>27.3</td>
<td>27.1</td>
<td>±3.3</td>
<td>0.1</td>
<td>-0.6 - -1.1</td>
</tr>
<tr>
<td>Cases (n=22)</td>
<td>29.9</td>
<td>30.0</td>
<td>±3.1</td>
<td>0.1</td>
<td>-1.3 - -1.4</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>25.7</td>
<td>25.2</td>
<td>±2.8</td>
<td>0.1</td>
<td>-1.6 - -0.7</td>
</tr>
<tr>
<td><strong>Waist/Hip</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cohort (n=59)</td>
<td>0.9</td>
<td>0.9</td>
<td>±0.1</td>
<td>-1.9</td>
<td>-0.1 - 0.0</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>0.9</td>
<td>0.9</td>
<td>±0.1</td>
<td>0.3</td>
<td>-0.01 - 0.03</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>0.9</td>
<td>0.9</td>
<td>±0.1</td>
<td>-2.8</td>
<td>-0.1 - -0.01</td>
</tr>
<tr>
<td><strong>IADL</strong></td>
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<tr>
<td>Total Cohort (n=58)</td>
<td>24.0</td>
<td>21.6</td>
<td>±3.5</td>
<td>-5.2</td>
<td>3.3 - 1.5</td>
</tr>
<tr>
<td>Cases (n=23)</td>
<td>23.7</td>
<td>20.1</td>
<td>±4.1</td>
<td>-4.2</td>
<td>5.3 - 1.8</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>25.7</td>
<td>25.2</td>
<td>±2.9</td>
<td>-3.4</td>
<td>2.7 - 0.7</td>
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<td><strong>PSMS</strong></td>
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<tr>
<td>Total Cohort (n=59)</td>
<td>23.1</td>
<td>22.4</td>
<td>±1.9</td>
<td>-2.9</td>
<td>-1.3 - 0.2</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>22.9</td>
<td>22.0</td>
<td>±2.0</td>
<td>2.3</td>
<td>-1.8 - 0.1</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>23.3</td>
<td>22.7</td>
<td>±2.0</td>
<td>-1.8</td>
<td>1.3 - 0.1</td>
</tr>
<tr>
<td><strong>CES-D</strong></td>
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<tr>
<td>Total Cohort (n=59)</td>
<td>4.4</td>
<td>8.6</td>
<td>±8.9</td>
<td>3.7</td>
<td>1.9 - 6.6</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>6.8</td>
<td>8.8</td>
<td>±9.9</td>
<td>1.0</td>
<td>2.2 - 6.2</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>2.7</td>
<td>8.5</td>
<td>±8.0</td>
<td>4.3</td>
<td>3.0 - 8.5</td>
</tr>
<tr>
<td><strong>HADS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cohort (n=59)</td>
<td>2.6</td>
<td>3.2</td>
<td>±3.1</td>
<td>1.4</td>
<td>-0.2 - 1.4</td>
</tr>
<tr>
<td>Cases (n=24)</td>
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<td>3.4</td>
<td>±3.2</td>
<td>0.1</td>
<td>-0.1 - 1.3</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>2.1</td>
<td>3.0</td>
<td>±3.0</td>
<td>1.2</td>
<td>0.1 - 1.9</td>
</tr>
</tbody>
</table>

*Statistically significant result
Table 39: Completed Group Cognitive Scores: Comparison Baseline and Follow Up

<table>
<thead>
<tr>
<th>n=59</th>
<th>Mean Time 1</th>
<th>Mean Time 2</th>
<th>Change in Mean</th>
<th>SD</th>
<th>t test</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMSE</strong></td>
<td></td>
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<tr>
<td>Total Cohort (n=59)</td>
<td>28.2</td>
<td>26.5</td>
<td>-1.7</td>
<td>±2.4</td>
<td>-5.5</td>
<td>-2.4 - -1.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>28.1</td>
<td>27.0</td>
<td>-1.1</td>
<td>±2.3</td>
<td>-2.4</td>
<td>-2.1 - -0.2</td>
<td>0.025*</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>28.3</td>
<td>26.1</td>
<td>-2.1</td>
<td>±2.5</td>
<td>-5.2</td>
<td>-3.0 - -1.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RBANS Total Index</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Total Cohort (n=58)</td>
<td>92.9</td>
<td>83.9</td>
<td>-9.1</td>
<td>±13.1</td>
<td>-5.3</td>
<td>-12.6 - -5.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>89.4</td>
<td>83.2</td>
<td>-6.2</td>
<td>±12.3</td>
<td>-2.4</td>
<td>-11.5 - -0.8</td>
<td>0.025*</td>
</tr>
<tr>
<td>Controls (n=34)</td>
<td>95.4</td>
<td>84.3</td>
<td>-11.1</td>
<td>±13.4</td>
<td>-4.8</td>
<td>-15.8 - -6.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RBANS Index I</strong></td>
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</tr>
<tr>
<td>Total Cohort (n=58)</td>
<td>98.1</td>
<td>86.0</td>
<td>-12.0</td>
<td>±15.8</td>
<td>-5.8</td>
<td>-16.2 - -7.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cases (n=23)</td>
<td>97.6</td>
<td>84.0</td>
<td>-13.6</td>
<td>±17.5</td>
<td>-3.8</td>
<td>-21.0 - -6.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>98.4</td>
<td>87.4</td>
<td>-11.0</td>
<td>±14.7</td>
<td>-4.4</td>
<td>-16.0 - -5.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RBANS Index II</strong></td>
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<td></td>
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<tr>
<td>Total Cohort (n=58)</td>
<td>94.6</td>
<td>94.0</td>
<td>-0.6</td>
<td>±17.7</td>
<td>-0.3</td>
<td>-5.3 - 4.1</td>
<td>0.796</td>
</tr>
<tr>
<td>Cases (n=23)</td>
<td>90.8</td>
<td>90.0</td>
<td>-0.8</td>
<td>±19.3</td>
<td>-0.2</td>
<td>-9.1 - 7.5</td>
<td>0.847</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>97.1</td>
<td>96.7</td>
<td>-0.5</td>
<td>±16.9</td>
<td>-0.2</td>
<td>-6.3 - 5.3</td>
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<td><strong>RBANS Index III</strong></td>
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</tr>
<tr>
<td>Total Cohort (n=59)</td>
<td>94.5</td>
<td>82.5</td>
<td>-12.1</td>
<td>±18.0</td>
<td>-5.1</td>
<td>-16.8 - -7.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>94.3</td>
<td>83.2</td>
<td>-11.0</td>
<td>±21.2</td>
<td>-2.5</td>
<td>-20.0 - -2.1</td>
<td>0.018*</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>94.7</td>
<td>81.9</td>
<td>-12.8</td>
<td>±15.7</td>
<td>-4.8</td>
<td>-18.2 - -7.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RBANS Index IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cohort (n=57)</td>
<td>92.8</td>
<td>88.1</td>
<td>-4.7</td>
<td>±15.5</td>
<td>-2.3</td>
<td>-8.8 - 0.6</td>
<td>0.027*</td>
</tr>
<tr>
<td>Cases (n=23)</td>
<td>83.0</td>
<td>86.5</td>
<td>3.4</td>
<td>±12.1</td>
<td>1.4</td>
<td>-1.8 - 8.6</td>
<td>0.186</td>
</tr>
<tr>
<td>Controls (n=34)</td>
<td>99.4</td>
<td>89.2</td>
<td>-10.1</td>
<td>±15.2</td>
<td>-3.9</td>
<td>-15.5 - -4.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>RBANS Index V</strong></td>
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</tr>
<tr>
<td>Total Cohort (n=59)</td>
<td>93.7</td>
<td>83.5</td>
<td>-10.3</td>
<td>±16.5</td>
<td>-4.8</td>
<td>-14.6 - -5.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cases (n=24)</td>
<td>93.8</td>
<td>84.5</td>
<td>-9.3</td>
<td>±16.2</td>
<td>-2.8</td>
<td>-16.1 - -2.5</td>
<td>0.010*</td>
</tr>
<tr>
<td>Controls (n=35)</td>
<td>93.7</td>
<td>82.7</td>
<td>-10.9</td>
<td>±16.9</td>
<td>-3.8</td>
<td>-15.7 - -5.2</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Statistically significant result
24.3 Regression Analysis

There was no independent association between Vitamin D and most measures of RBANS cognitive function. However, multivariate linear regression models showed a positive association between Vitamin D and the RBANS cognitive domain of attention (Index IV). Vitamin D deficient participants performed worse than controls at baseline on a range of indices but with supplementation of Vitamin D performance in cases on Index IV improved (though not to the level of controls at either time point) (Table 39).

When we compare the rate of change in RBANS IV to rate of change in Vitamin D level there is a positive association, which means that as Vitamin D level increases in the cases, Index IV performances also improved so Vitamin D increase was associated with improved performance in index IV.

Table 40: Association of 25(OH)D Concentration (<25nmol/L/>75nmol/L) and Change in RBAN

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBANS Total</td>
<td>19.3</td>
<td>-29.6 – 68.2</td>
<td>0.226</td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>-3.2</td>
<td>-13.9 – 7.6</td>
<td>0.559</td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>0.1</td>
<td>-12.1 – 12.2</td>
<td>0.993</td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>-1.6</td>
<td>-15.0 – 11.7</td>
<td>0.806</td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>19.7</td>
<td>10.1 – 29.3</td>
<td>0.003*</td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>1.1</td>
<td>-10.0 – 12.1</td>
<td>0.847</td>
</tr>
</tbody>
</table>

Model: Age 1, Gender, Education, Living Alone1 (Y/N), BMI 1, GSR, Vitamin D Supplementation1 (Y/N), Time to Assessment

Table 41: Association of Change in 25(OH)D Concentration and Change in RBANS

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBANS Total</td>
<td>0.1</td>
<td>-32.5 – 57.9</td>
<td>0.575</td>
</tr>
<tr>
<td>RBANS Index I</td>
<td>-0.04</td>
<td>-0.2 – 0.8</td>
<td>0.480</td>
</tr>
<tr>
<td>RBANS Index II</td>
<td>0.1</td>
<td>-0.1 – 0.2</td>
<td>0.993</td>
</tr>
<tr>
<td>RBANS Index III</td>
<td>-1.0</td>
<td>-0.2 – 0.1</td>
<td>0.499</td>
</tr>
<tr>
<td>RBANS Index IV</td>
<td>0.2</td>
<td>0.04 – 0.3</td>
<td>0.011*</td>
</tr>
<tr>
<td>RBANS Index V</td>
<td>-0.02</td>
<td>-0.1 – 1.0</td>
<td>0.847</td>
</tr>
</tbody>
</table>

Model: Age1, Gender, Education, Living Alone1 (Y/N), BMI, GSR1, Vitamin D Supplementation1 (Y/N)
Chapter 25: Conclusion

25.1 Discussion
In this nested case control study, Vitamin D deficient participants performed worse than controls at baseline on a range of cognitive indices. Vitamin D deficiency was associated with an improved performance in cases at follow-up assessment on Index IV of RBANS (attention domain). Serum Vitamin D levels were not associated with cognitive changes over time in the other four RBANS Indices. Supplementation of deficient levels of Vitamin D between baseline and follow up assessment may relate to this improvement.

The improvement in RBANS Index IV in patients who were initially Vitamin D deficient may have been a chance effect with regression to the mean or may reflect a benefit in cognitive function with repletion of deficient Vitamin D levels over time.

This study is a follow-up on previous cross-sectional work, which showed a positive association between Vitamin D and cognition in the TUDA population in younger older adults in RBANS Index II and subcomponents of Index IV in different cohorts from the study population (McCarroll, 2014).

The findings of a positive association between Vitamin D and the cognitive domain of attention are similar to prior studies in the literature, which have shown an association between Vitamin D and tests of executive function in cross-sectional (Buell et al., 2009, McCarroll, 2014) and longitudinal studies (Assmann et al., 2015, Darwish et al., 2015). However results from studies to date have been inconsistent (Karakis et al., 2016, Slinin et al., 2012), likely due to the differing populations and cognitive assessments used.

The findings from this study support an association between Vitamin D and certain cognitive domains and given Index IV (attention) improved over 5 years, it suggests that the association is ongoing and treating older people who are Vitamin D deficient might be worthwhile.
These findings are important as they may indicate a role for Vitamin D replacement in those older adults with deficient levels in the area of cognitive function. This study adds to this body of evidence supporting an association between Vitamin D and cognition and supports the need for further evaluation with larger randomised control trials.

25.2 Strengths and Limitations

This study has the advantage of detailed neuropsychological assessment, which has not been performed in many of the studies to date. Also there is a long follow-up period in this study, which is needed when trying to establish a real change in cognitive status over time.

Although there is evidence of an association between serum Vitamin D status and improvement in the cognitive domain of attention (RBANS Index IV) at follow-up, further larger studies are required to assess and further replicate this study’s findings.

One of the main limitations of this study is its small sample size, with only 108 (79%) participants completing the second assessment and full data available on only 59 (43%) participants.

Another limitation is the Vitamin D deficiency was treated at baseline (in keeping with ethical principles). This means that cases were not deficient at Time 2, which reduced the power to detect change.
Section 8: Thesis Conclusions
Chapter 26: Discussion

The aim of these studies was to investigate the prospective relationship between Vitamin D and cognition, resource utilisation including Emergency Department (ED) attendance and hospital admissions, and mortality in an Irish population of older community dwelling adults.

We found strong and consistent inverse associations between Vitamin D concentrations and resource utilisation. There was a negative association between Vitamin D levels and ED attendance and hospital admission rates. Participants who were deficient in Vitamin D were more likely to attend the ED and to be admitted than those with higher levels of Vitamin D. Those participants with lower levels who were admitted to hospital also had longer lengths of hospital stay (LOS). These relationships remained robust after confounding for multiple factors predictive of Vitamin D status and frailty and suggest that such associations cannot be attributed to known bone effects or confounding by frailty.

Vitamin D deficiency was associated with increased mortality rates over the 3.6 year study follow-up period. This inverse association once again, remained independent of multiple confounds, particularly markers of physical and cognitive frailty.

Finally, in our last study, we found a positive association between Vitamin D and cognition, in the domain of attention. Supplementation of deficient levels of Vitamin D between baseline and follow up assessments may relate to this improvement. Given attention scores improved over the five-year follow-up, this suggests the association is ongoing and treating older people who are Vitamin D deficient might be worthwhile. A significant strength of this study was the use of a detailed neuropsychological test battery, which can show subtle changes in cognition over time. This has not been used in the majority of studies to date.

Overall our studies show that Vitamin D deficiency appears to be associated with negative outcomes in relation to health, mortality and resource utilisation. Our results suggest that
supplementation of Vitamin D in older adults with deficient levels, may have beneficial effects beyond the known areas of falls and bone disease.

In our studies, when we found an association between Vitamin D and outcomes, the effects were not stronger when considered at different levels of Vitamin D, that is, there was no difference in effects at Vitamin D levels <25nmol/L compared with levels <50nmol/L. It is worth asking whether we should focus on certain Vitamin D concentrations. Our finding is noteworthy when some authors have suggested levels <25nmol/L as indicating deficiency (Pearce and Cheetham, 2010), but our findings would support considering levels <50nmol/L.

In Models 1 and 2 in each study, factors associated with Vitamin D deficiency and also physical and cognitive frailty was included. Model 3 in each study looked at whether Vitamin D might be associated with certain variables. It is worth reflecting that Model 3 results looked if any association can be explained by factors that might plausibly effect Vitamin D such as a history of falling (Dhaliwal and Aloia, 2017), cancer (Jeon and Shin, 2018), hypertension (Legarth et al., 2018), chronic kidney disease (Bellasi et al., 2017), atrial fibrillation (Alonso et al., 2016) and diabetes (Rafiq and Jeppesen, 2018). In our studies the association between Vitamin D and outcomes held even with Model 3 variables, suggesting Vitamin D plausibly affects outcome.

It is possible Vitamin D may be having effects beyond those we have adjusted for and via mechanisms which we do not yet understand. It is also possible Vitamin D is mediating its effects on mortality, ED attendance and hospital admissions over a longer time frame. In all cases, none of the associations were attenuated with any of the Model variables. It is also a possibility Vitamin D levels are correlated with patient frailty (Bruyere et al., 2017) and we may just be documenting that frail people have worse outcomes than their non-frail counterparts, though we went to some length to control for this. This will not be known until RCTs with appropriate sample sizes are completed.
Chapter 27: Implications for Future Research

We can say based on the results of our studies, Vitamin D deficiency appears to have a strong association with multiple negative outcomes, including increased rates of resource utilisation through increased numbers of Emergency Department attendances and hospital admission rates and mortality. Also Vitamin D supplementation appears to be associated with improved cognitive scores in specific sub-domains in those deficient in Vitamin D. Overall, our study findings suggest that optimisation of Vitamin D levels may be beneficial in ameliorating negative outcomes in older adults who are deficient in Vitamin D.

The time has come for large RCTs and there are a number on-going, including the post-intervention study of VITAL (Manson et al., 2018), the Australian D-health Trail (Neale et al., 2016) and the New Zealand ViDA study (Scragg et al., 2015) with results expected over the coming years.

Our study is one of the few studies to date to use a large sample size with a neuropsychological battery and I would suggest that future research should include a similar detailed neuropsychological battery to show subtle changes in cognition over time.

Our findings add to the body of work supporting the effects of Vitamin D beyond bone and support the need for further studies. In particular RCTs, similar to the study design by Avendell et al although this sample size may not have been large enough to show a treatment response (Avenell et al., 2012), with a large sample size and longer follow up periods than traditionally considered. I also recommend that further studies are restricted to participants with significant Vitamin D deficiency, though our research suggests levels <50nmol/L should be sufficient.
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VERLAAN 2017 Sufficient Levels of 25-hydroxyvitamin D and Protein Intake Required to Increase Muscle Mass in sarcopenic Older Adults - The PROVIDE Study. *Clinical Nutrition (in press).*


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Appendix
### The Mini-Mental State Exam

<table>
<thead>
<tr>
<th>Maximum</th>
<th>Score</th>
<th>Orientation</th>
<th>Registration</th>
<th>Attention and Calculation</th>
<th>Recall</th>
<th>Language</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>( )</td>
<td>What is the (year) (season) (date) (day) (month)?</td>
<td>Name 3 objects: 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. Count trials and record. Trials ________</td>
<td>Serial 7's. 1 point for each correct answer. Stop after 5 answers. Alternatively spell “world” backward.</td>
<td>Ask for the 3 objects repeated above. Give 1 point for each correct answer.</td>
<td>2 ( ) Name a pencil and watch. 1 ( ) Repeat the following “No ifs, ands, or buts” 3 ( ) Follow a 3-stage command: “Take a paper in your hand, fold it in half, and put it on the floor.” 1 ( ) Read and obey the following: CLOSE YOUR EYES 1 ( ) Write a sentence. 1 ( ) Copy the design shown.</td>
</tr>
</tbody>
</table>

Total Score

ASSESS level of consciousness along a continuum

Alert  Drowsy  Stupor  Coma

Appendix 2: Repeatable Battery for the Assessment of Neuropsychological Status

UK Adaptation
Record Form A

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Education Level</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Examiner</th>
<th>Date of Testing</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</table>

Observations:

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<th>Immediate Memory</th>
<th>Visuospatial/Constructional</th>
<th>Language</th>
<th>Attention</th>
<th>Delayed Memory</th>
<th>Total Scale</th>
</tr>
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<tbody>
<tr>
<td>Index Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence Interval</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentile</td>
<td>Percentile Rank</td>
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<td></td>
<td></td>
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<tr>
<td></td>
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<td>&gt;99.9</td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>&lt;0.1</td>
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Pearson
### Appendix 3: Lawton Instrumental Activities of Daily Living Scale

<table>
<thead>
<tr>
<th>Description</th>
<th>Value No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. TOILET</strong></td>
<td></td>
</tr>
<tr>
<td>Cares for self at toilet completely, no incontinence</td>
<td>4</td>
</tr>
<tr>
<td>Needs to be reminded, or needs help in cleaning self, or has rare (weekly at most) accidents</td>
<td>3</td>
</tr>
<tr>
<td>Soiling or wetting while asleep, more than once a week</td>
<td>2</td>
</tr>
<tr>
<td>Soiling or wetting while awake, more than once a week</td>
<td>1</td>
</tr>
<tr>
<td>No control of bowels or bladder</td>
<td>0</td>
</tr>
<tr>
<td><strong>2. FEEDING</strong></td>
<td></td>
</tr>
<tr>
<td>Eats without assistance</td>
<td>4</td>
</tr>
<tr>
<td>Eats with minor assistance at meal times, with help preparing food or with help in cleaning up after meals</td>
<td>3</td>
</tr>
<tr>
<td>Feeds self with moderate assistance and is untidy</td>
<td>2</td>
</tr>
<tr>
<td>Requires extensive assistance for all meals</td>
<td>1</td>
</tr>
<tr>
<td>Does not feed self at all and resists efforts of others to feed him</td>
<td>0</td>
</tr>
<tr>
<td><strong>3. DRESSING</strong></td>
<td></td>
</tr>
<tr>
<td>Dresses, undressed and selects clothes from own wardrobe</td>
<td>4</td>
</tr>
<tr>
<td>Dresses and undresses self, with minor assistance</td>
<td>3</td>
</tr>
<tr>
<td>Needs moderate assistance in dressing or selection of clothes</td>
<td>2</td>
</tr>
<tr>
<td>Needs major assistance in dressing but cooperated with efforts of other to help</td>
<td>1</td>
</tr>
<tr>
<td>Completely unable to dress self and resists efforts of others to help</td>
<td>0</td>
</tr>
<tr>
<td><strong>4. GROOMING</strong> (neatness, hair, nails, hands, face, clothing)</td>
<td></td>
</tr>
<tr>
<td>Always neatly dressed and well-groomed, without assistance</td>
<td>4</td>
</tr>
<tr>
<td>Grooms self adequately, with occasional minor assistance, e.g., in shaving</td>
<td>3</td>
</tr>
<tr>
<td>Needs moderate and regular assistance or supervision in grooming</td>
<td>2</td>
</tr>
<tr>
<td>Needs major assistance in dressing but cooperates with efforts of others to help</td>
<td>1</td>
</tr>
<tr>
<td>Actively negates all efforts to others to maintain grooming</td>
<td>0</td>
</tr>
<tr>
<td><strong>5. PHYSICAL AMBULATION</strong></td>
<td></td>
</tr>
<tr>
<td>Goes about grounds or city</td>
<td>4</td>
</tr>
<tr>
<td>Ambulates within residence or about one block distant</td>
<td>3</td>
</tr>
<tr>
<td>Ambulates with assistance of (check one): Yes another person, yes railing, yes cane, yes walker, or yes wheelchair; gets in and out without help; needs help in getting in and out</td>
<td>2</td>
</tr>
<tr>
<td>Sits unsupported in chair or wheelchair, but cannot propel self without help</td>
<td>1</td>
</tr>
<tr>
<td>Bedridden more than half the time</td>
<td>0</td>
</tr>
<tr>
<td><strong>6. BATHING</strong></td>
<td></td>
</tr>
<tr>
<td>Bathes self (tub, shower, sponge bath) without help</td>
<td>4</td>
</tr>
<tr>
<td>Bathes self, with help in getting in and out of tub</td>
<td>3</td>
</tr>
<tr>
<td>Washes face and hands only, but cannot bathe rest of body</td>
<td>2</td>
</tr>
<tr>
<td>Does not wash self but is cooperative with those who bathe him</td>
<td>1</td>
</tr>
<tr>
<td>Does not travel at all</td>
<td>0</td>
</tr>
<tr>
<td><strong>7. RESPONSIBILITY FOR OWN MEDICATION</strong></td>
<td></td>
</tr>
<tr>
<td>Is responsible for taking medication in correct dosages at correct time</td>
<td>2</td>
</tr>
<tr>
<td>Takes responsibility if medication is prepared in advance in separate dosages</td>
<td>1</td>
</tr>
<tr>
<td>Does not try to wash self, and resists efforts to keep him clean</td>
<td>0</td>
</tr>
</tbody>
</table>

**TOTAL SCORE**
Appendix 4: Physical Self Maintenance Scale (PSMS): Observer Rated

PHYSICAL SELF MAINTENANCE SCALE (PSMS)-caregiver

Subject’s Name_________________________Rated by _______________________ Date __________

Circle one statement in each category A-F that best describes the subject’s abilities.

A. Toilet
1. Cares for self at toilet completely, no incontinence
2. Needs to be reminded or needs help cleaning self, or has rare (weekly at most) accidents
3. Soiling or wetting while asleep more than once a week
4. Soiling or wetting while awake more than once a week
5. No control of bowels or bladder

B. Feeding
1. Eats without assistance
2. Eats with minor assistance at meal times and/or with special preparation of food, or help in cleaning up after meals
3. Feeds self with moderate assistance and is untidy
4. Requires extensive assistance for all meals
5. Does not feed self at all and resists efforts of others to help

C. Dressing
1. Dresses, undresses, and selects clothing from own wardrobe
2. Dresses, undresses self with minor assistance
3. Needs moderate assistance in dressing or selection of clothes
4. Needs major assistance in dressing, but cooperates with efforts of others to help
5. Completely unable to dress self and resists efforts of others to help

D. Grooming (facial, hair, nails, hands, face, clothing)
1. Always neatly dressed, well-groomed, without assistance
2. Grooms self adequately with occasional minor assistance (i.e. shaving)
3. Needs moderate and regular assistance or supervision in grooming
4. Needs total grooming care, but can remain well-groomed after help from others
5. Actively resists all efforts of others to maintain grooming

E. Physical Ambulation
1. Goes about grounds or city
2. Ambulates within residence or about one block distance
3. Ambulates with assistance of (circle one)
   i. Another person
   ii. Railings
   iii. Cane
   iv. Walker
   v. Wheelchair
   1. Gets in and out without help
   2. Needs help getting in and out

F. Bathing
1. Bathes self (tub, shower, sponge bath) without help
2. Bathes self with help in getting in and out of tub/shower area
3. Washes face and hands only, but cannot bathe rest of body
4. Does not wash self, but is cooperative with those who bathe him/her
5. Does not try to wash self, and resists efforts to keep him/her clean
### Appendix 5: Hospital Anxiety and Depression Scale (HADS)

#### Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.

Don't take too long over your replies; your immediate is best.

<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I feel tense or 'wound up':</td>
</tr>
<tr>
<td>1</td>
<td>I feel as if I am slowed down:</td>
</tr>
<tr>
<td>2</td>
<td>Nearly all the time</td>
</tr>
<tr>
<td>3</td>
<td>Very often</td>
</tr>
<tr>
<td>4</td>
<td>Occasionally</td>
</tr>
<tr>
<td>5</td>
<td>Sometimes</td>
</tr>
<tr>
<td>6</td>
<td>Not at all</td>
</tr>
<tr>
<td>7</td>
<td>I still enjoy the things I used to enjoy:</td>
</tr>
<tr>
<td>8</td>
<td>I get a sort of frightened feeling like 'butterflies' in the stomach:</td>
</tr>
<tr>
<td>9</td>
<td>Definitively</td>
</tr>
<tr>
<td>10</td>
<td>Not at all</td>
</tr>
<tr>
<td>11</td>
<td>Not quite as much</td>
</tr>
<tr>
<td>12</td>
<td>Occasionally</td>
</tr>
<tr>
<td>13</td>
<td>Quite Often</td>
</tr>
<tr>
<td>14</td>
<td>Very Often</td>
</tr>
<tr>
<td>15</td>
<td>I get a sort of frightened feeling as if something awful is about to happen:</td>
</tr>
<tr>
<td>16</td>
<td>I have lost interest in my appearance:</td>
</tr>
<tr>
<td>17</td>
<td>Very definitely and quite badly</td>
</tr>
<tr>
<td>18</td>
<td>Definitely</td>
</tr>
<tr>
<td>19</td>
<td>Not at all</td>
</tr>
<tr>
<td>20</td>
<td>Yes, but not too badly</td>
</tr>
<tr>
<td>21</td>
<td>Not at all</td>
</tr>
<tr>
<td>22</td>
<td>A little, but it doesn't worry me</td>
</tr>
<tr>
<td>23</td>
<td>I take just as much care as ever</td>
</tr>
<tr>
<td>24</td>
<td>Not at all</td>
</tr>
<tr>
<td>25</td>
<td>I can laugh and see the funny side of things:</td>
</tr>
<tr>
<td>26</td>
<td>I feel restless as I have to be on the move:</td>
</tr>
<tr>
<td>27</td>
<td>As much as I always could</td>
</tr>
<tr>
<td>28</td>
<td>Very much indeed</td>
</tr>
<tr>
<td>29</td>
<td>Not quite so much now</td>
</tr>
<tr>
<td>30</td>
<td>Quite a lot</td>
</tr>
<tr>
<td>31</td>
<td>Definitely not so much now</td>
</tr>
<tr>
<td>32</td>
<td>Not very much</td>
</tr>
<tr>
<td>33</td>
<td>Not at all</td>
</tr>
<tr>
<td>34</td>
<td>Worrying thoughts go through my mind:</td>
</tr>
<tr>
<td>35</td>
<td>I look forward with enjoyment to things:</td>
</tr>
<tr>
<td>36</td>
<td>As much as I ever did</td>
</tr>
<tr>
<td>37</td>
<td>Rather less than I used to</td>
</tr>
<tr>
<td>38</td>
<td>Definitely less than I used to</td>
</tr>
<tr>
<td>39</td>
<td>Hardy at all</td>
</tr>
<tr>
<td>40</td>
<td>I feel cheerful:</td>
</tr>
<tr>
<td>41</td>
<td>I get sudden feelings of panic:</td>
</tr>
<tr>
<td>42</td>
<td>Not at all</td>
</tr>
<tr>
<td>43</td>
<td>Very often indeed</td>
</tr>
<tr>
<td>44</td>
<td>Not often</td>
</tr>
<tr>
<td>45</td>
<td>Quite often</td>
</tr>
<tr>
<td>46</td>
<td>Sometimes</td>
</tr>
<tr>
<td>47</td>
<td>Not very often</td>
</tr>
<tr>
<td>48</td>
<td>Most of the time</td>
</tr>
<tr>
<td>49</td>
<td>I can sit at ease and feel relaxed:</td>
</tr>
<tr>
<td>50</td>
<td>I can enjoy a good book or radio or TV program:</td>
</tr>
<tr>
<td>51</td>
<td>Not at all</td>
</tr>
<tr>
<td>52</td>
<td>Often</td>
</tr>
<tr>
<td>53</td>
<td>Sometimes</td>
</tr>
<tr>
<td>54</td>
<td>Not often</td>
</tr>
<tr>
<td>55</td>
<td>Very seldom</td>
</tr>
</tbody>
</table>

Please check you have answered all the questions.

**Scoring:**

Total score: Depression (D) _________ Anxiety (A) _________

0-7 = Normal

8-10 = Borderline abnormal (borderline case)

11-21 = Abnormal (case)
Appendix 6: Centre for Epidemiologic Studies Depression Scale (CES-D)

Center for Epidemiologic Studies Depression Scale (CES-D)

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

<table>
<thead>
<tr>
<th>During the Past Week</th>
<th>Rarely or none of the time (less than 1 day)</th>
<th>Some or a little of the time (1-2 days)</th>
<th>Occasionally or a moderate amount of time (3-4 days)</th>
<th>Most or all of the time (5-7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I was bothered by things that usually don’t bother me.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>2. I did not feel like eating; my appetite was poor.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>3. I felt that I could not shake off the blues even with help from my family or friends.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>4. I felt I was just as good as other people.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>5. I had trouble keeping my mind on what I was doing.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>6. I felt depressed.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>7. I felt that everything I did was an effort.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>8. I felt hopeful about the future.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>9. I thought my life had been a failure.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>10. I felt fearful.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>11. My sleep was restless.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>12. I was happy.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>13. I talked less than usual.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>14. I felt lonely.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>15. People were unfriendly.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>16. I enjoyed life.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>17. I had crying spells.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>18. I felt sad.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>19. I felt that people dislike me.</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>20. I could not get “going.”</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>