Vitamin D and Ageing: An investigation into the role of vitamin D in cognitive and physical function of community-dwelling older adults

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Declaration

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

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Niamh Aspell

This thesis is dedicated to my dad, Joe Aspell, who embodies successful ageing. Your determination and strength inspires me every day.

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Summary

This thesis investigated the role of vitamin D as a potentially modifiable factor likely to support important aspects of ageing, including cognitive and physical health. Vitamin D deficiency has been associated with adverse health outcomes in older adults including increased risk of cognitive dysfunction and dementia, falls risk, disability and mortality. However, to date results from vitamin D supplementation studies in older adults, who are generally considered healthy, are inconclusive.

This thesis aimed to establish the prevalence and determinants of vitamin D deficiency in community-dwelling older adults, achieved through the analysis of a large population dataset, the English Longitudinal Study of Ageing (ELSA). Furthermore, we sought to investigate the effect of vitamin D_3 supplementation on cognitive and muscle function outcomes in adults aged 60+. This was achieved through a randomised double-blind placebo-controlled pilot study (Clinicaltrials.gov *identifier*: NCT02804841). Design of the RCT was informed in part by conducting a systematic review. The relationship between vitamin D deficiency and measures of physical function was further explored through an epidemiological analysis, in participants of the ELSA study.

A cross-sectional analysis of a large representative population of community-dwelling older adults residing at northerly latitudes revealed that 26.4% were vitamin D deficient (<30nmmol/L), and more than half (57.3%) had serum 25(OH)D concentrations <50nmol/L. Findings showed the prevalence of deficiency remained high even during the summer months when 1 in 6 (16.9%) was defined as vitamin D deficient and 45.2% were vitamin D insufficient. Vitamin D deficiency was most commonly seen in females, aged over 80 years and of non-white ethnicity. Protective factors included vitamin D supplementation, moderate physical activity and residing in the South. Supplement use was low (4.4%), but the protective effects were noted (Chapter 3).

In Chapter 4, a RCT of vitamin D_3 supplementation (2000IU/d given as 4000IU every 2 days) and pre-specified outcomes of global and domain-specific cognitive functions over a 6-month period was conducted. We demonstrated the effectiveness and safety of vitamin D_3 supplementation on serum 25(OH)D levels with a marked improvement in serum concentrations in the vitD_3 group after 6-months and no adverse events were

reported. No effect, however, was seen in tasks of global cognitive function and domain-specific tasks of executive function, attention, memory and visual reasoning. Exploratory analysis indicated a response in memory, attention and visual reasoning outcomes in participants who achieved adequate serum 25(OH)D status on study completion, however, should be interpreted with consideration of the subgroup analysis sample size.

In Chapter 5, pre-specified secondary outcomes for validated measures of physical function, including Timed-Up-and-Go test (TUG), and handgrip strength (HGS) showed no response to vitamin D_3 supplementation after a 6-month period. Exploratory analysis indicated a potential association with the lower-extremity measure of physical performance, TUG. We have also demonstrated in a cross-sectional analysis that vitamin D deficiency is associated with lower-extremity performance, measured by gait speed test in older adults aged over 60 years in the ELSA study. The need to identify factors associated with poor physical performance that may be modifiable targets for intervention is warranted.

In Chapter 6, we aimed to explore lower-extremity physical performance using a comprehensive measure, namely the SPPB. The prevalence of poor physical performance and low muscle strength was 12.7% and 30.6%, respectively. Overall the findings showed that older adults with the lowest serum 25(OH)D levels comprised the greatest proportion of adults with poor physical performance. Being aged 80+, female gender and low serum 25(OH)D levels (<30nmol/L) were identified as statistically significantly associated factors for poor physical performance. Poor muscle strength measured by handgrip strength was also demonstrated.

Future interventions should consider testing a combination of modifiable lifestyle factors, in larger, more diverse cohorts of older adults, using similar comprehensive assessments of muscle function and cognitive performance. It still remains uncertain whether vitamin D is most likely an associated marker of overall health and not a causal factor in disease. Nevertheless, at a minimum, it seems reasonable to aim for the prevention of vitamin D deficiency.

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List of Abbreviations

1,25(OH)2D	1,25 dihydroxyvitamin D
25(OH)D	25, dihydroxyvitamin D
3MS	Modified Mini-Mental State Examination
AD	Alzheimer's disease
ADRDA	Alzheimer's Disease and Related Disorders Association
AMNCH	Adelaide and Meath Hospital Dublin
ANOVA	Analysis of variance
APOE ε4	Apolipoprotein E ε4
AVLT	Auditory Verbal Learning Test
B	Baseline
BDT	Block Design Test
BMI	Body Mass Index
Ca	Calcium supplementation
Ca^{2+}	Calcium ions
CAB	Cognitive Assessment Battery
CAMDEX	Cambridge Mental Disorders of the Elderly Examination
CANTAB	Cambridge Neuropsychological Test Automated Battery
CASP-19	Control, Autonomy, Self-realisation, Pleasure
Cca	Corrected serum calcium
CDR	Cognitive Drug Research
CerebroVaD	Cerebrovascular disease
CESD	Centre for Epidemiological Studies Depression Scale
CI	95% Confidence Interval
CogTel	Cognitive Telephone Screening Instrument
CPRA	Competitive Protein Binding Assay
CSF	Cerebrospinal fluid
DBP	Vitamin D binding protein
DEQAS	Vitamin D External Quality Assessment Scheme
DMST	Digit Symbol Matching Test
DS-B	Digit Span-Backward
DS-F	Digit Span-Forward
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders

DSST	Digit Symbol Substitution Test
DVT	Digit Vigilance Test
EBMT	East Boston Memory Test
ELSA	English Longitudinal Study of Ageing
ES	Endocrine Society
EU	European Union
EWGSOP	European Working Group on Sarcopenia in Older People
FAB	Frontal Assessment Battery
GDNF	Glial-derived Necrosis factor
GDS	Geriatric Depression Scale
GP	General Practitioners
HADS	Hospital Anxiety and Depression Scale
HaPAI	Healthy and Positive Ageing Initiative
HGS	Handgrip strength
HPLC	High Performance Liquid Chromatography
HRB-CRF	Health Research Board-Clinical Research Facility
HRT	Hormone Replacement Therapy
IADL	Independent Activities of Daily Living
IL-6	Interleukin-6
IOM	Institute of Medicine
IPAQ-SF	International Physical Activity Questionnaire-Short Form
ITT	Intention-to-treat
IU	International Units
Kg	Kilogram
LC-MS/MS	Liquid Chromatography-Mass Spectrometry
LNS	Letter Number Sequencing
lwr	Lower-extremity measures
MCI	Mild Cognitive Impairment
MET-minutes	Metabolic minutes
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MO'S	Maria O'Sullivan
MrOS	Osteoporotic Fractures in Men

MRU	Memory Research Unit
MS	Muscle strength
n	Sample size
NA	Niamh Aspell (PhD candidate)
NART	National Adults Reading Test
NGF	Nerve Growth Factor
NHANES	National Health and Nutrition Examination Survey
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
NIST	National Institute of Standards and Technology
NOS	Newcastle-Ottawa Scale
NPAS	The National Positive Ageing Strategy
NR	Not-reported
NT-3	Neurotrophin-3
OR	Odds ratio
PA	Physical activity
PAL	Paired Associates Learning
PF	Physical function
PI	Principal Investigator
P-I	Post-Intervention
PL	Placebo
PP	Physical performance
PRISMA	Preferred Reporting of Items in Systematic Reviews and Meta-Analyses
PRM	Pattern Recognition Memory
Pro.VA	Old Seniors Project
PSS	Perceived Stress Scale
PTH	Parathyroid hormone
Q	Quartiles
RCF	Relative centrifugal force
RCI	Reliable change indicator
RCPM	Ravens Coloured Progressive Matrices
RIA	Radioimmunoassay

RT. var	Reaction Time variability
SACN	Scientific Advisory Committee on Nutrition
SART	Sustained Attention to Response Task
SD	Standard Deviation
SD (RT)	Standard Deviation of the Reaction Time
SDMT	Symbol Digit Modalities Test
SE status	Socioeconomic status
SENECA	Survey in Europe on Nutrition and the Elderly; A Concerted Action
Sig	Statistically significant association
SJH	St. James's Hospital
SMD	Standard mean difference
SPPB	Short Physical Performance Battery
SSS	Stanford Sleepiness Scale
SWM	Spatial Working Memory
T	Treatment
TCIN	Trinity College Institute of Neuroscience
T-Cog	Telephone Cognitive Screen
Ter	Tertiles
TICS	Telephone Interview for Cognitive Status
TILDA	The Irish Longitudinal Study on Ageing
TMT A&B	Trail Making Test Part A and B
TNF-α	Tumour Necrosis Factor-α
TUG	Timed-Up-and-Go
UVB	Ultraviolet B
VDR	Vitamin D Receptor
VR	Visual Reasoning
WHISCA	Women's Health Initiative Study of Cognitive Ageing
WHO	World Health Organisation
WLT	Word Learning test
WMS-DM	Wechsler Memory Scale- Delayed Memory
WMS-LM	Wechsler Memory Scale- Logical Memory
ZENITH	Zinc Effects in Nutrient Interactions and Trends in Health

	and Ageing
$\chi 2$	Chi-Square test

Chapter 1: General Introduction- Part A. A review of vitamin D status in ageing; a role in cognitive and physical function.

1.1. Successful Ageing

As we experience population ageing more of us can expect to reach and enjoy our old age. This demographic shift should be seen as a time of opportunity, however, the factors that impact successful ageing are complex and multifactorial. The Healthy Ireland framework published in 2013 sets out a vision to improve the health and wellbeing of the entire population of Ireland. At the same time, the National Positive Ageing Strategy (NPAS, 2014) was announced [(1)], the strategy envisaged;

"…a society for all ages that celebrates and prepares properly for individual and population ageing. It will enable and support all ages and older people to enjoy physical and mental health and wellbeing to their full potential. It will promote and respect older people's engagement in economic, social, cultural, community and family life, and foster better solidarity between generations."

In response, the Healthy and Positive Ageing Initiative (HaPAI) proposed a four-goal initiative to achieve successful ageing in Ireland [(2)]. With a primary goal to support people to maintain, improve or manage their physical and mental health and wellbeing as they age. To measure healthy ageing, multiple indicators were identified for functional capacity which is linked to a decline in both physical and cognitive function (including walking speed and frailty, mild cognitive impairment and physical activity). Multiple national data sources were analysed to identify the prevalence of these health indicators in adults aged over 50 years.

Key findings from HaPAI estimate;

- Almost half (48%) of people aged 50+ have a slow walking speed, a clinical indicator of frailty
- More than one in three (36%) people aged 50+ show evidence of mild cognitive impairment

These indicators can be used to measure changes in our ageing populations' health and allow for monitoring of progress in this area. In light of their high prevalence, and the growing number of older adults in the population, emphasis on evidenced-based preventative lifestyle strategies are becoming increasingly important in the quest of

achieving old age. Several modifiable lifestyle factors have been identified that may preserve cognitive and physical health into old age. These include good cardiovascular health, physical activity, low alcohol intake, avoid smoking and consuming a healthy diet. Emerging research highlights newer potential dietary and nutritional factors, for example calorie restriction [3] and dietary patterns, such as the Mediterranean diet [4]. In addition, multiple large interventions have investigated isolated supplements, such as omega 3 fatty acids, vitamin B and folic acid [5, 6], with emerging interest in vitamin D.

Successful Ageing [7]				
	Social Engagement	**Maintain Cognitive Function**	**Maintain Physical Function**	Avoid disease and risk for disease
	Optimise and reduce risk factors over the life course ↙ ↘			
	PROTECTIVE	RISK	PROTECTIVE	RISK
Birth		- APOE ε4 allele - SES factors	Physical stimulation-	- Malnutrition - Genetic factors
Early Life	Mental stimulation -	-Low education	Exercise- Balanced diet-	-Smoking
Mid life	Mental & Social stimulation-	-Hearing Loss -Hypertension -Obesity -Occupational Hazards	Exercise- Balanced diet-	-HRT -Smoking -Metabolic disease
Late Life	Moderate alcohol- Diet (Mediterranean)- Exercise-	-Social Isolation -Smoking -Vascular disease -Depression	Normal BMI- Exercise-	-Malnutrition -Poor mobility -Sensory loss
	POTENTIAL COGNITIVE FACTORS		**POTENTIAL PHYSICAL FACTORS**	
	VitD-supplementation	*-Low25(OH)D status*	*VitD-supplementation*	*-Low25(OH)D status*

Figure 1. 1: Protective and risk factors over the life course for cognitive and physical health.
The Lancet Commission 2017 [8]. Progression of functional limitations in relation to physical activity. A life course approach. European Review 2010 [9].

1.2. Vitamin D status in ageing

1.2.1. Vitamin D physiology

Vitamin D, as highlighted in Figure 1.1, may be a protective, modifiable factor in support of successful cognitive and physical health in ageing. Vitamin D is a precursor of the active form $1,25(OH)_2D$ (calcitriol) and is present in two forms; vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The main source in humans is via action of solar ultraviolet-B (UVB) radiation (270nm-300nm) on skin, converting 7-dehydrocholesterol (provitamin D_3) into pre-vitamin D_3, which is then rapidly converted to vitamin D_3 as illustrated in Figure 1.2. Vitamin D_2 is produced via UVB radiation on plant sources such as mushrooms and yeast. Natural dietary sources of vitamin D_3 are few, and include fish liver oils, oily fish, egg yolks and some fortified foods (e.g. some dairy and breakfast cereals) and supplements. Irrespective of how it is acquired, vitamin D offers limited function until it has been activated, a process which requires two hydroxylation steps [10, 11], as illustrated in Figure 1.2. First in the liver by a number of enzymes but primarily vitamin $D_3$25-hydroxylase (CYP2RA) [12], which converts it to the inactive precursor, 25-hydroxy vitamin D [25(OH)D], the prominent circulating form used to determine vitamin D status in humans. The second hydroxylation step occurs in the tubular cells of the kidney, by action of the enzyme 25vitamin D_3-1α-hydroxylase (CYP27B1), to the biologically active metabolite, 1, 25-dihydroxy vitamin D_3 [$1,25(OH)_2D_3$]. This active metabolite is an important modulator of calcium and phosphate homeostasis, importantly, however, both the activating enzyme CYP27B1 and the active form of vitamin D, $1,25(OH)_2D_3$, are present in many non-renal tissues including the human brain, immune cells, cardiovascular systems and pancreatic islets [13]. Due to its ability to be synthesised endogenously, vitamin D is widely accepted not as a classical vitamin but as a steroid hormone, furthermore, its synthesis in the human brain has led to it being commonly referred to as a neurosteroid [14].

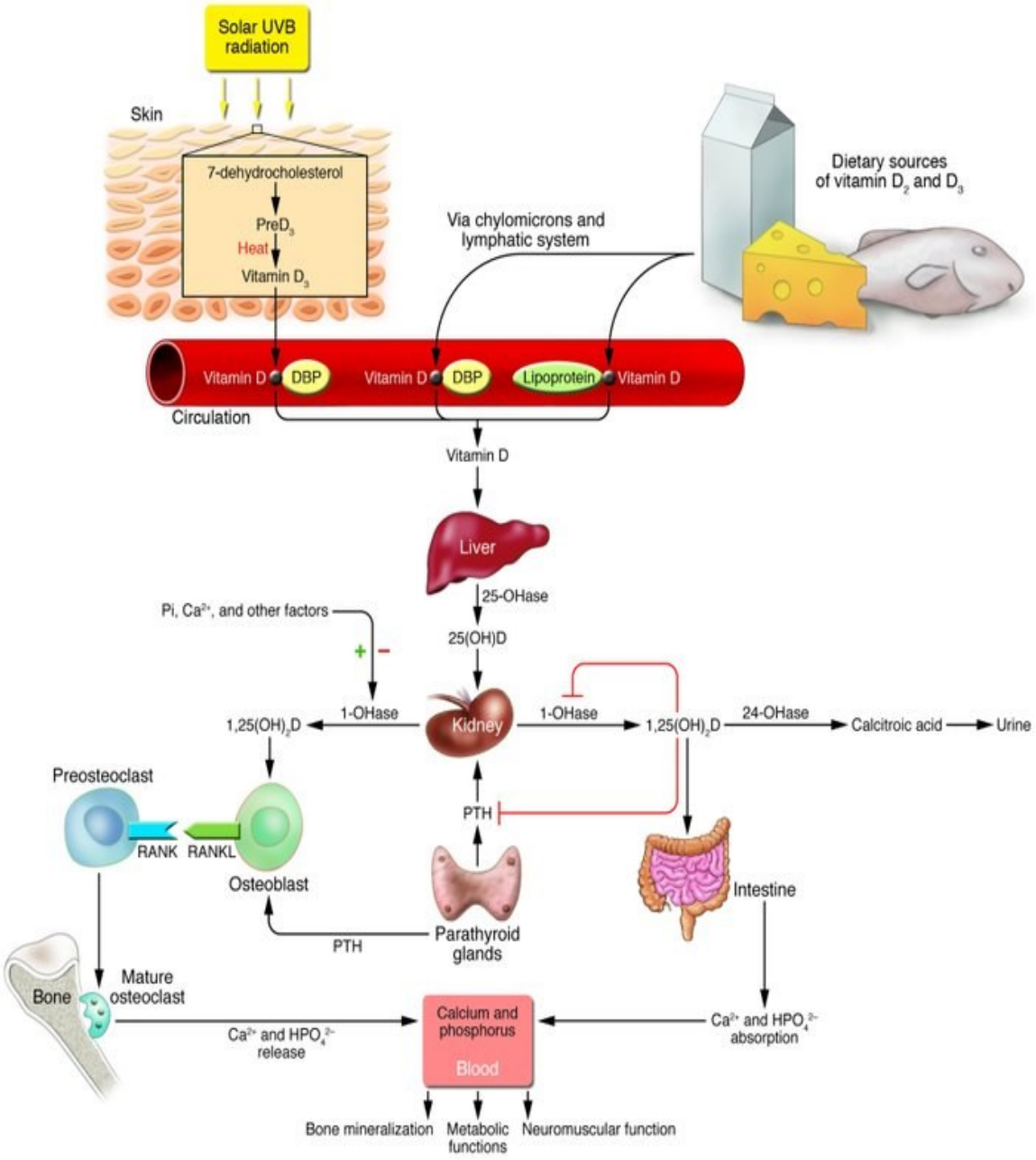


Figure 1. 2: Vitamin D metabolism and activation to 1, 25(OH)2D (15)

1.2.2. Factors influencing vitamin D status in ageing

Several factors influence vitamin D status including skin pigmentation, use of sunscreen or concealing clothing, season, latitude, being older or institutionalised, obesity, malabsorption, renal and liver disease and medication use. In general, UVB radiation on skin is the main source of vitamin D for humans. In countries north of the equator (40-60°N), vitamin D UVB doses are inadequate for 6 months of the year (October-March) [(16)]. Many other environmental factors impact on vitamin D UVB dose

availability including the ozone layer, cloud cover, air pollution, surface reflection and altitude [17]. With advancing age skin integrity decreases; skin thinness and a reduction in transdermal cholesterol reduce the efficiency of UVB vitamin D production, as much as 50% in older adults compared to younger adults [18]. Behavioural and social changes seen with advancing age further limit cutaneous vitamin D production. Less time spent outdoors due to ill health or limited mobility (institutionalised or homebound), medication use (loop diuretics, statins, glucocorticoids), changes in body composition (increase in fat and decrease in muscle), sun avoidance (melanoma risk), reduced skin exposure (clothing and colder temperatures) are all significant barriers that contribute to inadequate UVB vitamin D production in older adults [19-23].

The UK Scientific Advisory Committee on Nutrition (SACN) recommends an intake of 400IU/d (10ug/d) of vitamin D for older adults [24], a target difficult to achieve by dietary contribution alone unless oily fish is eaten daily. Other advisory bodies in the UK and Ireland, recommend that during the winter months and for those at risk of deficiency, vitamin D supplementation should be considered [25, 24]. Evidence suggests that vitamin D supplement uptake is typically low in the population, including older adults [26]. For the most part, dietary sources are limited and not generally consumed in adequate amounts by older adults [27, 26], with an exception for those residing in countries where vitamin D food fortification is in place (United States, Canada, Sweden, and Finland). The effects of age on intestinal absorption and decreased renal function may hinder vitamin D metabolism and availability [28, 29]. It has been hypothesised that metabolism of vitamin D may be reduced due to a decline of intestinal Vitamin D Receptor (VDR) distribution in older adults, however, few studies have been conducted in humans, with small samples and conflicting findings [30, 31].

1.2.3. Vitamin D deficiency and ageing

Serum 25(OH)D is accepted as the most accurate measure of vitamin D status, however, there is less agreement on how to define deficiency in the general population or specifically among older adults, as shown in Figure 1.3. The Institute of Medicine [32] advocate serum 25(OH)D concentrations ≥50nmol/L for bone health, and consider levels <30nmol/L to be deficient. The term 'vitamin D insufficiency' is used to describe serum levels of ≥30-50nmol/L [32]. In contrast, the Endocrine Society recommends

serum 25(OH)D levels ≥75nmol/L to maximise an effect on bone and muscle metabolism [33]. Suboptimal levels of vitamin D are much more common than clinical toxicity, which is rare [34].

Reported vitamin D deficiency rates in published studies vary widely, with considerable methodological differences including the definition of serum 25(OH)D status, sample size, population, gender-specific cohorts, latitude, season and mode of vitamin D assay. Prevalence estimates for vitamin D deficiency in community-dwelling older adults across the European Union (EU) are summarised in Table 1.1, along with the specific 25(OH)D cut-off criteria applied to define deficiency. As detailed in Table 1.1, deficiency ranged from 0-100%. Overall, however, it is apparent that suboptimal vitamin D status is widespread in older adults, irrespective of definition applied. For example, using IOM criteria, The Irish Longitudinal Study on Ageing (TILDA) shows the prevalence of deficiency to be 13.1% of the older adult population [35]. Other prevalence rates reported in Ireland in community-dwelling older adults almost double in cohorts characterised by disease. For example, those defined as cognitively impaired at 43.6% compared to hypertensive 27.3% and osteoporotic 13.8% cohorts [36]. In a recent study of Irish adults, a 10% higher prevalence of vitamin D deficiency was reported in adults aged 65 years and older compared to middle-aged adults, at 44.0% and 35.7% respectively [26].

DEFICIENT	INSUFFICIENT	SUFFICIENT	
Institute of Medicine			
<30	≥30<50	≥50	
Endocrine Society			
<50		≥50-72.5	≥72.5
Food Safety Authority of Ireland			
<30	≥30 <50	≥50	
Irish Hospital Pathology Lab			
<25	≥30 <50	≥80	
International Osteoporosis Federation			
<25	≥30 <50	≥75	

mmol/L

Figure 1. 3: Current national and international serum 25(OH)D status defining criteria

Table 1. 1: Prevalence of serum 25(OH)D deficiency in community-dwelling adults, aged over 50 years

nmol/L	*Lead Author*	*Year*	*Location*	*Cohort*	*N*	*% D-def*
<10	Cooper[34]	1989	UK	Hip fracture patients, females only	41	15
	Vir[35]	1978	UK	Outpatients	37	42
<15	Solanki[36]	1995	UK	Asian (n=37) White (n=19)	56	57 / 6
<20	Mavroeidi[37]	2010	UK	Females only, North UK white, South UK Asian	374	25 / 58·1
	Lips*[38]	2006	EU	Combined countries, females only	1020	0-3·1
	Lips[39]	1987	NL	Community-dwelling controls	74	16
<25	McCarroll[33]	2015	IRL	Disease defined cohorts; CVD, Osteo, MCI	4444	17·2, 8·6,32·8
	Hirani[40]	2010	UK	Health Survey Study (2005)	2070	13 (f) 8 (m)
	Forouhi[41]	2008	UK	MRC Ely Study (1990-2000)	524	5·5
	Hill[42]	2006	IRL	Community-dwelling, females only, late winter	95	7
	Hill[43]	2005	IRL	Community-dwelling, females only	59	S:4, W:17-36
	Hirani[44]	2005	UK	Health Survey England (2000)	1217	15 (f) 9·6 (m)
	Anderson[46]	2005	EU	OPTIFORD Study, females only	221	10, 25
	Woitge[47]	1998	DE	EVOS Study Population sample	580	24
<30	McCarroll[33]	2015	IRL	Disease defined cohorts; CVD, Osteo, MCI	4444	27·3, 13·8, 43·6
	Anderson[46]	2005	EU	OPTIFORD Study, females only	221	55-92
	Boonan[48]	1997	BEL	Males only, outpatients fracture clinic	40	18
	van der Wielan[49]	1995	EU	SENECA Study, wintertime blood samples	824	47 (f) 36 (m)
	Chapuy[50]	1997	FR	20 French cities (43°-51°N)	1569	14

	Chapuy[50]	1996	FR	EPIDOS Study, females only	440	39
<40	McDonald[51]	2008	SCT	APOSS Study, females only	3113	T:31·0,S:24·8,W:34·1
	McCarthy [52]	2006	IRL	Community-dwelling, females only	43	S:20, W:37·2
	Lips*[38]	2006	EU	Combined countries, females only	1020	3.3-28·6
<50	Cashman [24]	2012	IRL	NANS Age 51-64 (n=80), Age 65-84 (n=63)	143	35·7-44·0
	Brouwer-Brolsma[53]	2016	DNK	B-PROOF Study	2544	45
	McCarroll[33]	2015	IRL	Disease defined cohorts; CVD, Osteo, MCI	4444	66·0, 43·4, 75·0
	Forouhi[41]	2008	UK	MRC Ely Study	524	34·4
	Lips*[38]	2006	EU	Combined countries, females only	1020	12·7-40·8
	Hirani[44]	2005	UK	Health Survey England (2000)	1217	62·2(f) 50 (m)
	Hill[43]	2005	IRL	Community-dwelling, females only	59	S:17, W:53
<75	McDonald[51]	2008	SCT	APOSS Study, females only	3113	T:77.0,S:75.4,W:75·4
	Forouhi[41]	2008	UK	MRC Ely Study	524	76·9
	Lips*[38]	2006	EU	Combined countries, females only	1020	37·3-74·5
	Hirani[44]	2005	UK	Health Survey England (2000)	1217	86·6 (f) 79·5 (m)
<80	Hill 2005[43]	2005	IRL	Community-dwelling, females only	59	S:63, W:85
	Mavroeidi[37]	2010	UK	Females only, North UK white, South UK Asian	374	80·7-100

T, Total; S, Summer; W, Winter; m, male; f, female.

*Deficiency defined <22·5nmol/L

**Deficiency defined <37nmol/L

1.2.4. Vitamin D supplementation and toxicity

Both vitamin D_2 and D_3 are available in synthetic preparations, obtained "over the counter" or medically prescribed (most commonly for the treatment of osteoporosis), as a dietary supplement. In the absence of adequate UVB exposure government advisory committees recommend the use of vitamin D dietary supplements [24], despite the increase in public health initiatives, findings from population cohorts indicate uptake is low in older populations [37, 26, 38].

Intoxication of vitamin D presents clinically, as hypercalcaemia, normal or high serum phosphorous levels, normal or low alkaline phosphatase, high serum 25(OH)D concentrations, low PTH and high urine calcium/creatinine [39]. Physical side effects occur as a consequence of hypercalcaemia and include nausea, poor appetite, constant thirst and oliguria. Toxicity is believed to occur at serum 25(OH)D levels >250nmol/L, however, toxicity may not present until levels reach >750nmol/L [40]. Sun acquired levels in countries with high UVB exposure report normal concentrations of 100-200nmol/L, while 25(OH)D toxicity has not been reported from extended periods of UVB exposure. Although no studies have been conducted to test vitamin D intoxication in humans' circumstantial evidence is available [41]. An upper tolerable limit of 4000IU/d is in place. Some researchers support higher doses, Veith et al. advocates' levels up to 10000IU/d as safe [42]. Current guidelines appreciate that daily supplementation of 2000IU/d is generally acceptable for all adults, without medical supervision [43].

1.3. Vitamin D and cognition in ageing

1.3.1. Dementia – associated risk factors

The global incidence of dementia is increasing at a rate of one new case every 3 seconds, with associated medical, social and healthcare costs far exceeding the capacity of most countries [44, 45] . Dementia is a syndrome, usually progressive and chronic in nature, in which there is deterioration in cognitive function beyond what might be expected from normal ageing [46]. Due to the degenerative nature of the disease, sufferers lose their ability to perform routine tasks, experience poor quality of life and loss of autonomy. The drastic effects of dementia are also experienced by family members, who serve as primary caregivers. In 2017, Public Health England reported dementia to be the leading cause of death in older adults in England, overtaking cardiovascular disease, stroke and lung cancer for the first time. Similar trends have been expressed globally [47] which are largely driven by our increasing older adult population, longer life expectancies and improved diagnostic procedures [48, 49]. In the absence of curative treatments the focus on reducing the risk of developing dementia and delaying the onset is now a key priority for all public health authorities and governments [50].

Dementia risk increases with advancing age, family history, and genetic factors, for example carriers of apolipoprotein E ε4 (APOE ε4) genotype. Though several modifiable lifestyle risk factors have been identified that may preserve cognitive health including cardiovascular disease, diabetes, smoking and obesity [51], as illustrated in Figure 1.1. Indeed modifiable factors may promote resilience in a genetic risk factor, such as APOE ε4 gene carriers [52, 53].

1.3.2. Vitamin D and cognitive decline- mechanisms behind the link

There is a large body of evidence showing the link between vitamin D and cognition in ageing, derived from animal models and in vitro studies. Several author have contributed and reviewed this field including work by Eyles and Groves [54, 55]. The key mechanisms are outlined below and summarised in Figure 1.4. It is hypothesised that vitamin D exerts its effects via genomic and non-genomic pathways [56-58]. Exact mechanisms are unclear but evidence suggests vitamin D may protect against cognitive dysfunction through effects on neuroprotection, neurotransmission, synaptic plasticity,

immune modulation, neuronal calcium regulation, and enhanced nerve conduction [59-61]. With secondary protective effects on vascular systems and modulation of vascular risk factors [62].

1.3.2.1. Vitamin D metabolism and the central nervous system (CNS)

The discovery of 1α-hydroxylase and metabolic pathways for vitamin D in the human brain and cerebrospinal fluid (CSF) [63], support a localised anabolic and catabolic pathway for vitamin D in the CNS. The three enzymes necessary for complete synthesis and catabolism of active vitamin D has also been expressed [64, 65]. Serum 25(OH)D has also been evidenced to cross the blood-brain barrier, a characteristic shared by other neurosteroids [66].

1.3.2.2. Vitamin D receptor and the central nervous system

The nuclear functions of $1,25(OH)_2D_3$ are mediated through the VDR, a member of the nuclear receptor family identified in over 2700 genomic sites [67, 58]. Experimental scientists have demonstrated the extensive mapping of the VDR in the rat brain [68-71], showing a similar distribution in human brain tissues [63], with the earliest evidence in post-mortem brains of patients with Alzheimer's disease (AD) and Huntington's disease [63, 72]. The identification of the VDR distribution in neuronal and glial cells in the human brain followed thereafter, supporting a functional role of active vitamin D in the human brain [63]. Areas identified in both animal and human brain tissue show similar patterns [70], in peripheral neurons [73], and in several cell types of the CNS [74, 69, 71]. The action of $1,25(OH)_2D_3$ upon binding to the VDR has been linked with a diverse range of biological systems such as immune modulation, cell growth, and cell differentiation all of which impact brain function via interaction with target genes [75, 76].

1.3.2.3. Vitamin D and neuroprotection

Animal and cell culture evidence suggest that vitamin D may protect the structure and integrity of neurons through detoxification pathways and neurotrophin synthesis [77]. Vitamin D is known to regulate the expression of three of the four neurotrophins, namely; Nerve Growth Factor (NGF), Glial-Derived Nerve Factor (GDNF) and Neurotrophin 3 (NT-3) [78]. Treatment of vitamin D deficiency in adult rats resulted in increased GDNF expression and immunoreactivity after dopamine toxicity damage [79]. Furthermore, vitamin D_3 may attenuate neurotoxicity and administration of calcitriol has

been shown to increase levels of antioxidants, such as glutathione in the rat brain which protects oligodendrocytes and the integrity of nerve conduction pathways critical to mental processing [80]. Whilst in-vitro experiments show vitamin D treatment inhibits production of tumour necrosis factor- α (TNF-α) and interleukin 6 (IL-6) in microglial cell lines, suggesting an anti-inflammatory role [81].

1.3.2.4. Vitamin D and regulation of intraneuronal calcium

Elevated levels of calcium in the brain leads to neurotoxicity. Three calcium binding proteins have been shown to be modulated by vitamin D in brain tissues, calbindin, parvalbumin and calretinin [82]. All three are widely and uniquely distributed in the adult brain and are believed to serve a neuroprotective role as calcium buffers [83], as well as being involved in critical brain functions. In vitamin D deficient mice certain calcium channels are shown to be upregulated leading to an increase in calcium ions (Ca2+) [84] and in-vitro evidence has shown that vitamin D can down-regulate calcium channels [85]. The empirical work of Stumpf et al. and Eyles et al. [86, 63, 70] demonstrates the presence and localisation of vitamin D metabolites, related enzymes and the VDR in the brain, it has prompted the idea that vitamin D acts on neurosteroids in specific brain regions, particularly those related to learning and memory. Whilst mechanistic evidence suggests a potential target for vitamin D effects in the brain, evidence from human studies is lacking.

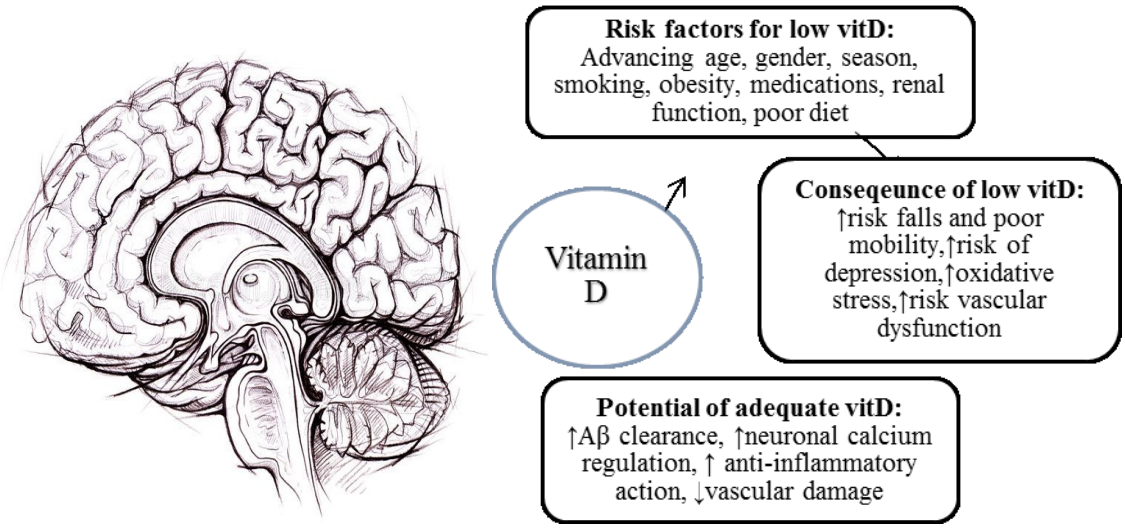


Figure 1. 4: Potential direct and indirect role of 25(OH)D in the central nervous system

1.3.3. Epidemiological evidence; low vitamin D status and cognitive performance in healthy older adults

In the decade succeeding the discovery of the VDR and 1-α-hydroxylase in the human brain, a plethora of evidence supporting a relationship between serum 25(OH)D and cognitive function has been presented. Data from healthy older adults without known cognitive impairment provides useful evidence in terms of evaluating the contribution of vitamin D in normal, non-pathological ageing, however, this information is less plentiful.

To date, the majority of cross-sectional studies support an association between hypovitaminosis D and poorer cognitive performance. Yet many use only brief measures of cognitive function or adjust for few or limiting confounding factors. A recent cross-sectional study demonstrated that older adults with low 25(OH)D levels (<30nmol/L) performed significantly worse than those with levels >75nmol/L, in tasks of processing speed and mental flexibility [87]. This evidence was drawn from a well-designed large national study of ageing conducted in Amsterdam, which employed an in-depth cognitive assessment and gathered detailed demographic and lifestyle information. In contrast, the largest cross-sectional study conducted to date found no association between serum 25(OH)D status and delayed memory [88], which comprised of 4831 participants of the National Health and Nutrition Examination Survey (NHANES III) [88]. The result may be attributed to the single domain-specific outcome measure used to assess cognitive function. Three large population cohorts have since demonstrated a significant relationship between low 25(OH)D levels (typically <30nmol/L) and poorer cognitive performance, using comprehensive global and domain-specific outcomes, compared to sufficient serum 25(OH)D levels (typically ≥75nmol/L) [89, 87, 90]. Other studies have demonstrated a relationship also, however, methodological issues were noted, with small sample sizes, limited analysis of confounding factors and single measures of cognitive performance [91-94]. Cross-sectional studies are considered to provide weak evidence, due to the known issue of reverse causality, in that poorer cognitive performance and the onset of dementia may influence vitamin D concentrations through behavioural and dietary changes.

Longitudinal studies suggest that vitamin D deficiency is associated with an increased risk of cognitive impairment and incidence of dementia and AD [95]. One such study conducted with 10186 older adults followed up over 30 years, revealed that those with serum 25(OH)D levels <25nmol/L had a greater combined risk of incidence dementia or vascular dementia than those with levels ≥50nmol/L [96]. Another prospective study revealed that those with measured serum 25(OH)D concentrations at baseline, and at 3 and 6 year follow-up performed worse in global function and executive functioning but not in tasks of attention [97]. Whilst most studies demonstrate a link between serum 25(OH)D deficiency and poorer cognitive performance or incidence of dementia using a measure of global or domain-specific cognitive function [98], it is not clear if vitamin D deficiency is a risk factor for cognitive impairments or a result of poor overall status and ill health [99].

Systematic reviews have attempted to analyse the current vitamin D evidence, however, no review has aimed to combine data exclusively of older adults, without cognitive impairment at baseline, as a way of exploring preventative strategies which may support successful ageing. This is addressed in Part B of this Introduction chapter.

1.3.4. Intervention evidence; the effects of vitamin D supplementation on cognitive performance

There are limited data from intervention studies. To date, 3 studies have been published reporting the effect of vitamin D supplementation on cognitive performance in adults. Overall 1 of the 3 studies reported a significant positive effect on cognitive measures, detailed in Table 1.2. The first prospective intervention, supplementing 4000IU of vitamin D_3 reported no significant improvement in domain-specific tasks of executive function, attention or memory in 82 community-dwelling adults [100]. Of note, was the heterogeneity between participants, as only 30% of this small sample were aged over 60 years, potentially masking an effect in the older participants.

Annweiler et al., however, reported a positive finding in a small retrospective study of 44 older adults [101]. Participants receiving on average 3333IU/d of vitamin D_3 performed better in one of two measures of global cognitive function (Cognitive Assessment Battery). Vitamin D supplemented participants also performed statistically significantly better in the Frontal Assessment Battery, a diagnostic cognitive screen

most frequently used to differentiate between dementias. Of note, participants were recruited through an outpatient memory clinic, however, were defined as free of cognitive impairment. Incomplete 25(OH)D data was available for all participants, so determining optimal levels for cognitive performance are unobtainable from this investigation.

In the largest supplementation study conducted to date in 4123 community-dwelling females across the United States, post-hoc analysis showed no effect for vitamin D_3 supplementation (400IU/d) and incidence of dementia or cognitive performance at follow-up (7.8 years) [102]. Whilst an in-depth cognitive assessment was used the study design was weak. In this female only cohort, participants were open to consuming their own vitamin D supplements during the study period, irrespective of treatment allocation. As well as this, serum 25(OH)D concentrations were not measured at the end of the study and the author reported poor study compliance. This left further ambiguity in terms of supplementation effects by providing no evidence for optimal serum 25(OH)D levels and cognitive function.

Overall evidence from intervention studies is in its early stages, whilst published studies to date are inconclusive this may be attributable to methodological weaknesses. Future interventions should be designed and implemented appropriately. RCTs which test the effects of vitamin D supplementation, in older adults free of cognitive impairment, employing in-depth cognitive assessment batteries may clarify a potential role for vitamin D in cognition.

Table 1. 2: Supplementation studies; effect of vitD supplementation and cognitive performance outcomes in community-dwelling older adults

Author	Study design	Intervention	Cohort	Outcome	B:25(OH)D (nmol/L)	PI: 25(OH)D (nmol/L)	Results (adjusted)	Effect
Peterson 2017 [100]	Randomised double-blind low dose placebo-controlled trial Duration: 18 weeks	T:4000IU/d D_3 PL:400IU/d D_3	Healthy adults Aged 20years + n=82*	SDMT DS-F, DS-B CANTAB PRM PAL	T: 67·2 PL:60·5	T:130·6 PL:85·9	No significant difference between groups at 18 weeks. Moderate effects seen for: PRM, p=0.11 (d=0.4), PAL, p=0.06 (d=0.4)	No
Annweiler 2012[103]	Supplementation Retrospective Duration: 16 months	T: 800IU/d or 100,000IU /month D_3	Memory Clinic Age 80·6yrs n=44	MMSE CAB FAB	T:42·0 PL:63·0	T: 75·0 PL:48·0	MMSE OR 3.8 (1.02-14.2), p=0.05 CAB OR 16.5, (2.51-108.6), p=0.004 FAB OR 8.0, (1.52-42.0), p=0.01	Yes
Rossom 2012 [102]	Supplementation Post-hoc analysis RCT Duration: 7.8years	T:400IU/d D_3 (+Ca 1000mg)	Healthy females Aged ≥65 years WHISCA Study n=4123	DSM-IV WHISCA cognitive battery	T:49·9 PL:19·2	Not obtained	No significant difference in incidence of probable dementia or MCI, and cognitive performance between groups.	No

B, baseline; PI, post-intervention; T, treatment; PL, placebo; SDMT, Symbol Digit Modalities Test; DS-F, Digit Span Forward; DS-B, Digit Span Backward; CANTAB, Cambridge Neuropsychological Test Automated Battery; PRM, Pattern Recognition Memory; PAL, Paired Associates Learning; MMSE, Mini-Mental State Examination; CAB, Cognitive Assessment Battery; FAB, Frontal Assessment Battery; OR, odds ratio; WHISCA, Women's Health Initiative Study of Cognitive Aging; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; MCI, Mild Cognitive Impairment.

*not entirely comprised of older adults.

1.4. Vitamin D and Muscle

1.4.1. Sarcopenia

With advancing age, even in the absence of disease, all physiological systems decline in both capacity and function [104]. The rate of decline is dependent on many factors including genetics, environment and lifestyle [105]. Asides from age-related declines in cognitive function adults will experience a reduction in overall muscle mass and strength with advancing age. This gradual decrease in lean muscle mass (0.5-1% per year), prominently of the lower extremities, begins in mid-life [106]. When the loss of muscle mass drops below a defined threshold (2 standard deviations below that of young adults), it is referred to as sarcopenia [107]. Age-related sarcopenia results in functional impairment, physical disability, and increased risk of falls and fractures [108]. Therefore, the preservation and potential reversibility of lost muscle mass and function are of major importance in terms of active and successful ageing. Sarcopenia is defined by the European Working Group on Sarcopenia in Older People (EWGSOP) as the loss of muscle mass and function (which is characterised by poor physical performance or low muscle strength) with advancing age, which is associated with disability and all-cause mortality [109]. The prevalence of sarcopenia is estimated to be 5-13% in older adults, increasing to 11-50% in the oldest old [110].

In order to directly measure a reduction in muscle mass, specialised whole or partial-body (mid-thigh) imaging by MRI or dual-X-ray absorptiometry would be required [111, 112]. Whilst these techniques are the gold standard, their use in routine practice or population research in older adults is not often feasible. EWGSOP recommends a number of methods to estimate muscle strength and performance including knee flexion/extension, handgrip strength, and the Short Physical Performance Battery (SPPB), Timed-Up-and-Go test (TUG) or usual gait speed for physical performance. Furthermore, EWGSOP considers anthropometric measures unreliable and not recommended in the diagnosis of sarcopenia.

1.4.2. Vitamin D and muscle function- mechanisms behind the link

With our growing older adult population, lifestyle factors that may prevent loss or improve muscle mass and strength have been tested, with strong evidence for exercise, especially resistance type training and dietary factors such as protein intake and omega 3 fatty acids [113, 5, 114]. There is strong evidence that vitamin D exerts additional actions on other 'non-classical' systems, such as skeletal muscle. Whilst adequate vitamin D status facilitates calcification of bone and supports mineral homeostasis, it is postulated that the link between vitamin D and fracture risk is mediated by enhanced skeletal muscle function and reduced incidence of falls [115].

1.4.2.1. Direct and indirect effects of vitamin D and muscle

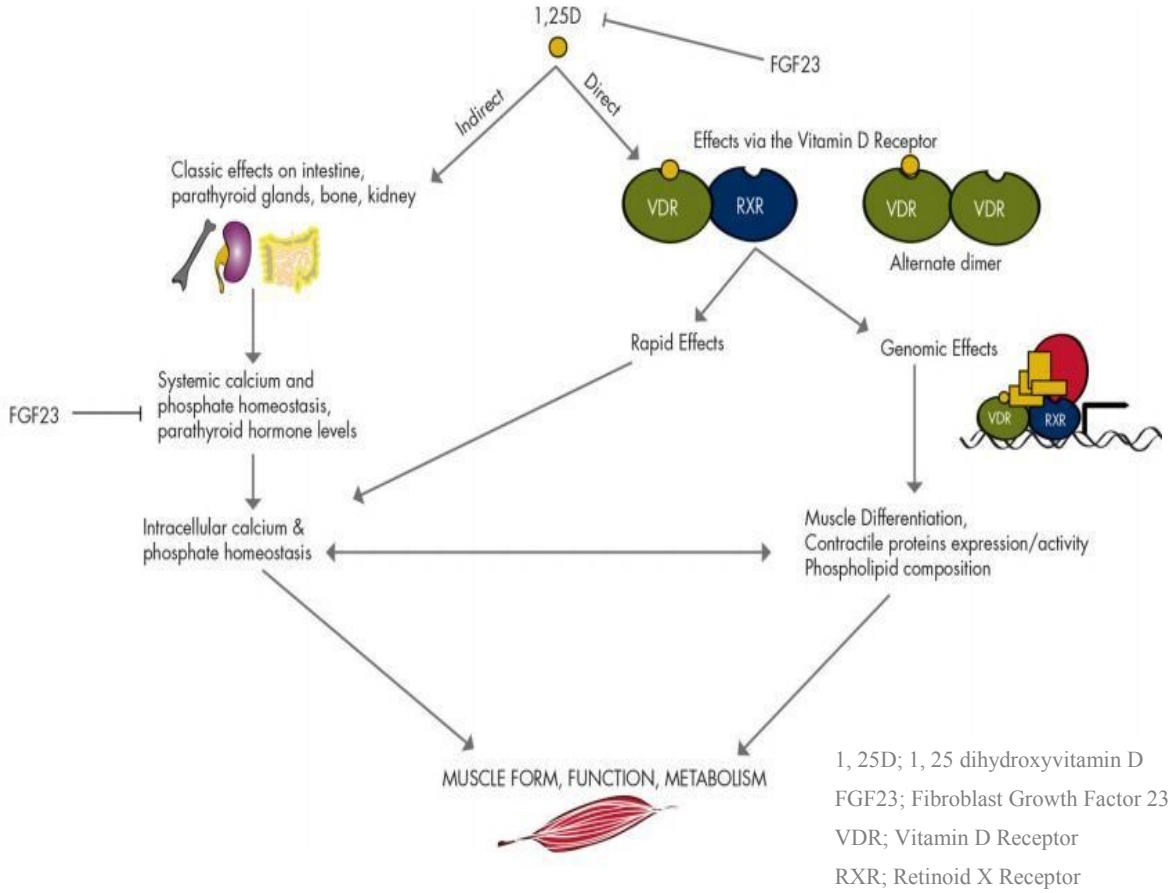
A number of mechanisms by which vitamin D impacts upon skeletal muscle function have been elucidated [116, 117]. As illustrated in Figure 1.5, these effects can be somewhat divided by direct (genomic) and indirect (non-genomic) actions.

It is generally accepted that the VDR is present in skeletal muscle [118], with experimental models demonstrating the presence of the VDR on animal and muscle cells. It is hypothesised direct effects occur upon binding $1,25(OH)_2D$ to the VDR resulting in gene transcription of a range of proteins, including those associated with calcium and insulin-like-growth hormones [119]. Differences in experimental techniques have yielded conflicting results [13], however, it is generally accepted that the VDR is present on skeletal muscle. The quantity of VDRs in muscle tissue appears to reduce with advancing age [120]. With evidence in older adults indicating treatment of vitamin D deficiency with supplementation of 4000IU/d of vitamin D_3 significantly increases the number of VDRs in type II muscle fibres by 30% [121]. In the same study, a 10% increase in cross-sectional muscle fibres (mid-thigh) was noted in response to vitamin D treatment.

Much of the research to date has focused on the indirect effects of vitamin D and muscle. While the exact mechanism of the non-genomic action of vitamin D continues to be debated, it is widely accepted that vitamin D levels have an effect on calcium channels of muscle cells in both animal and human tissues [119, 122, 123]. As calcium is an

important modulator of skeletal muscle function [124], it is proposed that vitamin D levels may have a significant impact on muscle function, performance and injury risk.

Vitamin D has been found to influence muscle metabolism [125, 126], low vitamin D levels result in increased PTH, which has been shown to enhance muscle protein breakdown [127]. Treatment with vitamin D supplementation has been evidenced to improve the electrical activity of skeletal muscle in serum 25(OH)D deficient older adults with osteomalacia, as far back as the 1970s [128]. The mechanism of action could be the effect 25(OH)D exerts on fast-twitch, type II muscle fibres [129], which are predominantly recruited in falls and implicated in osteomalacia [130, 131].



1, 25D; 1, 25 dihydroxyvitamin D
FGF23; Fibroblast Growth Factor 23
VDR; Vitamin D Receptor
RXR; Retinoid X Receptor

Figure 1. 5: Direct and indirect effects of vitamin D on muscle [117]

1.4.3. Epidemiological evidence; the association between vitamin D status and muscle function in ageing

Many studies have shown prolonged vitamin D deficiency is associated with muscle weakness and resulting disability [132], however, less evidence is available using direct measures of muscle strength and physical performance. Many correlations have been evidenced in older adult populations, however, no causal effect can be alluded.

Vitamin D has been associated with muscle weakness and pain, resulting in a loss of strength, balance and function [133]. The evidence shows that higher 25(OH)D levels are associated with better proximal function in older adults, irrespective of physical activity levels [134]. A number of observational studies have reported associations between vitamin D deficiency and poor upper and lower body strength [135], slower physical performance [136], lower body muscle mass [137] and frailty [138] in community-dwelling older adults.

This is exemplified by a recent meta-analysis connecting serum 25(OH)D levels and physical performance (walking speed), which concluded that Timed-Up-and-Go test (TUG) scores were slower (mean difference 0.48 seconds) among participants with serum 25(OH)D concentrations <25nmol/L compared to those with levels ≥75nmol/L. One of the largest observational studies conducted to date, comprising 4100 participants of the NHANES III study, demonstrated that serum 25(OH)D concentrations between 40-90nmol/L were associated with better lower extremity muscle function compared with levels <40nmol/L [136]. Similarly, evidence from the InCHIANTI study reported incremental changes in serum 25(OH)D concentrations were associated with better scores (mean difference of 0.61 points) in an assessment of physical performance, the Short Physical Performance Battery (SPPB) assessment [139]. Strong associations for a link between low serum 25(OH)D concentrations and poor muscle strength (handgrip strength) has also been evidenced when serum 25(OH)D concentrations drop below 25nmol/L [135].

Despite strong epidemiological evidence for vitamin D in physical functioning in community-dwelling older adults, the effects on muscle strength and performance remain inconclusive. As highlighted earlier, there were few published RCTs into the

effects of vitamin D supplementation in cognitive outcomes, in contrast, intervention studies investigating the potential effects of vitamin D on muscle function are available in abundance.

1.4.4. Intervention evidence; the effects of vitamin D supplementation on muscle function

Research investigating the potential effects that vitamin D supplementation may have on muscle function hypothesise that there is an increase in calcium concentrations, due to the increase in the amount of calcium-binding sites or altering how efficient they are. However, findings in older adults have shown inconsistent results. A summary of vitamin D intervention studies for muscle outcomes are detailed in Table 1.3.

To date, no RCT has yet shown an effect for vitamin D supplementation on grip strength. This agrees with the mechanistic and observational evidence, that vitamin D primarily affects the proximal muscles causing lower body myopathy in severe 25(OH)D deficiency. In the most recent RCT, Grimnes et al. reported no effect of vitamin D_3 (44000IU/week) supplementation in community-dwelling females over a 12 month period [(140)]. Muscle strength was measured in upper and lower body extremities using HGS, knee extension strength and muscle mass. Similar study design was employed by Glendenning et al. which tested the effectiveness of vitamin D_3 treatment of 150,000IU every 3 months on measures of HGS and physical performance (TUG), again reporting a non-significant result [(141)]. It has been expressed that this type of dosing regimen of vitamin D may have genomic effects which may negate beneficial effects of vitamin D and muscle metabolism [(142)]. However, these findings are consistent in older negative RCTs, supplementing vitamin D doses of 400IU and 1000IU per day in upper body measures of muscle strength [(143, 144)].

Positive effects of vitamin D supplementation on lower extremity physical performance has been shown in two RCTs, consistent with the mechanistic and observational evidence. Pfeifer et al. investigated an effect of 800IU/d of vitamin D_3 after 12 months in a sample 65 community-dwelling older males and noted a significant improvement in TUG test scores; however, no improvement was detected in lower body muscle strength (leg extension) and the sit to stand task. Interestingly, the two RCTs demonstrating a positive effect on physical performance supplemented with low to moderate doses of

vitamin D (400-800IU, in combination with calcium), and the treatment group in both studies improved serum 25(OH)D concentrations to sufficient levels (baseline treatment groups; 84nmol/L and 65nmol/L), whilst placebo groups remained largely unchanged post-intervention (baseline placebo groups; 57nmol/L and 36nmol/L). Evidence suggests vitamin D supplementation provides some benefit in physical performance in older adults, with less evidence for upper body muscle strength. The heterogeneity, however, of the interventions is notable, with most trials utilising combination treatments of varying doses of vitamin D, with or without calcium or resistance training, and predominantly in female-only populations.

Table 1. 3: Intervention studies; effect of vitamin D supplementation and physical outcomes in healthy older adults

Author	Study design	Intervention	Cohort	Physical outcomes	B: 25(OH)D	PI: 25(OH)D	Effect
Grimes 2017[140]	RCT 12 months	**Low:** $VitD_3$ 800IU/day&Ca **High:** same, plus 20 000IU $VitD_3$ twice weekly	n=297 Women Mean age: 62.8 years	HGS Knee extensor Balance test	High: 64.7 ± 21.4 Low: 64.1 ± 20.0	High: 164.1 Low: 81.8	No
Levis 2017[145]	RCT 9 months	**T:** $VitD_3$ 4000IU/d **PL:** matching placebo	n=130 Men B:Serum <75nmol/L	SPPB Gait speed	T:57.7 ± 12.5 PL:56.2 ± 13.2	T:115.3 ± 31.7 PL:59.9 ± 17.9	No
Hansen 2015[146]	RCT 12 months	**Low:** $VitD_3$ 800IU/d **High:** $VitD_3$ 800IU/d + 50000IU twice monthly	n=230 Women Aged ≤75 years	TUG Sit to stand Muscle mass	High: 52.2 ± 8 Low: 52.5 ± 8	High: 140 Low: 47.5	No
Woods 2014[147]	RCT 12 months	**T:** 400IU or 1000IU $VitD_3$ (BMI adjusted dose) **PL:** matching placebo	n=305 Women Aged 60-70 years	HGS Falls	Mean range across study groups 32.4-34.3	NR	No
Pirotta 2014[148]	RCT 10 weeks	**T:** 2000IU/d $VitD_3$ **PL:** matched placebo	n=26 B:Serum 25-60nmol/L	TUG Balance test Four square step test	T: 46 PL: NR	T: 81 PL:NR	No

Glendenning 2012[141]	RCT 9 months	**T:** 3-monthly dose of 150,000IU **PL:** matching placebo	n= 868 Community-dwelling Healthy Women Aged >70 years	Falls HGS TUG	Subgroup (n=40) 65.8 ± 22.7	~15nmol/L increase in vitD treated	No
Pfeifer 2009[149]	RCT 12 months 8 months WO	**T:** $VitD_3$ 800IU & Ca **PL:** Placebo	n=242 Community-dwelling adults Mean age 77±4 years	TUG Leg extensor Sit to stand Muscle mass	T: 54 ± 19 PL: 54 ±19	T: 84 ± 18 PL: 57 ± 20	Yes: TUG
Bunout 2006[143]	RCT 9 months	**T:** Resistance training w/o $VitD_3$ 400IU/& Ca **PL:** RT + Ca alone	n= 96 (86 females) Healthy adults Aged >70 years Serum <40.0nmol/L	MS(lwr) HGS SPPB TUG	T:31.6±6 PL: 32.8± 7	T:64.5±16 PL: 36.3±12	Yes: TUG
Kenny 2003[150]	RCT 6 months	**T:** $VitD_3$ 1000IU/d & Ca **PL:** Ca only	n=65 Community-dwelling males Age range: 65-87 years	HGS TUG	T: 65 ± 18 PL: 60 ± 8	T: 87.3 ± 14 PL: 56.5 ± 17	No

B, baseline; PI, post-intervention; RCT, randomised controlled trial; T, treatment; PL, placebo; $vitD_3$, vitamin D supplement, Ca, calcium supplement; w/o, with or without; HGS, handgrip strength; MS, muscle strength; SPPB, Short Physical Performance Battery; TUG, Timed-Up-and-Go; RT, resistance training; lwr, lower extremity measure; NR, not reported.

1.4.5. Vitamin D supplementation and muscle function- findings from systematic reviews and meta-analysis of vitamin D supplementation studies

Systematic review and meta-analysis of vitamin D intervention studies have shown a decreased risk of falls in older adults treated with vitamin D supplementation, which may be due to impaired neuromuscular function [(142)]. A summary of the findings for vitamin D supplementation outcomes and effects in physical function and muscle strength are summarised in Table 1.4.

Most recently in 2016, Rosendahl-Riise et al. conducted a systematic review and meta-analysis of vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older adults. The review included 15 RCTs, 9 of which concluded that supplementation with vitamin D, with or without calcium, did not have a beneficial effect on muscle strength or mobility; whilst an improvement in muscle strength and/or mobility was found in 6 studies for participants who were weakest or slowest at baseline or with low baseline 25(OH)D concentrations [(151)]. A meta-analysis was performed for the outcomes of HGS or TUG for which 10 of the 15 studies were included. A marginal effect of vitamin D supplementation was found for HGS after the elimination of significant heterogeneity by omitting 3 studies including subjects with vitamin D deficiency. A statistically significant deterioration in TUG test was also found. Overall the study concludes that vitamin D supplementation is of limited value in preserving or improving muscle strength and/or mobility in older adults. Study populations also vary in terms of baseline vitamin D status and baseline muscle strength and performance. Muir et al. noted that evidence of supplementation increasing serum vitamin D concentration above 75nmol/L was consistently associated with improvements in muscle strength and balance [(152)].

There is plausible evidence for a role of vitamin D in functional health in older adults, however, studies to date make it difficult to examine the true isolated effect of vitamin D treatment. Whilst most umbrella reviews conclude that evidence suggests vitamin D supplementation provides limited or no benefit in physical function in older adults, they also recognise the heterogeneity of the interventions included. If we are to focus on the comparison of studies which show the lowest heterogeneity, i.e. the meta-analysis

findings it seems reasonable to further investigate a measure of physical performance (TUG, gait, and/or walking speed) which appears to be influenced by vitamin D supplementation.

Table 1. 4: Review of systematic reviews of vitamin D supplement RCT and physical outcomes in older adults

Author	RCT(n)	Population	Intervention	Outcome	Results	Effect
Latham 2003[153]	13	Community-dwelling Inpatients	T:VitD, VitD analogs w/o Ca PL: Placebo or Ca	MP MS	Insufficient evidence for VitD alone, some benefit with combined VitD and Ca.	PP 3 MS 1
Annweiler 2009[154]	8	Community-dwelling Majority female	T: VitD or analog PL: NR	MP MS	Gait (1/3) Muscle strength (4/7)	PP 2 MS 4
Muir 2011 [152]	13	Aged ≥60 years Community-dwelling Institutionalised	T: Vit D w/o Ca PL: Placebo	1)TUG 2)MS (lwr)	1) -0.19 (CI -0.35, -0.02) P=0.03, I^2 0% 2) 0.05 (CI -0.11, 0.20) P 0.04, I^2 0%	PP 5 MS 4
Rejnmark 2011[155]	16	Community-dwelling One study in children	T: Vit D PL: PL or active comparator	1)TUG 2)Gait Speed 3)Chair stand 4)HGS	1) 2/7 show beneficial effect 2) 1/7 show beneficial effect 3) 0/2 show beneficial effect 4) 0/5 show beneficial effect	PP 2 MS 5
McCarthy 2015[156]	6	Community-dwelling	T: VitD w/o Ca PL: Placebo w/o Ca	MS (lwr)	2/6 show beneficial effect	MS 2
Rosendahl-Riise 2016[151]	15	Community-dwelling Aged >65 years Male to Female 1:9	T: VitD w/o Ca PL: Nutritional supplement (incl. D)	1)TUG 2)HGS	Meta-analysis 1) 0.3 (CI 0.1, 0.5) NS 2) +0.2 (CI -0.25, 0.7) Sig. worse effect	MS 6

T, treatment; PL, placebo; VitD, vitamin D supplement D_2 & D_3, w/o; with or without; Ca, calcium supplement; PP, physical performance; MS, muscle strength; NR, not reported; TUG, Timed-Up-and-Go; lwr, lower extremity measure; I^2; statistic % heterogeneity; CI, 95% confidence interval; HGS, handgrip strength; incl, including; NS, not significant.

1.5. Conclusion

In this introduction, I presented an overview of vitamin D physiology, the role of vitamin D in ageing and its potential importance in maintaining cognitive and physical health with advancing age. In recent years the emphasis on evidence-based, preventative strategies likely to support our growing older adult population has garnered considerable attention. Evidence now strongly links a role for serum 25(OH)D status in cognitive and physical health. Further research also suggests vitamin D may be associated with improved cognitive function and reduced risk of incidence dementia and mild cognitive impairment. However, there are gaps in the literature due to a lack of well-designed interventions testing the protective effects in healthy older adults. In terms of physical function, a substantial body of literature is available, which indicates physical performance and possibly physical strength may also benefit from vitamin D supplementation

Vitamin D in ageing is an interesting and valuable area, the evidence shows that deficiency rates are high due to increased risk in ageing. Supplementation is inexpensive and easily treats poor status and may be an effective means of maintaining functional abilities in later life. Randomised double-blind placebo-controlled trials are needed to explore the effect of vitamin D on cognitive performance and specific indicators of physical function in community-dwelling older adults as a preventative lifestyle approach for successful ageing.

1.6. Chapter 1- Part B: Systematic Review

In collating the evidence for my thesis it became apparent that no systematic review, focusing exclusively on community-dwelling older adults, without discernable cognitive impairment had been conducted. In order to test the effectiveness of vitamin D supplementation on cognition through a well-designed randomised controlled study, a systematic review would provide important considerations and was therefore deemed valuable. For this part of my thesis, I will discuss the findings from my systematic review.

Worldwide an estimated 46.7 million people are currently living with dementia and this figure is projected to rise to 131.5 million by 2050, highlighting this major public healthcare concern and its substantial associated healthcare costs [(157)]. With pharmacological treatments yet to reach fruition [(158)], the emphasis on evidence-based preventative lifestyle strategies is becoming increasingly important. Findings from systematic reviews largely support an association between hypovitaminosis D and poorer cognitive performance and incidence of dementia [(99)], however, findings remain challenging to interpret, for example in differentiating associations in established events such as AD versus earlier cognitive changes, in institutionalised versus community-dwelling adults, as well as the concentration of 25(OH)D associated with cognitive decline. There is a need to further address these important factors and to examine if low vitamin D may have a distinct impact on brain health.

The aims of this systematic review were to;

- Determine if vitamin D concentration (25(OH)D) was associated with cognitive outcomes in aged 50+ adults living independently in the community, without any discernable cognitive impairment
- Identify which, if any, specific cognitive domains or outcomes presented evidence of associations
- To explore what levels of 25(OH)D were associated with cognitive outcomes. We anticipate that findings from this review would assist the design of future randomised controlled trials

1.7. B. Methods

1.7.1. Search Strategy

A systematic search was conducted on the 15th of July 2016 in databases including PubMed, Medline, and ClinicalTrials.gov to identify cross-sectional, observational, randomised controlled trials, cohort studies, written in English and published between January 2006 and July 2016. This review was reported in accordance with the Preferred Reporting of Items in Systematic Reviews and Meta-Analysis (PRISMA) guidance [159].

1.7.2. Selection criteria

Articles were screened in title and abstract to exclude papers that did not meet the predefined criteria. The remaining papers were reviewed on full text by two reviewers (N.A & M.O'S.). The inclusion criteria for eligibility for full review were studies in which:

(i) Participants had to be at least 50 years of age at time of initial assessment
(ii) Participants were identified as community-dwelling
(iii) Participants did not meet the criteria for MCI defined by Petersons [160]
(iv) Participants had no other significant medical, psychiatric, or neurological problem
(v) Vitamin D was defined as the concentration of vitamin D in blood
(vi) Design was of cross-sectional, observational or intervention/trial design
(vii) Full English text was available

Studies were excluded if; i) the study population comprised fully or in part participants with known diagnosis of AD, other forms of dementia, MCI or self-reported memory complaint, (unless the paper reported results for healthy cognitive subgroups), ii) if vitamin D was defined as dietary vitamin D intake, iii) the study was perspective, molecular or animal in nature, expert opinion or review.

The outcome of interest was cognitive function, global cognition and/or domain-specific cognitive functions, such as, attention, memory, information processing and executive function.

1.7.3. Assessment of methodological quality

Two reviewers (N.A. & M.O'S.) independently assessed quality using the Newcastle-Ottawa Scale (NOS) [53] for cross-sectional and non-randomised studies [161]. Quality assessment was not used as exclusion criteria.

1.8. Results: Search results

Of the 722 originally identified titles, 49 articles met the initial inclusion criteria. Thorough examination excluded a further 33 of those 49 studies for reasons depicted in the study selection flow diagram, Figure 1.6. Overall, 13 of the 16 papers included in this review were published within the past 3 years. No intervention studies met the inclusion criteria.

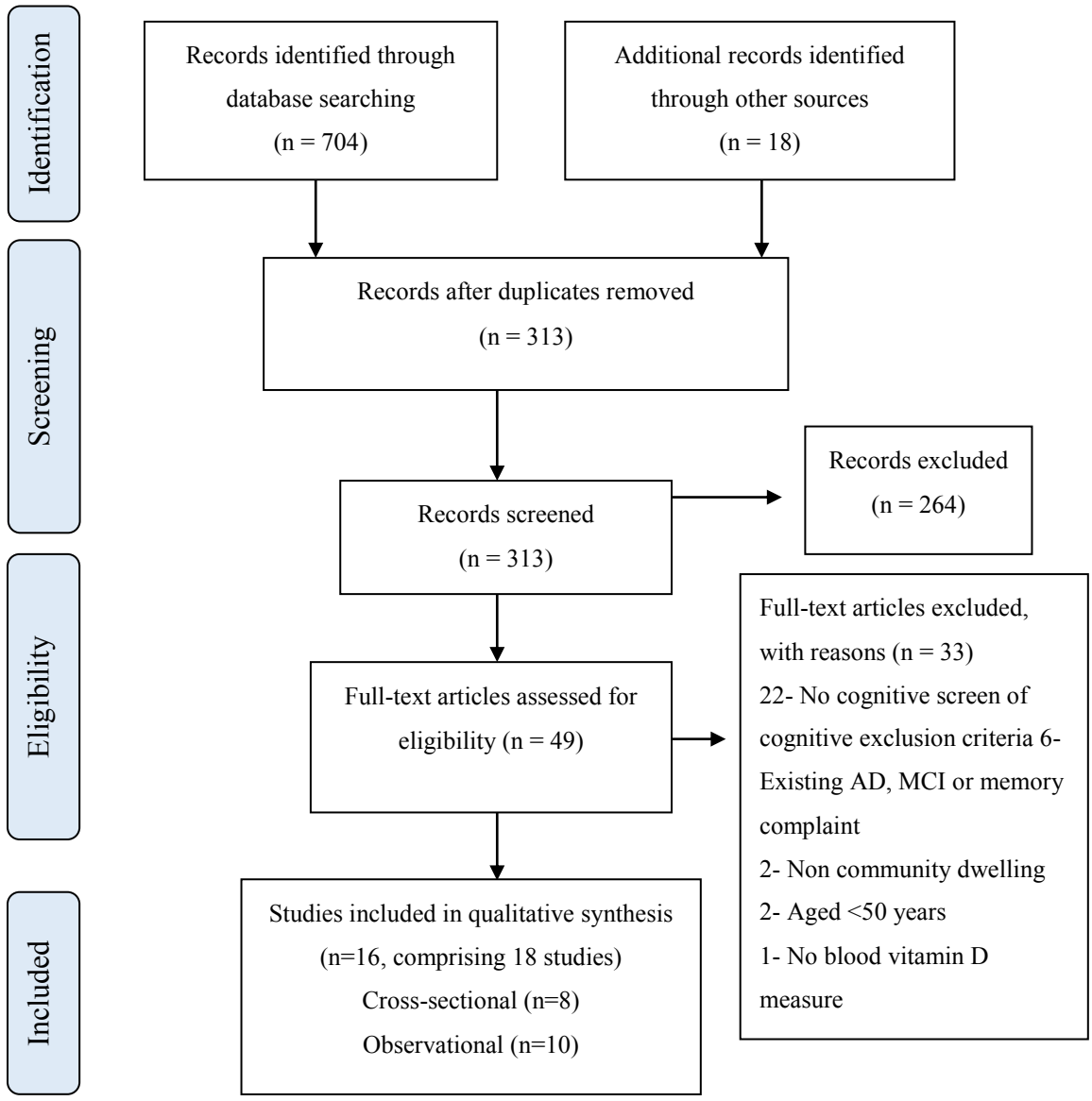


Figure 1. 6: PRISMA flow diagram

1.8.1. Study characteristics

The study characteristics are summarised in Table 1.5 (cross-sectional) and Table 1.6 (observational). A total of 18 studies (from 16 papers) were included for analysis, comprising 8 cross-sectional [89, 91-94, 88, 87, 90] and 10 observational [96, 89, 162-167, 90] studies. One study included both cross-sectional and observational data [89], while another included separate observational analysis of two population studies [163]. The study sample sizes ranged from 103 to 10,186. The total pooled population was 11,119 for cross-sectional studies and 25,880 for observational studies, with a mean follow up of 8.5 years (4-30 years) in the latter.

Age of participants ranged from 55 years [94] to 100 years [96], although most studies enrolled participants aged between 65-80 years [96, 89, 91, 92, 163, 93, 164-167, 88, 87, 90]. The majority included both genders, although two studies comprised only females [89, 165] and one only males [166]. Population studies were conducted in Europe [96, 91, 92, 163, 164, 94, 167, 87], the United States [89, 162, 163, 165, 166, 88, 90] and Australia [93], but were not ethnically diverse; ethnicity was adjusted for where relevant [163, 167, 90]; however, most participants were Caucasian. All studies were based on community-dwelling adults, in line with the inclusion criteria.

Based on the NOS tool all cross-sectional studies achieved 8 points from a maximum of 10 available, Table 1.5. Reason for deductions included a group of users poorly representative of the overall population (educated, professional females) [89], no description of response rate of characteristics of non-responders in population cohorts [91-94, 88, 90]. Quality assessments were scored to a maximum of 9 for observational studies, Table 1.6, of which 6 achieved the full score [96, 162, 163, 167, 90]. Points were deducted for poor follow-up rate [164] and studies with a selected group of users [89, 165, 166], thereby reducing the generalisability of results.

Table 1. 5: Systematic review of cross-sectional associations between circulating vitamin D and cognitive function in community dwelling older adults, included adjusted confounders

Author	n	Population	NOS Δ	Outcome cognitive assessment[a]	Exposure VitD status (nmol/L)	VitD assay	Age	Gender	Education	BMI	Season	CerebroVaD	Smoking	Alcohol	Depression	SE Status	PA	Global Sig.	Domain Sig.	Sig Ass.
Van Schoor 2016[87]	1253	LASA Study Amsterdam Age ≥65y	8	MMSE* RCPM Coding Task* AVLT	<30 30-50 50-75 >75¶	CPBA	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	Yes
Bartali (2014)[89]	1185	Nurses' Health Study USA Age 60-70y Females only	8	MMSE TICS EBMT Composite Z-score*	Q1. 35.19 (9.7-44.4) Q2. 50.9 (44.7-56.7) Q3. 61.6 (56.7-67.6) Q4. 74.1 (67.6-82.6) Q5. 95.8(82.9-184.7) ¶	RIA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		Yes
Wilson (2014)[90]	2777	Healthy ABC USA Age 70-79y	8	3MS* DSST*	<49.9 ¶ 50-75 (insufficient) >75 (sufficient)	RIA	✓	✓	✓	✓			✓	✓	✓		✓	✓	✓	Yes
Brouwer-Brolsma (B) (2013)[92]	127	Promuscle Study Dutch Age ≥65y	8	MMSE WLT* TMT A and B* Wechsler Digit Span RTT*	Ter1 13-38 ¶ Ter2 38-65 Ter3 65-163	LC-MS	✓	✓	✓	✓	✓		✓	✓			✓		✓	Yes
Brouwer-Brolsma (C)	103	SENECA Study Age 70-75 y	8	MMSE*	Ter1 0-34 ¶ Ter2 34-52 Ter3 52-125	CPBA	✓	✓	✓	✓	✓		✓	✓			✓	✓		Yes

(2013)[91]														
Menant (2012)[93]	463	Australia Memory and Ageing Study Age 70-90y	8	MMSE TMT A&B* DSMT BDT*	Mean: 62.2± 24.6 ≤50 (insufficient) >50 (sufficient)	RIA	✓		✓				✓	Yes
Tolppanen (2011)[88]	4831	NHANES III USA Age 60-90y	8	Delayed Memory	Mean; 69.0 (9-400)	RIA	✓	✓		✓		✓		No
Seamans (2010)[94]	380	ZENITH Study Europe Age 55–87y	8	CANTAB-PRM SWM* Attention	Mean76.2 ± 48.2 Ter1 <47.6 ¶ Ter2 47.61–85.8 Ter3 >85.81	ELISA	✓	✓		✓	✓		✓	Yes

NOS, Newcastle-Ottawa Scale for cross-sectional studies (score 0-9); CerebroVaD, cerebrovascular disease including stroke, transient ischemic attack and other vascular circulatory disease; SE Status, socio-economic status; PA, physical activity; Sig Ass, significant association in study p value <0.05; MMSE, Mini-Mental State Examination (0-30); RCPM, Ravens Coloured Progressive Matrices; AVLT, Auditory Verbal Learning Test; CPBA, Competitive protein binding assay; RIA, radioimmunoassay; TICS, Telephone Interview for Cognitive Status; EBMT, East Boston Memory Test; Q, Quartiles; T, Tertiles; GDS, Geriatric Depression Scale; CDR, Cognitive Drug Research; DVT, Digit Vigilance Task; CI, confidence interval; WLT, Word Learning Test; TMT, Trail Making Task; LC-MS/MS, Liquid Chromatography–Mass Spectrometry; SENECA, Survey in Europe on Nutrition and the Elderly; a Concerted Action; DSMT, Digit Symbol Matching Test; BDT, Block Design Test; 3MS, Modified Mini-Mental State Examination (0-100); DSST, Digit symbol substitution test; NHANES, National Health and Nutrition Examination Survey; ZENITH, Zinc Effects in Nutrient/Nutrient Interactions and Trends in Health and Ageing; CANTAB, Cambridge Neuropsychological Testing Automated Battery; PRM, pattern recognition memory; SWM, spatial working memory; ELISA, Enzyme linked immunosorbent assay.

*Tests which observed a statistically significant association. ¶ Circulating D reference for statistical tests.

Table 1. 6: Systematic review of longitudinal associations between circulating vitamin D and cognitive function in community dwelling older adults, included adjusted confounders

Author (year)	n	Population	NOS Δ	Outcome Cognitive Test	FU	Exposure Vitamin D Status (nmol/L)	VitD assay	Age	Sex	Education	BMI	Season	CerebroVa	Smoking	Alcohol	Depression	SE Status	PA	Baseline cog	Global	Domain	Sig Ass.
Kuzma 2016[163]	1612	CHS[a] USA Age ≥65y+	9	BVRT* 3MS*	18years	<25 ≥25<50 ≥50¶	LC-MS	✓	✓	✓	✓	✓		✓	✓	✓	✓			✓	✓	Yes
Kuzma 2016[163]	1074	LASA[b] Amsterdam Age ≥65y	9	MMSE* AVLT	13years	<25 ≥25<50 ≥50¶	CPBA	✓	✓	✓	✓	✓		✓	✓	✓	✓			✓		Yes
Karakis 2015[162]	1663	FramingH USA Age ≥60y+	9	DMS-IV NINCDS-ADRDA	9years	Continuous <24.96 >24.96 Percentiles	CPBA	✓	✓		✓	✓	✓									No
Afzal 2014[96]	10,186	Copenhagen City Heart Age 47-65years (baseline)	9	Incidence AD and/or VaD*	30years	Season specific Percentile ¶ <25 25-49.9 >50¶	RIA	✓	✓	✓	✓	✓		✓		✓		✓		✓		Yes
Bartali 2014 [89]	1185	Nurses' Health Study Aged 70+	8	MMSE TICS EBMT	6years	Q1. 35.19 Q2. 50.9 Q3. 61.6 Q4. 74.1	RIA	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓				No

		Females only				Q5. 95.8¶													
Perna 2014 [164]	527	ESTHER Study Age 65years	8	CogTel*	Mean: 4.6 years	Gender-specific	LC-MS	✓	✓	✓		✓	✓		✓	✓		✓	✓	✓	Yes
Toffanello 2014 [167]	1329	Pro.VA Study Mean age;73.9±7	9	MMSE*	Mean: 4.4 years	<50 50-75 ≥75 ¶	RIA	✓	✓	✓	✓		✓		✓			✓	✓	✓	Yes
Wilson 2014 [90]	1945	ABC Study USA Age 70-79	9	3MS* DSST	4 years	<49.9 50-75 >75	RIA	✓	✓	✓	✓		✓	✓	✓	✓			✓		Yes
Slinin 2012 [165]	5179	USA Female only Age 65years	8	MMSE* TMT part B	4 years	<25 severely deficient 25-49 deficient 50-74 insufficient >75 sufficient ¶	LC-MS	✓	✓	✓	✓	✓	✓		✓	✓		✓		✓	Yes
Slinin 2010[166]	1180	MrOS Study USA Males only Age 65years	8	3MS TMT Part B	Mean: 4.6 ± 0.4 years	≤49.7 49.9-62.6 62.7-74.4 ≥74.38 ¶	LC-MS	✓	✓	✓	✓	✓	✓	✓		✓					No

Δ NOS, Newcastle-Ottawa Quality Scale cohort studies (0-9); CerebroVaD, cerebrovascular disease including stroke, transient ischemic attack and other vascular circulatory disease; SE Status, socio economic status; PA, physical activity; Sig Ass, significant association in study; BVRT, Benton Visual Retention Test; 3MS, Modified Mini-Mental State Examination (0-100); LC-MS/MS, Liquid Chromatography–Mass Spectrometry; MMSE, Mini-Mental State Examination (0-30); AVLT, Auditory Verbal Learning Test: FramingH, Framingheart Study; CPRA,

Competitive Protein Binding Assay; DMS-IV, Diagnostic and Statistical Manual of Mental Disorders; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke; ADRDA, Alzheimer's Disease and related Disorders Association; AD, Alzheimer's disease; VaD, vascular dementia; RIA, radioimmunoassay; TICS, telephone interview for cognitive status; EBMT, East Boston Memory Test; CogTel, Cognitive Telephone Screening Instrument; Pro.VA, translates to "old seniors project"; DSST, digit symbol substitution test; TMT, Trail Making Task; MrOS, Osteoporotic Fractures in Men.

[a] CHS; Cardiovascular Health Study, [168] participants only.

[b] LASA; Longitudinal Aging Study Amsterdam participants only.

*Tests which observed a statistically significant association.

¶ Circulating D reference for statistical test.

1.8.2. Association between vitamin D and cognitive performance: overall findings

The overall findings demonstrated a statistically significant association between lower circulating 25(OH)D and poorer cognitive performance on one or more cognitive outcome measures in 14 of the 18 studies (77.7%).

Consistent with this, the majority of cross-sectional studies reported a statistically significant association between serum 25(OH)D and at least one cognitive outcome (6 of 8 studies), Table 1.5. Most frequently this association was demonstrated with a global cognitive function measure (assessed in 6 of 8 studies), 4 of which showed a positive association [89, 91, 87, 90], 2 no association [92, 93] and a further 2 studies did not measure global function. Higher vitamin D status was statistically significantly associated with better performance in a number of other cognitive domains, e.g. attention [92, 94, 90], executive function [92, 93] and information processing [93, 87]. Overall, 1 of 6 studies assessing memory (non-verbal, verbal and delayed) showed a significant association [92].

These findings were mirrored in the observational data, Table 1.6, although in 2 studies the cognitive outcome was diagnostic; combined incidence of dementia or vascular dementia [96] which reported associations with circulating vitamin D that were statistically significant and diagnosis of AD (based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association for definite, probable, or possible AD) which failed to reach a statistical significance [162].

1.8.3. Associations between vitamin D and cognition: diversity of cognitive outcomes

The positive relationship between circulating 25(OH)D and cognitive performance identified in 77.7% of studies was based on the reporting of at least one positive cognitive outcome for any test used. We identified considerable diversity in the cognitive outcomes applied across 18 studies. Global cognitive function was most frequently assessed (14/18 studies), performed using either the MMSE clinical screening measure or the modified version (namely the 3MS and the telephone screening tool TICS or Cogtel version A) [89, 91, 92, 163, 93, 164-167, 87, 90]. A high degree of

heterogeneity in the domain-specific outcome measures was noted; with 16 different tests identified across the 16 studies. These included assessment of executive function [92, 93, 165, 166], attention [92-94, 90], immediate, delayed, verbal and visual memory [89, 92, 163, 94, 88, 87], abstract reasoning and information processing [93, 87].

1.8.4. Associations between vitamin D and cognition: vitamin D levels and mode of analysis

All studies measured 25(OH)D concentrations, however, vitamin D cut off criteria applied across studies showed little consistency for deficiency (<30nmol/L, <49.9nmol/L, <25nmol/L), insufficiency (30-50nmol/L, 50-75nmol/L, ≤50nmol/L, 25-49nmol/L), and sufficiency (50-75nmol/L, >50 nmol/L, ≥75nmol/L). Furthermore approaches such as tertiles, quartiles, quintiles, <20th percentile, >80th percentile, gender and season-specific cut-off and continuous variables were also used to investigate the relationship between cognitive performance and vitamin D status. On synthesis of this data, it was not possible therefore to determine a specific 25(OH)D concentration or range associated with cognitive function. Overall, the results suggested that low levels, defined most frequently as <30nmol/L were more likely to demonstrate poorer cognitive performance than comparator cut off of >75nmol/L, typically the highest reference range, Table 1.5 and 1.6.

Vitamin D concentration may be influenced by the assay method [168], multiple laboratory techniques were applied across the studies; most frequently DiaSorin RIA (n=8), followed by Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) (n=5) considered by many as the "gold standard" [169], competitive protein binding assay (n=4), and enzyme linked assay (n=1) and IDS-iSYS technique (n=1). No apparent pattern could be determined in cognitive outcome according to the vitamin D assay applied.

1.8.5. Confounders

Multivariate regression models were applied in all studies and results for adjusted models were considered in this review, Table 1.5 and 1.6. All studies adjusted for age. Gender was adjusted for in 17 studies (by model or study design), one study did not adjust for gender, however, the sample was relatively balanced with no significant

between group difference, (53% female) [93], most studies adjusted for educational status (n=15) obtained or years of education, although few directly addressed for socio-economic status (n=4). Lifestyle factors were accounted for in many studies including BMI (kg/m²) (n=16), physical activity level (n=12) (high, medium, low or hours spent walking for exercise), smoking behaviour (n=13) and alcohol intake (g/week) (n=11). A further 11 studies adjusted for depression using validated tools (Geriatric Depression Scale) or medication inventory, an important variable from a cognitive perspective and 13/18 for season of blood draw, a potential confounder for circulating vitamin D analysis. Other important confounders that were less frequently applied included cerebrovascular disease including stroke (n=4), vitamin D supplementation, gait impairment and history of hypertension and renal disease.

1.8.6. Publication bias

Overall, 83.3% of the studies reported a positive association between vitamin D levels and at least one measure of cognitive function and remaining 16.7% of studies found no association. Of the 33 studies excluded at full text level 90.3% observed a statistically significant association. Of the 3 interventions, 75% found no effects of vitamin D supplementation on cognitive performance [170-172]. All (n=3) intervention studies recruited participants from either nursing homes, memory clinic outpatient services, younger adults and individuals diagnosed with mild to moderate AD, deeming them ineligible for this evaluation.

1.9. Discussion

We performed an up to date and methodologically rigorous systematic review of the literature to evaluate the evidence of an association between cognitive performance in older adults, without discernable cognitive impairment, and vitamin D status. With increasing interest and publications in this field an updated evaluation of the literature was merited, and 13 of the 16 papers analysed were published within the past 3 years. Overall this review suggests that low vitamin D status was significantly associated with lower scores in tasks of cognitive function, extending findings to healthy community-dwelling adults, free of cognitive impairment.

Our evaluation of cross-sectional studies showed that participants with low vitamin D status scored statistically significantly lower in at least one task of cognitive function (7

of 8 studies). Consistent with this, observational studies showed that cohorts with low vitamin D status at baseline, followed up overtime (4-30 years), had a greater risk of cognitive decline or incidence of cognitive impairment compared to those with high vitamin D status at baseline (8 of 10 studies). Collectively, our findings suggest a positive relationship between cognitive performance and circulating 25(OH)D in aged 50+ community-dwelling adults. While this has been previously demonstrated in samples with cognitive impairment [173], the present review now extends these findings to older adults considered to be cognitively healthy, insofar as could be determined.

There are few published interventions of vitamin D supplementation and cognition, the limitations of which have been detailed previously, in part A of this Introduction chapter.

1.9.1. Cognitive outcomes

This review highlighted the diversity in cognitive outcome measures used across published studies making comparison between results difficult. A variety of cognitive and dementia-related assessment tools have been utilised, on average 2 per study, no association was found in the only study which relied on 1 domain-specific tool [88] perhaps as this single domain cognitive assessment doesn't consider all possible subtypes of MCI. The importance of utilising multiple domains when assessing cognition has been highlighted in recent years [174]. In studies of cognitively healthy older adults this is particularly important, when detection of subtle cognitive changes in performance is being assessed. For example, a specific assessment of memory was not carried out in all studies identified by this review.

In the present review, the most frequently used cognitive test was a global measure of cognitive function namely the MMSE or modified versions, and consequently most positive associations with 25(OH)D were seen with a global measure; whilst this clinical screening test is widely used it has limited use in research, for detecting subtle differences and changes over time [175]. No studies considered assessment of pre-morbid IQ, which is highly correlated with most cognitive tests, since decline is essentially relative to pre-morbid ability. Unlike most clinical studies, which employ standardised comparable outcome measures, neuropsychological assessment in nutrition and ageing research appears less well-defined. Future studies of vitamin D and cognition, should

aim to explore finer aspects of cognitive function in healthy older adults, which may benefit from employing more sensitive established core outcome sets of measures.

1.9.2. Vitamin D Status and cognition

Overall, the results suggested that older adults with low 25(OH)D concentration, defined most frequently as <30nmol/L, were more likely to have poorer cognitive performance when compared to high levels of >75nmol/L, typically the highest reference range. The lack of consistency in reporting 25(OH)D levels meant it was not possible to determine a specific 25(OH)D concentration or range associated with cognitive function outcomes. There is a lack of consensus for cut-off levels for 25(OH)D status, not unique to cognitive studies as detailed in part A. In the US the Institute of Medicine suggests serum 25(OH)D concentrations of ≥50nmol/L are sufficient for general health [32], whereas the Endocrine Society recommends concentrations ≥75nmol/L for normal bone and mineral homeostasis [33] and there is ongoing debate regarding the optimal levels likely to mediate immune disease [176]. Less is known for other health outcomes including cognitive health [177].

1.9.3. Limitations

One of the key goals of this review was to focus on 25(OH)D concentrations and cognition in older adults without discernible cognitive impairment, insofar as this could be established. Establishing criteria for "normal" cognitive functioning versus MCI is challenging, particularly when deriving the data from existing published studies which apply different methodological and cognitive assessments. Some studies applied lower MCI cut-off values for MMSE scores [178] and therefore were deemed ineligible for inclusion in this analysis.

This review was based on cross-sectional and observational studies, the findings need to be addressed in intervention studies to establish whether low 25(OH)D concentration is merely a biomarker of poorer cognitive health. The lack of consistency in reporting 25(OH)D prevented the application of meta-analysis when reviewing results, equally availability of information on behavioural determinants of 25(OH)D status including vitamin D supplementation use, UVB exposure and sun protection factor use would enhance the scientific validity of future studies.

1.10. Conclusion part B

This systematic review demonstrated that lower circulating 25(OH)D status was associated with poorer cognitive performance in aged 50+ adults considered free from discernible cognitive impairment. The result extends previous findings in AD, dementia and MCI to populations of healthy older adults without cognitive impairment. Most frequently the associations were noted between low (<30nmol/L) versus high (>75nmol/L) 25(OH)D status, although an optimal 25(OH)D level for cognitive health could not be determined. The considerable heterogeneity in the number and types of cognitive assessment used and in reporting of vitamin D status, highlights the need for standardisation in future studies. Well-designed RCTs using valid comprehensive cognitive assessments, are required to determine if raising 25(OH)D concentrations through supplemental vitamin D improves cognitive health and contributes a viable component of lifestyle approaches to maintain brain health.

1.11. Overall aims and outline of the thesis

The main aim of this thesis work was to explore vitamin D status and to examine the potential effectiveness of vitamin D supplementation on cognitive and physical function in healthy community-dwelling older adults.

In order to investigate the effects of vitamin D supplementation, a RCT was conducted entitled 'D-activating Decline'. It was hypothesised that:

1) Vitamin D supplementation is beneficial in supporting cognitive performance in older adults without any discernible cognitive impairment
2) Vitamin D supplementation is effective in supporting muscle function in healthy older adults

Specific objectives of this thesis were as follows;

Vitamin D status and determinants of deficiency

- To explore the prevalence and determinants of vitamin D deficiency in community-dwelling older adults, with a specific focus on the contribution of northerly latitude. This was achieved by analysis of a large, representative sample of participants of the English Longitudinal Study of Ageing (Chapter 3)

Vitamin D and cognitive function;

- Firstly to extrapolate the existing evidence for vitamin D status in cognition in community-dwelling older adults without discernable cognitive impairment, through a systematic review, ultimately to inform the design of a RCT (Chapter 1 Part B)
- The aim of the intervention study was to investigate the effect of vitamin D_3 supplementation on cognitive performance in healthy community-dwelling older adults (Chapter 4). This was achieved through the design, set up and running of a randomised double-blind placebo-controlled pilot study. The study design and research protocol is detailed in Chapter 2
- Finally to understand the RCT participants experience of taking part in a brain health research study with particular interest to motivations and concerns for

participation. This was achieved through a mixed-methods feedback survey (Chapter 4 part B)

Vitamin D and physical function;

- To investigate the potential effect of vitamin D_3 supplementation on physical performance and muscle function in older adults (Chapter 5), in a randomised double-blind placebo controlled pilot study. This was a pre-specified secondary outcome of the cognitive RCT I in Chapter 4
- To further explore the association between vitamin D status and muscle function, in particular lower body extremity outcome measures. This was achieved through analysis of a large cohort of healthy community-dwelling older adults, based on the ELSA population data (Chapter 6)

General Discussion

- In chapter 7 the main findings of this thesis are summarised and discussed with respect to strengths and limitations of the study. Moreover, findings are placed in a broader perspective of current knowledge. Finally, recommendations for future research and implications for public health are given

Chapter 2: Methods

This chapter provides information on the materials and methods used for the two studies which form the core of this thesis. This includes a 6-month randomised double-blind placebo-controlled pilot trial (Chapter 4 and Chapter 5), and a large, nationally representative cross-sectional analysis of vitamin D deficiency (Chapter 3) and serum 25(OH)D concentrations and muscle function (Chapter 6). The aims of the 6-month randomised double-blind placebo-controlled pilot study was to assess the effect of vitamin D supplementation on cognitive and physical outcomes in healthy community-dwelling older adults, without cognitive impairment.

2.1 Study One: A randomised double-blind vitamin D supplementation placebo-controlled pilot trial (Chapter 4 and 5)

2.1.1 Ethical approval

Ethical approval was sought from the Joint Tallaght and St. James Hospital Research Ethics Committee. Copies of the letters of ethical approval and subsequent amendments are provided (Appendix 1-6).

2.1.2 Data storage

Confidential participant details and electronic data were stored under lock and key in the Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8. All data were treated as confidential and never disclosed to a third party. Randomisation was completed by minimisation procedure. The spreadsheet was created by an independent statistician (Dr. Cathal Walsh). The randomisation allocation key was secured in Trinity Centre for Health Sciences, St. James Hospital, Dublin 8, in line with ethical requirements.

2.1.3 Vulnerable subjects

Those aged less than 60 years were not included in this study, which excluded children and women of childbearing age. Intellectually impaired adults or those with brain injuries were not included.

2.1.4 Study sponsorship

The Ph.D. program was funded by a scholarship from the Irish Research Council (IRC). Best Formulations (California, USA) provided the vitamin D supplements and placebo for research detailed in Chapter 4 and 5. Neither the author nor the other investigators involved in the studies had any conflict of interest.

2.2 Pilot RCT Study design

2.2.1 Study design and intervention

This was a randomised, double-blind, placebo-controlled pilot study, with participant data collected at 0, 3 and 6 months follow-up. Active treatment comprised of gel capsules (gelatine; bovine and glycerine; vegetable), containing 4000IU (100ug) of vitamin D_3 comprised of cholecalciferol in corn oil and placebo comprised of soybean oil contained 0IU of cholecalciferol. All participants were directed to take 1 capsule, on alternating days for the study period. All packaging and capsules were identical. Compliance with the intervention was determined by capsule count at the end of the study period at 6 months.

2.2.2 Study protocols

Study protocols for the mixed-methods randomised double-blind placebo-controlled pilot trial are detailed in (Appendix 7) and were approved by the Joint Research Ethics Committee of Tallaght Hospital and St James's Hospital. Additional information was requested from the ethics board for measurement of serum calcium, for all research participants and the protocol was subsequently amended.

2.2.3 Participant recruitment

Participants were recruited through various community facing organisations. Multiple avenues were employed to ensure the largest catchment area was reached. Participants were recruited through advertisements in local parish newsletters, study advertisements in pharmacies, postal drops to general practitioners (GPs), ageing organisations such as;

Active Retirement Ireland, Age Action, Alone and through national press (Irish Examiner). The study advertisement is included as Appendix 8.

2.2.4 Study setting

A successful application was made to conduct all study assessments in the Wellcome Trust Health Research Board, Clinical Research Facility in St. James's Hospital. All study assessments took place in the purpose-built research facility. Associated costs included room booking (hourly rate), participant patient hospital registration (single cost) and nursing services (phlebotomy). Other small consumables were funded through the scholarship (blood tubes, cryovials and storage containers). The centre also provides access to blood processing facilitates and relevant training, provided by Dr.Joseph Mc Partlin.

2.2.5 Participant information leaflets and consent forms

Participant information leaflets *(*PIL) and consent forms were approved by the Joint Research Committee. (Appendix 9 and 10). Participation for this study was self-selecting, once participants expressed an interest in taking part, the study was explained in detail over the telephone. Subsequently, PIL and consent forms were posted to potential participants. After a 7 day period participants were contacted to complete a telephone pre-screen (detailed below). The GPs of the participants partaking in the RCT (Chapter 4 and 5) were also notified of their patients' involvement (Appendix 11). In line with ethical requirements participants of the trial were provided with an information sheet counselling vitamin D advice (Appendix 12).

2.2.6 Adverse events

Adverse events (AE) were to be documented in the participants Adverse Event Form and verbally brought to the attention of the lead physician (BL) or a member of medical staff in the centre on the day of reporting. At each contact with the participant the investigator sought information on adverse events by questioning. Information on adverse events were to be recorded immediately in participants' safety monitoring sheet. All clearly related signs symptoms and abnormal diagnostic procedures should be

recorded. High serum calcium is likely to occur with any of the following; nausea and vomiting, excessive thirst, frequent urination, constipation, abdominal pain, muscle weakness, confusion, and lethargy. No adverse event were reported.

2.2.7 Research team

The Ph.D. research was conducted under the supervision of Principal Investigator (PI) Associate Professor Maria O'Sullivan and Co-PI, Professor Brian Lawlor. Dr. Martin Healy, the lead biochemist in the Clinical Pathology Laboratory, St. James's Hospital, Dublin 8, performed the serum vitamin D and corrected calcium analysis of obtained serum samples. Minimisation procedures were created by statistician Dr. Cathal Walsh (University of Limerick). Dr.Joseph McPartlin provided laboratory training for serum sample processing and storage for batch analysis. I was responsible for all other aspects of study completion as detailed in Table 2.1.

Table 2. 1: Study logistics and required documentation

Contact	Reason	Goals	Required documents	Duration
1	Provide study information	Share study information and answer questions, obtain postal address and send PIL and consent forms.	Advertisement, PIL and consent form.	15mins
2	Pre-screen for enrolment	Explain study procedures and complete telephone screen, including cognitive screen, physical activity, and medical inventory, to assess for eligibility. Schedule screening, book appointment with HRB and post background questionnaire and map to facilities.	Telephone screen, background questionnaire, directions to clinical research facility.	25mins
3	Screening and assessment 1	Answer any questions and obtain consent. Obtain serum sample for 25(OH)D and corrected Ca analysis. Collect background questionnaire. Complete cognitive assessment, FFQ, physical assessment, mental health and wellbeing.	Assessment pack, consent form, background questionnaire.	80mins
4	Acknowledge study initiation and report serum levels	Contact participants regarding serum 25(OH)D status, notify GP of study inclusion and blood results	GP letter	15mins

5	Schedule 3mos. assessment	Contact participants to confirm a time and date for follow-up visit and serum sample. Schedule booking with the HRB. Email or text study appointment.	Assessment pack	20mins
6	Perform 3mos. assessment	Obtained serum sample. Repeat the same assessment as assessment number 1 above. Centrifuge, aliquot and freeze serum samples. Pill count.	Complete assessment pack.	75mins
7	Schedule 6mos. Assessment	Contact participants to confirm time and date for final assessment. Schedule booking with the HRB. Email or text appointment.	None	20mins
8	Perform 6mos. assessment	Obtained serum sample. Repeat the same assessment as assessment number 1 and 2 above. Centrifuge, aliquot and freeze serum samples. Pill count. De-brief participants.	Complete assessment pack. De-briefing information.	75mins
9	Acknowledge study completion	Contact participants regarding serum 25(OH)D status and group allocation. Notify GP of study completion and final serum 25(OH)D blood results.	GP letter	15mins
10	Feedback Survey	Contact participants to request permission to post an anonymous survey to gain their perspective of taking part in the research project.	Survey Questionnaire	15mins

All contacts were completed by Niamh Aspell.

Abbreviation: PIL: participant information leaflet, HRB; Health Research Board, Ca; serum calcium, FFQ: Food frequency questionnaire.

2.3 Screening for eligibility

2.3.1 Exclusion criteria

Participants were assessed for eligibility using a validated telephone screen for recruitment of participants to cognitive research [179]. Participants aged <60 or >80 years were not eligible to take part in this RCT. The screen included a Telephone Cognitive assessment (TCogs <23), reported history of stroke, epilepsy, schizophrenia, bipolar affective disorder, recurrent psychotic depression, and alcohol or drug abuse within the past 5 years; reported the use of anticonvulsants or antipsychotic medications; or self-reported memory complaint; history of any illness which caused permanent decrease in memory or other cognitive functions. The screening document was edited to include information regarding vitamin D; current vitamin D supplement use ≥800IU/day, endocrine disorders or renal disease (Appendix 13). Furthermore, those with measured serum 25(OH)D concentrations <15nmol/L or >125nmol/L or measured serum corrected serum calcium >2.55nmol/L, were also excluded.

2.3.2 Biological samples and serum 25(OH)D analysis

Blood samples were collected by a research nurse. Samples were centrifuged at 1,500 RCF for 10 minutes (9 acceleration, 9 brake) at 4°C following sufficient clot time (minimum 30 minutes; maximum 120 minutes) at room temperature. Serum was aliquoted in 1ml, appropriately labelled cryovials. Supernatants were stored at −80°C for batch analysis at 3 and 6 months.

Total serum 25(OH)D concentrations were quantified by measuring ergocalciferol ($25(OH)D_2$) and cholecalciferol ($25(OH)D_3$) using Liquid Chromatography-Mass Apectrometry (API 4000), considered the gold standard in the assessment of vitamin D [168]. A 3PLUS1® multilevel plasma calibrator set, ***Mass****Check*® controls and a National Institute of Standards and Technology (NIST) standard set (SRM 972) was also used for standardisation with an average of 97.8% recovery against the known concentration for the 4 standards. Furthermore, serum pools from the Vitamin D External Quality Assessment Scheme (DEQAS) were analysed using the LC-MS method. The intra-assay precision coefficient of variance (CV) for the method was < 3.5% for cholecalciferol.

All assays, included serum calcium, were performed at the Clinical Pathology Laboratory in St. James' Hospital, Dublin 8.

2.3.3 Vitamin D status

For this study, vitamin D status was defined by the Institute of Medicine guidelines [32] with serum concentrations <30nmol/L reflecting deficiency, vitamin D insufficiency was 30-50nmol/L and sufficiency defined as ≥50nmol/L, whilst levels >125nmol/L have not been evidenced to provide no additional benefit for some outcomes. We also considered cut-offs of ≥75nmol/L for comparison with other published evidence.

2.3.4 Randomisation

Minimisation is a widely accepted allocation tool for effective randomisation, particularly in studies of small sample size, as it ensures a balance between-groups for important prognostic factors [180]. Under the guidance of an experienced statistician (CW), the first participant is allocated a treatment at random, for each subsequent participant the spreadsheet determines which treatment would lead to a better balance between-groups based on pre-specified variables of interest. In this instance, factors considered as most important for this pilot study of small sample size included; age, gender and physical activity level (IPAQ-SF, category), considering the outcomes of interest, and to facilitate balance between-groups.

2.4 Study Assessments

Primary and secondary endpoints were pre-specified on clinicaltrials.gov. Primary measures included assessments of cognitive function and secondary outcomes included measures of muscle function. Detailed study assessments were completed at 0, 3 and 6 months, components of which are detailed in Figure 2.1.

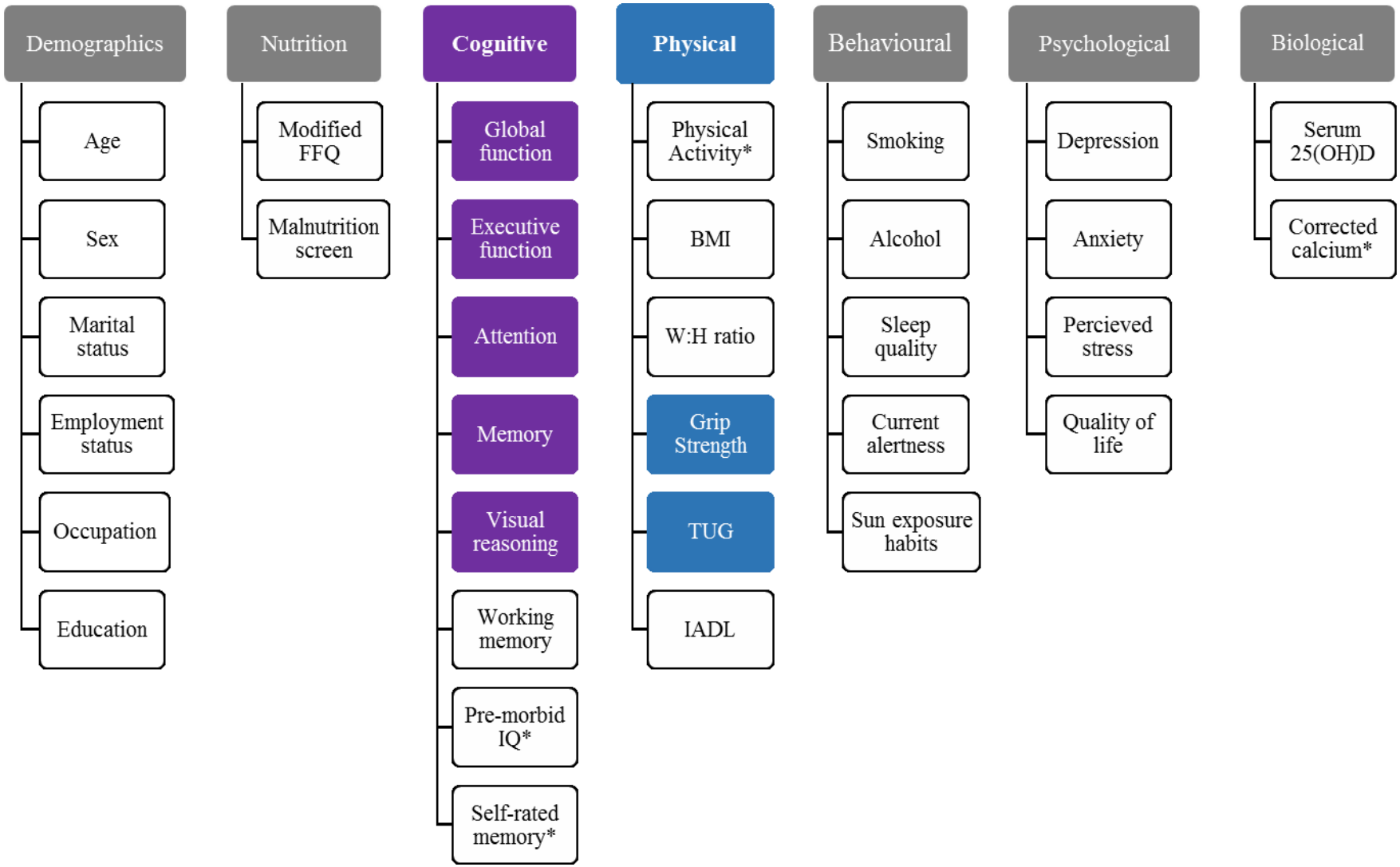


Figure 2. 1: RCT assessments conducted at each study assessment. * Baseline only. Pre-specified Primary and Secondary endpoints

2.4.1 Demographics

Demographic information was collected from all participants for age, gender, marital status (married, single, cohabiting, widowed, divorced), employment history (skill level and experience in years) and education level (years of education and highest level of education achieved) (Appendix 14).

2.4.2 Nutrition

A modified food frequency questionnaire (FFQ) used by another large nutrition and ageing studies [181] was completed to gather detailed information regarding the frequency of vitamin D containing foods were collected to allow for consideration of dietary intake for all participants and to monitor any changes in dietary D intakes over the course of the study. Participants were asked whether they consumed certain food types, and if so how frequently (Appendix 15). Detailed information on portion size was not collected as this was not an outcome for this study, as there are few high contributing food sources of vitamin D (naturally or fortified), and foods which provide good sources of vitamin D are not generally daily staple foods, it is anticipated that the contribution would be minimal. Nevertheless, important to acknowledge. Data on dietary supplement use was obtained during the telephone health screening.
The Mini Nutritional Assessment screening tool for malnutrition was included in the study design, which has been validated for use in older adult populations [182, 183].

2.4.3 Habitual vitamin D behaviour

No standard, validated sunlight questionnaires are routinely used to quantify sun exposure. Typical questions used to assess sunlight exposure are listed elsewhere and were included in our estimate of vitamin D acquired through sun exposure [184]. Sun exposure contributes more to serum 25(OH)D concentrations than food sources, and due to the timing of study initiation (Spring), sun habits were therefore deemed of importance. To assess the likely contribution of sun exposure to serum D concentrations, participants were asked multiple questions regarding sun avoidance, sun protection factor use, and sun holiday travel (Appendix 15).

2.4.4 Cognitive sphere- assessment of cognitive function

The term "cognitive functions" refers to a variety of brain functions and processes which includes; receiving external information, processing this internally and responding with a behaviour. It can be seen as a hierarchy, going from overall (global) to domain-specific cognitive functions. Domain-specific functions include memory, executive functioning, attention, perceptual functions, psychomotor abilities and language skills. The importance of utilising multiple domains when assessing cognition has been highlighted in recent years [174]. In order to establish overall performance within a domain it is therefore important to conduct a series of tests that will evaluate key components.

How to measure cognitive performance?

Neuropsychological performance tests are the method most commonly used in nutrition and lifestyle studies. Cognitive function can be assessed objectively by a number of validated pen-and paper or computer-based tasks, illustrated in Figure 2.2. Each test involves varying degrees of researcher involvement which requires a level of expertise to administer and extensive training is generally required. Computer based tasks overcome this with standardised procedures which accurately measures fine details such as processing speed and reaction time.

As the evidence for an underlying link between diet and or dietary components and cognitive performance remains inconclusive, the best approach is to include a battery of cognitive tests that cover a variety of domains, this may help identify the specific cognitive processes involved. For this approach to be effective the design must also consider an appropriate test duration, considering test fatigue which may be manipulated or exacerbated with running order of each test.

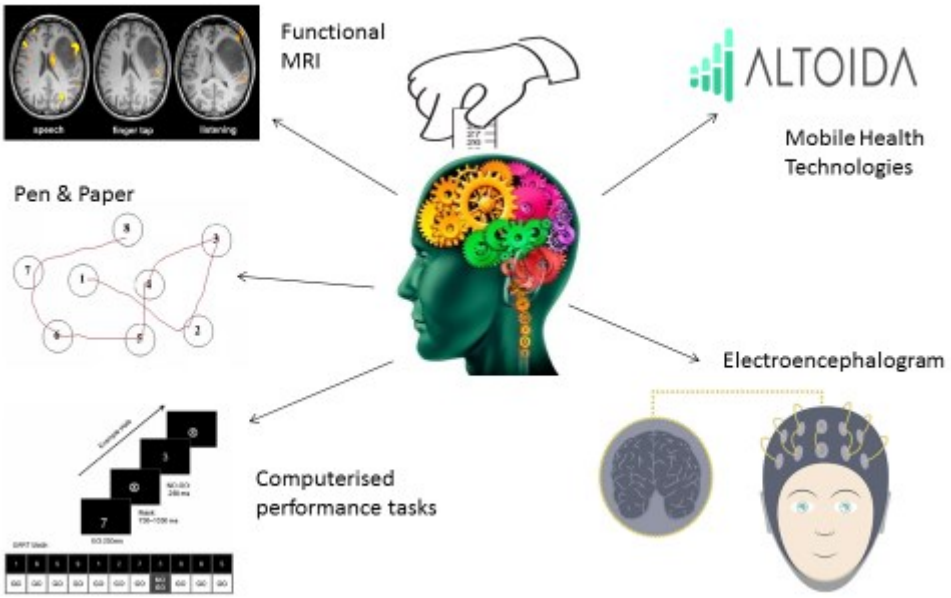


Figure 2. 2: Direct and indirect methods of measuring brain and cognitive function

By testing multiple cognitive domains and examining a range of cognitive abilities we could assess the effect of vitamin D supplementation and cognitive function in different brain areas. We anticipated the findings would replicate the cognitive module in population studies to provide comparability with nationally representative (TILDA) and other longitudinal studies. During discussions at protocol development stages, a senior clinical neuropsychologist (RC) and the study Co.PI (BL), the final cognitive sphere for the present RCT included global and domain-specific measures of executive function, attention, memory and visual reasoning. Details of the assessments are detailed below.

2.4.5 Global function- Montreal Cognitive Assessment (MoCA)

The MoCA is a quick screening instrument for mild cognitive dysfunction [(185)]. It assesses different cognitive domains, including attention and concentration, executive functions, memory, language, visuo-constructional skills, conceptual thinking, calculations and orientation, providing an overall composite score, ranging from 0-30. Various cut-offs can be applied depending on normative data available for the study population. A score <23 is generally considered indicative of cognitive impairment, in healthy community-dwelling older adults [(186)]. The MoCA is widely used in population studies and is administered as part of the cognitive battery in TILDA.

2.4.6 Executive function- Trails Making Task, part B (TMT-B) and B minus A (B-A)

Trail Making Task [187] has two components in part B the participant draws a line to join encircled numbers and letters in alternating numeric and alphabetic order (i.e. 1-A-2-B), as quick as possible, whilst instructed not to lift the pencil from the page. The numbers are placed in a somewhat random order, so the line should not overlap. Generally errors are not reported as they are reflected in the time to complete the test. Cut-offs for older adults populations for part B are 300 seconds, as this is the maximum time allowed to complete the test. Performance on TMT part B is considered a sensitive indicator of neurological impairment. Part B involves higher level cognitive skills and is more difficult due to increased visually interfering stimuli than part A [188], however, the difference in B minus A (B-A) may provide an additional measure of mental flexibility and executive functioning [189].

Studies have demonstrated activation in areas of the brain believed to be sensitive to executive functioning, motor control and cognitive flexibility [190]. Sensitivity to subcortical white matter hyper-intensities (WMH) in healthy older adults have also been shown [191]. A common feature of neuropsychological instruments is the susceptibility to practice effects, short intervals of 6-weeks may reflect practice effects [192]. The TMT has excellent inter-rater reliability [193].

2.4.7 Attention- Sustained Attention to Response Task

Sustained attention is measured using a computer-based Sustained Attention to Response Task (SARTfixed) [194]. SART assesses executive control of behaviour. It is sensitive to transient lapses of attention, and challenges the ability to endogenously maintain an alert state [195]. SART activates the sustained attention network and is sensitive to frontal lobe dysfunction [196].

It is a continuous performance reaction-time (RT) task. It requires participants to respond to digits, '1' through '9' which appear on screen sequentially, over a period of approximately 5 minutes. Totalling 225 digits, which vary in size but not in screen position. Participants are instructed to press the left control pad button on presentation

of each digit (GO-trial) with the exception of the 25 occasions when the digit '3' (NO GO trial) appears, when they are required to withhold their responses. For each trial a digit is presented for 150ms followed by an inter-stimulus interval (ISI) [53] that varies randomly between 1000 and 1500ms. This variable interval is introduced to prevent participants from succumbing to a speed accuracy trade off that can occur when ISIs are evenly paced [53].

Commission errors (responding to NO-GO trials) reflects lapses of sustained attention, and omission errors (failure to respond to GO trials) reflects task disengagement and corresponds to lapses in attention. In older adults, fewer errors and slower mean RT represents a speed accuracy trade off, however, more SART errors accompanied by slower mean RT indicates reduced cognitive processing speed and/or lapses in sustained attention [197, 198]. Greater RT variability (standard deviation [SD] of RT) is associated with attentional deficits, slower RT and increased SART errors in older adults [199].

We anticipated that those allocated vitamin D supplementation would display quicker RT, lower RT.var and less combined SART errors (SART error scores) than those allocated to placebo. The SART was selected as it has been widely used in studies in Trinity College Institute of Neuroscience (TCIN) and was used in the TILDA cognitive battery.

2.4.8 Attention- Trail Making Task, part A

In part A of the Trail Making Task the participant is asked to draw a line to connect circled numbers in a numerical sequence (i.e., 1-2-3, etc.) as rapidly in possible [200, 201]. TMT-A is primarily a test of visual sustained attention, simple perceptual tracking and processing speed. Similar to TMT-B, errors are not reported as they are reflected in the time taken to complete the task.

2.4.9 Memory and Recall- Weschler Memory Scale III, Logical Memory I and II

The test assesses narrative memory under a free recall condition. Two short stories are orally presented. For older adults, one story is presented twice. The participant is asked to recall each story from memory immediately after hearing it (Logical Memory I).

Following a minimum 20 minute interval, the participant is once again asked to retell each story from memory for a delayed recall condition (Logical Memory II). The stories administered depend on age, for ages 16-69 (Adult Battery) are story B and C; and the stories for ages 65-90 (Older Adult) are story A and B. In line with other ageing studies conducted in the Memory Research Unit [202] in TCIN, on the basis of advice from (RC), the cut off for the adult battery was 69, and the older adult version is administered to those aged 70 years and older. Final scores were then calculated from indexed scaled scores to give a comparable result irrespective of the participants' age and version administered.

2.4.10 Visual Reasoning

The visual reasoning test from the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) battery, having also been used in TILDA and the MRU, and therefore allowing for comparability with nationally representative data was included in the cognitive battery.

Test administration: This test comprises 6 trials consisting of a grid of four boxes, three of which contain a shape, and one of which is empty. Six shapes are presented below the grid, marked A-F, and the participant is asked to select which of these options should go into the empty box in order to form a pattern.

2.4.11 Working Memory

Letter Number Sequencing (LNS) is used as an auditory working memory subtest as part of the Wechsler Adult Intelligence Scale-III (WMS-III) working memory index. An assumption in the cognitive literature is that performance on recall tasks is somewhat unaffected by ageing, whereas tasks that require the reorganisation of items prior to recall are more age-sensitive. This is presumed to be because manipulation tasks require greater prefrontal involvement than maintenance tasks, a view supported by current neuroimaging evidence [203]. We decided to include this task as an exploratory cognitive component in support of the general memory section.

Test administration: A series of alternating numbers and letters are presented to the participant at a rate of 1 item per second (e.g. B, 4, P, 7), and the participant must then recall the numbers first in ascending order followed by the letters in alphabetical order.

2.4.12 Composite scores

Composite scores for each cognitive domain were calculated by obtaining standardised Z scores for each cognitive test and combining them to create total scores for; executive function (TMT-B, TMT B minus A, visual reasoning), attention (TMT A, SART RT, RT.var., SART errors), and memory (logical memory I & II and LNS working memory).

Using Z scores provides a more-reliable estimate of the effect of each domain, reduces the number of single tests reported, and allows the performance for each domain to be compared in a standardised way. Therefore an estimate of the effect size for each domain can be compared to differences in individual tests using the population standard deviation for each test.

2.5 Other factors important in cognitive performance assessments

2.5.1 Pre-morbid IQ

The inclusion of pre-morbid IQ is extremely important when assessing cognitive function. IQ is highly correlated with most cognitive tests, and therefore measures taken on their own, without consideration for pre-morbid functioning, can result in false correlations. When assessing cognitive decline a score within the "normal" range of a cognitive test can represent cognitive decline for an individual with extremely high levels of pre-morbid functioning. The National Adult Reading Test (NART) is the most commonly used reading test, and therefore allows for maximum comparability with existing literature. We choose the Short NART, which allows for discontinuation procedures, to avoid causing undue distress to participants who are failing a significant amount of words early on in the test which becomes progressively more difficult. The short form discontinues at 25 words for participants who fail on 5 or more words, for those who score <12 out of 25 the assumption is their score would be no different than to failing the full NART. For scores between 12 and 25 a formula is provided to calculate full scores. The accuracy of the short NART method was found to be equivalent to the full NART [(204)]. The assumption behind the use of the reading test is the pronunciation.

2.5.2 Self-reported memory and forgetfulness

A subjective measure of self-reported memory was included to provide an indication of whether the participant is worried about their memory. This was included at the baseline assessment, and took the form of a 5 point likert scale. These self-report items have been included in the MRU and TILDA.

2.5.3 Behaviour

For behavioural influences alcohol intake [Do you drink alcohol? How often do you drink alcohol? Units per week (g) were reported] and problematic alcohol behaviour was assessed using the self-report CAGE questionnaire.

The **CAGE questionnaire** asks the following questions:

1. Have you ever felt you needed to **C**ut down on your drinking?
2. Have people **A**nnoyed you by criticising your drinking?
3. Have you ever felt **G**uilty about drinking?
4. Have you ever felt you needed a drink first thing in the morning (**E**ye-opener) to steady your nerves or to get rid of a hangover?

2.5.4 Sleep and sleep quality

Measures of sleep were included in the protocol to allow for exploration of the potential effects of insufficient sleep on cognitive outcomes. Researchers have demonstrated that both sleep quality and sleep duration are significantly associated to cognitive performance [205]. Two aspects of sleep were measured; sleep quality (Pittsburgh Sleep Quality Index-PSQI) and current sleepiness (Stanford Sleepiness Scale).

The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire that assesses sleep quality over a one month time period. The measure consists of 19 individual items, creating 7 components that produce one global score. The component scores consist of subjective sleep quality, sleep latency (i.e., how long it takes to fall asleep), sleep duration, habitual sleep efficiency (i.e., the percentage of time in bed that one is asleep), sleep disturbances, use of sleeping medication, and daytime dysfunction. Each item from the PSQI was combined to create a total score to measure overall sleep

quality which is highly correlated with cognitive performance, particularly tasks of attention and processing speed.

The Stanford sleepiness scale is a 7 point scale, 1 represents "Feeling active, vital, alert or wide awake" to 7 "No longer fighting sleep, sleep onset soon; having dream like thoughts". A cut-off of 4 or more indicates the participant is likely to be suffering from a lack of sleep.

2.5.5 Psychological health and wellbeing

As psychological wellbeing is highly correlated with cognitive status we also collected multiple measures to control for this.

2.5.5.1 Depression

The Centre for Epidemiologic Studies Depression Scale (CES-D) was selected to assess depression [206]. This scale was also used in TILDA and the MRU. The CES-D is a 20-item scale that is designed to assess depressive symptoms in the general population and measures the major components of depressive symptomatology, including depressive mood, feelings of guilt and worthlessness, psychomotor retardation, loss of appetite and sleep disturbance. Participants are asked to rate the frequency of symptoms along a 4-item scale.

2.5.5.2 Anxiety

The Hospital Anxiety Depression Scale – Anxiety subscale (HADS-A) [207], was also included in the study design. The HADS-A is a widely used 7-item self-report scale designed to briefly measure current anxiety symptoms in non-psychiatric hospital patients. The HADS-A has been validated for use in research and was also used in TILDA. Another advantage of this scale is that it is quick and easy to administer.

2.5.5.3 Perceived stress

There is substantial evidence demonstrating that stress may have a significant impact on cognitive functioning, and that prolonged exposure to stress may be relevant to cognitive deficits seen in many older adults [208].
The Perceived Stress Scale [209] measures the degree to which situations in one's life are perceived as stressful, attempting to measure overall stress rather than an individual's

response to particular stressors/events. The scale contains items designed to assess how unpredictable, uncontrollable and overloaded participants find their lives. The 10 item PSS (PSS-10) has maximum reliability, however, a 4 item version (PSS-4) is available for use. The PSS-4 was also included in the TILDA protocol, and use of this version therefore allows for comparability with nationally representative data.

2.5.5.4 Quality of life

The CASP-19 was included in the study design, CASP: "Control" "Autonomy" "Self-realisation" "Pleasure". The CASP measure is based on the theory of needs satisfaction, and defines quality of life by the degree to which an individual's needs are met across these domains [210]. These scales were developed with an older population in mind.

2.6 Physical assessments

2.6.1 Physical Activity

Physical activity was considered important as it reflect current physical abilities and there is considerable evidence for a relationship between cognitive function and physical activity [211]. The International Physical Activity Questionnaire-Short Form (IPAQ-SF) is a standardised objective measure designed for use in populations studies [212], and has been used in The Irish Longitudinal Study of Ageing. The IPAQ-SF provides a measure of energy expenditure – by allowing for the calculation of volume of activity by weighting each type of activity by its energy requirements defined in METs to yield a score in MET-minutes. METs are multiples of the resting metabolic rate, and a MET minute is computed by multiplying the MET score of an activity by the number of minutes for which this activity is performed. Both categorical and continuous variables are achievable. Vigorous, moderate and walking categories were employed and collected at pre-screening only.

2.6.2 Weight, Height and Body Mass Index (kg/m^2)

Participants height was measured using a wall mounted stadiometer. Footwear, heavy outer clothes (coat, jackets) and head wear (hat, scarf and other hair accessories) were removed. The participant was asked to stand with his/her back to the measuring rod

facing forward and straight ahead, feet together and knees straight. The head stop was lowered along the rod until it touched the participant's head.

Weight (kg) was measured using a calibrated seca weighing scale (with light clothes on, empty pockets and without shoes). BMI was calculated as weight (kg) divided by height (m^2) and was classified according to the World Health Organisation (WHO) criteria.

2.6.3 Waist to Hip Ratio (W:H)

To assess distribution of body fat, waist (narrowest curve of the trunk) and hip circumference (widest point) were measured using a seca measuring tape to calculate waist to hip ratio. W: H ratio is a good indicator of health risk, similar to the quantification of obesity by BMI.

2.6.4 Muscle strength- handgrip strength

In the present study handgrip strength (HGS) was a pre-specified secondary outcome measure for muscle strength for the physical component of the RCT. HGS affects everyday function and declines with age, and poor grip strength is associated with higher morbidity and mortality [213]. Measurement of handgrip strength was considered the most informative measure of muscle strength and has been previously demonstrated to be sensitive to changes in muscle strength in older adult populations and has been used in other large ageing studies including TILDA. Grip strength was measured using the Baseline Hydraulic hand dynamometer with an analogue reading scale in kilograms (kgs).

Participants with pain or swelling, inflammation, recent injury to their hand/wrist did not complete the test or if possible completed the test on one hand only. Each participant was asked to indicate their dominant hand, "What hand do you write with?" HGS was assessed according to standard procedures, in brief participants removed any jewellery before the test, the upper arm was kept tight against their body, sitting in an upright chair, and the participant was asked to squeeze the handle with maximum force for a few seconds. The value to the nearest whole number in kgs was recorded. As with

the TILDA study, two tests were recorded for each hand, alternating between hands, and starting with the dominant hand, as noted in Table 2.2.

Table 2. 2: Measures of Grip Strength

GRIP D1	*Grip strength 1 for dominant hand (kg)*
GRIP ND1	Grip strength 1 for non-dominant hand (kg)
GRIP D2	Grip strength 2 for dominant hand (kg)
GRIP ND2	Grip strength 2 for non-dominant hand (kg)
GRIP D mean	Mean grip strength for dominant hand (kg)
GRIP ND mean	Mean grip strength for non-dominant hand (kg)

Gender and BMI-specific cut-offs for weak handgrip strength were applied to assess poor handgrip status [214], criteria is detailed in Table 2.3.

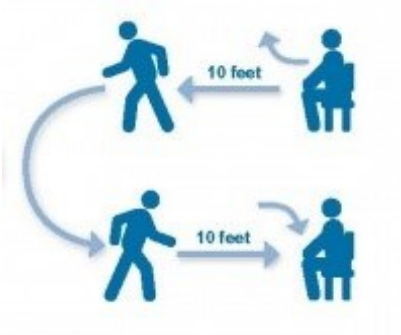
Table 2. 3: Gender and BMI- specific cut-offs for poor muscle function

	Criteria 1	*Criteria 2*	*Criteria 3*	*Criteria 4*
		Males		
BMI	≤24.04	24.05-26.04	26.05-28.04	>28
HGS (kg)	≤29	≤30	≤30	≤32
		Females		
BMI	≤23.04	23.05-26.04	26.05-29.04	>29
HGS (kg)	≤17	≤17.3	≤18	≤21

2.6.5 Muscle performance- Timed-up-and-Go test

To assess physical performance the "Timed-Up-and-Go" test (TUG) was employed. This was a secondary outcome of the RCT and assessed at 0, 3 and 6 months. TUG assesses proximal muscle strength (standing from a chair), balance (turning), and executive function (following the five stage command), in addition to gait speed. Time taken to complete this task was recorded using a stop watch in sec.ms. TUG is supported by the EWGSOP as a primary measure of sarcopenia and has been included in the physical assessment of other national ageing studies, such as TILDA [215].

The TUG test is designed to assess functional ability and falls risk and the cut-offs varying depending on the population being tested and the outcome of interest. The cut-offs for TUG in community-dwelling older adults range from 13.5-23.7 for falls risk [216]. A cut-off to predict incident disability in healthy, community-dwelling older adults of 13.0 seconds, with others indicating <12 seconds reflecting normal mobility. Researchers recognise the choice depends on the average ability of the sample population. A cut-off of >10 seconds was deemed as impaired performance for highly functioning older adults [217].



2.6.6 Muscle function – concurrent low HGS and poor physical performance (TUG)

A combined scores was created to categorise participants by functional ability. Poor muscle function was defined as low HGS (BMI and gender cut-offs as detailed in Table 2.2) AND slow TUG scores (>10 seconds).

2.7 Participant feedback survey

Once the trial was completed, anonymous questionnaires (Appendix 16) were posted to collect qualitative and quantitative data on trial participants. Areas of interest included participant motivations, concerns, and discouraging factors in the form of open ended questionnaires. Questionnaires were written using plain English and all study participants were deemed highly literate by assessment of premorbid IQ using the National Adults Reading Test. All participants gave written informed consent to take part in the follow-up study. Final serum 25(OH)D blood results were sent to participants GP, on study completion and after unblinding (Appendix 17).

2.8 Statistics

Descriptive statistics and differences are reported as mean ± standard deviations (SD) for all variables unless otherwise stated. Normality for continuous variables was checked visually using gladder plots and Shapiro-Wilk statistical testing. Box plots were utilised to identify significant outliers with corresponding lv analysis of mild or extreme values. Comparison of baseline characteristics was conducted using an independent t-test or Chi-Square for normally distributed data and Mann-Whitney U for non-parametric variables. Paired t-tests were applied to assess within-group differences and McNemar's test was used to check for statistical proportional differences within matched group pairs. ANCOVA was used to adjust for relevant factors between-groups at 6 months. When significance was detected, at an alpha value of <0.05, or close <0.10, corresponding effect sizes were reported as Cohen's *d*.

All analyses were performed using STATA Corp 14.0 software (Stata Corp LP).

2.9 Study Two. The ELSA Study (Chapter 3 and Chapter 6)

The ELSA Study is an ongoing nationally representative study of health in England. ELSA consists of men and women born on or after the 29 February 1952. The Health Survey of England [13] recruited participants using multistage stratified probability sampling with postcode sectors selected initially and household addresses thereafter. The ELSA study sample was selected from the HSE sample in 1998, 1999, and 2001 and biennial follow-up is ongoing since. Further details of ELSA have been reported previously [218].

In Chapter 3 and 6, cross-sectional data from Wave 6 of the ELSA study was used (2012-2013), as this was the first time 25(OH)D concentrations were collected for the new ELSA study sample. This large population dataset was used to investigate the prevalence and determinants of vitamin D deficiency in older adults (Chapter3) and in a further study to explore serum 25(OH)D concentrations and muscle parameters in older adults (Chapter 6).

2.9.1 Ethical approval

The ELSA study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the London Multicentre Research Ethics Committee (MREC 01/2/91). https://www.elsa-project.ac.uk/ Written informed consent was obtained from all participants.

2.10 Study outcome measures

2.10.1 Serum 25(OH)D measurement, deficiency criteria and related factors

Serum 25(OH)D was assessed from fasting blood sample during collected during the nurses' visit of Wave 6. All blood sampling occurred from January 2012- July 2013. The analysis of blood samples were carried out at the Royal Victoria Infirmary (Newcastle upon Tyne, UK). Concentrations of 25(OH)D was analysed using DiaSorin Liaison 25-hydroxyvitamin D immunoassay, which yields a lower detection limit of 3.0nmol/L[219]. The CV values ranged from 8.7 to 9.4%. All assays were performed in duplicate. The laboratory responsible for analysis participated in the Internal and the Vitamin D External Quality Assessment Schemes (DEQAS). Vitamin D deficiency was

defined according to two criteria, the IOM guideline [220] for serum 25(OH) D levels (<30nmol/L; termed deficiency) and the cut-off <50nmol/L termed deficiency by Endocrine Society guidelines (ES), to allow for comparability with other reported prevalence rates globally. Extreme outliers were defined as those with 25(OH)D >250nmol/L (>100ng), none were identified in the population, therefore, all 25(OH)D samples were included. Season was categorised using the extended vitamin D calendar for summer (April-Sept) and winter (Oct-March) [14].

Other health behaviours which may explain 25(OH)D status was available and included; sun holiday travel within the past 12 months (yes/no) and supplement defined as prescribed vitamin D and calcium use as required for treatment of osteoporosis. No information on other dietary supplements, including multivitamin and 'over the counter' vitamin D supplements was available in the dataset.

2.10.2 Handgrip strength

Gender and BMI-specific cut-offs for weak handgrip strength were applied to assess weak handgrip status, in line with the RCT study as detailed in Table 2.3. The test was completed by all participants who were willing to take it, but with certain exclusions (if participants had swelling or inflammation, severe pain or a recent injury, or if they had had surgery to the hand in the preceding 6-months). If there was a problem with only one hand, measurements were taken using the other hand. Three values were recorded for each hand, starting with the non-dominant hand and alternating between hands. The dynamometer used was the 'Smedley's for Hand' Dynamo Meter, scale 0–100 kg.

2.10.3 Short physical performance battery (Chapter 6 only)

The short physical performance battery (SPPB) was used as a primary outcome for muscle performance and included in cross-sectional analysis in Chapter 6. An investigation into vitamin D status and muscle function in community-dwelling older adults, aged >60 years. Details of the test are listed below. The SPPB evaluates balance, gait, strength and endurance by examining an individual's ability to complete at task of balance (a), walk 8ft (b) and rise from a chair 5 times or more (c) [221]. Each individual test is scored and combined to give a final composite score (Table 2.4), which is

approved as a validated measure of physical performance in the diagnosis of sarcopenia with meaningful changes are available for older adult populations [214, 222].

Results from each test are scored according to the criteria outlined below, in Table 2.4.

Static Balance Test

Test score were measured for; A: Side-by-Side B: Semi-Tandem C: Full-Tandem

Chair Rise

No hands chair rise, fold arms across chest and rise to standing as fast as possible.

Rise 5 times if aged ≥70 years. Rise 10times if aged <70 years.

Gait Speed

Participants were asked to walk, at their "normal" pace, not sprint, for a distance of 8 metres. Once they crossed the start line, a stop watch recorded in seconds, the time to reach the finish line.

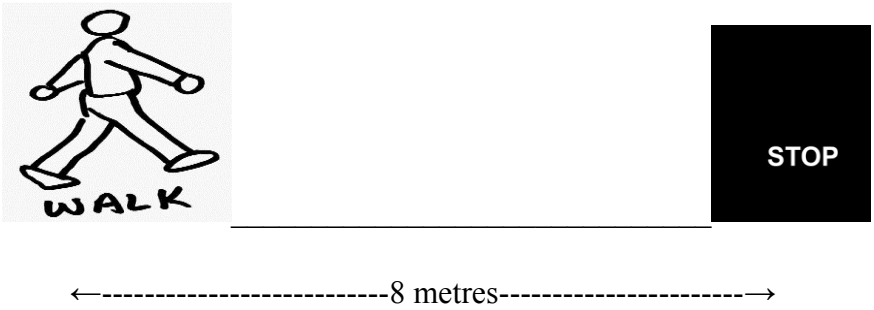


Table 2. 4: Short Physical Performance Battery scoring criteria (Chapter 6 only)

Static Balance Domain				
	Side-by-side	**Semi-tandem**	**Full-tandem**	
Points	1	2	3	4
Time	10	10	3,9	10
Gait Speed				
	8ft. Walking test			
Points	1	2	3	4
Time	≥5.7	4.1-5.6	3.2-4.0	≤3.1
Chair Rise Domain				
	Rise to stand			
Points	1	2	3	4
Time	≥16.7	16.6-13.7	13.6-11.2	≤11.1

Time: measured in seconds; Total score: 0-6; poor performers, 7-9; at risk, 10-12; high performers

A short overview of particular methods relevant to each chapter are presented within each results chapter also. Statistical analysis is detailed in full within each chapter.

Chapter 3: An investigation of the prevalence and associated determinants of vitamin D deficiency in community-dwelling older adults, residing at latitudes 50-55°N.

3.1 Introduction

We have described in Chapter 1 part A that several known age-related adverse health outcomes, including bone loss, fracture risk and falls incidence is associated with suboptimal serum 25-hydroxyvitamin D [25(OH)D] status [223-225] with further evidence for non-skeletal roles in carcinogenesis, immune function, cardiovascular disease, dementia and all-cause mortality [226, 227]. Cross-sectional studies demonstrate that vitamin D concentrations decline with increasing age, and deficiency is more commonly observed in older adults [228], largely attributable to physiological, social and lifestyle changes [134]. Despite increased emphasis on the importance of initiatives to improve vitamin D status in older populations [229], prevalence rates for vitamin D deficiency continue to be of concern [228, 230].

The reported prevalence of vitamin D deficiency in older populations across Europe and the UK vary considerably, with studies suggesting rates between 0-77% [228, 231, 232]. This variance is attributable in part to methodological differences across studies including definitions of vitamin D deficiency, geographic location, season, sample size, vitamin D assay and characteristics of study cohorts such as health status, gender, age and institutional and community settings. Serum 25(OH)D is considered the most appropriate biomarker to quantify vitamin D status, as it measures both dietary vitamin D (acquired from foods and dietary supplements) as well as endogenously formed cholecalciferol (vitamin D_3) by the action of solar UVB radiation on 7-dehydrocholesterol [233]. The cut-off criteria for 25(OH)D, however, remains under debate. The Institute of Medicine defines vitamin D deficiency as measured serum 25(OH)D levels <30nmol/L, for prevention of metabolic bone diseases [220]. Many researchers argue this cut-off is overly conservative for general health and application of the higher criteria for deficiency, namely <50nmol/L is widely used. Recently, the prevalence of hypovitaminosis D among community-dwelling adults was estimated at 17.5% (<30nmol/L) and 48.4% (<50nmol/L) in the Longitudinal Ageing Study Amsterdam [162, 87].

Vitamin D deficiency in older adults is multifactorial, influenced by gender, season, supplement use, age-related behavioural changes including clothing and less time spent outdoors, health status and age-related changes in vitamin D metabolism [234, 235, 36, 236-

[238]. In general, for white-skinned individuals living in northerly countries (UK and Ireland) where food fortification is not mandatory, the majority of vitamin D comes from UVB exposure and to a lesser extent from the diet [(169)]. In Northern Europe, countries of latitudes greater than 40°N, comparable to the north of Madrid, have inadequate UVB exposure for cutaneous vitamin D synthesis for 6 months of the year (October to March)[(14)], this includes England located at 50-55°N. The influence of geographic location and latitude of residence on vitamin D status in older populations have been reported, though regional and north-south variation within countries is less clear, since the latter may be confounded by differences in lifestyle and social behaviours [(239, 240, 36)].

In the present chapter, we investigated vitamin D status in a large population of community-dwelling adults aged over 50 years residing at northern latitudes, based on data from the English Longitudinal Study of Ageing (ELSA).

The specific aims of the present study were;

- To establish the prevalence of serum 25(OH)D deficiency in older adults, overall and according to gender, vitamin D supplement users, and season
- To determine the prevalence of serum 25(OH)D deficiency by region of residence
- To investigate the determinants of vitamin D deficiency, with a particular interest in the contribution of latitude

3.2 Subjects and Methods particular to this chapter

3.2.1 Study design and population

The current study includes participants from the ELSA Study, ethics and the general study design are detailed in the methods Chapter 2. For the present analysis cross-sectional data from Wave 6 was used (January 2012-July 2013), as this was the first time 25(OH)D concentrations were collected for the new ELSA study sample.

A total of 10601 subjects were interviewed at Wave 6. For the present analysis, participants were included if they were aged <50 years old, identified as a resident in England and had a measured concentration of 25(OH)D, the latter requiring a blood sample collected by nurses visit. In total, 7731 completed the nurse visits, with the most common reason of missing or unavailable biological data to be an unwillingness to consent or ineligibility (bleeding disorders or anticoagulant use). After excluding those who did not complete the core and nurses data collection at Wave 6, or had missing data for other key variables, the final analytical sample comprised of 6004 subjects, which is illustrated in a schematic flowchart in Figure 3.1.

3.2.2 Study measurements

3.2.2.1 Serum 25(OH)D measurement, deficiency criteria and related factors

Serum 25(OH) D was assessed from fasting blood sample collected during the nurses' visit of Wave 6. All blood sampling occurred from January 2012-July 2013. Concentrations of 25(OH)D were analysed using DiaSorin Liaison 25-hydroxyvitamin D immunoassay. Full details regarding serum sampling and processing are detailed in Chapter 2.

Vitamin D deficiency was defined according to two criteria, the IOM guidelines [220] for serum 25(OH)D levels (<30nmol/L; termed IOM deficiency) and the Endocrine Society cut-off (<50nmol/L termed ES deficiency), to allow for comparability with other reported prevalence rates globally. Extreme outliers were defined as those with 25(OH)D >250nmol/L (100ng), none were identified in the population, therefore, all 25(OH)D samples were included. Season was categorised using the extended vitamin D calendar for summer (April-Sept) and winter (Oct-March) [14]. Other health behaviours which may explain 25(OH)D status was available and included; sun holiday travel

within the past 12 months (yes/no) and medically prescribed vitamin D supplement use as required for treatment of osteoporosis.

3.2.2.2 Classification by region of residence and latitude

To protect the anonymity of the research participants, detailed postcode information was not available for this analysis. However, Government Offices for the Region (GOR) data were available for each participant, established by the national archives, which is regarded as the primary classification for the presentation of regional statistics [241]. Each participant has been classified by GOR region for location, to which we applied a central most point of latitude, established by corresponding latitudes from weather stations by the Centre for Environmental Data Analysis (CEDA) and Google Earth. England ranges in latitude, from Lizard point as the most Southerly point on the mainland (49°57'30"N) and Marshall Meadows Bay most northerly (55° 48' 18.3"). Potential maximal distance from the central point within each region was 1°. GOR divides England into nine regions, for the present study regions were combined to compare between the North, Midlands and the South. Those residing in the North East, North West and Yorkshire and The Hummer were defined as the North; those in East Midlands, West Midlands and East England were defined as the Midlands; and those in the South East, South West and London were defined as the South. Those identified as residing in Scotland, Wales and North Ireland were not included in this analysis (n=21).

3.2.2.3 Covariates

From the wave 6 data, we identified important demographic, socioeconomic, lifestyle and clinical contributors relevant to vitamin D status, older adults and location of residence as covariates in the analyses. Age was recorded as a continuous number until 90 years (with ages above 90 collapsed to the value of 91 to ensure anonymity), age category was also considered an important variable [age ≥60 years, retirement age (≥65 years), sex, marital status (married, single never married, divorced/separated or widowed), education level obtained (minimum O-level education including foreign equivalents), and employment status (employed, unemployed, retired) were considered important socio-demographic factors. Ethnicity was considered, however, 99% are White English.

Health behaviours related to alcohol consumption [within the last 12 months (categorised to less than daily, daily, and times per week); smoking status (current smoker, past smoker, never smoked); subjective physical activity levels (sedentary, mild, moderate and vigorous ≥1 time per week). Polypharmacy defined as the use of ≥5 daily medications and self-reported health (poor, fair, good, very good and excellent). Whether the participant had a long standing illness that was limiting was also considered.

Anthropometric measures included BMI, calculated using the standard formula (kg/m²) from weight and height nurse obtained measures. Physical performance was measured using the 8ft gait speed walking test (seconds), available only for a subgroup of participants (those aged ≥60 years of age).

3.2.2.4 Data analysis

Descriptive statistics are presented as means and SD or counts and proportions for demographic and lifestyle characteristics. Overall prevalence rates are presented as crude and weighted results. The weighting strategy aimed to minimise any bias from differential non-response in obtaining a blood sample. Differences between the groups were examined by independent student t-tests, $\chi 2$ or ANOVA as appropriate. Normality was assessed visually using histogram matrix plots and ladder tables to identify appropriate transformations [242], and Monte Carlo simulations to identify outliers [243].

Weighted multiple logistic regression analysis was used to explore the determinants of 25(OH)D deficiency and presented as odds ratios (OR) and 95% confidence intervals (CI) to determine the direction of the association between 25(OH)D deficiency and relevant covariates. Tests for linear relationships were performed by the inclusion of continuous predictor variables to the model and were reported as OR for a single unit change with 95% CI. In adjusted models covariates were selected based on previous research findings and following stepwise regression to test the contribution of each predictor (excluded $p>0.2500$). The contribution of covariates to deficiency was examined by including each set of covariates as models in the following order; model 1, IOM vitamin D deficiency defined as <30nmol/L and socio-demographics, health-related behaviours, physical factors and health conditions. In model 2, ES vitamin D deficiency defined as <50nmol/L and socio-demographics, health-related behaviours,

physical factors and health conditions. Corresponding unadjusted results are also reported.

All analyses were performed using STATA Corp 14.0 software (StataCorp LP).

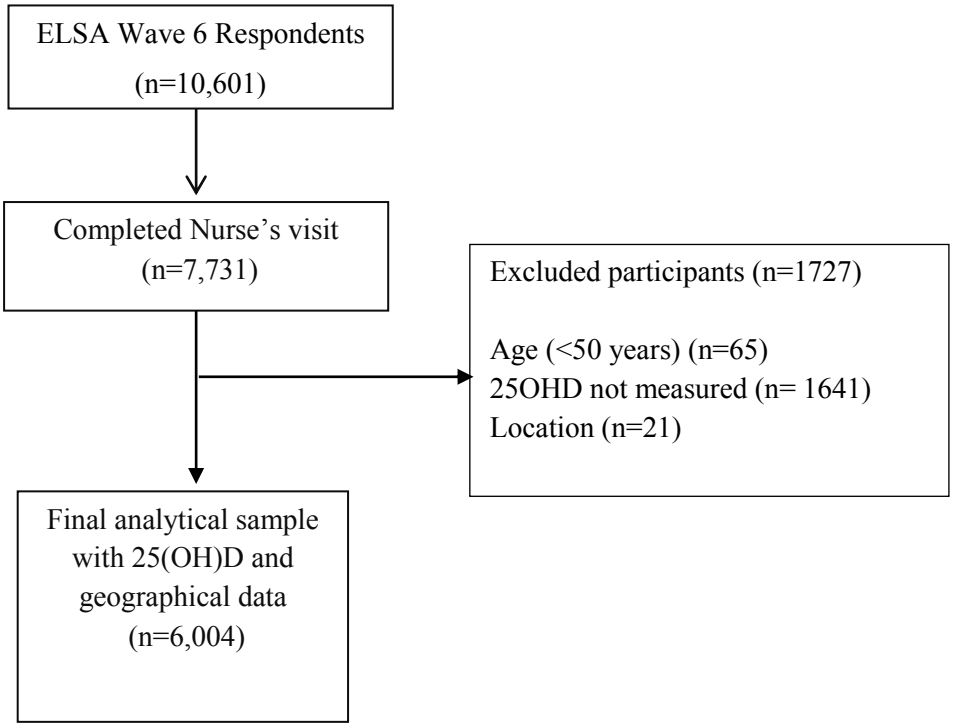


Figure 3.1: Participant exclusion for this study, with reasons

3.3 Results

3.3.1 Characteristics of study population

A total of 6004 individuals aged 50 years or older were included in this study. The mean sample age was 66.4 (SD 8.8) years, with those aged 60-69 representing the largest age category (41.0%). Table 3.1 and Table 3.2, show the characteristics of the study population overall, stratified by vitamin D deficiency and by region of residence. Overall, 55% were female and more than 73% had achieved a minimum O-level education or equivalent. The majority of individuals (76.4%) rated their health as 'good' or 'better than good', a quarter (25.7%) reported the use of 5 or more medications daily and 31% reported a longstanding limiting illness. Consumption of alcohol at least once per week was estimated at 63.9% and current smoking status at 12%. Overweight and obese BMI was prevalent at 42.3% and 29.7%, respectively. Few study participants (4.4%) reported taking a prescribed vitamin D supplement, which was indicated for osteoporosis treatment; habitual vitamin D supplements intakes were not captured.

Across regions, in this cohort, the largest sample resided in South East England (n=1047) and the fewest in London (n=483), as detailed in Table 3.4. Characteristics of the cohort by region were significantly different for both educational attainment and health indicators. Those residing in the South of England had the highest proportion of participants with a minimum of O-level qualifications (78.6%), detailed in Table 3.2, the highest frequency of moderate physical activity (67.9%) and of 'excellent' self-reported health (14.4%) and the lowest rates of obesity (26.6%), limiting illness (28.3%) and polypharmacy (32.7%). There were, however, no statistically significant regional differences for vitamin D factors, namely season of blood draw, sun holiday travel or vitamin D supplement use.

Table 3. 1: Participant characteristics stratified by vitamin D deficiency (IOM and ES)

	Overall (n=6004)	**<30nmol/L (n=1423)**	**<50nmol/L (n=3317)**
	N (%) ± SD	**N (%)** ± SD	**N (%)** ± SD
Socio-demographic factors			
Age, years	66·4 ± 8.8	66·4 ± 9.5	66·3 ± 9.1
50-59	1476 (24·6)	399 (28·0)***	865 (26·1)**
60-69	2463 (41·0)	528 (37·1)***	1305 (39·3)**
70-79	1553 (25·9)	335 (23·5)*	830 (25·0)
80+	512 (8·5)	161 (11·3)***	317 (9·6)***
Female	3291 (54·8)	832 (58·5) **	1855 (55·9)
Education ≥O level	4392 (73·2%)	979 (68·8)***	2382 (71·8)**
No qualification	1340 (22·4)	383 (26·9)***	554 (20·6)**
Marital Status			
†Married	3997 (66·6)	830 (58·3)***	1925 (71·6)***
Single	320 (5·3)	96 (6·8)**	121 (4·5)**
Widow	763 (12·7)	240 (16·9)***	283 (10·5)***
Health and Lifestyle factors			
BMI kg/m^2	28·0 ± 4·9	28·9 ± 5·6***	27·2 ± 4·4***
Underweight	50 (0·9)	17 (1·2)	26 (0·9)
Normal	1578 (27·1)	311 (21·9)***	815 (31·2)***
Overweight	2461 (42·3)	501 (35·2)***	1180(45·1)***
Obese	1731 (29·7)	521 (36·6)***	595 (22·7)***
Physical Activity			
Vigorous (>1/wk)	1359 (22·6)	194 (13·6)***	773 (28·8)***
Moderate (>1/wk)	3933 (65·6)	768 (53·9)***	1917 (71·3)***
Mild (>1/wk)	4975 (82·9)	1114 (78·3)***	2285 (85·0)***
Current smoker	695 (11·6)	267 (18·8)***	213 (7·9)***
Alcohol, 5-6day/wk	356 (5·9)	62 (4·4)**	188 (6·9)**
No alcohol	646 (10·8)	243(17·1)***	423(12·8)***
SR Health			
Excellent	772 (12·9)	122 (8·6)***	436 (16·2)***
Very good	1908 (31·8)	368 (25·9)***	898 (33·4)**

Good	1901 (31·8)	442 (31·1)	853 (31·7)
Fair	1062 (17·7)	332 (23·3)***	387 (14·4)***
Poor	360 (6·0)	159 (11·2)***	114 (4·2)***
§**Limiting illness**	1887 (31·4)	554 (38·9)***	735 (27·4)***
Polypharmacy	1546 (25·7)	436 (30·6)***	932 (28·1)***
Vitamin D and related factors			
25(OH)D, nmol/L	48·7 ± 23·4	21·3 ± 5·1	31·7 ± 10·6
Winter blood	3564 (59·4)	1009 (70·9)***	1354 (50·4)***
‡Sun travel	3457 (57·6)	663 (46·6)***	1676 (62·4)***
VitD supp user	262 (4.4)	27 (10.3)***	72(27.5)***

SD, standard deviation; kg/m^2, kilograms per metre squared; SR, self-reported; winter, October to March.

†includes those first time, remarried and legally recognised civil partnership.

‡sun holiday within the last 12 months.

§ If longstanding illness is limiting.

Independent student t-test and χ^2 comparison between groups defined as deficiency <30nmol/L versus ≥30nmol/L and <50nmol/L versus ≥50nmol/L.

P value denoting significant levels between groups *P*<0.05*, *P*<0.01**, *P*<0.001***

Table 3. 2: Participant characteristics stratified by region of residence in England (n=6004)

	North (n=1692)	Midlands (n=2036)	South (n=2276)
	N (%) ± SD	**N (%)** ± SD	**N (%)** ± SD
Socio-demographic factors			
Age, years	66.3 ± 8.8	66.6 ± 8.9	66·3 ± 8.8
Female	950 (56·2)	1114 (54·7)	1227 (53·9)
Education ≥O level	1183 (69·9)	1420 (69·7)	1789 (78·6) ***
No qualification	429 (25·4)	521 (25·6)	390 (17·1)***
Marital Status			
†Married	1111(65·7)	1357 (66·7)	1566 (68·8)*
Single	99 (5·9)	116 (5·7)	168 (7·4)*
Widow	250 (14·8)	270 (13·3)	279 (12·3)*
Health and lifestyle factors			
BMI(kg/m²)	28·3 ± 5.1	28·2 ± 4.9	27·7 ± 4·8***
Underweight	21 (1·3)	11 (0·6)	18 (0·8)
Normal	417 (25·2)	512 (26·2)	649 (29·4)*
Overweight	674 (40·8)	832 (42·5)	955 (43·2)
Obese	541 (32·7)	601(30·7)	589 (26·6)***
Physical Activity			
Vigorous (>1/wk)	376 (22·2)	451 (22·2)	532 (23·4)
Moderate (>1/wk)	1055 (62·4)	1332 (65·4)	1546 (67·9)***
Mild (>1/wk)	1392 (82·3)	1714 (84·1)	1871 (82·2)
Current smoker	205 (12·1)	244 (11·9)	246 (10·8)
Alcohol 5-6 days/week	101 (5·9)	99 (4·8)	156 (6·9)
SR Health			
Excellent	189 (11·2)	256 (12·6)	327 (14·4)**
Very good	494 (29·2)	610 (29·9)	804(35·3)***
Good	545 (32·2)	664 (32·6)	692 (30·0)
Fair	349 (20·6)	369 (18·1)	344 (15·4)***
Poor	114 (6·7)	137 (6·7)	109 (4·8)**
§**Limiting illness**	576 (34·0)	668 (32·8)	643 (28·3)***
Polypharmacy	636 (37·6)	749 (36·8)	745 (32·7)***

Vitamin D related factors			
Winter blood	1011 (59·7)	1219 (59·9)	1334 (58·6)
‡Sun holiday travel	953 (56·3)	1200 (58·9)	1304 (57·3)
VitD supp user	84 (4.9)	85(4.2)	93(4.1)

SD, standard deviation; SR, self-reported; winter, October to March.

† Married includes those first time, remarried and legally recognised civil partnership.

‡ Within the last 12 months.

§ If self-reported long standing illness is limiting.

ANOVA comparison between North and Midlands using South as the reference group.

P value denoting significant levels between groups $p<0.05$*, $p<0.01$**, $p<0.001$***

Table 3. 3: Prevalence of vitamin D deficiency, stratified by age and gender (n=6004)

IOM Deficiency <30nmol/L (n=1423)				
%	**50-59** (n=469)	**60-69** (n=492)	**70-79** (n=325)	**80+** (n=137)
Overall	27.4*	20.4***	22.2***	32
Males	28.1	19.3	17.3	26.3
Female	26.9	21.5	26.1	36.8
†Season-Winter	32.9	24.3	27.8	35.6
ES Deficiency <50nmol/L (n=3317)				
%	**50-59** (n=1007)	**60-69** (n=1251)	**70-79** (n=785)	**80+** (n=273)
Overall	58.9	52.1***	53.6***	63.8
Males	59.0	51.8	50.1	58.2
Female	58.9	52.3	56.4	68.4
†Season-Winter	66.7	58.7	60.3	69.1
Prevalence of vitamin D deficiency, stratified by age and D supplement users‡ (n=262)				
N (%)	**50-59** (n=33)	**60-69** (n=105)	**70-79** (n=90)	**80+** (n=34)
<30nmol/L (n=27)	9 (27.3)	8 (7.6)	8 (8.9)	2 (5.9)
<50nmol/L (n=72)	15 (45.4)	25 (23.8)	19 (21.1)	13 (38.2)

IOM, Institute of Medicine; ES, Endocrine Society. †Winter defined by extended vitamin D calendar October to April.‡Reported taking prescribed Vitamin D supplement for osteoporosis treatment.

P value denoting significant levels between groups $p<0.05$*, $p<0.01$**, $p<0.001$***

3.3.2 Prevalence of 25(OH)D deficiency

Overall prevalence of vitamin D deficiency <30nmol/L and deficiency <50nmol/L was high at 23.7% (weighted 26.4%) and 55.3% (weighted 57.3%) respectively. For older age categories of ≥60 years and ≥65 years, vitamin D deficiency (<30nmol/L) and deficiency (<50nmol/L) were similar at 22.6% and 22.5% (weighted: 25.3% and 25.3%) and 54.2% and 53.9% (weighted: 56.1% and 55.9%) respectively. The highest prevalence of vitamin D deficiency (IOM) was noted in females aged 80 years and older (36.8%), with the lowest rates in males aged between 70-79 years (17.3%), detailed in Table 3.3. Mean 25(OH)D was 48.7 SD 23.5 (range; 9-239nmol/L).

Seasonal variation was demonstrated, as expected, with significantly higher prevalence of vitamin D deficiency (IOM, ES) in winter (28.3% and 62.0%) than summer (16.9 % and 45.2%) respectively. Consistent with this, seasonal variation in mean 25(OH)D status was 8.9nmol/L [54.1nmol/L vs. 45.1nmol/L (p<0.0001) for summer and winter season]. The majority of older adults classed as vitamin D deficient (IOM) were assessed in winter (71.0%), with the proportion matched (50.4% winter assessment) for those classed as deficient by the ES <50nmol/L cut-off.

Although the number of medically prescribed vitamin D supplement users were low (n=262, 4.4%), mean 25(OH)D was significantly higher in supplement users compared to non-users (65.8nmol/L vs. 47.9nmol/L, p<0.0001).

Table 3. 4: Serum 25(OH)D status by region in England, findings from the ELSA Study (n=6004). Prevalence (%) vitamin D deficiency (IOM) and vitamin D deficiency (ES), stratified by season‡ and region

					Vitamin D deficiency (IOM) <30nmol/L			Vitamin D deficiency (ES) <50nmol/L		
Region	**Overall (n=6004)**	**°N Latitude**	**†Mean 25OHD**	**SD**	**Overall** N=1423 (23·7%)	**Winter** N=1009 (28·3%)	**Summer** N= 414 (16·9%)	**Overall** N=3316 (55.2%)	**Winter** N=2210 (62.0%)	**Summer** N= 1106 (45.2%)
North East	375	54·9	46·4	23·3	28·8	36·4	20·6	61·6	70.3	52.2
Yorkshire & H	656	53·7	47·9	22·0	25·9	28·9	14·8	56.3	60.3	48.7
North West	661	53·6	47·5	23·9	25·9	29·4	20·3	60.2	66.4	51.3
East Midlands	658	52·8	48·1	23·0	24·9	30·5	17·6	54.6	62.0	44.7
West Midlands	655	52·5	45·4	24·9	29·8	24·5	21·3	61.2	68.1	48.9
East England	723	52·2	50·6	23·7	29·6	31·3	16·4	52.0	56.5	45.6
London	483	51·5	45·6	23·7	29·6	31·3	26·4	62.1	65.9	54.6
South East	1047	51·1	52·3	23·5	17·9	21·2	13·8	48.9	56.0	39.7
South West	746	50·4	50·6	22·1	19·6	26·6	10·3	49.6	60.7	34.9

†25(OH)D, nmol/L.

‡ Season defined by vitamin D calendar; summer April-October.

3.3.3 Vitamin D status by region of residence and latitude

Vitamin D deficiency for regions across England is summarised in Table 3.4. The north-south gradient was noted for the prevalence of vitamin D deficiency (IOM), deficiency (ES) and serum 25(OH)D concentrations. The frequency of vitamin D deficiency (IOM) was significantly higher in residents of the north (p<0.004) and the midlands (p<0.001) compared to the south of England. Consistent with this, mean serum 25(OH)D was significantly lower the north (p<0.001), and the midlands (p<0.002) compared to the south. Similarly, when examined by latitude, mean 25(OH)D increased with decreasing latitude. Data from London, however, did not follow this pattern, with average 25(OH)D status of 45.6nmol/L in Table 3.4.

The highest prevalence of vitamin D deficiency (IOM) was identified in the North East region during winter (36.4%) and the lowest in the South West in summer (10·3%) as detailed in Table 4.4. Similarly, vitamin D deficiency (ES <50nmol/L) was highest in the North East in winter (70.3%), though remained high at 53.2% during summer. The lowest prevalence (34.9%) of deficiency (ES) was noted in the South West during summer. The prevalence increased from the most Southerly region (50.4°N) to the most Northerly (54.9°N) by a similar magnitude of ~10%, irrespective of the season as shown in Table 3.4.

3.3.3 Determinants of vitamin D deficiency

After adjusting for pre-defined covariates the multivariate logistic regression analysis in model 1, revealed that being aged 80+, female, of non-white ethnicity, widowed, obese, current smoker and self-reporting fair or poor health was statistically significant negative correlates for vitamin D deficiency (IOM) as detailed in Table 3.5. Factors such as being retired [0.79 (0.65, 0.95)], normal BMI [0.81 (0.67, 1.00)], regular vigorous physical activity [0.69 (0.59, 0.83), vitamin D supplement use [0.24 (0.18, 0.33)], sun travel [0.86 (0.75, 0.99)] and summer season [0.47 (0.40, 0.56)] were statistically significant positive correlates of vitamin D deficiency (IOM). Similar variables contributed to the model for vitamin D deficiency (ES) as shown in Table 3.6, apart from female gender (p<0.27), which was no longer a statistically significant determinant. Whereas residing in the midlands and moderate alcohol consumption and

latitude were positive correlates of vitamin D deficiency (ES) can be seen in Table 3.6. Among the independent correlates, non-white ethnicity was the strongest indicator of vitamin D deficiency (ES); compared with White English [4.67 (2.57, 8.51)].

Residing in the south of England was associated with a 22.8% lower risk of deficiency (IOM) [0.78 (0.64-0.95) (p<0.003)] in Table 3.5. For each 1° increase in latitude, individuals were 11% more likely to be vitamin D deficient (IOM) [1.11 (1.04-1.17, p<0.001)] and 9% more likely to be vitamin D deficient (ES) [1.09 (1.04, 1.15), p<0.001]. This model included important environmental determinants of vitamin D status including season of blood draw, sun holiday travel, vitamin D supplement use which was statistically significantly associated with vitamin D deficiency, as shown in Table 3.5 and 3.6.

Models 1 and 2 were further applied to a subgroup of the population aged ≥60 years. Of note, poorer physical function assessed by walking speed was available in participants aged over 60 years, was a significant contributor to the model, these findings are detailed in Table 3.7. Results showed that for each 1-second increase in walking speed (i.e. indicating slower performance) there was a 17% increased risk of vitamin D deficiency (IOM) [1.17 (1.09, 1.26) (p<0.001)]. Poor physical performance remained a statistically significant predictor in model 2 in Table 3.8. In Chapter 6 of this thesis, we investigate a potential relationship between serum 25(OH)D concentrations and muscle function in this subsample (aged >60 years) in greater detail.

Table 3. 5: Weighted logistic regression for IOM vitamin D deficiency (<30nmol/L) in ELSA study participants (n=6004)

	Unadjusted Model 1		Adjusted Model 1	
Demographic variables	**OR**	**(95%CI)**	**OR**	**(95%CI)**
Female	1·21**	(1·04, 1·41)	1·23**	(1·04, 1·44)
Age -50-59		1		1
60-69	0·71***	(0·59, 0·84)	0·85	(0·69, 1·05)
70-79	0·79*	(0·66, 0·97)	0·89	(0·68, 1·16)
80+	1·31*	(1·01, 1·69)	1·42*	(1·01, 1·96)
Non-white ethnicity	3·59***	(2·4, 5·4)	3·8***	(2·39, 6·05)
Marital Status- Widow	1·59***	(1·31, 1·93)	1·44***	(1·15, 1·79)
Single	1·37*	(1·04, 1·82)	1·33	(0·98, 1·81)
Employment-retired	0·79**	(0·69, 0·92)	0·79*	(0·65, 0·95)
Region- North		1		1
Midlands	0·93	(0·78, 1·11)	0·95	(0·79, 1·14)
South	0·79*	(0·66, 0·96)	0·78**	(0·64, 0·95)
†**Latitude** (°N)	1·11***	(1·05,1·17)	1·11***	(1·04, 1·17)
Modifiable Health and Lifestyle factors				
BMI -Normal	0·73***	(0·61, 0·87)	0·81*	(0·67, 1·00)
Obese	1·59***	(1·36, 1·86)	1·32**	(1·09, 1·58)
Current smoker	2·18***	(1·78, 2·67)	1·88***	(1·51, 2·34)
SR health-Excellent		1		1
Very good	1·16	(0·86, 1·56)	1·02	(0·75, 1·37)
Good	1·47	(1·01, 1·96)	1·16	(0·86, 1·57)
Poor	2·26**	(1·67, 3·06)	1·49*	(1·08, 2·07)
Fair	3·49***	(2·43, 5·02)	1·99***	(1·33, 2·96)
VitD supplement use	0·39***	(0·25, 0·64)	0·28***	(0·17, 0·45)
Season (summer)	0·52***	(0·45, 0·61)	0·47***	(0·40, 0·56)
Sun travel	0·54***	(0·47, 0·63)	0·74***	(0·63, 0·86)
PA- Vigorous (>1/wk)	0·46***	(0·37, 0·57)	0·68***	(0·55, 0·86)
Moderate (>1/wk)	0·5***	(0·43, 0·58)	0·74***	(0·62, 0·88)

OR, odds ratios; CI, confidence interval; SR, self-reported; PA, physical activity.

†Replaced region in the adjusted model.

P value denoting significant levels between groups $p<0{\cdot}05$*, $p<0{\cdot}01$**, $p<0{\cdot}001$***.

Table 3. 6: Weighted logistic regression for (ES) vitamin D deficiency (<50nmol/L) in ELSA study participants (n=6004)

	Unadjusted Model 2		Adjusted Model 2	
Demographic variables	**OR**	**(95%CI)**	**OR**	**(95%CI)**
Female	1·06	(0·93, 1·20)	1·08	(0·78, 1·24)
Age 50-59		1		1
60-69	0·76***	(0·65, 0·88)	0·94	(0·78, 1·12)
70-79	0·82*	(0·69, 0·96)	1·03	(0·83, 1·29)
80+	1·28*	(1·00, 1·64)	1·67***	(1·23, 2·28)
Non-white ethnicity	4·38***	(2·57, 7·44)	4·67***	(2·57, 8·51)
Marital Status- Married	0·72***	(0·63, 0·81)	0·89	(0·78, 1·04)
Widow	1·46***	(1·22, 1·74)	1·27*	(1·01, 1·60)
Single	1·39**	(1·08, 1·81)	1·29	(0·94, 1·77)
Retired	0·78***	(0·69, 0·89)	0·73***	(0·63, 0·86)
Education- O-level min.	1·22**	(1·06, 1·41)	0·93	(0·79, 1·09)
Region- North		1		1
Midlands	0·84*	(0·72, 0·98)	0·83*	(0·69, 0·97)
South	0·81**	(0·69, 0·94)	0·79**	(0·66, 0·93)
†**Latitude** (°N)	1·09***	(1·04, 1·15)	1·09***	(1·04, 1·15)
Modifiable Health and Lifestyle factors				
BMI- normal	0·68***	(0·59, 0·79)	0·81**	(0·69, 0·96)
obese	1·78***	(1·54, 2·05)	1·53***	(1·30, 1·79)
Alcohol intake 5-6 week	0·72**	(0·56, 0·94)	0·98	(0·75, 1·28)
3-4week	0·65***	(0·55, 0·78)	0·77**	(0·64, 0·95)
Current smoker	1·97***	(1·59, 2·42)	1·8***	(1·45, 2·25)
SR Health- Excellent		1		1
Very good	1·36**	(1·09, 1·68)	1·21	(0·97, 1·52)
Good	1·59***	(1·29, 1·96)	1·29*	(1·02, 1·62)
Fair	2·24***	(1·78, 2·82)	1·59***	(1·23, 2·05)
Poor	2·48***	(1·79, 3·43)	1·55*	(1·09, 2·23)
VitD supplement use	0·32***	(0·23, 0·43)	0·24***	(0·18, 0·33)
Sun travel	0·66***	(0·58, 0·75)	0·86*	(0·75, 0·99)
Season (summer)	0·51***	(0·45, 0·58)	0·47***	(0·41, 0·54)

PA- Vigorous (>1/wk)	0·54***	(0·46, 0·63)	0·69***	(0·59, 0·83)
Moderate (>1/wk)	0·62***	(0·55, 0·71)	0·86	(0·74, 1·01)

[†]Replaced region in the adjusted model.

OR, odds ratios; CI, confidence interval; SR, self-reported; PA, physical activity.

P value denoting significant levels between groups $p<0·05$*, $p<0·01$**, $p<0·001$***.

Table 3. 7: Weighted logistic regression for IOM vitamin D deficiency (<30nmol/L) in ELSA participants, aged ≥60 years (n=4528)

	Adjusted Model <30nmol/L	
Demographic variables	**OR**	**(95%CI)**
Female	1·35	1·13, 1·63***
Age, years	1·01	0·99, 1·02
Non-white ethnicity	2·54	1·41, 4·58**
Marital Status		
Widow	1·47	1·16, 1·87**
Single	1·32	0·89, 1·94
Employment, retired	0·72	0·58, 0·89**
Region- North		
Midlands	0·89	0·71, 1·09
South	0·8	0·65, 0·99*
Modifiable Health and Lifestyle factors		
BMI - Normal	0·83	0·66, 1·04
Obese	1·27	1·04, 1·56*
Current smoker	2·26	1·74, 2·94***
SR Health - Excellent		1
Very good	1·49	1·08, 2·08*
Good	1·38	0·99, 1·92*
Fair	1·65	1·15, 2·36**
Poor	1·9	1·15, 3·15*
VitD supplement use	0·17	0·09, 0·32***
Sun travel	0·66	0·56, 0·79***
Season (summer)	0·45	0·37, 0·54***
PA- Vigorous (>1/week)	0·59	0·46, 0·76***
Moderate (>1/week)	0·89	0·74, 1·09
Gait Speed (seconds)	1·17	1·09, 1·26***

OR, odds ratios; CI, confidence interval; SR, self-reported; PA, physical activity. *P* value denoting significant levels between groups $p<0·05$*, $p<0·01$**, $p<0·001$***.

Table 3. 8: Weighted logistic regression for ES vitamin D deficiency (<50nmol/L) in ELSA participants, aged ≥60 years (n=4528)

	Adjusted Model 2 <50nmol/L	
Demographic variables	**OR**	**(95%CI)**
Female	1·12	0·97, 1·29
Age, years	1·01	1·00, 1·03*
Non-white ethnicity	2·92	1·67, 5·13***
Marital Status- Married	0·79	0·66, 0·94**
Widow	1·25	0·97, 1·61
Single	1·38	0·96, 1·99
Employment, retired	0·69	0·59, 0·83***
Region- North		1
Midlands	0·89	0·74, 1·07
South	0·8	0·67, 0·96*
Modifiable Health and Lifestyle factors		
BMI- Normal	0·86	0·72, 1·03
Obese	1·59	1·34, 1·89***
Alcohol intake, 5-6 days/week	0·85	0·63, 1·16
3-4 days/week	0·78	0·64, 0·96*
Current smoker	2·1	1·62, 2·73***
SR Health Excellent		
Very good	1·58	1·25, 2·00***
Good	1·42	1·12, 1·80**
Fair	1·64	1·24, 2·17***
Poor	1·89	1·19, 2·89*
VitD supplement use	0·16	0·11, 0·24***
Sun travel	0·81	0·69, 0·94**
Season (summer)	0·47	0·41, 0·54***
PA-Vigorous (>1/week)	0·67	0·56, 0·80***
Moderate (>1/week)	0·89	0·76, 1·06
Gait Speed (seconds)	1·1	1·03, 1·18**

OR, odds ratios; CI, confidence interval; SR, self-reported; PA, physical activity. *P* value denoting significant levels between groups *P*<0·05*,

$P<0{\cdot}01$**, $P<0{\cdot}001$***.

3.4 Discussion

In the present study we found that year-round vitamin D deficiency, irrespective of definition, remains highly prevalent in a nationally, representative sample of community-dwelling adults, aged over 50 years, residing in England. We observed that 26.4% (1 in 4) were vitamin D deficient by IOM criteria and more than half were identified as vitamin D deficient (57.3%) by the ES criteria. Prevalence of deficiency remained high even during the summer months when 1 in 6 (16.9%) was defined as vitamin D deficient (IOM) and 45.2% were vitamin D deficient (ES). We also demonstrated a clear gender difference as the prevalence of deficiency was highest in females, particularly those aged over 80 years (36.8%), whilst males aged 70-79 represented the lowest rates of deficiency (17.3%). Supplement use was low (4.4%), but the protective effects were noted as supplement users had sufficient 25(OH)D status (65.8nmol/L). Additionally, we showed that even within a country of short ranging latitude (50.4-54.9°N), north-south gradient and incremental changes in latitude appeared to influence 25(OH)D status.

The prevalence of vitamin D deficiency identified in this study appeared to be higher in the context of similar published findings [239, 230]. To our knowledge, this is the largest nationally representative study of vitamin D status conducted in community-dwelling older adults in England and Europe. Previous figures from National Diet and Nutrition Survey (NDNS) in 1995, report prevalence rates of vitamin D deficiency (using a lower cut-off, <25nmol/L) at 19% for those aged over 65 years [244], and the Health Survey England in 2000, using the same deficiency criteria, established rates of 15%, increasing to 21% in 2005[230]. Most recent figures from 2011-2012 are reported at 9% overall. For comparison, when considering those from our study aged 65 years and older, at <25nmol/L, prevalence rates remain similar at 15%. The ELSA sample replaced and expanded the original HSE study sample and collected serum vitamin D samples for the first time in 2012-2013. These figures are higher than those reported in similar cohorts outside of the UK, with recent findings from The Irish Longitudinal Study of Ageing (TILDA) reporting the prevalence of vitamin D deficiency (<30nmol/L) to be 13.1% [35] and The Longitudinal Ageing Study Amsterdam, though lower are most comparable to our findings, at 17.5% (<30nmol/L) and 48.4% (<50nmol/L) [87]. Similarly, these studies identified the increased risk of deficiency in

those aged over 80 years, which show a marked increase in the oldest old (>85 years) [245]. Taken together, this highlights an important high-risk group for vitamin D deficiency and targeted interventions.

Our findings show a seasonal variation in vitamin D status with the prevalence of deficiency (IOM) increasing from 16.9% in summer to 28.3% during the winter months and vitamin D deficiency (ES) increased from 45.2% in summer to 62.0% in winter blood. We also noted an 8.9nmol/L seasonal difference in 25(OH)D concentration in this population. A variance of this amount is the difference of adequate status in this population [54.1nmol/L vs. 45.1nmol/L (p<0·0001)], demonstrating the adverse influence of the extended winter months on vitamin D status. Vitamin D supplement use was low at 4.4%, this figure is similar to other reported prevalence rates in the older adult populations [246]. Interestingly, although supplement use was low, serum 25(OH)D concentrations in supplement users were within the sufficient range commonly defined as ≥50-75nmol/L (65.8nmol/L), indicating the effectiveness of utilising vitamin D supplements for prevention of deficiency.

Our findings show a distinct north-south gradient in deficiency rates and serum 25(OH)D concentrations, with those residing in the south identified as less likely to be vitamin D deficiency (IOM) (0.78, CI 0.64-0.95) and vitamin D deficient (ES) (0.79 CI 0.66-0.93). And those in the midlands were also at reduced risk of vitamin D deficiency (0.83, CI 0.69-0.97). Previous research has identified trends in UVB availability decreasing as you move from north to south Europe [247], the results here reflect this trend but extend the findings within a small region such as England. Latitude is a known determinant of 25(OH)D status [248], findings from longitudinal data suggest an increased risk for females residing at two points in the UK, in Aberdeen, Scotland (57°N) when compared to similar data in Surrey, South England (51°N) [240]. Early analysis from HSE survey in 2005 reported the regional data to be a predictor of vitamin D deficiency but used London as the comparator which represents a more diverse cohort. Baseline data from a randomised controlled trial, conducted in two centres in Ireland showed a significant difference in 25(OH)D status for those residing in Cork (51.0°N) versus those in Northern Ireland (55°N) in older adults aged ≥64 years, although the sample size was small [249]. Prevalence rates for 25(OH)D deficiency across Europe are contradicting, with deficiency rates higher in southern Europe

compared to northern Europe were UVB radiation is lower [231]. It has yet to be clarified if this contradiction is likely due to increased consumption of vitamin D rich foods such as oily fish and cod liver oil in Scandinavian countries, mandatory food fortification, or if genetic adaption to UVB radiation has occurred in these populations. The contribution of latitude is evident but not entirely clear.

We have identified multiple determinants of vitamin D deficiency in the older adult population. Consistent with other studies, we found that females, those of oldest age, dark skin, and smoking were negative determinants of serum 25(OH)D status [250, 251, 239, 230, 231, 238]. A reoccurring observation in population studies of older adults is the reported high prevalence rate of overweight or obese subjects, which is present in our findings also. We showed that obesity increased the risk of vitamin D deficiency (IOM) by 30%, increasing to 50% for ES criteria of vitamin D deficiency. Whilst being of normal BMI lowered the risk by 20%, irrespective of defining criteria which have been reported previously [36, 238]. We have confirmed and extended these findings showing that walking speed, a clinical indicator of frailty, was statistically significantly associated with a 17% increased risk of vitamin D deficiency (IOM) 1.17 (1.09-1.26) in adults aged over 60 years. Furthermore, retirement and poor self-rated health were also inversely related to deficiency. Those less likely to be deficient, irrespective of definition, resided in the South, reported having good or excellent self-reported health, engaged in regular physical activity, consumed alcohol in moderation, and engaged in sun holiday travel. We also demonstrated that latitude contributes significantly to 25(OH)D status, even within countries of short ranging latitude, each 1° northerly increase resulted in an 11% increased risk of vitamin D deficiency(IOM) (CI, 1.04-1.17).

Our study has many strengths and potential limitations which need to be considered. We were able to report the most recent prevalence rates of vitamin D deficiency, over the largest nationally representative study of community-dwelling older adults, to date. These findings provide an important comparison to previously reported figures, as they confirm that vitamin D deficiency continues to be a problem. We have extended the findings showing that location is an important factor in vitamin D status as well as demonstrating the contribution of other lifestyle factors, which could be considered for future intervention. Whilst scientist followed standardised protocols to assess serum

measurements of 25(OH)D, the method used, DiaSoirin Liaison immunoassay is considered to be a lesser technique when quantifying vitamin D status. Secondly, the effect of UVB exposure can be influenced by other many factors, such as sunscreen use and other sun exposure habits, for which we were unable to include in this analysis. We were also unable to investigate the contribution of dietary vitamin D intakes, supplement dose or medications that might interfere with vitamin D metabolism. These limitations are frequently reported in studies of this size, whilst the addition of vitamin D behavioural assessments would improve our understanding they may not be feasible in large population studies.

Prevalence rates identified in this cohort exceed many other European figures, again warranting increased awareness within the ageing population in England, especially for those most at-risk; including older females, of non-white ethnicity, and residing in the North. Vitamin D supplement use is low and likely underreported, but we have evidenced the protective contribution it plays in maintaining sufficient serum 25(OH)D levels. Over the past 30 years, there has been a notable increase in the number of studies investigating vitamin D deficiency and chronic diseases, many of which have demonstrated adverse outcomes in musculoskeletal function and health in ageing. It is disappointing that vitamin D deficiency remains high in the older adult population when the effectiveness of vitamin D supplementation is known. At a population level, whilst supplement uptake remains low, food fortification may have the widest reach in tackling vitamin D deficiency, which has been successful in other north European countries. Whilst many researchers continue to focus on establishing an optimal 25(OH)D level for health, it seems at a minimum we should first aim to prevent deficiency.

In summary, more than half of adults aged >50 years had 25(OH)D concentrations <50nmol/L, with 1 in 4 categorised as deficient, according to IOM criteria. We identified that those at most risk of deficiency were of non-white ethnicity, residing in the north of England, and aged over 80 years with clinical indicators of frailty. Wintertime negatively influenced 25(OH)D status irrespective of location. Supplement use, maintaining a healthy BMI, engaging in physical activity and sun travel were identified as being protective. These findings highlight the importance of public health strategies throughout midlife and older age aimed at preventing vitamin D deficiency. It

would not be unreasonable for clinicians to routinely check serum vitamin D levels, particularly in the oldest old. Given supplementation is well-tolerated and an inexpensive means of decreasing general health risk.

Chapter 4: Effects of vitamin D_3 supplementation on cognitive performance in community-dwelling, older adults. Results from a randomised double-blind placebo-controlled pilot study.

4.1 Introduction

We have evidenced in Chapter 1 part B, through a systematic review that low serum 25(OH)D concentration was inversely associated with poorer cognitive performance and increased risk of cognitive impairment and dementia in community-dwelling older adults. A meta-analysis conducted by Etgen et al. supports these findings showing vitamin D deficient participants had over twice the risk of cognitive impairment [OR 2.39, 95%CI (1.91-3.00), $p<0.0001$)] [252]. Whilst observational evidence supports a link, only a limited number of supplementation studies have been conducted in terms of preventative lifestyle strategies likely to support cognitive function in ageing.

A recent RCT reported no effect of vitamin D_3 supplementation (4000IU daily), over an 18 week period, on tasks of executive function, attention and memory, in 82 adults [100]. However, the study sample was not entirely comprised of older adults and serum 25(OH)D status in the treatment group at baseline was near levels deemed desirable for cognitive function in epidemiological evidence (67.2nmol/L) [54]. The point in the life course when vitamin D status is most important for cognition is not established. It has been suggested that the impact of vitamin D deficiency appears in early and late life, with a greater proportion of observational studies reporting associations in older adults compared to young and middle-aged adults [253].

Of the two vitamin D supplementation studies conducted in healthy adults aged over 60 years, one was a post-hoc analysis of females enrolled in an RCT investigating fracture risk, whilst the second was 'before-after' in study design in older adults attending a memory clinic [101, 102]. The largest study, a post-hoc analysis, comprised entirely of postmenopausal females, who were supplemented 400IU of vitamin D_3 plus calcium daily, whilst the placebo group were also permitted to take their own vitamin D and calcium supplements throughout the study period. Compliance was reported to be poor and serum 25(OH)D concentrations were not measured at the end of the study, providing no evidence for optimal serum 25(OH)D levels and cognitive function or to distinguish between vitamin D or calcium effects [102]. Other limiting factors inherent to vitamin D intervention studies include various definitions of serum 25(OH)D deficiency, mode of serum analysis and form and duration of dosing. Although heterogeneity between intervention studies is notable and contributes to inconsistencies

within vitamin D and cognitive research literature, it also highlights important considerations for future investigations.

Whether cognitive impairment in ageing populations is influenced by vitamin D supplementation remains unknown. Well-designed interventions, which incorporate in-depth cognitive assessments in community-dwelling older adults without discernable cognitive impairment will offer valuable evidence which may confirm the mechanistic and observational findings. Furthermore, vitamin D deficiency is highly prevalent in community-dwelling older adults [134], as detailed in Chapter 3, further warranting exploration.

Based on the literature, we decided to investigate vitamin D supplementation and cognitive function in community-dwelling older adults over a 6-month period in a randomised double-blind, placebo-controlled pilot study. First, we wanted to investigate whether vitamin D supplementation has an effect on overall cognitive function, measured as a global assessment underlying several cognitive assessments. Secondly, we wanted to evaluate whether there was a difference between vitamin D supplement users and the placebo group in domain-specific tasks of executive function, attention, memory and visual reasoning.

Hypothesis 1: Participants supplementing on average 2000IU daily of vitamin D_3 will perform better at 6 months than those receiving placebo in tasks of overall cognitive function.

Hypothesis 2: Participants supplementing on average 2000IU daily of vitamin D_3 will perform better at 6 months in domain-specific tasks of cognitive function, including executive function, attention, memory and visual reasoning.

4.2 Methods particular to this chapter

4.2.1 Study details

Detailed methods regarding study design, the intervention, and ethical approval are detailed in methods Chapter 2 (Study 1). A brief overview is outlined below.

4.2.2 Participant enrolment

- Aged between 60 and 80 years
- Able to provide informed written consent
- No positive screen for suspected MCI, TCogs <23 [179]
- No other condition likely to affect cognitive function or vitamin D metabolism
- Not consuming supplemental vitamin D of ≥800IU/day
- Serum 25(OH)D concentrations ≥15nmol/L or ≤125nmol/L
- Normal corrected serum calcium level (<2.55nmol/L)

4.2.3 Study design and intervention

Participant data were collected at 0, 3 and 6 months.
Active treatment comprised of 4000IU of vitamin D_3 and matching placebo. All participants were directed to take 1 tablet, on alternating days for the study period. Compliance with the intervention was determined by tablet count at the end of the study period at 6 months.

4.2.4 Randomisation

Participants were randomly assigned to a study group by minimisation procedure (based on gender, age category, and physical activity level).

4.3 Assessment of cognitive function

A battery of pre-specified cognitive outcomes is listed on clinicaltrials.gov, as described in Chapter 2 and detailed in Table 4.1. Global function was assessed using the MoCA and domain-specific measures included tasks of executive functioning, attention, memory and visual reasoning.

Additionally, a broad inventory of factors relating to cognitive performance was obtained including the ability to perform daily tasks (IADL), subjective memory (self-reported memory), pre-morbid IQ (National Adults Reading Test, NART), and overall indicators of mental health and wellbeing, which are described in Chapter 2 and detailed in Table 4.3.

Table 4. 1: Overall and domain-specific primary and exploratory cognitive outcome measures

Domain	Test name	Description
Global	MoCA (0-30points)	Pen and paper task
Executive Function	TMT B (seconds)	Pen and paper task
	TMT B-A (seconds)	Pen and paper task
Attention	TMT A (seconds)	Pen and paper task
	SART RT (milliseconds)	Computerised task
	SD (RT) (milliseconds)	Computerised task
	SART error score, n	Computerised task
	SART RT var. [(SD)/RT]	Computerised task
Visual	CAMDEX VR (0-6)	Visual matrix task
Memory	WMS-IV Logical	Verbal pen and paper task
	WMS-IV Delayed	Verbal pen and paper task
Secondary exploratory outcome		
Memory	WMS-III Working	Verbal pen and paper task

MoCA, Montreal Cognitive Assessment; TMT B, Trail Making Task part B; TMT B-A, TMT part B minus part A; TMT A, TMT part A; SART, Sustained Attention to Response Task; RT, reaction time; SD(RT), standard deviation of reaction time; n, combined number of SART commission and omission errors; RT var., reaction time variability; (SD)/RT, SD of RT divided by mean RT; CAMDEX VR, Cambridge Mental Disorders of the Elderly Examination Visual Reasoning; WMS; Wechsler Memory Scale.

4.3.1 Serum vitamin D measurement and status criteria applied

Blood samples were collected by a research nurse at 0, 3 and 6 months. Serum 25(OH)D samples were analysed by Liquid Chromatography-Mass Spectrometry (LC-MS).

For this study, vitamin D status was defined using the IOM guidelines [220]. Deficiency is defined as serum 25(OH)D concentrations <30nmol/L, for insufficiency serum concentrations, ranged between 30nmol/L and <50nmol/L, and serum 25(OH)D concentrations ≥50nmol/L was defined as sufficient.

For exploratory analysis, in line with findings from the systematic review in Chapter 1 part B, we also considered serum 25(OH)D concentrations ≥75nmol/L.

4.3.2 Statistical Analysis

The study is a pilot trial, therefore, our sample size was not aimed at achieving appropriate power regarding the effectiveness of the intervention, but rather to obtain sufficient effect sizes to allow preliminary statements to be made regarding the impact of this type of intervention.

The following statistical approach was agreed;

1. Determine the effectiveness of 2000IU/d of vitamin D_3 at increasing serum 25(OH)D concentration at 3 and 6 months in the vitD_3 group (paired *t*-test).

2. Investigate the differences in mean scores of global function, executive function, attention, visual reasoning and memory between the vitD_3 and PL groups at 6 months, following an *intention-to-treat* (ITT) approach (independent *t*-test, ANCOVA and Cohen's *d if applicable*).

3. Conduct exploratory, cross-sectional analysis to investigate the changes in global and domain-specific composite measures of cognitive performance after 6 months, in a subgroup of participants who were defined as serum 25(OH)D deficient at baseline (<30nmol/L) and randomised to the PL group (paired *t*-test, Cohen's *d if applicable*).

4. Finally, to investigate whether those who achieved sufficiency at 6 months (≥75nmol/L vs. <75nmol/L), performed better in tasks of global and domain-specific cognitive performance at 6 months, irrespective of treatment allocation (independent *t*-test and Cohen's *d if applicable*).

All results are presented as mean ± standard deviation (SD) (all suitable variables) or numbers and proportions. Analyses were based on ITT and corresponding *p*-values were considered statistically significant at an alpha level of <0.05. Statistical test values <0.10 were deemed of potential interest and corresponding effect sizes were reported (Cohen's *d*). For ITT analysis, last observation carried forward was applied for any missing data recorded at <5%. ITT model 1 represents an independent *t*-test result for differences between the $vitD_3$ group and PL at 6 months. ITT model 2 represents an analysis of covariance (ANCOVA) for differences between-groups at 6 months whilst controlling for age, BMI, sun exposure habits since the 3-month visit, and alertness at time of the test, depression, anxiety, quality of life and perceived stress. Analyses were pre-planned based on clinical considerations and previous findings. To account for practice effects, a reliable change index (RCI) is reported for significant results. If RCI is >1.96 then the difference is deemed reliable, a change of that magnitude would not be expected due to the design of the measure in terms of practice effects [254].

All analyses were performed using STATA Corp 14.0 software (Stata Corp LP).

4.4 Results

4.4.1 Study population and baseline characteristics

The CONSORT diagram for participant recruitment and attainment is shown in Figure 4.1. A study retention rate of 98.3% (59/60) was achieved. No adverse events were reported by either group. In total, 2 participants were unable to attend the interim 3 months follow-up visit (vitD_3 group) but continued to adhere to treatment procedures and completed the post-intervention assessment at 6 months. One dropout occurred after 1 month (PL group), in a participant who no longer wished to take part.

Baseline characteristics of the study participants are presented in Table 4.2. The average age of participants was 68.5 ± 4.9 (range; 60.0-80.5 years), female gender was 53.3% (n=32), and all participants were of Caucasian ethnicity. Educational attainment was high, 85% (n=51) of participants were educated to secondary level at a minimum. Occupation status for highly-skilled and skilled employment was reported for 96.7% (58/60) of participants, indicating the abilities' of the cohort. Highly skilled and skilled employment categories were defined in the questionnaire by titles, such as "CEO, high management, professor, surgeon, engineer, nurse, tradesman, and teacher". There was no statistically significant difference between study groups for employment history. The groups were well matched for age, gender, socioeconomic status, health behaviours, psychological well-being, cognitive function including primary cognitive outcome measures, and pre-morbid IQ as detailed in Table 4.2 and Table 4.3.

4.4.2 Health profile of participants

Medication use was reported at 72.4% overall, the average number of medications taken was 1.4, with a low incidence of polypharmacy 3.3% (n=2). There were no statistical differences between groups for medication use and polypharmacy. Independent activities of daily living (IADL) indicated no issues for study participants, with the majority scoring full marks of 8. Nutritional status was normal for both groups, with 97.7% achieving scores of "no risk" for malnutrition. Alcohol consumption was moderate by units consumed per week, and smoking was minimal 1.6% (n=1). Mean BMI (kg/m^2) scores were similar between groups and fell within the category of overweight, according to WHO definition (overweight ≥25.0 <30.0 kg/m^2).

4.4.3 Vitamin D related factors at baseline

No statistically significant difference was observed between study arms for sun behaviour including sun avoidance, SPF use and time spent outdoors between 10-3pm during sunny months as detailed in Table 4.2. In line with the inclusion criteria all participants had normal-corrected serum calcium levels (2-2.55 nmol/L), [vitD_3; 2.30 (0.1), PL; 2.28 (0.1), p=0.26]. Adherence to allocated treatment was measured by counting the number of tablets returned at the end of the study. Between-group differences of 2.0 tablets resulted in a compliance rate of 99.0%.

4.4.4 Psychological and behavioural characteristics at baseline

Between-group differences are reported in Table 4.3. Overall, pre-morbid IQ identified 73.3% (n=44) of participants as having high intelligence (IQ; 120-140) with proportions balanced between study groups (p<0.56). Other factors that are important in the assessment of cognitive function were well balanced between the study groups, as detailed in Table 4.3. There was no statistically significant difference between groups for self-rated memory.

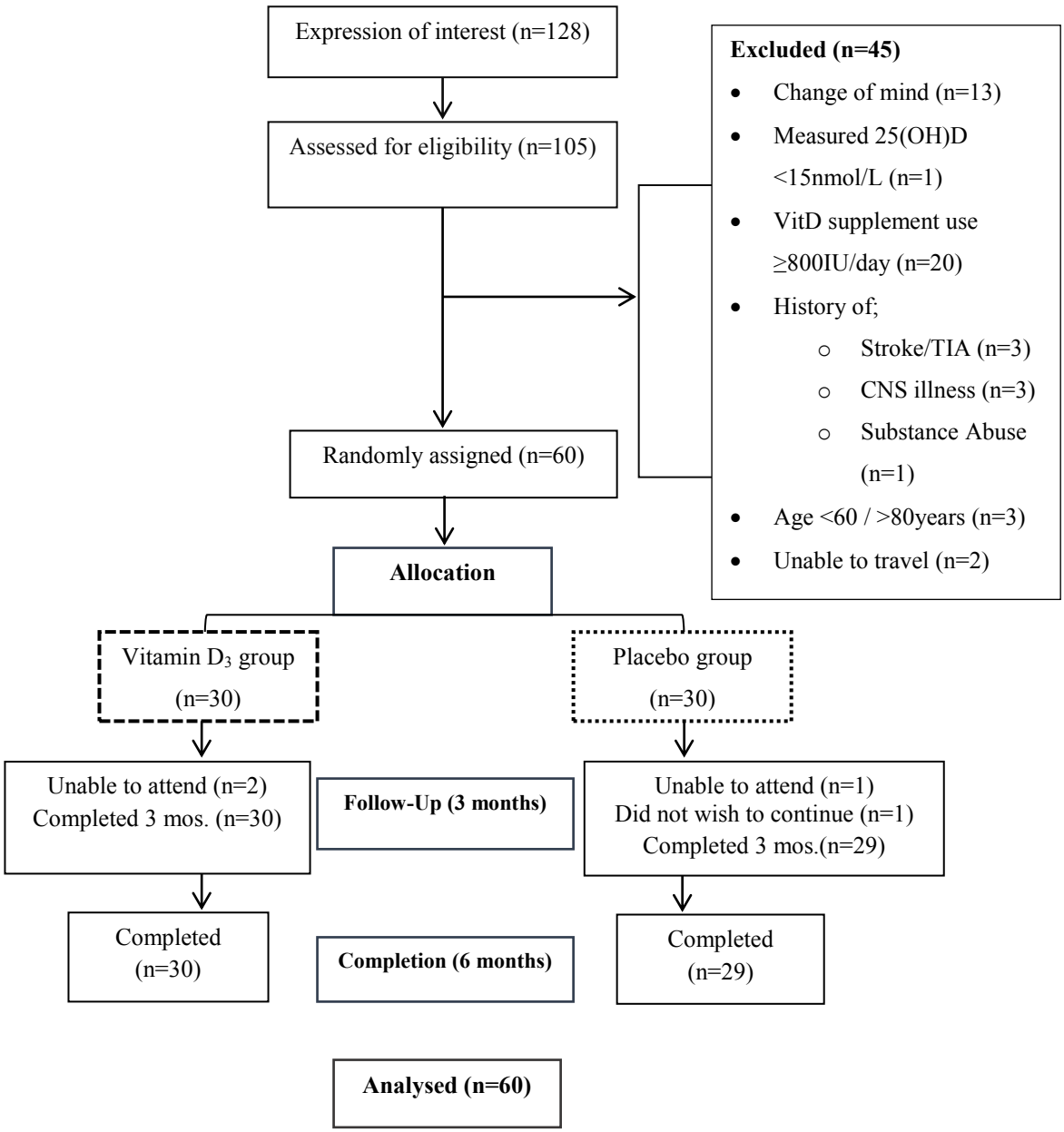


Figure 4. 1: CONSORT diagram for participant recruitment and attainment

Table 4. 2: Baseline characteristics of RCT participants, by treatment allocation

	VitD$_3$ (n=30)	PL (n=30)	*p*
Demographics			
Age (years)	68.6 ± 4.9	68.3 ± 5.1	0.82
Females, n	17	15	0.61
Caucasian, n	30	30	1
Education (years)	16.2 ± 3.91	15.2 ± 3.78	0.28
§*Secondary level*	23	28	0.45
Employment - *Highly skilled*	9	11	0.58
- *Skilled*	21	17	0.28
Physical Measures			
BMI (kg/m^2)	26.6 ± 2.85	27.4 ± 3.79	0.33
At risk of malnutrition (MNA), n	2	0	0.15
TUG, seconds	11.1 ± 3.4	10.8 ± 2.9	0.64
HGS, dominant hand (kg)	26.1 ± 9.2	27.4 ± 11.4	0.64
Health Behaviours			
Alcohol intake- units per week	9.0 ± 7.4	12.7 ± 12.3	0.31
Smoking, n	1	0	0.27
Medication use, (Y/N)	19	23	0.11
Polypharmacy, n	0	2	0.15
Vitamin D related factors			
Blood draw (Spring), n	30	30	1
Serum 25(OH)D (nmol/L)	51.8 ± 8.1	49.4 ± 22.5	0.65
Serum C. calcium (nmol/L)	2.30 ± 0.1	2.28 ± 0.1	0.26
VitD supplement use (<400 IU/d), n	5	4	0.72
Time outdoors (10-3pm), mins	136.7 ± 73.9	140.5 ± 78.2	0.85
SPF use (always/usually), n	21	19	0.58
Sun travel (previous 6months), n	10	5	0.14

Values are expressed as mean ± SD, unless otherwise stated. Between-group differences were analysed by independent *t*-tests and χ^2 or non-parametric equivalents. Statistical significance was reported at $p < 0.05$.

PL, placebo; vitD$_3$, vitamin D$_3$ treatment group. Employment, highly skilled (e.g. CEO,

engineer, professor, judge, surgeon); skilled (e.g. nurse, craftsman, teacher); MNA, Mini Nutritional Assessment; TUG, Timed Up and Go; Polypharmacy, 5 or more medications; Serum C. calcium, corrected serum calcium; Time spent outdoors, minutes spent outdoors between 10am-3pm on a sunny day; SPF, sun protection factor.

[§] Completed secondary level education at minimum.

Table 4. 3: Baseline cognitive status and related factors

Cognitive status and ability	VitD$_3$ (n=30)	PL (n=30)	*p*
TCogS (0-27)	26.6 ± 0.8	26.3 ± 1.2	0.55
NART, Pre-morbid IQ	122.96 ± 4.32	122.19 ± 5.25	0.67
Self-rated memory - *Excellent/V. good*	15	14	0.79
Good	12	11	0.82
IADL (0-8), *achieved full score*	30	29	0.32
Neuropsychological factors			
Depression (CES-D) (0-60)	1.2 ± 1.9	2.5 ± 3.4	0.09
Anxiety (HADS)	1.1 ± 1.8	1.2 ± 1.9	0.93
Perceived stress (PSS-4) (0-16)	1.0 ± 1.8	0.9 ± 1.6	0.88
Quality of Life (CASP-19) (0-57)	53.7 ± 3.9	51.8 ± 6.7	0.18
Sleep Quality (PSQI) (0-21)	3.3 ± 1.6	4.6 ± 4.0	0.09
Current alertness (SSS) (0-7)	1.6 ± 0.7	1.9 ± 1.1	0.26

Values are expressed as mean ± SD, unless otherwise stated. Between-group differences were analysed by independent *t*-tests and χ^2 or non-parametric equivalents. Statistical significance was reported at $p < 0.05$.

VitD$_3$, vitamin D$_3$ treatment; PL, placebo; treatment group; TCogS, Telephone Cognitive Screen; NART, National Adult Reading Test; IADL, Independent Activities of Daily Living; CES-D, Centre for Epidemiological Studies Depression Scale (score over 16 indicates clinical depression); HADS, Hospital Anxiety Disorders Scale, (higher scores greater levels of anxiety); CASP-19, "Control", "Autonomy", "Self-realisation" and "Pleasure" (lower scores indicate lower quality of life); PSQI, Pittsburgh Sleep Quality Index (higher scores indicate difficulty with various areas of sleep quality); SSS, Stanford Sleepiness Scale, "1=feeling vital, alert".

4.5 Serum 25(OH)D analysis

4.5.1 Baseline serum vitamin D status

There was no statistically significant difference in mean serum 25(OH)D concentrations between groups at baseline [vitD$_3$; 51.8 (18.1), PL; 49.4 (22.5), p=0.65], as detailed in Table 4.2. In total, 18.3% (11/60) were deemed vitamin D deficient (<30nmol/L) at baseline. There was a statistically significant difference between the proportion of participants deemed serum vitamin D deficient and insufficient between the study groups, with a p-value of 0.02 for both. There was no difference between groups for vitamin D sufficiency at baseline, which can be found in Table 4.4.

4.5.2 Serum vitamin D levels in response to vitamin D supplementation at 6months

This study initiated data collection in early spring, a period where serum vitamin D concentrations are more likely to be low due to limited subcutaneous vitamin D production during the winter months in the absence of adequate UVB radiation. Serum vitamin D concentrations increased in response to supplementation. There were a statistically significant increase in mean (SD) serum 25(OH)D concentrations in the vitD$_3$ group at 3 months [+55.9 (23.9) nmol/L, p<0.0001)] and at 6 months [+24.8 (34.8) nmol/L, p<0.0001)] from baseline 51.8 ± 18.1nmol/L. Fluctuations were observed in both groups at 3 months, due to seasonal influences, a statistically significant decrease in serum 25(OH)D concentration was noted in the vitD$_3$ group at 6 months [-27.3 (26.9), p<0.0001], compared to levels achieved at 3 months during the summer blood sample.

Table 4. 4: Vitamin D status by IOM guidelines at 0, 3 and 6 months, between study groups

IOM criteria (nmol/L)	**0 months**			**3 months**		**6 months**		
	PL (n=30)	$vitD_3$ (n=30)	Overall	PL (n=28)	$vitD_3$ (n=28)	PL (n=29)	$vitD_3$ (n=30)	Overall
Deficient (<30)	9	2	11 (18.3%) *	3	0	6 (-3)	2 (0)	8
Insufficient (≥30<50)	7	16	23 (38.3%) *	11	0	16 (+9)	3 (-13)	19
Sufficient (≥50<75)	9	9	18 (30%)	7	2	4 (-5)	7 (-2)	11
Optimal ǂ (≥75<100)	5	3	8 (13.3%)	5	6	3 (-2)	10 (+7)	13
High (≥100)	0	0	0 (0%)	2	20	0 (0)	8 (+8)	8

χ^2 statistical analysis for proportions.

* Statistical significance was reported at $p < 0.05$.

ǂ Exploratory cut-off, hypothesised as optimal for overall health including age-related physical and cognitive function.

IOM, Institute of Medicine; PL, placebo; $vitD_3$, vitamin D_3 treatment group.

At 0 months reported as an overall number of participants and (proportion) by vitamin D status.

At 6 months reported as a number of participants and (change in count from baseline).

4.5.3 Seasonal influence on serum 25(OH)D concentrations at 3 and 6 months

Mean (SD) serum 25(OH)D concentrations (nmol/L) peaked at 3 months for both groups, however, the vitD$_3$ group was statistically significantly higher [vitD$_3$; 107.0 (23.9), PL; 57.7 (24.7), p<0.0001], as illustrated in Figure 4.2. A large proportion of participants, mostly in the vitD$_3$ group (n=20), had serum levels over >100nmol/L at 3 months (range 103-158nmol/L). Some participants achieved serum levels >100nmol/L at 6 months (n=8), all of which were in the vitD$_3$ treatment group (range 102-125nmol/L). At 6 months, the mean serum 25(OH)D concentrations were PL; 42.3 (18.4), vitD$_3$; 76.7 (29.2) nmol/L, p<0.0001. Irrespective of season, there was a statistically significant increase in mean 25(OH)D concentrations within the vitD$_3$ group and a significant decrease in the PL group from baseline [vitD$_3$; +24.8 (34.4), PL; -6.9 (23.2), nmol/L p<0.0001].

4.5.4 Changes in serum vitamin D status at 3 and 6 months

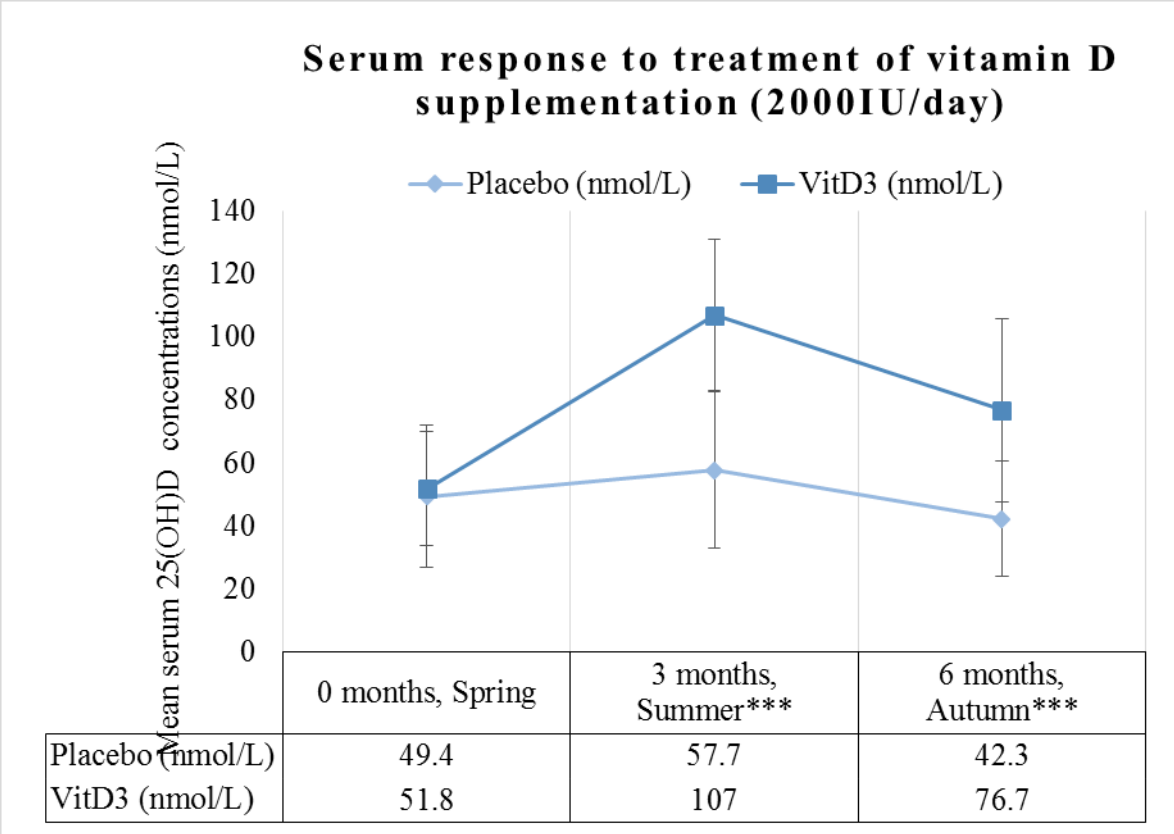
At 3 months, which coincided with the vitamin D summer season, all of those in the vitD$_3$ group had a serum level in the sufficient range, whereas, 50% of the placebo group did not, as outlined in Table 4.4.

Overall 11 participants were deemed vitamin D deficient (0 months). Of these, 2/11 had been randomised to the vitD$_3$ group and both corrected their deficiency and achieved sufficient vitamin D status at 3 and 6 months. Of the 9/11 participants from the placebo group who were vitamin D deficient at 0 months, 1/9 achieved sufficient status at 3 months during the summer visit. At 6 months, 8/9 were identified as vitamin D deficient or insufficient (serum concentrations ranged from; 20.0-38nmol/L at 6 months).

In the placebo group, the majority did not reach sufficiency (22/29) at 6 months. Although, 5 participants did not achieve sufficient (≥50nmol/L) serum vitamin D status in the vitD$_3$ treatment group.

Using the higher serum 25(OH)D 'optimal' criteria of concentrations ≥75nmol/L, over half of the vitD$_3$ treatment group (18/30) achieved this, whereas, only a small number were observed in the placebo group (3/29).

However, in the treatment group at 6 months, 2 other participants were deemed vitamin D deficient after the intervention, both of which had insufficient levels at baseline.



	0 months, Spring	3 months, Summer***	6 months, Autumn***
Placebo (nmol/L)	49.4	57.7	42.3
VitD3 (nmol/L)	51.8	107	76.7

Figure 4. 2: Mean serum 25(OH)D concentrations and standard deviations at 0, 3 and 6 months

*** Statistically significant difference between groups and within-groups from baseline, and at 3 and 6 months ($p<0.001$)

4.6 Results: Cognitive performance at 0 and 6 months

4.6.1 Baseline cognitive performance between treatment and placebo groups

There were no statistically significant differences between groups at baseline for global or domain-specific cognitive functions, detailed in Table 4.5. The mean score for global function was within the published MoCA criteria for a "normal" score [185], in both group [PL; 26.8 (2.4), vitD_3; 27.5 (2.2), p=0.27]. In tasks of executive function and tests of attention both groups were well matched at baseline. For all other domain-specific tests, mean scores were consistently similar, with no statistically significant differences detected. In tasks of logical and delayed memory, no statistically significant differences were observed. The same can be explained for scores of visual reasoning between the groups [PL; 4.2 (1.3), vitD_3; 4.1 (0.9), p=0.73]. In an exploratory outcome of working memory, we again note that there is no statistically significant difference between the groups (p=0.35).

Overall this cohort of community-dwelling older adults was well-matched for cognitive performance on all pre-specified and exploratory cognitive outcome measures at baseline.

Table 4. 5: Primary and exploratory cognitive outcome scores at baseline, between study groups

Domain	Test	PL (n=30)	VitD$_3$ (n=30)	*p*
Global	MoCA (0-30 score)	26.8 ± 2.4	27.5 ± 2.2	0.27
Executive Function	TMT B sec	82.7 ± 41.7	72.4 ± 30.3	0.30
	TMT B-A sec	53.5 ± 36.7	44.0 ± 28.0	0.26
Attention	TMT A sec	29.2 ± 9.2	28.4 ± 1.2	0.69
	SART RT (m/sec)	332.6 ± 100.9	299.9 ± 67.4	0.41
	SD (RT) (m/sec)	97.6 ± 77.2	77.8 ± 55.3	0.33
	SART error score, n	15.6 ± 14.2	10.3 ± 8.9	0.09
	SART RT var.	0.27 ± 0.12	0.25 ± 0.13	0.59
Visual	CAMDEX VR (0-6)	4.2 ± 1.3	4.1 ± 0.9	0.73
Memory	WMS-IV Logical	10.4 ± 3.0	9.9 ± 3.2	0.56
	WMS-IV Delayed	10.7 ± 3.1	10.0 ± 4.2	0.51
Secondary exploratory outcome				
Memory	WMS-III Working	11.8 ± 2.8	12.5 ± 3.4	0.35

MoCA, Montreal Cognitive Assessment; TMT B, Trail Making Task part B; TMT B-A, TMT part B minus part A; TMT A, TMT part A, SART RT, Sustained Attention to Response Task; RT, reaction time; SD(RT), standard deviation of reaction time; n, combined number of SART commission and omission errors; RT var., reaction time variability; (standard deviation of the

reaction time divided by mean reaction time); CAMDEX VR, Cambridge Mental Disorders of the Elderly Examination Visual Reasoning subtest; WMS, Wechsler Memory Scale. Independent *t*-test or non-parametric equivalent Mann Whitney-U. *Statistical significance was reported at $p < 0.05$.

4.6.2 Effect of vitamin D supplementation on primary cognitive outcome measures at 6 months

Cognitive performance scores at 6 months are detailed in Table 4.6 and Figure 4.3 and Figure 4.4.

4.6.2.1 Global cognitive performance- MoCA

Considering the pre-determined ITT analysis, we noted no statistically significant difference between the $vitD_3$ and PL group, with little to no effect size (ITT $d = 0.04$). Consistent with this finding, the exploratory analysis showed no statistically significant change in MoCA scores from baseline between the two groups (Δ p=0.19).

4.6.2.2 Executive function- TMT B and TMT B minus A

For TMT B and B minus A, no differences were observed between the $vitD_3$ and PL in either task of executive function, after controlling for important factors associated with cognitive performance e.g. mood and wellbeing at the time of assessment (adjusted TMT B; p= 0.67 and TMT B-A; p= 0.82).

4.6.2.3 Attention – SART and TMT A

No statistically significant differences were detected between the $vitD_3$ and PL group at 6 months in assessments of attention or sustained attention. RT var., which explains much of the test difference in the SART task, was similar in both groups at the end of the intervention [PL; 0.26 (0.14), $vitD_3$; 0.24 (0.16), p=0.80].

4.6.2.4 Visual Reasoning

In a test of visual reasoning participants allocated to the $vitD_3$ group performed better at 6 months, whilst the outcome achieved a moderate effect size of d=0.45, it did not reach statistical significance, p=0.07. Once adjusted for confounding factors (e.g. alertness, anxiety and mood), no meaningful between-group difference was evident, p=0.31.

4.6.2.5 Memory

For logical memory (verbal, immediate memory), participants in the $vitD_3$ group performed better at 6 months according to the unadjusted model [ITT 1, PL; 13.1 (2.7), $vitD_3$; 14.4 (2.0), p=0.05, d=0.51], however, this finding was lost in adjusted model 2

after controlling for confounders (ITT 2, p=0.09). There were no statistically significant differences for scores of delayed memory at 6 months between groups, p=0.66.

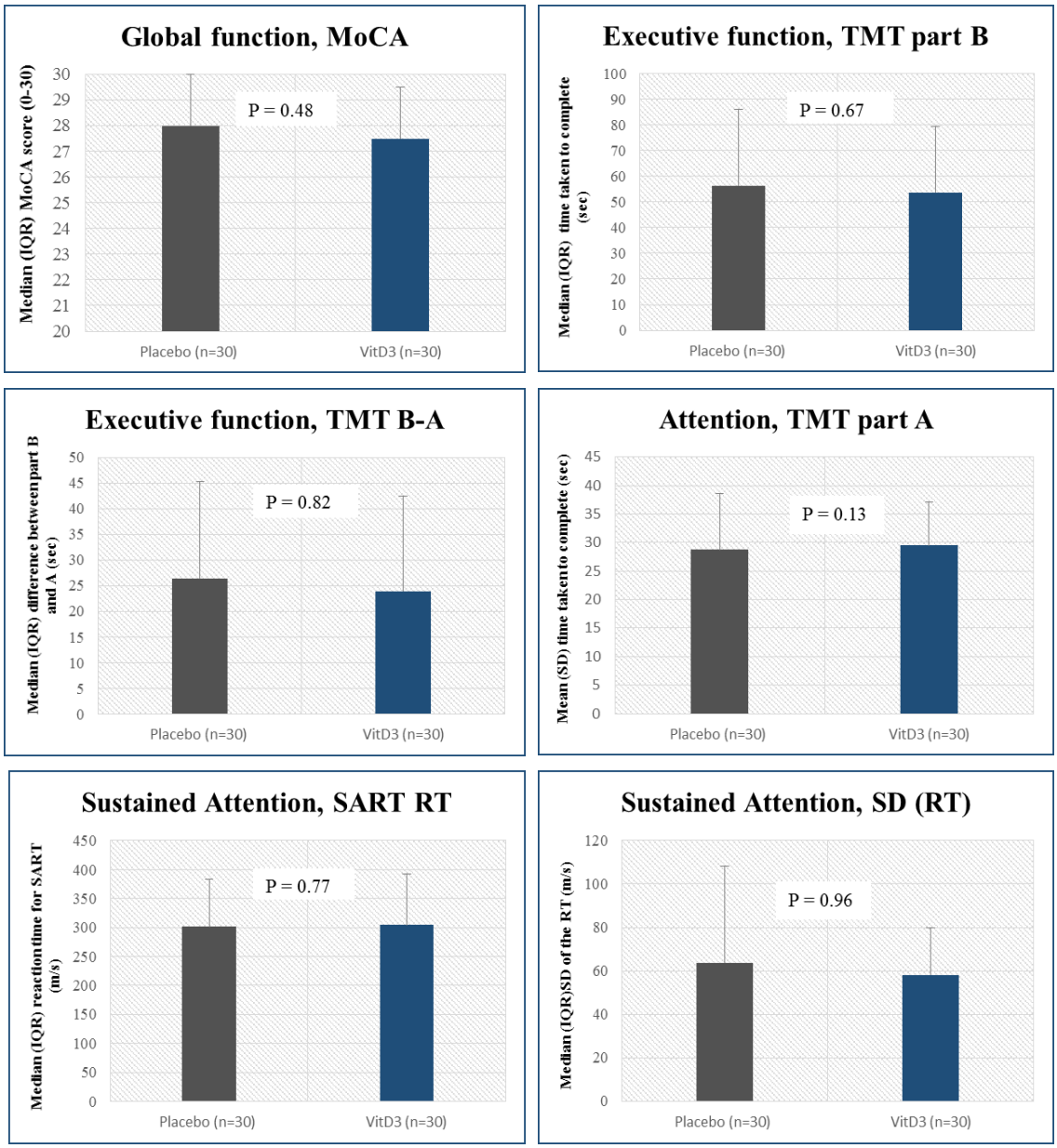


Figure 4. 3: Differences in global, executive function and attention outcome variables between placebo and treatment (vitD3) groups at 6 months.

Corresponding ITT model 2 results, performed using ANCOVA. Non-parametric data are presented as median (IQR).

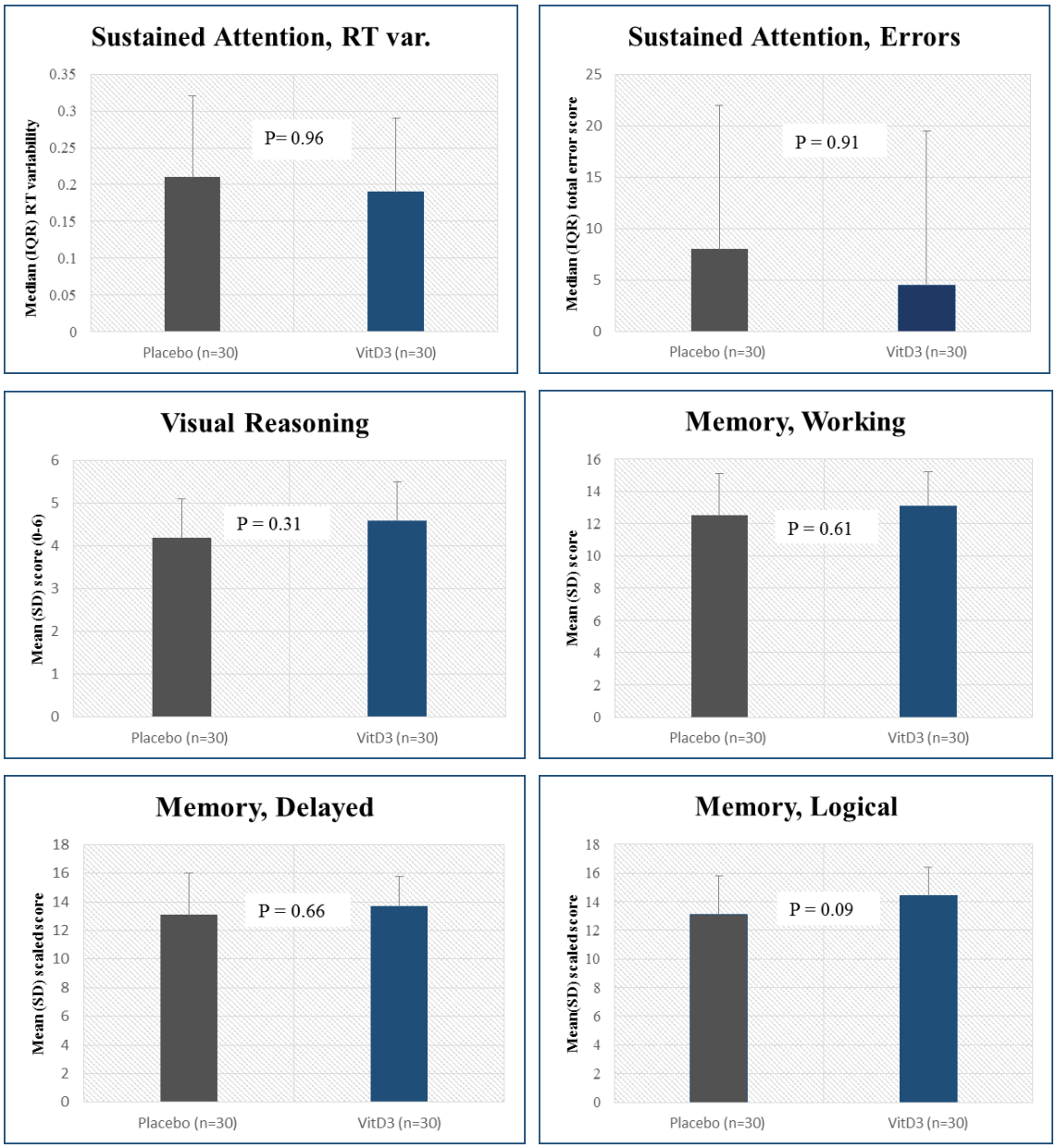


Figure 4. 4: Differences in sustained attention, visual reasoning and memory outcome variables between placebo and treatment (vitD$_3$) groups at 6 months.

Corresponding ITT model 2 results, performed using ANCOVA. Non-parametric data are presented as median (IQR).

Table 4. 6: Primary and exploratory cognitive outcome measures after 6 months

		Placebo (n=30)ǂ		VitD$_3$ (n=30)				Treatment effect (n=60)	
Domain	Test name[§]	6 months	Δ	6 months	Δ	*Δ p*	*ITT d*	*ITT*1 *(unadjusted)*	ITT2 *(adjusted)*
Global	MoCA (0-30 score)	27.7 ± 1.9	0.9	27.6 ± 1.6	0.17	0.19	0.04	0.68	0.48
Executive	TMT B sec	62.4 ± 25.9	-21.3	58.1 ± 22.0	-14.3	0.38	0.18	0.44	0.67
Function	TMT B-A sec	33.7 ± 20.7	-19.8	28.6 ± 17.3	-15.4	0.52	0.26	0.23	0.82
	TMT A sec	28.7 ± 9.9	-0.51	29.5 ± 7.6	1.12	0.34	0.09	0.73	0.13
	SART RT (m/sec)	316.7 ± 82.1	-15.4	310.4 ± 64.1	8.2	0.18	0.09	0.92	0.77
Attention	SD(RT) (m/sec)	88.2 ± 79.3	-7.9	79.3 ± 69.8	2.6	0.56	0.12	0.43	0.96
	SART Error Score, n	13.2 ± 16.9	-2.0	11.4 ± 14.7	1.3	0.31	0.11	0.39	0.90
	SART RT.,var	0.26 ± 0.14	-0.0	0.24 ± 0.16	-0.0	0.99	0.13	0.35	0.88
Visual	VR (0-6 score)	4.2 ± 0.9	0.03	4.6 ± 0.9	0.6	0.08	0.45	0.07	0.31
Memory	WMS-IV Logical	13.1 ± 2.7	2.7	14.4 ± 2.0	4.4	0.05	0.51	0.05	0.09
	WMS-IV Delayed	13.1 ± 2.9	2.5	13.7 ± 2.1	3.7	0.18	0.23	0.39	0.66
Secondary exploratory outcome									
Memory	WMS-III Working	12.5 ± 2.6	0.7	13.1 ± 2.1	0.6	0.91	0.23	0.27	0.61

Test units; sec, seconds; m/sec, milliseconds

Results reported as mean ± SD.

[§]Description and abbreviations in Table 4.1 and 4.2.

ǂ Last observation carried forward for ITT analysis.

Δ mean difference within-group from baseline.

* Statistical significance was reported at $p < 0.05$.

Δ *p*: significant mean difference from baseline, between groups.

ITT *d*: Cohen's *d* effect size for between-group differences at 6 months.

ITT (unadjusted) model 1 represents independent *t*-test results or Mann Whitney-U for differences between treatment groups at 6 months.

ITT (adjusted) model 2 represent ANCOVA controlling for age, BMI, sun exposure habits, perceived stress, current alertness, quality of life, depression and anxiety.

4.7 Analysis according to serum 25(OH)D levels

We compared cognitive performance scores at 6 months, within a subgroup of participants with vitamin D deficiency at baseline in Table 4.7. Furthermore, the data were analysed for differences between those who achieved serum 25(OH)D levels ≥75nmol/L and those who did not, at the 6 month assessment. The results are presented in Table 4.8.

4.7.1 A comparison of cognitive performance scores at 6 months, in participants defined as serum 25(OH)D deficient (<30nmol/L) in the placebo group at 0 months

Evidence from subgroup analysis in other vitamin D intervention studies suggests that people who are serum vitamin D deficient at enrolment, are more likely to improve in the outcome measure(s) of interest, in response to vitamin D supplementation [255, 256]. Due to the small sample size, subgroup analysis was performed using composite scores for executive function, attention and memory, comprising multiple single-tests, as outlined in Table 4.7. Global function was assessed by the MoCA, which is a composite test by design, for overall cognitive function.

The overall vitamin D deficiency rate at baseline was 18.3% (11/60), however, most (9/11) were in the placebo group, limiting this analysis. We were unable to test these results against the vitD_3 group, as only 2/11 were deemed vitamin D deficient at baseline and both corrected their deficiency and achieved levels >75nmol/L at 3 and 6 months. Of the 9 participants from the placebo group who were deficient at baseline, 8 were identified as vitamin D deficient or insufficient at 6 months (mean serum 25(OH)D; 20.0-38nmol/L).

Analysis of placebo group participants, who were vitamin D deficient at enrolment (n=9), showed no statistically significant difference in any composite domain score from baseline which is documented in Table 4.7.

4.7.2 An exploratory comparison of participants who achieved serum 25(OH)D concentrations ≥75nmol/L on study completion

In Chapter 1 part B, observational findings indicated that community-dwelling older adults with serum vitamin D concentrations ≥75nmol/L, were more likely to perform better on tasks of cognitive performance than those with levels <75nmol/L. To test this hypothesis in participants of the present RCT, we conducted cross-sectional analysis, irrespective of treatment, detailed in Table 4.8.

Global function scores at 6 months were similar in participants with serum levels <75 or ≥75nmol/L (p=0.48). For tasks of executive function those with levels <75nmol/L at 6 months showed a faster performance compared to those ≥75nmol/L, however, results were not statistically different, in TMT B. Similar findings were found for attention tasks such as RT var., which achieved a moderate effect size when comparing serum vitamin D levels <75 versus ≥75nmol/L, at 6 months. (p=0.08, d= 0.59).

In tasks of memory, those with serum 25(OH)D concentrations ≥75nmol/L performed statistically significantly better in tasks of delayed memory (p=0.04, d=0.59) and moderate effect sizes were noted for tasks of immediate memory at 6 months (p=0.07, d=0.55). No statistically significant difference was seen in a task of visual reasoning (VR) when comparing those <75 or ≥75nmol/L at 6 months (p=0.06, d=0.52). There were no statistically significant differences noted at 6 months in a task of working memory.

Table 4. 7: Global and domain-specific cognitive composite scores. Mean changes at 6 months, in vitamin D deficient (<30nmol/L) participants, allocated to the placebo group (n=9)

		Intra-group difference at 6 months Placebo group <30nmol/L at baseline ǂ			
Cognitive Domain	**Z scores combined**	**0 months (n=9)**	**6 month score (n=9)**	***p***	***d***
Global function	MoCA (raw scores)	26.7 ± 3.0	27.2 ± 2.6	0.30	-
Executive function	TMT B, TMT B-A, VR	0.8 ± 2.7	0.1 ± 1.0	0.58	-
Attention	TMT A, SART RT, SD(RT), Errors	1.5 ± 5.5	0.51 ± 5.6	0.52	-
Memory	WMS-III & IV Logical, delayed & working memory	-0.2 ± 1.7	-1.84 ± 4.0	0.22	-

Paired *t*-test for within-group difference at 6 months.

Z, composite score of each standardised test score

MoCA, Montreal Cognitive Assessment; TMT B, Trail Making Task part B; TMT B-A, TMT part B minus part A; TMT A, TMT part A, SART RT; Sustained Attention to Response Task reaction time; SD(RT), standard deviation of reaction time; n, combined number of SART commission and omission errors; (SD)/RT, standard deviation of the reaction time; VR, Visual Reasoning subtest; WMS, Wechsler Memory Scale.

ǂ Overall 11 participants were identified as serum 25(OH)D deficient at baseline, of those 9/11 were allocated to PL and 2/11 to vitD_3 treatment. The analysis is presented only for the PL participants (n=9) as inadequate counts are available for comparison with vitD^3.

Table 4. 8: Cross-sectional analysis of cognitive outcome measure at 6 months, between-group analysis of participants who achieved serum 25(OH)D concentrations ≥75nmol/L at 6 months

		Between-group difference at 6months			
Domain	**Test name**	**<75nmol/L** (n=38)	**≥75nmol/L** (n=22)	***p***	***d***
Global	MoCA (0-30)	27.8 ± 1.7	27.5 ± 1.8	0.48	-
Executive Function	TMT B sec	61.6 ± 25.0	58.3 ± 22.8	0.61	-
	TMT B-A sec	31.9 ± 19.7	30.1 ± 18.7	0.74	-
Attention	TMT A sec	29.7 ± 9.8	28.1 ± 6.9	0.51	-
	SART RT (m/s)	319.9 ± 80.6	302.9 ± 58.2	0.39	-
	SD (RT) (m/s)	94.2 ± 82.5	63.7 ± 37.3	0.12	-
	SART error score, n	14 ± 16.9	9.4 ± 13.5	0.28	-
	RT var.	0.28 ± 0.18	0.20 ± 0.07	0.08	0.59
Visual	**CAMDEX** VR	4.2 ± 0.9	4.7 ± 1.0	0.06	0.52
Memory	**WMS-IV** Logical	13.3 ± 2.6	14.6 ± 2.1	0.07	0.55
	WMS-IV Delayed	12.9 ± 2.7	14.3 ± 1.9	0.04*	0.59
Secondary exploratory outcome					
Memory	**WMS-III** Working	12.6 ± 2.3	13.2 ± 2.4	0.29	-

MoCA, Montreal Cognitive Assessment; TMT B, Trail Making Task part B; TMT B-A, TMT part B minus part A; TMT A, TMT part A, SART RT; Sustained Attention to Response Task; RT, reaction time; m/s, milliseconds; SD(RT), standard deviation of reaction time; n,

combined number of commission and omission errors; RT.var, reaction time variability; (SD)/RT, standard deviation of the reaction time divided by mean reaction time; CAMDEX VR, Cambridge Mental Disorders of the Elderly Examination Visual Reasoning subtest; WMS, Wechsler Memory Scale.

Independent student t-test for between-group differences.

* Statistical significance was reported at $p < 0.05$.

4.8 Discussion

In the present pilot RCT study, we investigated the effect of vitamin D_3 supplementation (2000IU/d given as 4000IU every 2 days) compared to placebo on cognitive performance in a sample of community-dwelling older adults, after 6 months. The primary outcomes were validated pre-specified assessments of global and domain-specific cognitive functions (Clinicaltrials.gov *identifier*: NCT02804841). We have demonstrated the effectiveness of vitamin D_3 supplementation on serum 25(OH)D levels with a marked improvement in serum concentrations (nmol/L) in the vitD_3 group after 6 months [51.8 (18.1) to 76.7 (29.2), $p<0.0001$], whereas the placebo group experienced an overall drop in serum 25(OH)D concentrations by 6 months [-6.9 (34.4)].

Whilst cross-sectional and observational evidence from the systematic review (Chapter1 part B) largely supports an association between low serum 25(OH)D concentrations and poorer cognitive performance, findings from the present study demonstrate no significant effect for vitamin D_3 supplementation and pre-specified measures of global cognitive function and domain-specific tasks of executive function and attention. Some minor findings were observed in tasks of logical (verbal) memory, delayed memory and visual reasoning, which may merit further investigation.

For tasks of logical memory and visual reasoning, whilst the significance levels were relatively small (unadjusted ITT; logical, $p=0.05$, $d=0.51$; visual, $p=0.07$, $d=0.45$) and potentially underpowered, it is notable that these domains achieved moderate effect sizes. With a similar trend in our cross-sectional analysis for participants who achieved serum 25(OH)D concentrations ≥75nmol/L (attention RT var., $p=0.08$, $d=0.59$; logical, $p=0.07$, $d=0.55$; visual, $p=0.06$, $d=0.52$; and delayed memory, $p=0.04$, $d=0.59$).

To our knowledge, the present study is the first RCT to investigate the effect of vitamin D supplementation on cognitive function in healthy older adults living in the community. Our findings are similar to those reported from a recent RCT, of cognitive function in healthy adults, some of which were older adults; the author found no effect of high dose vitamin D supplementation (4000IU/d) against a low dose comparator

(400IU/d) [100]. However, there are a number of important methodological differences between the present study and that reported by Petterson et al. A point, which the authors acknowledge as a limitation, is that over half of the relatively small cohort were aged less than 60 years old (n=48/82, aged 20-59 years). This may potentially mask an effect in the older participants as results were analysed collectively [253].

Pettersen et al. report no effect for high dose (4000IU/d) vitamin D_3 supplementation in validated domain-specific assessments of executive function, attention and memory (nonverbal, verbal and working memory). Similar to the present study, no indication for an effect of high dose vitamin D supplementation was noted for executive function and attention. Unfortunately, global function was not assessed by Pettersen, so we are unable to compare overall global cognitive performance between the studies. Unexpectedly, the authors report a statistically significant improvement in tasks of non-verbal memory (Pattern Recognition Memory-delayed) in the low dose vitamin D placebo group. Serum 25(OH)D levels achieved in the low dose group post-intervention (85.9nmol/L) are commonly reported as 'optimal' for cognitive function in observational evidence, as evidenced in the systematic review in Chapter 1 part B. Whereas those in the high dose treatment group had mean serum concentrations of 130nmol/L post-intervention, which are potentially adverse [257]. Furthermore, the author reports a statistically significant cross-sectional association, irrespective of treatment, for the same non-verbal memory assessment in those with serum levels ≥75nmol/L.

RCTs for vitamin D and cognitive function in healthy older adults are a relatively new potential component for healthy ageing research. Observational studies comprise the majority of the evidence base to date. It is only quite recently that large population studies of community-dwelling older adults have assessed cognitive performance and vitamin D status using validated domain-specific cognitive outcome measures. The design of the present study sought to include both global cognitive function and domain-specific assessments which have been indicated in the research literature. The majority of positive associations between serum vitamin D and cognitive function identified from observational evidence have been derived using global cognitive measures such as the Mini-Mental State Examination (MMSE) [163-167, 90]. Among 1253 (≥65 years) older adults from the Longitudinal Ageing Study in Amsterdam (LASA)

[87], participants with serum vitamin D status <30nmol/L had significantly lower scores in overall cognitive functioning (MMSE) than those with serum 25(OH)D levels ≥75nmol/L. As the field has developed, domain-specific measures have become the focus and interestingly the most recent associations were observed in domain-specific tasks of visuospatial abilities, executive function and memory [163, 94].

This is evident in a combined sample of community-dwelling older adults (n=1612), who took part in the Cardiovascular Health Study in the US and the LASA Study [163]. Participants with vitamin D deficiency (<25nmol/L) performed worse in a task of visual memory than those with levels >50nmol/L. These findings are consistent with prospective studies of cognitive dysfunction, which demonstrate a similar decline in logical and delayed memory in vitamin D deficient participants compared to levels >50 to 125nmol/L [258]. Some minor findings were observed in the present study in tasks of logical verbal memory, delayed memory and visual reasoning, which may merit further investigation.

Interestingly, a meta-analysis of domain-specific tasks of executive function and memory demonstrated that lower vitamin D concentration predicts executive dysfunction and to a lesser extent deficits in episodic memory in community-dwelling older adults [259]. In the present study we found no effect of vitamin D supplementation in the same tasks of executive function (TMT-B; mental shifting and TMT-A information processing speed), but an indication towards memory specific tasks. A more recent review conducted by the same authors [253], reported that there was insufficient evidence for episodic memory yet evidenced this in an adolescent population from NHANES III [88]. Contradictory, within the same review the author demonstrates a clear age-dependent threshold for vitamin D status in cognitive function, with positive associations found largely in studies of adults aged >65 years only. Whilst early reviews suggest memory outcomes are notably poorer in participants who are older >60 years with low vitamin D levels than associations observed for any other cognitive domain [260]. To make firm conclusions, future studies should not depend on single domain-specific tasks but utilise a battery of cognitive domains similar to the present study, to clarify, what domain, if any, is likely implicated.

The vitamin D dose in the present study was on average 2000IU/d in community-dwelling older adults. This was higher than one of the three vitamin D supplementation and cognitive function studies and lower than two, as detailed in Table 1.3. Despite this, post-intervention serum 25(OH)D levels in the vitD$_3$ group of the present study are similar to those reported in the only significant supplementation study conducted by Annweiler et al. [103], with mean post-intervention serum levels in the treatment group of 75nmol/L. It is important to note, in the study conducted by Annweiler participants (n=44, mean age 80.6 years) were deemed 'free of cognitive impairment', however, were recruited through a memory clinic. The author reported, in a short article, that those receiving no treatment compared to treatment, on average, 3333IU/d, had poorer outcomes in the Cognitive Assessment Battery, a clinical screening tool assessing global cognitive function (OR 16.5, CI 2.51, 108.6). In the earliest vitamin D supplementation study in a large cohort of post-menopausal females, Rossom et al. conducted post-hoc analysis for overall cognitive function and multiple domain-specific cognitive outcomes including executive function, attention and memory. The author reported no significant finding for incidence dementia or poorer performance in any domain-specific task after 8 years. No post-intervention serum vitamin levels were reported, and the treatment dose was relatively low (400IU/d), furthermore, placebo participants were able to take their own vitamin D and calcium supplements during the study period and poor compliance was reported overall, therefore it is possible sufficient levels were not achieved [102].

In recent years emerging evidence suggests that vitamin D deficiency may predict a response in outcomes of vitamin D interventions [140, 255] and that once serum levels reach high concentrations a U-shaped association is frequently reported [261, 262, 245]. However, purposefully recruiting people, especially, vulnerable groups such as older adults, with vitamin D deficiency, who may be allocated placebo, presents a major ethical issue. Consequently, this limits the study design of vitamin D supplement interventions. In the present study, for examples, we excluded participants with serum 25(OH)D levels <15nmol/L at baseline. Overall 18.3% (n=11) were vitamin D deficient at baseline (<30nmol/L) thus representing a small sample size; Moreover, only 2 of which were classed as vitamin D deficient at baseline were allocated to the treatment group after unblinding; therefore the sample was too small to conduct meaningful analysis of treatment effects. We explored the subgroup of participants deemed vitamin

D deficient at baseline and allocated placebo (n=9/11). Analysis of cognitive composite cognitive scores showed no statistically significant difference within this subgroup for any domain-specific score at 6 months, or changes in serum 25(OH)D concentrations with mean serum 25(OH)D levels of 31.6nmol/L, at 6 months. Larger, overall sample sizes in the future may allow pre-specified and powered subgroup analysis to investigate this further. It is anticipated that the potential for subgroup analysis will be possible in findings of larger intervention studies, which will be reported in the near future (DO-Health, VITAL-Cog).

A point of note was that educational attainment and employment status documented in the present study was high at 85% overall for leaving certificate education at minimum and 96.7% reporting occupational status as highly skilled or skilled. Reflecting figures higher than those reported in national ageing studies such as TILDA [263]. This is somewhat reflected in the subjective quality of life scores, which are highly correlated with economic factors. By contrast, a mean score of 52.6/57 was reported in this study compared 42.7 reported in the TILDA study population [263]. Overall this indicates a highly functioning cohort which may not be comparable to the general older adult population. Future interventions may benefit from longer follow-up periods, aimed at detecting cognitive decline as the outcome of interest.

4.8.1 Strengths and limitations

A number of limitations in the present study should be acknowledged, including the relatively small sample size, although there was a strong 98.3% participant retention rate. Ideally, although not always logistically feasible, conducting the RCT during the vitamin D winter season may be less conflicting in demonstrating a true treatment effect, by reducing the influences of UVB exposure during the intervention period. Furthermore daily supplemental vitamin D is believed to be most effective, however, this comparison is generally in relation to bolus doses of vitamin D [264].

There are several strengths of the present study, as a well-designed RCT, it investigated the effects of vitamin D supplementation on cognition based on a sample defined as healthy community-dwelling older adults (i.e. without known cognitive impairment). This study applied a comprehensive valid battery to assess cognition; moreover, it demonstrated that including this core cognitive outcome set in a vitamin D and ageing

intervention study was feasible and acceptable, with a high retention and compliance rate achieved. A short report detailing the study participants' feedback regarding all aspects of trial involvement is detailed at the end of this chapter. Both the placebo and vitamin D treatment group were well-matched at baseline for important variables relevant to cognition, vitamin D and other important factors such as mental health and well-being.

The present study represents the first RCT, to the authors' knowledge, to investigate the effects of vitamin D supplementation on cognition among community-dwelling older adults without cognitive impairment. We have demonstrated that vitamin D_3 supplementation is an effective and safe strategy for improving serum 25(OH)D levels and correcting vitamin D deficiency.

In conclusion, vitamin D_3 supplementation over a 6 month period showed no statistically significant effect on global or domain-specific tasks of cognitive function in a sample of community-dwelling older adults, aged over 60 years. Minor findings in relation to logical memory and visual reasoning were interesting but should be interpreted with caution. Furthermore, we found that those who achieved serum vitamin D concentrations ≥75nmol/L performed statistically significantly better in a task of delayed memory and moderate effect sizes were noted in reaction time variability of the sustained attention task, logical memory and visual reasoning, compared to levels <75nmol/L at 6 months. Although interesting these findings cannot be directly linked to the intervention.

4.9 Chapter 4- Part B: Participation survey and feedback from a randomised double-blind placebo-controlled pilot study of vitamin D and cognition.

In line with good research practice, particularly RCTs, we conducted a participant feedback survey once the intervention was complete.
The main aim of this research was to explore participants' motivations for taking part in this type of research, the participants' experience, and to gain perspective on aspects of the trial design. The findings would be useful in the development of participant centred guidelines which may support the design and implementation of future nutrition health and lifestyle interventions concerning cognition and successful ageing.

4.10 Methods: Mixed methods feedback

Full trial details are available in Chapter 2. Open-ended questionnaires were completed after the intervention was conducted. All qualitative responses (n=48) were analysed using content analysis [265], to identify themes, by two independent researchers (NA, RF). Quantitative feedback was generated using rank-based questionnaires, evaluating the practical aspects of the study process from excellent to fair.

4.11 Results

4.11.1 Feedback participant characteristics

The response rate to the feedback survey was high at 81.4%, 48 of the 59 participants' who completed the vitamin D and cognition RCT, returned their feedback responses. The feedback sample consisted of 29 females (60.4%) of an average age of 67.6 years (range 60-80 years). The majority of participants were new to research (77.1%) and rated there overall experience highly (9.4 ± 0.8 out of 10). Although, 11 (22.9%) reported previously partaking in research as detailed in Table 4.9.

Study experience was highly rated, of the three 60-minute visits across the 6 month study period, most participants rated the time commitment and study processes as "excellent" or "very good" (Table 4.9)

Table 4. 9: RCT Participant characteristics who completed the feedback survey

Demographic	**Overall (n=48)**
Age (mean ± SD)	67.6 ± 5.2
Gender (female), n (%)	29 (60.4)
Location of Residence	
Dublin City (<10km to site)	8 (16.7)
Dublin Greater Area (>10km to site)	31 (64.6)
Rural (>50km to site)	9 (18.7)
Research Experience	
Have you volunteered in research before? *Yes n, (%)*	11 (22.9)
Overall experience? (0-10)	9.4 ± 0.8
Recommend to a friend? (0-10)	9.6 ± 0.7
How would you evaluate the study procedures in regards to; ("Excellent or very good")	
Time taken to complete each assessment (60mins) (%)	92.7
Number of visits required [266] (%)	90.2
Number of questionnaires, n (%)	87.5
Number of memory assessments conducted (6), n (%)	85.4

Results reported as mean ± SD or counts and proportions.

Following the interpretation of participants' feedback results, reoccurring themes were identified for the motivating factors (Table 4.10); concerns (Table 4.11); and changes for future trials (Table 4.12).

Table 4. 10: What motivated you to take part in this project? (Themes and Quotes)

Make a contribution
"I like helping people"
"Interested in positive, healthy ageing and also like helping the community"
"Hopefully contribute in some small way to making it a worthwhile project"
"Wish to contribute to worthwhile research project of benefit to the community"
"Had just retired so wanted to do something positive in helping society"
"Easy way to help out in research in a small way"
"Good bit of excellent treatment for health issues, wanted to give something back"
Interest
"Contribute in a small way towards finding answers to medical unknowns"
"Help with research"
"Help further a scientific project"
"My career background was scientific research"
"Interested in assisting in this field of research"
Topic of interest
"Worthwhile project to participate in"
"My sister and I used to get a dessertspoon of cod-liver oil a day - hence my interest"
"In favour of this research that will help healthy ageing"
"Vitamin D was the study. Hopefully improvement in normal bone and muscle function"
"An interest in preserving health, memory, and general health and well-being as I get older"
"Wanted to find out the benefits of vitamin D"
"Interested in positive, healthy ageing"
"Curious about vitamin D levels and how to correct any deficiencies and maintain it at an appropriate level"
"In favour of research especially vitamin D"
"We don’t get enough vitamin D in our climate"

Table 4. 11: Did any aspect of the study concern you, prior to starting or during your time on the project? (Themes and Quotes)

No Concerns
A total of 36/48 participants expressed they had "No", "None", "No concerns" or justification of no concerns (below) in taking part in the project. "Checked several studies on Google scholar, confident this study would be well run" "No part of this study caused concern" "Explained the procedure perfectly so I was happy"
Memory and Health
"Particularly concerned about memory loss" "Disappointed that I didn't feel my overall health improve" "I was afraid I would not be able to do tests e.g written or memory" "Was worried project might identify some aspect of my own personal health of which I was unaware" "I feared somewhat that I might discover some serious deterioration in mental capacity" "I may be diagnosed as having signs of Alzheimer. If I had I wanted to know though" "Not knowing what the tests would involve"
Study Procedure
"Medication at the start, but I was reassured by X that everything was above board"
Involvement
"Perhaps fear of the unknown, was put at ease with more information" "Was worried had I anything to offer" "Still working full time so I was concerned about time.. Was accommodated every time"

Table 4. 12: During your time with the researcher, are there any aspects of the assessment you would have change? (Themes and Quotes)

No changes
37/48 participants expressed they would make “No" or "No changes"
"Times we were given were accommodating"
"Content of the project was particularly interesting as were the memory tests"
"Researcher was at all times respectful, understanding, gentle and explained all aspects of the tests thoroughly and clearly"
"Researcher was particularly pleasant to meet and converse with"
"I thought the questions were balanced and worthwhile"
"I found everything easy to deal with"
"I was very pleased throughout the assessment and always felt that there was no pressure on me"
"Researcher was always very helpful and put you at ease before each test"
"Researcher was kind and courteous, overall pleasant experience"
Cognitive tests
"In regard to 'recall', I didn’t expect the same stories to be used in 1st and last assessment"
"Everything was easy"
"Cognitive testing was of good variety"
"Memory tests were intimidating, found it much easier on the second occasion"
"Study could have been more comprehensive and comparing to other memory studies"
"None except for the question - have one minute to say all words beginning with 'F', could only think of the 'bad' word"
"It was challenging, it was enjoyable and I understood what each test was about (I think)"
Facilities
"The use of a room which one could not hear other people conducting interviews -the effect on concentration this may have provided results that did not accurately reflect the tests"
"Had to use Luas as parking in hospital is nearly impossible"
"Hospital was a bit difficult to get to and car parking was difficult"
Personal health
"Other areas in assessing risk factors e.g. blood glucose/diabetes/PSA/Prostate cancer"

4.12 Summary

4.12.1 Motivations

Self-selecting participants recruited to cognitive ageing studies have also been shown to be more likely to have a family history of AD [267], it has been suggested that those with a family history or personal motivation may be more likely to volunteer for research due to increased personal interest in prevention of cognitive decline [267], a suggestion which was supported by our mixed-methods feedback survey.

> *"An interest in preserving health, memory, and general health and well-being as I get older"*

Overall most participants were motivated to take part in this health intervention as they had a personal interest in contributing to research, helping others, or had a specific interest in the topic of ageing, dementia or vitamin D.

4.12.2 Concerns

Most participants reflect that they had no concerns about taking part, particularly as the study information was comprehensive. Most common concerns were that the study would result in the identification of a disease or to the general program of research.

> *"Was worried had I anything to offer"*
>
> *"Was worried project might identify some aspect of my own personal health of which I was unaware"*
>
> *"I may be diagnosed as having signs of Alzheimer. If I had I wanted to know though"*

Concern was highlighted in regards to the potential difficulty of cognitive testing before deciding to take part and some were concerned they would find it too challenging. Interestingly none of the participants were concerned with taking a vitamin supplement or placebo and being blinded to the allocation. In contrast to initial anxieties around the cognitive assessment it was also highlighted by the research participants that the assessments were too easy, and got easier at each visit;

"Memory tests were intimidating, found it much easier on the second occasion"

"Everything was easy"

4.12.3 Suggested changes

In contrast to comments of discouragement for location of the site it was also highlighted in "further comments?" that many participants were happy with the site location and the facilities available;

> *"SJH was very convenient to the frail and was easily accessible - have free travel so was easy to get to and from the hospital"*

Furthermore, 77% of the participants who provided feedback expressed they would recommend no changes or had no suggestions for change.

4.12.4 Important factors

Respondents consistently reported the importance of their **interaction with the researcher** conducting the assessments.

> *"Assessments conducted by researcher in a courteous and punctual fashion, very important to me"*
>
> *"Calm and friendly…being friendly and making a connection was v.important"*
>
> *"Researcher was at all times respectful, understanding, gentle and explained all aspects of the tests thoroughly and clearly"*

The role of the researcher to provide a calm and supportive environment is particularly important when conducting tasks of cognitive function which are highly correlated with mood. This may influence attrition rates.

4.13 Key findings

- Participants were mainly healthy, of high socio-economic status as per profile in Table 4.2.
- Feedback survey response was high at 80%; some 22.9% (11) participants had previously taken part in study;
- Some participants found the cognitive assessments intimidating at the beginning, however, there were no concerns regarding vitamin D supplement use or potential allocation to placebo.
- Overall experience was rated as high for a time commitment of 3 visits of 60 minutes duration over a 6-month period, and for the study process; with 6 assessments forming the cognitive battery and multiple health and wellbeing questionnaires.
- Important factors included flexibility in scheduling appointments and feeling relaxed and comfortable with the researcher, which may influence the overall retention rates of which the RCT achieved 98.3%.
- Future research needs to establish ways of recruiting more diverse study groups.

Chapter 5: Effect of vitamin D_3 supplementation on muscle strength and physical performance in community-dwelling, older adults. Results from a randomised double-blind placebo-controlled pilot study.

5.1 Introduction

Sarcopenia can be defined as a progressive loss of skeletal muscle mass that occurs in conjunction with a loss of muscle strength or performance, or both (i.e. muscle function) [109]. This functional decline that accompanies sarcopenia can adversely affect mobility, the risk of falls and fractures, quality of life, a decline in activities of daily living, loss of independence, and disability [107, 268, 269]. Figures from the US, estimate the direct healthcare costs to be $18.5 billion, more than the estimated cost for osteoporotic fractures of $16.3 billion [270].

The prevalence of sarcopenia in older adults varies depending on the study population and the definition of muscle loss applied (5-33%) [271]. Estimates in community-dwelling older adult populations, aged between 60-80 years, are 5-13% [272, 137], the equivalent of 50 million people globally in 2010, and a projected 200 million by 2050 as a result of population ageing [109]. Studies show the prevalence of sarcopenia is highly correlated with advancing age [273, 274]. Other contributing factors include physical inactivity, poor nutritional status, inflammation, a reduction in hormones and neuromuscular dysfunction [275-277], and many intervention studies have been conducted to try overcome one or more of these associated factors [278, 279].

Following the discovery of the vitamin D receptor (VDR) on non-renal tissues researchers have tested the effectiveness of vitamin D supplementation in various chronic diseases including carcinogenesis, inflammatory diseases, cardiovascular disease, and all-cause mortality [280-285]. An abundance of evidence is also available for an effect on muscle strength, physical performance, and muscle function, however, the findings are inconsistent [286, 223, 225, 152].

Primary measurements of sarcopenia include indicators of muscle mass, muscle strength and physical performance [109]. To determine muscle strength and performance multiple techniques and cut-offs can be used. Low handgrip strength (HGS) is associated with premature death and morbidity and other health-related difficulties in older adults [287], it is strongly associated with lower extremity muscle power and it is a superior predictor of clinical outcomes than low muscle mass [274]. Whilst physical performance can be measured by the Timed-Up-and-Go test (TUG) which is an indicator of incident

mobility, falls risk and is useful in following clinical change over time [288-290]. Both measures, HGS and TUG, are supported by the European Working Group on Sarcopenia in Older People (EWGSOP) as primary measures of sarcopenia.

Animal and laboratory models demonstrate a plausibility for vitamin D and muscle function in ageing [291, 292]. Observational studies largely support an association between low serum 25(OH)D concentrations and poorer muscle strength, poorer physical performance and overall muscle function [136, 139, 135, 138]. Randomised controlled trials (RCTs) have yet to confirm the cross-sectional and longitudinal evidence. A recent systematic review and meta-analysis reported a small, but positive effect for vitamin D supplementation and overall muscle strength with greatest benefit in those who had serum 25(OH)D concentrations <30nmol/L at baseline [286]. Whilst other meta-analysis report heterogeneity in study design [151], most notably, the intervention of bolus vitamin D dosing, which has adverse effects on falls risk and is considered inappropriate for use in studies of physical outcomes [130].

The aim of the present study was to investigate vitamin D supplementation and physical function outcomes in community-dwelling older adults. Based on pre-specified secondary outcomes of the vitamin D supplementation and cognitive function RCT in Chapter 4. Outcomes of interest include; muscle strength, physical performance and overall muscle function.

Hypothesis 1: Individuals who are supplementing daily with an average of 2000IU of vitamin D_3, will perform better at 6 months than those allocated to placebo in a task of physical performance as assessed using the Timed-Up-and-Go test (TUG).

Hypothesis 2: Individuals who are supplementing daily with an average of 2000IU of vitamin D_3, will perform better at 6 months in a task of muscle strength as assessed by handgrip strength (HGS).

5.2 Methods particular to this chapter

5.2.1 Study details

Detailed methods regarding study design, the intervention, and ethical approval are detailed in methods Chapter 2 (Study 1). A brief overview is outlined below.

5.2.2 Participant enrolment

- Aged between 60 and 80 years
- Able to provide informed written consent
- No positive screen for suspected MCI [179]
- No other condition likely to affect cognitive function or vitamin D metabolism
- Not consuming supplemental vitamin D of ≥ 800IU/day
- Serum 25(OH)D concentrations ≥15nmol/L or ≤125nmol/L
- Normal corrected serum calcium level (<2.55nmol/L)

5.2.3 Study design and intervention

Participant data was collected at 0, 3 and 6 months.
Active treatment comprised of 4000IU of vitamin D_3 and matching placebo. All participants were directed to take 1 tablet, on alternating days for the study period. Compliance with the intervention was determined by tablet count at the end of the study period at 6 months.

5.2.4 Randomisation

Participants were randomly assigned to a study group by minimisation procedure (based on gender, age category, and physical activity level).

5.2.5 Assessment of physical performance, strength and overall function

Full rational for assessment measures and defining criteria are presented in the methods Chapter 2.

In short, specific outcomes of the present study include;

- Physical performance was assessed using the Timed-Up-and-Go test (TUG) of walking speed measured in seconds to completion.
- Muscle strength was measured using a dynamometer to assess handgrip strength. HGS was measured for both dominant and non-dominant hands, mean results over 2 readings for the dominant hand were used. Gender and BMI specific cut-offs were applied as described in the methods Chapter 2, Table 2.2 and Table 2.3.
- Exploratory analysis included the assessment of poor muscle function defined as concurrent low HGS (gender and BMI-specific cut-offs) and poor physical performance (TUG >10 seconds).
- Body weight and height were measured without shoes and with light clothing at 0, 3 and 6 months. BMI categories were based on WHO criteria.

5.2.6 Serum vitamin D measurement and status criteria applied

Blood samples were collected by a research nurse at 0, 3 and 6 months. Serum 25(OH)D samples were analysed by Liquid Chromatography-Mass Spectrometry (LC-MS). For this study, vitamin D status was defined using the IOM criteria [32]. Deficiency was defined as serum 25(OH)D concentrations <30nmol/L, for insufficiency serum concentrations ranged between 30nmol/L and <50nmol/L, and serum 25(OH)D concentrations ≥50nmol/L was defined as sufficient. For comparison, we also considered levels ≥75nmol/L and ≥100nmol/L for exploratory analysis.

5.2.7 Statistical Analysis

The present study is a pilot investigation and therefore the following statistical approach was agreed;

1. Determine the effectiveness of 2000IU/d vitamin D_3 at increasing serum 25(OH)D concentration at 3 and 6 months in the vitD_3 treatment group (paired *t*-test).

2. Investigate the between-group differences for physical performance and muscle strength at 6 months, following an *intention-to-treat* (ITT) approach (independent *t*-test, Cohen's *d if applicable*).

3. Conduct exploratory analysis to investigate the mean difference from baseline in muscle parameters within and between the study groups after 6 months (independent *t*-test, paired *t*-test, Cohen's *d if applicable*).

All results are presented as the mean ± standard deviation (SD) (all suitable variables) or numbers and proportions. Analyses were based on ITT and corresponding p-values were considered statistically significant at an alpha level of <0.05. Statistical test values <0.10 were deemed of potential interest and corresponding effect sizes were reported (Cohen's *d*). For ITT analysis, last observation carried forward was applied for any missing data recorded at <5%.

All analyses were performed using STATA Corp 14.0 software (Stata Corp LP).

5.3 Results

5.3.1 Study population and baseline characteristics

The CONSORT diagram is included in the primary analysis of this RCT in Chapter 4. A study retention rate of 98.0% was achieved. No adverse events were reported. In total, one participant withdrew from the study after 1 month from the placebo group, who no longer wished to continue.

There were no statistically significant differences between the vitamin D and placebo groups at baseline for demographic, health and lifestyle factors or vitamin D related factors as detailed in Table 5.1. The overall study group comprised of 60 participants (age range; 60-80.5 years, mean 68.5 SD 4.9). All participants were of Caucasian ethnic background. As anticipated, due to minimisation randomisation design, both groups were balanced for age, gender, and physical activity level. The study group were educated, and most participants completed at minimum leaving certificate level education. Occupation status was generally high-skilled or skilled. Most self-reported to be in 'excellent' or 'very good' health. Cognitive status was not indicative of cognitive impairment in line with the inclusion criteria, detailed in the methods Chapter 2. This is supported by the high number of instrumental activities of daily living achieved by all participants. Overall quality of life scores were high and comparable between the two groups, all of which are detailed in Table 5.1.

Table 5. 1: Baseline characteristics of RCT study participants

	VitD$_3$ (n=30)	PL (n=30)	*p*
Demographics			
Age (years)	68.6 ± 4.9	68.3 ± 5.1	0.82
Females, n	17	15	0.61
Caucasian, n	30	30	1
Education (years)	16.2 ± 3.91	15.2 ± 3.78	0.28
Secondary level[§]	23	28	0.45
Employment - Highly skilled	9	11	0.58
Skilled	21	17	0.28
Anthropometry			
BMI (kg/m^2)	26.6 ± 2.85	27.4 ± 3.79	0.33
BMI - normal (18.5 <24.9)	8	11	
- overweight (≥25.0 <29.9)	19	11	0.09
- obese (≥30)	3	8	
Waist circumference, cm	91.2 ± 11.0	96.5 ± 12.8	0.09
Physical health			
At risk of malnutrition (MNA), n	2	0	0.15
IADLs (0-8)	8.0 ± 0.0	7.9 ± 0.3	0.32
[ɣ] Physical activity -Low	9	10	
-Moderate	16	17	0.75
-High	5	3	
Health behaviours			
Alcohol intake, units per week	9.0 ± 7.4	12.7 ± 12.3	0.31
Smoking, n	1	0	0.27
Medication use, n (Y/N)	19	23	0.11
Polypharmacy, n	0	2	0.15
Vitamin D related factors			
Blood draw (Spring), n	30	30	1
Serum 25(OH)D (nmol/L)	51.8 ± 8.1	49.4 ± 22.5	0.65
Serum C. calcium (nmol/L)	2.30 ± 0.1	2.28 ± 0.1	0.26
VitD supplement use (<400IU/d), n	5	4	0.72

Time outdoors (10-3pm), mins	136.7 ± 73.9	140.5 ± 78.2	0.85
SPF use (always/usually), n	21	19	0.58
Sun travel (previous 6 months), n	10	5	0.14
Mental health and well-being			
TCogS (Score 0-27)	26.6 ± 0.8	26.3 ± 1.2	0.55
QoL (CASP-19, 0-57)	53.7 ± 3.9	51.8 ± 6.7	0.18
Depression (CES-D) (0-60)	1.2 ± 1.9	2.5 ± 3.4	0.09

Values are expressed as mean ± SD or counts, unless otherwise stated.

PL, placebo; vitD3, vitamin D_3 treatment group; employment, highly skilled (e.g. CEO, engineer, professor, judge, surgeon); skilled (e.g. nurse, craftsman, teacher); IADL, Instrumental Activities of Daily Living, a higher score indicates high functioning; MNA, Mini Nutritional Assessment; Polypharmacy, 5 or more medications; Serum C. calcium; corrected serum calcium; Time spent outdoors, minutes spent outdoors between 10am-3pm on a sunny day; SPF, sun protection factor; TCogS, Telephone Cognitive Screen; QoL (CASP-19), Quality of Life "Control" "Autonomy" Self- realisation" and "Pleasure"; CES-D, Centre for Epidemiological Studies Depression Scale.

[§] Completed secondary level education at minimum.

[ɣ] International Physical Activity Questionnaire-Short Form (IPAQ-SF) MET-minutes categories (metabolic minutes).

Between-group differences were analysed by independent *t*-tests and χ^2 or non-parametric equivalents.

Statistical significance was reported at $p < 0.05$.

5.3.2 Physical factors, health and lifestyle behaviours at baseline between vitamin D treatment and placebo group

The groups were well matched at baseline for anthropometric and physical health variables, as detailed in Table 5.1. Mean BMI (kg/m^2) indicated the average participant in this study was overweight. Mean waist circumference, an indicator of central adiposity, was above the WHO cut-off for increased metabolic risk in both groups [293]. Physical activity levels categorised by metabolic minutes (MET-minutes), which was derived from the International Physical Activity Questionnaire-Short Form (IPAQ-SF), which shows the majority of participants in both groups engaged in moderate physical activity on a weekly basis. Medication use was high, however, polypharmacy (≥5 medications) was low for both groups. The majority of medications reported were for the management of hypertension and hyperlipidaemia. Almost all participants scored full marks in the Instrumental Activities of Daily Living (IADL) scale meaning they had no issues with performing tasks of daily living (e.g. bathing, shopping, preparing meals, managing finances and taking medication). Most were at 'no risk of malnutrition' according to the MNA screening tool. Regarding lifestyle behaviours, alcohol consumption was moderate and within the safe limits for both study groups. Smoking was not a substantial behaviour in this study sample (1/60).

5.3.3 Serum 25(OH)D concentrations and vitamin D status in response to vitamin D_3 supplementation

Full results are detailed in the primary outcomes of the same RCT in Chapter 4. The vitD$_3$ and PL groups were comparable at baseline for serum 25(OH)D concentrations (nmol/L) [(vitD$_3$, 51.8 (18.1); PL, 49.4 (22.5); p=0.65], as illustrated in Figure 5.1. There was a statistically significant increase in mean serum 25(OH)D concentrations within the vitD$_3$ group and a significant decrease in the PL group at 6 months from baseline [vitD$_3$, +24.8 (34.4); PL, -6.9 (23.2); p<0.0001]. At 6 months, the mean serum 25(OH)D concentrations were [vitD$_3$, 76.7 (29.2); PL, 42.3 (18.4); p<0.0001]. Taken together this shows that vitamin D$_3$ supplementation raised 25(OH)D concentrations in the treatment group at 6 months.

Fluctuations were observed in both the $vitD_3$ and PL groups at 3 months, due to seasonal influences, a statistically significant decrease in serum 25(OH)D concentrations was noted in the $vitD_3$ group at 6 months (autumn) compared to levels achieved at 3 months (summer) [-27.3 (26.9), $p<0.0001$].

Vitamin D status for the $vitD_3$ and PL groups, before and after the intervention, is detailed in Chapter 4, Table 4.4. Overall, 18.3% (11/60) were deemed deficient (<30nmol/L) at baseline. At 3 months, all of those in the $vitD_3$ group were deemed sufficient, whereas, half of the PL group were deficient or insufficient (14/28).

At baseline, 2 participants from in the $vitD_3$ treatment group were deficient, both corrected their deficiency at 6 months. However, in the treatment group at 6 months, 2 other participants were deemed vitamin D deficient after the intervention, both of which had insufficient levels at baseline. Of the 9 participants from the PL group who were deficient at baseline, 8 were identified as deficient or insufficient (serum concentrations ranged from; 20.0-38nmol/L) after the intervention. The proportion of vitamin D sufficiency was higher in the $vitD_3$ group after the intervention (n=25) compared to PL (n=7).

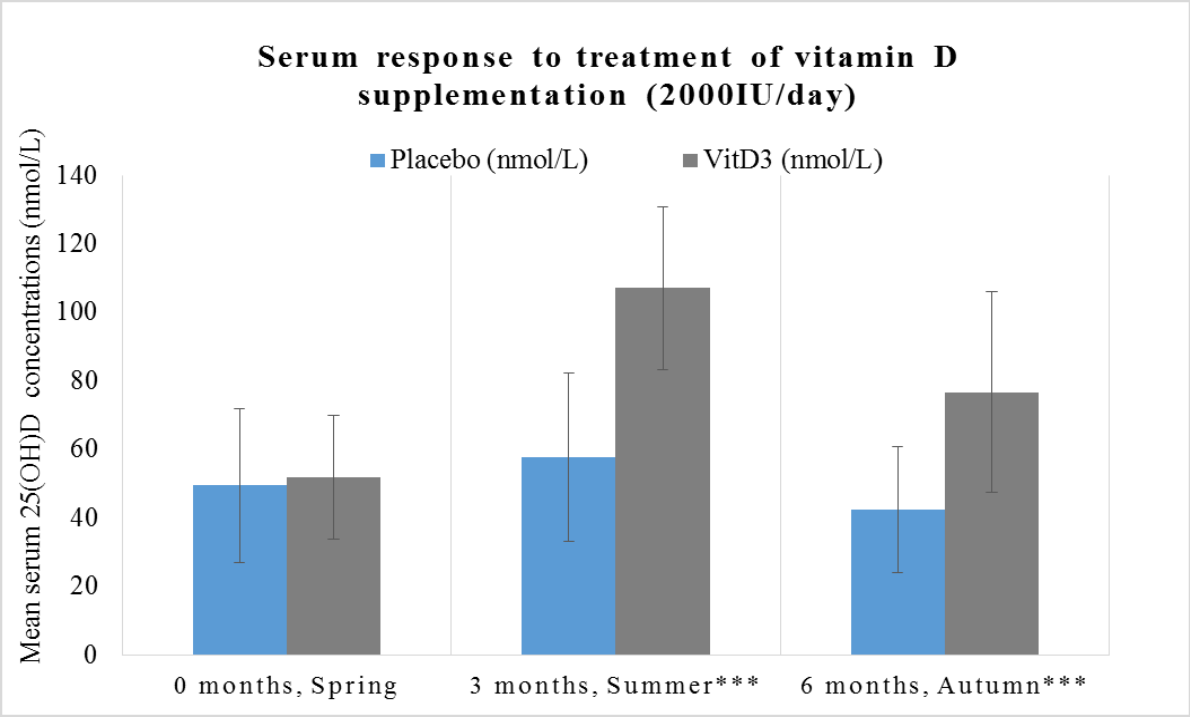


Figure 5. 1: Serum 25(OH)D in response to treatment of 2000IU (daily average) compared to placebo at 0, 3 and 6 months.

***Statistical significance between and within-group difference was reported at $p<0.0001$.

5.3.4 Muscle strength, physical performance and muscle function at baseline

Overall the prevalence of poor muscle strength, poor physical performance and poor muscle function was high at 36.7%, 51.7% and 13.3% respectively. The prevalence between study groups was similar, as illustrated in Figure 5.3. We found no statistically significant difference between the vitD$_3$ group and the PL group at baseline for measures of muscle strength (HGS, p=0.64), physical performance (TUG, p=0.64), or muscle function [concurrent; poor performance in TUG and low HGS, (p=1.00)] as detailed in Table 5.2.

Poor muscle strength was based on gender and BMI-specific cut-offs (Table 5.2), the groups were well matched, with n=11 participants each in the vitD$_3$ and placebo group, p=1.00.

As described in more detail in the methods Chapter 2, TUG cut-offs vary and are generally applied based on the outcome of interest and the overall ability of the sample population. Our overall mean (SD) TUG score was 10.9 (3.1) seconds, similar to that reported in the TILDA study population 10.1 (3.2) [290]. Therefore, for comparability, poor physical performance on the TUG test was defined as a score >10 seconds [217]. The groups were similar for the prevalence of poor physical performance (p=0.45), detailed in Table 5.2 and Figure 5.3.

The number of participants with poor muscle function was low overall at 13.3%, and prevalence was the same in both groups (n=4, 13.3%), as illustrated in Figure 5.3.

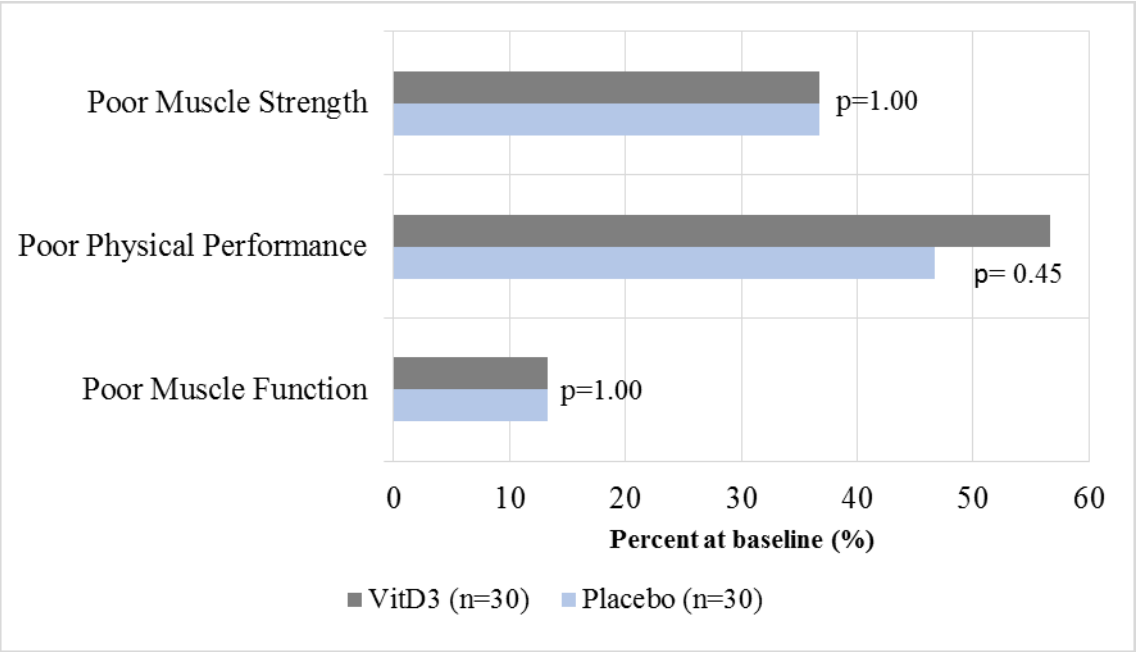


Figure 5. 2: Baseline prevalence of poor muscle strength (HGS), poor muscle performance (TUG >10 seconds) and poor muscle function (concurrent poor muscle strength and poor physical performance).

5.3.5 Effects of vitamin D supplementation on muscle strength- handgrip strength (HGS)

Muscle strength was assessed on both the dominant and non-dominant hands. At 6 months, there were no statistically significant differences in HGS (dominant hand) between the $vitD_3$ and the PL group for this measure of muscle strength (kg) [$vitD_3$, 25.9 (9.2); PL, 26.5 (11.1), p=0.82], detailed in Table 5.2. Consistent with this, no difference was detected based on the analysis of the non-dominant arm measures. The prevalence of poor muscle strength (gender and BMI specific cut-offs) was not altered in response to treatment, $vitD_3$ (n=10, 33.3%) versus PL (n=11, 36.7%).

5.3.6 Effects of vitamin D supplementation on physical performance- Timed-Up-and-Go (TUG)

Physical performance was assessed using the TUG test, results are reported in Table 5.2. Higher scores indicate slower and poorer performance. There was no statistically significant difference between the $vitD_3$ and PL group in mean TUG scores at 6 months [$vitD_3$, 10.7 (2.1); PL, 11.3 (3.0), p=0.39].

Consistent with this, when the criteria for poor physical performance were applied using the TUG cut-off score >10 seconds, the proportion of those defined as poor performers did not statistically differ between the $vitD_3$ and PL groups (p=0.28).

Table 5. 2: Mean change in muscle function, performance and impaired physical performance between groups at 6 months

Physical outcomes measures	**Placebo (n=30)**		**VitD$_3$ (n=30)**		***P***	**ITT *P***
	Baseline	6 months	Baseline	6 months	Baseline	6 months
Physical Performance						
TUG (seconds)	10.8 ± 2.9	11.3 ± 3.0	11.1 ± 3.4	10.7 ± 2.1	0.64	0.39
Poor performance (TUG >10 sec)	14 (46.7)	21 (70)	17 (56.7)	17 (56.7)	0.45	0.28
Muscle Strength						
HGS- dominant (kg)	27.4 ± 11.4	26.5 ± 11.1	26.1 ± 9.2	25.9 ± 9.2	0.64	0.82
non-dominant (kg)	25.4 ± 11.4	24.5 ± 11.1	25.3 ± 10.3	24.5 ± 8.9	0.98	0.98
Poor muscle strength, n (%)	11 (36.7)	11 (36.7)	11 (36.7)	10 (33.3)	1	0.79
Muscle Function						
Poor MF (HGS&TUG), n (%)	4 (13.3)	9 (30.0)	4 (13.3)	6 (20.0)	1	0.37

Results are reported as mean ± standard deviation or counts and proportions (%).

ITT, Intention-to-treat; TUG, Timed-Up-and-Go, higher scores indicate poorer performance; poor performance, proportion of participants with a TUG score > 10 seconds; HGS, handgrip strength, reported as mean of 2 readings for both hands; poor muscle strength; impaired muscle strength based on gender and BMI specific cut-offs; poor MF, poor muscle function defined as poor physical performance (TUG >10 seconds) AND poor muscle strength (gender and BMI cut-offs).

Independent student *t*-test for continuous data or non-parametric equivalent (Mann Whitney-U) and χ^2 for categorical variables.

*Significance was reported at $p<0.05$.

5.3.7 Exploratory analysis of the effect of vitamin D_3 supplementation on muscle function- concurrent poor muscle strength and poor physical performance

The prevalence of poor muscle function increased at 6 months; $vitD_3$ (n=6, 20.0%) versus PL (n=9, 30.0%). No statistically significant difference was observed between-groups (p=0.37), as detailed in Table 5.2.

5.3.8 An exploratory analysis of intra-group changes from baseline in muscle strength, physical performance and muscle function at 6 months

The data were further analysed to determine whether a statistically significant change occurred in either the $vitD_3$ or PL group from baseline, to 6 months, results are detailed in Table 5.3.

Within-group changes were not statistically significantly different for physical performance scores (TUG). The PL group were on average 0.51 seconds slower at 6 months and the $vitD_3$ group were faster. The overall mean difference between the $vitD_3$ and PL groups was 0.95 seconds and statistically significant (p=0.03).

Considering muscle strength, as measured by HGS, there was an increase in strength observed in both groups at 6 months, with a greater improvement in the placebo groups, however, this did not reach statistical significance (p=0.31). In line with this, the overall change between-groups was also not significant (p=0.46).

Considering muscle function, the proportions defined as having poor functional status were moderately high. Whilst statistical analysis was not significant, descriptively it is worth noting from baseline an additional 5 participants were deemed to have poor muscle function in the PL group, an increase was also observed in the $vitD_3$ group (+2).

Table 5. 3: Between and within-group change in muscle outcomes from baseline to 6 months

			Baseline	6 months	SMD	*P*
Performance	**TUG (seconds)**	Δ PL	10.8 ± 2.9	11.3 ± 3.0	+0.51	0.11
		Δ vitD$_3$	11.1 ± 3.4	10.7 ± 2.1	-0.44	0.17
	Between-group Δ difference				0.95	0.03*
Strength	**HGS dom (kg)**	Δ PL	27.4 ± 11.4	26.5 ± 11.1	+0.82	0.31
		Δ vitD$_3$	26.1 ± 9.2	25.9 ± 9.2	+0.13	0.79
	Between-group Δ difference				0.95	0.46
Function	**Poor MF, n**	Δ PL	4	9	+5	0.28 ǂ
		Δ vitD$_3$	4	6	+2	0.89 ǂ

Δ Within-group change from baseline to 6 months (paired t-test).

MF, poor muscle function defined as poor physical performance (TUG >10 seconds) AND poor muscle strength (gender and BMI cut-offs).

Between-group difference independent *t*-test or Mann Whitney-U.

ǂ McNemar test for matched binary pairs.

*Significance was reported at $p<0.05$.

5.4 Discussion

The aim of this study was to determine the effect of vitamin D_3 supplementation (daily average of 2000IU) on muscle strength and physical performance in community-dwelling older adults. Physical function was assessed as a pre-specified secondary outcome of the 6-month pilot RCT, with the primary cognitive outcomes previously presented in Chapter 4.

We demonstrated that vitamin D concentrations did significantly increase in response to supplementation in the $vitD_3$ treatment group compared to the placebo group after 6 months. We found that individuals who supplemented with vitamin D_3 for 6-months did not perform better in measures of muscle strength or physical performance compared to those who received a placebo (PL). Pre-specified functional outcomes were measured by gait speed for physical performance (Timed-Up-and-Go test, TUG) and handgrip for muscle strength (HGS). An improvement in TUG speed for the $vitD_3$ group was observed whilst the PL group showed slower performance. Irrespective, neither change was deemed meaningful [294]. There was no difference in muscle strength between the $vitD_3$ and PL group at 6 months. Additionally, no statistically significant difference was found within-groups as a measure of change overtime or for sub-group analysis based on vitamin D status.

The $vitD_3$ and PL group were homogenous for baseline characteristics in this study. No statistically significant difference in demographic or physical health variables was identified, reflecting the success of utilising minimisation techniques for randomisation. This high comparability between groups was ideal for analysis of treatment effects.

The age profile, gender balance and physical attributes of this study sample are comparable with other nationally representative studies of ageing populations such as TILDA or ELSA [295, 218]. Compared to previous RCTs on vitamin D supplementation and muscle function in older adults, the mean age of this study was lower on average [143, 141, 144, 149], however, this was due to our inclusion criteria of 60-80 years. This age range was chosen as the overall aim of this thesis to investigate preventative strategies in healthy older adults, and research has suggested earlier interventions are most

advantageous [296]. Furthermore, potential critical periods for vitamin D and physical and cognitive health are modelled to be most influential in young old (60-70 years) and older old adults (70-80 years) [297, 50].

Health behaviours reported in this sample were lower than national figures, with a clear absence of smoking behaviour. Overall alcohol consumption was classified as moderate [298], as per average units consumed per week. Physical activity levels were similar in this study population compared with TILDA [295]. No participant in this study reported depressive symptomology, whereas prevalence rates of 11.9-18% have previously been reported for community-dwelling older adults residing in Ireland [299, 295].

Mean serum 25(OH)D levels and prevalence rates of deficiency and insufficiency in this study were comparable with other Irish figures [35]. Whilst mean serum 25(OH)D levels were similar to the ELSA study findings, reported in Chapter 3, the prevalence of deficiency was lower in this study at 18.3% compared to 23.7% in the ELSA study. It is likely that those with vitamin D deficiency at baseline are more likely to respond to vitamin D treatment and therefore incur a beneficial effect, if any, in muscle parameters. Potentially the low prevalence of vitamin D deficiency in this sample cohort was too small to detect a noticeable change. Baseline serum 25(OH)D concentrations in this present study are comparatively lower than most of the physical RCTs reviewed in Chapter 1 part A [141, 140, 144, 145, 149]. Two studies recruited participants with low serum 25(OH)D concentrations at baseline [143, 148]. Bunout et al. for example, reported a significant improvement in TUG test scores after a 9-month intervention of vitD_3 (400IU/d) and calcium [143]. Of note, the author reported the mean serum 25(OH)D concentrations in the placebo group to be in the insufficient range at study completion. The other positive RCT finding conducted by Pfeifer et al. again reported an improvement in TUG test scores, however, serum 25(OH)D concentrations were in the sufficient range for both the treatment and placebo group at the end of the study period [149]. Therefore providing little evidence for a potentially optimal serum 25(OH)D concentration for physical functioning.

In the present study the prevalence of poor muscle strength, poor physical performance and poor muscle function was high at 36.7%, 51.7% and 13.3% respectively. Baseline measures of handgrip strength and TUG test score vary greatly among the RCTs

published to date. Comparison of baseline handgrip strength with studies included in the overview of published systematic reviews in the introduction Chapter1 part A, revealed that mean handgrip strength at baseline was stronger in our study population compared with three other studies [143, 141, 147] and reduced compared to others [150, 145]. Mean TUG was slower in this present study compared with four other studies [141, 300, 150, 149] and quicker than just one other study [143], included in Chapter 1 part A. However, direct comparisons are complicated by different measurement techniques and equipment used. Unpublished figures from the TILDA study identified a prevalence for weak handgrip strength of 22.9%, slow TUG test of 19.4% and combined impairment of 9.6%. This means that compared to this nationally representative data, the present RCT detected a high degree of impairment.

This present study found no statistically significant difference in mean HGS in the $vitD_3$ and PL group, indicating no effect of vitamin D supplementation on muscle strength at 6-months. This finding is consistent with other published RCT evidence to date were few studies found an effect (summarised in Chapter 1 part A, Table 1.4). No study found an effect of vitamin D supplementation on HGS as detailed in Table 1.5.

For physical performance, the $vitD_3$ group improved and the PL group got slower at 6 months although, the changes were small at ~0.5 seconds in both cases. The mean difference in the change in TUG between $vitD_3$ and PL groups was statistically significant with a moderate effect size ($p = 0.03$, Cohen's $d = 0.57$). This mean change from baseline in TUG score between groups did not translate to a reduction in the prevalence of slow TUG (cut-off >10 seconds) in the $vitD_3$ group. According to the literature it is suggested the minimal detectable change for TUG test score is 4 seconds [294], whilst others argue TUG test score is not a significant predictor of adverse clinical outcomes such as falls, but more so an accurate predictor based on cut-offs only [288].

A recent systematic review and meta-analysis into the effects of vitamin D on muscle parameters found a worsening effect of vitamin D supplementation on TUG test results but this was based on a small number of studies and the authors declare this should be interpreted with caution due to a high degree of heterogeneity [151]. The author found that among those studies which reported an improvement in either HGS or TUG, the finding was observed in subgroups with pre-existing low levels of 25(OH)D or for

participants who had muscle measures classed as weakest and slowest at baseline. In the present study, the size was a limiting factor for this analysis as sub-group samples were small, as detailed in the cognitive outcomes of this RCT in Chapter 4.

5.4.1 Strengths and limitations

The defining strength of this present study is the double-blind, placebo-controlled design and the successful randomisation process which led to no observed statistically significant differences in mean demographic, health and lifestyle factors (including physical activity) and muscle outcome measures for the vitD_3 versus PL group at baseline. Additionally, the compliance rate with treatment was high (99%) among participants and no adverse events were reported. Thus, evidencing the effectiveness and safety of raising serum 25(OH)D levels in community-dwelling older adults.

There are a few limitations of note in relation to this study trial. Muscle outcomes were the secondary pre-specified endpoint of the pilot study, with a primary endpoint of cognitive function, and of modest sample size. We have good evidence to provide conclusions for future studies regarding the feasibility and the development of larger scale RCTs. A longer study duration extending into the winter season, along with more detailed assessment of muscle outcomes, would have facilitated a more in-depth analysis.

5.5 Conclusion and future research

The results of this present study provide insufficient evidence to support vitamin D supplementation alone as an intervention for improving muscle strength (as measured by handgrip strength). This consensus is widely supported among existing vitamin D and muscle intervention studies in older adults [140, 145, 147]. Interestingly, a small but statistically significant overall change in TUG test score was found in this present study, in a positive direction for vitamin D_3 supplementation. This exploratory finding is consistent with the fact that among the existing vitamin D supplementing studies, more studies have found a significant effect for TUG compared with handgrip.

Further consideration could be given to the usefulness of physical performance measures to clarify the role of vitamin D and muscle. Overall, studies examining the effect of vitamin D on muscle outcomes are lacking a standardised measure for muscle strength. Some authors have suggested the use of a common physical performance battery, such as the Short Physical Performance Battery [301, 302, 222]. It is possible that subpopulations of older adults, who are vitamin D deficient or with a higher prevalence of muscle impairment, may show greater improvement in response to short term vitamin D supplementation.

Trials with large sample sizes are needed to further investigate the potential benefit of vitamin D supplementation in older adults with low levels of serum 25(OH)D and/or low muscle strength or poor physical performance at enrolment. Studies to date have agreed that physical activity is an effective intervention for improving muscle mass and muscle function in healthy older adults. Equally other factors such as omega 3 fatty acids, protein intake, physical activity and resistance training are important considerations for multimodal approaches to maintain muscle function in ageing.

Chapter 6: Vitamin D and muscle function in community-dwelling older adults. Findings from the English Longitudinal Study on Ageing

6.1 Introduction

In Chapter 5 we aimed to test the hypothesis that participants randomised to a treatment of, on average, 2000IU/d of vitamin D_3 would perform better in assessments of muscle strength and physical performance than those randomised to placebo. The 6-month pilot study comprised of 60 community-dwelling older adults, aged over 60 years. We found no significant effect of vitamin D_3 supplementation on measures of muscle strength (handgrip strength, HGS) or physical performance (Timed-Up-and-Go test, TUG) at 6 months. We did note an indication towards poorer physical performance (TUG score) in the placebo group, from baseline, compared to the vitamin D_3 group. Whilst the sample size may have been underpowered to detect potential treatment effects, the results are consistent with the current evidence base [303-307, 145, 308].

A recent supplementation study (800IU D_3 daily) reported improved muscle strength and physical performance in community-dwelling older females [309]. After a 6-month period, a statistically significant increase was noted in a validated in-depth physical performance battery (Short Physical Performance Battery) and in measures of appendicular muscle strength (HGS and knee extension). Results from observational studies suggest low serum 25(OH)D concentrations (<50nmol/L) have an adverse effect on multiple measures of muscle strength and physical performance in older adults, in particular, lower body strength, slower physical performance and lower body muscle mass [136, 139, 135, 138].

As detailed in the introduction in Chapter 1 part A, vitamin D deficiency is highly prevalent in older adults, particularly in populations at northerly latitudes. Whilst *in vitro* studies indicate a direct and indirect role for serum 25(OH)D and 1, 25(OH_2)D [116, 126, 117, 292] in promoting muscle function, evidence in human studies is less consistent [116, 126, 117, 292, 156, 152, 151]. With respect to homogeneity, a number of differences in observational and intervention studies are apparent, including the study sample profile (e.g. many include female-only cohorts), vitamin D factors (e.g. serum status criteria, mode of analysis, dosing regimens) and outcome measures used. Our own RCT, for example, investigating vitamin D supplementation on muscle function used TUG as the outcome measure, described in Chapter 5. Only a few studies have used in-depth

assessments of physical performance (SPPB) [136, 307], which warrants further investigation.

Therefore the aim of the present study was to investigate a link between serum vitamin D concentrations and physical performance (SPPB) and muscle strength (HGS) in a large sample of community-dwelling older adults aged over 60 years.

Hypothesis: Low serum 25(OH)D concentrations are associated with poorer performance in assessments of muscle strength and physical performance.

6.2 Methods particular to this chapter

6.2.1 Study design and population

The current study includes 10601 participants from ELSA, ethics and the general study design are detailed in the methods Chapter 2. For the present analysis cross-sectional data from Wave 6 was used (2012-2013), as this was the first time 25(OH)D concentrations were collected for the new ELSA study sample.

In total, 7731 completed the nurse visit, with the most common reason of missing or unavailable biological data to be an unwillingness to consent or ineligibility (bleeding disorders or anticoagulant use). Inclusion criteria were participants aged 60+ years, with valid measures of BMI, serum 25(OH)D, handgrip strength and physical performance (Short Physical Performance Battery). After excluding those who did not complete the core and nurses data collection at Wave 6, or had missing data for other key variables, the final analytical sample comprised of 4157 subjects, which is illustrated in Figure 6.1.

6.2.2 Study measurements

6.2.2.1 Serum 25(OH)D measurement, deficiency criteria and related factors

Serum 25(OH)D was assessed from fasting blood samples during collected during the nurses' visit of Wave 6. Concentrations of 25(OH)D were analysed using DiaSorin Liaison 25-hydroxyvitamin D immunoassay. All blood sampling occurred from January 2012- July 2013. Vitamin D status was determined by IOM guidelines [32]. Season was categorised using the extended vitamin D calendar for summer (April-Sept) and winter (Oct-March) [14].

6.2.2.2 Measures of physical performance and muscle strength

- Physical performance was measured using the Short Physical Performance Battery (SBBP), which was available only for a subgroup of ELSA participants (those aged ≥60 years of age), as detailed in the methods Chapter 2. In short, the SPPB is a composite battery of 3 physical tasks: an 8-metre gait speed test measured in seconds, a repeated chair rise measured as the time taken to complete multiple raises and a static balance test measured by successfully holding varying stances for a defined period of time.

- Handgrip strength was measured by mean grip strength of three readings using the dominant hand, where possible. Gender and BMI specific cut-offs were applied to assess muscle function, using the same criteria applied to the physical outcomes of the RCT in Chapter 4 and following the criteria detailed in methods Chapter 2.

6.2.2.3 Potential confounders and related factors

Socio-demographic factors included; gender, educational attainment and employment status (employed/retired). Anthropometric factors; BMI, categorised by WHO guidelines and waist circumference measured in centimetres [310]. Health-related factors; self-reported health; excellent, very good, good, fair and poor. Self-reported long standing illness and if self-reported long-standing illness was limiting. History of falls (has had 1 fall, multiple falls, or a number of falls) and medication use (polypharmacy ≥5 medications). Lifestyle factors; physical activity categorised as mild, moderate and vigorous activity more than once per week. Alcohol intakes; high alcohol frequency (drinks alcohol most days), and current smoking status (Yes/No). Other health behaviours which may explain serum 25(OH)D status was available and included; travel abroad within the past 12 months (Yes/No) and vitamin D supplement use defined as prescribed vitamin D and calcium use as required for treatment of osteoporosis.

6.2.3 Data analysis

Descriptive statistics are presented as means and SD or counts and proportions for demographic and lifestyle characteristics. Overall prevalence rates are presented as crude results. The weighting strategy for cross-sectional blood analysis, applied in Chapter 3, was not deemed appropriate in the current study as only a subsample of ELSA participants who provided a blood sample were included in this analysis. Differences between the groups were examined by independent student t-tests or Mann Whitney-U for ordered variables and non-parametric continuous data, $\chi 2$ or ANOVA for proportions and mean difference as appropriate. For serum 25(OH)D concentrations, extreme outliers were defined as those with serum 25(OH)D levels >250nmol/L (100ng), none were identified in the population, therefore, all 25(OH)D samples with corresponding physical assessment were included.

Normality was assessed visually using histogram matrix plots and ladder tables to identify appropriate transformations [242], and Monte Carlo simulations to identify outliers [243]. Multiple logistic regression analysis was used to explore the determinants of poor physical performance and poor muscle strength, presented as odds ratios (OR) and 95% confidence intervals (CI) to determine the direction of the association between functional parameters and relevant covariates. In adjusted models covariates were selected based on previous research findings and following stepwise regression to test the contribution of each predictor (excluded $p>0.2500$). The contribution of covariates to muscle function was examined by including each set of covariates as models in the following order; model 1, poor physical performance defined as a score of 0-6 in the SPPB, socio-demographic factors, health-related behaviours, physical factors and health conditions. In model 2, low HGS strength defined by gender and BMI specific cut-offs. Corresponding unadjusted results are also reported.

All statistical analyses were conducted with STATA 14.0 software (Stata Corp LP).

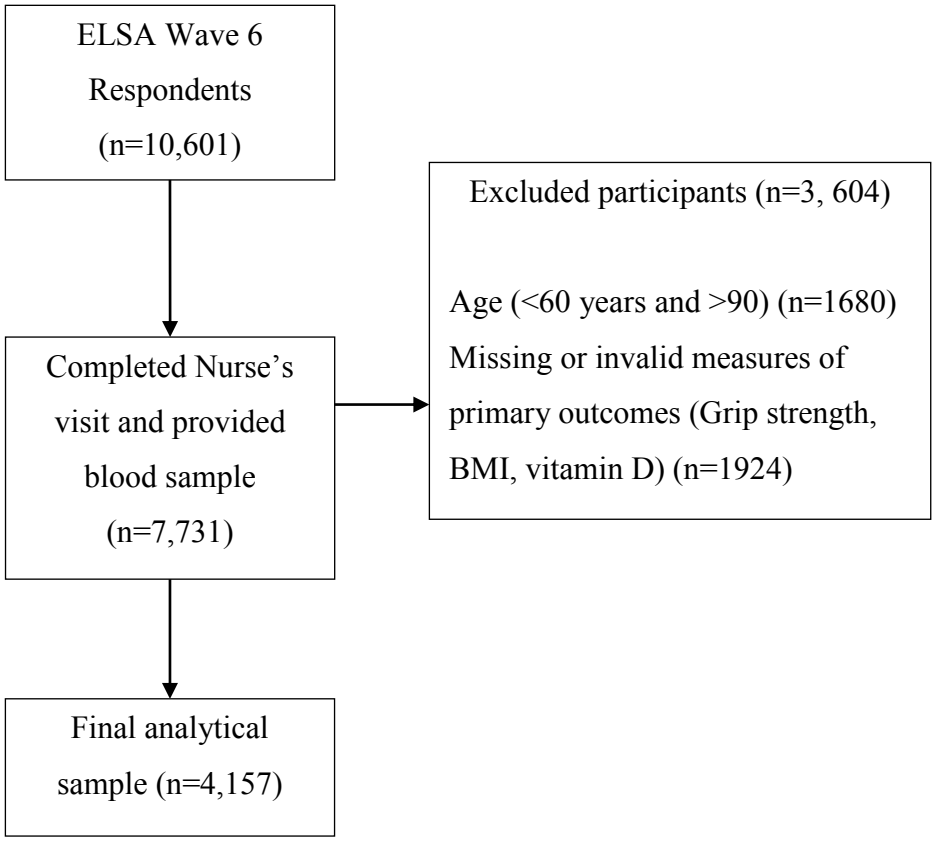


Figure 6. 1: Participant exclusion for this analysis, with reasons.

6.3 Result

6.3.1 Characteristics of study population

A total of 4157 adults aged 60 years or older were included in the present study. The mean sample age was 69.8 (SD 6.9) years. Table 6.1 shows the characteristics of participants overall and by gender. Overall, 54.9% were female and 74.9% of participants had a minimum O-level education or equivalent, with a statistically significantly larger proportion of males 79.5% compared to females (71.1%). Overall, 31.8% reported their health to be 'very good', and 11.7% to be 'excellent', compared to others their own age.

Between genders, in this sample, a greater proportion of males were yet to retire 22.9% compared to 14.4% of females. Multiple characteristics were statistically significantly different for both anthropometric measures and health and lifestyle factors. Whilst no significant difference was noted in mean BMI (kg/m^2), a greater proportion of females were of normal (28.6%) and obese (31.4%) BMI, whereas half of the male participants were classified as overweight (50.2%). Smoking behaviour was similar between genders and 9.4% overall, high alcohol consumption was low at 6.1% overall, with a statistically higher rate in males 7.9%. The frequency of moderate and vigorous physical activity was higher in male participants, at 68.5% and 49.3% respectively, whereas the proportion of females engaging in regular mild physical activity, such as walking, was high at 88.2%.

Overall 15.8% reported having a single fall and 10.5% reporting multiple falls in the last 12 months. A greater prevalence of single falls (17.1%) and recurrent falls (11.6%) was noted in females. In total, 80.5% of male fallers compared to 69.9% of female fallers were more likely to require or seek medical attention after a fall. Self-reported long-standing illness was reported at 54.3% overall, a higher proportion of females (61.7%) reported their illness to be limiting compared to 55.3% of males. Almost a third of participants reported taking 5 or more medications daily (29.1%). Less than one-fifth of participants reported poor quality of life (CASP-19, score <36) (18.6%), with a statistically significantly greater proportion seen in males 20.2%.

Table 6. 1: ELSA study participant characteristics overall and stratifed by gender (n=4,157)

	Overall (n=4157)		Males (n=1874)		Females (n=2283)	
	N (%)	**SD**	**N (%)**	**SD**	**N (%)**	**SD**
Socio-demographic factors						
Age, years	69.8	6.9	69.7	7	69.8	6.9
60-69	2268 (54.6)		1033 (55.1)		1235 (54.1)	
70-79	1450 (34.9)		641 (34.2)		809 (35.4)	
80+	439 (10.6)		200 (10.7)		239 (10.5)	
ǂ **Education** ≥ O Level	3099 (74.9)		1483 (79.5)		1616 (71.1)	
Employment- retired	3090 (74.3)		1362 (72.7)		1728 (75.7)***	
Ethnicity (non-white)	87 (2.1)		46 (2.5)		41 (1.8)	
Health and Lifestyle factors						
BMI(kg/m²) WHO	28.0	4.8	27.9	4.1	28.1	5.3
Under (<18.5)	37 (0.9)		7 (0.4)		30 (1.3)***	
Normal (≥18.5 to <25)	1085 (26.1)		432 (23.1)		653 (28.6)***	
Overweight (≥25 to <30)	1823 (43.9)		940 (50.2)		883 (38.7)***	
Obese (≥30)	1212 (29.1)		495 (26.4)		717 (31.4)***	
Physical activity						
Vigorous (>1/wk)	1739 (41.8)		923 (49.3)		816 (35.7)***	
Moderate (>1/wk)	2703 (65.0)		1283 (68.5)		1420 (62.2)***	

Mild (>1/wk)	3463 (83.3)		1450 (77.4)		2013 (88.2)***	
Current smoker	391 (9.4)		174 (9.3)		217 (9.5)	
Alcohol- 5-6 days/week	254 (6.1)		148 (7.9)		106 (4.6)***	
SR Health						
Excellent	485 (11.7)		212 (11.3)		273 (11.9)	
Very good	1323 (31.8)		607 (32.4)		716 (31.4)	
Good	1365 (32.8)		617 (32.9)		748 (32.8)	
Fair	770 (18.5)		346 (18.5)		424 (18.6)	
Poor	213 (5.1)		92 (4.9)		121 (5.3)	
Long-standing illness	2258 (54.3)		1007 (53.7)		1251 (54.8)	
-*limiting*[§]	1328 (58.8)		557 (55.3)		771 (61.7)**	
Polypharmacy (≥4)	1209 (29.1)		548 (29.2)		661 (28.9)	
CASP-19	42.5	7.8	42.3	7.8	42.6	7.7
Poor QoL (CASP <36)	772 (18.6)		378 (20.2)		394 (17.3)*	
Fall/s (one fall)	658 (15.8)		267 (14.3)		391 (17.1)**	
Multiple falls	438 (10.5)		173 (9.2)		265 (11.6)**	
ǂ Medical attention	813 (74.2)		354 (80.5)		459 (69.9)***	

Results reported as mean (SD), or counts and %. SR, self-reported; kg/m^2, kilograms/metre squared;

†married includes those first time, remarried and legally recognised civil partnership

[§]if long-standing illness it limiting. ǂ Whether medical attention was sought for fall.

P-value denoting significant levels between groups *P*<0.05*, *P*<0.01**, *P*<0.001***

6.3.2 Muscle function and vitamin D status

6.3.2.1 Muscle strength and physical performance

Findings for muscle strength and physical performance in the present study are detailed overall and by gender, in Table 6.2. The overall mean handgrip strength (HGS-dominant hand), was 27.9 (10.6) kg, as anticipated, a clear gender difference was observed; males 36.1 (9.2) kg compared to females 21.4 (6.2) kg. The overall prevalence of low HGS (gender and BMI specific cut-offs) was 30.6%, with a greater prevalence in females (33.6%) compared to males (26.9%).

Poor physical performance, defined by EWGSOP as a score of ≤ 6 points in the SPPB, identified 12.7% of participants overall. Almost 50% higher prevalence was noted in females (14.7%) compared to males (10.3%). The majority of participants were identified as "high performers", with a score of 10-12, with an overall prevalence rate of 67.9%. Again males showed better performance at this cut-off, with a prevalence of 71.6% identified as high performers as opposed to 65.0% of females.

6.3.2.2 Serum vitamin D levels and status

Serum 25(OH)D and vitamin D status are detailed in Table 6.2. Overall the mean serum 25(OH)D concentrations were 49.7 (23.5) nmol/L. A minimal, but statistically significant difference was observed between genders [males, 50.5 (23.1); females, 49.1 (23.8) nmol/L]. Overall prevalence of vitamin D deficiency (<30nmol/L) was reported at 21.8%, consistent with overall prevalence of vitamin D deficiency in Chapter 3. Prevalence of vitamin D deficiency was higher in females at 24.1%. Almost half of the study population had serum levels within the sufficient range (45.7%), with a statistically significantly higher proportion of males (47.5%). High serum 25(OH)D levels (≥125nmol/L) were reported for a small proportion of participants (0.5%). Vitamin D supplement use, with or without calcium for treatment of osteoporosis, was low at 5.1% (3.8% of which were female), and sun travel within the previous 12 months was reported by 48.6% of participants.

Table 6. 2: Muscle strength (HGS) , physical performance (SPPB) and vitamin D status overall and stratified by gender

	Overall (n=4157)		Males (n= 1874)		Females (n= 2283)	
	N (%)	**SD**	**N (%)**	**SD**	**N (%)**	**SD**
Muscle Strength and Physical Performance						
HGS Dominant (kg)	27.9	10.6	36.1	9.2	21.4***	6.2
Non-dominant (kg)	25.8	938	33.4	8.6	19.6***	5.5
ǂ **Low HGS %**	1271 (30.6)		505 (26.9)		766 (33.6)***	
SPPB Poor (≤ 6)	527 (12.7)		192 (10.3)		335 (14.7)***	
At risk (7-9)	804 (19.3)		340 (18.1)		464 (20.3)***	
Best (10-12)	2826 (67.9)		1342 (71.6)		1484 (65.0) ~	
Vitamin D status and related factors						
25(OH)D nmol/L	49.7	23.5	50.5	23.1	49.1*	23.8
IOM guidelines						
<30nmol/L	908 (21.8)		359 (19.2)		549 (24.1)***	
>30 and <50nmol/L	1326 (31.9)		618 (32.9)		708 (31.0)	
≥50 and <125nmol/L	1901 (45.7)		890 (47.5)		1011 (44.3)*	
≥125nmol/L	22 (0.5)		7 (0.4)		15 (0.7)	
§VitD supp use, n (%)	212 (5.1)		24 (1.3)		188 (8.2)***	
¶ Sun holiday travel	1904 (48.6)		858 (48.9)		1046 (48.4)	

Results are reported as means and SD or counts and proportions, n (%).

HGS, handgrip strength; SPPB, Short Physical Performance Battery; IOM, Institute of Medicine.

‡ Gender and BMI specific cut-offs, detailed in methods Chapter 2.

§ Vitamin D supplement use, with or without calcium, as directed for use to treat osteoporosis.

¶ Travel abroad within the previous 12 months, yes or no.

~ p= 0.08

P value denoting significant levels between groups $p<0.05$*, $p<0.01$**, $p<0.001$***

6.3.2.3 A comparison of muscle strength and physical performance outcomes relative to vitamin D concentrations

Vitamin D concentrations were divided into quintiles to investigate if serum 25(OH)D concentrations were associated with functional outcomes as detailed in Table 6.3 and illustrated in Figure 6.2 and Figure 6.3.

A statistically significant decrease in the prevalence of poor physical performance (SPPB ≤6) was evident as serum 25(OH)D increased. Poor performance was almost three times higher in participants with 25(OH)D concentrations ≤28nmol/L compared to those with levels ≥50nmol/L. A similar, but less pronounced trend was seen for those deemed "at risk", with a statistically significantly greater proportion in the lowest D quintile. The prevalence of "high performance" a score at the higher end of the performance battery (10-12 points), was substantially greater, and statistically different in the highest serum vitamin D quintile compared to the lowest D quintile.

Again, as illustrated in Figure 6.3, participants in the lowest serum vitamin D quintile performed worse in a measure of muscle strength (low HGS), with a statistically significant decrease in poor muscle strength in participants with serum concentrations >50nmol/L.

Table 6. 3: Muscle function and serum 25(OH)D quintiles

nmol/L	Q1 ≤28	Q2 29-41	Q3 42-54	Q4 55-69	Q5 ≥70
Quintiles, n (%)	856 (20.6)	836 (20.1)	845 (20.3)	821 (19.8)	799 (19.2)
Low HGS %	346 (40.4)***	268 (32.1)	236 (27.9)	207 (25.2)***	214 (26.8)**
SPPB (0-12)					
Poor (≤6)	185 (21.6)***	115(13.8)	90(11.9)	65 (7.9)***	72 (9.0)***
At risk (7-9)	200 (23.4)***	171 (20.5)	149 (17.6)	146 (17.8)	138 (17.3)
High (≥10)	471 (55)***	550 (65.8)	606 (71.7)	610 (74.3)***	589 (73.7)***
ǂ Falls (≥1)	99 (11.6)	86 (10.3)	84 (9.9)	90 (10.9)	79 (9.9)

Q, quintiles (nmol/L); low HGS, low handgrip strength established by BMI and gender-specific cut-offs; SPPB, Short Physical Performance Battery.

ǂ Falls, reported to have had multiple falls.

P value denoting significant levels between groups p<0.05*, p<0.01**, p<0.001***

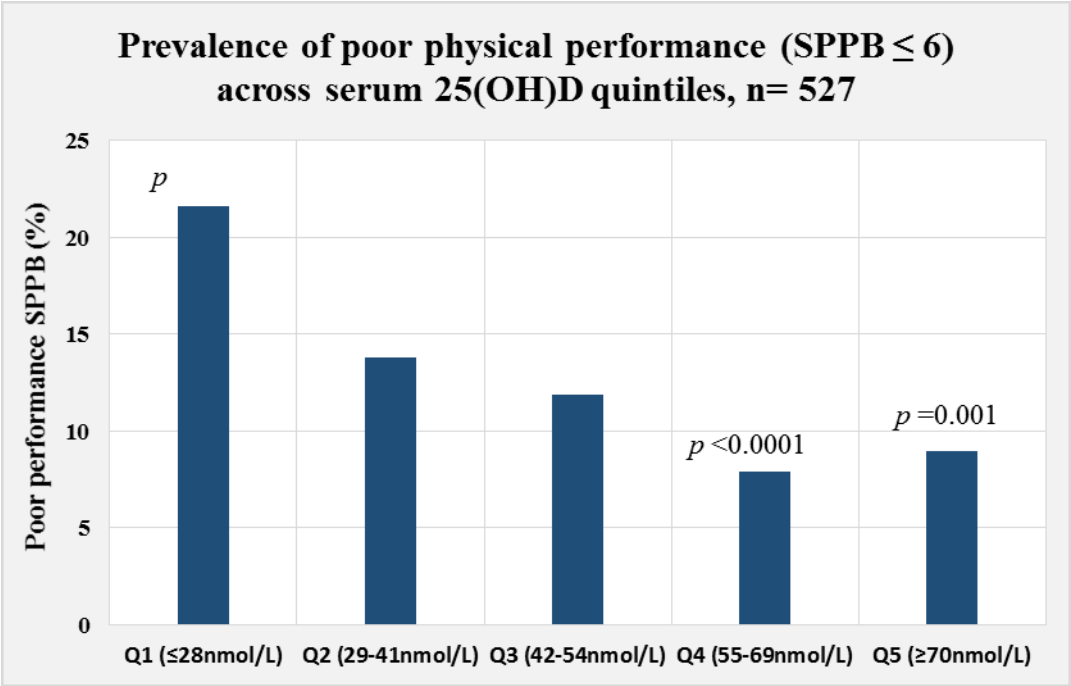


Figure 6. 2: Prevalence of poor muscle performance according to serum 25(OH)D quintiles.

A statistically significant proportion of poor physical performers are noted in the lowest quintile relative to those in quintiles 4 and 5.

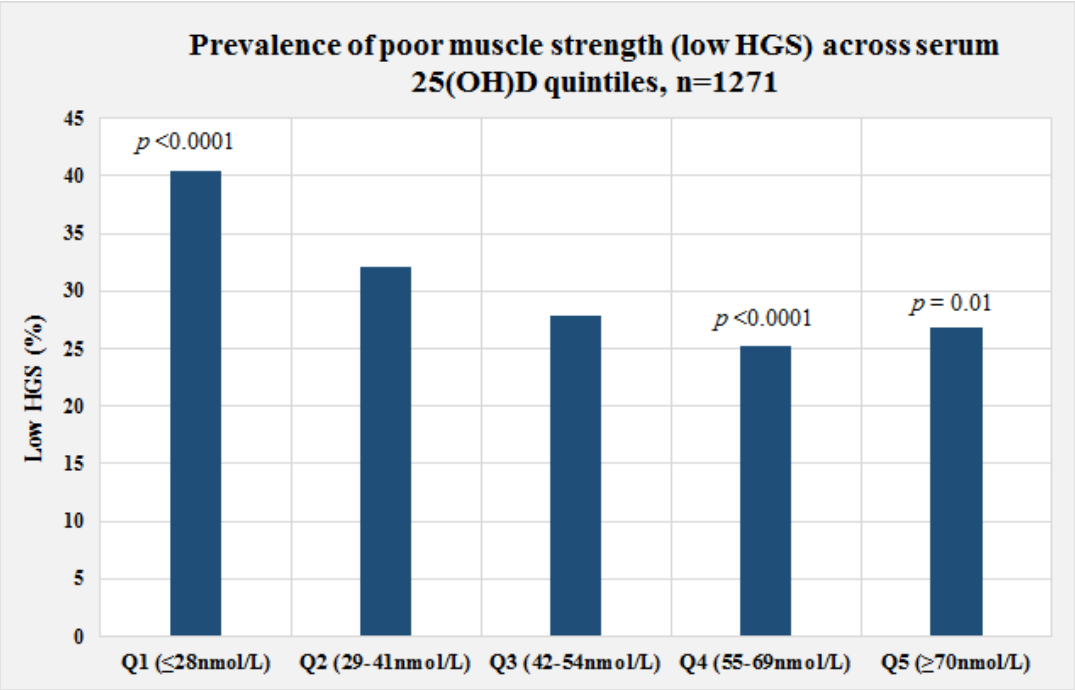


Figure 6. 3: Prevalence of poor muscle strength according to serum 25(OH)D quintiles. A statistically significant proportion identified as having low muscle strength have serum concentrations in quintile 1 relative to quintile 4 and 5.

6.3.2.4 Determinants of poor physical performance in community-dwelling older adults aged over 60 years.

After adjusting for pre-defined covariates, the multivariate logistic regression analysis in model 1 (SPPB), revealed that being aged 80+, female, retired, and self-reporting fair general health was statistically significantly adversely correlated with poor physical performance, defined by a score ≤6 in the Short Physical Performance Battery. Full details are available in Table 6.4 and illustrated in Figure 6.4. Asides those of oldest age, reporting multiple falls was the second strongest negative indicator of poor physical performance [2.55(1.94, 3.36), p<0.001] followed by polypharmacy [2.42 (1.93, 3.02), p<0.001] and low muscle strength as measured by handgrip strength [2.17 (1.73, 2.72), p<0.001]. Vitamin D deficiency (<30nmol/L) was also identified as a statistically significant negative correlate of poor physical performance [1.65 (1.31, 2.09), p<0.001]. BMI was not a statistically significant correlate of poor physical performance and was omitted from the stepwise regression. Unadjusted OR for obese BMI were [1.87 (1.46, 2.39), p<0.001], however, once adjusted the findings were no longer significant [1.03 (0.71, 1.25), p=0.84]. The only statistically significant factor that positively correlated with poor performance was regular moderate physical activity [0.30 (0.24, 0.38), p <0.001].

Table 6. 4: Adjusted OR with (95% CI) for poor physical performance (SPPB ≤6) in the English Longitudinal Study of Ageing, adults aged over 60 n=4157

	Unadjusted Poor SPPB (≤ 6)			Adjusted Poor SPPB (≤ 6)		
Socio-demographic factors	**OR**	**(95%CI)**	***P***	**OR**	**(95%CI)**	***P***
Female	1.50	(1.25, 1.82)	<0.001	1.39	(1.11, 1.74)	0.004
Age 60-69¶	-	-	1	-	-	1
70-79	1.86	(1.49, 2.31)	<0.001	1.29	(0.99, 1.69)	0.050
80-89	7.38	(5.76, 9.47)	<0.001	3.78	(2.78, 5.15)	<0.001
Employment-retired ¥	4.78	(2.94, 7.69)	<0.001	1.98	(1.18, 3.34)	0.010
Health and Lifestyle factors						
Vitamin D deficiency (<30nmol/L)	2.43	(1.99, 2.95)	<0.001	1.65	(1.31, 2.09)	<0.001
§ Vitamin D supplement use	2.10	(1.56, 2.88)	<0.001	1.37	(0.91, 2.05)	0.134
Self-reported health- Poor	3.25	(2.67, 3.96)	<0.001	1.55	(1.22, 1.97)	<0.001
Physical Activity-Moderate	0.16	(0.13, 0.19)	<0.001	0.30	(0.24, 0.38)	<0.001
ǂ Falls (>1)	3.78	(3.00, 4.75)	<0.001	2.55	(1.94, 3.36)	<0.001
Polypharmacy (≥ 5 medications)	4.90	(4.1, 5.9)	<0.001	2.42	(1.93, 3.02)	<0.001
Low HGS	5.10	(4.2, 6.1)	<0.001	2.17	(1.73, 2.72)	<0.001
Poor QoL (CASP <36)	1.95	(1.58, 2.39)	<0.001	1.25	(0.97, 1.61)	0.080

OR, odds ratio; 95% CI, 95% confidence interval; low HGS, Low handgrip strength; QoL, Quality of Life; CASP-19, Control, Autonomy, Self-realisation, Pleasure.

[¥] Retired, compared to those currently in employment. [§] Use medically directed for treatment of osteoporosis. [ǂ] Falls, reported to have had multiple falls.

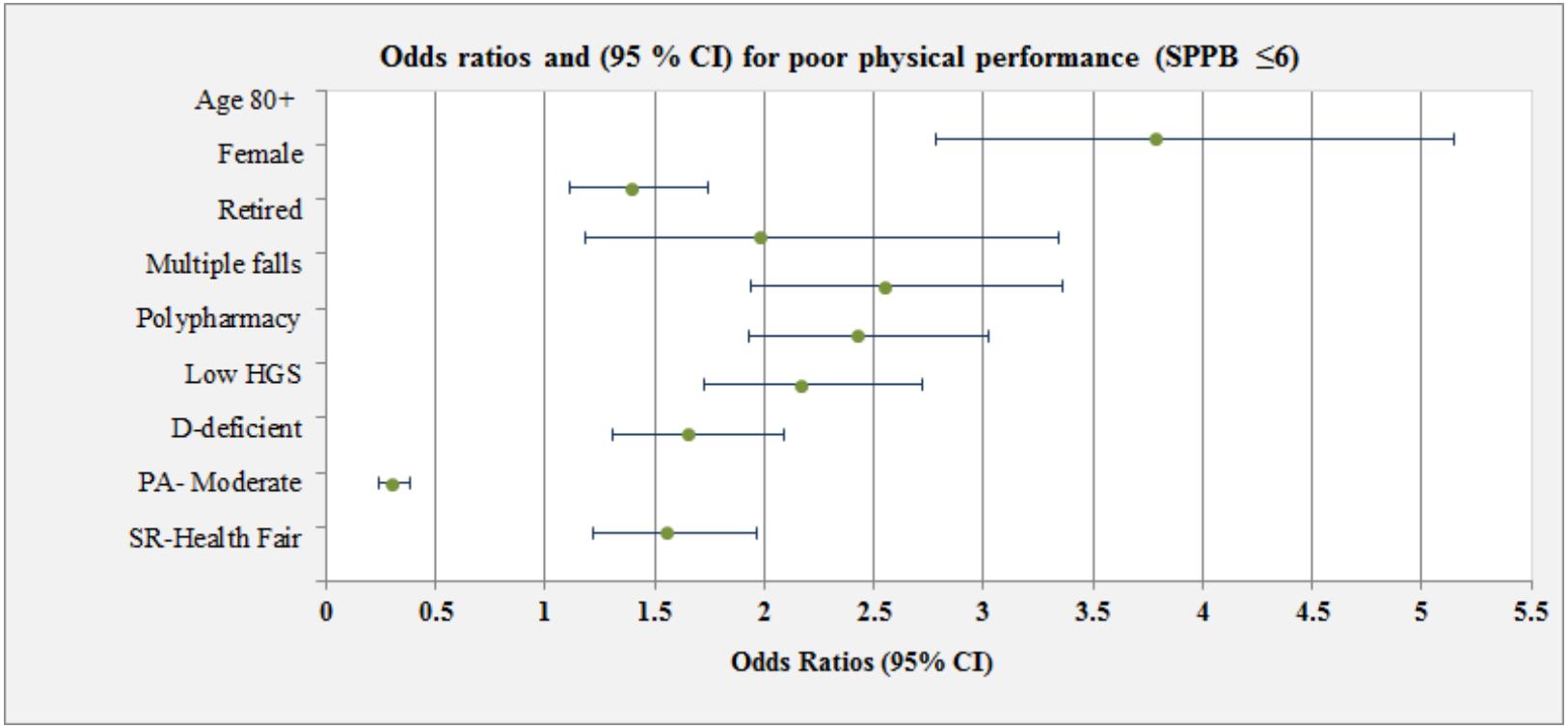


Figure 6. 4: Statistically significant odds ratio and 95% confidence intervals for socio-economic, health and lifestyle factors associated with poor physical performance.

Corresponding results and variable abbreviations detailed above in Table 6.4.

6.3.2.5 Determinants of poor muscle strength in community-dwelling older adults aged over 60 years.

After adjusting for pre-defined covariates, the multivariate logistic regression analysis in model 2, revealed that being 70+, female, vitamin D deficient (<30nmol/L), overweight or obese BMI, poor physical performance, taking multiple medications (≥5), multiple falls, retired, and self-reporting fair health was statistically significantly negatively correlated with low muscle strength. Engaging in moderate physical activity was the only positive correlate of muscle strength [0.65 (0.58, 0.79)]. Vitamin D supplement use did not contribute to the model, when included for exploratory analysis it was not a statistically significant factor in poor muscle performance (p=0.15). However, the direction of association indicates vitamin D supplement use is adversely correlated with low HGS 1.26 (0.92, 1.73). The only factor included in the model, and was not correlated with low HGS was poor quality of life. Full results are detailed in Table 6.5 and illustrated in Figure 6.5.

6.3.2.6 Vitamin D supplementation, poor muscle strength and poor physical performance

When vitamin D supplementation was included in both adjusted models, it was not statistically significantly associated with either poor physical performance or muscle strength. However, the direction of the relationship was negative for physical performance [1.37 (0.91, 2.05), p=0.134] and poor muscle strength [1.26 (0.92, 1.73), p=0.148]. Supplement use was reported for those with osteoporosis and this likely conflicts the interpretation of findings and was therefore unsuitable for inclusion in this analysis. Two-thirds of those identified as having osteoporosis reported taking a vitamin D (with or without calcium) supplement (n=212/313).

Table 6. 5: Adjusted OR with (95% CI) for low handgrip strength in the English Longitudinal Study of Ageing adults aged over 60 n=4157

	Unadjusted Low HGS			Adjusted Low HGS		
Demographic variables	**OR**	**(95%CI)**	***P***	**OR**	**(95%CI)**	***P***
Age 60-69¶	-	-	1	-	-	1
70-79	2.32	(2.00, 2.69)	<0.001	1.92	(1.62, 2.26)	<0.001
80-89	7.49	(6.00, 9..35)	<0.001	4.71	(3.69, 6.02)	<0.001
Female	1.37	(1.19, 1.57)	<0.001	1.25	(1.08, 1.46)	0.003
Employment-retired	2.81	(2.19, 3.62)	<0.001	1.49	(1.15, 1.96)	0.003
Health and Lifestyle factors						
Vitamin D deficiency (<30)	1.79	(1.54, 2.09)	<0.001	1.44	(1.21, 1.71)	<0.001
Self-reported health- Poor	2.20	(1.87, 2.59)	<0.001	1.39	(1.15, 1.67)	0.001
BMI -overweight	1.29	(1.09, 1.55)	0.003	1.31	(1.08, 1.59)	0.006
obese	2.42	(2.02, 2.91)	<0.001	2.26	(1.84, 2.78)	<0.001
Physical Activity- Moderate	0.41	(0.36, 0.48)	<0.001	0.68	(0.58, 0.79)	<0.001
Falls (>1) ǂ	2.08	(1.69, 2.54)	<0.001	1.47	(1.17, 1.86)	0.001
Polypharmacy (5+ medications)	2.63	(2.29, 3.03)	<0.001	1.51	(1.28, 1.78)	<0.001
Poor SPPB (≤ 6 points)	5.07	(4.18, 6.15)	<0.001	2.22	(1.77, 2.77)	<0.001
Poor QoL (<36 points)	1.36	(1.16, 1.61)	<0.001	0.97	(0.81, 1.17)	0.785

OR, odds ratio; 95% CI, 95% confidence interval; low HGS, Low handgrip strength; QoL, Quality of Life; CASP-19, Control, Autonomy, Self-realisation, Pleasure.

ɣ Retired, compared to those currently in employment.

§ Use medically directed for treatment of osteoporosis.

ǂ Falls, reported to have had multiple falls.

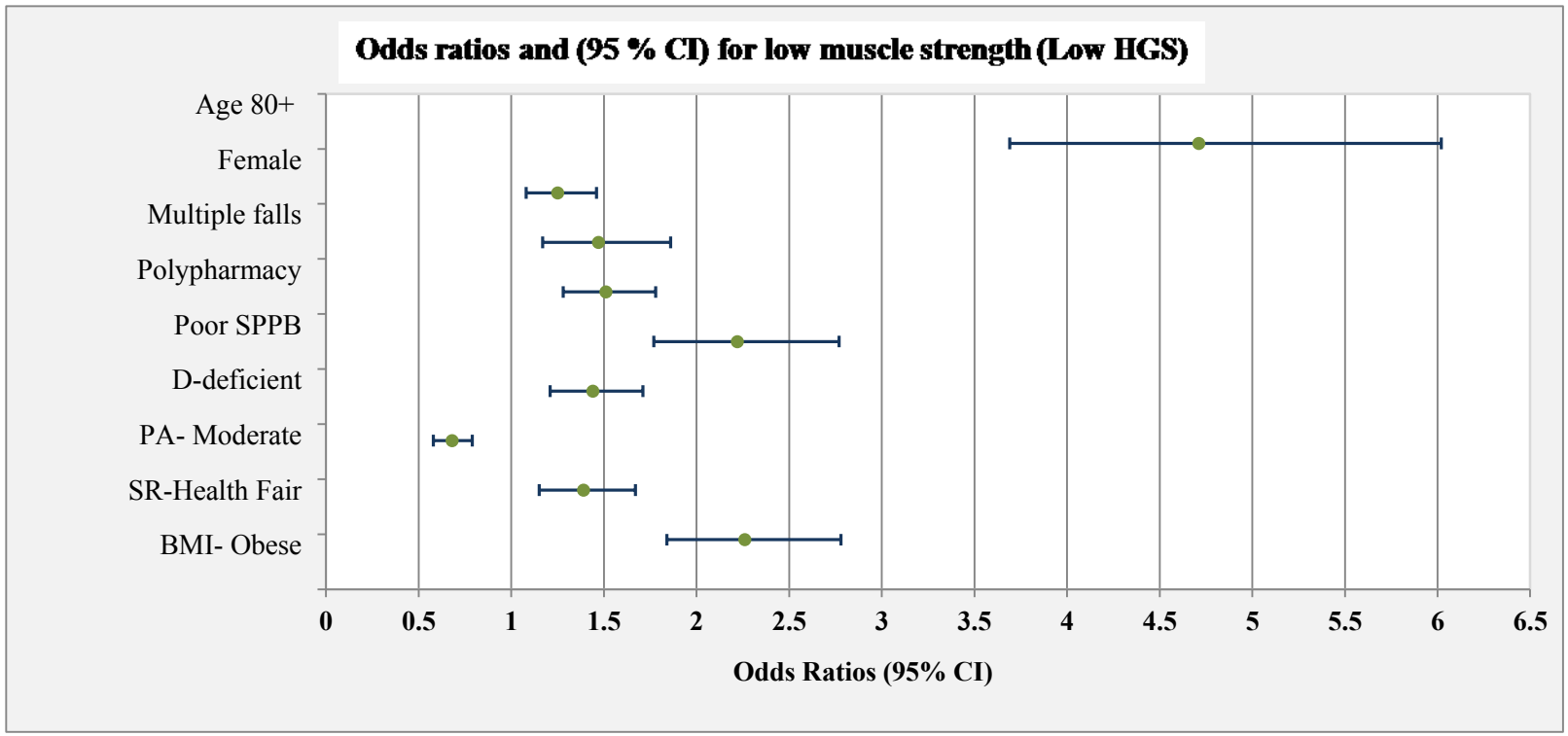


Figure 6. 5: Statistically significant odds ratio and 95% confidence intervals for socio-economic, health and lifestyle factors associated with low muscle strength.

Corresponding results and variable abbreviations detailed in Table 6.5.

6.4 Discussion

In the present study, we aimed to investigate the relationship, if any, between serum vitamin D concentrations and muscle function in a large sample of community-dwelling older adults aged over 60 years. Amongst other important correlates of poor muscle function, we evidenced that low serum 25(OH)D levels (<30nmol/L) are associated with poor physical performance and low muscle strength. Additionally, we observed that females, aged 80+, taking multiple medications and reporting multiple falls were more likely to perform worse in a battery of lower extremity physical performance and in muscle strength.

Prevalence of poor physical performance and low muscle strength was observed in 12.7% and 30.6%, respectively. A clear gender difference in physical parameters was evident, with a greater proportion of females identified as poor physical performers (14.7%) and having low muscle strength (33.6%). The prevalence of poor muscle function identified in this cohort is similar to findings previously observed in the TILDA study at 22.6% for poor muscle function (using the same criteria applied the present study, HGS) and 19.4% for poor physical performance (assessed by the TUG test) [311]. In our own RCT detailed in Chapter 5, we noted substantially higher rates of poor muscle function (weak HGS, 36.7%) and poor physical performance (slow TUG >10seconds, 51.7%).

The prevalence of vitamin D deficiency (<30nmol/L) was 21.8% overall, again higher in females than males (24.1%). Our findings show a clear trend towards a risk of functional disability in participants with serum vitamin concentrations in the lowest serum 25(OH)D quintile (<28nmol/L). Whilst those with serum 25(OH)D levels within the highest quintiles (>55nmol/L and >70nmol/L) had the lowest rates of poor muscle strength (assessed by HGS) and poor physical performance (assessed by SPPB). This is further supported by logistic regression analysis as serum vitamin D deficiency was statistically significantly associated with a greater risk of poor physical performance and low handgrip strength. These findings are consistent with previous associations identified in participants of the InCHIANTI study, early findings by Houston et al. in 2006 identified that both males and females with serum 25(OH)D levels <25nmol/L had

significantly lower SPPB scores. Furthermore, those with serum 25(OH)D concentrations <50nmol/L had lower handgrip strength [312]. In the same prospective cohort, recent evidence from Shardell et al. in 2015 again demonstrates that serum 25(OH)D concentrations <25nmol/L was positively related to lower-extremity physical performance (SPPB) in this large cohort of community-dwelling older adults [139]. Furthermore, Bischoff-Ferrari et al. observed comparable findings in a large sample of community-dwelling older adults in the US (n=4100) [313], revealing that participants with serum 25(OH)D concentrations <40nmol/L had worse lower-extremity function, as assessed by 8ft. walk test and sit-to-stand test.

In the present study, a significant association was detectable for muscle strength for those with low serum 25(OH)D concentrations in the lowest vitamin D quintile (<28nmol/L). Recent findings from Granic et al. observed similar results in adults from the Newcastle 85+ Study [307], a cohort of the oldest old and those at the highest risk of deficiency and low muscle strength and performance. In measures of HGS, those defined as having low and high vitamin D status based on season-specific quartiles were associated with weaker HGS for both males and females, after a 5-year follow-up. Only a significant decline in HGS was seen in males within the lowest season-specific quartile, compared with those in the middle quartile ranges of vitamin D status. Granic et al. also report that females had worse scores in TUG, again in the lowest and highest quartiles, but the rate of decline in physical performance was not detected over the 5-year follow-up period. A decline over time is seen particularly in males, a steeper decline in muscle strength was noted in those with lowest vitamin D quartiles over 5-years and for those not supplemented with vitamin D [307].

In support of our findings, Van Schoor et al. demonstrated in a reanalysis of participants of the LASA study, an association between low serum 25(OH)D levels and poorer performance in the SPPB [308]. Since the first publication [314], the serum vitamin D concentration had been standardised using the Vitamin D Standardisation Program, as part of the ODIN project [315], and the influence of the standardisation did not change the overall results, that low serum 25(OH)D was associated with reduced physical function, falls and fractures. When using the original values, the author notes an increase in total SPPB score up to vitamin D levels of 60–70nmol/L after this point scores plateau. When using the standardized values, this occurred sooner at a vitamin D

level of 50–60nmol/L, indicating a shift in threshold. The association between serum 25(OH)D and decline in SPPB was no longer statistically significant after VDSP was applied. The author acknowledged this may be attributable to the small prospective numbers included. Van Schoor et al. conducted a post-hoc analysis using a cut-off value of 50nmol/L. In this analysis, those with serum levels <50nmol/L had a 1.50 times higher odds of decline in the SPPB compared with to serum 25(OH)D levels >50nmol/L. The relevance of this findings and our own, is the original mode of assay was also used in the current ELSA study, therefore supporting our finding irrespective of the serum analysis used [308]. It would be advantageous to confirm our findings if standardisation was performed in the ELSA study samples.

We have identified in the present study, multiple factors associated with poor physical performance and low muscle strength in the older adult population. Consistent with previously published findings females, of oldest age, recurrent fallers, taking 5 or more medications daily, and those who rate their health as fair were negative determining factors of poor physical performance [316-319]. We showed that low muscle strength was also an adverse correlate of poor lower extremity physical performance. Furthermore, retirement was also inversely related to poor physical performance. Whilst BMI and self-reported quality of life were not identified as factors related to poor physical performance. Importantly, regular, moderate physical activity was shown to have a positive association with physical function which may inform interventions to support optimal physical functioning in old age. Multiple determinants were also observed for those identified as having poor muscle function, defined by low handgrip strength. The strongest negative correlate was those aged 80+, followed by obese BMI, poor physical performance, recurrent falls, and vitamin D deficiency (<30nmol/L). As with poor physical performance, those who engage in moderate physical activity are 32.0% less likely to demonstrate low muscle strength.

6.4.1 Strengths and limitations

The present study was derived from a large population of community-dwelling older people, with a comprehensive assessment and validated measures of muscle function and physical performance. A number of limitations which need to be considered when interpreting our findings. As highlighted in Chapter 3, the serum measurement

technique for serum 25(OH)D concentrations used in ELSA was DiaSorin Liaison radioimmunoassay, rather than the 'gold standard' LC-MS. However, findings from the LASA study demonstrate standardisation of serum 25(OH)D concentrations inferred little influence on vitamin D status and re-analysed measures of muscle function. In line with all cross-sectional association studies, the findings need to be considered in the context of reverse causality. Nevertheless, the findings indicate the potential usefulness of the SPPB for further studies. This outcome measure is clinically important as it's a strong predictor of falls risk, hospitalisation and disability in older adults [320-322].

6.4.2 Summary

We were able to demonstrate the prevalence of poor muscle function and physical performance in a large cohort, of similar proportions of community-dwelling males and females, aged over 60 years. These findings highlight the prevalence of functional impairment in a cohort of healthy older adults, which are commonly associated with overall health and wellbeing and indicate an increased risk of morbidity and mortality. We have demonstrated that serum 25(OH)D status is an important factor in physical health of older adults, as well as numerous important health-related factors, which could be considered for future interventions.

6.4.3 Conclusion

We have demonstrated that serum 25(OH)D status is an important factor in physical health of older adults, which in consideration of the prevalence of older adults residing in northerly latitudes identified as being vitamin D deficient (Chapter 3), highlights this potential public health concern. We identified a high prevalence of low muscle strength Although we were unable to demonstrate an effect for vitamin D supplement use and muscle function through an RCT (Chapter 5), we did demonstrate an effect of vitamin D supplementation and improved serum 25(OH)D status, which was both safe and well-tolerated. In light of the present findings, it seems sensible to advise vitamin D supplementation to correct vitamin D deficiency in the older adult population. It was interesting to note that while vitamin D deficiency was a risk factor for poor muscle performance, the key protective factor identified was physical activity. Taken together, these findings suggest that multi-modal approaches including treatment of vitamin D

deficiency and increasing physical activity, with other lifestyle factors in older adults may have the greatest potential to support physical function.

Chapter 7: General Discussion

7.1 Summary of findings

With our growing older population, there is an increasing need to identify potentially modifiable characteristics or interventions that are intended to enhance the functioning of older adults who could be described as ageing normally. Vitamin D has been hypothesised to play a role in cognitive and physical function in older adults and this thesis sought to explore vitamin D as a preventative strategy for healthy ageing.

The main aims of this thesis work were to explore vitamin D status in ageing and to examine the potential effect of vitamin D supplementation as a viable component to support of cognitive and physical function. In order to investigate the potential contribution of vitamin D in successful ageing a number of studies were conducted. Firstly, to identify the prevalence of serum 25(OH)D deficiency in order to understand the current status in mid-life and in older adults through a large population dataset (Chapter 3). Further aims included testing the effectiveness of vitamin D supplementation through a randomised double-blind placebo-controlled pilot study in healthy older adults with cognitive performance the primary outcome of interest (Chapter 4) and physical function as the secondary outcome (Chapter 5). Finally, to further explore the association between serum 25(OH)D status and muscle function, in particular, lower-extremity physical performance, in a large cohort of healthy community-dwelling older adults (Chapter 6).

The first aim of this thesis work was to establish the prevalence of vitamin D deficiency and explore the determinants of deficiency in older adults (Chapter 3). We investigated vitamin D status in a large population of community-dwelling adults aged over 50 years residing at northerly latitude, based on data from the English Longitudinal Study of Ageing (ELSA). Year-round vitamin D deficiency was highly prevalent in this large, representative sample of community-dwelling adults with 26.4% (1 in 4) found to be vitamin D deficient (<30nmol/L); more than half were identified as vitamin D deficient (57.3%) (<50nmol/L). Prevalence of deficiency remained high even during the summer months when 1 in 6 (16.9%) was defined as vitamin D deficient (IOM) and 45.2% were vitamin D deficient by the Endocrine Society criteria. These figures are higher than those reported from The Irish Longitudinal Study of Ageing (TILDA) reporting the prevalence of vitamin D deficiency (<30nmol/L) to be 13.1% [35]. The Longitudinal

Ageing Study Amsterdam (LASA), are most comparable to our findings, at 17.5% (<30nmol/L) and 48.4% (<50nmol/L) [87]. Similarly, these studies identified the increased risk of deficiency in those aged over 80 years, which have also been shown in the oldest old (>85 years) from the Newcastle 85+ Study [245]. Taken together, this highlights an important high-risk group for vitamin D deficiency and targeted interventions.

We identified that those at most risk of deficiency were of non-white ethnicity, aged 80+, residing in the north of England and with clinical indicators of frailty. Supplement use was low (4.4%), but the protective effects were noted as supplement users had adequate 25(OH)D status (65.8nmol/L). Additionally, we showed that even within a country of short ranging latitude (50.4-54.9°N), north-south gradient and incremental changes in latitude appeared to influence 25(OH)D status. For comparison to Ireland, it is of note that England is of similar latitude to the Republic of Ireland. The significance of these findings needs to be considered in relation to our changing demographic profile in Ireland. Research carried out by the Economic and Social Institute (ESRI) in 2017 [323], projected population increases are greatest for older ages. The population aged 80 and over is projected to increase by between 89% (0.128 million) and 94% (0.135 million) from 2015 to 2030. As well as our ever-expanding older adult population, Ireland is fast becoming a more ethnically diverse country, ethnic and racial minorities make up ~12% of the population of Ireland a proportion that doubled in the first decade of the 21st century [324]. The literature supports a role for vitamin D deficiency and adverse outcomes beyond bone health, most notably in carcinogenesis, immune function, cardiovascular disease and all-cause mortality [226, 227], this thesis focused on cognitive and physical functioning.

To investigate the role of vitamin D in successful ageing, we investigated through a randomised double-blind placebo-controlled pilot trial the effect of vitamin D_3 supplementation (2000IU/d given as 4000IU every 2 days) and primary outcomes of cognitive function in a sample of community-dwelling older adults over a 6-month period. First, we wanted to investigate whether vitamin D_3 supplementation has an effect on overall cognitive function, measured with a pre-specified assessment of global function. Secondly, we wanted to evaluate whether there was a difference between vitamin D supplement users and the placebo group in pre-specified domain-specific

tasks of executive functioning, attention, memory and visual reasoning (Clinicaltrials.gov *identifier*: NCT02804841). We demonstrated the effectiveness and safety of vitamin D_3 supplementation on serum 25(OH)D levels with a marked improvement in serum concentrations in the vitD_3 group after 6-months and no adverse events were reported. The vitD_3 and PL group were homogenous for baseline characteristics in this study. We found no effect of vitamin D_3 supplementation and the pre-specified measures of global cognitive function and domain-specific tasks. Our findings extend the understanding of vitamin D as a preventative strategy in cognitive health research as the first RCT to exclusively recruit older adults, without cognitive impairment at baseline. Our findings are similar to those reported from a recent RCT, of cognitive function in healthy adults, some of which were older; the author found no effect of high dose vitamin D supplementation [(100)]. Some minor findings were observed in both the present study and the study conducted by Pettersen et al. for tasks of memory. Additionally, minor findings were observed for both delayed memory and visual reasoning, which may merit further investigation. RCTs for vitamin D and cognitive function in healthy older adults are a relatively new potential component for healthy ageing research, however, provide a worthy topic for future dementia prevention strategies in Ireland. In keeping with the recommendations of the Lancet Dementia Commission 2017, researchers and government bodies should be ambitious about dementia prevention [(8)], identifying multiple modifiable risk factors which could potentially delay or prevent a third of dementia cases [(8)].

Whilst more research is needed regarding the contribution of vitamin D in multimodal interventions and brain health, we conducted a participant feedback survey to identify the barriers when implementing this types of interventions (Chapter 4 part B). The mixed-methods feedback survey found that the RCT participants were highly motivated to take part as they had a personal interest in contributing to research or on the topic of successful ageing, vitamin D or dementia. Their overall experience was rated as high and their initial concerns included anxiety regarding the cognitive assessments, however, once explained in detail they no longer had concerns. Interestingly, participants expressed no concern in taking the study tablet and the possibility of being allocated to placebo. The group overall were highly-educated, highly-skilled, of high socioeconomic profile and all were of Caucasian ethnicity. The retention rate of the trial was almost 100%, which may reflect the high motivation noted in the survey. This

also highlights the broader issue of studies, including ELSA and TILDA, which are often based on healthy, well-educated motivated participants which limits the generalisability of the findings.
The idea that vitamin D may provide a beneficial role in ageing, beyond bone health, is a relatively new concept. There is an ongoing debate regarding how relatable neuropsychological testing is to "real world" situations, and the use of performance measures that test real-life situations may offer a new opportunity. Emerging mobile technologies which obtain real-time information regarding abilities to perform activities of daily living, which are highly correlated with neuropsychological tests, may change the way cognitive research is conducted particularly in healthy older adults in way of preventative strategies for dementia and physical disability [325].

In Chapter 5, we investigated pre-specified secondary outcomes from the primary RCT in community-dwelling older adults detailed in Chapter 4. The aim of the secondary analysis was to investigate vitamin D_3 supplementation and physical function outcomes. We hypothesised that individuals who were supplementing daily with an average of 2000IU of vitamin D_3 will perform better after 6-months than those allocated to placebo in a task of physical performance as assessed by Timed-Up-and-Go test (TUG), and muscle strength as assessed by handgrip strength (HGS). The results showed that the vitD_3 group did not perform better in either measure of muscle strength or physical performance compared to the placebo (PL) group. A non-significant finding for weak muscle strength is consistent with previous RCTs [143, 141, 140, 144, 147]. Discrepancies among other RCTs conducted to date may be a result of baseline vitamin D levels and the vitamin D dosing regimen used [303, 304, 286, 153, 152]. An improvement in TUG speed for the vitD_3 group was observed compared to the PL group which showed slower performance. Irrespective, neither change was deemed meaningful [294]. RCTs have shown that treatment with vitamin D can improve physical outcomes in older adults [136, 326, 327], particularly in relation to lower-extremity performance [225, 328]. We have also demonstrated in Chapter 3 that vitamin D deficiency is associated with lower-extremity performance, measured by 8-metre gait speed test in older adults aged over 60 years in the ELSA study.

In relation to the study design, the vitD_3 and PL group were homogenous for baseline characteristics in this study. No statistically significant difference was found within-

groups as a measure of change overtime or for sub-group analysis based on vitamin D status. The vitD_3 and PL group were homogenous for baseline characteristics in this study. No statistically significant difference in demographic or physical health variables was identified, reflecting the success of utilising minimisation techniques for randomisation. This high comparability between groups was ideal for analysis of treatment effects. In the present study the prevalence of poor muscle strength, poor physical performance and poor muscle function was high at 36.7%, 51.7% and 13.3% respectively. The prevalence is higher than those observed in the TILDA study population at 22.6% for poor muscle function (using the same criteria applied the present study, HGS) and 19.4% for poor physical performance (assessed by the TUG test) [311], and the ELSA study in Chapter 6, revealed the prevalence of poor physical performance and low muscle strength was observed in 12.7% and 30.6%, respectively. Therefore a high level of physical impairment was noted in the RCT participants in our study, which may benefit from more comprehensive assessments of physical function. RCTs have shown that treatment with vitamin D can improve muscle function in older adults at risk of vitamin D deficiency, specifically institutionalised [225], and patient groups [327]. With some evidence for a reduction of falls risk and improvements in balance [223, 326, 225, 328, 149]. However, findings in community-dwelling older adults remain inconsistent [286, 152]. Beyond physical activity and resistance type training interventions, there are limited demonstrated options for preventing physical performance decline in older adults. The need to identify factors associated with poor physical performance that may be modifiable targets for intervention is central to successful ageing research.

To explore lower-extremity physical performance, the thesis work went on to investigate the link between serum vitamin D concentrations using an in-depth composite assessment of physical performance, the Short Physical Performance Battery (SPPB), in community-dwelling older adults aged over 60 years, using the ELSA study population. Muscle strength was also assessed for comparison of previously published findings and our own observations in Chapter 5. In this study, the prevalence of poor physical performance and low muscle strength was observed in 12.7% and 30.6%, respectively. Overall the findings showed that older adults with lowest serum 25(OH)D levels comprised the greatest proportion of adults with poor physical performance (SPPB score 0-6). This was the case for muscle strength also. Whilst those with serum

25(OH)D levels within the sufficient range (>50nmol/L to <125nmol/L) had the lowest rates of poor muscle strength and physical performance. Being aged 80+, female gender and low serum 25(OH)D levels (<30nmol/L) were identified as significantly associated factors with poor physical performance and low muscle strength. As identified earlier (Chapter 3), being aged 80+, female and at risk of vitamin D deficiency, seems an important group for future health strategies to include in healthy ageing interventions. In protective terms the only positive factor identified was regular, moderate physical activity. Our findings show a clear trend towards a risk of functional disability in participants with serum vitamin concentrations <28nmol/L. Physical activity is a well-recognised intervention for maintenance of muscle function and prevention of sarcopenia [329, 305], equally the evidence that keeping physically fit offers protective effects for brain health in ageing is also well demonstrated [330, 331, 211]. Likely beneficial future approaches should be multimodal in design, employing interventions which target multiple risk factors.

7.2 Public health implications

Overall the thesis findings demonstrate that vitamin D deficiency is prevalent and remains a major public health issue, evidenced in the largest study of vitamin D status in community-dwelling older adults, to our knowledge. We have identified important determinants of vitamin D deficiency, such as, the oldest old, females, of non-white ethnicity, and physical activity and vitamin D supplement appear to be protective. The Food Safety Authority of Ireland recommends that during the winter months and for those at most risk of deficiency [25], that vitamin D supplementation should be considered, however, uptake remains low [35].

A role for vitamin D in the aetiology of cognitive impairment and dementia is plausible, supported by substantial mechanistic and epidemiological data summarised in part B of Chapter 1. Whilst much effort is being made to standardise reporting of vitamin D status similar standardisation of cognitive assessments in nutrition research would progress our understanding. Future studies of vitamin D and cognition should aim to explore finer aspects of cognitive function in healthy older adults, which may benefit from employing more sensitive established core outcome measures, as demonstrated in the

RCT in Chapter 4. We have presented evidence throughout this thesis that indicates levels >70nmol/L may support cognitive and physical function in healthy older adults and that supplementation is a safe and effective strategy for correction and maintenance of year-round vitamin D levels, particularly in the absence of mandatory food fortification in Ireland. Large ongoing interventions such as DO-Health and VITAL-Cog which will test the effectiveness of many lifestyle and dietary behaviours may offer new insights for brain health research.

Most recent census figures in Ireland show that since 2011 the population aged over 65 years increased by 19% to 638,000, and is expected to increase to some one million people by 2030 [332]. There is also a significant increase in the oldest old. By 2041, it is projected that the number of people aged 85 years or older will increase from 74,000 to 356,000. The success of population ageing is attributable to several factors including improvements in health care, nutrition, education and economic welfare [333, 334]. However, while people are living longer and many are living healthier lives into old age, this is not universal. There are still considerable health and social challenges to overcome to ensure we all have the opportunity to live a healthy, active and fulfilling old age. To a certain extent, cognitive and physical decline is a direct consequence of ageing, however, the rate and severity of decline are experienced differently for all older adults depending on the exposure of multiple non-modifiable and modifiable risk factors.

The prognosis is that the number of older adults in Ireland with dementia will increase from 47,000 in 2011 to 132,000 in 2041 [335]. It has long been considered that dementia was neither preventable nor treatable but this view is changing. The projected effect of a 10-25% reduction in seven identified risk factors (diabetes, midlife hypertension, midlife obesity, smoking, depression, cognitive inactivity or low educational attainment, and physical inactivity) could prevent up to 50% of all cases of AD [336]. The Dementia Commission 2017, demonstrated the impact of these key risk factors, across the life course, following similar public health advice for cardiovascular disease prevention, earliest intervention including many modifiable risk factors, is most protective. Whilst we have attempted to demonstrate the contribution of vitamin D supplementation as a preventative strategy for successful ageing, it is likely if any effect is present it may be most pronounced in combination with other modifiable risk factors. A view recently

supported through meta-analysis of vitamin D and physical outcomes in older adults [304]. The contribution of moderate physical activity on cognitive and physical health provides the strongest evidence to date.

The thesis findings showed that 1 in 4 older adults (26.4%) were serum 25(OH)D deficient, most commonly seen in females, aged 80+, with indicators of physical frailty and residing in northerly latitudes, such as Ireland. The RCT demonstrated that vitamin D supplementation of 2000IU/d was safe and effective in improving vitamin D status, however, population uptake of vitamin D supplement use remains low [35]. In 2011, the Food Safety Authority of Ireland proposed that all Irish adults should take a daily vitamin D supplement as neither dietary sources consumed or sun exposure obtained in summer is sufficient to maintain adequate vitamin D status throughout the winter months. Taken together these findings indicate the need for targeted strategies within sections of the older population to improve vitamin D status. Furthermore, vitamin D deficiency was associated with worse physical performance. On the basis of the results of this RCT, a single component intervention of vitamin D_3 supplementation showed no effect on cognitive and physical performance in healthy older adults. Although an indication, similarly reported by others [143, 100, 149], in memory and lower-extremity physical performance was noted.

7.3 Strengths and limitations

Strengths of this body of work include the large population dataset included and the RCT design with pre-specified outcomes of interest. A number of limitations should be mentioned. This study aimed to recruit a diverse group of older adults through various community outreach strategies, however, due to the self-selecting nature of recruitment, and the majority of participants were very highly educated and had above-average levels of cognitive function. It is possible that the study population was subject to 'volunteer bias', which occurs when individuals who volunteer for a study differ from those who do not volunteer in characteristics relevant to the research question [337]. Whilst muscle impairment was noted, especially in lower-extremity function (51.7%), overall maybe the RCT participants were too healthy and frailer populations may be an

area of important future work. Furthermore, it's limited in generalisaibity to a predominantly white population at latitudes 50-55°N.

This thesis aimed to investigate effects of vitamin D on cognitive and physical function, but participants in the current study exhibited relatively little, if any, change in cognitive measures over the 6-month period. Interventions for vitamin D require a minimum 3-month period to appropriately evaluate responses to treatment [109], whilst the overall profile of physical dysfunction changed from baseline, our pilot sample was likely underpowered to detect a significant change and was a secondary outcome. Similarly few were vitamin D deficient at baseline and analysis by vitamin D deficiency could not be performed in a meaningful way. Low vitamin D status has been shown to predict a response in other health outcomes, for example in respiratory disease [256]; again this is a known issue and we by ethical consideration excluded those with very low serum 25(OH)D levels. Vitamin D deficiency is often a feature associated with ethnicity, poorer health and lower socioeconomic status. With respect to the population data, the sample size was large and included robust measures for defining vitamin D deficiency, and physical performance using a comprehensive indicator of poor physical function, the SPPB [338, 321, 322]; however, as with cross-sectional study design only association, and not causation, can be inferred.

7.4 Conclusion and future directions

Vitamin D deficiency is highly prevalent (26.4%) in community-dwelling older adults, those most at-risk include females, aged 80+, of non-white ethnicity and residing in northerly latitudes. Whilst vitamin D supplementation and physical activity were positive correlates of low serum 25(OH)D status. Supplementation of 2000IU/d of vitamin D_3 is a safe and effective means of improving vitamin D status, however, it showed no effect on cognitive or physical performance in the first RCT of healthy older adults.

Future interventions should consider testing a combination of modifiable lifestyle factors, in larger, more diverse cohorts of older adults, using more comprehensive assessments of muscle function and cognitive performance. Larger interventions will

also allow for pre-specified subgroup analysis of individuals with low vitamin D concentrations.

Future prevention of functional impairments in healthy older adults may depend on a combination of diet or lifestyle habits. Is still remains uncertain whether vitamin D is most likely an associated marker of overall health and not a causal factor in disease. Nevertheless, at a minimum, it seems reasonable to aim for the prevention of vitamin D deficiency. Future approaches to designing and evaluating multimodal diet and lifestyle approaches in support of successful ageing are most likely to provide best evidence.

Biography

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Conference Poster Presentations as a result of this thesis work

An example of 3 conference posters are indexed at the end of this section.

- **Aspell, N**., Zgaga, L., Healy, M., Lawlor, BA., O'Sullivan, M. Prevalence and determinants of vitamin D deficiency in older adults residing at northerly latitudes: a population based study" accepted for presentation at The ODIN Conference: Vitamin D & Health in Europe: current and future perspectives, Sept 2017.
- **Aspell, N**., Healy, M., McPartlin, J., Lawlor, BA., O'Sullivan, M. A randomised controlled trial of the effects of vitamin D on muscle strength and performance in healthy community dwelling older adults" accepted for presentation at The ODIN Conference: Vitamin D & Health in Europe: current and future perspectives, Sept 2017.
- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Effects of vitamin D supplementation on cognitive function in healthy, community dwelling older adults: results from a randomised double-blind placebo-controlled pilot trial" accepted for poster presentation at The Nutrition Society Postgraduate Meeting, Queens University, Belfast, Norther Ireland, June 2017.
- **Aspell, N**., Zgaga, L., Healy, M., Lawlor, BA.,O'Sullivan, M. "Severe vitamin D deficiency is highly prevalent in older adults residing at northerly latitudes-considerations for policy and practice" accepted for poster presentation at The Irish Society for Clinical Nutrition and Metabolism Conference, Ballsbridge, Dublin 4, March 2017. **Manuscript under review**
- **Aspell, N.,** Healy, M., Loughrey, D., Lawlor, BA., O'Sullivan, M. "Circulating 25-hydroxyvitamin D and cognitive performance in healthy older adults: a systematic review" accepted for poster presentation at The Irish Society for Clinical Nutrition and Metabolism Conference, Dublin 4, March 2017.
- **Aspell, N.,** Zgaga, L., Healy, M., Lawlor, BA., O'Sullivan, M. "Circulating 25-hydroxyvitamin D and cognitive performance in healthy older adults; a systematic review" accepted for poster presentation at the Psychology, Health and Medicine Conference, Royal College of Surgeons, Dublin 2, March 2017.
- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Associations between vitamin D status and cognition in community dwelling older Irish adults" poster accepted

for the International Association of Gerontology and Geriatrics European Region Congress, Dublin 1, April 2015.

- **Aspell, N.,** O' Sullivan, M., Lawlor, BA. "Low serum concentrations of 25-hydroxyvitamin D is associated with impaired cognitive function in community dwelling older Irish adults" poster accepted for the The Irish Society for Clinical Nutrition and Metabolism Conference, Dublin, March 2015.

Conference oral presentations as a result of this thesis work

- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Vitamin D status in ageing and its potential impacts on cognitive health" accepted for oral presentation at The Nutrition Society Postgraduate Meeting, Dublin, February 2017.
- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Vitamin D- ageing, physical and cognitive health" accepted for oral presentation at The Irish Gerontological Society, Postgraduate Meeting, Our Ladies Hospice, Harolds Cross, Dublin 8, April 2017.
- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Is there a role for vitamin D in ageing?" accepted for oral presentation at The Nutrition Society Postgraduate Meeting, Queens University, Belfast, Northern Ireland, June 2017

Peer-reviewed journal articles

- **Aspell, N**., O' Sullivan, M., Lawlor, BA. "Vitamin D in ageing and its potential impacts on cognitive health" Annual Nutrition Society postgraduate awardee, Proceedings of the Nutrition Society, Queens University, Belfast, Northern Ireland, June 2017- manuscript under review.

Awards and Scholarships relating to the thesis

- Irish Research Council scholarship awardee 2014 (€72,000).
- Nutrition Society Travel bursary awardee 2017.
- Nutrition Society Irish Postgraduate Competition Winner 2017.

Appendix 1

Claire Hartin Ph: 4142199
email: claire.hartin@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
THE NATIONAL CHILDREN'S HOSPITAL

TALLAGHT, DUBLIN 24, IRELAND
TELEPHONE +353 1 4142000

Ms. Niamh Aspell
Postgraduate Student
The University of Dublin
Trinity College
Department of Clinical Medicine
Trinity Health Centre
St. James's Hospital
Dublin 8

20th May 2015

RE: D-activating Decline-Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study

REC Reference: 2015-05 Chairman's Action (13)
(please quote reference on all correspondence)

Dear Ms. Aspell,

Thank you for your initial application to SJH/AMNCH Research Ethics Committee in which you requested ethical approval for the above study and your follow up response to the query of the Chairman.

The Chairman, having reviewed this proposal has given ethical approval on behalf of the Ethics Committee.

The following documents were reviewed

- Standard application Form
- Text for Information Leaflet
- Poster
- Background questionnaire
- D-Activating Decline Participant Phone Screen
- Protocol, Version 1, dated 28/04/2015

Yours sincerely,

Claire Hartin
Secretary
SJH/AMNCH Research Ethics Committee

Appendix 2

The University of Dublin
Trinity College
Department of Clinical Medicine
Trinity Health Centre
St. James's Hospital
Dublin 8

23/10/2015

The Adelaide and Meath Hospital
Tallaght
Dublin 24

Dear Dr. Peter Lavin,

Re: REC Reference: 2015-05 Chairman's Action (13)

Study Title: D-activating Decline- Exploring the effects of vitamin D3 Supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.

Amendment No: 1 Minor change in form of delivery of supplement.

We wish to make a minor amendment to the ***form of delivery of the study supplement.*** Initially we stated treatment of 2000IU/d of vitamin D3. We wish to adjust the delivery slightly to 4000IU every two days. Current clinical guidelines from the Endocrine Society state repletion of vitamin D levels can be achieved safely with doses of 50,000IU/week (6,000IU/d). For this study the overall monthly intake (60,000IU) remains unchanged and this level stays within the upper limits of 4000IU/d for maintenance of vitamin D levels in adults. Delivery of placebo will mirror this adjustment also.

Please contact me if you have any further queries.

Kind regards,

Niamh Aspell

Ms. Niamh Aspell (PhD Candidate)
Tel: *0831729337* Email: *niamha@tcd.ie*

Appendix 3.

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH
Department of Clinical Medicine; Human Nutrition and Dietetics.

NEIL Neuro-Enhancement for Independent Lives

The University of Dublin
Trinity College
Department of Clinical Medicine
Trinity Health Centre
St. James's Hospital
Dublin 8

24/10/2016

The Adelaide and Meath Hospital
Tallaght
Dublin 24

Dear Dr. Peter Lavin,

Re: REC Reference: 2015-05 Chairman's Action (13)

Study Title: D-activating Decline- Exploring the effects of vitamin D3 Supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.

Amendment No: 3 Minor change in participant study conclusion.

We wish to make a minor amendment to the ***participant completion stage,*** we have designed a study debrief and feedback questionnaire (attached), participants who have verbally agreed to receive this questionnaire during their final study visit will be asked to complete and return to the research team (via stamped addressed envelope).

Please contact me if you have any further queries.

Kind regards,

Niamh Aspell

Ms. Niamh Aspell (PhD Candidate)
Tel: *0831729337* Email: *niamha@tcd.ie*

Appendix 4.

The University of Dublin
Trinity College
Department of Clinical Medicine
Trinity Health Centre
St. James's Hospital
Dublin 8

10/04/2016

The Adelaide and Meath Hospital
Tallaght
Dublin 24

Dear Dr. Peter Lavin,

Re: REC Reference: 2015-05 Chairman's Action (13)

Study Title: D-activating Decline- Exploring the effects of vitamin D3 Supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.

Amendment No: 1 Minor change in participant exclusion criteria.

We wish to make a minor amendment to the ***participant exclusion criteria.*** Initially we stated exclusion for measured vitamin D levels <25nmol/mL or >60nmol/mL. Considering the Institute of Medicine guidelines on vitamin D status we wish to adjust the parameters to <15nmol/mL and >125nml/mL, ideally capturing those with low levels and excluding those unlikely to benefit from supplementation, continuing to measure calcium and vit D at each 3 time points, in line with our ethics.

In the original ethics application we requested prior permission to contact the participants GP to notify of participation, which we have updated on the consent form. (attached- point 7).

Please contact me if you have any further queries.

Kind regards,

Niamh Aspell

Ms. Niamh Aspell (PhD Candidate)
Tel: *0831729337* Email: *niamha@tcd.ie*

Appendix 5.

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hartin Ph: 4142199
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Ms. Niamh Aspell
Irish Research Council Postgraduate Candidate
Department of Clinical Medicine
Trinity Centre for Health Sciences
St. James's Hospital
Dublin 8

28th April 2016

Re: D-activating Decline-Exploring the effects of vitamin D3 Supplementation on cognitive function in community dwelling health older adults - A Pilot Study

REC Reference: 2016-04 List 13 (14)
(Please quote reference on all correspondence)

Dear Ms. Aspell,

Thank you for your recent correspondence to SJH/AMNCH Research Ethics Committee in which you requested an amendment to the above referenced study.

The Chairman, Dr. Peter Lavin, on behalf of the Research Ethics Committee, has reviewed this request and grants permission for this amendment.

Yours sincerely,

Claire Hartin
Secretary-SJH/AMNCH Research Ethics Committee

Appendix 6.

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hartin Ph: 4142199
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Ms Niamh Aspell
The University of Dublin
Trinity College
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St. James's Hospital
Dublin 8

11th November 2016

Re:D-activating Decline-Exploring the effects of viamin D3 Supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study

REC Reference: 2016-11 List 41 (4)
(Please quote reference on all correspondence)

Dear Ms. Aspell,

Thank you for your correspondence to SJH/AMNCH Research Ethics Committee, in which you requested an amendment in relation to the above referenced study.

The Chairman, Dr. Peter Lavin, on behalf of the Research Ethics Committee, has reviewed this request and grants permission for this amendment.

Yours sincerely,

Claire Hartin
Secretary
SJH/AMNCH Research Ethics Committee

Appendix 7

D-Activating Decline: Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.

Protocol Version: 1 **Date:** 28/04/2015

Current Research Team:

Ms. Niamh Aspell
Postgraduate Researcher,
Nutrition**,** Dept. of Clinical Medicine,
Medicine, Trinity Centre for Health Sciences,
Health Sciences, St. James's Hospital, Dublin 8.

Prof. Maria O'Sullivan, PhD,
Associate Professor in Human
Dept. of Clinical
Trinity Centre for
St. James's Hospital, Dublin 8, ROI.

Prof. Brian Lawlor
Conolly Norman Professor of Old Age Psychiatry (TCD),
Consultant Psychiatrist of Old Age Psychiatry,
Mercer's Institute for Research on Aging,
St. James's Hospital, Dublin 8, ROI.

Current Sponsors: The University of Dublin, Trinity College.

Irish Research Council Government of Ireland Postgraduate Scholarship

Current Study Site: St. James's Hospital, Dublin 8, ROI.

Table of Content

Abbreviations

vitD- Vitamin D

MMSE- Mini Mental State Examination

NSAID's – NonSteroidal Anti Inflammatory Drug

VDR- Vitamin D Receptor

DβH- Dopamine beta - hydroxylase

NGF- Nerve Growth Factor

GDNF- Glail Derived Nerve Factor

NT3- Neurotrophin 3

GTP- Guanosine-5'-Triphosphate

TNFα- Tumor Necrosis Factor Alpha

MRNA- messenger Ribonucleic Acid

PTH-Parathyroid Hormone

ATP- Adenosine Triphosphate

RCT- Randomised Controlled Trial

IPAQ- International Physical Activates Questionnaire

WMSIII- Wechsler Memory Scale- Third Edition

SART- Sustained Attention Response Task

TILDA- The Irish LongituDinal Study on Ageing

CAMDEX- Cambridge Examination for Mental Disorders of the Elderly

WAIS- Wechsler Adult Intelligence Scale

TMT- Trail Making Task

MoCA-Montreal Cognitive Assessment

NART- National Adults Reading Test

TRIL- Technology Research for Independent Living

CES-D-Centre for Epidemiologic Studies Depression Scale

HADS-Hospital Anxiety and Depression Scale

PSS.4-Percieved Stress

IADL-Activities of Daily Living

MET- Metabolic Equivalent of Task

NEIL- Neuro-Enhancement for Independent Lives

TCIN- Trinity College Institute of Neuroscience

CRF- Case Record Form

SAE-Serious Adverse Event

GCP- Good Clinical Practice

Study Summary

Title	D-Activating Decline: Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.
Primary Objective	Primary objectives are to determine any measurable effects of vitamin D3 supplementation on cognitive performance and incidence of cognitive decline in community dwelling older adults.
Study Design	A randomised double-blind placebo-controlled pilot trial with follow up of 6 months.
Primary Endpoints	Improved cognitive performance as measured through a validated neuropsychological assessment battery.
Inclusion/ Exclusion Criteria	Inclusion: • ≥60 years of age and ≤80years of age • Community Dwelling • Free of cognitive impairment (defined by MMSE score >23) • Ability to provide written consent Exclusion: • Measures **low or high 25 (OH) D status**, defined as < 25nmol/L or >60nmol/L • Current use of supplemental vitD >800IU/d • Self-reported known hypersensitivity to vitD • Self-reported known hypercalcaemia (corrected serum calcium > 2.7nmol/l • Self-reported Hyperparathyroidism • Known Renal disease / dysfunction/failure • History of Stroke • Epilepsy (self-report/ medication list) • History of major psychiatric or neurological condition. • Treated for alcohol and/or drug abuse within the past 5 years • Participation in a concurrent clinical trial • Current use the following medications: ✓ Anti-convulsants ✓ Psychoactive medications ✓ Hormone replacement therapy

Section 1: Introduction

1.1. Background

There is a growing body of evidence which supports vitamin D in the aetiopathology of several chronic diseases, beyond its classical role in the endocrine system.

In the ageing literature, vitamin D status and exercise have been independently linked with brain health and the delay of cognitive decline. Whilst vitamin D is obtained from the diet, it is remarkable amongst vitamins in that 90% is produced in the skin in response to ultraviolet B radiation (NIH, 2007). Due to physiological changes in ageing, a 70-year old has an estimated 70% reduced ability to produce vitamin D via sunlight compared to a 20-year old. In northern latitudes, such as Ireland, sunlight intensity is insufficient during winter months, this coupled with changing lifestyles means that older people will likely need some vitamin D from supplementation.

Vitamin D is commonly accepted as a secosteroid hormone that directly or indirectly regulates over 1000 genes acting mainly via the vitamin D receptor (VDR) (Holick et al., 2007). The VDR and vitamin D metabolites are widely found in the human body including the brain and central nervous system. Vitamin D may reduce cognitive decline through a number of proposed mechanisms including effect on neuro-protection, vasculo-protection, modulation of vascular risk factors, anti-oxidation, neuronal calcium regulation, immuno-modulation, enhanced nerve conduction and detoxification mechanisms (Buell et al., 2009).

Whilst several studies support the ameliorative and preventative potential of vitamin D and successful cognitive ageing the results are inconsistent and do not provide conclusive evidence of a cause and effect.

1.2. Preclinical Data

Vitamin D Receptor and the Brain

VDR is expressed widely in the adult brain in temporal, orbital and cingulated cortices, cerebellum, mesopontine area, thamalus, hypothalamus, in the accumbens nuclei, parts of the stria terminalis and amygdala and widely throughout the olfactory system. It is also expressed in the pyramidal neurons of the hippocampal regions CA1 ,CA2, CA3, CA4 in rats (Stumpf et al., 1982) as well as in humans and is most highly expressed in the substantia nigra (Eyles et al., 2005). VDR has been reported in several cell types of

the central nervous system including astrocytes, oligodendrocytes, microglia and Schwann cells (Cornet et al., 1998), (Baas et al., 2000). VDR is densely expressed in the subventricular zone in neonatal rat brains, the area representing the active site for proliferation in the embryo and throughout life (Stumpf et al., 1982).

25OHD can cross the blood brain barrier (Kaleuff et al., 2006) and may also be synthesised in the brain (Eyles et al., 2005). The discovery of the 1ά- hydroxylase and biosynthetic / biodegradative pathways for vitamin D in neurones and glial cells (Neveu et al., 1994), (Eyles et al., 2005) in co-location with the VDR supports a functional role for vitamin D in the human brain.

Vitamin D Targets in Brain

VitD is known to have multiple gene targets in the brain, whose products play an important role in neuro-protection, neuronal differentiation and brain function.

Vitamin D and Noradrenaline

The human brain is loaded with VDRs that regulates over 1000 genes, including those that promote the expression for enzymes and receptors key to the serotonin neurotransmitter system vital for mood, motivation and memory. VitD deficiency may directly affect the balance of various neurotransmitters responsible for brain functioning. Noradrenaline is believed to improve alertness during sustained attention tasks, vitD provides enzymes essential for creating catecholamine neurotransmitters (noradrenaline, serotonin, norepinephrine) with imbalances associated with sustained attention system. Animal research models provide evidence of an interaction with Alzheimer's disease and the enzyme required to convert dopamine to noradrenaline, Dopamine β-hydroxylase (BβH), with vitamin D believed to be essential in the conversion phase.

Vitamin D and Neuroprotection

Studies have shown that vitamin D may protect the structure and integrity of neurons through detoxification pathways and neurotrophin synthesis. Neurotrophins are proteins necessary for neuronal survival in ageing. Vitamin D is known to effect the expression of three of the four neurotrophins in mammals including, Nerve Growth Factor (NGF), Glial Derived Nerve Factor (GDNF) and Neurotrophin 3 (NT3), (Neveu et al. 1994), (Naveilhan et al., 1996).

Vitamin D and Enzyme Regulation

1,25 dihydroxyvitamin D inhibits inducible nitric oxide synthetase (Garcion et al., 1997), an enzyme that is up regulated during ischaemic events and in patients with Alzheimer's and Parkinson's Disease (Buell et al., 2008). It is responsible for generating nitric oxide which is known to cause damage to neurons and oligodendrocytes at high concentrations.

VitD enhances innate antioxidant pathways. It is known to up regulate Gamma Glutamyl Transpeptidase (Garcion et al. 1999), an enzyme that leads to increased production of glutathione (a potent antioxidant) which protects oligodendrocytes and the integrity of nerve conduction pathways critical to mental processing.

Choline acetyltransferase, an enzyme responsible for the synthesis of acetylcholine and which is known to play a role in memory is also upregulated by vitD (Sonnenberg J et al. 1986).

Vitamin D and Neuronal Calcium Regulation

Three calcium binding proteins have been shown to be modulated by vitD in brain tissues, calbindin, parvalbumin and calretinin (Alexianu et al., 1998), (DeViragh et al., 1989). All three are widely and uniquely distributed in adult brain and are believed to serve a neuroprotective role as calcium buffers (Fierro et a.,1996) as well as being involved in critical brain functions.

Vitamin D and Cytokines

VitD is a potent immunosuppressant and is considered an anti-inflammatory agent with profound effects on T cell function (van Etten and Matthieu et al, 2005). Vitamin D (calcitriol) is known to inhibit the synthesis of pro-inflammatory cytokines in the microglial cell line (Lefbvre d'Hellencourt et al., 2003). It may decrease the production of TNFά in the brain, which is thought to play a pathogenic role in neurodegenerative disorders such as Alzheimer's and Parkinson's Disease (van Etten and Matthieu et al, 2005). Oral treatment with 25-hydroxyvitamin D has been shown to reduce interleukin-b production in rat hippocampus (Moore et al., 2005).

Vitamin D & Alzheimer's Disease

In human subjects with Alzheimer's, a reduction in VDR mRNA in specific regions of the hippocampus (CA1 and CA2) was found compared to controls (Sutherland et al.,1992). Hippocampal cell loss and neuronal aging have been attributed to elevated L type voltage calcium channels and glucocorticoid neurotoxicity (Kimura et al., 1998). Vitamin D (calcitriol) is known to down regulate the expression of voltage sensitive calcium channel transcripts in rat hippocampal cells. Treatment of aged rats with vitamin D restores L-type voltage activity to that seen in younger animals (Brewer et al., 2006).

1,25-dihydroxyvitamin D (calcitriol) may play a part in amyloid clearance, as it has been shown to stimulate β-amyloid phagocytosis while protecting against apoptosis (Masoumi et al., 2009). Higher frequency of VDR polpymorpisms has been found in Alzheimer brains versus aged matched controls (Gezen-Ak et al.,2007). Several studies report low levels of 25(OH)D in the community in patients with Alzheimer's disease (Kipen et al.,1995), (Sato et al., 1998), (Buell et al., 2010).

Vitamin D & Vascular Cognitive Impairment

Vitamin D may help reduce vascular related brain injury by mediating the effects of inflammation, calcium dysregulation and increased oxidative stress (Buell et al., 2009). During transient ischaemic events TGF and GDNF are up regulated in hippocampal cells to promote survival. REF Vitamin D increases innate antioxidant defences by upregulating glutathione and GDNF concentrations (Naveilhan et al.,1996) which has been shown to attenuate ischaemic brain disease in rodents (Wang et al.,2001).

In animal and in vitro models of cerebral ischaemia, vitamin D inhibits antigen cell maturation (Carthy et al.,1989), down regulates NF-kb (Kong et al.,1999) and stimulates anti-flammatory cytokine production (Timms et al., 2002). Vitamin D insufficiency (level of 25-50 nmol/l) has been associated with large vessel infarcts and white matter hyperintensity volume and severity (Buell et al., 2010). Vitamin D may also play a role in cerebrovascular protection through its possible effect on blood pressure, heart function and diabetes.

Vitamin D / Parathyroid hormone and Cognition

Vitamin D deficiency is a known cause of secondary hyperparathyroidism and an inverse relationship exists between serum 25(OH)D and PTH levels. It is plausible that PTH and

calcium levels may be part of a complex relationship between vitamin D and cognitive function.

Receptors for PTH have been found in numerous organs including the brain and it is widely distributed in the central nervous system (Usdin TB et al.,1995). PTH appears to pass the blood brain barrier and may therefore affect cerebral function (Joborn et al., 1991). PTH increases intracellular calcium concentration in nerve cells which could have potentially harmful affects like inhibited mitochondrial oxidation and ATP production (Massry SG et al.,1995), (Smogorzewski et al., 2001). Incubation with PTH in one study caused increased intracellular calcium concentration and cell deterioration in rat hippocampal slices (Hirasawa et al., 2000). High PTH levels were associated with low performance in 10 out of 13 tests of cognition in the 5th Tromso Study (Jorde et al., 2006). In elderly patients with primary hyperparathyroidism, dementia is frequently found and often alleviated after parathyroidectomy (Ohrvall U et al.,1994). Impairments on tests of cognitive function in patients with primary hyperparathyroidism includes verbal memory, nonverbal abstraction (Walker MD et al. 2009) and spatial learning (Roman SA et al, 2005). PTH was associated with impaired cognition at baseline (as measured by Mini Mental State Examination and Clinical Dementia Rating Scale) and with a five-year cognitive decline in a general aged population independently of calcium and renal function (Bjorkman et al., 2010).

1.3. Work Leading up to this study

There are few published RCTs that have investigated the effect of vitamin supplementation on cognitive function in older adults. Studies addressing this concept vary considerably in methodological approaches, for example differences in study cohorts (dementia vs healthy), measurements of cognitive function, as well as doses and duration of vitamin D treatment. We a) conducted a systematic review of vitamin D and cognition in older adults and b) tested these findings in our own cross sectional cohort.

Establishing evidence for the level of 25OHD associated with improvements in cognitive outcomes

Based on systematic review of observational data we noted that overall mean 25(OH) D level of >68.9nmol/L compared to levels <50.85nmol/L were associates with better

cognitive performance, On systematic review of RCTs, we also noted that was further confirmed through two intervention studies were the overall average serum level >61.5nmol/L was associated with better outcomes compared to levels <41nmol/L. An average increase of 18.5nmol/L was observed following vitamin D supplementation in these studies. This suggests, a potential value in targeting vitamin D intervention to older adults with low-moderate levels of 25OHD. This rational for targeting those with low levels is consistent with recent opinion (Heany et al., 2014) and with recent findings in well conducted RCTs in other diseases, such as TB (Martineau, 2015). Targeting low vitamin D levels may seem obvious, however it has not been a pre-defined approach in previous intervention studies, as raising levels to a postulated 'therapeutic' zone of >75-100nmol/L has been argued**.**

Outcome; for recruitment screen out high 25OHD (target lower levels)

Establishing evidence for which cognitive domains may be influenced by vitamin D – what measures to include?

Based on systematic review, we sought to identify specific aspects of cognition shown to improve in response to vitamin D. Many studies are limited, in that they rely on a single global assessment such as the Mini Mental State Examination (MMSE). We noted that Annweiler et al. 2012, showed an 11% improvement in executive function task, Frontal Assessment Battery and Dhesi et al 2004 demonstrated a 15% improvement in a test of specific executive function, choice reaction task, following supplementation of 3,333IU/day vitamin D3, for a 6month follow up period. Cross sectional research appears to supports this, with executive function and attention task showing significant association with circulating vitamin D. Based on previous published data by Annweiler et al. 2012, n=44, we estimate that raising 25(OH) D by 25-30nmol/L would result in 15% change in executive function in the vitamin D treated group.

We have further explored executive function in our own existing cross sectional ageing cohort (TRIL). Based on our preliminary data we estimated a 14% difference in executive function in older adults with sufficient 25OHD levels (>50nmol/L and <75nmol/L) (n=99) versus deficient/insufficient levels (12.5nmol/l to <50nmol/L) (n=184). (Aspell *et al*., 2015**).**

The primary outcome in the pilot RCT will be differences in cognitive variables in response to vitD versus placebo at 6 months, our key focus is change in 'executive function' based on the validated Trail Making Task (TMT) and 'attention 'assessed by the validated Sustained Attention to Response Task. We will also test for overall cognitive performance using global measures of cognition. Moreover, an in-depth cognitive battery will be included for exploratory work, these have been extensively validated and used by the study team, in the Memory clinic in St James's and in large studies such as TILDA and the MRU in Trinity College Institute of Neuroscience and similar ageing research in TCD.

Outcome: suggests value in the assessment of executive function and a global tool (with a battery for exploratory analysis)

Evidence for effects on functional outcomes

There is considerable work on the effect of vitD supplementation on muscle function, muscle mass, mobility and falls prevention in older adults, while positive effects have been demonstrated the data remain inconclusive. Our recent pilot study (Raftery et al., 2014) of vitD on muscle function in inflammation and a larger RCT suggest a significant improvement in muscle function, possibly through a reduction in inflammation. Interesting, increased / maintained muscle mass is also associated with better insulin sensitivity. Both inflammation and insulin resistence are associated with cognitive decline.

1.4. Dose Rationale and Risk/Benefits

Section 2: Study Aims and Objectives

AIMS AND OBJECTIVES

Primary aim:

To determine whether older adults benefit greater cognitively from vitD supplementation (2000IU/d), over a 6 month follow-up period with a particular focus on executive function and attention specific cognitive domains. And to test if this effect is greater in those classified as physically active relative to less physically active comparisons.

Primary Objective:

To determine measurable effects of vitamin D3 supplementation on cognitive performance and incidence of cognitive decline in healthy older adults.
We will conduct a randomised double-blind placebo-controlled trial in healthy older adults over a 6 month follow up period, to investigate any measurable differences in cognitive performance at 0,3 and 6 months.

Secondary Objective:
To determine measurable effects of vitamin D3 supplementation on functional outcomes in physically active healthy older adults.
We will conduct a randomised double-blind placebo-controlled trial in healthy older adults over a 6 month follow up period, to investigate any measurable differences in functional ability such as muscle function and frailty at 0,3 and 6 months.

Section 3: Study Design

3.1. General Design

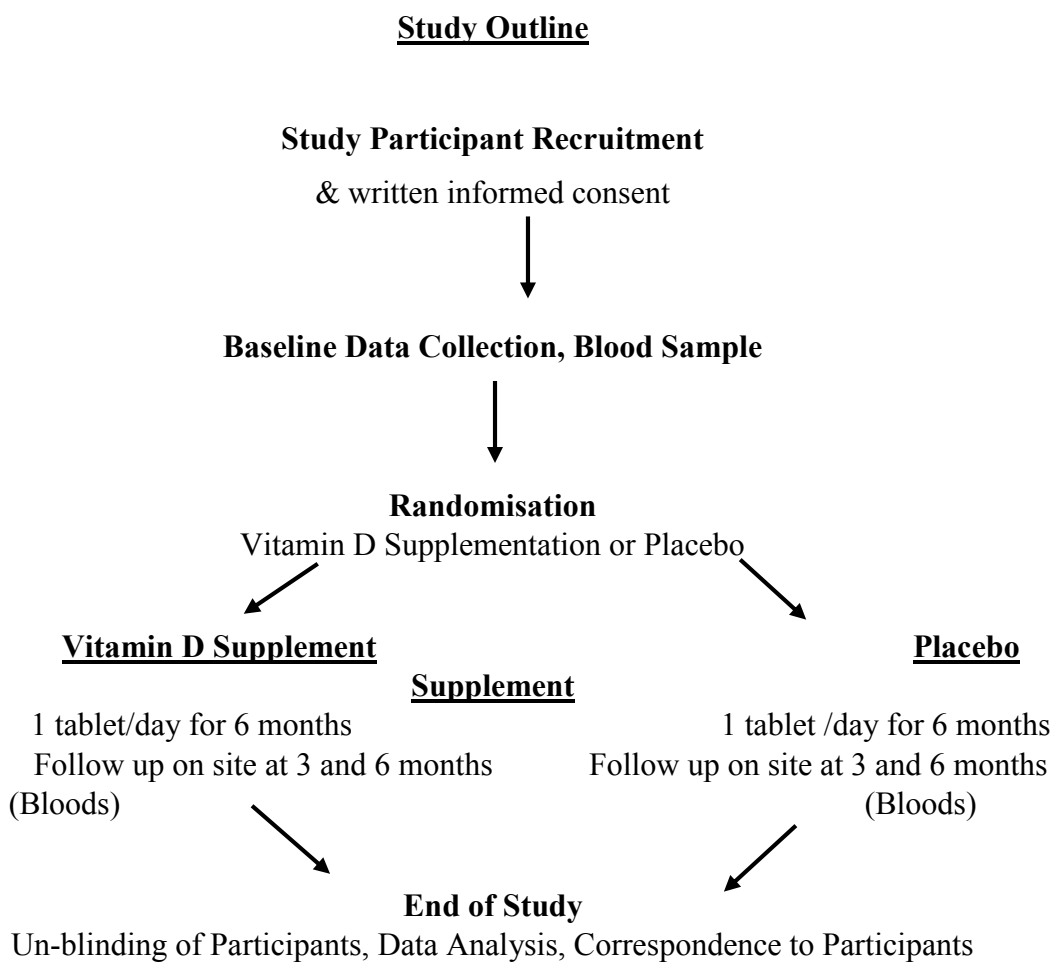
The current trial is a randomised, double-blind, placebo-controlled clinical trial in community dwelling, healthy older adults. Half of each group will be randomly assigned to receive 2000IU/d of vitD3, and the other half will randomly be assigned to receive placebo. Participants are requested to remain on the assigned intervention/placebo for 6 months and will be required to attend for assessments at 0, 3 and 6 months.
For pre-defined sub analysis the group will be stratified according to regular levels of physical activity, to explore cognitive parameters if those with higher levels of physical activity in the vitD treatment group vs sedentary group. Based on the TRIL data, of a similar cohort of community dwelling adults, the group split evenly into sedentary and moderately active without pre-stratification.

3.2. Power Calculation

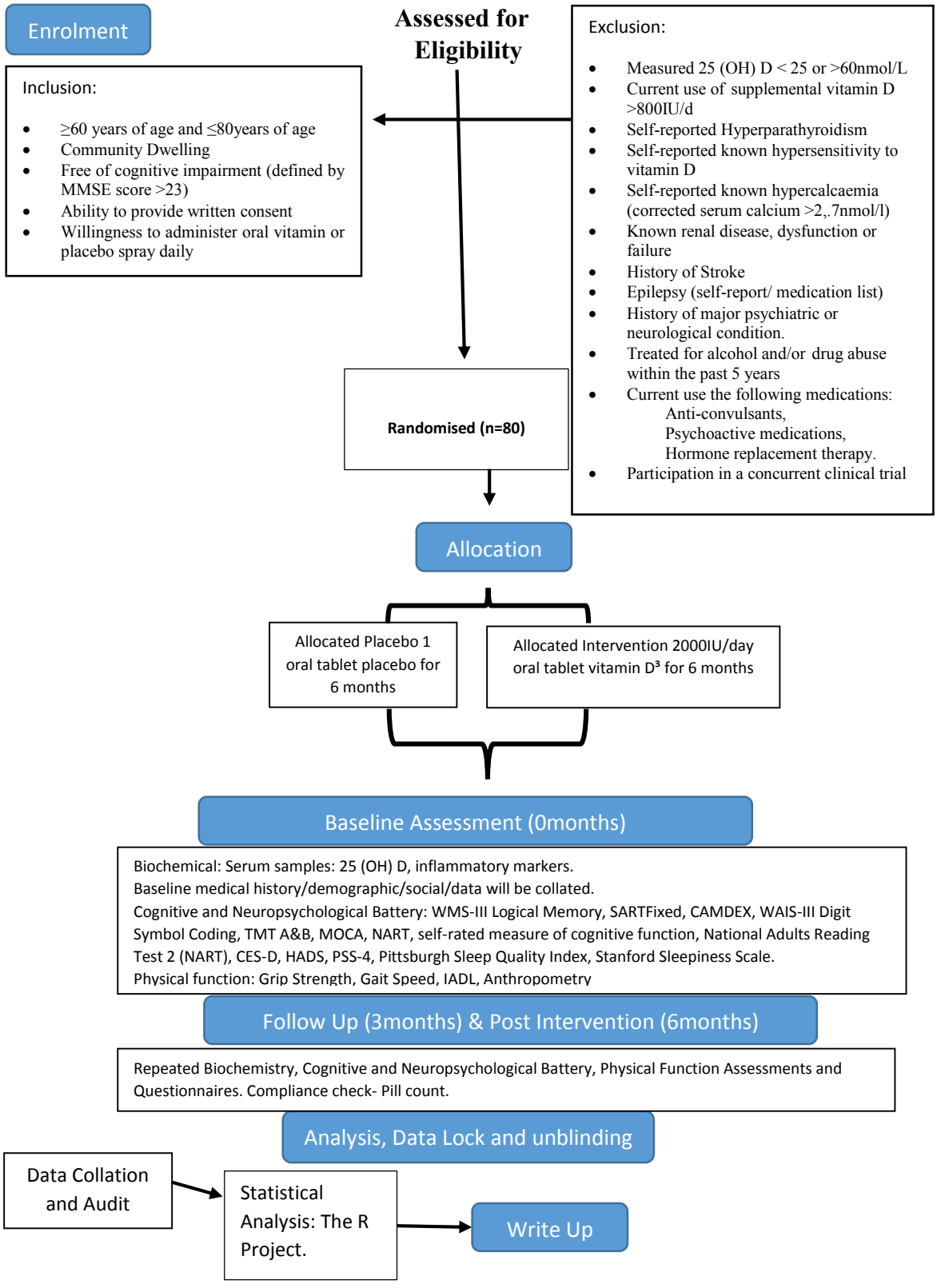
Based on our systematic review of vitD and cognition in ageing, the limited number of previous RCTs available have used samples sizes of 44 and 123 participants. These studies detected changes of 11% and 15% in measures of executive function. Therefore

a sample of 80 was considered appropriate for this pilot study, with a vitamin D dose of 2000 IU/d.

3.3. Schematic Representation



STUDY DESIGN



3.4. Primary Study Endpoints:

- Cognitive Performance [Time frame: Baseline, 3months, 6months]
    - Predefined analysis by physical activity levels
- Physical Function [Time frame: Baseline, 3months, 6months]

3.5. Exploratory Analysis:

- Sleep Quality and Mood [Time frame: Baseline, 3months, 6months]

3.6. Primary Safety Endpoints:

- Hypercalcaemia (corrected serum calcium > 2.55nmol/l) ***

Section 4: Subject Selection and Withdrawal

4.1. Inclusion Criteria

Subjects must fulfill all of the below:

- ≥60 years of age and ≤80years of age
- Community Dwelling
- Free of cognitive impairment (defined by MMSE score >23)
- Ability to provide written consent
- Ability to swallow study capsule

4.2. Exclusion Criteria

If any one of the below criteria are present the subject cannot partake in the trial:

- Measured serum 25 (OH) D <15nmol/L or > 125nmol/L
- Current use of supplemental vitD >800IU/d
- Self-reported known hypersensitivity to vitD
- Self-reported known hypercalcaemia (corrected serum calcium > 2.55nmol/l)
- Self-reported Hyperparathyroidism
- Renal dysfunction/failure
- History of Stroke
- Epilepsy (self-report/ medication list)
- History of major psychiatric or neurological condition.

- Treated for alcohol and/or drug abuse within the past 5 years
- Current use the following medications:
    - ✓ Anti-convulsants
    - ✓ Psychoactive medications
    - ✓ Hormone replacement therapy
- Participation in a concurrent clinical trial

4.3. Subject Recruitment and Screening

We aim to recruit 80 participants through several established channels such as outreach talks within the community, advertisements in local parishes, community centres and social networking sites; and through current links within the Neuro-Enhancement for Independent Lives (NEIL) Programme in Trinity College Institute of Neuroscience (TCIN) and though HSE community links in central and North Dublin. Previous agreement with the Director of South Dublin Senior Citizen's Club has also been established.

Individuals interested in taking part will be given a copy of the information leaflet (Appendix 2), which will provide information about the study in a simple manner. This information sheet will have a removable section which potential participants can give contact information and sign to agree to be contacted by a member of the research team about participation in the study. Or, interested individuals can contact the researcher on the telephone or via the email contact details given on the advertising sheet.

A researcher will then contact potential participants by phone. The project will be explained again and the researcher will answer any questions the participant may have. Those who indicate that they are interested will be sent an information pack, containing a cover letter (Appendix 3), consent form (Appendix 4), and study information sheet (Appendix 5). Once participants have signed consent forms and returned them the researcher will contact them again by phone, to administer a short health screen (Appendix 6) and to take an inventory of the participant's medication, in order to screen for any conditions or medications that may render them unsuitable for participation. The screening should take 15 minutes. Following a telephone screen potential participants will be invited to visit the study site do a trained phlebotomist can obtain a serum 25 (OH) D sample.

Individuals who screen negative will not be included in the study and will be told that they are unlikely to benefit from the intervention and therefore the study may not be

suitable for them. As a duty of care any participants who fall below 25nmol/L will not be included in this intervention and with their consent a letter will be sent to their GP to highlight the need for appropriate treatment. The option to be added to the NEIL Programme mailing list will be given if they wish to hear about future research that may be more suitable. The participants who successfully complete the screen will be scheduled for baseline assessment.

4.4 Subject Discontinuation

4.4.1. When and how to withdraw subjects

Subjects may have the study treatment withdrawn if one or more of the following occurs:

- If the participant at any time makes a voluntary decision to withdraw.
- The study is terminated.

Should a subject decide to voluntarily withdraw from the trial they are asked to contact Niamh Aspell, or local coordinator to advise same either telephone call or in writing. A note of same, including the time and date the communication was made should be documented on the participants Case Record Form (CRF). If a subject elects to cease taking treatment, follow up visits and data collection should continue as scheduled for as long as possible.

If an adverse event occurs (i.e. hypercalcaemia, impaired renal function), a clinician can withdraw the participant immediately (a study adverse event form will be completed).

Subjects are advised that a clinician can withdraw them from the trial at any time they wish upon enrolment.

4.4.2 Data collection and follow-up for withdrawn subjects

In the case of subject withdrawal, the following actions are required;

Participants who voluntarily withdraw, or who are withdrawn from the trial by a clinician are asked to attend their scheduled follow-up appointments, by letter, primarily to obtain a blood sample for safety purposes.

Patients will also be asked to bring to their next scheduled follow-up appointment the remainder of the oral spray (intervention/placebo) given to them for safe disposal.

The participant will be asked to volunteer any information they wish to provide regarding their decision to withdraw, particularly if they report any negative side-effects of treatment.

All identifiable data and identifiable human biological tissue samples will be destroyed either when the participant withdraws or when the research participant specifically requests that this occur (follow local ethics board guidelines).

If the participant discontinues treatment due to an adverse event, the participant should be followed until the event resolves, or there is a return to a clinically acceptable medical status.

Participant deaths or Serious Adverse Events, which occur within 30 days of discontinuation, should be reported to the P.I.

4.4.3. Lost to follow up

In order to deem a subject as truly lost to follow-up the following actions are required to have taken place;

1. A telephone conversation with the subject. If necessary up to 3 attempts to contact the subject by telephone is required.
2. A certified letter requesting the subject attend their next scheduled appointment for the purpose of obtaining a blood sample for safety monitoring purposes.

4.4.4 Replacement of Subjects Withdrawn or truly lost to follow-up.

Patients truly lost to follow up may be replaced.

Section 5: Study Intervention

5.1 Description

5.1.1 Investigational Product

The investigational product comes in the form of one 2000IU vitamin D3 tablet. The 2000IU vitamin D3 tablet also contains Magnesiumstearat and Cellulose microcrystalline 102EP and a coating of Sepifilm suspension (Hydroxypropylmethylcellulose, cellulose microcrystalline and diacetylized monoglyceride). 1 tablet per day should be taken with meals (a dosage which has been used in previous clinical trials and is deemed safe, dosage at this level are available for

commercial use and are well tolerated, non-invasive and do not require a hospital visit for administration).

5.1.2 Placebo.

The placebo is Cellulose microcrystalline 102EP, Magnesium stearat and is coated with Hydroxypropylmethylcellulose, Titandioxid, Talcum, Magnesium stearat and olive oil. BioVinci (Denmark) are supplying the intervention and placebo.

5.2 Treatment Regimen

Subjects will be requested to take 1 tablet per day, preferably at approximately the same time every day and preferably with main meal, for 6 months. If a subject forgets to take a tablet, they will be instructed not to double up on missed doses and to report the number of doses missed on their next clinic visit.

5.3 Randomisation

Tablets will be supplied from BioVinci randomised and in containers with 1 month supply in each. Subjects recruited will randomly be assigned to one of two treatment conditions by a stratified minimisation procedure which ensures that the two groups do not differ significantly in a) gender, b) age, c) physical activity levels. All subjects will be assigned a unique randomisation number and no number will be repeated twice. Neither the participant nor the researcher supplying the capsules will know which preparation is administered, thus ensuring double-blind administration.

5.4 Preparation and administration of study treatment

Tablets will be supplied from BioVinci randomised and in containers with 1 month supply in each.

5.5 Subject compliance monitoring

Compliance will be evaluated by patient interview at each visit and by a pill count of the remaining tablets on the last visit. Participants will also be reminded to take the supplement periodically by either a telephone call by a researcher, or a standard text messaging service such as textmagic.com. An a priori decision to include only those patients who exceeded 85% compliance (Cashman et al, 2009).

5.6 Blinding of the study treatment

All subjects and research staff will be blinded as to whether the subject is assigned to placebo or intervention until after database lock.

Codes linking the randomisation number for each subject to actual treatment will be secured in a sealed opaque envelope and maintained in a locked drawer in the research centre.

5.7 Receiving, Storing, Dispensing and Return

5.7.1 Receipt of Investigational product and placebo

- Upon receipt of the study treatment supplies from BioVinci. an inventory will be performed.
- Records or logs will include the following:
- Amount received from the manufacturer
- Label ID Numbers or batch numbers
- Dates of treatment movement
- Unique participant identifier
- Amount dispensed and returned to each participant.
- Initials of the person responsible for each treatment inventory entry
- Note only authorised personnel can dispense the study treatment.
- Any change in the quality of the goods must be reported to BioVinci Ltd. within 7 days after arrival.
- It is the responsibility of the investigator to ensure that a record of study deposition is maintained.

5.7.2 Storage

The product will be stored in line with optimal storage conditions provided; i.e. the product should be kept in an air conditioned area at a temperature of 15-30 degrees C and below 50 percent relative humidity during bulk storage. Storage near radiators, steam pipes, or in direct heat of sun, or in refrigerators should be avoided. The product should be repackaged in a moisture-vapour proof packages in a temperature and humidity controlled environment.

5.7.3 Dispensing of the Study treatment

The study drug will be dispensed by Niamh Aspell, or local researcher. The investigator will dispense the appropriate amount of a 3 month supply at each assessment of the study drug (intervention or placebo as per randomisation list) to the research participants on site. After dispensing the study drug- the unique identifier no. will be placed with on the inventory list alongside the batch number and dated.

5.7.4 Return of the study treatment

After completion of the study, there will be a final reconciliation of the treatment supplies shipped, consumed and treatments remaining. Any discrepancies will be noted, investigated and resolved. After appropriate accounting, unused study treatments (tablets and containers) will be will be handed to pharmacy for disposal and the date of this action will be documented in the study files.

Section 6: Safety, Patient Monitoring and Adverse events

6.1 Definitions

Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject who has been administered a medicinal product (and which does not necessarily have causal relationship with this treatment) (IRCRIN/MMI, 2010).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example) symptom, or disease temporarily associated with the use of a medicinal (investigational or marketed) product.

In this study, that includes any illness, sign, or symptom, or clinically significant laboratory test abnormality that has appeared or worsened during the course of the clinical trial, regardless of causal relationship to the treatment(s) under investigation. Following the questioning and examination of the participant, all AEs, must be recorded and described on the appropriate Adverse Event Form.

Adverse Reaction (AR)

If the AE has a reasonable causal relationship with the treatment, it is defined as an adverse reaction.

Serious adverse event (SAE)

Certain categories of AEs are defined as serious adverse events (SAE). A SAE is any untoward medical occurrence that is results in

- Death
- Is life threatening?
- Requires hospitalisation, or prolongation of existing hospitalisation
- Results in persistent or significant disability
- Is a congenital anomaly / birth defect (IRCRIN/MMI 2010)

An SAE for the purpose of the current trial is new hypercalcaemia requiring treatment and/or new elevated serum creatinine.

All adverse events which do not meet the criteria for a SAE should be considered as non-serious adverse events.

Adverse Event Recording Period

The study period during which adverse events must be reported is the period from initiation of the supplementation and to the end of the study treatment follow-up.

Pre-existing condition

A pre-existing condition is one that is present at the start of the study. A pre-existing condition should be recorded as an adverse event if the frequency, intensity, or character of the condition worsens during the study period.

Post Study Adverse Event

All unresolved adverse events should be followed up by the medical team until the event(s) are resolved, the subject is truly lost to follow up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subjects physician believes might reasonably be related to participation in the study.

Hospitalisation, prolonged hospitalisation or surgery

And adverse event that results in hospitalisation or prolonged hospitalisation should be documented and reported as an SAE.

However, neither the condition, hospitalisation, prolonged hospitalisation, nor surgery is reported as an adverse event if the event of the following circumstances;

- Hospitalisation or prolonged hospitalisation for diagnostic or elective surgical procedures for a pre-existing condition.

- Hospitalisation or prolonged hospitalisation for therapy of the target disease of the study, unless I is a worsening or increase in frequency of hospital admissions related to participation in the trial as judged by the physician.

6.2 Recording of an Adverse Event.

All Adverse Events (AE) are to be recorded on the Adverse Event Form.

Timely and complete reporting of all AEs is necessary to identify events that are treatment related or potentially treatment related, thereby allowing: (1) a greater understanding of the overall safety profile of the treatment; (2) recognition of dose related toxicity; (3) appropriate modification of the study protocol and (4) adherence to GCP.

Recording an adverse event

At each contact with the subject the investigator must seek information on adverse events by questioning. Information on adverse events should be recorded immediately in participant's safety monitoring sheet. All clearly related signs symptoms and abnormal diagnostic procedures should be recorded.

High serum calcium is likely to occur with any of the following; nausea and vomiting, excessive thirst, frequent urination, constipation, abdominal pain, muscle weakness, confusion, lethargy with

The clinical course of each adverse event should be followed until resolution, stabilisation or until it has been determined that the study treatment or participation is not the cause. Any serious adverse event that occurs after the study period and is considered possibly related to the study treatment or study participation should be recorded and reported immediately.

6.3 Reporting of Adverse Events, Serious Adverse Events and Unanticipated Problems.

An AE will be documented in the patients Adverse Event Form and verbally brought to the attention of the physician in the clinic the patient is attending.

6.3.1 Reporting a SAE: Investigator Reporting to Consultant Psychiatrist**

A SAE will be documented on an Adverse Event Form and will be verbally communicated with the Registrar immediately or participants GP (below). A written

account should include the following information; subject name and subject Number, a description of the event, whether treatment was discontinued, the reason why the event is classed as an SAE, date of onset, current status.

6.3.2 Reporting a SAE: Investigator Reporting to G.P.

A SAE will be communicated to the patients GP within 5 working days. A description of the event, the reason why the event is considered a SAE, date of onset, current status must be included and signed off by a physician.

6.3.3. Reporting a SAE: investigator reporting to local Ethics Committee

Reports of all SAE (including follow-up information) must be submitted to the Ethics Committee within 10 working days. Copies of the report to the Ethics Committee will be filed in the Clinical Trial Master file. SAEs require expeditious handling.

6.4 Unblinding

6.4.1. Unblinding in a non-emergency

Participants will be unblinded following completion of follow-up for all randomised participants (i.e. after the last study participant completes follow-up).

6.4.2. Unblinding in an emergency

Breaking the blind will be avoided whenever possible in order to protect the integrity of the study. Breaking the blind should only be considered in circumstances in which knowledge of the treatment assigned is essential for determining the course of treatment required. If an emergency necessitates that the blind be broken, only the Research Statistician will have access to the unblinding codes. If contacted regarding breaking the blind only the Principal Investigators will act according to his/her best judgement in deciding whether or not to break the blind for that subject.

The request to unblind must be authorised first by one of the Primary Investigators. Details of unblinding must be recorded in the master clinical trials folder, including; dates of unblinding, reason of unblinding, authorisation for unblinding, and the randomisation/treatment pack number.

6.5 Safety Monitoring Plan

Safety monitoring includes careful assessment and appropriate reporting of adverse events. A safety monitoring plan will be implemented and data will be reviewed by an internal data and safety monitoring board. This will include assessment of the number and nature of serious adverse advents at 3 and 6 months.

Section 7: Data Safety, Data Handling and Record Keeping

7.1 Data Safety and Monitoring Committee.

A Data Safety and Monitoring Committee (DMC) made up of independent experts will monitor the study data when complete baseline and 3 month data is available on the 80 participants. The study data will be made available to the DMC by means of a study report. 2 meetings will take place. They will open with an open forum for which the Investigators can discuss general aspects of the trial and a closed forum which will include the study statistician and the DMC only. The remit of the DMC is will be to assess the safety and efficacy aspects of the trial. The DMC should specify what information they require and decide which statistical approach to data monitoring they wish to use.The DMC provide an advisory role to the PIs.

7.2 Confidentiality

Precautions will be taken to protect the privacy of research subjects and the confidentiality of their personal data. Data collected will be coded, the key for which Niamh Aspell will retain. All data will be collected and maintained according to the Data protection Acts 1988, and 2003 and according to the Data Protection Guidelines on research in the Health Sector, 2007.

7.3 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the evaluation of the trial. Source data is contained in source documents; examples of which include; laboratory notes, recorded data from automated instruments

7.4 Case Record Forms

A case record form (CRF) is the primary data collection instrument used in the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space is left blank because a procedure was not done for example, N/D should be written,

or N/A if that is the case. All entries should be printed legibly in black ink. Id any error has been made, to correct such an error, draw a single line through the incorrect entry and write the correct entry above it. All such changes must be initialled and dated. Do not erase or white out errors.

7.5 Record Retention

Essential documentation should be retained for at 10 years after completion of the trial.
If any investigator with records in their possession withdraws from the study (e.g. relocation) the records shall be transferred to a mutually agreed designee (i.e. another investigator).

Section 8: Study Monitoring, Auditing and Inspecting

8.1 Study Monitoring, auditing and inspecting plan

The investigator will allocate adequate time for such monitoring activities, including reviewing safety of data, data entry is timely and data is accurately inputted to the database (cross check percentage of data etc.)

Section 9: Ethical Considerations

This protocol and any amendments will be submitted to the AMNCH/SJH ethics committee for formal review. The study is conducted according to the international standard of Good Clinical Practice (GCP) and the Declaration of Helsinki 2008. All documentation given to participants or that participants will see, is first approved by the ethics committee.
All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Appendices for a copy of the Subject Informed Consent Form (Appendix 4). This consent form will be submitted with the protocol for review and approval by local ethics committee for the study. The formal consent of a subject, using the EC approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

Section 10: Administration

10.1 Adherence to the protocol

Except for change that is intended to eliminate an immediate hazard to participants, the approved protocol shall be conducted as described. Any significant deviation from the protocol must be reported in the case report form with an explanation.

Section 11: Study Finances

11.1 Funding Sources PhD Student:

Irish Research Council Government of Ireland Postgraduate Scholarship

11.2 Conflict of Interest

None

11.3 Subject Payments

None

Appendix 8

Help us D-Activate Decline
Volunteers Aged 60+ Needed

Researchers in the D-Activating Decline project are happy to invite you to volunteer to take part in a research study that aims to understand how low vitamin D levels can be improved for older adults and how this may benefit our health as we age.

In order to do this, we need volunteers to take part in our research. There are a few different parts to our research project. One part involves answering some questions about lifestyle and completing some tasks that measure various aspects of mental function. We also measure grip strength, weight, height, and walking speed. Finally we measure vitamin D levels from a standard blood sample. Our research involves taking a study tablet once a day, this may contain 2000IU of vitamin D3 or it may be a placebo or "dummy" tablet for a 6 month period, neither the volunteer of the research team will know which you are receiving. We do this so we to determine whether any changes experienced are directly linked to the vitamin itself and also sometimes other researchers have found that knowing if someone is receiving a supplement can influence the assessment process.

Our researchers are interested in how vitamin D supplements might improve our health as we age. To find out, we'll need you to visit us 4 times in total over a 6 month period, to ask you some questions about lifestyle and measures of mental function project and also to take a blood sample to check your vitamin D levels.

Our research volunteers are very important to us, because without them we cannot find out more about how nutrition and lifestyle behaviours might be meaningful in preserving our health as we age.

Are ***you*** interested in taking part in our research? If you are you interested in taking part in our research please contact:

Ms. Niamh Aspell, Ph.D. Candidate, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 7. **Email:** niamha@tcd.ie. **Phone:** (01) 896 4039.

Appendix 9

Study Information Sheet

D-Activating Decline: Vitamin D Interventions for Older Adults

Research Team: Ms. Niamh Aspell, Prof. Maria O'Sullivan, Prof. Brian Lawlor.

What is the project?

We would like to invite you to participate in a study that aims to investigate how vitamin D levels are important for our health as we age and how we can use supplements to reverse low vitamin D levels, which as a result may support healthy ageing.

If you agree to take part you will be asked answer some background questions over the phone – to provide us with some information about your life (for example, your occupation, your living situation, and your health).

You will then be invited to visit our research team in St. James's Hospital were a blood sample will be obtained by a trained phlebotomist. We will wait for the results of this and contact you once we receive them (usually 3-5 days). If your results are within a suitable range for this project you will be invited to visit us again for an assessment. A member of the research team will ask you some questions about your lifestyle, mood, and will also ask you to complete some tasks that rely on mental functioning. These are paper and pen tests. We will also measure your height and weight, grip strength and the time that it takes you to walk a short distance.

We will use a hydraulic hand dynamometer (pictured right) to measure your grip strength. You will be required to squeeze the handle of this dynamometer for a few seconds. There are no risks associated with using this machine, and any participant with pain or swelling in their wrists, or any other problems with their wrists, will not be asked to take this test.

The researcher will complete these assessments with you again after a further 12 weeks and a further 26 weeks. During the 26 weeks you be required to take 1 study capsule per day, this will either be vitamin D or a "dummy" tablet, neither the research team nor the volunteer can know which has been assigned until the study is complete.

All of the information that you provide will be treated in the strictest confidence. You will be assigned a participant ID code, and all of the information you provide will be stored only under this ID Code, not under your name or any contact details. You are free to decline to provide some or all of the information requested in the questionnaires or at any time during the session. The information we collect during this study will never be made available to anyone other than members of the research team or their assistants.

You are free to decline any of the tasks or measurements that you do not wish to complete.

What are my rights if I join the study?

Participation in the study is entirely voluntary and if you agree to participate you have the following rights:

1. The information from this study will be kept strictly confidential and will not be made available to any other people.

2. We will aim to publish our results in scientific journals but any information we have will be completely anonymous and presented as a group.

3. As participation is completely voluntary, you are free to withdraw from the study at any time. You are also free to withdraw your data at the conclusion of your participation should you so wish.

4. Under the Freedom of Information Act you can have access to any information we store about you, if requested.

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Prof. Brian Lawlor
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CONSENT FORM

Appendix 10

Title of research study: D-activating Decline- Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling healthy older adults- A Pilot Study.

Research Team: Ms. Niamh Aspell, Professor Maria O'Sullivan, Professor Brian Lawlor.

You have received this consent form because you indicated that you wish to take part in research at the D-Activating Decline project in the Trinity Centre for Health Sciences, St James's Hospital. This study and this consent form have been explained to me. The researcher has answered all my questions to my satisfaction. I believe that I understand what will happen if I agree to be part of this study.

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

What does the study involve?

If you consent to take part in this research, you will be asked to attend four testing sessions in St. James's Hospital, Dublin. The first session will involve obtaining a blood sample from the arm by a trained phlebotomist. Be assured that the phlebotomist will ensure that you are comfortable with the procedure before it begins and you are free to withdraw at any point. After the assessment, you will then be provided with the oral tablet (either vitamin D3 or placebo) and asked to take it every 2 days for 26 weeks. This allows the researchers to determine whether any changes experienced by participants are directly attributable to the vitamin itself rather than other aspects of the study or supplement. Neither you nor the researcher will know whether it is the vitamin D3 supplement or placebo you are taking. You will be sent reminders periodically to help you take the supplement consistently over the 26 week period. After 12 weeks, you will be asked to return once more to St. James's Hospital, where you will be asked to give a blood sample

and complete some questionnaires and tasks that provide us with information about your lifestyle, your mood and mental processes like memory and attention, similar to the tasks you completed during your first assessment. Finally at 26 weeks, you will be asked to complete the same procedure. Again, these tasks are relatively straightforward and you will be provided with clear instructions by the researcher. This session should no longer than ninety minutes. There will be regular breaks during the session, you are free to terminate the session at any time and can decline to provide some or all of the information requested.

What are my rights if I join the study?

Participation in the study is entirely voluntary and if you agree to participate you have the following rights:

1. The information from this study will be kept strictly confidential and will not be made available to any other people.
2. We will aim to publish our results in scientific journals but any information we have will be completely anonymous and presented as a group.
3. As participation is completely voluntary, you are free to withdraw from the study at any time. You are also free to withdraw your data at the conclusion of your participation should you so wish.
4. Under the Freedom of Information Act you can have access to any information we store about you, if requested.

Once I have signed this consent I understand that:

1. I will be assigned a unique ID code, under which all of my data will be stored.
2. I consent to provide information to the researchers involved. I understand that I can decline to provide some or all of the information requested. I understand that this information will never be made available to anyone other than members of the research team of their assistants.
3. Any data obtained through my participation in this research will be treated as confidential and processed only in accordance with the Data

Protection Acts, and that they will be used only for the purposes of research. I understand that I can request any information stored about me, under the terms of the Freedom of Information act.

4. I have read the attached information leaflet on the above project and have been given a copy to keep. The information has been explained fully to me and I have been given opportunity to ask questions about the project and understand why the research is being done and any foreseeable risks or consequences involved.
5. I agree to give a sample(s) of blood for research in the above project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason. If I withdraw my consent I understand that my sample will be destroyed unless I otherwise authorise. I understand that I may ask for my samples to be destroyed and that this will be without my medical treatment or legal rights being affected. I agree that the samples I have given and the information gathered by me can be stored and looked after by the (SJH/TCD).
6. I understand that that the blood samples I provide will be stored under the unique ID code for use in future analyses.
7. I agree to allow the researcher to notify my GP of my inclusion in the study and in the event of abnormal blood results I accept that this report will be sent to my GP for monitoring and possible treatment.
8. I understand that I will not benefit financially in any way by taking part in this research.
9. I know how to contact the research team if I need to.
10. I will not be requested to participate in any study that could be foreseen to be detrimental to a person's well-being, under normal circumstances, and that every study that I will be invited to participate in will have received prior approval from the relevant ethics committees of Trinity College Dublin

…… …….. ……

D-AD ID Number: _____

Name of participants Date Signature

Contact Details for Next of Kin Name: _____

Relationship to you: _____

Mobile number: _____

Home phone: _____

Address: _____

Name and contact details of GP:

Statement for Researcher: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered these questions. I believe the participant understands my explanation and has freely given informed consent.

…… …….. ……

Name of researcher Date Signature

Niamh Aspell
niamha@tcd.ie
Tel: +3531896 4039

Prof. Maria O'Sullivan
MOSULLI@tcd.ie
Tel: +3531896 4039

Prof. Brian Lawlor
blawlor@stjames.ie

D-AD ID Number: _____

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH
Department of Clinical Medicine; Human Nutrition and Dietetics.

NEIL Neuro-Enhancement for Independent Lives

Appendix 11

Date:

Dear _____,

Re: Vitamin D Supplementation and Cognition in Healthy Community Dwelling Older Adults.

I write to inform you that _____ who has listed you as their GP, has enrolled in a study in a vitamin D study at the Department of Clinical Medicine in Trinity College Dublin and agreed for us to send you this information.

The study is being conducted in the Clinical Research Facility in St. James's Hospital, Dublin and involves vitamin D supplementation and tasks of cognitive and physical function. In addition, a simple blood sample will be taken to measure vitamin D levels and calcium. In the event we receive any abnormal laboratory results for these the team will forward these to your clinic. A summary of the study is attached also for your interest.

Yours Sincerely,

_____

Ms. Niamh Aspell (Study Coordinator)
Study mobile: 085 2114649

Research Team: Associate Professor Maria O'Sullivan BSc. PhD, INDI
Professor Brian Lawlor MD, FRCPI, FRCPsych, DABPN.
Ms. Niamh Aspell (PhD Candidate/Study Coordinator) BSc.

IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH
Department of Clinical Medicine; Human Nutrition and Dietetics.

NEIL Neuro-Enhancement for Independent Lives

Title: Vitamin D Supplementation and Cognition in Healthy Community Dwelling Older Adults. D-Activating Decline Study

Vitamin D deficiency is common, especially among older adults, and may be associated with poor cognition and muscle function.

Design: Placebo controlled Randomised Controlled Study
Duration: 6months
Treatment: 2000IU/day vitamin D3 **Placebo:** Oil Capsule containing no vitamin D3
Assessment: Computer and written tasks assessing cognitive function, psychological wellbeing, physical function and diet and lifestyle. Blood sample. (0weeks, 12weeks, 26weeks)

Inclusion Criteria:

- ≥60 years of age and ≤80years of age
- Community Dwelling
- Free of cognitive impairment (defined by MMSE score >23)
- Ability to provide written consent
- Willingness to administer oral vitamin capsules.

Exclusion Criteria:

- Measured 25 (OH) D < 15nmol/mL (ie very low)
- Current use of supplemental vitamin D >800IU/d
- Self-reported Hyperparathyroidism
- Self-reported known hypersensitivity to vitamin D
- Self-reported known hypercalcaemia (corrected serum calcium >2.7nmol/l) – (also tested in the study)
- Known renal disease, dysfunction or failure
- History of Stroke
- Epilepsy (self-report/ medication list)
- History of major psychiatric or neurological condition.
- Treated for alcohol and/or drug abuse within the past 5 years
- Current use the following medications:
  - Anti-convulsants,
  - Psychoactive medications,
  - Hormone replacement therapy.
- Participation in a concurrent clinical trial.

*This study has received ethical approval from Tallaght Hospital / **St. James's Hospital** Joint **Research Ethics Committee** (REC) and will be conducted in the Health Research Board, Clinical Research Facility, St. James's Hospital, Dublin 8.*

IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn

Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8. Ms Niamh Aspell, IRC Ph.D. Candidate, Email: niamha@tcd.ie, Tel: 085 2114649

Appendix 12

The D-lightful Vitamin

Vitamin D in Ireland

In Ireland vitamin D deficiency was believed to have been eradicated after World War Two, due to better nutrition. However studies in the Irish population show that low vitamin D status is extremely common particularly in the winter months. Researchers in Ireland have reported a prevalence of inadequate vitamin D levels as high as 48% of the older population. At present vitamin D recommendations in Ireland are being revised.

So why aren't we getting enough?

Despite its name, vitamin D is not a regular vitamin, it is more commonly accepted as a hormone as we obtain it primarily via exposure to sunlight. During the summer months, UV-B rays from the sun can create all the vitamin D we require if our skin gets adequate exposure to sunlight. Although during winter months the further away from the equator you are the less chance you have of being exposed to UV-B radiation. Due to our northerly latitude we can't make any vitamin D from sunlight exposure from November to February.

But excessive sun exposure increases the risk of skin cancer?

The key to responsible sunlight exposure is to make sure that you don't burn. Take 5-15 minutes of casual sunlight exposure on the hands, face and arms two to three times a week during the summer months. This type of exposure can create as much as 20,000IU of vitamin D in your system, as vitamin D can be stored in the body for 30-60 days. This has to be midday sun exposure, early morning and evening sun light generates UV-A radiation, which doesn't generate any vitamin D and can be harmful. Also using an SPF of 15 will decrease your ability to make vitamin D by 99%. So 5-15 minutes of unprotected sun exposure during the summer months is safe and effective! After this amount of time sunscreen should be applied.

Where else can I get it?

Approximately 90% of our vitamin D intake comes from sunlight whilst the remaining 10% is contributed by the diet. The foods which contain vitamin D are not commonly consumed by

the Irish population, so although we rely on our diets to provide us with vitamin D in the winter months, in reality, this can be difficult to achieve. Previous studies have reported low daily intakes of 184IU in older Irish adults far from the recommendations of 400IU of vitamin D.

Food Sources of vitamin D

High 400-1000IU
200g Wild Fresh Salmon or Mackeral
1tsp Cod Liver Oil
100g Portobello or Dried Shitake Mushrooms

Moderate 160-250IU
Tinned Tuna or Sardines in oil
200ml of Supermilk
Cheese

Low 40-100
*Other Fortified Foods- 125g Yogurts, Margarine , 35g Ready to Eat Cereals
1 Egg including the yolk

Source of data: Food Safety Authority of Ireland, 2015. IU: International units.
Irish Osteoporosis Society recommends 800-1000IU per day, for men and women over the age of 50 years. *Not all milk, yogurts and ready to eat cereals are fortified with vitamin D. Read the label.

Who is most at risk?

A 70year old has a 70% reduced ability to produce vitamin D via sunlight compared to a 20 year old due. A number of risk factors contribute to an increased frequency of vitamin D deficiency in older adults: medication use, skin ageing, changing lifestyle factors such as reduced outdoor activity or clothing. Obesity also increases your requirement 2-3 times. And people with darker skin need significantly more sunlight then Caucasians. So the older we the more likely we will need vitamin D supplementation.

Do I have enough?

Vitamin D status is determined by a simple blood test. You ideally want to aim for levels above 50nmol/ml. Researchers believe the optimal range is likely between 60-80nmol/ml. To achieve optimal levels current clinical guidelines form the Endocrine Society state that repletion of low levels of vitamin D can be achieved in the form of supplementation up to 6000IU per day followed by a maintenance regime of 4000IU. Whilst vitamin D recommendations are under review for older adults in Ireland, Canada is of similar latitude therefore it is useful to recognise their guidelines of 1500-2000IU/day for optimal health.

Author: Ms. Niamh Aspell, BSc. Human Nutrition. Irish Research Council funded PhD Candidate. D-Activating Decline Study. Email: niamha@tcd.ie. November 2015. References on request.

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

ST. JAMES'S HOSPITAL
James's. St. Dublin 8. Ireland.

IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn

Appendix 13

D-Activating Decline
Participant Phone Screen

Questionnaires

1. IPAQ (Short Form)
2. Health Screen Questionnaire
3. TCogs (MMSE)
4. Medication Checklist

Participant ID: _____

Assessed by: _____

Date: _____

DA_D ID: _____

Hello, my name is _____. I am a Research Assistant from the D-Activating Decline project at the Trinity Centre for Health Sciences in St James' Hospital, Co. Dublin. I am calling you to thank you for returning the signed consent for our study 'D-Activating Decline: Exploring the effects of vitamin D3 supplementation on cognitive function in physically active healthy older adults', and to ask you some questions about your health? It will take a few minutes is now a good time?

[***If not a good time*:** Could I arrange a time that would suit you to call back? (*Arrange suitable time*) Ok, I will speak to you then. Thank you for taking the time to talk to me.

***IF YES*:** Is there anything that you would like to ask me about the study before we begin?

Are you happy for me to ask you some questions, about your physical abilities?

If it's ok with you I'd like to ask you a few further questions to make sure that you are eligible for involvement in our research.

Background Information	
Age and Date of birth of participant	
Are you currently taking a vitamin D supplement? If yes, could you tell me what dose you are currently taking? (List of medications)	
Have you been experiencing significant memory problems, or have you been told that you have a dementia?	

DA_D ID: _____

Short Last 7 Days Telephone IPAQ

Short Last 7 Days Telephone IPAQ (Craig et al., 2003)
Researcher: I am going to ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.
Now, think about all the vigorous activities which take hard physical effort that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time. **1. During the last 7 days, on how many days did you do vigorous physical activities?**
Days per week _____ Don't Know/Not Sure Refused
[Interviewer clarification: Think only about those physical activities that you do for at least 10 minutes at a time.] [Interviewer note: If respondent answers zero, refuses or does not know, skip to Question 3]
2. How much time did you usually spend doing vigorous physical activities on one of those days?
Hours per day ____ Minutes per day ____ Don't Know/Not Sure Refused
[Interviewer clarification: Think only about those physical activities you do for at least 10 minutes at a time.] [Interviewer probe: An average time for one of the days on which you do vigorous activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: **"How much time in total would you spend over the last 7 days doing vigorous physical activities?"**
Hours per week _____ Minutes per week _____ Don't Know/Not Sure Refused
Now think about activities which take moderate physical effort that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may

include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities?

Days per week_____
Don't Know/Not Sure
Refused

[Interviewer clarification: Think only about those physical activities that you do for at least 10 minutes at a time] [Interviewer Note: If respondent answers zero, ***refuses or does not know, skip to Question 5****]*

4. How much time did you usually spend doing moderate physical activities on one of those days?

Hours per day _____
Minutes per day _____
Don't Know/Not Sure
Refused

[Interviewer clarification: Think only about those physical activities that you do for at least 10 minutes at a time.] [Interviewer probe: An average time for one of the days on which you do moderate activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: ***"What is the total amount of time you spent over the last 7 days doing moderate physical activities?"***

Hours per week _____
Minutes per week _____
Don't Know/Not Sure
Refused

Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

Days per week _____
Don't Know/Not Sure
Refused

[Interviewer clarification: Think only about the walking that you do for at least 10 minutes at a time.] [Interviewer Note: If respondent answers zero, ***refuses or does not know, skip to Question 7]***

6. How much time did you usually spend walking on one of those days?

Hours per day _____
Minutes per day _____

Don't Know/Not Sure Refused
[Interviewer probe: An average time for one of the days on which you walk is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: **"What is the total amount of time you spent walking over the last 7 days?"**
Hours per week _____ Minutes per week _____ Don't Know/Not Sure Refused
Now think about the time you spent sitting on week days during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. **7. During the last 7 days, how much time did you usually spend sitting on a week day?**
Hours per weekday_____ Minutes per weekday _____ Don't Know/Not Sure Refused
[Interviewer clarification: Include time spent lying down (awake) as well as sitting] [Interviewer probe: An average time per day spent sitting is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: **"What is the total amount of time you spent sitting last Wednesday?"**
Hours on Wednesday_____ Minutes on Wednesday _____ Don't Know/Not Sure Refused

My next set of questions ask for a few background things about you, and also some questions about your health, if that's ok. This is to make sure that you are eligible for involvement in our research.

Christensen et al (1992) Health Screening Questionnaire	
Question	**Response (circle)**

1. Have you ever had a stroke or T.I.A.? (Probe: T.I.A. Stands for transient ischemic attack more commonly referred to as a mini stroke)	Yes/No
2. Do you have difficulty understanding conversations because of your hearing even if you wear a hearing aid?	**Yes/No**
3. a) Have you ever had a problem due to abuse of drugs or medications? **If yes;** **b) Was this within the past 5 years?**	Yes/No Yes/No
4. Have you been hospitalised for mental or emotional problems in the past 5 years?	Yes/No
5. Have you ever been treated for alcohol or drug abuse?	Yes/No
6. Have you ever had seizures?	**Yes/No**
7. Have you ever had any illness that caused a permanent decrease in memory or other mental functions?	**Yes/No**
8. Are you receiving kidney dialysis?	**Yes/No**
9. Do you have a liver disease?	**Yes/No**
10. Do you have any blood-borne infectious diseases (e.g. HIV/AIDS, Hepatitis A, Hepatitis B?)	**Yes/No**
11. Do you have any contact-based infectious diseases (e.g. herpes, MRSA, scabies, rubella, mumps, or ringworm?)	**Yes/No**
12. Do you currently have any infectious airborne diseases (e.g. flu, tuberculosis, chicken pox, or measles?)	**Yes/No**

Self-reported Health Screening Questionnaire	
13. Do you currently have any swallowing difficulties? (Would you be able to administer a tablet similar to that of a vitamin tablet, without any difficulty?)	**Yes/No**
14. Have you been diagnosed or received treatment for hyperparathyroidism (otherwise known and an overactive thyroid- medication, radioactive treatment)?	**Yes/No**

DA_D ID: _____

15. Have you ever had an adverse reaction to vitamin or multivitamin use?	**Yes/No**
16. Has your GP ever told you that you have high levels of calcium in the blood or are you presently taking calcium tablets?	**Yes/No**

Telephone ALFI-MMSE (Newkirk et al., 2004)

Next, I am going to ask you to answer a series of questions that measure your memory, attention and orientation. These questions are designed to test people of all different abilities, they are designed to be challenging so don't worry if you have any difficulty.

Award 1 point for each correct response.

ORIENTATION:

Keypunch # Wrong Right

(107)	0	1
(108)	0	1

25. What is the **year**? _____

26. What **season** of the year is it? _____

(During March, winter or spring is acceptable; during June, spring or summer is acceptable; during September, summer, fall, or autumn is acceptable; during December, fall, autumn, or winter is acceptable).

(109)	0	1
(110)	0	1
(111)	0	1
(112)	0	1
(113)	0	1
(114)	0	1
(115)	0	1

27. What is the **date** or **day** of the month? *(+/- 1 date is acceptable)*_____

28. What is the **day** of the week?_____

29. What is the **month**?_____

30. Can you tell me where you are right now? For instance, what **state** are you in?___

31. What **county** are you in?_____ **32.** What **city/town** are you in? _____ **33.** IF IN A PRIVATE HOUSEHOLD, ASK:

What is the **address** you are at?_____

Correct street name and house number must be given.

Zip code is not necessary for a correct response.

OR

IF INSTITUTIONALIZED, ASK:

DA_D ID: _____

What is the name of the place where you are staying?_____ *Name of institution must be given to receive credit.*

(116)	0	1

34. What is your **telephone number** (there/at home or where you usually can be reached)?_____

REGISTRATION:

35. I am going to name three objects. After I have said them, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.

The three objects are: **"Apple", "Table",** and **"Penny"**.

Could you repeat the three objects for me?

The words should be read at a rate of 1 per second, speaking clearly and audibly. You are allowed to read the words only once before scoring.

Score on first trial

(117)	0	1
(118)	0	1
(119)	0	1

Apple _____
Table _____
Penny _____

Repeat the three words until: 1) the subject correctly repeats all three

or

2) 3 total trials have been presented (including initial presentation).

Number of trials:_____

ATTENTION:

36. Now, I am going to give you a word and ask you to spell it forwards and backwards. The word is **WORLD.** Spell **WORLD** forwards.

(If the subject is unable to spell the word, spell it out loud, and ask the subject to repeat the spelling. Continue until it has been spelled successfully or until you have spelled it to the subject three times.

Now spell the word **WORLD** backwards: ___ ___ ___ ___ ___

D L R O W

(120)	0	1	2	3	4	5

Score 5 points for a correct sequence. Count 1 error for each omission, letter transposition (switching adjacent letters), insertion (inserting a new letter), or misplacement (moving W,O,R,L,D *by more than one space).*

RECALL:

37. Now, what were the 3 objects I asked you to remember?

(This should be administered as soon as the "world backwards" item is completed. Cueing is allowed if the subject is not able to recall words, but credit is not given for any word recalled after a cue).

(121)	0	1
(122)	0	1
(123)	0	1

Apple_____
Table_____
Penny_____

LANGUAGE:

38. I would like you to repeat a phrase after me exactly as I say it. The phrase is:

(124)	0	1

"No ifs, ands, or buts".

It is very important to speak loudly and enunciate clearly as you read this phrase. One repetition of the phrase is permissible if it is clear that the phrase was not adequately heard. Otherwise, repetition is not allowed.

39. Tell me, what is the thing called that you are speaking into as you talk to me?

(125)	0	1

Correct: Telephone, Receiver, Mouthpiece, etc…

40. Now I'd like you to do these three things.

Say hello, tap the mouthpiece of the phone 3 times, then say I'm back.

(126)	0	1
(127)	0	1
(128)	0	1

Hello_____

Tap 3 times_____

I'm back_____

Total Score (26 Max):_____
(Entering the sum is optional)

PIP (predicted in person) Score: _____

DA_D ID: _____

Medication Inventory

Lastly, do you mind if I ask you some questions about your medications if you take any?

Name of Medication	Dose (mg)	How often?

***IF YES*: Would you be able to get those medications, and read out the names of them to me? If it does not suit you to do this now, I can call back at another time, and you could have the medications ready by the phone.** [**If they want to call back, arrange time: Ok, I will speak to you then. Remember to have your medications by the phone, so you can read the names of them out to me. Thank you for taking the time to talk to me.**]

Could you please state the medication name, dosage and how often you take it?

Thank you for answering those questions.

If screen is ok- Could we arrange a date and time for you to visit our centre to take a blood sample? *(arrange appointment).*

If screen is not ok-

We believe that potential participants with vitamin D levels >125nmol, which is classified as vitamin D sufficiency, are unlikely to benefit from involvement in our study, as your levels would be deemed as healthy, it would be unlikely you would receive any additional benefit from taking part. If your levels are <125nmol/L your name will enter a draw with the rest of our participants, and half of all our

participants will go on to receive our vitamin D intervention and half will receive a "dummy" tablet. The research team are blind to this, this means we also don't know which tablet you are receiving throughout your involvement, this is so we can complete your assessments in an unbiased manner, ensuring are results will not be . (answer any questions)

Our researchers will invite you to visit us at 3 points after we have received your blood sample results. We will give you three months supply at the first assessment, and three at the second, which should last for your 6 month involvement if you administer 1 tablet per day. We will arrange 2 more visits, at 3 month and at 6 months. We will remain in touch throughout the process to arrange these assessments so don't worry about remembering these details, we will be in touch throughout and available on phone and email if you have any queries or need to re schedule appointments.

I will send you out a letter today to confirm your first appointment, this will include a map of our location. I will meet you at reception prior to your assessment and show you to the testing room.

So we will see you on [date] at [time]. I will send you out the letter today. Thanks again for taking the call and for participating in our research.

Do you understand that there may be a chance that you won't be included in this project? is this ok? (answer any questions)

The next thing to do is that we will post you a background questionnaire to fill out. We will collect this from you when we visit. Feel free to contact us in the meantime if you have any issues completing this questionnaire.

(answer any questions)

Thanks again for taking the call and for participating in our research.

DA_D ID: _____

Appendix 14

Background Questionnaire

D-Activating Decline: Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling healthy older adults.

Participant ID code: Date:

This questionnaire is designed to provide us with some information about aspects of your life that may be related your health in later life. We greatly value your participation in our research, and we hope that you will find this questionnaire interesting to complete. Your answers are extremely important to us. Please answer all questions honestly. All of your responses will be treated in the strictest confidence.

If you would prefer not to answer any question, please skip it and move on to the next item. If you are unsure of how to complete any item, please leave it blank and we can help you to complete it when we visit.

Please complete this questionnaire at home, and we will at your next visit.

1. Are you male or female?	Male Female
2. What is your date of birth?	
3. What is your age?	
4. Are you….	Married? Living with a partner as if married? Single (never married)? Separated? Divorced? Widowed?
5. What is the highest level of	Some primary (not completed)

education you have completed?	Primary or equivalent Intermediate/junior/group certificate or equivalent Leaving certificate or equivalent Diploma/certificate Primary degree Postgraduate/higher degree None
6. In total, how many years of education have you completed?	

We would like to collect some information about the types of occupations you have had during your life. Five categories of occupation are listed below. For each job you have had during your life, please choose the category of occupation it fits into, and indicate the number of years you spent working in this category. For example, someone who spent 10 years working as an electrician, and 5 years working as a driver, both of which fall under the first category, 'low skilled manual work', would put 15 in the box beside this category. Alternatively, someone who spent 5 years working as a call centre operator (which falls under the first category ' low skilled manual work', and 10 years working as a real estate agent (which falls under the second category skilled non manual work), would put '5' in the box beside category 1, and '10' in the box beside category 3.

Please indicate how many years you have spent working in each of the categories below during your lifetime.	**Years:**
1. Low skilled manual work (e.g. agricultural worker, gardener, housekeeping (hotel), waiter, driver, mechanic, plumber, call centre operator, electrician, etc.)	
2. Skilled manual work (e.g. craftsman, clerk, cook, shop assistant, tailor, nurse, professional soldier, barber/hairdresser etc.)	
3. Skilled non-manual work (e.g. shopkeeper, white-collar worker,	

priest, monk or nun, sales representative, real estate agent, nursery school teacher, musician, etc.)	
4. Professional occupation (e.g. CEO of small company, qualified freelancer, teacher, contractor, lawyer, engineer, etc.)	
5. Highly responsible or intellectual occupation (e.g. CEO of large company, judge, university professor, top manager, politician, surgeon, etc.)	

Health

Would you say your health is;	a) Excellent b) Very good c) Good d) Fair e) Poor
In general, compared to other people your age, would you say that your health is;	a) Excellent b) Very good c) Good d) Fair e) Poor
Would you say that your emotional or mental health is;	a) Excellent b) Very good c) Good d) Fair e) Poor
In the past year, have you lost 10 pounds (4.5kg) or more in weight when you weren't trying to, for example, because of an illness?	a) Yes b) No
How would you rate your day-to-day memory at the present time? Would you say it is…	a) Excellent b) Very good c) Good d) Fair e) Poor

Appendix 15

Study ID: _____ Date: _____ Assessment No: _____ Assessor: _____

Diet and Lifestyle

Section 1: Food Frequency

1. **Do you consume fortified foods? (Refer to aide memoire)**

   Yes ☐ No ☐

If YES, please specify- times per day/week/month and name of product:

Fortified Breakfast cereals: _____

Fortified Breakfast bars: _____

Fortifies Bread: _____

Fortified Spreads: _____

Fortified Drinks:_____

Other: _____

2. **Do you eat any of the following foods?**

If YES, please specify- times per day/week/month:

Meat (beef, liver): **Yes ☐ No ☐**

Poultry: **Yes ☐ No ☐**

Oily Fish (Salmon, Mackerel, Tuna): **Yes ☐ No ☐**

Eggs (including yolk): Yes ☐ No ☐

Cheese: Yes ☐ No ☐

Appendix 15

Section 2: Sun Exposure

1. **During the sunny months, if outdoors, do you stay in the sun or sit in the shade?**

- Avoid direct sunshine ☐
- Sometimes stay in sunshine ☐
- I enjoy staying in the sunshine ☐

2. **During the sunny months would you apply sun protection factor?**

Never ☐ Rarely ☐ Sometimes ☐ Usually ☐ Always ☐
Other_____

What sun protection factor do you usually apply? _____

3. **Have you been on a sun holiday in the past 6 months?**

Yes ☐ No ☐

If YES,

Total number of days_____

During which month _____

Where to? _____

4. **Apart from the last 6 months, do you generally tend to go on sun holidays?**

Yes ☐ No ☐

If YES, how often? _____

5. **Do you use a sunbed regularly?**

Yes ☐ No ☐

If YES, how often? _____

6. **Ethnic Background** _____

Section 3: Alcohol Intake

1. **Do you think alcohol?**

☐ Yes, currently

Appendix 15

☐ No, but I have in the past

☐ No, never

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

3. **How many units of alcohol do you consume per week?** (1 unit= ½ pint of beer, 1 glass of wine, 1 measure of spirits)

Beer _____ Wine _____ Spirits _____

Section 4: Smoking

1. **What is your smoking status?**

Current (past month) ☐

Past ☐

Never ☐

2. **Have you ever smoked cigarettes regularly (at least 1/day) for a period longer than 6 months?**

Yes ☐

No ☐

If YES, at what age did you start? _____

3. **Are you still smoking?**

If YES: How many cigarettes do you smoke per day? _____

If No: What age did you stop? _____
How many cigarette did you smoke on average per day? _____

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Department of Clinical Medicine; Human Nutrition and Dietetics.

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Appendix 16

D-Activating Decline: Exploring the effects of vitamin D3 supplementation on cognitive function in community dwelling older adults- A Pilot Study.

Dear _____,

THANK YOU FOR TAKING PART!!

From here, we will now be able to explore and evaluate the information collected. This will allow us to investigate how vitamin D levels are important for our health as we age and how we can use supplements to reverse low vitamin D levels, which as a result may support healthy ageing.

Without research participants like you, it would not be possible for us to do this work, so we really appreciate you taking the time to get involved in our research. We have begun analysing our data and will share our findings with you when we have completed.

We hope that you enjoyed participating in this study, and found it to be a positive experience. However, if you found any aspect of the experience to be distressing, or have any questions about your participation, please feel free to discuss this with a member of our team. Their contact details are provided below.

Thank you again for being a part of our research.

Yours sincerely,

_____

Ms Niamh Aspell, Ph.D. Candidate
(083) 1729337

Prof Maria O'Sullivan Email: MOSULLI5@tcd.ie Phone: (01) 8964039

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Department of Clinical Medicine; Human Nutrition and Dietetics.

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Participant Feedback

For the attention of participants of the D-Activating Decline Study

You have received this short questionnaire because you have been a research participant in our pilot study and indicated that you would be happy to provide feedback. To do this we ask if you could provide us with some more information about your experience on the project, your role as a research participant and any recommendations you may have if more studies, like this one, were be completed.

What happens to my data?
All of the information that you provide will be treated in the strictest confidence. You are free to decline to provide some or all of the information requested. The information we collect during this study will never be made available to anyone other than members of the research team or their assistants.

If you are happy to complete this questionnaire we would ask that you tick the below "opt in" box to indicate your consent in taking part.

Opt in ☐

We will ask you not to write your name or other identifiable information on this questionnaire to ensure all the questionnaires remain anonymous.

If you require any further information, please contact:

Niamh Aspell
niamha@tcd.ie
(083) 1729337

Prof Maria O'Sullivan
mosulli5@tcd.ie
(01) 8964039

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1. What motivated you to take part in this project?

2. Did any aspect of the study concern you, prior to starting or during your time on the project?

3. During your visits with the researcher are there any aspects of the assessment you would have changed?

Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8. Ms Niamh Aspell, IRC Ph.D. Candidate,
Email: niamha@tcd.ie, Tel: 0831729337

4. What factors would encourage **and** discourage you from taking part in a similar project in the future?

Further comments:

5. Have you volunteered as a research participant prior to taking part in this study?

Yes ☐ No ☐

6. On a scale of 1-10, how would you rate your overall experience as a research participant (1=very low- 10=very high)? _____

7. On a scale of 1-10, how likely is it that you would recommend volunteering to a family member or friend? (1=very low - 10=very high)? _____

Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8. Ms Niamh Aspell, IRC Ph.D. Candidate,
Email: niamha@tcd.ie, Tel: 0831729337

8. How would you evaluate the study procedures in regards to;

	Excellent	V. good	Good	Fair	Poor
Easy to understand study information					
Time taken to complete each visit					
Number of visits					
Number of questionnaires					
Number of blood samples taken					
Number of memory assessments conducted					

Thank you for taking the time to complete this questionnaire, we appreciate your time and honesty in answering the questions. When convenient to you, please post the questionnaire back in the enclosed stamped addressed envelope.

Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8. Ms Niamh Aspell, IRC Ph.D. Candidate,
Email: niamha@tcd.ie, Tel: 0831729337

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Department of Clinical Medicine; Human Nutrition and Dietetics.

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Date:

Appendix 17

Dear_____ ,

Re: Vitamin D Supplementation and Cognition in Healthy Community Dwelling Older Adults.

Following previous correspondence I write to inform you that _____ has completed participation on the vitamin D supplementation study, in which they were allocated _____ for 6 months.

We have received the laboratory report which shows the final vitamin D levels were _____, a change from their initial assessment in Spring of 2016 _____. According to the guidelines in our laboratory serum vitamin D levels <50nmol/mL are deemed deficient.

I have spoken with the participant and suggested, in line with current recommendations, that it would be beneficial to take vitamin D supplements (400-800IU per day), during the winter months. They have given permission for the results to be sent to you for follow up, if required.

Any further questions please feel free to contact me.

Yours Sincerely,

_____

Ms. Niamh Aspell (Study Coordinator)
Study mobile: 083 172 9337

Research Team: Associate Professor Maria O'Sullivan BSc. PhD, INDI
Professor Brian Lawlor MD, FRCPI, FRCPsych, DABPN.
Ms. Niamh Aspell (PhD Candidate/Study Coordinator) BSc.

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Poster 1

Prevalence and Determinants of Vitamin D Deficiency in Older Adults Residing at Northerly Latitudes: a Population Based Study

Aspell N[1], Healy M[2], Lawlor BA [3]O'Sullivan M[1],
Department of Clinical Medicine[1], Trinity Centre for Health Science, St. James's Hospital (SJH), Dublin, Department of Biochemistry, SJH, Dublin 8[2], Trinity Institute of Neuroscience, The University of Dublin, Trinity College, Dublin[3].

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

ELSA English Longitudinal Study of Ageing

Background and Aims

Vitamin D deficiency is associated with adverse outcomes in older adults living in Northern Europe(1). Circulating 25-hydroxyvitamin D (25(OH) D) status predominately relies on UV exposure(2), however the extent of which northerly latitude exasperates deficiency is less explored.
The specific aims of this study were:

I. to establish the prevalence of serum 25(OH) D deficiency in older adults, overall and according to gender, vitamin D supplement users, and by season
II. to determine the prevalence of serum 25(OH)D deficiency by region of residence
III. to investigate the determinants of vitamin D deficiency, with a particular interest in the contribution of latitude.

Methods

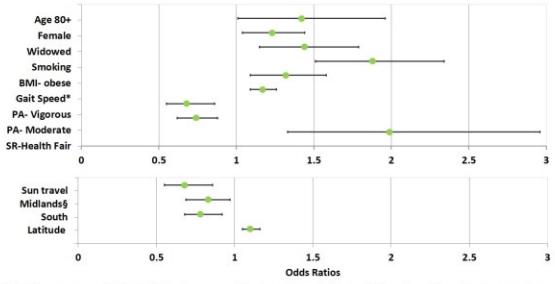
This study is based on a sample of 6,004 adults, aged >50years, with measured serum 25(OH)D, from Wave 6 of the **English Longitudinal Study of Ageing** (ELSA). Serum 25(OH)D levels were measured between (January 2012- July 2013) and analysed by Diasorin Liaison immunoassay to quantify total circulating 25(OH)D level. Deficiency was defined according to the Institute of Medicine's guideline for serum 25OHD levels (<30nmol/L; deficiency) and also the widely used cut off of <50nmol/L. Data for latitude was established using Government Office Region (GOR) data. Multivariate logistic regression models were applied to potential predictors of 25(OH)D status. All analysis were performed using STATA V14.

Table1. Prevalence of vitamin D deficiency, stratified by age and gender (n=6004)

Severe deficiency <30nmol/l (n=1423)				
Age category	50-59 (n=469)	60-69 (n=492)	70-79 (n=325)	80+ (n=137)
Overall	27.4*	20.4***	22.2***	32
Males	28.1	19.3	17.3	26.3
Female	26.9	21.5	26.1	36.8
†Winter	32.9	24.3	27.8	35.6
Deficiency <50nmol/l (n=3317)				
Age category	50-59 (n=1007)	60-69 (n=1251)	70-79 (n=785)	80+ (n=273)
Overall	58.9	52.1***	53.6***	63.8
Males	59.0	51.8	50.1	58.2
Female	58.9	52.3	56.4	68.4
†Winter	66.7	58.7	60.3	69.1
Vitamin D deficiency, stratified by age and D supplement users‡ (n=262)				
N (%)	50-59 (n=33)	60-69 (n=105)	70-79 (n=90)	80+ (n=34)
<30nmol/L (n=27)	9 (27.3)	8 (7.6)	8 (8.9)	2 (5.9)
<50nmol/L (n=72)	15 (45.4)	25 (23.8)	19 (21.1)	13 (38.2)

†Winter defined by extended vitamin D calendar October to April. ‡Reported taking prescribed Vitamin D supplement for bone treatment

Figure 2. Weighted, adjusted odds ratios with (95 % CI) for severe vitD deficiency (<30nmol/L) and vitamin D deficiency (<50nmol/L) §



Multivariate logistic regression weighted with blood measure derived sampling weight, adjusted for other factors including socio-demographic and lifestyle factors; PA: physical activity, no of times reported in a week, vitamin D factors (sun holiday travel in the last 12 months, supplement use, season). Gait speed measured by 8 metre walking speed (seconds) for which data was collected for adults over 60years old. Predictors significant at p<0.01.

Table 2. 25(OH)D status by region, Prevalence (%) severe vitamin D deficiency and vitamin D deficiency, stratified by season and region.

					<30nmol/L		<50nmol/L	
Region England	Overall (n=6004)	°N Latitude	Mean 25OHD	SD	Winter N=1009 (28·3%)	Summer N= 414 (16·9%)	Winter N=2210 (62.0%)	Summer N= 1106 (45.2%)
North East	375	54·9	46·4	23·3	36·4	20·6	70.3	52.2
Yorkshire and Humber	656	53·7	47·9	22·0	28·9	14·8	60.3	48.7
North West	661	53·6	47·5	23·9	29·4	20·3	66.4	51.3
East Midlands	658	52·8	48·1	23·0	30·5	17·6	62.0	44.7
West Midlands	655	52·5	45·4	24·9	24·5	21·3	68.1	48.9
East England	723	52·2	50·6	23·7	31·3	16·4	56.5	45.6
London	483	51·5	45·6	23·7	31·3	26·4	65.9	54.6
South East	1047	51·1	52·3	23·5	21·2	13·8	56.0	39.7
South West	746	50·4	50·6	22·1	26·6	10·3	60.7	34.9

25OHD, nmol/L. Season defined by vitamin D calendar; summer April-October.

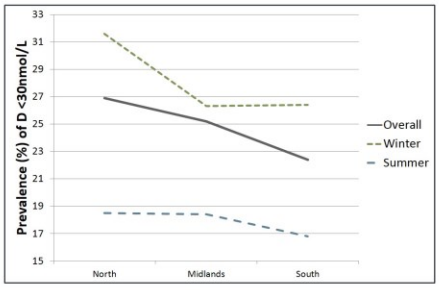


Figure 1. Prevalence of vitD deficiency (<30nmol/L), stratified by region and season

Results

Mean age was 66.4 ± 8.8years and 55% were female. Only 4·4% of the study population reported taking a prescribed vitamin D supplement (osteoporosis treatment). The overall health profile was improved in the South compared to the North and Midlands. No statistically significant differences were seen across regions for season of blood draw, sun holiday travel or vitamin D supplement use. **Table 1** & **Table 2**, show the prevalence of 25(OH)D deficiency by age, gender, supplement users, by GOR and season. **Figure 1** shows the seasonal prevalence of severe vitamin D deficiency by region of England.

Multivariate logistic regression showed that women; aged 80+; widowed; with obese BMI and with fair self-reported health, were more likely to be severely vitD deficient **(Figure 2).** North-south variation was evident, those residing at latitudes in the South (50.4-52.1°N) were less likely to be deficient than those in Northerly regions (54.9°N).

Conclusion

- More than half of community dwelling older adults had 25(OH)D concentrations <50nmol/l, with 1 in 4 categorised as severely deficient.
- Small northerly increments in latitude appear to be an important contributor to vitD status within countries of northerly latitude such as Ireland and England, after adjustment for socioeconomic and vitamin D factors.
- VitD awareness, supplementation and food fortification could be useful public health strategies for improving vitD status in mid and later-life.

References:
1. Lips P. Vitamin D status and Nutrition in Europe and Asia J Steroid Biochem Mol Biol. 2007;103:620-625.(2007).
2. Theodoratou E *et* al. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials BMJ. 2014;348: 2035.
3. Lips P, van Schoor and de Jonagh RT. Diet, sun and lifestyle as determinants of vitamin D status. Ann AY Acad Sci. 2014;1317:92-98.

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Poster 2

Does Vitamin D Supplementation Improve Cognitive Performance? A Randomised Double-Blind Placebo-Controlled Pilot Trial in Healthy Older Adults

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Aspell N[1], Healy M[2], Lawlor B[3], O'Sullivan M[1]
[1]Department of Clinical Medicine, Department of Biochemistry[2], Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland and [3]Institute of Neuroscience, Trinity College, Dublin 2, Republic of Ireland.

Clinical trial identifier: NCT02804841[4]

Background and Aim

Low vitamin D (vitD) status has been associated with impaired cognitive function and incidence of Alzheimer's disease and dementia[1-2]. However, the effects of vitD supplementation on cognitive performance in healthy older adults- free of cognitive impairment, has yet to be tested in a RCT.

Primary aim: to test the effect of vitamin D3 supplementation on cognitive performance in healthy older adults.

Methods

Participant inclusion criteria (n=60)

- Age ≥60 years
- Free of cognitive impairment (TCog >24, Health Screen for cognitive studies[3])
- Community dwelling
- Measured serum vitD >15nmol/L and <125nmol/l

Study design

- Minimisation randomisation 1:1 (Age, Gender & Physical Activity Level- International Physical Activity Questionnaire)
- Double-blind
- Vitamin D3 (50ug/d) or Placebo
- Duration: 6 months
- Statistics: predefined Intention to treat analysis (ITT)
- All analysis were conducted using STATA V14.

Study Assessments: 0, 3 and 6months **Duration:** 60 minutes

Primary outcome:
Global and domain specific Cognitive performance

Global Function	• **Montreal Cognitive Assessment**
Executive Function	• **Trails B, Trails B-A,** Letter Number Sequencing
Attention	• **Sustained Attention to Response *fixed* Task, Trails A**

Other related factors:

Detailed information was gathered at each assessment for related health behaviours, mental health and wellbeing, sun exposure habits, vitamin D behaviours, and physical function.

Results

Baseline

Mean age was 68.5years SD 4.9 and 53.3% were female. In total, 18.3% were deemed deficient at baseline according to Institute of Medicine guidelines[5] .

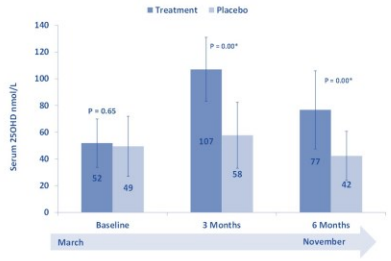
There were no significant baseline differences between groups for socio-demographic, health and lifestyle factors, vitamin D and sun exposure or mental wellbeing.

Post Intervention

At 6 mos. 25(OH) D concentrations were significantly higher in those whom in the vitD3 group (51.8 to 76.7nmol/L) compared to the placebo group (49.4 to 42.3) **(Figure 1)**.

VitD supplementation did not show effect on global cognitive or domain specific tasks of executive function and attention **(Table 1)**.

Figure 1. Serum 25 (OH)D response to vitamin D3 treatment



Compliance rate of 99.0%

Retention rate 98.3%

No adverse events reported

Table 1. Change in cognitive performance scores in global and domain specific tasks at 6 months by study group

		Placebo (n=30)		Vitamin D3 (n=30)				Treatment effect	
		Post intervention 6mo	Δ	6mo	Δ	Δp	ITT d	ITT model 1	ITT model 2
Global	MoCA (0-30)	27.7 ± 1.9	0.9	27.6 ± 1.6	0.17	0.19	0.04	0.88	0.49
Executive Function	TMT A (sec)	28.7 ± 9.9	-0.51	29.5 ± 7.6	1.12	0.34	0.09	0.73	0.23
	TMT B (sec)	62.4 ± 25.9	-21.3	58.1 ± 22.0	-14.3	0.38	0.18	0.44	0.93
	TMT B-A	33.7 ± 20.7	-19.8	28.6 ± 17.3	-15.4	0.52	0.26	0.23	0.63
Attention	RT (sec)	316.7 ± 82.1	-15.4	310.4 ± 64.1	8.2	0.18	0.09	0.92	0.71
	SD(RT)	88.2 ± 79.3	-7.9	79.3 ± 69.8	2.6	0.56	0.12	0.52	0.6
	CE	3.3 ± 2.9	-1.1	3.6 ± 4.7	-0.1	0.32	0.07	0.36	0.74
	OE	9.9 ± 15.2	-0.9	7.8 ± 11.3	1.4	0.38	0.19	0.32	0.42

Mean ± SD. Δ delta change from baseline, d; Cohen d for effect size ITT model 1. Intent to treat; ITT model 1 represents independent sample t-test results for differences between treatment groups at 6 months. ITT model 2 included covariates for demographic characteristics, sun exposure habits, depression, anxiety, sleep quality, current sleepiness, quality of life, and stress

Conclusion

While this pilot study demonstrated that short term vitD3 intervention improved vitD status and that embedding a core cognitive outcome set, within the vitD RCT was feasible and acceptable, no short term changes in cognitive outcome measures were detected.

References

1. Van der Schaft J, Koek HL, Dijkstra E, *et al.* (2013) *J Ageing res rev* **12**, 1013-23.
2. Aspell N, Lawlor B, O'Sullivan M. (2017) *Psychology, Health and Medicine Conference, RSCI*, Dublin 2.
3. Christensen (1992). *Psych&Ag*.
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5. Institute of Medicine (2010) National Academy Press.

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BEST FORMULATIONS

Poster 3

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Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

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at St. James's Hospital

IRISH RESEARCH COUNCIL
An Chomhairle um Thaighde in Éirinn

A Randomised Controlled Trial of the Effects of Vitamin D on Muscle Strength and Performance in Healthy Community Dwelling Older Adults

Aspell N[1], Healy M[2], Lawlor B[3], O'Sullivan M[1]
[1]Department of Clinical Medicine, Department of Biochemistry[2], Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland and [3]Institute of Neuroscience, Trinity College, Dublin 2, Republic of Ireland.

Background and Aim

Low vitamin D (vitD) status has been associated with impaired muscle strength and physical performance in older adults. Evidence indicates that poor skeletal muscle function has negative impacts in healthy ageing, including risk of falls.

The aim was to test the effect of vitamin D3 supplementation on muscle strength and performance in community dwelling older adults.

Methods

Participant inclusion criteria (n=60)

- Age ≥60 years
- Community dwelling
- Measured serum vitD >15nmol/L and <125nmol/l

Study design

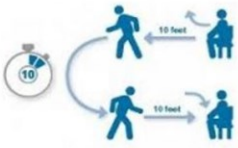
- Minimisation randomisation 1:1 (Age, Gender & Physical Activity Level- International Physical Activity Questionnaire)
- Double-blind
- Vitamin D3 (50ug/d) or Placebo
- Duration: 6 months
- Statistics: predefined Intention to treat analysis (ITT)
- All analysis were conducted using STATA V14.

Primary outcome:

Muscle Strength: Hand Grip Strength (kg)

Muscle performance: Timed Up and Go walking Speed (sec)

Muscle function: (HGS: gender/BMI cut-offs) **(Table 1)** and impaired TUG; (test-units >13.5sec) combined.



Gender and BMI- specific cut off for low muscle function				
	Criteria 1	Criteria 2	Criteria 3	Criteria 4
Males				
BMI (kg/m²)	≤24.04	24.05-26.04	26.05-28.04	>28
HGS (kg)	≤29	≤30	≤30	≤32
Females				
BMI (kg/m²)	≤23.04	23.05-26.04	26.05-29.04	>29
HGS (kg)	≤17	≤17.3	≤18	≤21

Results

Baseline

Mean age was 68.5±4.9years and 53.3% were female. In total, 18.3% were deemed deficient at baseline(4) . There were no significant baseline differences between groups for important factors. Prevalence of reduced muscle strength, performance and function were 36.7%, 20% and 6.7%, respectively.

Post Intervention

At 6 mos. 25(OH) D concentrations were significantly higher in those whom in the vitD3 group (51.8 to 76.7nmol/L) compared to the placebo group (49.4 to 42.3) **(Figure 1)**. Compliance rate of 99.0%. Retention rate of 98.3%. No adverse events.

Vitamin D showed no effect for muscle performance (TUG), however, a statistically significant change from baseline was observed between groups (**Table 2**).

No effect on muscle strength of function was observed at 6months **(Table 1)**.

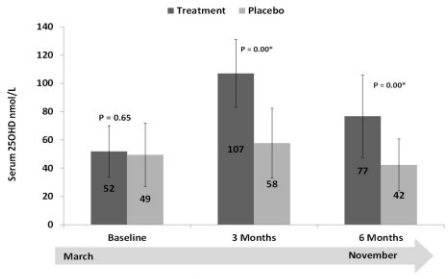


Figure 1. Serum 25 (OH)D response to vitamin D3 treatment

Table 2. Mean change in muscle function, performance and impaired physical performance between groups at 6 months

	Baseline (n=60)				Post Intervention 6 months (n=60)			
	Vit3(n=30)	SD	PL(n=30)	SD	VitD3 (n=30)	SD	PL (n=30)	SD
HGS- mean (kg)	26.1	9.2	27.4	11.8	-0.13	2.8	-0.84	4.4
TUG- mean (sec)	11.1	3.4	10.8	2.9	-0.44	1.7	0.53*	1.7
Impaired **Muscle Perf.** (%)	26.7	-	13.6	-	-16.7 (p=0.08)	-	+6.7 (p=0.08)	-

HGS; Hand grip strength mean of 2 readings using dominant hand, Timed Up and Go; TUG measured in seconds, higher scores indicated poorer performance, Impaired muscle performance; % defined as impaired if TUG score was >13.5secs and the proportion change at 6 months, from normal to impaired (+) and impaired to normal (-) .
*Significant mean difference P<0.05, independent samples t-test.

Conclusion

This pilot study indicated a modest improvement in muscle performance in response to vitamin D3 supplementation. No differences in overall muscle strength and performance were detected. A small but statistically significant positive effect of vitamin D supplementation on TUG test performance was detected. This is consistent with vitamin D intervention studies. Further consideration could be given to the usefulness of physical performance measures to clarify the role of vitamin D and muscle. Larger intervention studies are merited.

References
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BEST FORMULATIONS