Vitamin D Status and Biomarkers of Functional Health and Ageing in Very-Old Adults:

Analysis of the Newcastle 85+ Study

A Thesis submitted to Newcastle University for the degree of *Doctor of Philosophy*

By

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Dedication

I dedicate this PhD thesis to my family - my mum and dad, Amal and Hesham, my sisters and brother, my husband Anas, and my daughters, Zaineh and Masa, for their unconditional love and support.

Acknowledgment

First of all, I thank God for the inspiration and strength He has given me during my PhD journey. I also wish to express a great deal of appreciation to the Saudi Arabian Government and to Umm Al Qura University for offering me this scholarship, and for giving me the opportunity to study this PhD. A warm thanks to my family for all their love and encouragement; I would not have finished this PhD without my parents' prayers for me. In addition, this journey would not have been easy without my daughters' admiration and belief in me, which gave me a reason to wake up every morning, read, learn, work hard and be a better person. My kindest thanks to my aunt and uncle, Layla and Osama, for the boost they gave to me and my family. This PhD would not have been possible without the unconditional support and patience from my beloved husband. Also, a special thanks to my friends, Abrar, Shaimaa, Haya and Duaa, for making this journey joyful and an unforgettable memory. Kind thanks go to local general practitioners and staff of in addition to the research, management and administrative teams of The Newcastle 85+ study and of course the study participants and their family for providing the data. Generous thanks go to Wasim Iqbal for his collaborative work in the systematic review and to the authors of the "the association between 25-hydroxyvitamin D concentration and disability trajectories in very-old adults" study. Finally, a sincere and heartfelt thank you goes to Tom, Nuno and Terry for their supervision, feedback and input of this thesis.

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Abstract

The number of those aged over 80 years-old (the "very-old") will increase from 5% of the population in European countries in 2010, to more than 10% of the population by 2050 (OECD, 2013). Very little is known about the nutritional intake, nutritional status and its association with health and wellbeing in the very-old. Due to its diverse biological effects, vitamin D has gained immense interest recently as a potential modifier for a range of health outcomes This PhD systematically reviewed the available literature to provide an accurate snapshot of vitamin D status in the very-old. It also used a unique dataset from the Newcastle 85+ Study, a longitudinal community-dwelling study of health trajectory and outcomes conducted in over 800 people from the North-East of England aged 85 years. The overall aim of using this data were to explore vitamin D association with a range of functional and ageing biomarkers in the very-old. Vitamin D status [25(OH)D] was available for 775 participants, and measured by immunoassays at baseline only and divided to the following concentration: <25 nmol/l (low), 25-50 nmol/l (moderate), and >50 nmol/l (high). Disability was measured using a questionnaire on the difficulty of performing 17 Activities of Daily Living at baseline, 1.5, 3 and 5 years. NTproBNP was measured using an electrochemiluminescent sandwich immunoassay. The HbA1c was measured using a Tosoh Eurogenetics automated HLC-723G7 HPLC analyser. Telomere Length was measured as an abundance of telomeric template vs. a single gene by quantitative real-time PCR. Spirometry and peak flow measurements were to obtain three technically satisfactory maximal effort 'blows' to generate reproducible FEV1and FVC. Results of the systematic review showed that prevalence of deficiency varies by latitude and living conditions of the participants, and that vitamin D deficiency is widespread in many regions, particularly in Europe. Using the Newcastle 85+ Study data also showed a high prevalence of vitamin D deficiency (>30%) was found amongst very-old adults. Findings of this thesis indicate that participants with low 25(OH)D concentration (<25 nmol/l) were more likely to have a poorer disability trajectory over 5 years compared with those with moderate concentration (25–50 nmol/l) (OR= 3.12, 95% CI= 1.6–5.8, p= 0.001), although physical activity was the strongest predictor of disability trajectories. However, this thesis could not prove the protective effect of vitamin D in regards to metabolic and cardiopulmonary health biomarkers (NT-proBNP, HbA1c, FEV1, FVC and diastolic blood pressure) in fully

adjusted models at baseline or over 18 and 36 months. Finally, high 25(OH)D concentration is positively associated with Telomere Length (95%CI= 12.0-110.3, B= 61.2±25.0, p=0.015) but does not have protective effects over 18 and 36 months. In conclusion, this thesis highlights that vitamin D deficiency is very common in very-old adults and that low vitamin D status is associated with at least some functional and ageing biomarkers in this under studied age group.

List of Abbreviations

1,25(OH)D	1,25 dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
ADL	Activities of daily living
B Cell	B lymphocytes cell
BADL	Basic activities of daily living
BMD	Bone mineral density
BMI	Body mass index
BNP	Brain natriuretic peptide
CBP	CREB-binding protein assay
CHD	Coronary heart disease
CLIA	Chemiluminescence immunoassay
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CVD	Cardiovascular diseases
CYP2R1	Vitamin D 25-hydroxylase
CYP24A1	Vitamin D 24-hydroxylase
CYP27B1	1-alpha-hydroxylase
DBP	Vitamin D binding protein
DEQAS	Vitamin D External Quality Assessment Scheme
DNA	Deoxyribonucleic acid
DRV	Dietary reference values
EC-Immunoassay	Electrochemiluminescence immunoassay
EFSA	European Food Safety Authority
eNOS	Endothelial cell nitric oxide synthase
ERA	Estimated dietary allowance
Euronut SENECA	European study of nutrition and health in the elderly
FEV1	Forced expiratory volume in one second
FFM	Fat-free mass
FVC	Forced vital capacity

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDS	Geriatric depression scale
GFR	Glomerular filtration rate
GP	General practice
HbA1c	Glycated haemoglobin
HPLC	High-performance liquid chromatography assay
HPLC-APCI-MS	High-pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry
IADL	Instrumental activities of daily living
ICAM-1	Intercellular Adhesion Molecule 1
IL-2	Interleukin-2
IoM	Institute of Medicine
IU	International unit
kb	Kilo-base
LTL	Leucocyte Telomere Length
MCP-1	Monocyte chemoattractant protein-1
MCPD	Metabolic and cardiopulmonary diseases
mg	Milligram
mcg	Microgram
nmol/l	Nanomoles Per Litre
MS	Liquid chromatography part of LC-MS assay
NT-proBNP	N-terminal pro-type B natriuretic peptide
PA	Physical activity
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PTH	Parathyroid hormone
Q-PCR	Quantitative polymerase chain reaction
Q-FISH	Quantitative fluorescence in situ hybridization
RAS	Renin angiotensin system
RCT	Randomize control trail
RIA	Radioimmunoassay

RNI	Reference nutrient intakes
RXR	Retinoic acid X receptor
SACN	Scientific Advisory Committee on Nutrition
SMMSE	Standardised Mini-Mental State Examination
SPF	Sun protection factor
T Cell	T lymphocyte cell
T2DM	Type 2 diabetes millets
TNF-α	Tumor necrosis factor
us-CPR	Ultrasensitive CRP
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
UVB	Ultraviolet B
VCAM-1	Vascular cell adhesion molecule 1
VDR	Vitamin D receptor

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Chapter 1: Introduction

Ageing

1.1. Demographics transition

Life expectancy has increased worldwide since the nineteenth century (Oeppen and Vaupel, 2002), and due to the declining birth and death rates, the population is shifting from being classed as younger to becoming very-old. Consequently, both developed and developing countries are undergoing changes to their population's age structure (Harper, 2014, Dong et al., 2016). As a result, the number of those aged over 80 years-old will increase from 5% of the population in European countries in 2010, to more than 10% of the population by 2050 (OECD, 2013). Life expectancy has already exceeded 75 years-old in 57 countries since 2011 (Marengoni et al., 2011), and this trend is expected to continue. By 2050, it is estimated that 379 million people will be aged 80 and above, while almost 10% of the population in developed countries will be aged above 80 (WHO 2012; OECD, 2013). In addition, approximately 10% of the world's population was aged over 60 in 2000, while other figures highlight that in Europe, those aged above 80 will constitute around 12% of the population by 2060. Moreover, around half of England's population is now expected to live until their mid-80s (Harper, 2014); European Commission, 2015). This ageing phenomenon is not exclusive to Western countries. From 2000 to 2005, adults aged 60 and above made up approximately 6.5% of the Arab population, and this percentage is expected to grow to 18% of the population by 2050 (Yount and Sibai, 2009).

This ageing phenomenon has altered the demographics of the population, and has brought with it health, economic, social and personal challenges. Therefore, living well for longer has become the key goal in achieving lower level of morbidity, fewer years of disability and greater quality of life among the older population (Baugreet et al., 2017).

1.2. Economic, personal and social implications of ageing

Longer life expectancy has an associated financial burden on both the individual and on populations (Vaupel and Kistowski, 2005). In relation to the level of healthcare, ageing is positively associated with chronic diseases and, with every additional chronic

disease, the spending on Medicare and medication significantly increases (Lehnert et al., 2011). Therefore, it is necessary to alter the allocation of health resources (Mason, 2005) and maintain healthcare for the very-old for as long as possible, which means reducing the long-term health, financial and social care needs (Harper, 2014). Additionally, one of the main effects of the very-old population is the sector of work. The retirement age has changed in many countries; retirement among people aged 60 to 69 years-old has decreased from 51% to 44%, but the quantity and quality of productivity has been affected (Harper, 2014), which subsequently has a negative effect on their health and the economy. However, there is no doubt about the positive benefits of increasing the retirement age.

Ageing also brings with it social and personal costs. This is because the very-old need to be taken care of, and since the size of the younger population has reduced, healthcare workers are often needed in order to provide such care (Harper, 2014). Moreover, lifestyle and biological changes also exist, such as social isolation, financial constraints, mobility reduction and independency (Valtorta and Hanratty, 2012). Nevertheless, dealing with ageing differs between developed and developing countries. For instance, in European countries, the very-old link retirement with leisure and satisfaction, even though it could affect their health behaviour and physical activity (PA) (Stenholm et al., 2016). Conversely, the very-old in Arab countries miss having access to the amenities they were previously accustomed to, and they complain about the absence of specialized services, as well as a lack of social and economic support (Yount and Sibai, 2009).

1.3. Nutritional needs of very-old adults

With ageing, the possibility of developing chronic diseases as well as functional and cognitive impairment increases (Marengoni et al., 2011). As a result, demands for specific macronutrients, such as protein, or micronutrients, such as potassium, vitamin B12 and vitamin D, may also increase (Chernoff, 2005). However, there is virtually no scientific evidence on the nutritional needs of very-old adults and most public health dietary and nutritional recommendations are for the younger old (typically 65+ years-

old). Besides, what constitutes an ‘adequate’ nutrient status for very-old adults remains unknown.

Generally speaking, the process of ensuring (and providing) an adequate micronutrient intake for old adults appears challenging. Therefore, those who fall into the very-old category are at high risk of suffering from malnutrition and nutrition-related health problems because of inadequate food consumption (Ahmed and Haboubi, 2010). To elaborate, people aged 73 years-old feel less hunger compared to younger adults (aged 26 years-old) for a number of reasons (Giezenaar et al., 2016). For instance, the very-old experience poor appetite, dental problems, chewing and swallowing difficulties, taste loss and food texture concerns, all of which are strongly associated with malnourishment (Hickson, 2006). Moreover, physical impairments can lead to the limited purchase and preparation of food that will also impact upon their food consumption (Pillsbury et al., 2010).

In addition to this, multimorbidity and polypharmacy, both of which are highly prevalent in the very-old, can cause physiological changes that influence the body’s ability to digest, absorb, transport and metabolise food (Hickson, 2006). Medication and special medical conditions do affect the food choices and variety in old adults, which contributes to lower food intake and nutrient deficiency (Mazahery and von Hurst, 2015). Additionally, social issues and financial status may prevent this category of people from following a well-balanced diet and consuming sufficient intake (Leslie and Hankey, 2015).

What follows in this chapter offers an insight into the need for vitamin D amongst very-old adults; more specifically, it provides a detailed review of the vital requirements for vitamin D intake and the consequences of deficiency and insufficiency of it for very-old adults.

Vitamin D

1.4. Vitamin D metabolism and the associated changes with ageing

Vitamin D is fat soluble vitamin that can be stored in the liver as well as adipose tissue. It has the effect of both a vitamin and a hormone (Afrika, 2010). Calcitriol (1,25(OH)D), which is produced by the kidney to be a steroid hormone, originates from cholesterol and functions in a similar way to other steroid hormones, in that it responds to various physiological signals by interacting with its vitamin D receptor (VDR).

Metabolism vitamin D3 (from sunlight exposure) and ingested vitamin D2 (from food and supplements) both undergo an obligatory two-step metabolism for the production of the biologically active form. First, vitamin D is released from vitamin D-binding protein (DBP) to the liver, and hydroxylases in the liver by D-25-hydroxylase (CYP2R1) to 25(OH)D. After that, it travels to the kidneys to be converted into 1,25(OH)D (biologically-active form) by 1α-hydroxylase (CYP27B1) (Figure 1.1). This step is controlled by the parathyroid hormone (PTH). Once formed, 1,25(OH)D travels and binds to a nuclear receptor or plasma membrane receptor at the target organs, such as the small intestine (Bikle, 2014). Both hydroxylase enzymes, together with VDR, have been identified in over 30 different extra-skeletal tissues, which means that the cells in these tissues have the potential to produce biological responses (see figures 1.2 and 1.3), depending on the availability of appropriate amounts of vitamin D (Høyer-Hansen et al., 2010).

It is well-documented that the concentration of 25(OH)D is associated with many factors, such as skin synthesis (due to skin pigmentation, sunscreen use, age, season, latitude and sun exposure), bioavailability (due to skin synthesis reduction, intestinal malabsorption, increased body fat mass and hepatic or urinary dysfunction), and catabolism (due to medication and disease) (Pramyothin and Holick, 2014). Very-old adults are at an increased risk of developing vitamin D deficiency due to many age-related factors that will be discussed below (Janssen et al., 2013).

Most of the changes in ageing skin occurs as a result of a combination of endogenous (e.g., gene mutations, cellular metabolism, hormone environment) and exogenous factors (e.g., chemicals, toxins, pollutants, ultraviolet (UV), and ionizing

radiation) (Makrantonaki and Zouboulis, 2007). Consequently, the concentration of 7-dehydrocholesterol (provitamin D) reduces with ageing (MacLaughlin and Holick, 1985). As a result, the dermal efficacy for forming vitamin D, and the dermal capacity for synthesizing vitamin D, when exposed to UV light, start to decline (Mazahery and von Hurst, 2015, Lanske and Razzaque, 2007). Moreover, the epidermis undergoes gradual atrophy between the ages of 30 and 80 years, which results in skin thinning by 10–50% (Makrantonaki and Zouboulis, 2007). Therefore, a 70 years-old person can only synthesize 25% of the vitamin D that a 20 years-old person would synthesise when exposed to the same amount of sunlight (Holick, 2004a). Clinical observations have also indicated that older osteoporotic women have thin, frail skin (Calleja-Agius et al., 2007), where the amount of pre-vitamin D3 produced in the skin of subjects aged over 77 years-old was found to be half that produced in the skin of subjects less than 18 years-old (MacLaughlin and Holick, 1985).

Renal function declines with ageing, and this is accompanied by a decrease in renal 1α-hydroxylation (Gallagher, 2013). Thus, it has been hypothesised that ageing is related to renal hydroxylation reduction (Gallagher, 2013). Hirani et al. (2015) found that the concentration of 1,25(OH)D in the blood declined significantly with age, but the 25(OH)D concentration did not, which suggests a reduction in the kidney to hydroxylase 25(OH)D to 1,25(OH)D (Tsai et al., 1984). Moreover, the production of CYP24A1, which is responsible for the catabolism of 1,25(OH)D in the kidneys, was found to be increased in the blood (Christakos et al., 2016).

A decrease in the intestinal responsiveness to 1,25(OH)D also occurs with ageing (Ebeling et al., 1992). This is due to a decline in intestinal VDR (Christakos et al., 2016), which subsequently leads to a resistance to vitamin D metabolite in the bowel mucosa.

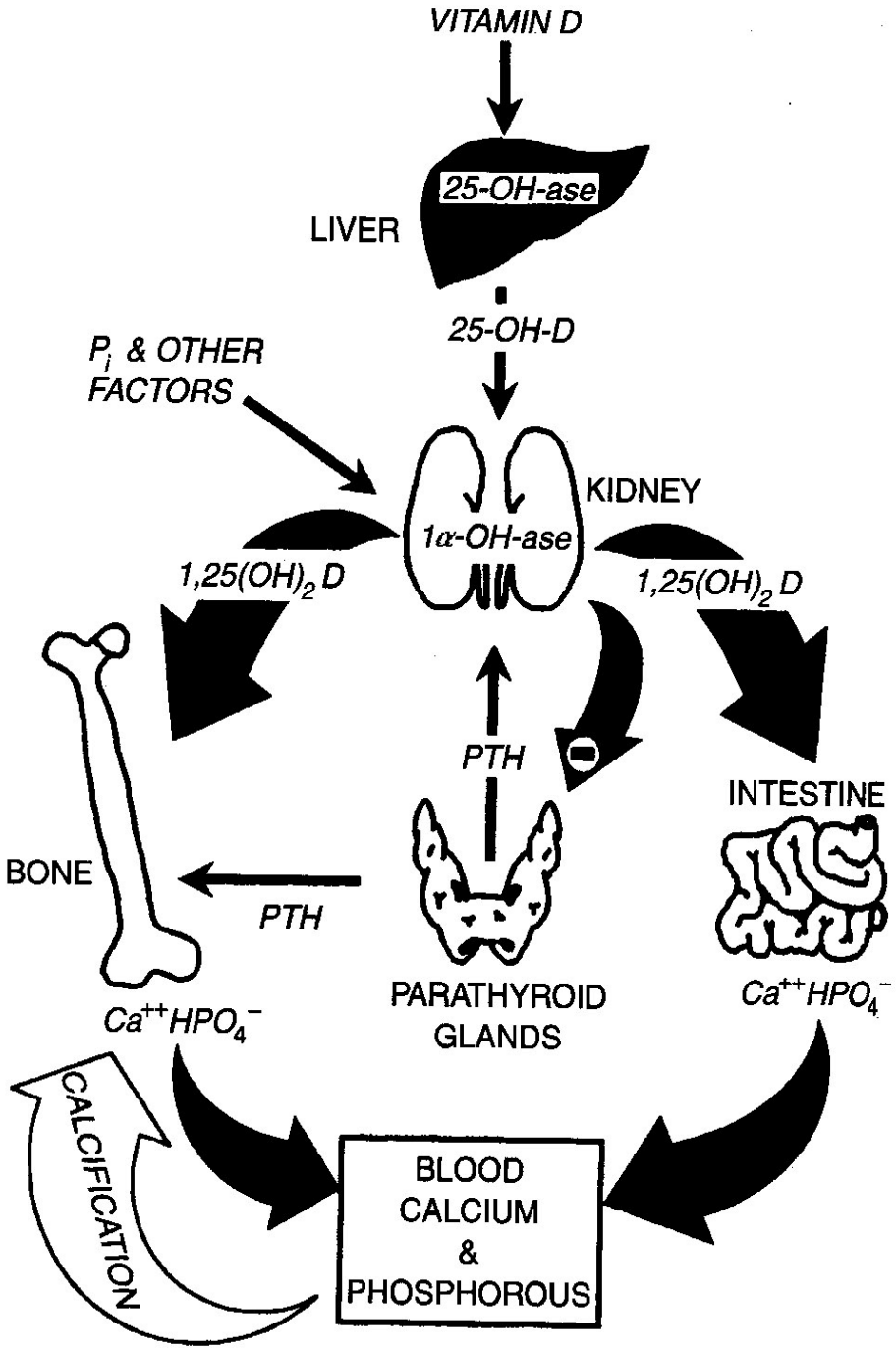


Figure 1. 1.Vitamin D Metabolism and the biologic actions of 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2$ D_3) (modified from Holick (2004a)).

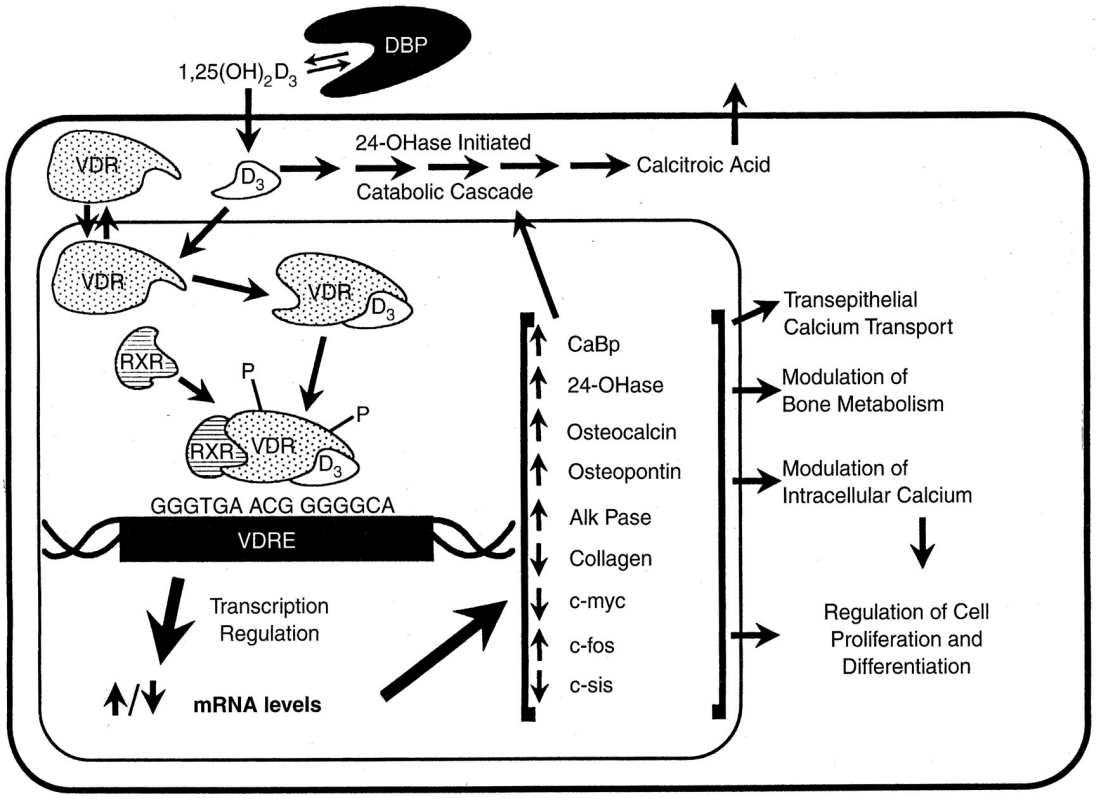


Figure 1. 2. Proposed mechanism of action of 1,25 (OH)2D3 in target cells, resulting in a variety of biologic responses. The free form of 1,25 (OH)2D3 enters the target cell and interacts with its nuclear vitamin D receptor (VDR), which is phosphorylated (P). The 1,25 (OH)2D3-VDR complex combines with the retinoic acid X receptor (RXR) to form a heterodimer, which in turn interacts with the vitamin D-responsive element (VDRE) to enhance or inhibit transcription of vitamin D-responsive genes such as 25 (OH) D-24-hydroxylase (24-OHase) (Holick, 1998).

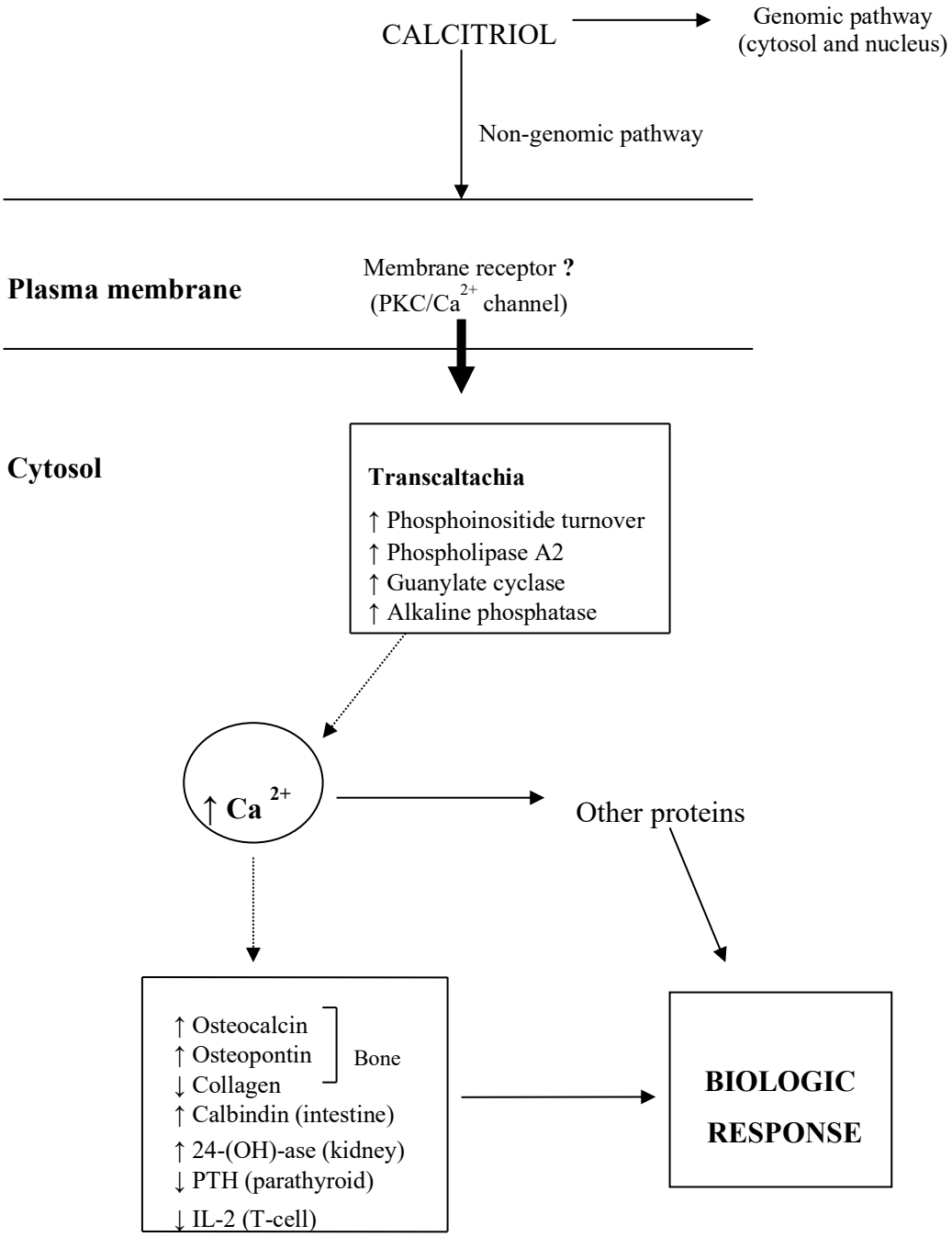


Figure 1. 3. Schematic illustration of the non-genomic effects of calcitriol. Calcitriol regulates intra-cellular signalling processes through its interaction with plasma membrane receptors, which results in a rapid increase in cytosolic calcium (transcaltachia) and subsequent biologic responses. [PKC, Protein Kinase C; IL-2, Interleukin-2] (Taken from Lal et al. (1999)).

1.5. Assessment of vitamin D status

Vitamin D in the body can be assessed using many indicators in the blood, such as 25(OH)D, 1,25(OH)D, PTH, and calcium. The circulating concentration of 25(OH)D was commonly used as the best indicator of vitamin D status in the body, because it is the main circulatory and storage form of vitamin D (Prentice et al., 2008). It reflects the total amount of vitamin D synthesized in the skin, in addition to that which has been consumed via diet (Prentice et al., 2008). Additionally, the circulating half-life of 25(OH)D concentration is 2-3 weeks compared to the 4 hours of 1,25(OH)D (Holick, 2009). Moreover, vitamin D biologically converts to 25(OH)D quickly, while only a small fraction of it is converted to 1,25(OH)D (Holick, 1990).

Alternatively, 1,25(OH)D is the biologically active form of vitamin D that is used in the body, so it can also be used to assess the status of vitamin D (Holick, 2009). However, the evidence shows that a 1,25(OH)D concentration should not be used to diagnose hypovitaminosis D, and the main reason for this is that a very low concentration of 25(OH)D can be converted to 1,25(OH)D, even in patients with vitamin D deficiency. As a result, 25(OH)D can be low or undetectable, while 1,25(OH)D can show a normal or even high circulating concentration, which could lead to a misdiagnosis (Holick, 1990). In addition, 1,25(OH)D does not usually show any improvement after the consumption of supplements (Biancuzzo et al., 2013). Therefore, 25(OH)D is considered the best biomarker to reflect vitamin D concentration in the body.

Many clinical and non-clinical based assays are used to assess 25(OH)D concentration in serum and plasma samples (Carter et al., 2018, Binkley et al., 2004). Each assay has its own merits and drawbacks. Mass spectrometry approaches such as GC-MS or LC-MS may be considered a ‘gold standard’ but these methods require a high degree of technical knowledge and the equipment is very expensive. Radioimmunoassay (RIA) has historically been a popular method to assess 25(OH)D including in NDNS surveys and at the time of baseline blood collection for the Newcastle 85+ study (2006/2007) was the most common method for assessing 25(OH)D globally. The RIA method was used for the purposes of this thesis as well. However, different labs and assays report different values of 25(OH)D concentration in certain samples. For instance,

the mean 25(OH)D of 10 healthy adults was reported to be from 42.5 to 87.5 nmol/l by different laboratories, and different results were noted for the same subjects depending on the laboratory concerned (Binkley et al., 2004). What this therefore highlights, is just how vital it is to improve the accuracy of the available assays and to define an international standard assay. Therefore, the Vitamin D External Quality Assessment Scheme (DEQAS) was incorporated to monitor the analytical performance and reliability of 25(OH)D and 1,25(OH)D assays. Overall, the accuracy and precision has improved over recent years. In 2016, the mean method bias and coefficient of variation of most assays was within or close to ± 5% and 10% respectively (Carter et al., 2018).

1.6. Vitamin D nutritional requirements: International variation in the set criteria for adequacy

As mentioned earlier, the nutritional requirements are not specifically set for very-old adults but are based on the younger old (those aged over 65+ years-old), even though their requirements could be far greater than those of younger adults (Ahmed and Haboubi, 2010) due to the reasons discussed earlier in the chapter. As vitamin D deficiency is expected among very-old adults, a high estimated intake of vitamin D may be required to maintain a sufficient concentration of 25(OH)D for this group (Baraké et al., 2010). There is no general agreement on what the adequate concentration of 25(OH)D or the required daily intake of vitamin D should be (see Tables 1.1 and 1.2). This thesis focuses on the guidelines of nutritional adequacy and requirements by the United States of America (USA) Institute of Medicine (IoM), the United Kingdom (UK) Scientific Advisory Committee on Nutrition (SACN) and The European Food Safety Authority (EFSA). Throughout the thesis, a 25(OH)D concentration <25 nmol/l is considered as low, concentration between 25-50 nmol/l is considered as moderate and concentration >50 nmol/l is considered as high.

1.6.1. Institute of Medicine (IoM) - North America, United States of America and Canada

The IoM (2011) have defined vitamin D deficiency as a concentration of 25(OH)D <30 nmol/L and vitamin D inadequacy as a concentration of 30-50 nmol/L (Ross et al., 2011). This is based on evidence for both skeletal and extra-skeletal outcomes. IoM summarizes the new Dietary Reference Intakes (DRIs), which takes into account the determination of the Estimated Average Requirement (EAR), the median intake needs of the population and the calculation of the Recommended Dietary Allowance (RDA); the level of intake that would meet the requirements of at least 97.5% of the population. The DRI also specifies the tolerable upper intake level (UL); the highest daily intake (Table 1.1). The IoM recommends an EAR of 400 IU/d (10 mcg) and a RDA of 600 IU (15 µg) for people over 70 years-old, and for those at risk of vitamin D deficiency, it should be 1500-2000 IU/d (40- 50 mcg). However, they do emphasize that the upper concentration should not exceed 4000 IU (100 mcg) and 10,000 IU (250 mcg) for the RDA and treatment respectively (Holick, 2011, Ross et al., 2011) (Table 1.2).

1.6.2. Scientific Advisory Committee on Nutrition (SACN) - United Kingdom

SACN have set the cut-off point of <25 nmol/l for vitamin D deficiency (SACN, 2016), However, 25 nmol/l is not a clinical threshold for diagnosing musculoskeletal diseases; it is an indicator of the increased risk of poor musculoskeletal health at concentration below 25 nmol/l. Therefore, a 25(OH)D concentration of ≥ 25 nmol/L was selected as the basis for the reference nutrient intakes (RNI) for vitamin D for 97.5% of the population (Table 1.1). The SACN report that the proposed RNI for people at risk, including adults aged 65+, of 400 IU/d (10 mcg) would be required for individuals in the UK to achieve a 25(OH)D concentration ≥ 25 nmol/L when the UVB sunshine exposure is minimal (Public Health England, 2017). RNI refers to the average intake over a period of time (e.g. a week) and takes account of day-to-day variations in vitamin D intake (Table 1.2).

1.6.3. European Food Safety Authority (EFSA)

The EFSA panel considers a serum 25(OH)D concentration of 50 nmol/l is a suitable target value for all population groups, in view of setting the adequate intakes (AIs) for all population groups. Considering the evidence on the relationship between serum 25(OH)D concentration and musculoskeletal health outcomes in adults, infants and children, and adverse pregnancy-related health outcomes (Table 1.1). EFSA sets 15 mg/day as dietary reference values (DRVs) for vitamin D for adults, based on a meta-regression analysis and considering that, at this intake, the majority of the population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/l (Table 1.2). The DRV for vitamin D are based on the assumption of minimal exposure to the sun with resulting limited level of synthesised vitamin D to ensure that European consumers take in sufficient level of vitamin D, irrespective of their geographic location and exposure to sun light (EFSA Panel on Dietetic Products and Allergies, 2016).

Generally, limited studies have evaluated vitamin D intake and 25(OH)D concentration in very-old adults. The maximum age of the participants in most studies is 80 years-old, and rarely do studies include participants aged 90 or 100 years-old (Brouwer-Brolsma et al., 2016, Veugelers et al., 2015). Since vitamin D may play an important role in the prevention and treatment of skeletal and non-skeletal diseases, such as cancer, cardiovascular disease, infections and autoimmune disease (Bikle, 2014), it is necessary to evaluate this group's intake and status regarding vitamin D.

Table 1. 1: International definitions of 25(OH)D status to ensure the nutritional adequacy of vitamin D

IoM*:

- <30 nmol/l (Deficient)
- 40 nmol/l (Basis for EAR)
- ≥50 nmol/l (opted for RDA)

SACN**:

- <25 nmol/l (Deficient)
- ≥25 nmol/l (Basis for RNI)

EFSA***:

- <50 nmol/l (Deficient)
- ≥50 nmol/l (opted appropriate for AI)

ref. *Ross et al. (2011),
**SACN (2016),
***EFSA Panel on Dietetic Products and Allergies (2016).
µg:.microgram. IoM: Institute of Medicine. SACN: Scientific Advisory Committee on Nutrition. EFSA: European Food Safety Authority. ERA: estimated average requirement. RDA: recommended dietary allowances. RNI: reference nutrient intakes. AI: adequate intake.

Table 1. 2: International dietary vitamin D requirements (*µg/ day)*

Age	Recommended intake (*µg* /day)		
	IoM* *(RDI)*	SACN** *(RNI)*	EFSA*** *(AI)*
Children (0-12 months)	---	8.5-10	10
Children (1-18 years)	15	10	15
Adults (19-70 years)	15	10	15
Older adults (>70 years)	20	10	15
Pregnancy/ Lactation	15	10	15

ref. *Ross et al. (2011),
**SACN (2016),
***EFSA Panel on Dietetic Products and Allergies (2016).
µg:.microgram. IoM: Institute of Medicine. SACN: Scientific Advisory Committee on Nutrition. EFSA: European Food Safety Authority. RDI: dietary reference intake. RNI: reference nutrient intakes. AI: adequate intake.

1.7. What is the vitamin D status of very-old adults across the globe?

According to a new British Nutrition Foundation (BNF), the prevalence of vitamin D deficiency in different age groups within the UK showed biphasic association between vitamin D status and age (Lanham-New et al., 2011). At younger age, vitamin D status was good, but it decreases between 19 to 24 years-old. Then, it starts to increase again until age 65 years-old. After this point (after the age of 65), the 25(OH)D concentration begins to decrease (van Schoor et al., 2014) and then, after the age of 70, both men and women experience an annual decline in 25(OH)D concentration of 0.025 nmol/L and 0.032 nmol/L, respectively (Maggio et al., 2005). Similarly, research by Ageing Study Amsterdam (ASA) has shown that the 25(OH)D concentration increased by 4 nmol/L in people aged 55 to 65 years-old during a period of 6 years, but then started to decrease by 4 nmol/L in those aged 65 to 88 years-old (de Jongh et al., 2017).

The Euronut SENECA study reported that a 25(OH)D concentration below 30 nmol/L was relatively widespread among very-old adults (>75 years-old) in Europe in Winter (van der Wielen et al., 1995). A systematic review of 25(OH)D concentration in people over 60 years-old in central European countries reported a mean of 25(OH)D concentration, ranging from 32.5 nmol/l in Ukraine to 72.5 nmol/l in Hungary in Winter (Pludowski et al., 2014). In an observational study by Andersen et al. (2005), it was stated that the median concentration of 25(OH)D for Northern European women with a mean age of 71 years-old was 40.7 nmol/l and for 17% of them, the 25(OH)D concentration was below 25 nmol/l, while more than 67% of them had a 25(OH)D concentration that was below 50 nmol/l in Winter. Further to this, a recent systematic review found that the lowest 25(OH)D concentration of postmenopausal women aged >70 years-old in Southern European countries was 33.7 nmol/l in Italy, whereas the highest concentration was 81.7 nmol/l in Spain across the season (Manios et al., 2018).

Besides, those who are institutionalised are at a higher risk of vitamin D deficiency, of which up to 80% of the very-old institutionalised population has been reported to have a concentration of 25(OH)D <25 nmol/l (Spiro and Buttriss, 2014). Generally, limited studies assess 25(OH)D concentration of very-old adults and evaluate the prevalence of deficiency among this sector.

1.8. Vitamin D and health outcomes in older adults

1.8.1. Musculoskeletal and Physical Health

Several pieces of evidence support the association between 25(OH)D concentration and skeletal and muscle strength and function (Hamilton, 2010). 1,25(OH)D increases the absorption of essential minerals across the intestine, such as calcium, phosphorus, magnesium, zinc and manganese. It also enhances the net renal reabsorption of calcium and phosphorus (New, 1999). Thus, the most significant role of 1, 25(OH)D is to maintain calcium and phosphate homeostasis (Fernandes and Barreto, 2017) (see figure 1.2). 25(OH)D concentration takes 15-50 days to fall from 100 nmol/L to 50 nmol/L in Winter (the time of less sun exposure) (Ashwell et al., 2010). Consequently, only 10-15% of calcium and 50-60% of phosphorus are absorbed when vitamin D is low (Holick, 2006). This shows that it is important to consider the 25(OH)D concentration in the very-old category of people, in order to maintain and improve their musculoskeletal health.

Vitamin D and Bone Health: Mechanistic evidence

Bone metabolism always includes two processes of bone formation and bone resorption which simultaneously increase or reduce. When bone metabolism is interrupted, and bone resorption is more efficient than bone formation, bone mineral density (BMD) reduction and osteoporosis occur (Christakos et al., 2016). Vitamin D deficiency results in a decreased concentration of ionized calcium, which is immediately recognized by the calcium sensor in the parathyroid glands (Veldurthy et al., 2016). This results in the increased expression, production and secretion of PTH. PTH helps to maintain calcium metabolism by increasing the tubular reabsorption of calcium in the kidneys, enhancing the production of 1,25(OH)D, and interacting with osteoblasts to increase the receptor activator of the nuclear factor- B ligand system (Bouillon et al., 2014). Therefore, it is not a low calcium concentration that causes rickets in children and osteomalacia in adults; rather, vitamin D deficiency, which causes secondary hyperparathyroidism, results in a PTH-induced loss of phosphorus into the urine and decreased intestinal phosphorus absorption (Holick, 2004a).

Vitamin D and Bone Health: Epidemiological Evidence

Skeletal bone mass is determined by a combination of endogenous factors (i.e. genetic, hormonal) and exogenous factors (i.e. nutritional, physical activity) (New, 1999). Even though approximately 50% of the variance in peak bone mass may be determined genetically, vitamin D is essential for bone mineralization (McGuigan et al., 2002).

A significant positive association has been found between 25(OH)D concentration and BMD in old adults with osteomalacia (Bischoff-Ferrari et al., 2004b). Olmos et al. (2016), El-Desouki et al. (2004), Bhambri et al. (2006) have all confirmed that a positive association exists between vitamin D and hip BMD and the lumbar spine. When analysing the statistics for fracture rates, they show that, for every 1,000 people over 65 years in Germany, the fracture rate was 8%, 36%, and 35% every year for people with normal (>75 nmol/l), insufficient (50-75 nmol/l) and deficient (>50 nmol/l) 25(OH)D concentrations, respectively (Rothenbacher et al., 2014). According to Steingrimsdottir et al. (2014), the estimated risk of hip fracture was twice as high in individuals with a 25(OH)D concentration below 30 nmol/L. Another study found that men aged 65 years and older, with a total 25(OH)D concentration <50 nmol/l, had a significantly increased the risk of suffering subsequent hip fractures during the next five years compared to men with a concentration of >70 nmol/l (Cauley et al., 2010). However, little evidence reports that the concentration of 25(OH)D did not differ significantly between participants with hip fracture and those without, which was the case for both sexes (Chan et al., 2012, Nakamura et al., 2007).

Vitamin D and Bone Health: Clinical Trials Evidence

The majority of studies agree with the effect of vitamin D supplementation in reducing incidents of hip fracture (Avenell et al., 2005, Avenell et al., 2014). Similarly, a daily dose of 700-800 IU of vitamin D reduces the hip fracture risk by 26% and the non-vertebral fracture risk by 23% (Bischoff-Ferrari et al., 2005, Bischoff-Ferrari et al., 2012). In contrast to this, however, even though bone loss at the hip was found to be significantly greater for the placebo and the 400 IU vitamin D groups (compared with the 1000 IU vitamin D group), the BMD change at the lumbar spine was not significantly

different between the treatment groups (Macdonald et al., 2013). Other studies showed that low doses of vitamin D3 supplementation (less than 800 IU) - *without calcium* - did not reduce the risk of fractures in five trials (Cranney et al., 2008).

Similarly, in a meta-analysis of 11 observational studies, the 25(OH)D concentration was associated with an adjusted rate ratio for any fracture of 0.93 and an adjusted rate ratio for hip fracture of 0.80 (Yao et al., 2019). However, a meta-analysis of 11 RCTs of vitamin D supplementation alone (a daily or intermittent dose of 400-30 000 IU) did not find a reduced risk of any fracture or hip fracture, but these trials were constrained by infrequent intermittent dosing, low daily doses of vitamin D, or an inadequate number of participants (Yao et al., 2019). In contrast, a meta-analysis of six RCTs of combined supplementation with vitamin D (daily doses of 400-800 IU) and calcium (daily doses of 1000-1200 mg) found a 6% reduced risk of any fracture and a 16% reduced risk of hip fracture (Yao et al., 2019).

From all the above, we can conclude that maintaining a "moderate" 25(OH)D concentration (30-50 nmol/l) is positively associated with skeletal health. However, the definition of an adequate (moderate) concentration is conflicting between the studies. On the other hand, high doses of vitamin D supplementation may not positively influence bone health.

Vitamin D and Muscle Health: Mechanistic evidence

After the hydroxylation of 25(OH)D, 1,25(OH)D binds to its nuclear receptor, which is identified in a large number of tissues, including the muscles. Once it enters and the nucleus binds to its VDR, it complexes with the retinoic acid X receptor (RXR) to form a heterodimeric complex (Haussler et al., 2011). Once the 1,25(OH)D-VDR-RXR complex binds to the vitamin D-responsive element, a variety of transcriptional signalling occurs. This process influences the protein synthesis in the muscle, muscle calcium uptake and type II muscle fibre size and number (Ceglia, 2009). Two potential mechanisms have been suggested to explain the association between vitamin D deficiency and poor muscle mass. The first mechanism is that the decline in VDR expression by parathyroid gland leads to impaired muscle response to 1,25(OH)D, while

the second mechanism is that the reduction in 1,25(OH)D reduces the stimulation of VDR expression by the muscles (Bischoff-Ferrari et al., 2004a). With advanced age, expression of VDR by parathyroid glands is decreased which may relate to reduced 25(OH)D concentrations leading to reduced gene expression of VDR.

Vitamin D and Muscle Health: Epidemiological Evidence

An approximate estimate of 15% muscle mass loss occurs per decade after the age of 70 (Keller and Engelhardt, 2013). Moreover, muscle strength is found to be 20-40% lower at the age of 70 compared to young adults (Gomes et al., 2017). Several studies have described the positive association between a low 25(OH)D concentration and poor muscle strength. That is, a concentration of 25(OH)D was found to be significantly associated with arm and leg strength in women; likewise, those with hypovitaminosis D had significantly lower arm and leg strength compared to women with a normal concentration of 25(OH)D (>37.5 nmol/l) (Zamboni et al., 2002). Reduced muscle function is independently associated with an increased risk of falls. More than half of people aged 80 and over experience a fall every year (Duval et al., 2017), and subjects who fell more often were significantly older and had a low 25(OH)D concentration (<25 nmol/l) (Snijder et al., 2006). A low 25(OH)D concentration (<25 nmol/l) was also positively associated with falls when considering the potential confounders in participants with a mean age of 85 years-old, although the 25(OH)D concentration did not significantly differ between fallers and non-fallers (Duval et al., 2017).

Vitamin D and Muscle Health: Clinical Trials Evidence

Generally, studies with doses of 800-1000 IU of vitamin D per day proved the beneficial effects on balance and lower extremity muscle strength (Muir and Montero-Odasso, 2011). Additionally, an achieved 25(OH)D concentration of 60 nmol/l or more resulted in a 23% fall reduction (Bischoff-Ferrari et al., 2009), while a high dose of vitamin D (700 IU/day) saw a reduction in the risk of falling by 19% (Bischoff-Ferrari et al., 2009). Other evidence has suggested that there was no reduction in the incidence of

fractures or falls in the vitamin D treated group (Law et al., 2006). Similarly, a systematic review of 14 studies has only found a small benefit of supplementation with regard to falls (Cranney et al., 2008). A meta-analysis of 13 RCTs studies including participants >60 years-old concluded that supplemental vitamin D with daily doses of 800-1,000 IU consistently demonstrated beneficial effects on strength and balance (Muir and Montero-Odasso, 2011).

In contrast, very high doses of vitamin D supplements had a reverse effect on falls and fractures. An RCT that used 300,000 IU ergocaliferol injections did not find any significant effect of vitamin D treatment on the frequency of falls or against fractures at any site (Smith et al., 2007). Indeed, participants receiving annually a very high-dose oral cholecalciferol (placebo OR 500000 IU for three to five years) experienced 15% more falls and 26% more fractures than the placebo group (Sanders et al., 2010).

In summary, there is consistent evidence that maintaining 25(OH)D above 25 nmol/l is associated with improved musculoskeletal outcomes, particularly, muscle strength and function and falls risk reduction. However, whether maintaining higher 25(OH)D concentrations e.g. 50 nmol/L or > 75 nmol/l is beneficial for these outcomes remains unclear. Trial evidence supports a benefit of vitamin D supplements on musculoskeletal health when baseline 25(OH)D is low. The dose of vitamin D which has a benefit is typically > 15-20 μg/day.

1.8.2. Metabolic and Cardiopulmonary Functional and Blood Biomarkers

Metabolic and cardiopulmonary disease (MCPD) is a term used throughout this thesis to describe a varied group of serious disorders that can affect the heart and lungs. The most common MCPDs are hypertension, diabetes, stroke, coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD). Evidence shows a positive association between 25(OH)D concentration and MCPD, whereby a low 25(OH)D concentration was found to be common amongst COPD patients, especially those with CHD (Zhang and Yuan, 2016). It has also been observed that the hospital admission of all cardiovascular diseases (CVD) and respiratory diseases had Winter peaks, which, as previously mentioned, coincides with a time when vitamin D deficiency occurs (Douglas

et al., 1996). That said, whether or not we can state conclusively that vitamin D is the cause or a result of MCPD disorders still remains unclear.

In relation to this topic, the experimental and clinical evidence indicates that a link exists between 25(OH)D concentration and cardiovascular health (Ginde et al., 2009, Pekkanen et al., 2015). The lowest quartile of 25(OH)D concentration (<24.5 nmol/l) was associated with twice the risk of suffering a stroke compared to those in the highest 25(OH)D quartile (>53.7 nmol/l) (Busch et al., 2011). However, from these studies, there is no way of telling if having heart failure, along with low PA and sun exposure, lowers the 25(OH)D concentration, unconnected to the pathophysiology of heart failure, or if the 25(OH)D concentration was directly involved in worsening the functional capacity (Boxer et al., 2010). The direct effect could be via the effect of 25(OH)D on several risk factors for CVD, including elevated hypertension, metabolic syndrome (diabetes) (both will be discussed in detail below), hyperlipidemia and inflammation due to the elevation in the plasma inflammatory markers (Messenger et al., 2012, Cigolini et al., 2006).

In addition, the community-dwelling studies pertaining to this area of study have highlighted an association between 25(OH)D concentration and lung function (Hughes and Norton, 2009). A high 25(OH)D concentration was also associated with less airway responsiveness and improved glucocorticoid response (Pfeffer and Hawrylowicz, 2012), while a low 25(OH)D concentration was associated with upper respiratory tract infection (Finklea et al., 2011). However, there is a possibility that the association of vitamin D deficiency with increased respiratory infection occurs only in those patients with the most severe pulmonary illnesses (Finklea et al., 2011).

A. Vitamin D and Cardiopulmonary Function

Systolic and Diastolic Blood Pressure: Mechanistic evidence

Generally, vitamin D affects hypertension as a result of its ability to reduce the renin–angiotensin system (RAS) in the kidney (Holick, 2004b); thus, the inappropriate stimulation of the RAS has been associated with hypertension. Several clinical and experimental studies have demonstrated that a 1,25(OH)D was one of the most potent

negative endocrine regulators of the RAS (Kendrick et al., 2009, Chopra and Davis Cherian, 2011). One may also note the associated link between PTH and hypertension (Zittermann, 2006). A community-dwelling study of 1205 older men and women found that vitamin D was not significantly associated with blood pressure; however, they did demonstrate that higher PTH was significantly associated with a higher systolic and diastolic blood pressure (BP), as well as a higher prevalence of hypertension (Snijder et al., 2007).

Systolic and Diastolic Blood Pressure: Epidemiological Evidence

High BP or hypertension is a risk factor for CVD (Fuchs and Whelton, 2020). Observational studies have found an inverse association between vitamin D and hypertension (Norman and Powell, 2014). A study by Pekkanen et al. (2015) proposed that a low 25(OH)D concentration may indicate an increased risk of systolic and diastolic dysfunction. Moreover, the higher risk associated with vitamin D deficiency was particularly evident among individuals with hypertension, in whom 25(OH)D concentration <37.5 nmol/l was associated with a two-fold risk of cardiovascular events (Wang et al., 2008).

Systolic and Diastolic Blood Pressure: Clinical Trials Evidence

Experimental studies mostly rely on a combination of vitamin D and calcium supplements to improve the BP. For example, a daily dose of 800 IU (20 mcg) of vitamin D supplement, combined with 1200mg of calcium, decreased the systolic BP in women over 70 years-old by 9.3% (Mosekilde, 2005). A meta-analysis of 17 RCTs, that included participants aged 18-75 years-old, concluded that vitamin D supplements reduced the systolic BP of those who had low 25(OH)D concentration and over 50 years-old (He and Hao, 2019). A meta-analysis of 11 cohort studies that included a general population (≥18 years-old) suggested that the risk of hypertension increased substantially below 75 nmol/L of 25(OH)D and remained significant over the range of 75–130 nmol/L of 25(OH)D (Zhang, 2020). On the other hand, the pooled results of 27 RCTs showed that

there was no significant reduction in systolic or diastolic BP after vitamin D intervention, which indicates that supplementation with vitamin D does not lower BP in the general population. However, the included studies did not record changes in diet, sun exposure or latitudes, genetic factors or educational status, which were factors that would modify the effect of the intervention. Besides, several of the included trials failed to achieve enough power (they were below 80%) to detect any weak differences between the intervention and placebo groups because of the small sample size and high rate of noncompliance (Zhang, 2020).

Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) as biomarkers of lung function: Mechanistic evidence

The association between vitamin D deficiency and reduced lung function could depend on the calcaemic effects of vitamin D kyphosis and cause a limitation in rib mobility and inspiratory muscle function, and so was consequently associated with a reduction in forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) (Herr et al., 2011).

Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) as biomarkers of lung function: Epidemiological Evidence

FEV1 and FVC are lung function tests that are measured during spirometry. FEV1 is the most important measurement of lung function. It is the amount of air that can be forced from the lungs in one second, while FVC is the total amount of air exhaled during the FEV1 test. Studies showed an inconsistent association between vitamin D and COPD. Some researchers note a positive association between the 25(OH)D deficiency and the severity of COPD (Finklea et al., 2011). The concentration of 25(OH)D was positively associated with the volume-related lung function parameters FEV1 and FVC. Other studies, however, indicate that a higher 25(OH)D concentration was associated with an increased rate of COPD (Rafiq et al., 2018), although Moberg et al. (2014) did not find any association between selected COPD phenotypes and 25(OH)D deficiency.

No experimental studies to date confirm the effect of vitamin D supplements on FEV1 and FVC.

Glycated haemoglobin (*HbA1c): Mechanistic evidence*

The mechanism of action for vitamin D in type 2 diabetes mellitus (T2DM) is thought to be mediated in direct and indirect ways (Kumar et al., 2018, Szymczak-Pajor and Śliwińska, 2019). The indirect way is through the regulation of plasma calcium level, which regulate insulin synthesis and secretion, whereas the direct way is through its action on the pancreatic tissue. VDR and DBP are presented in the pancreatic tissue, β-islet cells, and 1,25(OH)D enhances insulin production and secretion via its action on the VDR (Kumar et al., 2018). Moreover, the 1,25(OH)D improves insulin sensitivity by preventing the excessive synthesis of inflammatory cytokines (Szymczak-Pajor and Śliwińska, 2019).

Glycated haemoglobin (*HbA1c): Epidemiological Evidence*

Glycated haemoglobin (HbA1c) is the most important laboratory parameter indicating glycaemic control (Organization, 2011). The general target of HbA1c is ≤7% for glycaemic control. A study proposed that the incidence of diabetes could be reduced by 41% by being in the top rather than the bottom quartile of 25(OH)D (Forouhi et al., 2012). One may therefore conclude that vitamin D deficiency may influence T2DM. However, no association between vitamin D and glycaemic control was found in the study that enrolled a total of 128 patients with diabetes mellitus, aged 57.7±10. The mean HbA1c values of the patients were 9.18±2.52, and 98.4% of the diabetic patients had insufficient vitamin D values (Olt, 2015).

Glycated haemoglobin (*HbA1c): Clinical Trials Evidence*

A meta-analysis of 11 prospective studies involved a total of 3,612 cases of T2DM and 55,713 non-diabetic participants aged 40-75 years-old. It suggested a strong inverse association between 25(OH)D concentration (<50 nmol/l) and incident T2DM

(Forouhi et al., 2012). Another meta-analysis included 47 RCTs involving participants aged 20-77 years-old. The median trial duration was four months and the median dose of vitamin D supplements was 4000IU/d. The results showed that vitamin D supplementation significantly reduced the fasting glucose by 0.11 mmol/L and fasting insulin by 1.47 mIU/l, while no significant effects of the supplementation were observed on the insulin secretion or beta cell function indexes. They concluded that vitamin D supplementation might improve glucose and insulin metabolism without affecting the risk of T2DM among nondiabetic adults (Tang et al., 2018). The conflicting results could be due to the inclusion of a wide age range of participants, and including trials with and without calcium treatment.

Brain natriuretic peptide (*BNP) as a biomarker of cardiac function: Mechanistic evidence*

In the heart, the association between 25(OH)D and N-terminal pro brain natriuretic peptide (NT-proBNP) could be explained by a direct and indirect mechanism. The direct mechanism is via the promoter region of the brain natriuretic peptide (BNP) gene that contains various transcription factors binding sites, including VDR in the heart (Santhekadur et al., 2017). Chen et al. (2008) demonstrated that VDR has the capacity to bind directly to the BNP gene promoter. The indirect mechanism is via PTH, calcium or inflammatory effect of 25(OH)D on the heart (Chen et al., 2008). As a result of low 25(OH)D and VDR, heart failure occurs and the plasma BNP increases.

In the lungs, the association between 25(OH)D and BNP could be explained indirectly due to the influence of low 25(OH)D on the lungs. Consequently, BNP is increased for vasodilation and antiproliferative actions (Leuchte et al., 2006). However, there is a suggestion that pulmonary dysfunction is related to the severity of heart failure. Therefore, the increases in NT-proBNP level cause a significant decrease in pulmonary volume (Nazemiyeh et al., 2015).

Brain natriuretic peptide (*BNP) as a biomarker of cardiac function: Epidemiological studies*

The BNP is a hormone that is synthesised by the cardiomyocytes (ventricles) as a pre-pro BNP. It undergoes cleavage and modification by corin and furin enzymes to be converted from pro-BNP to BNP. Pre-pro BNP modification results in the formation of a signal peptide and a propeptide (pro BNP, 108 amino acids), which is stored as a mature hormone in the human heart. The N-terminal fragment is the biologically inactive form (Santhekadur et al., 2017). The plasma half-life of BNP is 20 minutes while the plasma NT-proBNP's half-life is 120 minutes. This explains why the NT-proBNP blood values are six times higher than the BNP values (Weber et al., 2006). The main stimulus responsible for increasing BNP and NT-proBNP synthesis and secretion is myocardial wall stress, which causes a strong vascular relaxation and stimulates natriuresis (Nazemiyeh et al., 2015). It has been reported to indicate ventricular dysfunctions more efficiently than other natriuretic peptides (NPs) (Yoo, 2014). The systematic effects of BNP includes vasodilatation, increased urine output with a high sodium level, and the inhibition of the nervous system and the renin-angiotensin-aldosterone system (Nazemiyeh et al., 2015).

Studies have also suggested that NT-proBNP may be used as a screening test for pulmonary hypertension (Leuchte et al., 2006) and as a diagnosis for acute pulmonary embolism (Pruszczyk et al., 2003). A high level of NT-proBNP was also observed among patients with severe COPD (Chi et al., 2012). Previous observational studies reported three major connections between BNP and lung disease. First, NT-proBNP increases significantly with the severity of COPD (Chi et al., 2012). Second, NT-proBNP level is associated significantly with chronic respiratory failure (Leuchte et al., 2006) and third, plasma NT-proBNP level is associated significantly with systolic pulmonary artery pressure (Nazemiyeh et al., 2015).

Two observational studies, involving young CHD patients, failed to detect such an association between 25(OH)D concentration and NTproBNP level, as well as between PTH and NT-proBNP level (Passeri et al., 2016, Wetmore et al., 2011). Nevertheless, in one study, the presence of CHD frequently occurred in those with vitamin D deficiency

and mild hyperparathyroidism (Passeri et al., 2016). However, this would not be the case among very-old adults due to age-related changes.

BNP is dependent on renal function for clearance so it is reasonable that age-dependent BNP elevation as well as 25(OH)D reduction may be partly due to the decline of glomerular filtration rate (GFR) with ageing (Wiley et al., 2010). Besides, it is well documented that the BNP level increases with ageing (Keyzer et al., 2014) while VDR expression decreases with ageing (Bischoff-Ferrari et al., 2004a). However, no studies have yet assessed the effect of vitamin D supplementation on NT-proBNP level. Besides, no studies have yet explored the association between 25(OH)D and NT-proBNP in very-old adults.

B. Vitamin D and ageing: Telomere Length

Telomeres are the specific DNA protein structures found at both ends of each chromosome to protect the genome from nucleolytic degradation, unnecessary recombination, repair and interchromosomal fusion (Pusceddu et al., 2015). Each DNA replication causes Telomeres shortening, and when the Telomere Length reaches a critical limit, the cell undergoes senescence and/or apoptosis (Shammas, 2011). This situation can be reversed by the enzyme named Telomerase, that is responsible for Telomere Length maintenance (Muzumdar and Atzmon, 2012). Higher Telomerase activity would increase Telomere Length while lower activity would result in a shorter Telomere (Muzumdar and Atzmon, 2012). As part of normal ageing, most human somatic tissues and adult stem cells undergo Telomere attrition and do not express sufficient amounts of Telomerase to maintain the Telomere Length indefinitely (Pusceddu et al., 2015). Telomere Length may therefore serve as a biological clock to determine the lifespan of a cell and an organism (Shammas, 2011). As a normal cellular ages, the Telomere Length decreases. Leukocyte Telomere Length (LTL) and peripheral blood mononuclear cells (PBMCs) Telomere Length are mostly used in studies.

Telomere Length (Predictors)

Telomere attrition in human somatic cells is mostly due to the "end-replication" problem and low Telomerase activity. The rate of attrition differs between individuals and tissues and is influenced by multiple factors including donor age, sex, genetic, environment, social and lifestyle (Shammas, 2011, Muzumdar and Atzmon, 2012).

Oxidative stress and chronic inflammation are considered the major contributors of Telomere attrition (Xu et al., 2009). Consistent with that, insulin resistance, TNF-a and CRP were found to be significantly inversely associated with LTL in premenopausal women (Aviv et al., 2006a). Due to the expression of these biomarkers by inflammation and oxidative stress, Telomerase activity and Telomere Length were reduced in the leukemic cells (Aviv et al., 2006a). General exposure to stressful physiological conditions can accelerate t=Telomere loss and also cause more extensive oxidative damage to the cells and tissues. Stressed women were found to have a shorter Telomere Length equivalent to ten years of life compared to unstressed women, indicating that the women under stress were at risk of early onset age-related health problems (Shammas, 2011). Manestar-Blazic took a step backwards and suggested that Telomere Length and longevity depend on the Telomere state in the germ line of the parents at conception (Muzumdar and Atzmon, 2012).

Women usually have a longer mean Telomere Length compared to men (Muzumdar and Atzmon, 2012). Even though the Telomere Length does not differ between men and women new-borns, it is longer in adult women than in adult men (Benetos et al., 2001). Estrogen was suggested to be related to the leukocyte Telomere in premenopausal women through its anti-inflammatory and antioxidant affects as well as its ability to stimulate telomerase (Aviv et al., 2006a). However, because Estrogen declines with age, its role as a Telomere regulator is limited in postmenopausal women (Muzumdar and Atzmon, 2012). Other evidence claims that sex does not have any significant effect on the rate of Telomere loss (Shammas, 2011).

Body composition and dietary factors are related to LTL in women (Cassidy et al., 2010). The Telomeres in obese women have been shown to be significantly shorter than those in lean women in the same age group, and the loss of Telomeres in obese

individuals was equivalent to 8.8 years of life (Shammas, 2011). Likewise, body mass index (BMI) and waist circumference were found to be inversely associated with LTL (Cassidy et al., 2010). Regarding diet, regular multivitamin use, as well as vitamins E, C and beta-carotene were all related to longer Telomere Length in women aged 35–74 in a cross-sectional study (Xu et al., 2009, Shammas, 2011). Moreover, polyunsaturated fatty acid intake was negatively associated, while dietary fibre was positively associated with LTL in a large cross-sectional study of middle-aged and older women (Cassidy et al., 2010). On the other hand, smoking and exposure to pollution were associated with increased oxidative stress and the rate of Telomere shortening (Shammas, 2011).

Telomere Length-related Diseases

A Telomere Length for the specific age group that is shorter than average has been associated with an increased incidence of age-related disease and/or decreased lifespan in humans (Shammas, 2011). An evaluation of Telomere Length in old adults shows that individuals with shorter Telomeres have a far higher rate of mortality than those with longer Telomeres (Shammas, 2011). Similarly, the mortality rate from infectious diseases was eight times higher for individuals in the bottom 25% of the Telomere Length distribution than for individuals in the top 75%, while individuals in the bottom half of the Telomere Length distribution had a heart disease mortality rate over three times that of those in the top half of the distribution (Cawthon et al., 2003). Moreover, several studies have shown that a high rate of Telomere attrition is associated with an elevated risk of coronary artery disease, myocardial infarction and heart failure (Pusceddu et al., 2015).

Telomere Length: Mechanistic evidence

There are potential mechanisms that could explain an association between Telomere Length and vitamin D concentration. Activated vitamin D exerts anti-inflammatory and anti-proliferative actions, which would affect the turnover rate of leukocytes by: (1) reducing the expression of inflammatory cytokines, such as TNF-α, IL-

2 and CRP; (2) expressing VDR on T and B lymphocytes cells and (3) regulating the ROS level by controlling the expression of cellular antioxidants. Consequently, vitamin D would reduce the rate of Telomere Length attrition (Mazidi et al., 2017, Richards et al., 2007, Berridge, 2017).

Telomere Length: Epidemiological Evidence

Vitamin D status could plausibly affect the maintenance of Telomere Length directly or via its effects on inflammation and/or the rate of cell proliferation (Mazidi et al., 2017). Little evidence has been found to support the association between vitamin D and Telomere Length. However, this association has not yet been confirmed by RCT studies. In a large population of women (n= 2160, age= 18–79 years-old), a higher 25(OH)D concentration was associated with a longer LTL. Although this association does not prove causality, it does suggest that 25(OH)D concentration may play an important role in the modulation of LTL (Richards et al., 2007). Likewise, a large cross-sectional study (n= 4347) aimed to determine the association between 25(OH)D and Telomere Length in both sexes aged 18-80 years-old. The participants were free of any history of chronic disease and the results revealed an association between 25(OH)D concentration and Telomere Length in limited-adjusted models, which suggests a possible role of 25(OH)D concentration in the maintenance of Telomere Length (Mazidi et al., 2017). Similarly, a study that included a large group of men and women (aged 20–>60 years-old, n= 4260) found a positive association between the concentration of 25(OH)D and LTL. Those with optimal 25(OH)D concentration showed a 0.13-kbp longer LTL than their counterparts with 25(OH)D concentration <50 nmol/L. The association was independent of other variables, such as age, sex, race/ethnicity, BMI, total energy and sugar intake, calcium intake, socioeconomic status, consumption of milk and dietary supplements, and PA (Beilfuss et al., 2017). However, all of these studies were cross-sectional and included a wide age range of participants.

On the other hand, a cross-sectional study of white men did not observe any association between vitamin D biomarkers (25(OH)D and 1,25(OH)2D) and LTL (Julin et al., 2017). Liu et al. (2016) also found no significant association between 25(OH)D

concentration and long LTL for the overall analysed population (aged 48–93 years-old) or the subgroups (men, women, black, white). However, they found that non-deficient white women were the only group who showed a significant association between 25(OH)D concentration ≥30 nmol/l and longer LTL.

These conflicting results may be attributed to the difference in the ages of the participants. The age range for white women in one study was 48–93 years-old (mean age= 62·8) (Liu et al., 2016), whereas Richards et al. (2007) studied younger women aged 18–79 years-old (mean age= 49·4 years-old). Besides, some studies had a smaller number of subgroup participants (white women=373, compared to the total number of the participants=4,117) and therefore a lower statistical power for examining the association within this subgroup (Liu et al., 2016).

1.9. General Objectives

The overall objective of this PhD thesis is to explore the association between the vitamin D status in very-old men and women from the ninth decade and beyond, and both functional and age-specific biomarkers using a unique cohort of older people from the Newcastle 85+ Study. More specifically, this PhD aimsed to:

Chapter 3

1. Assess the distribution of 25(OH)D concentration worldwide.
2. Consider the variations in the geographical and living conditions of very-old adults.
3. Identify the regions with a lack of research on 25(OH)D concentration in very-old adults.

Chapter 4

1. Explore the vitamin D status association factors in very-old men and women independently based on SACN recommendations.

Chapter 5

1. Explore the association between 25(OH)D concentration and the disability trajectory over five years.
2. Investigate whether there is a threshold concentration of 25(OH)D above which the disability trajectory among very-old adults slows.

Chapter 6

1. Assess the association between 25(OH)D and the metabolic and cardiopulmonary health biomarkers of heart and lung function over three years.
2. Define the adequate concentration of 25(OH)D to improve metabolic and cardiopulmonary health.

Chapter 7

1. Examine the association between 25(OH)D and Telomere Length over three years.

2. Investigate whether differences exist between the outcomes for men and women.
3. Explore the optimal concentration of 25(OH)D with regard to Telomere Length.

Chapter 2: General Methodology

Methodology:

2.1. Systematic review (Chapter 3):

The methodology of the systematic review is based on the protocol we created and published on PROSPERO "Serum25(OH)D levels in the very-old (>80 years) people across the globe: a systematic review [CRD42018117158] (registered 29/11/2018)." The systematic review was undertaken and completed by the PhD researcher of this thesis.

2.1.1. Search method for identification of the studies

A. Electronic search

The initial search terms were created to identify the best key terms, and two reviewers (SH and WI) worked independently to select the eligible studies. No discrepancies were found. Databases such as Medline, ProQuest, PubMed and Web of Science were used for the search. The search terms were divided into population, intervention, condition and outcomes (PICO), and were separated using "AND" and "OR" commands. Key words were implemented to search the databases are shown in Table 2.1.

Unrestricted searches produced a vast amount of results (PubMed database for example produced 3,486,488), so the first terms of each search strategy were restricted to titles only.

Table 2.1: Search Terms used

	AND	AND	AND	AND	AND
Or	○ "Vitamin D" ○ 1 25 dihydroxycholecalciferol ○ 1 25(OH)D ○ 25 hydroxycholecalciferol ○ 25OH ○ calcitriol ○ calcidiol ○ ergocalciferol	○ Elderly ○ age* ○ old* ○ 80 year ○ 80+ ○ adult	○ residential institutional ○ nursing ○ hospital* ○ facility	○ fractures ○ falls ○ bone mineral density ○ bone loss hyperparathyroidism ○ muscle strength ○ muscle loss muscle ○ sarcopenia ○ osteoporosis ○ parathyroid vitamin D status ○ deficiency	○ bservational ○ cohort ○ cross-sectional ○ case-control ○ longitudinal

After this initial search, the results were then refined throughout based on the databases' thesauruses. Mesh terms were used for PubMed and Medline, and ProQuest terms were used for ProQuest and Web of Science, since Web of Science does not have a thesaurus. References to relevant reviews and studies were also screened (n=89) (see Figure 3.1).

2.1.2. Criteria for considering studies for this review

A. Type of studies

All of the observational studies, including cohort and cross-sectional studies, were collected, with no restriction on region, year or language. Cohort studies (only the baseline wave) also reported data for the total population of very-old adults at baseline, which can be considered cross-sectional data (Lauretani et al., 2012, Diekmann et al., 2013). Case-control studies were not included, as the authors agreed that the control group was not considered representative of the general population.

B. Participants

All participants were considered and included, regardless of whether they were institutionalised or community- dwelling, or any demographic factor, sex or ethnicity. The primary criterion was that they were ≥80 years-old.

C. Outcomes

The main and only outcome is a 25(OH)D concentration. 25(OH)D was reported as a mean ± variance (square root of SD) in nmol/l. Studies that reported 25(OH)D as a mg/l were converted to nmol/l. To gain an insight into the overall concentration of 25(OH)D, the mean 25(OH)D of the included studies was compared with the SACN cut-off: </≥25 nmol/l.

2.1.3. Data collection and analysis

A. Selection of the studies

The titles and abstracts were screened to evaluate the eligibility of the studies for inclusion, based on the age of the participants and measurement of the concentration of 25(OH)D. Studies were not excluded solely because that they did not report the outcome, but only if they did not measure it. However, 52 studies were excluded because of the following reasons: 4 studies were case-control, 7 studies were not representative, 3 studies were overlap with another included study, 1 study did not report the mean age of the participants and 36 studies included participants less than 80 years-old (see Figure 3.1).

Studies examining specific diseases were not included, as these were unrepresentative of a general population. In addition, studies that included participants with a mean age <80, or those who did not include the mean age of the participants, were excluded, even if the age range was higher than 65 years-old. RCTs and case-control studies were also excluded.

B. Data extraction and management

The results for each individual search were recorded and all the results were exported to reference manager software (ENDNOTE X9). A master file was then created, containing all of the results of the search. From here, the extracted data were saved on an Excel sheet on (Microsoft Excel), recording the outcome measures, type of study, type of participants, age (mean and range), location, PA, health condition, supplements use, type of 25(OH)D measurement (Assay) and months of recruitment. Standard criteria for recording, grouping the data and converting the units (if necessary) were used for the data extraction. Lastly, unless the groups were divided into men and women in the actual study, the data were combined.

C. Dealing with duplicate studies

Duplicates were subsequently removed using the in-built function within the software. If the population of a study overlapped that of other studies, the most recent study with the largest population size was retained and the others were excluded.

2.1.4. Data synthesis

A. Qualitative summary

The extracted data were tabulated and summarized in the main document of the thesis.

2.2. The Newcastle 85+ Study (Chapters 4-7):

2.2.1. The Newcastle 85+ Study: Population sample and design

The Newcastle 85+ Study is a socio-demographically representative study of the general UK very-old population. It comprises both community-dwelling and institutionalised older adults under the following criteria: if they were born in 1921, aged 85 years-old at recruitment, living in Newcastle-upon-Tyne and North Tyneside (North East England), and registered with a participating general practice (GP) in Newcastle or North Tyneside Primary Care Trusts. In the UK, patients are registered with a single GP, which acts as a gatekeeper to secondary care, as well as holding a record of all hospital admissions and outpatient attendances. The review of GP records included hospital correspondence, in order to ensure that all recorded disease diagnoses were extracted, irrespective of where and when the diagnosis was made by trained nurse. The study's initial aim was to provide a comprehensive awareness of very-old adults and explore their health trajectory and outcomes at different phases over the five-year follow-up period (Collerton et al., 2009). All data collection and laboratory analysis were undertaken by the Newcastle 85+ Study team. The hypothesis and concepts related to vitamin D and the study outcomes as well as data manipulation and statistical analysis was done by the researcher for the PhD thesis.

2.2.2. Recruitment profile

From the 64 GPs in the Newcastle and North Tyneside Primary Care Trusts, 53 (83%) agreed to participate in this study. The recruitment and baseline assessment took place over a 17-month period during 2006-2007. All of those who met the inclusion criteria were sent invitation letters to participate (n= 1459). Only those individuals with end stage terminal illness (n= 11) were excluded. Of those invited, 1,042 were recruited and 845 (319 men and 526 women) had both a multi-dimension health assessment and GP record review data. A detailed flowchart of the recruitment profile is shown in Figure 2.1.

A health assessment - which comprised questionnaires (socioeconomic, health, dietary intake and lifestyle variables), measurements, function tests (anthropometry, cognition, physical function, pulmonary and cardiovascular) and a fasting blood sample - was carried out at the participants' usual residence. GP medical records were also reviewed to extract data on diagnosed diseases and prescribed medication. Both the health assessment and data extraction were conducted by one of the 11 trained research nurses following a standard protocol. Participants could decline elements of the protocol. The data collection was spread over three main interviews that lasted about 90 minutes each, with an additional short visit for weight and body composition measurement, and to collect a blood sample (Figure 2.2) (Collerton et al., 2009).

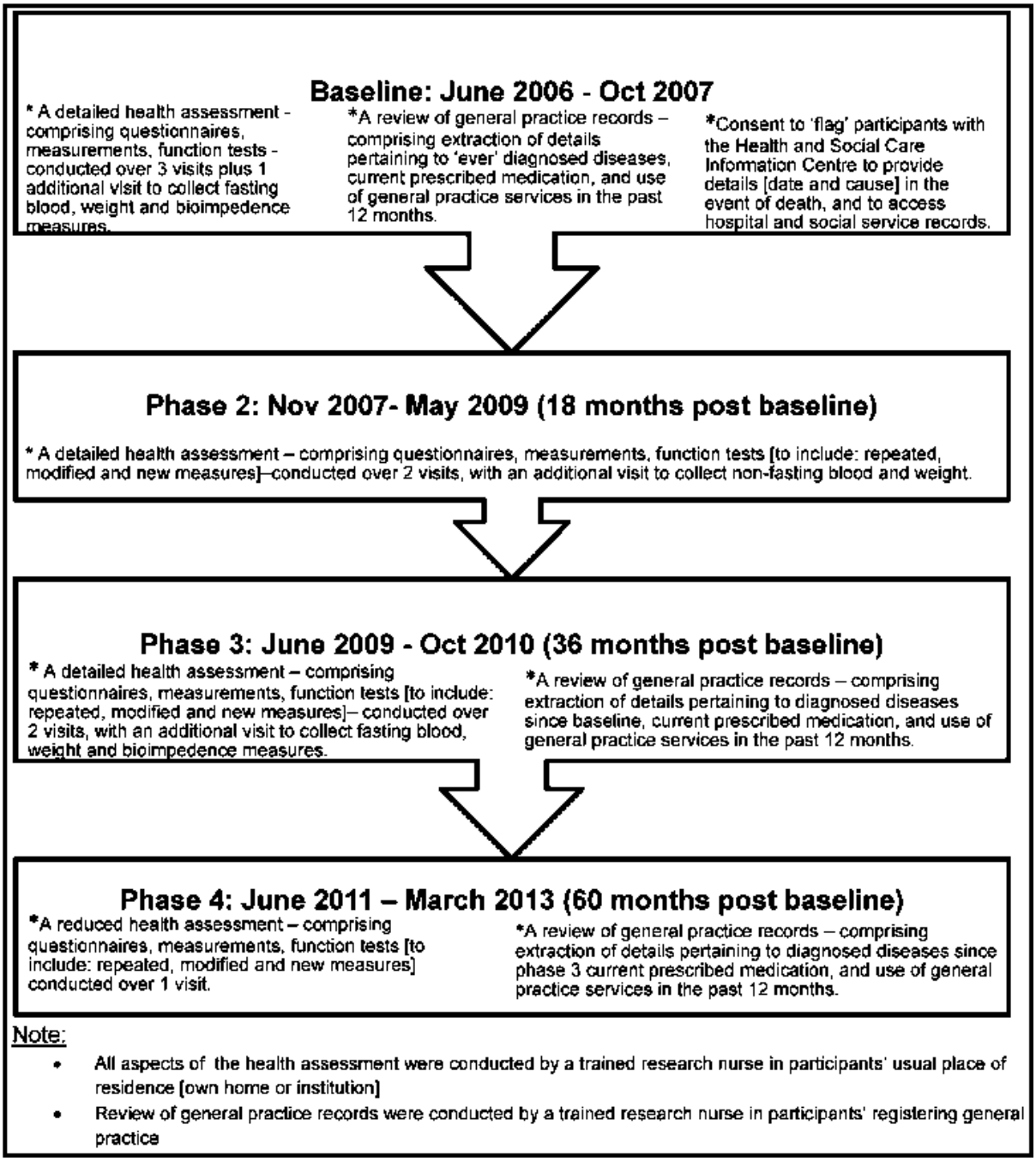
2.2.3. Assessment and retention profile

The follow-up phases took place at 18, 36 and 60 months from baseline (Collerton et al., 2009, Davies et al., 2010, Davies et al., 2014). It was noted that the attrition rates decreased during these phases. That is, from Phase 1 to Phase 2, 25% (n= 215) of the participants withdrew or died, while Phase 2 to Phase 3 saw a decrease of 23% (n= 147) and, from Phase 3 to Phase 4, there was a 29% (n= 139) decrease. Therefore, in total, out of the 845 participants who were included at the baseline of the study, 631 participants continued to Phase 2, 484 participants to Phase 3 and, lastly, 344 participants continued to Phase 4 (Davies et al., 2010).

2.2.4. Ethical Approval

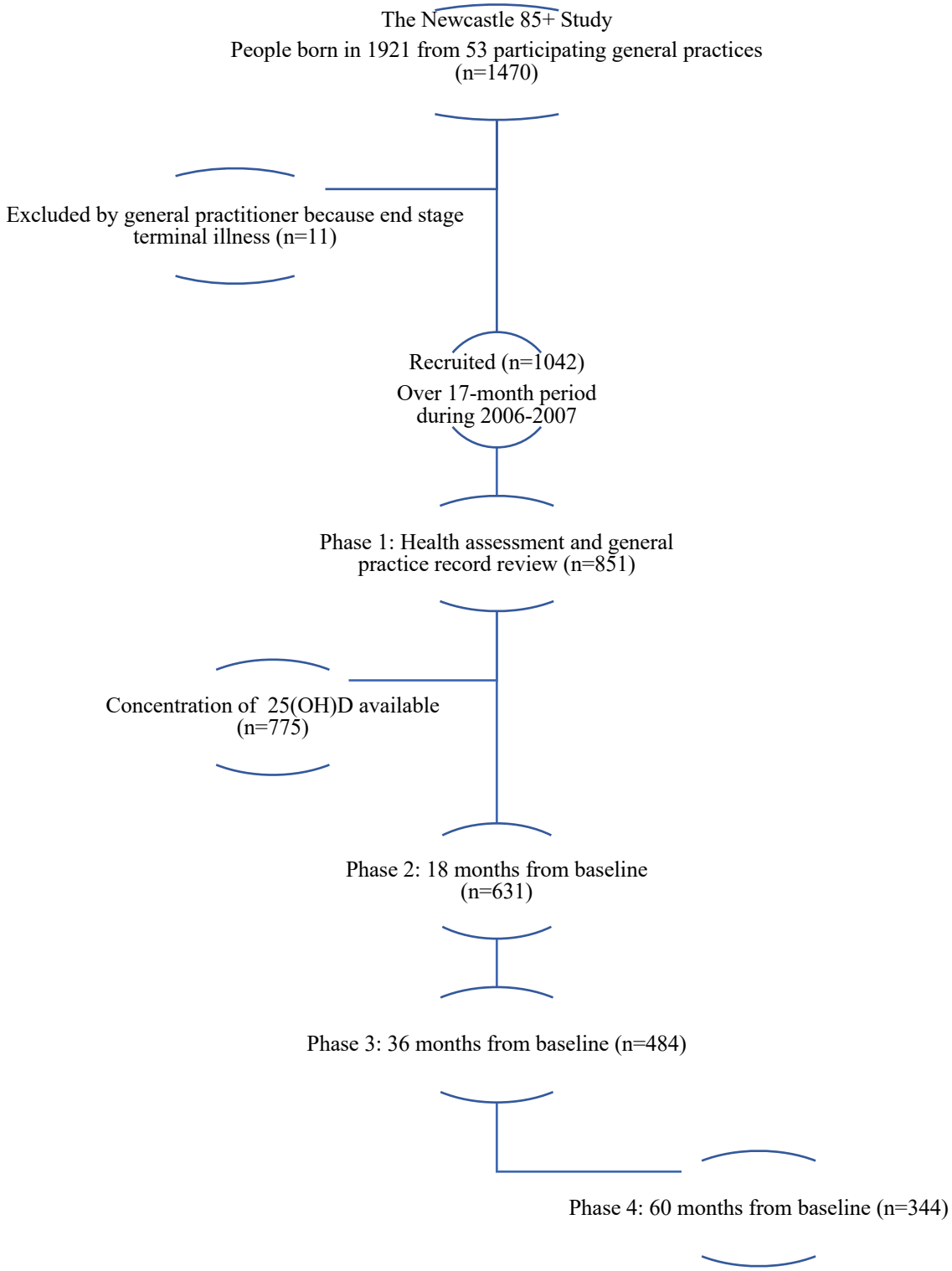
The research complied with the requirements of the Declaration of Helsinki. Ethical approval was obtained from the Newcastle and North Tyneside 1 Research Ethics Committee (reference number 06/Q0905/2) and written informed consent was obtained from the participants. In circumstances where people lacked the capacity to give their consent (i.e. due to cognitive impairment), a formal written consent was sought from a relative or carer (Collerton et al., 2007).

Figure 2.1: Assessment and retention profile



*Reference (Davies et al., 2014)

Figure 2.2: The Newcastle 85+ Study recruitment



2.2.5. 25(OH)D Assay and Definition of Vitamin D Status

Following an overnight fast, 40 ml of blood was drawn from the participants via the antecubital vein between 7:00 am and 10:30 am. Great attention was paid to ensuring that the blood samples were sent to the laboratory as quickly as possible, and 95% of the samples was received for processing within 1 hour of venipuncture. After extraction and precipitation, the total 25(OH)D concentration was determined by the DiaSorin radioimmunoassay (RIA) kit (DiaSorin Corporation, Stillwater, MN) according to the manufacturer's recommendations, using 25(OH)D-specific antibodies and 125I-labelled 25(OH)D (Diasorin Corporation) as a tracer. The minimum detectable concentration of 25(OH)D was 5 nmol/L, and the inter-assay coefficients of variation ranged from 8.4% to 12.6% (Martin-Ruiz et al., 2011). The concentration of 25(OH)D was only measured at baseline.

The blood sampling took place between June 2006 and August 2007. During 2006, the number of participants sampled during each month was: June (15), July (66), August (45), September (57), October (66), November (56) and December (34). During 2007, the number of participants sampled during each month was: January (66), February (65), March (65), April (56), June (76), July (74) and August (33).

SACN cut-off, which is used for the dietary recommendations for the UK population, are used throughout this thesis. The concentration of 25(OH)D <25 nmol/L was used to indicate the risk of vitamin D deficiency (SACN, 2016), (more information will be discussed in Chapter 4).

2.2.6. Disability Measures and Scores

Data on disability level were obtained via a nurse-administered questionnaire. At baseline and the follow-up assessments, the participants were asked about their ability to perform 17 activities comprising basic activities of daily living (BADLs), instrumental activates of daily living (IADLs) and mobility items (Table 2.1); these were taken predominantly from the Groningen Activity Restriction Scale (Kempen et al., 1996). A loss of ability for individual items formed a single hierarchy, that was similar for both

men and women. A disability score was calculated based on the total number of activities of daily living (ADL) that were performed with difficulty or requiring an aid/appliance or personal help (Collerton et al., 2009). A disability score of 0 was given for each item that was reported to be performed *without* difficulty, and a score of 1 for each item that was performed *with* difficulty (maximum score is 17). The participants were classified as having a disability if they had difficulty with one or more items, or no disability if they had no difficulty with any of the items.

The ability to perform 17 ADLs was self-reported. The participants were also asked to answer three questions on mobility (Table 2.1). Each question was framed by the phrase '*can you*' rather than '*do you*', in order to gain a greater capacity to assess the true level of disability accounting for situational responses.

Table 2.2: Self-reported activities of daily living

BADL	IADL	Mobility items	Response categories
- Feeding self (including cutting up of food) - Washing face and hands - Washing all over - Getting in and out of bed - Getting on and off the toilet - Getting in and out of a chair - Dressing and undressing - Cutting own toenails	- Light housework - Heavy housework - Preparing and cooking a hot meal - Shopping for groceries - Taking medication - Managing money	- Getting around the house - Going up and down stairs/steps - Walking at least 400 yards	- Can do on own without difficulty - Can do on own but with difficulty - Can do on own but only with aid or appliance - Unable to do without personal help

BADL: basic activities of daily living. IADL: instrumental activates of daily living.
*Reference (Jagger et al., 2011).

2.2.7. Biomarkers of Physical Function and Ageing

A total of 74 candidate biomarkers were investigated and analysed, including novel candidates (ageing-specific), as well as classical and disease-specific candidates (multi-morbidity, cognitive impairment, disability and short term mortality (1.5 year)) according to the previously reported methods (Martin-Ruiz et al., 2011). Each biomarker was measured on a continuous scale and the values were dichotomized into 'deficit' or 'no deficit' using empirical cut-off point (Mitnitski et al., 2015).

A. NT-proBNP measurement

Plasma samples for the NT-proBNP measurement were aliquoted on the day of collection and stored at -80°C. The NTproBNP was measured by an electrochemiluminescent sandwich immunoassay using the Modular Analytics E170 system (Roche Diagnostics, Lewes, UK). The between batch coefficient of variation was 1.5-3.5% from 122-4322 ng/l, with an analytical range of 5-35000 ng/l. The laboratory performing the NT-proBNP assay and the echocardiologist were blinded to the echocardiographic and NT-proBNP data, respectively.

B. HbA1c measurement

The glycosylated haemoglobin (HbA1c) was measured using a Tosoh Eurogenetics automated HLC-723G7 HPLC analyser (Martin-Ruiz et al., 2011).

C. Blood pressure measurement

Diastolic and systolic BP was measured using a digital blood pressure monitor - Omron HEM 705-IT (Omron Healthcare UK Ltd., Milton Keynes, UK). Three measurements were taken at two minute intervals; the average of the second and third measurements was used (Martin-Ruiz et al., 2011).

D. Lung function measurements

Spirometry and peak flow measurements were performed at the participants' place of residence by a trained research nurse using MicroLab Spirometer and Spida V.5 software (Micro Medical, Rochester, UK). The aim was to obtain three technically satisfactory maximal effort 'blows' to generate reproducible FEV1, FVC and peak expiratory flow measurements (PEF); the blows were repeated until this was achieved or the maximum effort was reached.

E. Telomere Length

Telomere Length (PBMCs) was measured as an abundance of telomeric template versus a single gene (GAPDH) by quantitative real-time PCR. The intra-assay coefficient of variation was 2.7% while the inter-assay coefficient of variation was 5.1%. Four internal control DNA samples were run within each plate to correct for plate–to-plate variation. Measurements were performed in quadruplicate. All PCRs were carried out on an Applied Biosystems 7900HT Fast Real Time PCR machine with a 384-well plate capacity (Martin-Ruiz et al., 2011).

2.2.8. Other Socioeconomic, Health and Lifestyle Variables

A. Socioeconomic

The multidimensional health questionnaire included: sex (men or women), housing type (standard, sheltered or institutional), living arrangements (living alone, with spouse, with others), years of full education, and social class according to the National Statistical Socio-Economic Classification (NS-SEC) (high, middle, low) based on past main occupation (Collerton et al., 2009).

B. Health

Information on health and morbidity (diagnosis of disease, cognitive status, Standardised Mini-Mental State Examination (SMMSE) score, systolic & diastolic blood pressure) were only collected from GP medical records. Diseases were recorded via a predetermined list of key diseases (Table 2.2). All diagnoses of listed diseases were scored as *present* (score 1) or *absent* (score 0), together with the date of first diagnosis. A simple disease count was used (maximum score 18) from selected chronic diseases (Table 2.2). The participants were included only if all of the variables were scored as *present* or *absent*. Relevant health assessment data were only used to obtain information on atrial fibrillation or flutter, renal impairment and anaemia (Collerton et al., 2009).

Cognitive impairment was assessed via the SMMSE, with a score ranging from 0 (impaired) to 30. Depression was assessed via the geriatric depression scale (GDS), and then checked against the GP records if a particular diagnosis was recorded (Granic et al., 2015b, Vertesi et al., 2001).

Table 2.3: List of diseases collected from GP medical records

- **Hypertension**
- **Ischaemic heart disease**
- **Cerebrovascular disease**
- **Peripheral vascular disease**
- **Heart failure**
- **Atrial flutter or fibrillation**
- **Arthritis (osteoarthritis or cervical or lumbar spondylosis or rheumatoid arthritis or other arthritis or non-specified arthritis)**
- **Osteoporosis**
- **Chronic obstructive pulmonary disease or asthma**
- **Other respiratory disease**
- **Diabetes**
- **Hypothyroidism or hyperthyroidism**
- **Cancer diagnosed within five years (exclusion non-melanoma skin cancer)**
- **Dementia**
- **Parkinson's disease**
- **Renal impairment**

* Reference (Collerton et al., 2009).

C. Lifestyle

Anthropometry measurements included: weight and height (which were used to calculate BMI in kg/m^2 and categorised by <18.5, 18.5-24.9, 25-29.9, >30), body composition, and fat free mass (which measured by Tanita body composition analyser). Lifestyle factors included: smoking, alcohol consumption and PA. A PA questionnaire (PAQ) was designed using data from the Newcastle 85+ pilot study, and then trialled in this age group prior to implementation. The PAQ categorised the participants into *low* (scores 0–1), *moderate* (scores 2–6) and *high* (scores 7–18) PA categories, according to the frequency and intensity of PA carried out per week (supplementary data Box S1, available in Age and Ageing online) (Innerd et al., 2015).

Nutrient intake including vitamin D intake from food was determined using two 24 hour dietary recalls on separate days (Mendonça et al., 2015). As vitamin D intakes were very low (typically below 2 μg/day in many of the participants) and in our previous analysis (Hill et al, 2016) did not impact circulating 25(OH)D, I did not include the dietary vitamin D intake in this thesis. Instead I included use of vitamin D supplements (not quantified in the study and a simple yes, no response on the health questionnaire) was included as supplement use was a significant predictor of 25(OH)D concentrations in previous analysis (Hill et al., 2016)

Supplements use was divided into two categories: no supplements users and supplements users. The information on supplements use was limited to the type and brand, therefore micronutrient-containing supplements were assumed to be taken according to the manufacturer's specifications. Supplements containing vitamin D use (yes/no) was obtained from the interviewer-administrated questionnaire and prescribed vitamin D medication from GP records (Mendonça et al., 2015).

2.2.9. Statistical analysis

The normality of the distributions was assessed by reviewing the histograms and Q-Q plots, and the Shapiro-Wilk test was applied. The normally-distributed, continuous variables were presented as means and standard deviations (SD), while the non-Gaussian

distributed variables were presented as medians and interquartile ranges (IQR). The categorical data were presented as percentages (with the corresponding sample size). Mann-Whitney and kruskal-wallis tests were used for ordered and non-normally distributed continuous variables, and a $\chi 2$ test for the categorical variables.

To determine the association between potential determinants and 25(OH)D concentration, a binary logistic regression was used. Multinomial logistic regression was used to determine the association between disability and 25(OH)D concentration. To examine the association between 25(OH)D with baseline NT-proBNP, FEV1 and FVC, linear regression was used separately for each independent variable. To examine the association between 25(OH)D with prospective Telomere Length, linear regression was used. More details about the statistical analysis of the variables will be provided in the methodology section of each chapter.

2.2.10. General Characterization of the Included Population

The analysis of this thesis was restricted to those with an available measurement of their 25(OH)D concentration (n= 775). The population included in the analysis consisted of 304 men (39%) and 471 women (61%). The majority of them (40%) had a normal BMI, of whom 42% were moderately active, and 77% of whom rated their health as good or excellent with a mean disease of 5, while only 16.5% of them used vitamin D-containing medication and 19.5% of them used supplement. Their mean 25(OH)D was 45.43 nmol/l. Moreover, out of the entire sample, 21.2%, 15.6%, 39.7% and 23.1% had their blood taken in Winter, Spring, Summer and Autumn, respectively.

2.2.11. Strengths and Weaknesses of the Newcastle 85+ Study

The Newcastle 85+ Study is a unique cohort that exclusively focused on one age group, which minimizes the effect of age variability. Additionally, it includes a large number of participants and an extensive amount of multidimensional health data. The participants were all from Newcastle upon Tyne and North Tyneside and from a predominantly white background, including those who were institutionalised and

cognitively impaired very-old adults. Even though it was a representative study of the UK population, generalisations to other geographical locations and to population with different ethnicities should be undertaken with caution. The rapid processing of the blood samples following venepuncture was another strength of this study.

Chapter 3: A systematic review of observational studies reporting vitamin D status in very-old adults across the world

Key words: 25(OH)D, community, institutionalised, latitude, aged, 80 and over

3.1.Abstract

***Objective**:* This study aimed to assess the worldwide 25(OH)D concentration in very-old adults (aged over 80 years) in the available literature, and to explore any association with living condition (Community living or Institutionalised) and geographical location.

***Design**:* Systematic review of cross-sectional studies to assess 25(OH)D concentration in very-old adults.

***Setting**:* Four databases were searched: Medline, ProQuest, PubMed and Web of Science.

***Participants**:* Community-dwelling and institutionalised very-old participants with a reported concentration of 25(OH)D.

***Results**:* A total of 23 studies were included in this systematic review. Of them, 20 studies reported 25(OH)D concentration. The other three were only reported the prevalence of deficiency. Four studies were from the USA, 11 from Europe, three were from China and Japan, and three from Australia and New Zealand. The highest concentration of 25(OH)D was reported in community-dwelling very-old adults from the USA (mean= 82 (74.6-89.4) nmol/l), while the lowest concentration was reported in institutionalised very-old adults from Austria (mean= 17.8 (16.6-19.1) nmol/l). Using the concentration 25 nmol/l threshold to define vitamin D deficiency (used in 8 of the 18 studies), the lowest percentage of deficiency was found amongst the very-old in New Zealand (2.4%), while the highest percentage was in the UK (22%).

***Conclusion**:* 25(OH)D concentration and the prevalence of deficiency vary between very-old adults depending on latitude and their living conditions.

3.2.Introduction

Very-old adults, who are defined as those aged over 80 years, are the fastest growing age group (Collerton et al., 2007). Ageing brings about notable changes in physiological and behavioural factors, which puts older adults at risk of poor health conditions, including poor vitamin D status (Pramyothin and Holick, 2014). To date, the majority of studies assessing vitamin D status in very-old adults have included the 'young-old or those aged less than 80 years of age. Even though the vitamin D requirements of very-old adults are probably different to those of younger older adults, there is a lack of knowledge regarding their precise requirements.

The young-old tend to be retired, enjoy good health and have more time to go outside and gain exposure to sunlight (thus keeping their concentration of 25(OH)D stable (>30 nmol/l) (Barnett et al., 2012)). According to the National Diet and Nutritional Survey (NDNS) report, only 15–27% and 4–9% of those aged 65 years and over have a 25(OH)D concentration below 25 nmol/l in the Winter and Summer months, respectively, in the UK. On the other hand, those aged 80 years or older tend to be less active, have more chronic diseases (Collerton et al., 2009) and their skin's ability to synthesise vitamin D is reduced, which subsequently increases their risk of developing low vitamin D status (MacLaughlin and Holick, 1985). Using SACN cut-off, approximately 51.7% of those with a mean age of 85 years-old (including young-old adults) had a concentration of 25(OH)D less than 25 nmol/l (Maier et al., 2013). In addition, those who were institutionalised were at a higher risk of vitamin D deficiency; up to 80% of the very-old institutionalised population (aged >40 years-old) have been reported as having a concentration of 25(OH)D <25 nmol/l (Spiro and Buttriss, 2014).

There have been few studies that have evaluated 25(OH)D concentration in very-old adults. In most studies, the maximum age of the participants is 80 years-old, and rarely do studies include participants aged 90 or older. For example, only 14% of the participants were over 80 years-old in a study that aimed to assess vitamin D deficiency in old adults (Nakamura et al., 2011). Furthermore, the majority of studies do not stratify the concentration of 25(OH)D by participants' age. For instance, 29% of the participants in a study by Rothenbacher et al. (2014) were aged ≥80 years-old, but their 25(OH)D

concentration was not reported separately. Since vitamin D may play an important role in the prevention and treatment of skeletal and non-skeletal diseases, such as cancer, cardiovascular disease, infections and autoimmune disease (Bikle, 2014), it is necessary to evaluate this group's concentration of 25(OH)D. Therefore, this review aims to:

- Assess the distribution of 25(OH)D concentration worldwide.
- Consider the geographical and living conditions variation (Community living or Institutionalised) amongst very-old adults.
- Identify the regions with a lack of research on 25(OH)D concentration in very-old adults.

3.3. Methodology

The data collection process for the systematic review was illustrated in detail in Chapter 2. Briefly, all of the observational studies, including cohort and cross-sectional studies, were collected, with no restriction on region, year or language. Cohort studies (only the baseline wave) also reported data for the total population of very-old adults at baseline. All participants were also considered and included, regardless of whether they were institutionalised or community- dwelling, or any demographic factor, sex or ethnicity. The primary criterion was that they were ≥80 years-old.

3.4. Results

Study collection

In total, 5835 titles and abstracts were screened (Figure 3.1) and, following the eligibility criteria, 113 were recorded. The full-text of these articles was assessed in terms of quality criteria. The population of four studies overlapped with other studies included in the analyses. The latest studies with the largest population size were retained and the others were excluded (Chen et al., 2007, Zochling et al., 2002, Veronese et al., 2014). As a result of this, the final number of studies included was 20, from each of which the data were extracted. Of the 20 studies, 18 studies reported 25(OH)D concentration and the

other two reported the prevalence of deficiency. Several studies included multiple age groups of participants (De Rui et al., 2014, Schilling, 2012, Hirani and Primatesta, 2005).

Summary of the studies' characteristics

A summary of the characteristics of the 23 studies is shown in Tables 3.1-3.4. The majority of the studies included both sexes, with two focusing solely on men (Center et al., 1999, Delos Reyes et al., 2017) and two focusing solely on women (Bruyère et al., 2014, Terabe et al., 2012). Only four studies assessed participants who were both community-dwelling and institutionalised (Bruyère et al., 2014, Hill et al., 2016, Hirani and Primatesta, 2005, Matheï et al., 2013), while four studies included participants who were institutionalised only (Muschitz et al., 2015, Terabe et al., 2012, Schilling, 2012, Passeri et al., 2003) and the remainder included only community-dwelling participants.

A total of 10 studies (Hill et al., 2016, Giuliani et al., 2018, Bacon et al., 2016, Looker et al., 2002, Bolland et al., 2006, Passeri et al., 2003, Delos Reyes et al., 2017, Terabe et al., 2012, Schilling, 2012, Kupisz-Urbańska et al., 2020) reported that they recruited the participants and collected their blood throughout the year, while the other 11 failed to report the time of year when they collected the 25(OH)D of the participants. Only two studies specified the months of collecting the blood samples (Ten Haaf et al., 2019, Tanabe et al., 2019). Nine studies (Bacon et al., 2016, Bruyère et al., 2014, De Rui et al., 2014, Hill et al., 2016, Jacques et al., 1997, Johnson et al., 2008, Ning et al., 2016, Tanabe et al., 2019, Kupisz-Urbańska et al., 2020) mentioned that 18-40% of their participants took supplements. Moreover, the majority of the included studies failed to report the health condition and PA of the participants, with the exception of five studies, who reported the percentage of participants with chronic disease (Hill et al., 2016, Matheï et al., 2013, Passeri et al., 2003, De Rui et al., 2014, Haslam et al., 2014) and the level or type of the PA (Hill et al., 2016, Jacques et al., 1997, Matheï et al., 2013, Passeri et al., 2003, De Rui et al., 2014), respectively.

Summary of the concentration of the 25(OH)D across the world

The mean 25(OH)D concentration of the very-old adults from the 18 studies is shown in Tables 3.1-3.4. The highest concentration of 25(OH)D was reported in community-dwelling very-old adults from the USA (mean= 82 (74.6-89.4) nmol/l) (Jacques et al., 1997), while the lowest concentration was reported for institutionalised very-old from Austria (mean= 17.8 (16.6-19.1) nmol/l) (Muschitz et al., 2015). That is, the highest concentration was reported in a country that is located at a latitude of 42.2, where it is legislated for milk to be fortified with vitamin D (Jacques et al., 1997), while the lowest concentration was reported by institutionalised participants living at a latitude of Austria (47.5) (Muschitz et al., 2015).

Our findings also showed that the prevalence of vitamin D deficiency varied between different latitudes. There was heterogeneity between the studies regarding how vitamin D deficiency was defined. Using a 25(OH)D concentration of <25 nmol/l to define vitamin D deficiency, 9 of the 21 studies reported that the lowest prevalence of deficiency was among very-old New Zealand participants (2.4%) (Bacon et al., 2016), which could also be explained by the use of vitamin D supplements (18% of the participants). However, the study did not mention whether these supplements were over-the-counter or prescribed. Another explanation could be the type of participants. It is possible that those who participated in the study or provided blood samples differed in terms of vitamin D status from those who did not. In the other words, those who agreed to provide blood reported less difficulty with performing their daily living tasks independently, and were also younger, leaner and more active, which facilitated their exposure to outdoor sunlight. In contrast the highest prevalence was among very-old United Kingdom participants (22%) (Hirani and Primatesta, 2005), which is located at a latitude of 54.9, with less than 19% of the participants taking supplements (Hill et al., 2016).

a. ***Studies from Europe included in the systematic review***

Among the 13 European included studies (Table 3.1), the range of 25(OH)D varied between the countries. Ten of them reported the 25(OH)D concentration. The

lowest concentration of 25(OH)D reported was 17.8 (16.6-19.1) nmol/l in Austria (Muschitz et al., 2015), while the highest concertation reported was 79.2 (74.4-84.0) nmol/l in Italy (De Rui et al., 2014) and 81.7 (52.1-111.3) nmol/l in Spain (Bruyère et al., 2014). Different cut-offs were used to define a deficiency in 25(OH)D. Using the 25 nmol/l threshold, the proportion of deficiency was around 18-22% (Giuliani et al., 2018, Hirani and Primatesta, 2005).

The study by Bates et al. (2003) included 119 community- dwelling participants of both sexes. Of these, 24 participants had a low 25(OH)D concentration. The study did not report the mean 25(OH)D concentration of the participants. The study by De Rui et al. (2014) included 132 men with a mean concentration of 90.5 (56.5-121.27) nmol/l and 210 women with a mean concentration of 47.5 (29-68) nmol/l. The study by Hill et al. (2016) included 526 women and 249 men living in the community or institutions. Overall, the mean 25(OH)D concentration of the participants was 42 (11-73) nmol/l and 255 of them had a concentration <30 nmol/l. The study by Hirani and Primatesta (2005) included 62 men and 71 women living in the community and 128 men and 369 women living in institutions. Their mean 25(OH)D concentration was 47.5 (43.5-51.5) nmol/l, 44.7 (42.3-42.3) nmol/l, 37.1 (35.3-35.3) nmol/l and 36.6 (35.6-37.6) nmol/l, respectively. The study by Kupisz-Urbańska et al. (2020) included 81 men and 16 women living in the community with mean 25(OH)D concentration 18.4 (13.7-30.8) nmol/l. The study by Matheï et al. (2013) included 133 men and 234 women living in the community and institutions. Approximately 32.7% of them had a concentration <25 nmol/l (which considered low). The researchers did not report the mean of the 25(OH)D concentration. The study by Muschitz et al. (2015) included 790 men and 2,577 women, who reported a mean 25(OH)D of 37.4 nmol/l and 44 nmol/l, respectively. The study by Passeri et al. (2003) included 90 women and 14 men with a mean 25(OH)D concentration of 20.35 (11.54-29.25) nmol/l. The study by Delos Reyes et al. (2017) included 136 participants of both sexes with a 25(OH)D concentration of 55 (29-79) nmol/l, and 19.4% of them had a concertation <25 nmol/l. The study by Schilling (2012) included 1,578 participants of both sexes, with a mean 25(OH)D concentration of 23 nmol/l. Finally, the study by Ten Haaf et al. (2019) included 19 men and 6 women living in the community and their mean 25(OH)D concentration was 77.8 (59.2- 96.4) nmol/l.

Focusing on the study by Bruyère et al. (2014), it included women from nine European countries, as shown in Table 3.1. Overall, the total number of women included in the study was 1,984, with a mean 25(OH)D concentration of 53.3 (26.6-80) nmol/l, and 44.5% of them had a concentration <50 nmol/l. The highest concentration was reported in Spain of 81.7 (52.1-111.3) nmol/l, while the lowest concentration was reported in Belgium of 45.7 (24.9-66.5) nmol/l. Using the 50 nmol/l threshold, the prevalence of deficiency varied between 9.8% in Spain to 22.3% in the UK (Bruyère et al., 2014) (Figure 3.2).

b. ***Studies from Australia and New Zealand included in the systematic review***

Among the three included studies from New Zealand and Australia (Table 3.2), the concentration of 25(OH)D varied from 44.2 (24.4- 64) nmol/l (Bolland et al., 2006) to 66.5 (38-95) nmol/l (Bacon et al., 2016), which is considered a sufficient concentration. The prevalence of deficiency cannot be compared due to the diversity of the cut-off used by the various studies (see Figure 3.4).

As shown in Table 3.2, the study by Bacon et al. (2016) included 199 and 180 Maori and non-Maori women, and 90 and 177 Maori and non-Maori men, respectively. The 25(OH)D concentration was 59 (33-85) nmol/l, 74 (43-105) nmol/l, 58 (31-85) nmol/l and 76 (47-105) nmol/l, Maori and non-Maori women and men, respectively. Using 25 nmol/l, only 12 (2%) participants recorded deficiency. Only 6 men and 125 women were included in the Bolland et al. (2006) study and their 25(OH)D concentration was 80.5 (60-110) mol/l and 44.2 (15-135) nmol/l, respectively. The researchers did not report the prevalence of deficiency among the participants. Finally, among the 23 men who participated in the study by Center et al. (1999), the 25(OH)D concentration was 65 (49.8- 78.2) nmol/l, and 37.5% of them had a concentration <58 nmol/l. Giuliani et al. (2018) study included 1,987 men and 5,668 women with a mean 25(OH)D concentration of 57.5 (21.3-93.7) nmol/l and 68 (26.5-109.5) nmol/l, respectively. Using the <25 nmol/l threshold, 19.7% of the men and 17% of the women had a deficiency.

c. ***Studies from North America included in the systematic review***

Among the four included studies from North America (the USA and Canada) (Table 3.3), the 25(OH)D concentration was around 60 nmol/l (Looker et al., 2002) to 75.1 nmol/l (Johnson et al., 1995), which is considered a sufficient concentration. Three studies used the 25 nmol/l threshold and the prevalence of deficiency was 2-7% (Looker et al., 2002, Johnson et al., 1995, Jacques et al., 1997) (Figure 3.5).

A total of 160 of the 194 participants were women in the study by Haslam et al. (2014). Overall, the mean age of the participants was 100.3 years-old. The mean 25(OH)D concentration was 68.4 nmol/l and 35.1% of them had a concentration <50 nmol/l. The study by Jacques et al. (1997) included 92 women and 59 men with 63.2 (57.3-69.1) nmol/l and 82 (74.6-89.4) nmol/l, respectively. Using 25 nmol/l threshold, 3 and 7 % of them, respectively had deficiency. The study by Johnson et al. (1995) included 27 men and 53 women. The mean 25(OH)D concentration of the men was 74.2 (41-107.4) nmol/l and 18.5% of them had a concentration <50 nmol/l. The 25(OH)D concentration of the women was 75.5 (40.4- 110.6) nmol/l and 24.5% of them had a concentration <50 nmol/l. Finally, 553 men were included in the study by Looker et al. (2002) with a 25(OH)D concentration of 68 nmol/l, and only 3% had a concentration <25 nmol/l. The same study also included 605 women with a 25(OH)D concertation of 60 nmol/l and only 4% had a concentration <25 nmol/l.

d. ***Other studies included in the systematic review***

Only three other studies were found and included in this section (Table 3.4); one from China (Ning et al., 2016) and two from Japan (Terabe et al., 2012). The Chinese study included both sexes living in the community and used EC-Immunoassay (electrochemiluminescence immunoassay). One Japanese study included only women and used a RIA assay (Terabe et al., 2012) and the other one included both sex and used 25(OH)D total assay (Tanabe et al., 2019). However, the 25(OH)D concentration was 37.2 (11.2- 63.2) nmol/l and 40.68 (28.98- 52.38) (Ning et al., 2016) (Terabe et al., 2012), respectively. Two studies used the 63 nmol/l threshold and the prevalence of deficiency was 87.1% is China (Ning et al., 2016) and 78.1% in Japan (Terabe et al.,

2012) (Figure 3.6). The last study used 50 nmol/l threshold and the prevalence of the deficiency was 72% (Tanabe et al., 2019)

Impact of Living Conditions (Community living or Institutionalised ***on estimated vitamin D status***

Of the studies that included community-dwelling participants, two was from China (Ning et al., 2016, Tanabe et al., 2019), one from Australia (Center et al., 1999), six from Europe (Giuliani et al., 2018, De Rui et al., 2014, Delos Reyes et al., 2017, Kupisz-Urbańska et al., 2020, Ten Haaf et al., 2019, Bates et al., 2003), two from New Zealand(Bacon et al., 2016, Bolland et al., 2006), and four from the United States of America (USA) (Looker et al., 2002, Haslam et al., 2014, Jacques et al., 1997, Johnson et al., 2008). Using the SACN cut-off, the community-dwelling participants had an adequate concentration of 25(OH)D, especially those from the USA, New Zealand, and Australia. The participants from Asia [China] reported the lowest 25(OH)D concentration, but this still lay within the sufficient range (mean= 37.2 (35.3-39.0) nmol/l). However, the men participants showed the highest concentration, especially those from from the USA, New Zealand and Italy. No studies were found in Africa, India or the Middle East.

Of the studies that included institutionalised participants, only one was from Japan (Terabe et al., 2012) and three from Europe (Muschitz et al., 2015, Schilling, 2012, Passeri et al., 2003). The institutionalised participants, on the other hand, showed a low concentration of 25(OH)D, except for those from Asia [Japan] (mean= 40.6 (39.2-42.1) nmol/l). That said, all of the other institutionalised participants were from Europe and reported a mean 25(OH)D concentration <25 nmol/l.

Impact of sex on estimated vitamin D status

Generally, men had a higher concentration of 25(OH)D than women, regardless of living conditions and latitude. Focusing on the studies that included one sex, only two studies included solely men and two included solely women. The male-only studies were

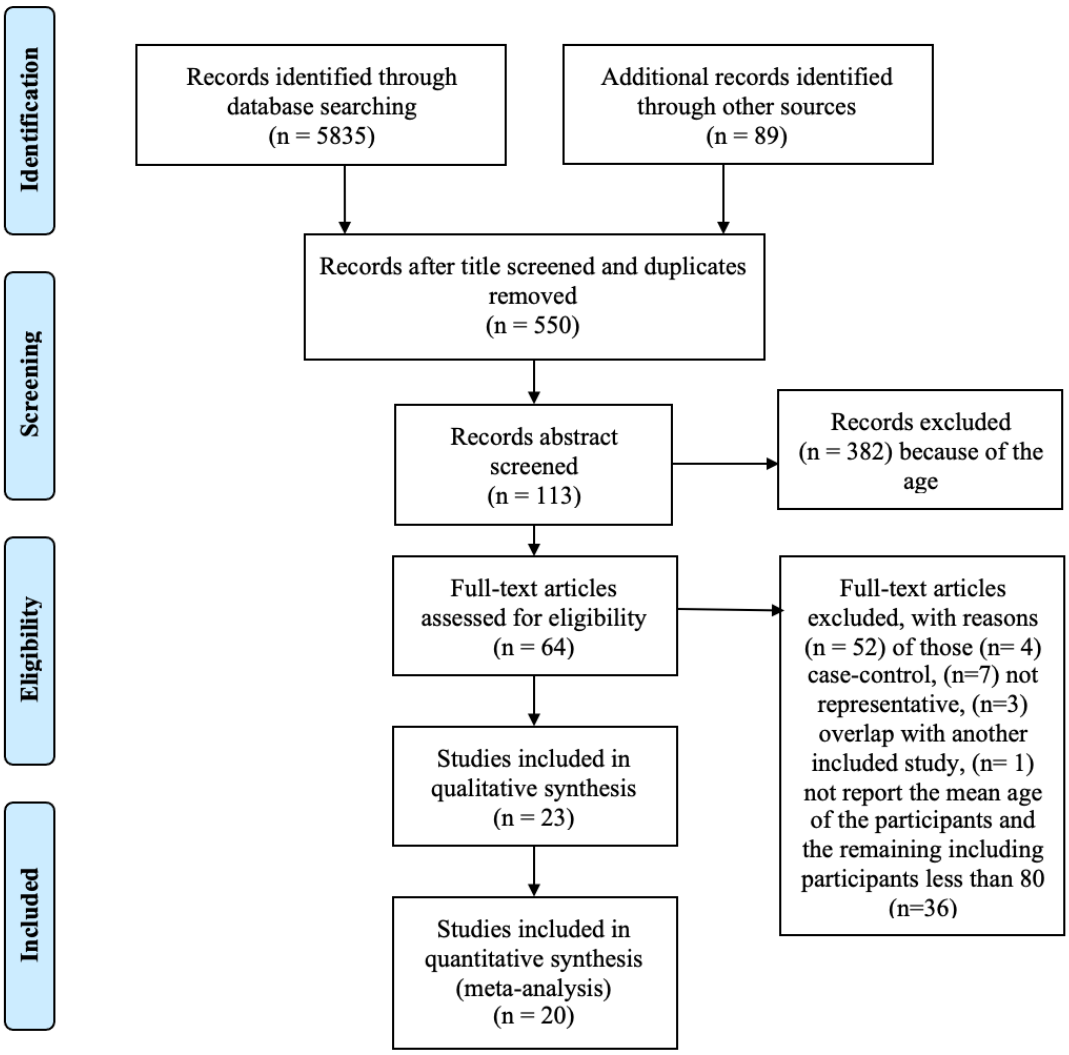
from Australia (Center et al., 1999, Delos Reyes et al., 2017) and Ireland (Center et al., 1999, Delos Reyes et al., 2017) and both included community-dwelling participants. The female-only studies were from nine European countries (Bruyère et al., 2014, Hill et al., 2016) and Japan (Terabe et al., 2012) and included both community-dwelling and institutionalised participants.

Impact of assays for 25(OH)D on estimate of vitamin D status

Nine different assays were used to measure the 25(OH)D concnetration in the current review. Even though the assays that were used to measure 25(OH)D varied between the studies, the majority of the studies used the Radioimmunoassay (RIA), with the exception of two studies, that used the mass spectrometry (MS) assay (Schilling, 2012, Delos Reyes et al., 2017, Tanabe et al., 2019), Bacon et al. (2016), who used high-performance liquid chromatography (HPLC) assay, Hirani and Primatesta (2005) and Matheï et al. (2013), who used the chemiluminescent immunoassay (CLIA) assay, Jacques et al. (1997), who used CBP, Ning et al. (2016), who used the EC assay, Muschitz et al. (2015), who used the Abbott Architect platform assay, Tanabe et al. (2019) who used 25(OH)D total assay and finally, Kupisz-Urbańska et al. (2020) who used ELISA.

The highest concentration (66.5 nmol/l) was measured by HPLC (Bacon et al., 2016) while the lowest concentration (17 nmol/l) was mesured by Abbott Architect platform (Muschitz et al., 2015). However, the participants were community-dwelling from New Zealand and institutionalised from Austria, respectively.

Figure 3.1: Flow diagram of the screening procedure that was followed to identify eligible studies



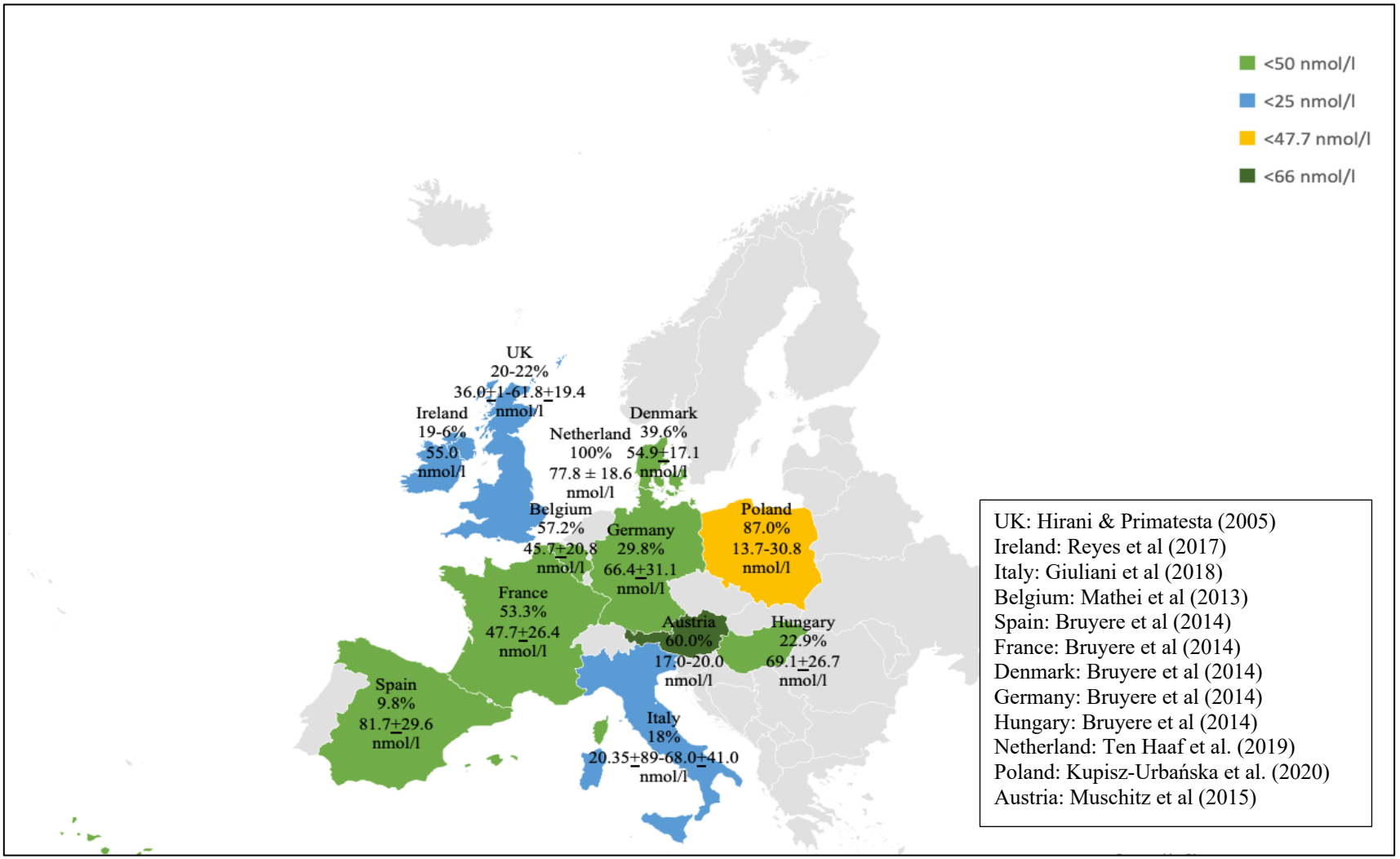


Figure 3.2.: Prevalence of 25(OH)D concentration deficiency in Europe using the cut-offs of the study (prevalence of deficiency % and mean ±SD) Mean ± SD (nmol/l). SD: standard deviation. Cut-offs were set by the studies.

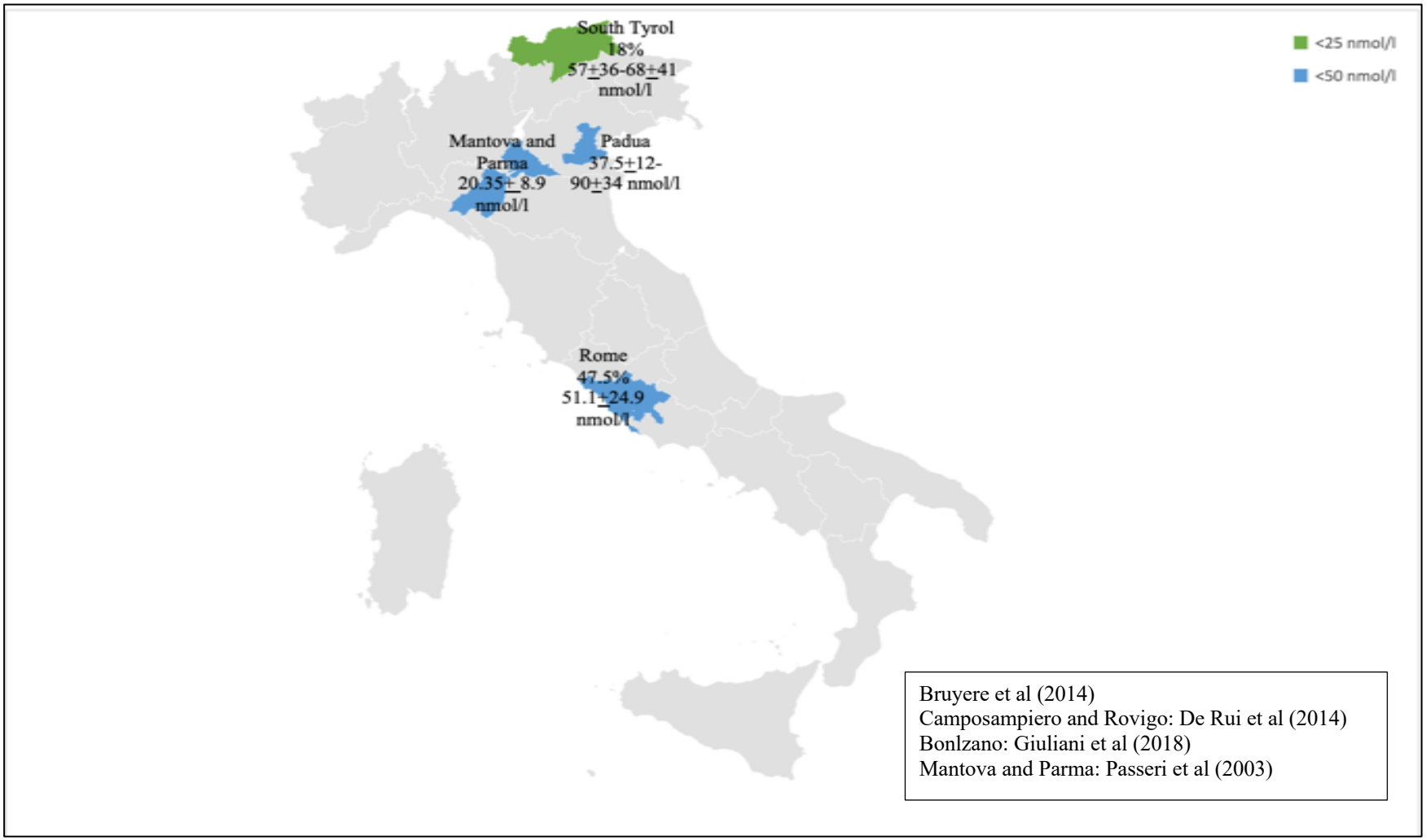


Figure 3.3: Prevalence of 25(OH)D concentration deficiency in Italy using the cut-offs of the study (prevalence of deficiency % and mean ±SD nmol/l). Mean ± SD (nmol/l). SD: standard deviation. Cut-offs were set by the studies.

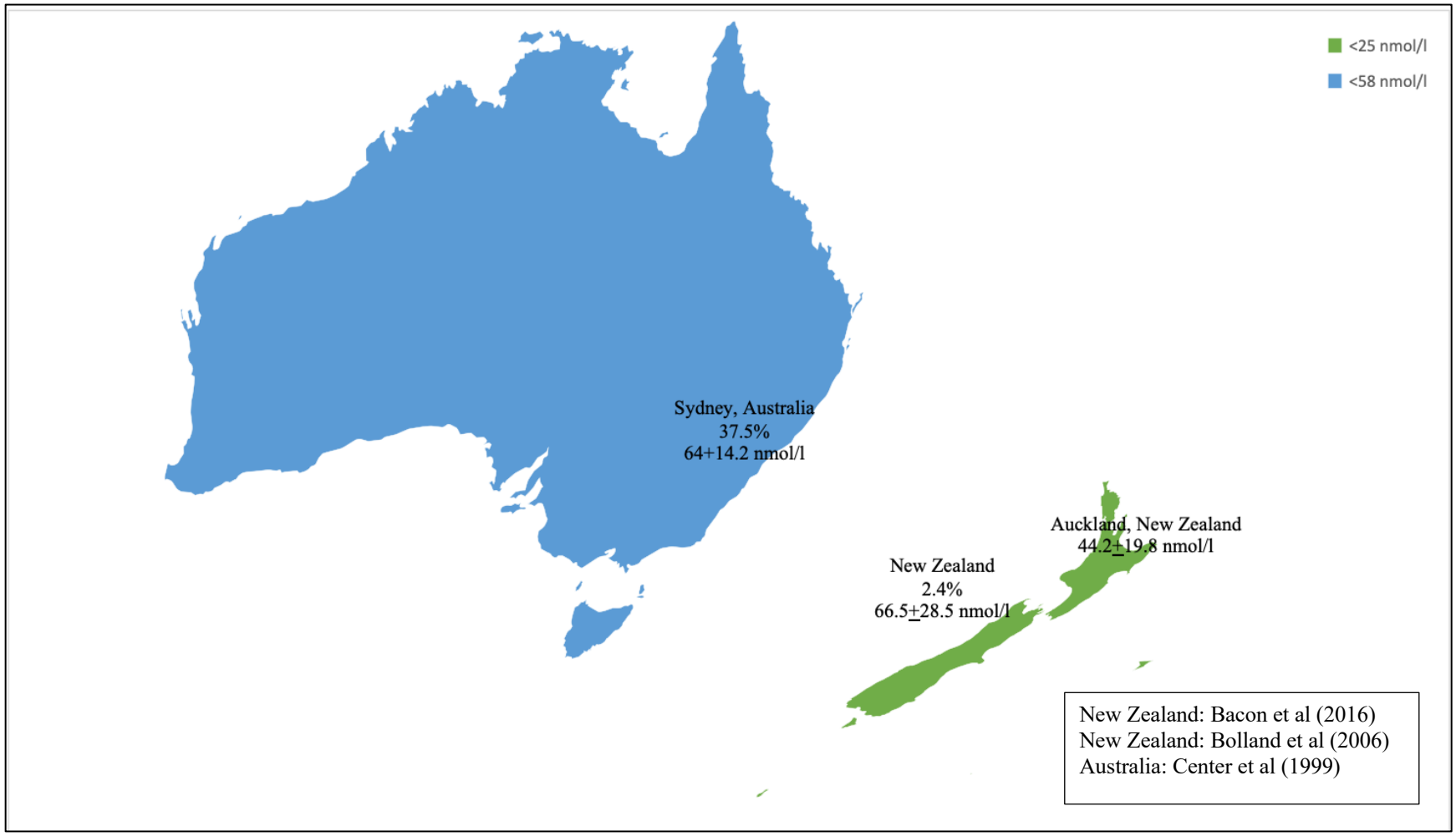


Figure 3.4: Prevalence of 25(OH)D concentration deficiency in New Zealand and Australia using the cut-offs of the study (prevalence of deficiency % and mean ±SD nmol/l).
Mean ± SD (nmol/l). SD: standard deviation. Cut-offs were set by the studies.

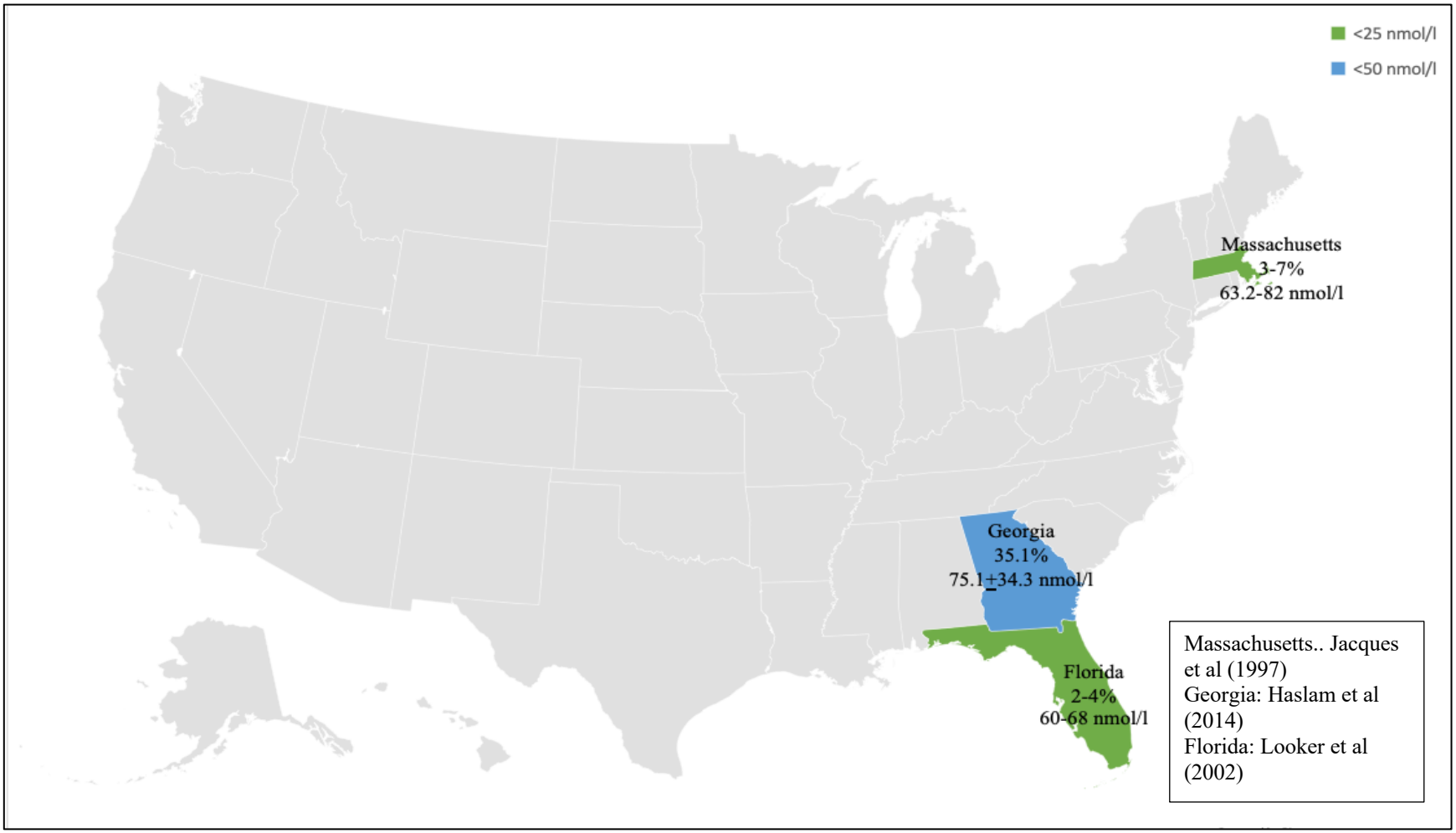


Figure 3.5: Prevalence of 25(OH)D concentration deficiency in North America using the cut-offs of the study (prevalence of deficiency % and mean ±SD nmol/l).
Mean ± SD (nmol/l). SD: standard deviation. Cut-offs were set by the studies.

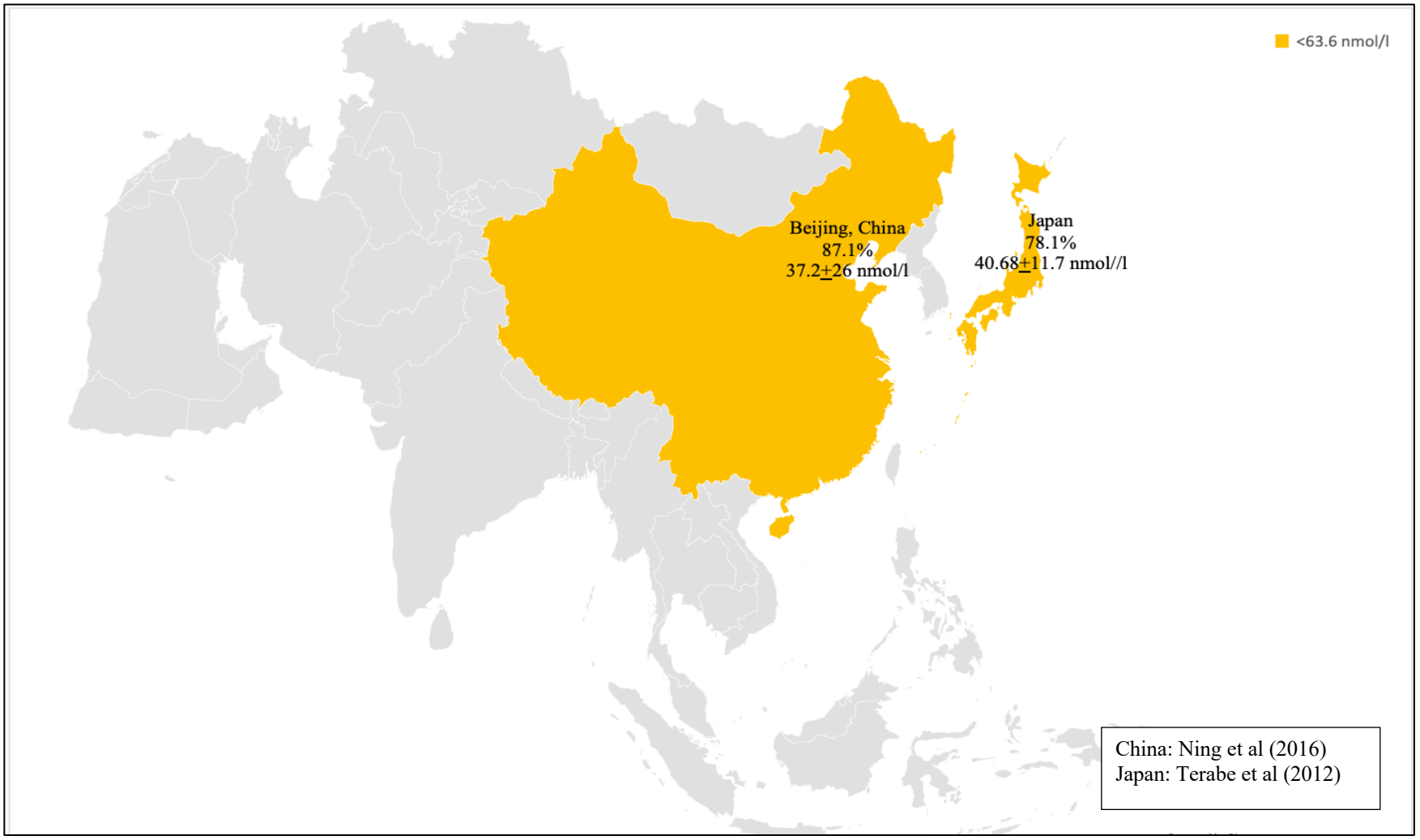


Figure 3.6: Prevalence of 25(OH)D concentration deficiency of the other studies using the cut-offs of the study (prevalence of deficiency % and mean ±SD nmol/l) . Mean ± SD (nmol/l). SD: standard deviation. Cut-offs were set by the studies.

Table 3.1: Summary of European studies included in the systematic review reporting the prevalence of vitamin D deficiency and the mean of 25(OH)D concentration

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Health condition	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
Bates et al (2003)	England, UK (52.3)	119	M/F	NA	Community	NA	NA	NA	<25 nmol/l	20%	NA	RIA
Bruyere et al (2014)	Europe:	1984	F	NA	Community + Institutionalised	NA	NA	Yes	<50 nmol/l	44.5%	53.3±26.7	RIA
	Belgium (50.5)	430								57.2%	45.7±20.8	
	Denmark (65.2)	53								39.6%	54.9±17.1	
	France (46.2)	821								53.5%	47.7±26.4	
	Germany (51.1)	47								29.8%	66.4±31.1	
	Hungary (47.1)	48								22.9%	69.1±26.7	
	Italy (41.8)	101								47.5%	51.1±24.9	
	Poland (51.9)	179								27.4%	64.4±29.0	
	Spain (40.4)	102								9.8%	81.7±29.6	
	UK (55.3)	203								22.3%	61.8±19.4	
De Rui et al (2014)	Camposampiero and Rovigo, Italy (45)	676	M/F	NA	Community	Depression, cognitive impairment, CVD, neurodegenerative, osteo-	27-37% independent	Yes	<50 nmol/l	NA	40.0-80.0	RIA

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Health condition	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
						articular osteoporosis, cancer, diabetes, chronic pulmonary						
Giuliani et al (2018)	Bonlzano, Italy (46.4)	74235	M/F	All year	Community	NA	NA	NA	<25 nmol/l	18.0%	57-68± 36-41	RIA
Hill et al (2016)	Newcastle, UK (54.9)	775	M/F	All year	Community + Institutionalised	Mean disease count= 3.2	33% independent	Yes	<30 nmol/l	33.0%	42±3	RIA
Hirani & Primatesta (2005)	England, UK (52.3)	630	M/F	NA	Community + Institutionalised	NA	NA	NA	<25 nmol/l	22.0%	36-47	CLIA
Kupisz-Urbańska et al. (2020)	Warsaw, Poland (52.2)	97	M/F	All year	Community	NA	NA	Yes	<47.7 nmol/l	87.0%	18.4 (13.7–30.8)	ELISA
Mathei et al (2013)	Wallonia, Brussels and Flanders, Belgium (50.5)	376	M/F	NA	Community + Institutionalised	NA	29.5% Independent	NA	<25 nmol/l	32.7%	NA	CLIA
Muschitz et al (2015)	Austria (47.5)	3367	M/F	NA	Institutionalised	NA	NA	NA	<66 nmol/l	60.0%	17-20	Abbott Architech platform

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Health condition	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
Passeri et al (2003)	Mantova and Parma, Italy (44.8)	4	M/F	All year	Institutionalised	Hypertension dementia, Cardio, diabetes, malignant tumours.	38% independent	NA	NA	NA	20.3± 8.9	RIA
Reyes et al (2017)	Galway, Ireland (53.2)	701	M	All year	Community	NA	NA	NA	<25 nmol/l	19.6%	55.0	MS
Schilling et al (2012)	Trier, Germany (51)	1578	M/F	All year	Institutionalised	NA	NA	NA	<39 nmol/l	2.0%	NA	MS
Ten Haaf et al. (2019)	Netherland	25	M/F	March & July	Community	NA	Sports activities, hr/wk 3.5 ± 7.8	Yes	<50 nmol/l	100.0%	77.8 ± 18.6	MS

M: men, F: women, NA: not applicable, PA: physical activity. RIA: radioimmune assay, CLIA: chemiluminescent immunoassay, MS: mass spectrometry.

Table 3.2: Summary of New Zealand and Australia studies included in the systematic review reporting the prevalence of vitamin D deficiency and the mean of 25(OH)D concentration

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Disease	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
Bacon et al (2016)	New Zealand (38)	566	M/F	All year	Community	NA	NA	Yes	<25 nmol/l	2.4%	66.5±28.5	HPLC
Bolland et al (2006)	Auckland, New Zealand (36)	131	M/F	All year	Community	NA	NA	NA	<50 nmol/l	NA	44.2±19.8	RIA
Center et al (1999)	Sydney, Australia (33.8)	23	M	NA	Community	NA	NA	NA	<58 nmol/l	37.5%	64±14.2	RIA

M: men, F: women, NA: not applicable, PA: physical activity. HPLC: high-performance liquid chromatography, RIA: radioimmuno assay.

Table 3.3: Summary of North America studies included in the systematic review reporting the prevalence of vitamin D deficiency and the mean of 25(OH)D concentration

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Disease	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
Haslam et al (2014)	Georgia, USA (32)	194	M/F	NA	Community	Congestive heart failure, COPD	NA	No	<50 nmol/l	35.0%	68.4	RIA
Jacques et al (1997)	Framingham, USA (42.2)	147	M/F	NA	Community	53-66% no disease	NA	Yes	<25 nmol/l	3-7%	63.2-82.0	CBP
Johnson et al (2008)	Georgia, USA (32)	317	M/F	NA	Community	NA	NA	Yes	<25 nmol/l	5.0%	75.1±34.3	RIA
Looker et al (2002)	USA (25-47)	1155	M/F	All year	Community	NA	NA	No	<25 nmol/l	2-4%	60-68	RIA

M: men, F: women, NA: not applicable, PA: physical activity., COPD: chronic obstructive pulmonary disease. RIA: radioimmune assay, CBP: Comparative binding protein assay.

Table 3.4: Summary of other studies included in the systematic review reporting the prevalence of vitamin D deficiency and the mean of 25(OH)D concentration

Reference	Country (latitude)	Sample Size (n)	Sex	Month	Living setting	Disease	PA	Supplement	Cut-off Deficiency	Prevalence below 25(OH)D threshold %	Concentration of 25(OH)D (nmol/l) mean ± SD	Assay
Ning et al (2016)	Beijing, China (39.9)	738	M/F	NA	Community	NA	NA	Yes	<63.3 nmol/l	87.1%	37.2±26.0	EC-Immunoassay
Terabe et al (2012)	Japan (36.2)	403	F	All year	Institutionalised	NA	NA	NA	<63.3 nmol/l	78.1%	40.6±11.7	RIA
Tanabe et al. (2019)	Nakano, Japan (35.7)	209	M/F	May-August	Community	pneumonia, urinal tract infection, gastrointestinal, bone fractures	NA	NA	<50 nmol/l	72.0%	NA	25(OH)D total assay

M: men, F: women, NA: not applicable, PA: physical activity., RIA: radioimmune assay, EC: electrochemiluminescence immunoassay.

3.5. Discussion

Main findings

Based on the findings of this study, latitude, living conditions and the assays used to measure concentration of 25(OH)D are the main predictors of vitamin D status among very-old adults. The lowest prevalence of vitamin D deficiency was found in New Zealand, while the highest was found in the UK. Overall, the community-dwelling participants reported a sufficient 25(OH)D concentration, whereas the institutionalised participants reported a deficient 25(OH)D concentration, regardless of their latitude. The highest and lowest 25(OH)D concentration and prevalence of deficiency were measured by different assays.

Evidence from other studies

The highest concentration of 25(OH)D was reported in the USA, while the lowest was reported in Austria. That is, the highest concentration was reported in a country that is located at a latitude of 42.2, where it is legislated for milk to be fortified with vitamin D (Jacques et al., 1997), while the lowest concentration was reported by institutionalised participants living at a latitude of Austria (47.5) (Muschitz et al., 2015). Our findings were consistent with previous studies that showed the influence of latitude on 25(OH)D concentration (Webb et al., 1988). Similarly, two studies in the USA (latitude of 42.2) involved participants with a mean age of ≥80 years-old, who reported a concentration of 94 nmol/l and 87 nmol/l amongst both community-dwelling and institutionalised participants, respectively (Peterson et al., 2012, Schwartz et al., 2018). Conversely, two studies from Austria included institutionalised participants with a mean age of ≥80 years-old, who reported a concentration of 17 nmol/l and 22 nmol/l, respectively (Pilz et al., 2012, Trummer et al., 2012).

Our findings also showed that the prevalence of vitamin D deficiency varied between different latitudes. The lowest prevalence of deficiency in vitamin D status was found in New Zealand (Bacon et al., 2016), which could also be explained by the use of vitamin D supplements (18% of the participants). However, the study did not mention whether these supplements were over-the-counter or prescribed. Another explanation could be the type of participants. It is possible that those who participated in the study or provided blood samples differed in terms of vitamin D status from those who did not. Sunlight and latitude could also potentially explain these findings. For example, the participants were drawn from Northern New Zealand which has a latitude of 37°South. Dermal vitamin D production is possible for

most of the year at the subtropical climate of the North Island of New Zealand. In contrast, the highest prevalence of deficiency in vitamin D status was found in Newcastle, UK, which is located at a latitude of 54.9, with less than 19% of the participants taking supplements (Hill et al., 2016).

Moreover, overall, the findings showed that very-old community-dwelling participants have a sufficient 25(OH)D concentration across the world. However, the community-dwelling participants from China, where the latitude is 39.9 degrees, reported the lowest mean concentration, 25(OH)D (37 nmol/l), but, according to the Ning et al. (2016) study, the participants' vitamin D intake was less than 100 IU/d (2.5 µg/d). Besides, food products are rarely fortified with vitamin D in China and vitamin D-rich foods are not a common part of the daily staple diet for Beijing residents. Moreover, Chinese women have a preference for having fairer skin, so they usually avoid exposure to sunlight (Ning et al., 2016).

Contrary to this, our results were consistent with the previous studies, which found that institutionalised very-old adults are at risk of a low vitamin D status, regardless of the latitude at which they live. For example, community-dwelling participants from Italy reported a concentration of 79.2 (74.4-84.0) nmol/l (De Rui et al., 2014), while institutionalised participants from Italy reported a concentration of 20.3 (16.2-24.4) nmol/l (Passeri et al., 2003). A further two studies from Australia showed that institutionalised participants (a mean age of <80 years-old) reported a concentration lower than 30 nmol/l (Sambrook et al., 2004, Stein et al., 1999). However, the current review found that Japanese institutionalised very-old adults (latitude 36.2) reported the highest concentration, even though they were women, and approximately 20% of all participants were thought to have decreased vitamin D activating ability by their kidneys (Terabe et al., 2012). That said, it should be noted that this study did not report the supplementation intake of the participants nor their mean intake of vitamin D.

The assays that were used to measure the 25(OH)D concentration were inconsistent and so the results may have been over- or under-estimated. As mentioned earlier in Chapter 1, different labs and assays report different values of 25(OH)D concentration in certain samples. For example, high variability was observed between the HPLC, RIA and CLIA assays (Snellman et al., 2010). The study stated that the highest accuracy was found with the high-pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS) and lowest with the CLIA assay. Generally, HPLC can discriminate concentration of 25(OH)D2 and 25(OH)D3 metabolites, whereas RIA and CLIA

assays measure total 25(OH)D concentration (Snellman et al., 2010). Another study demonstrated that RIA assay showed a performance comparable to MS. However, most immunoassays had difficulties measuring low concentration. For example, below 8 μg/L (Farrell et al., 2012)

In the current review, the majority of the studies used RIA to measure the concentration of 25(OH)D. However, it is reassuring that the highest concentration of 25(OH)D, which was reported in the USA, was measured by comparative binding protein assay.(CBP) assay (Jacques et al., 1997), whereas the lowest concentration, which was reported in Austria, was measured by the Abbott Architect platform (Muschitz et al., 2015). Furthermore, the highest prevalence of vitamin D deficiency, found in the UK, was measured by an RIA assay (Hill et al., 2016), while the lowest prevalence of vitamin D deficiency, found in New Zealand, was measured by an HPLC assay (Bacon et al., 2016). Among the institutionalised participants, the concentration of 25(OH)D was measured by an MS assay (Schilling, 2012) and the Abbott Architect platform (Muschitz et al., 2015) in two studies based in Europe, which both reported very low concentration. The HPLC assay identified 22% (Hirani and Primatesta, 2005) and 32.7% (Matheï et al., 2013) of participants who had concentration lower than 25 among European community-dwelling participants. In contrast, two studies in the current review, both based in the UK, used RIA and CLIA assays and reported a 20% and 22% deficiency, respectively, using a 25 nmol/l cut-off (Bates et al., 2003, Hirani and Primatesta, 2005). Therefore, the true status of vitamin D and the prevalence of its deficiency remain debatable.

In relation to the included studies, either they did not report the months when the 25(OH)D was collected or they collected it throughout the year. Moreover, the current review failed to find an association between 25(OH)D and sex. However, the results of the included studies were inconsistent with regards to detecting a association between 25(OH)D, season and sex, after the age of 80 years-old. For instance, Bacon et al. (2016) found that seasonal variations were only observable in men but not women participants. However, these seasonal variations disappeared among those who used vitamin D supplementation. Likewise, Giuliani et al. (2018) found that the 25(OH)D concentration was significantly lower among men than women. They also reported that, after the age of 80, the seasonal variation is constant. Delos Reyes et al. (2017) agreed that the seasonal variation is steadier among very-old adults. Conversely, Looker et al. (2002) demonstrated that the effect of age was weaker in Winter and at a lower latitude but that, after the age of 80, this seasonal variation disappeared. In

contrast, Jacques et al. (1997) claimed that age and 25(OH)D concentration was strongly associated in the wintertime only. Bolland et al. (2006) maintained that age is a predictor of 25(OH)D concentration in women, but not in men. Similarly, Bruyère et al. (2014) observed an inverse association between age and 25(OH)D in women. However, Johnson et al. (2008) reported that the risk of having a poor vitamin D status increases by 2-3-fold amongst those aged 100 years-old compared to those aged 80 years-old. The differences between the men and women may be explained by the differences in body composition between the sexes (i.e. women have more body fat compared to men). They may also be explained by the variance in the preferred activities of men and women, as women tend to engage in more indoor activities (e.g. housework) compared to men. Very-old adults are also prone to engage in limited PA and cover up their bodies, which lowers their chance of exposure to sunlight, even during different seasons.

Even though some studies claimed the necessity of increasing the 25(OH)D concentration in very-old adults to above 75 nmol/l in order to improve their general health and physical performance (Brannon et al., 2008), other studies indicated an adverse effect of a high concentration ($\geq$50 nmol/l) among very-old adults (Grant et al., 2016). Previous published studies based on the Newcastle 85+ Study found a U-shaped association between 25(OH)D concentration and cognitive status, mortality, muscle strength and physical performance in very-old adults (Granic et al., 2015a, Granic et al., 2017, Granic et al., 2015b).

One of the findings of the current review is that no studies were identified from Africa or the Middle East. This may be due to the low prevalence of very-old adults in these countries compared to western countries. Also, in many communities without birth certification, ages are difficult to elicit, especially for older adults. However, the fact that there exists only a low number of very-old adults does not mean that this group can be overlooked. Previous studies from Africa and the Middle East showed a poor vitamin D status (with a mean varying between 10-30 nmol/l) among adults and old adults (>65 years-old) (El-Rassi et al., 2012). This subsequently increases the demand to assess the vitamin D status among the very-old adults group in these countries. Additionally, the current review identified only three studies from Asia, although the prevalence of very-old adults in Asian countries is high. For instance, the percentage of those aged >65 in Asian countries will account for 20-36% of the whole population by 2060 (ESCAP, 2011) while, in Japan, those

aged 80 years and over will account for more than 15% of the whole population by 2050 (OECD, 2013).

Strengths and limitations

This systematic review has a number of both strengths and limitations. The main strength is that it is the first review to pool studies of individuals aged ≥80 years-old. In contrast, the limitations include the fact that the observational studies considered low quality evidence and, therefore, we were unable to take into account statistical heterogeneity because not all studies reported the confounding factors (e.g. dietary intake, supplements, sun exposure). Finally, different studies measured 25(OH)D in different ways, which limited the generalisability of the results.

3.6. Summary

Among the 20 studies that reported the 25(OH)D concentration, the highest concentration of 25(OH)D was reported in the community-dwelling very-old from the USA, while the lowest concentration was reported in the institutionalised very-old from Austria. Generally, using the SACN cut-off, the community-dwelling participants have an adequate concentration of 25(OH)D, especially those from the USA, New Zealand, and Australia, whereas, the institutionalised participants showed a low concentration of 25(OH)D, except for those from Japan.

Chapter 4: The prevalence and determinants of vitamin D deficiency in the Newcastle 85+ Study using UK-specific definitions

Key words: serum 25-hydroxyvitamin D, deficiency, determinants, aged 80 and over, SACN, cohort study

4.1. Abstract

Introduction: SACN dietary recommendations are specified for the UK population. A 25(OH)D concentration <25 nmol/L is used to indicate a risk of vitamin D deficiency based on the RNI when the UVB exposure is minimal.

Aims: This study aimed to determine the prevalence of vitamin D deficiency using current UK SACN specific 25(OH)D cut-offs and explore the predictors (sex, BMI, season, lifestyle, supplement and medication use) of vitamin D deficiency.

Methods: Concentration of 25(OH)D was analysed in 775 participants from the baseline phase of the Newcastle 85+ cohort study. The season of the blood sampling, health, lifestyle and anthropometric data were collected and included as potential predictors of vitamin D status in binary regression models using SACN cut-off stratified by sex.

Results: The prevalence of vitamin D deficiency, according to the SACN guidelines (a concentration of 25(OH)D <25 nmol/L), varied significantly with season, with the highest prevalence observed in Spring (46%) and the lowest in Autumn (16%) (P<0.001). In the multivariate binary regression models, the Winter and Spring blood sampling months, using vitamin D containing medication, supplements and PA were associated with a lower 25(OH)D concentration in the very-old men and women.

Conclusion*:* There is an alarming high prevalence of vitamin D deficiency (<25 nmol/L) in 85 years-old adults living in North East England at all times of the year but particularly during Winter and Spring. The season of blood sampling, using vitamin D containing medication and supplements and PA appeared to be the strongest predictors of 25(OH)D concentration in the very-old men and women.

4.2. Introduction

As mentioned earlier in the literature review, there is variety of recommendations regarding the adequate concentration of 25(OH)D or the required daily intake of vitamin D (see Tables 1.1 and 1.2). IoM and SACN were popular guidelines in North America and Canada and the UK, respectively. The IoM recommendations were based on EAR. They used bone health as the basis for developing Dietary Reference Intakes (DRIs) for vitamin D. On the other hand, SACN (which offers dietary recommendations for the UK population) used a 25(OH)D concentration <25 nmol/L to indicate a risk of vitamin D deficiency (SACN, 2016). The SACN recommendations were based on the RNI, which is two standard deviations above the EAR. It is set for groups considered at risk of vitamin D deficiency and was based on RCTs carried out during the Winter. The RNI, therefore, represents an average amount (10 µg/d (400 IU/d)) that is needed by the majority of the UK population to maintain a 25(OH)D concentration ≥ 25 nmol/L when the UVB exposure is minimal (taking into account the day-to-day variations in vitamin D intake). This amount is enough, or more than enough, for approximately 97.5% of the population throughout the year, in order to protect musculoskeletal health (SACN, 2016).

In the UK, NDNS rolling programme (RP) (a continuous survey of diet and nutrition in adults and children older than 18 months) provides nationally representative data of the UK population regarding vitamin D intake and 25(OH)D concentration (PHE, 2016). NDNS. The NDNS data (2016) indicated that 29% of those over 65 years-old in the UK have a 25(OH)D concentration <25 nmol/L in the Winter. Generally, in the UK, mean intakes from food sources were 2.8 µg/day in older persons aged 65 years and over. Inclusion of intakes from dietary supplements brought their mean intake up to 5.1 µg/day, remaining well below the RNI of 10 mcg/day. The group of those aged 75+ included participants over 85 years-old but the NDNS did not report their number. However, blood samples were obtained from only 61 of those aged 75 years and over (PHE, 2016).

Understanding the prevalence of vitamin D deficiency in very-old UK people is important when devising tailored public health policy/guidelines to address this phenomenon within this age group. Therefore, in order to maximize our knowledge about vitamin D status in this population group, this study aimed to determine the prevalence of vitamin D deficiency using current UK SACN specific 25(OH)D cut-offs and explore the predictors (sex, BMI, season, lifestyle, supplement and medication use) of vitamin D deficiency.

4.3. Methods

Statistical methods

Four seasons were defined as follows: Spring (March-May), Summer (June-August), Autumn (September-November) and Winter (December-February). The concentration of 25(OH)D was not normally distributed (and could not be normalized by transformation). For this study, the concentration of 25(OH)D was categorised by the SACN cut-offs: <25 nmol/L (low) and ≥25 nmol/l (adequate). Participants were compared across the two 25(OH)D groups using the Mann-Whitney test for ordered and non-normally distributed continuous variables, and the $\chi 2$ test for the categorical variables.

To determine the association between the potential determinants and the 25(OH)D concentration, binary logistic regression was used, first fitting explanatory factors (demographic and health characteristics) singly (unadjusted), and then by a forward stepwise procedure for the final multivariable model. Factors for which the unadjusted odds ratios (ORs) had an associated P value <0.05 were eligible for inclusion in the multivariable model. The odds ratios and corresponding 95% CI were used to describe the influence of the potential predictors on 25(OH)D concentration. The performance of the model was assessed using classification plots, the Hosmer and Lemeshow test and the Nagelkerke R Square. All analyses were performed using IBM SPSS Statistics, software version 19 (IBM, New York).

4.4. Results

Prevalence of low and adequate vitamin D status based on SACN recommendations

The proportions of participants with vitamin D deficiency (<25 nmol/L) and adequacy (≥25 nmol/l) according to the SACN thresholds were stratified by season and are shown in Table 4.1. The proportions of participants with vitamin D deficiency were: 46%, 22%, 16% and 33% during Spring, Summer, Autumn and Winter, respectively (Table 4.1). Using the threshold of 25 nmol/L (basis of the RNI) to define vitamin D deficiency, the proportion of participants with a low vitamin D concentration was markedly high, even in Summer (22%).

Characteristics of participants stratified by concentration of 25(OH)D group based on the SACN recommendations

The participants in the 'low' 25(OH)D group (<25 nmol/L) were more likely to be sampled in the Winter and Spring seasons, non-users of vitamin D containing medication and have the lowest level of PA (Table 4.2). The participants in the 'adequate' 25(OH)D group (≥25 nmol/L) were more likely to be sampled during the Summer and Autumn seasons, smokers, users of vitamin D containing preparations and more physically active.

Factors associated with low and adequate 25(OH)D status in very-old adults

Several candidate factors were associated with 25(OH)D concentration in the unadjusted binary regression model in the very-old adults (Table 4.2). A lower vitamin D status was more likely to occur in the very-old adults who were sampled during the Winter months compared to the Spring months, with those who were not vitamin D containing medication and supplements users, not obese alcohol consumers, and had a lower SMMSE score. However, disability score was negatively associated with 25(OH)D concentration.

The factors for which the unadjusted OR had an associated P value <0.05 were eligible for inclusion in the multivariable model. In the multivariate binary logistic regression model, the independent variables are adjusted simultaneously for the other variables in the model (Table 4.4). Also in the very-old adults, the season of blood sampling, using vitamin D containing medication and supplements and PA were independently associated with a low 25(OH)D concentration.

Factors associated with low and adequate 25(OH)D status in very-old men

Several candidate factors were associated with 25(OH)D concentration in the unadjusted binary regression model in the very-old men (Tables 4.2 and 4.3). Lower vitamin D status was more likely to occur in very-old men, who were sampled during the Autumn and Winter months compared to the Spring months, with those who were low physically active, alcohol consumers and had a lower SMMSE score. Disease count and disability score were negatively associated with 25(OH)D concentration. However, taking vitamin D containing medication (n=23) did not show a significant association with 25(OH)D concentration in very-old men.

Factors for which the unadjusted odds ratio (OR) had an associated P value <0.05 were eligible for inclusion in the multivariable model. In the multivariate binary logistic regression model, the independent variables are adjusted simultaneously for the other variables in the model (Table 4.4). The season of blood sampling, using supplements and PA were independently associated with a low 25(OH)D concentration in men. None of the other variables was significantly associated with vitamin D status.

Factors associated with low and adequate 25(OH)D status in very-old women

Several candidate factors were associated with 25(OH)D concentration in the unadjusted binary regression model in the very-old women (Tables 4.2 and 4.3). A lower vitamin D status was more likely to occur in the very-old women, who were sampled during the Winter months compared to the Spring months, with those who were not vitamin D containing medication and supplements users and who were obese. However, none of the other variables was significantly associated with vitamin D status in very-old women.

The factors for which the unadjusted OR had an associated P value <0.05 were eligible for inclusion in the multivariable model. In the multivariate binary logistic regression model, the independent variables are adjusted simultaneously for the other variables in the model (Table 4.4). Also in the very-old women, the season of blood sampling, using vitamin D containing medication and supplements and PA were independently associated with a low 25(OH)D concentration.

Table 4.1: Percentages of participants with 25(OH)D concentration above and below the SACN 25(OH)D thresholds within each season.

Concentration of 25(OH)D threshold	Spring (*n*=121) (March-May)	Summer (*n*=309) (June-August)	Autumn (*n*=180) (September-November)	Winter (*n*=165) (December-February)	Overall (*n* =775) All year
<25 nmol/L % (n)	46.3 (56)	22.0 (68)	16.1 (29)	33.3 (55)	26.8 (208)
≥25 nmol/L % (n)	53.7 (65)	78.0 (241)	83.9 (151)	66.7 (110)	73.3 (567)

SACN: Scientific Advisory Committee on Nutrition. 25(OH)D: <25 nmol/l (low) and ≥25 nmol/l (adequate).

Table 4.2: Characteristics of Newcastle 85+ Study participants stratified by SACN cut-offs

Characteristic	Concentration of 25(OH)D								
	All		p	Men			Women		
	<25 nmol/L (n=208)	≥25 nmol/l (n=570)		<25 nmol/L (*n*=76)	≥25 nmol/l (n=228)	*p*	<25 nmol/L (*n*=132)	≥25 nmol/l (n=342)	*p*
Women % (*n*)	63.3 (132)	60.0 (342)	0.428	-	-	-	-	-	-
Institutionalised % (*n*)	8.2 (47)	9.6 (20)	0.547	5.3 (4)	4.4 (10)	0.752	12.1 (16)	10.8 (37)	0.687
Season of blood testing *%* (*n*)			<0.0001			<0.0001			<0.0001
Spring	26.9 (56)	11.5 (65)		30.3 (23)	11.4 (26)		25.0 (33)	11.4 (39)	
Summer	32.7 (68)	42.5 (241)		27.6 (21)	41.5 (97)		35.6 (47)	42.1 (144)	
Autumn	13.9 (29)	26.6 (151)		17.1 (13)	28.1 (64)		12.1 (16)	25.4 (87)	
Winter	26.4 (55)	19.4 (110)		25.0 (19)	18.0 (41)		27.3 (36)	21.1 (72)	
Vitamin D containing medication % (n)	0.5 (1)	22.3 (127)	<0.0001	0.0 (0)	10.1 (23)	0.004	0.8 (1)	30.4 (104)	<0.0001
Supplements users % (n)	4.8 (10)	25.0 (142)	<0.0001	3.9 (3)	21.9 (50)	<0.0001	5.3 (7)	27.1 (92)	<0.0001
BMI % (*n*)			0.008			0.460			<0.0001
Underweight	22.4 (44)	32.5 (172)		21.9 (16)	27.7 (61)		23.3 (28)	35.9 (111)	
Normal	43.5 (84)	43.5 (230)		53.4 (39)	46.4 (102)		37.5 (45)	41.4 (128)	
Overweight	19.2 (37)	16.3 (86)		19.2 (14)	16.4 (36)		19.2 (23)	16.2 (50)	
Obese	14.5 (28)	7.8 (41)		5.5 (4)	9.5 (21)		20.0 (24)	6.5 (20)	
PA % (*n*)			0.002			<0.0001			0.364
Low	27.2 (56)	20.3 (115)		32.9 (25)	15.5 (35)		23.8 (31)	23.5 (80)	
Moderate	47.6 (98)	41.3 (234)		36.8 (28)	31.4 (71)		53.8 (70)	47.9 (163)	
High	25.2 (52)	38.3 (217)		30.3 (23)	53.1 (120)		22.3 (29)	28.5 (97)	
Smoking status *%* (*n*)			0.238			0.937			0.282
Current smoker	4.9 (28)	8.2 (17)		5.3 (4)	4.0 (9)		9.8 (13)	5.6 (19)	
Alcohol consumption % (*n*)			0.015			0.038			0.351
Low	30.6 (63)	29.8 (168)		17.1 (13)	15.6 (35)		38.5 (50)	39.2 (133)	
Moderate	29.6 (61)	41.0 (231)		39.5 (30)	54.9 (123)		23.8 (31)	31.9 (108)	
High	11.7 (24)	9.9 (56)		21.1 (16)	18.3 (41)		6.2 (8)	4.4 (15)	
Disease count mean (SD)	4.9 (0.1)	4.8 (0.0)	0.325	5.1 (0.2)	4.4 (0.1)	0.012	4.8 (0.1)	5.08 (0.1)	0.150
Disability score mean (SD)	5.2 (0.3)	4.3 (0.1)	0.001	5.2 (0.7)	3.3 (0.3)	0.001	5.5 (0.5)	4.9 (0.2)	0.429
SMMSE score mean (SD)	25.3 (0.3)	26.3 (0.1)	0.001	25.2 (0.7)	26.7 (0.2)	0.047	25.1 (0.6)	26.4 (0.2)	0.210

Vitamin D groups are derived based on SACN recommendations. SACN: Scientific Advisory Committee on Nutrition. 25(OH)D: <25 nmol/l (low) and ≥25 nmol/l (adequate). BMI: body mass index. PA: physical activity. SMMSE: mini mental state examination. Mann-Whitney test for ordered and non-normally distributed continuous variables and χ^2 test for categorical variables were used to compare participants across 25(OH)D groups.

Table 4.3: Unadjusted binary logistic regression analysis of the association factors with concentration of 25(OH)D stratified by sex

Characteristic	All			Men			Women		
	Odds ratio (95% CI)	***B***	***P***	**Odds ratio (95% CI)**	***B***	***P***	**Odds ratio (95% CI)**	***B***	***P***
Institutionalised	0.84 (0.48-1.46)	-0.16	0.547	0.82 (0.25-2.71)	-0.19	0.752	0.88 (0.47-1.64)	-0.12	0.687
Season of blood testing (v's Spring)			…			<0.001			<0.001
Summer	0.59 (0.31-1.09)	-0.52	0.092	0.52 (0.24-1.14)	-0.64	0.105	0.59 (0.32-1.09)	-0.52	0.092
Autumn	1.53 (0.91-2.57)	0.42	0.107	2.14 (1.04-4.39)	0.76	0.038	1.53 (0.91-2.57)	0.42	0.107
Winter	2.71 (1.39-5.29)	1.00	0.003	2.28 (1.01-5.11)	0.82	0.045	2.71 (1.39-5.29)	1.00	0.003
Vitamin D containing medication (vs non-user)	0.01 (0.00-0.12)	-4.08	<0.001	…			…		
Supplements users (vs non-user)	0.15 (0.07-0.29)	-1.88	<0.001	0.00	-20.21	0.998	0.01 (0.00-0.12)	-4.04	<0.001
BMI (v's underweight)			…			0.467			<0.001
Normal	2.67 (1.48-4.78)	0.98	0.001	0.72 (0.21-2.41)	-0.32	0.602	4.76 (2.30-9.81)	1.56	<0.001
Overweight	1.87 (1.08-3.21)	0.62	0.024	0.49 (0.16-1.54)	-0.69	0.227	3.41 (1.72-6.76)	1.22	<0.001
Obese	1.58 (0.85-2.93)	0.46	0.141	0.49 (0.14-1.68)	-0.71	0.257	2.60 (1.20-5.64)	0.95	0.015
PA (v's low)			…			0.001			0.365
Moderate	0.49 (0.31-0.76)	-0.70	0.002	0.26 (0.13-0.53)	-1.31	<0.001	0.77 (0.42-1.38)	-0.25	0.386
High	0.57 (0.39-0.84)	-0.05	0.004	0.48 (0.26-0.90)	-0.72	0.024	0.69 (0.42-1.14)	-0.36	0.156
Smoker (vs non-smoker)	0.41 (0.15-1.09)	-0.88	0.076	0.56 (0.08-3.93)	-0.57	0.562	0.62 (0.24-1.59)	-0.47	0.323
Alcohol consumption (vs non-consumer)	2.37 (1.40-4.01)	0.86	0.001	3.58 (1.57-8.15)	1.27	0.002	1.79 (0.89-3.58)	0.58	0.100
Disability	0.96 (0.92-0.99)	-0.04	0.016	0.91 (0.86-0.97)	-0.86	0.003	0.98 (0.94-1.02)	-0.01	0.515
Disease count	0.95 (0.85-1.06)	-0.05	0.374	0.77 (0.63-0.94)	-0.25	0.013	1.05 (0.92-1.21)	0.05	0.433
SMMSE score	1.04 (1.00-1.07)	0.03	0.012	1.07 (1.01-1.13)	0.07	0.014	1.02 (0.98-1.06)	0.02	0.176

Concentration of 25(OH)D were categorized into 2 categories of vitamin D status: <25 nmol/l (low) and ≥25 nmol/l (adequate). CI: confidence interval. BMI: body mass index. PA: physical activity. SMMSE: mini mental state examination. The unadjusted binary odds ratios were calculated for each variable; the reference group for each variable is given in parentheses.

Table 4.4: Adjusted multivariate binary logistic regression analysis for SACN 25(OH)D cut-offs stratified by sex

Sex	Characteristic	Odds ratio (95% CI)	*B*	*P*
All (n=775)	Vitamin D containing medication (none user v's user)	0.01 (0.00-0.07)	-4.62	<0.001
	Supplements users	3.71 (1.77-7.79)	-2.13	<0.001
	Season of blood testing (v's Spring)			
	Summer	0.52 (2.49-1.11)	-0.64	0.095
	Autumn	1.76 (0.96-3.21)	0.56	0.630
	Winter	3.71 (1.77-7.79)	1.31	0.001
	PA (low)			
	Moderate	0.36 (0.17-0.73)	-1.01	0.005
	High	0.51 (0.29-0.90)	-0.66	0.021
Men (n=304)	Vitamin D containing medication (none user v's user)	0.00	-20.56	0.998
	Supplements users	0.16 (0.04-0.57)	-1.84	0.005
	Season of blood testing (v's Spring)			
	Summer	0.53 (0.22-1.30)	-0.64	0.168
	Autumn	2.79 (1.24-6.25)	0.76	0.012
	Winter	3.07 (1.26-7.50)	0.82	0.014
	PA (low)			
	Moderate	0.20 (0.09-0.44)	-1.58	<0.001
	High	0.35 (0.17-0.70)	-1.04	0.003
Women (n=471)	Vitamin D containing medication (none user v's user)	0.01 (0.00-0.07)	-4.62	<0.001
	Supplements users	0.11 (0.05-0.27)	-2.13	<0.001
	Season of blood testing (v's Spring)			
	Summer	0.52 (0.24-1.11)	-0.64	0.095
	Autumn	1.76 (0.96-3.21)	0.56	0.063
	Winter	3.71 (1.77-7.79)	1.31	0.001
	PA (low)			
	Moderate	0.36 (0.17-0.73)	-1.01	0.005
	High	0.51 (0.29-0.90)	-0.66	0.021

SACN: Scientific Advisory Committee on Nutrition. Concentration of 25(OH)D were categorized into 2 categories of vitamin D status: <25 nmol/l (low) and ≥25 nmol/l (adequate). CI: confidence interval. SMMSE: mini mental state examination The adjusted binary odds ratios were calculated with all variables in the table included simultaneously in the model; the reference group for each variable is given in parentheses.

4.5. Discussion

Main findings

The results showed that vitamin D deficiency was high at all times of the year when using the SACN threshold (27% of the participants). Moreover, it confirmed the effect of the seasons on vitamin D status. The season of blood sampling, using vitamin D containing medication and supplements and PA were strong predictors of 25(OH)D concentration in the very-old adults.

Evidence from other studies

This descriptive study confirmed the results of the previous study by Hill et al. (2016). Previous research on the Newcastle 85+ Study used the IoM recommendations to describe vitamin D status among the very-old adults and showed that 33% of the participants have a concentration <30 nmol/l (Hill et al., 2016). The study concluded that vitamin D deficiency was highly prevalent in very-old adults living in North East England at all times of the year. It also highlighted the differences between vitamin D status based on season and living conditions, and demonstrated that only Winter and Spring blood sampling were associated with a lower 25(OH)D concentration, as well as not using supplements is associated with lower 25(OH)D concentration (Hill et al., 2016).

Both studies were consistent with the results of the studies illustrated previously in Chapters 1 and 3. For example, the observational study by Andersen et al. (2005) stated that the median concentration of 25(OH)D for Northern European women with a mean age of 71 years-old was 40.7 nmol/l and that, for 17% of them, the 25(OH)D concentration were below 25 nmol/l, while more than 67% of them had a 25(OH)D concentration below 50 nmol/l. Moreover, in our systematic review, a 25(OH)D concentration below <25 nmol/l applied to approximately 18-22% of the very-old participants in Europe (Hirani and Primatesta, 2005, Bates et al., 2003, Giuliani et al., 2018).

The association between 25(OH)D concentration and season was well documented (Elizondo-Montemayor et al., 2017). This study also confirmed a seasonal effect on 25(OH)D concentration. For instance, the highest prevalence of vitamin D deficiency among the participants in the study by Giuliani et al. (2018) was during Winter and Spring. Likewise, Looker et al. (2002) demonstrated that vitamin D deficiency was more common in Winter

compared to Summer. All of these studies supported the effect of season on 25(OH)D concentration. Generally, men and women showed the same predictors of vitamin D status. This study also proposed that PA is a significant predictor of 25(OH)D concentration. However, the association between 25(OH)D concentration and PA will be discussed later in this thesis (see Chapter 5 for more details).

We could not prove that taking vitamin D containing medication improved the concentration of 25(OH)D in men because of the low number of consumers (only 23 of the men took vitamin D containing medication). However, they all had a 25(OH)D concentration >25 nmol/l. In contrast, the association between 25(OH)D and taking vitamin D containing medication was significant in the women.

Strengths and limitations

This descriptive study has both strengths and limitations. First, it used different cut-off to describe vitamin D status among the very-old men and women (SACN, which is currently used in the UK) in comparison to the one used in the study by Hill et al. (2016). Moreover, it stratified the analysis by sex to maximize our knowledge about this sector. Finally, the models were adjusted to suit the demographic and health characteristics factors to make it possible to consider the predictors associated with vitamin D status. On the other hand, the limitation included a low number of the participants incorporated into some of the predictors; for example, there were only 23 vitamin D containing medication users among the men. Furthermore, the models did not include dietary or behavioural variables.

4.6. Summary

The results confirmed that vitamin D deficiency [as defined by a 25(OH)D concentration <25 nmol/L] is high at all times of the year, particularly during the Winter and Spring months in 85+ years-olds in North East England. The season of blood sampling, using vitamin D containing medication and supplements and PA were strong predictors of 25(OH)D concentration in the very-old men and women. However, low PA, a low SMMSE score, a high disease count, supplement, alcohol consumption and high disability scores were all associated with low 25(OH)D concentration in the men, while using vitamin D containing medication and supplements were associated with higher 25(OH)D concentration in the women.

Chapter 5: The association between 25-hydroxyvitamin D concentration and disability trajectory in very-old adults: The Newcastle 85+ Study

Key words: vitamin D status, disability, very-old.

5.1. Abstract

***Introduction**:* Poor vitamin D status is common in very-old adults, which may have adverse consequences for muscle function, a major predictor of disability. However, there is a dearth of data which has examined the association between vitamin D status and the trajectory of disability in very-old adults.

***Aims**:* To explore the association between the concentration of 25(OH)D and disability trajectory in very-old adults and to determine whether there is an 'adequate' 25(OH)D concentration, which might protect against a poorer disability trajectory.

***Methodology**:* A total of 775 participants from the Newcastle 85+ Study whose 25(OH)D concentration at baseline was available were included. A 25(OH)D concentration of <25 nmol/l, 25–50 nmol/l and ≥50 nmol/l were used as the cut-offs to define low, moderate and high vitamin D status, respectively. Disability was defined as difficulty in performing 17 activities of daily living, at baseline and after 18, 36 and 60 months. The trajectories were derived by group-based trajectory modelling. The association between the 25(OH)D cut-off and disability trajectory was examined by multinomial logistic regression.

***Results**:* A three-trajectory model was derived (low-to-mild, mild-to-moderate and moderate-to-severe). In partially adjusted model, participants with a 25(OH)D concentration <25 nmol/l were more likely to have a moderate and severe disability trajectory compared with those with concentration of 25–50 nmol/l, after adjusting for sex, living in an institution, season, cognitive status, BMI, and vitamin D containing medication usage. However, this association disappeared after further adjustment for physical activity.

***Conclusion**:* This observation study showed that physical activity rather than vitamin D status predicted the disability trajectory in very-old adults.

5.2. Introduction

Life expectancy is increasing worldwide. By 2050, it is predicted that there will be 379 million people aged 80 and above, and almost 10% of the population of developed countries will be aged ≥80 years (OECD, 2013). Disability is defined as experiencing difficulty in performing activities that are essential for independent living. Such activities comprise the basic activities of daily living (BADL), such as getting up and washing one's hands, and instrumental activities of daily living (IADL), such as shopping for groceries and doing housework (Gobbens and van Assen, 2014). The frequency of activities of daily living (ADL) disability is higher among very-old adults (those aged 80 years and older) (Yu et al., 2016). Difficulty with performing ADL is a predictor of longer hospital stays and of additional GP visits (Millán-Calenti et al., 2010). Furthermore, disability increases the risk of mortality by 2–3 times among very-old adults (Majer et al., 2011). Generally, disability raises the amount of benefits paid for assistance programs and care facilities in developed countries; for example, it increases the cost of care by 22% in the UK (Ali, 2014).

Very-old adults are more likely to have a lower circulating concentration of 25(OH)D (Janssen et al., 2013). This is due to many reasons, including the decreased production of vitamin D by the skin, low exposure to sunlight, and low vitamin D intake as well as catabolism factors, such as medication and disease (Gallagher, 2013). Following the hydroxylation of 25(OH)D in the kidneys, 1,25(OH)D binds to its nuclear receptor (VDR) which is expressed in multiple tissues, including the muscles. It then influences protein synthesis in the muscles, muscle calcium uptake and type II muscle fibre size and number (Ceglia, 2009). Two potential mechanisms have been suggested to explain the association between 25(OH)D concentration and the muscles. First, the age-related reduction in 1,25(OH)D reduces the stimulation of VDR expression by the muscles. Second, the decline of VDR expression upon ageing leads to an impaired muscle response to 1,25(OH)D (Bischoff-Ferrari et al., 2004a).

Maintaining a moderate concentration of 25(OH)D may protect against disability in terms of both musculoskeletal and cognitive functions; the few studies that have assessed this association have found an inverse association between 25(OH)D concentration and the risk of disability (Oliveira et al., 2017, Valderrama-Hinds et al., 2017, Zamboni et al., 2002, Semba et al., 2000). However, these studies have several limitations, including: the use of different definitions of low 25(OH)D concentration; being cross-sectional rather than longitudinal;

recruiting those aged 65 and over, with few studies of the very-old; being unrepresentative because they recruited women only (Zamboni et al., 2002, Semba et al., 2000), targeting a specific ethnic group (Valderrama-Hinds et al., 2017), or involving patients with a specific disease (Oliveira et al., 2017). Consequently, there is a need for longitudinal studies of the association between 25(OH)D concentration and disability trajectory , which focus on very-old adults, including those living in institutions. This study explored the association between the 25(OH)D concentration and disability trajectory over five years in very-old adults (initially aged 85 years-old). It also investigated whether there is a threshold concentration of 25(OH)D, above which the disability trajectory among very-old adults slows. We hypothesized that a poor 25(OH)D concentration (lower than 25 nmol/l) was associated with a faster disability trajectory in very-old adults.

5.3. Methodology

Population Sample

The participants were taken from the Newcastle 85+ Study, which included both community-dwelling and institutionalised older adults who were 85 years-old at recruitment and living in Newcastle-upon-Tyne and North Tyneside. Both a health assessment, comprising questionnaires, measurements and function tests, and a fasting blood sample, as well as general practice medical records to extract data on diagnosed diseases and prescribed medication were available for 851 participants. This analysis included all Newcastle 85+ Study participants (n=775) for whom data on their health assessment, general practice records and 25(OH)D concentration were available at baseline. From this initial group, data on their health assessment and general practice records were available for 631, 484 and 344 participants in Phases 2 (18 months), 3 (36 months), and 4 (60 months), respectively (more details were provided in Chapter 2) (Collerton et al., 2009).

Disability Measures and Scores

The data on disability level was obtained by a trained research nurse-administered questionnaire. At the baseline and follow-up assessments, the participants were asked about their ability to perform 17 activities comprising of BADLs and IADLs and mobility items (Table 2.1); these were taken predominantly from the Groningen Activity Restriction Scale

(Kempen et al., 1996). A loss of ability regarding individual items formed a single hierarchy, that was similar for men and women. A disability score was calculated based on the total number of ADL that were performed with difficulty or requiring an aid/appliance or personal help (Collerton et al., 2009). A disability score of 0 was given for each item that was reported to be performed *without* difficulty, and a score of 1 for each item that was performed *with* difficulty (maximum score is 17). The participants were classified as having disability if they had difficulty with one or more item, or no disability if they did not have any difficulty with any of the items.

The ability to perform 17 ADLs was self-reported. The participants were also asked to answer three questions on mobility (see Table 2.1 in Chapter 2). Each question was framed by the phrase '*can you*' rather than '*do you*', in order to gain a greater capacity to assess the true level of disability accounting for situational responses.

Statistical analysis

The group-based trajectory model (GBTM) is an analysis that is used to investigate population differences in developing courses of behaviour or outcomes over a period of time or age (Jones and Nagin, 2013). It was used in this study to derive distinct clusters of the participants' disability trajectories from baseline over the subsequent 60 months. It was adjusted for those who dropped out. Bayesian information criteria (BIC) were used to assess the best number of trajectories within the model. The model was then further assessed by the posterior probability of group membership >75%. Any differences between the disability trajectory groups were tested using the Kruskal-Wallis test for ordered non-normally distributed continuous variables (weight, BMI, fat-free mass, concentration of 25(OH)D, chronic disease count) and the $\chi 2$ test for the categorical variables (sex, PA, alcohol drinker, smoker, 25(OH)D concentration, impaired cognitive status, living in an institution).

Multinomial regression was used to determine the association between disability and 25(OH)D concentration in both cross-sectional and longitudinal analysis. The concentration of 25(OH)D was not normally distributed, so non-parametric analysis was used. The following cut-offs were used in the analysis: <25 nmol/l (low), 25 to 50 nmol/L (moderate) and $\geq$50 nmol/l (high) (Vieth and Holick, 2018). Important confounders were selected based on their clinical and theoretical relevance as well as univariate analysis with the disability trajectory. These confounders were then fitted, removed and refitted until the best possible

but parsimonious model was achieved while checking for model fit statistics throughout, using 10% of the change-in-estimate. The multi-collinearity between the confounders was assessed using VIF (variance inflation factor). Model 1 was an unadjusted model. Model 2 was adjusted for sex, living in an institution and the season of the blood collection. Model 3 was adjusted further for cognitive status, BMI and vitamin D containing medication usage. Model 4 was adjusted further for PA. The models were stratified by sex.

The statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics software version 24 (IBM, New York, USA) except for the disability trajectory, that was derived using STATA v15.0 (package *traj*) (Stat Corp., College Station, TX).

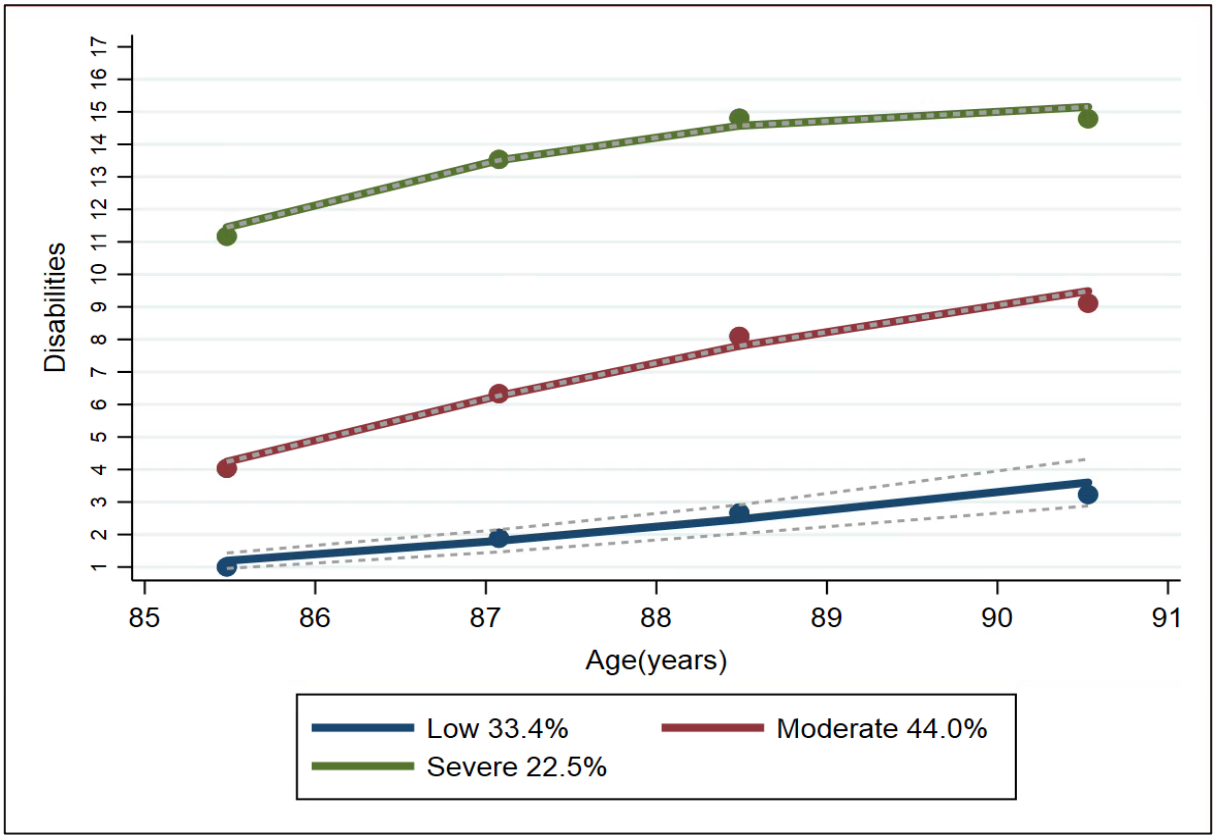
Sensitivity analysis

To investigate the effects of grip strength, FFM (fat-free mass) and disease count, the models were further adjusted for each of these variables. The models were rerun, excluding those participants with evidence of cognitive impairment (MMSE score <26) (more details in Chapter 2). The models were also stratified by the season of the blood collection using the same categories as mentioned in Chapter 2.

5.4. Results

Disability trajectory

The disability trajectory (DT; one linear and two quadratic) from age 85 to 90 years-old were best presented by a triple-group model. The trajectories are plotted in Figure 5.1 and the characteristics of the participants with each of these trajectories are described in Table 5.1. DT1 represents a low-to-mild disability trajectory (group size: 33.4%), DT2 represents a mild-to-moderate disability trajectory (group size: 44%) and DT3 represents a moderate-to-severe disability trajectory (group size: 22.5%). The participants with a low-to-mild disability trajectory had a slightly increased disability trajectory over five years, while the participants with a mild-to-moderate or moderate-to-severe disability trajectory showed a serious trajectory with advancing age, with the score (number of activities that the participants were unable to undertake unaided) increasing from four to 9.5 and from 11 to 15, respectively.



The percentages represent the group size. Disabilities based on calculating the basic activities of daily living (BADLs), instrumental activities of daily living (IADLs) and mobility.

Figure 5.1: Disability trajectory with 95% confidence intervals in participants from the Newcastle 85+ Study with available 25(OH)D data

The differences in socioeconomic, lifestyle and health factors between the disability trajectories

Body weight, total number of years in education, fat-free mass and smoking did not differ significantly between the participants in each of the three disability trajectories. The participants in the three groups showed significant differences regarding their BMI, PA level, alcohol intake, vitamin D containing medication usage, number of chronic diseases, cognitive status, SMMSE score and living in an institution. However, the moderate-to-severe DT group was characterised by a higher percentage of women, a lower proportion of alcohol drinkers, living in an institution, being less physically active, having a higher number of chronic diseases, being cognitively impaired and having a lower score for SMMSE (Table 5.1). Although there were no significant differences in the median 25(OH)D concentration between the DT groups, the distribution of the participants across the three categories of vitamin D adequacy based on 25(OH)D concentration (low, moderate and high) differed significantly across these three trajectories. However, the highest percentage of participants in the low-to-mild and mild-to-moderate groups had a concentration of 25(OH)D between 25 and 50 nmol/l.

Table 5.1: Participant characteristics by the three disability trajectories

	Low-to-mild (n= 249)	Mild-to-moderate (n= 351)	Moderate-to-severe (n= 175)	*p*
Women % (n)	48.4 (121)	56.6 (231)	69.3 (122)	<0.001
Weight (kg) mean (SD)	63.9 (11.8)	63.5 (13.4)	63.9 (14.3)	0.732
BMI mean (SD)	23.8 (3.8)	24.7 (4.4)	24.9 (5.2)	0.029
Fat-free mass (kg) mean (SD)	46.5 (9.2)	44.4 (9.1)	45.0 (8.9)	0.151
Total number of years in education % (n)	61.9 (153)	62.1 (213)	70.3 (111)	0.241
0–9 years	23.9 (59)	24.2 (83)	22.2 (35)	
10–11 years	14.2 (35)	13.7 (47)	7.6 (12)	
12–20 years				
PA				
Low % (n)	2.4 (6)	27.3 (68)	70.3 (175)	<0.001
Medium % (n)	15.8 (55)	58.7 (205)	25.5 (89)	
High % (n)	63.2 (110)	33.9 (59)	2.9 (5)	
Alcohol drinkers % (n)	80.0 (156)	72.4 (168)	55.3 (52)	<0.001
Smoking % (n)	3.6 (9)	8.0 (28)	4.5 (8)	0.124
Vitamin D containing medication % (n)	10.0 (25)	13.4 (47)	31.8 (56)	<0.001
Supplements users % (n)	23.3 (58)	20.8 (73)	12.0 (21)	0.012
Concentration of 25(OH)D nmol/l median (IQR)	42.0 (29-59)	36.0 (23-58)	39.0 (21-70)	0.178
25(OH)D				0.002
<25 nmol/l % (n)	26.4 (66)	36.0 (90)	37.6 (94)	
25-50 nmol/l % (n)	34.7 (122)	31.8 (112)	33.5 (118)	
≥50 nmol/l % (n)	38.1 (67)	19.9 (35)	42.0 (74)	
Chronic disease count mean (SD)	4.1 (1.5)	4.9 (1.75)	5.6 (1.9)	<0.001
Impaired cognitive status % (n)	12.0 (30)	23.3 (82)	57.5 (100)	<0.001
Living in institution % (n)	0.4 (1)	3.4 (12)	30.7 (54)	<0.001

BMI: body mass index. PA: physical activity. *p,* p-value: Kruskal-Wallis test for continuous non-normally distributed variables or $\chi 2$ test for categorical variables. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate), ≥50 nmol/l (high).

25(OH)D concentration and disability at baseline

A cross-sectional analysis of the association between the 25(OH)D cut-off and disability baseline data reveals a U-shaped association between 25(OH)D and disability. A significant association was found between 25(OH)D concentration and disability score at baseline, with (p=<0.001) and (p= 0.002) for the low (<25 nmol/l) and high (≥50 nmol/l) concentrations, respectively.

25(OH)D concentration and disability trajectory

The results of the analysis show that participants with a low concentration of 25(OH)D (<25 nmol/l) were more likely to have a mild-to-moderate disability trajectory (OR= 2.01, 95% CI= 1.29–3.14, p= 0.002) or a moderate-to-severe disability trajectory (OR= 3.39, 95% CI= 1.99–5.76, p= 0.001) than a low-to-mild disability trajectory compared to those with a moderate concentration in the unadjusted model, after adjusting for sex, living in an institution and season (OR= 2.01, 95% CI= 1.27–3.19, p= 0.003) and (OR= 3.02, 95% CI= 1.70–5.38, p= 0.001) and after further adjustment for cognitive status, BMI and vitamin D containing medication usage (OR= 1.97, 95% CI= 1.22–3.17, p= 0.005) and (OR= 3.12, 95% CI= 1.67–5.85, p= 0.001), respectively. However, this association disappeared after adjustment for PA (Table 5.2). The results also show that participants with a high concentration were more likely to have a moderate-to-severe disability trajectory compared to those with a moderate concentration over five years but only in the unadjusted model (OR= 1.94, 95% CI= 1.23–3.06, p= 0.004). However, in the adjusted models, no association was found between a high concentration and disability trajectory.

25(OH)D concentration and disability trajectory by sex

Men with a low concentration of 25(OH)D were more likely to have a mild -to-moderate disability trajectory (OR= 3.55, 95% CI= 1.56–8.09, p= 0.003) than a low-to-mild disability trajectory compared to those with a moderate concentration in the unadjusted model, even after adjusting for living in an institution and season (OR= 4.42, 95% CI= 1.79–10.90, p= 0.001) and after further adjustment for cognitive status, BMI and vitamin D containing medication usage (OR= 3.83, 95% CI= 1.44–10.17, p= 0.007). However, this association disappeared after future adjustment for PA (Table 5.3).

Women with a low concentration were more likely to have a mild-to-moderate and moderate-to-severe disability trajectory than a low-to-mild disability trajectory compared to those with a moderate concentration in the unadjusted model (OR= 1.87, 95% CI= 1.03–3.39, p= 0.039) and (OR= 3.03, 95% CI= 1.50–6.13, p= 0.002), respectively. This association was maintained even after adjusting for sex, living in an institution and season (OR= 2.06, 95% CI= 1.12–3.83, p= 0.020) and (OR= 2.58, 95% CI= 1.21–5.50, p= 0.014), respectively. It also continued after further adjustment for cognitive status, BMI and vitamin D containing medication usage (OR= 1.95, 95%CI= 1.02–3.72, p= 0.041) and (OR= 2.70, 95% CI= 1.16–6.27, p= 0.020), respectively, but disappeared after future adjustment for PA. However, women show a U-shaped association between 25(OH)D and a moderate-to-severe disability trajectory but only in the unadjusted model (OR= 2.29, 95% CI= 1.25–4.17, p= 0.007).

Sensitivity analysis

Using the same models with further adjustment for grip strength, fat-free mass and disease count separately, no association was found between 25(OH)D concentration and disability trajectory. However, when PA was removed from the model, after adjusting for grip strength, fat-free mass and disease count, participants with a low concentration were more likely to have mild-to-moderate and moderate-to-severe disability trajectory (Table B in Appendix).

The models were also rerun excluding individuals with cognitive impairment (SMMSE <26). Participants with normal cognitive status (n= 561) who had a low 25(OH)D concentration were more likely to have a moderate-to-severe disability trajectory (OR= 2.30, 95% CI= 1.15–4.58, p= 0.017) than a low-to-mild disability trajectory in the unadjusted model. This was also maintained in the adjusted models for the same confounders: sex, living in an institution and season (OR= 2.14, 95% CI= 1.04–4.39, p= 0.038) and even after adjustment for BMI and vitamin D containing medication usage (OR= 2.44, 95% CI= 1.13–5.27, p= 0.022) (Table 5.4). This association disappeared after further adjustment for PA.

The participants' characteristics by season have been described previously. When stratifying the analysis by season, no association was found between 25(OH)D concentration and disability trajectory in Spring (n= 121). Participants with a low concentration were more likely to have a moderate-to-severe disability trajectory compared to those with a moderate concentration in the unadjusted model for Summer (n= 309) and Autumn (n= 180) (OR=

2.96, 95% CI= 1.17–7.48, p= 0.021) and (OR= 6.03, 95% CI= 1.47–24.77, p= 0.013), respectively. However, the association disappeared in the adjusted models. For Winter (n= 165), participants with low concentration were more likely to have a mild-to-moderate disability trajectory (OR= 5.83, 95% CI= 1.81–18,74, p= 0.003) compared to a moderate concentration in the unadjusted model and after adjustment for sex and living in an institution (OR= 6.44, 95% CI= 1.79–23.12, p= 0.004) and after further adjustment for cognitive status, BMI and vitamin D containing medication usage (OR= 5.10, 95% CI= 1.28–20.37, p= 0.021). This association disappeared after adjustment for PA. On the other hand, participants with a high concentration were more likely to have a moderate-to-severe disability trajectory (OR= 6.92, 95% CI= 2.23–21.43, p= 0.001) compared to a moderate concentration in the unadjusted model and after adjustment for sex, living in an institution and season (OR= 4.51, 95% CI= 1.24–16.37, p= 0.022). After adjustment for cognitive status, BMI and vitamin D containing medication usage, the association disappeared; however, it was continued after adjustment for PA (OR= 6.11, 95% CI= 1.01–36.75, p= 0.048) (Table 5.5).

Table 5.2: Association between 25(OH)D concentration and disability trajectories

Trajectory	25(OH)D	Model 1			Model 2			Model 3			Model 4		
		OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Low-to-mild	(ref)	(ref)			(ref)			(ref)			(ref)		
Mild-to-moderate	<25 nmol/l	2.01	1.29–3.14	0.002	2.01	1.27–3.19	0.003	1.97	1.22–3.17	0.005	1.61	0.95–2.74	0.074
	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
	≥50 nmol/l	1.05	0.73–1.52	0.774	0.94	0.64–1.38	0.771	0.92	0.61–1.38	0.707	1.07	0.69–1.67	0.749
Moderate-to-severe	<25 nmol/l	3.39	1.99–5.76	0.001	3.02	1.70–5.38	0.001	3.12	1.67–5.85	0.001	1.95	0.94–4.06	0.071
	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
	≥50 nmol/l	1.94	1.23–3.06	0.004	1.34	0.80–2.22	0.254	0.83	0.45–1.55	0.577	1.02	0.49–2.12	0.945

CI: confidence interval. BMI: body mass index. PA: physical activity. OR: odd ratio. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and ≥50 nmol/l (high). Model 1 is the unadjusted model. Model 2 is further adjusted for sex, living in an institution and season. Model 3 is further adjusted for cognitive status, BMI and vitamin D containing medication. Model 4 is further adjusted for PA.

Table 5.3: Association between different 25(OH)D cut-offs and disability trajectories by sex

Sex	Trajectory	25(OH)D	Model 1			Model 2			Model 3			Model 4		
			OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*
Men (n= 304)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
		<25 nmol/l	1.96	0.99-3.89	0.052	1.94	0.97-3.89	0.060	2.01	0.98-4.09	0.054	1.58	0.71-3.49	0.255
	DT2	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	0.46	0.46-1.46	0.512	0.82	0.45-1.48	0.518	0.84	0.44-1.57	0.586	1.26	0.63-2.51	0.500
		<25 nmol/l	3.55	1.56-8.09	0.003	4.42	1.79-10.90	0.001	3.83	1.44-10.17	0.007	1.67	0.53-5.25	0.378
	DT3	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.14	0.53-2.46	0.726	0.79	0.32-1.96	0.625	0.41	0.14-1.23	0.115	0.76	0.20-2.81	0.687
Women (n= 471)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
		<25 nmol/l	1.87	1.03-3.39	0.039	2.06	1.12-3.82	0.020	1.95	1.02-3.72	0.041	1.59	0.77-3.28	0.202
	DT2	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.13	0.68-1.86	0.622	1.10	0.66-1.82	0.699	1.01	0.58-1.77	0.946	0.96	0.52-1.77	0.896
		<25 nmol/l	3.03	1.50-6.13	0.002	2.58	1.21-5.50	0.014	2.70	1.16-6.27	0.020	2.05	0.77-5.46	0.150
	DT3	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	2.29	1.25-4.17	0.007	1.69	0.89-3.23	0.107	1.18	0.53-2.62	0.684	1.10	0.43-2.80	0.838

DT1, low-to-mild disability trajectory; DT2, mild-to-moderate disability trajectory; DT3 moderate-to-severe disability trajectory. BMI: body mass index. PA: physical activity. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref), ≥50 nmol/l (high). OR, odd ratio. CI, confidence interval. *P*, p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for living in an institution and season. Model 3 is further adjusted for cognitive status, BMI, and vitamin D containing medication. Model 4 is further adjusted for PA.

Table 5.4: Association between 25(OH)D concentration and disability trajectories of people with normal cognitive status (n= 561)

Trajectory	25(OH)D	Model 1			Model 2			Model 3			Model 4		
		OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*
DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
DT2	<25 nmol/l	1.45	0.90-2.35	0.124	1.49	0.90-2.47	0.114	1.40	0.83-2.35	0.203	1.11	0.62-1.99	0.718
	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
	≥50 nmol/l	0.98	0.65-1.46	0.923	0.90	0.59-1.37	0.642	0.87	0.56-1.35	0.536	1.03	0.63-1.67	0.892
DT3	<25 nmol/l	2.30	1.15-4.58	0.017	2.14	1.04-4.39	0.038	2.44	1.13-5.27	0.022	1.41	0.58-3.40	0.444
	25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
	≥50 nmol/l	1.37	0.74-2.55	0.310	1.18	0.62-2.25	0.608	0.89	0.43-1.86	0.773	1.11	0.47-2.59	0.807

DT1, low-to-mild disability trajectory; DT2, mild-to-moderate disability trajectory; DT3 moderate-to-severe disability trajectory. BMI: body mass index. PA: physical activity. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref), ≥50 nmol/l (high). OR, odd ratio. CI, confidence interval. *p,* p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex, living in an institution and season. Model 3 is further adjusted for BMI, and vitamin D containing medication. Model 4 is further adjusted for PA.

Table 5.5: Association between different 25(OH)D cut-offs and disability trajectories by season

season	Trajectory	25(OH)D	Model 1			Model 2			Model 3			Model 4		
			OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*
Spring (n= 121)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
	DT2	<25 nmol/l	1.77	0.68-4.55	0.236	1.73	0.66-4.49	0.258	1.71	0.63-4.65	0.290	1.76	0.54-5.72	0.342
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	0.85	0.27-2.62	0.778	0.72	0.22-2.32	0.583	0.80	0.51-4.28	0.800	1.14	0.17-7.47	0.891
	DT3	<25 nmol/l	2.83	0.97-9.13	0.081	2.98	0.81-10.95	0.100	4.71	0.92-24.11	0.063	3.52	0.49-24.95	0.208
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	2.15	0.59-7.89	0.245	0.97	0.19-4.95	0.973	1.75	0.14-21.78	0.662	3.31	0.13-83.02	0.466
Summer (n= 309)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
	DT2	<25 nmol/l	1.97	0.92-4.25	0.080	1.80	0.83-3.92	0.136	1.86	0.83-4.15	0.128	1.26	0.50-3.15	0.618
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	0.98	0.56-1.71	0.945	0.94	0.53-1.65	0.831	0.99	0.54-1.82	0.989	1.13	0.56-2.27	0.714
	DT3	<25 nmol/l	2.96	1.17-7.48	0.021	2.53	0.95-6.72	0.062	2.56	0.90-7.28	0.076	1.17	0.33-4.15	0.800
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.35	0.65-2.80	0.420	1.07	0.48-2.35	0.865	0.88	0.344-2.26	0.794	0.94	0.30-2.85	0.913
Autumn (n= 180)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
	DT2	<25 nmol/l	3.43	0.90-13.12	0.071	3.80	0.96-15.10	0.057	3.81	0.70-20.71	0.121	2.24	0.34-14.63	0.398
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.01	0.48-2.14	0.963	0.93	0.43-2.01	0.857	0.83	0.36-1.93	0.680	1.11	0.42-2.92	0.832
	DT3	<25 nmol/l	6.03	1.47-24.77	0.013	3.09	0.66-14.40	0.149	4.26	0.65-27.80	0.129	1.38	0.14-13.46	0.778
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.49	0.62-3.53	0.366	0.95	0.37-2.44	0.921	0.30	0.08-1.06	0.062	0.26	0.049-1.43	0.123
Winter (n= 165)	DT1	(ref)	(ref)			(ref)			(ref)			(ref)		
	DT2	<25 nmol/l	2.15	0.91-5.07	0.079	2.08	0.88-4.95	0.094	2.09	0.84-5.19	0.109	2.05	0.77-5.47	0.148
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	1.43	0.59-3.44	0.422	1.26	0.51-3.10	0.606	1.16	0.41-3.22	0.774	1.65	0.27-4.95	0.367
	DT3	<25 nmol/l	5.83	1.81-18.74	0.003	6.44	1.79-23.12	0.004	5.10	1.28-20.37	0.021	4.38	0.96-19.97	0.056
		25-50 nmol/l	(ref)			(ref)			(ref)			(ref)		
		≥50 nmol/l	6.92	2.23-21.43	0.001	4.51	1.24-16.37	0.022	3.82	0.79-18.35	0.094	6.11	1.01-36.75	0.048

DT1, low-to-mild disability trajectory; DT2, mild-to-moderate disability trajectory; DT3 moderate-to-severe disability trajectory. BMI: body mass index. PA: physical activity. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref), ≥50 nmol/l (high). OR, odd ratio. CI, confidence interval. *p*, p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex, living in an institution. Model 3 is further adjusted for cognitive status, BMI, and vitamin D containing medication. Model 4 is further adjusted for PA.

5.5. Discussion

Main findings

For the current analysis, the disability trajectories model was best presented by the triple-group model. These trajectories differed from two previously derived disability trajectory in different samples of the Newcastle 85+ Study (Mendonça et al., 2018, Kingston et al., 2015). To our knowledge, this is the first longitudinal study to suggest a U-shaped association between 25(OH)D concentration and disability in very-old adults, which means participants with 25(OH)D concentration <25 nmol/l as well as concentration >50 nmol/l were more likely to have moderate-to-severe disability trajectory. We showed that, in limited adjusted model, people aged 85+ years with a sufficient 25(OH)D concentration (25–50 nmol/l) were more likely to have less disability at baseline and a slower disability trajectory over the following five years. However, our results did not prove the protective effect of 25(OH)D concentration ≥50 nmol/l over five years, even though the cross-sectional analysis and unadjusted regression analysis showed a U-shaped association between 25(OH)D concentration and disability onset over five years.

Evidence from other studies

The current analysis suggests that a low concentration of 25(OH)D <25 nmol/l can partly predict the onset and progression of disability in very-old adults; this is inconsistent with the findings of recent cross-sectional and prospective cohort studies that investigate the association between disability and 25(OH)D concentration in those age 65 years and over. For example, two studies found that a 25(OH)D <50 nmol/l increased the risk of disability in arthritis and multiple sclerosis patients, respectively (Valderrama-Hinds et al., 2017, Oliveira et al., 2017). Likewise, Semba et al. (2000) found that a 25(OH)D <50 nmol/l was associated with a higher possibility of disability in women aged over 65 years and living in the community. However, our findings were insufficient either to invalidate or prove the effects of a higher concentration (≥50 nmol/l) in terms of slowing the disability trajectory over five years.

The higher risk of a disability trajectory amongst participants with a 25(OH)D concentration higher than 50 nmol/l in the cross-sectional analysis and unadjusted model could be driven largely by those with cognitive impairment or those taking vitamin D containing medication or prescribed medication. First, our overall analysis showed that the

association between a high concentration and disability trajectory disappeared after adjusting for these variables. Moreover, excluding participants with low SMMSE scores supports this finding. On the other hand, participants with a normal cognitive status did not show an association between high concentration and disability trajectory. In addition, there is no general agreement amongst researchers regarding the optimal concentration of 25(OH)D in relation to disability trajectory. The IoM (2011) defines vitamin D deficiency as a concentration of 25(OH)D <30 nmol/l, and vitamin D adequacy as a concentration of 30–50 nmol/l for all age groups based on integrating data from several health outcomes and PTH (Ross et al., 2011). In contrast, SACN sets the cut-offs at <25 nmol/l and ≥25 nmol/l for vitamin D deficiency and sufficiency, respectively (SACN, 2016). However, previous results have documented a U-shaped association between 25(OH)D and various health outcomes, including muscle strength and performance (Granic et al., 2017).

Generally, the main role of vitamin D is to support musculoskeletal health. Therefore, maintaining an adequate 25(OH)D concentration is essential in order to slow the effect of ageing on the bones and muscles. Ageing is accompanied by a redistribution of the cortical and trabecular bone (Bouxsein and Karasik, 2006). Moreover, a low 25(OH)D concentration increases osteoblastic activity and bone turnover (van de Peppel et al., 2018, Lips and van Schoor, 2011). A significant positive association has also been documented between 25(OH)D concentration, BMD (Bischoff-Ferrari et al., 2004b) and type II muscle fibre (Ceglia, 2008) in older people. In addition, the VDR expression is reduced in the muscles as part of ageing (Bischoff-Ferrari et al., 2004). A positive association between 25(OH)D concentration and muscle strength has been reported (Ceglia, 2008). Therefore, a lack of VDR, which is expected in very-old adults, leads to reduced muscle mass and strength, as explained previously. Furthermore, studies in rats have demonstrated that a high PTH, due to a low concentration of 25(OH)D, induces muscle catabolism and reduces calcium transport in the skeletal muscle (Ceglia, 2008), thereby leading to poor muscle strength. Combined, this can explain the effect of a low concentration of 25(OH)D on the onset and progression of disability.

In addition, the association between a moderate 25(OH)D concentration and physical performance and strength has been confirmed previously (Visser et al., 2003). Kotlarczyk et al. (2017) found that slower gait speed and poorer IADL scores were associated with low 25(OH)D concentration. Moreover, a positive association between the 8-foot walk test and the sit-to-stand test, with a concentration of 25(OH)D, was also found (Bischoff-Ferrari et al.,

2004c). These results indicate that a low concentration of 25(OH)D (<40 nmol/l) was associated with poor muscle strength, which is a predictor of disability. Consistent with our results, Granic et al. (2017) demonstrated that a concentration of >30 nmol/l maintains muscle strength, but a concentration of ≥50 nmol/l did not have muscular or musculoskeletal benefits in the very-old adults.

Physical activity is clearly a predictor of disability (Oliveira et al., 2017), although the association between PA and 25(OH)D was conflicted between the studies. First, a high concentration of 25(OH)D can positively influence the intensity of PA (Al-Eisa et al., 2016). However, a converse association is also suggested (Van den Heuvel et al., 2013). In the same vein, a study analysing the data from NHANES reported that PA is generally associated with a high concentration of 25(OH)D, whether this activity occurs indoors or outdoors (Fernandes and Barreto, 2017). This theory is also supported by the findings of Hill and others (Hill et al., 2016), who showed that the participants in the lowest quintile of the 25(OH)D from the Newcastle 85+ Study had the lowest level of PA. Therefore, restricted PA, which is associated with disability, can have an adverse effect on 25(OH)D concentration, possibly due either to a defect in metabolism or limited exposure to sunlight. Besides, PA is accompanied by improved health, stronger muscles and a lower BMI, which are all associated with 25(OH)D concentration (Semba et al., 2000, Stewart et al., 2009, Toffanello et al., 2012). Furthermore, the progression of disability is accompanied by a greater risk of feeding disability onset (Dunlop et al., 1997); this contributes to the risk of nutrient deficiency, including vitamin D. Our results show that the association between 25(OH)D and the disability trajectory disappeared after adjusting for PA. This suggests that the association between 25(OH)D concentration and disability could be due the effect of PA rather than 25(OH)D concentration. This means those with a higher PA have a better vitamin D status and, obviously, those with a high PA have less disability.

Age-related changes also result in body composition changes. For that reason, a lean body mass is significantly lower in older adults compared to younger ones - a change that accelerates after the age of 60 (Kyle et al., 2001). The univariate analysis of our data showed an association between fat-free mass and disability trajectory. However, the evidence demonstrated that the amount of fat mass but not fat-free mass was associated with muscle function and disability. For instance, Sternfeld et al. (2002) and Visser et al. (1998a) agreed that there was no association between physical disability and total body skeletal muscle mass, while a high percentage of fat mass was associated with physical disability.

Indeed, fat-free mass was not a significant predictor of mobility-related disability (Visser et al., 1998b). The association between 25(OH)D concentration and fat and lean mass could be explained by the escalation in fat mass, which may enhance the storage of vitamin D and, consequently, lower the circulating 25(OH)D (Stewart et al., 2009). However, the adjustment for BMI in the model, and for FFM in the sensitivity analysis, did not affect the association between 25(OH)D concentration and disability trajectory in the current study.

Our results also suggest that in limited adjusted models, men with a low concentration of 25(OH)D were more likely to develop only a severe disability trajectory, while women with a low concentration were more likely to develop either a moderate or a severe disability trajectory. This could be explained by the findings of Granic et al. (2016), who demonstrated that men had better muscle strength and physical performance (measured by grip strength and timed-up-and-go), but a steeper decline in both grip strength and timed-up-to-go over five years. Similarly, Millán-Calenti et al. (2010) reported that older men and women (80+ years) have a higher risk of being dependent (OR= 1.10) using ADL and IADL compared to younger adults (65+), but the risk among women is even higher (OR= 2.48). Conversely, our results are inconsistent with the findings of Semba et al. (2000), who demonstrated that only women with a low concentration were at risk of having a disability. This difference could be due to the smaller number of men compared to women in their study. However, across all studies including mine, the number of men is smaller than the number of women which may have implications for the interpretation of the research findings.

The association between 25(OH)D concentration and disability trajectory in the current study varies by season. In Winter, a U-shaped association was found between 25(OH)D concentration and disability trajectory. This conflicting results between the seasons could be explained by the differences between the participants' cognitive status, PA and vitamin D containing medication usage. The data showed that, in the Winter, of the 165 participants, 14 were cognitively impaired and took vitamin D containing medication; nine of the cognitively impaired participants were physically active compared to the 52 participants who had a normal cognitive status and were physically active. Therefore, a potential negative effect of the highest 25(OH)D quartile on disability trajectory could be partly driven by those who have an impaired cognitive status, that influences their PA, and by those who have reached a higher concentration through taking vitamin D containing medication shortly before the baseline assessments.

Strengths and limitations

The study has several strengths, including its prospective design, representation of the UK population due to the large number of participants, the five-year follow-up to measure disability, the robustness of the clustering technique (GBTM) used to derive the disability trajectory, and the adjustment for several potential confounders associated with disability and 25(OH)D concentration. PA and season, which could reflect UV exposure, were also considered in the models. In addition, determining disability by using 17 ADLs that compromise BADL, IADL and mobility items is also a strength of this study. Moreover, our study used the prevalent cut-offs to determine the concentration required to predict the onset and progress of the disability trajectory.

That said, the findings reported here should be interpreted with caution due to the following limitations. First, the concentration of 25(OH)D was only measured at baseline, so they may change during the subsequent five years depending on sun exposure, season, vitamin D containing medication usage, PA and disease. Another limitation was that the frequency or dosage of the vitamin D containing medication used, as well as UV exposure, were not measured. Finally, it is possible that some disability transitions were not fully captured during the follow-up phases, as these took place 18 or 24 months apart.

5.6. Summary

Sex, living in an institution, season, cognitive status, BMI and vitamin D containing medication usage were found to be predictors of disability trajectory. In models adjusted for the main confounders, we found an association between 25(OH)D concentration and disability trajectory. The main finding from the present study points to the role of 25(OH)D in the rate of increased disability among the participants. Maintaining aa adequate concentration, between 25 nmol/l and 50 nmol/l, may be protective against the onset of disability and the progress of the disability trajectory in the very-old adults. Finally, we could not find a beneficial effect of a concentration >50 nmol/l in protecting against a disability trajectory. Indeed, to the contrary, a high 25(OH)D (>50 nmol/l) concentration was predicted to be related to the inception of disability.

Chapter 6: The association between 25-hydroxyvitamin D concentration and metabolic and cardiopulmonary health in the very-old: The Newcastle 85+ Study.

Key words: ‘aged 80 and over’, 25(OH)D, NT-proBNP, FEV1, FVC

6.1. Abstract

***Introduction**:* Ageing is accompanied by multiple alterations and damage within the molecular pathways, which results in an increased risk of developing chronic diseases. A positive association was found between 25(OH)D concentration and cardiac and pulmonary diseases, which could be explained by locally synthesized 25(OH)D in the heart and lungs, and its receptors, that work in an autocrine/paracrine manner within these tissues. However, no study has yet examined the effect of 25(OH)D on MCPD in very-old adults.

***Aims**:* This study aims to use metabolic markers [Glycated haemoglobin (HbA1c)], cardiac markers [N-terminal brain natriuretic peptide (NT-proBNP) and diastolic blood pressure)] and respiratory markers [forced expiratory volume in one second (FEV1) and forced vital capacity (FVC)] to examine the effect of 25(OH)D on heart and lungs.

***Methodology**:* Data of 775 participants (with available 25(OH)D measurements) were included from the Newcastle 85+ Study. The participants were eligible for inclusion in the cohort study if they were born in 1921 and were registered with one of the general practitioners in Newcastle and North Tyneside. Both the health assessment (compromised questionnaires, measurements, function tests and fasting blood sample) and medical record data extraction were conducted by a trained research nurse. A concentration of 25(OH)D <25 nmol/l was considered low, 25-50 nmol/l was considered moderate and >50 nmol/l was considered high. To examine the association between 25(OH)D and NT-proBNP, HbA1c, FEV1, FVC and diastolic blood pressure, linear regression was used separately for each independent variable at baseline, 18 and 36 months.

***Results**:* A linear regression showed that low 25(OH)D concentration (<25 noml/l) was positively associated with good NT-proBNP, while 25(OH)D concentration between 25-50 noml/l was negatively associated with good FEV1 and FVC measurements, and finally, high 25(OH)D concentration (>50 noml/l) was negatively associated with good HbA1c. However, all of these association disappeared after adjusting for PA. No association was found between 25(OH)D concentration and diastolic blood pressure. In addition, no association was found between 25(OH)D concentration and any of the biomarkers at 18 and 36 months.

***Conclusion**:* Even though 25(OH)D may be associated with good metabolic and cardiopulmonary health biomarkers, our results could not prove its protective effect in the fully adjusted models at baseline or over 18 and 36 months.

6.2. Introduction

Ageing is accompanied by multiple alterations and damage within the molecular pathways (Wagner et al., 2016). This results in an increased risk of developing chronic diseases, such as MCPD. MCPD is a medical term used to describe a varied group of serious disorders that affect the heart and lungs. The most common types of MCPD are diabetes, hypertension, stroke, CHD and COPD. Therefore, biomarkers are used to monitor and predict health status and identify those susceptible to developing a particular health problem (Crimmins et al., 2008). However, there is no single biomarker that provides a valid measure of the biological process among an ageing group.

For example, NT-proBNP has been identified as a biomarker of age-related myocardial dysfunction (Wagner et al., 2016). It is a prohormone of BNP that is synthesised and released from the stressed wall of the cardiomyocytes (Nazemiyeh et al., 2015). Circulating NT-proBNP is a cardiac biomarker for the diagnosis, prognosis and therapeutic monitoring of heart failure (Passeri et al., 2016). Several studies have also suggested that NT-proBNP may be used as a screening test for pulmonary hypertension (Leuchte et al., 2006) and also as a diagnosis for acute pulmonary embolism (Pruszczyk et al., 2003). Moreover, FEV1 and FVC are lung function tests that are used as lung functional biomarkers. FEV1 is the amount of air that can be forced from the lungs in one second, while FVC is the total amount of air exhaled during the FEV1 test. Lastly, HbA1c is the most important laboratory parameter indicating glycaemic control.

Vitamin D plays an important role in controlling the metabolic, cardiac and pulmonary functions. First, various extra-renal tissues, including many of the cell types involved in MCPD, such as cardiac myocytes, fibroblasts and alveolar type II cells, express the converting enzyme (CYP27B1), and are therefore able to produce 1,25(OH)2D (Norman and Powell, 2014). Typically, the ability to generate the 1,25(OH)2D in these cells is dependent on the availability of 25(OH)D derived from the circulating blood and also on the availability of CYP27B1. Besides, VDRs were identified in over 30 different extra-skeletal tissues, including vascular smooth muscle cells (VSMC), endothelial cells (EC), cardiac myocytes, airway smooth muscles and the majority of immune cells (Norman and Powell, 2014). The effect of vitamin D is mediated by VDR, and as a result, the locally synthesized 1,25(OH)2D in these tissues binds to the intranuclear VDRs in an autocrine/paracrine manner. Given these effects, the evidence shows a positive association between VDR, 25(OH)D concentration and MCPD. A low 25(OH)D concentration was common among

COPD patients, especially those with CHD (Zhang and Yuan, 2016). A deficiency of VDR causes the severe progression of heart failure post-myocardial infraction because patients lack cardio-protective signaling through the VDR pathway. It has also been observed that the hospital admission of all respiratory and CVD had Winter peaks (Douglas et al., 1996). Furthermore, in a large community-dwelling sample of elderly individuals without cardiovascular diseases, a higher circulating 25(OH)D concentration was associated with lower insulin resistance (Danziger et al., 2013).

To our knowledge, no study has yet assessed the association between 25(OH)D concentration and MCPD in very-old adults. Therefore, this study aims to assess the association between 25(OH)D and metabolic and cardiopulmonary health that is identified amongst the Newcastle 85+ Study participants, using different physical and physiological biomarkers of heart, lung and metabolism functions. We also aim to define the adequate concentration of 25(OH)D to improve metabolic and cardiopulmonary health. We hypothesised that the concentration of 25(OH)D <25 nmol/l was related to poor level of NT-proBNP, HbA1c, FEV1, FVC and diastolic blood pressure.

6.3. Methodology

Population Sample

The participants were taken from the Newcastle 85+ Study, which included both community-dwelling and institutionalised older adults, who were 85 years-old at recruitment and living in Newcastle-upon-Tyne and North Tyneside. Both the health assessment, which comprised of questionnaires, measurements, function tests and a fasting blood sample, as well as general practice medical records to extract data on diagnosed diseases and prescribed medication, were available for the 851 participants. This analysis included all of the Newcastle 85+ Study participants (n=775) for whom data on health assessment, general practice records and 25(OH)D concentration were available at baseline (as detailed in Chapter 2) (Collerton et al., 2009).

NT-proBNP measurement

The blood samples for the NT-proBNP measurement were aliquoted on the day of collection and stored at -80°C. NTproBNP was measured by an electrochemiluminescent

sandwich immunoassay using the Modular Analytics E170 system (Roche Diagnostics, Lewes, UK). The between batch coefficient of variation was 1.5% - 3.5% from 122- 4322 ng/l, with an analytical range of 5-35000 ng/l. The laboratory performing the NT-proBNP assay and the echocardiologist was blinded to the echocardiographic and NT-proBNP data, respectively (Martin-Ruiz et al., 2011).

HbA1c measurement

Glycosylated haemoglobin (HbA1c) was measured using a Tosoh Eurogenetics automated HLC-723G7 HPLC analyser (Martin-Ruiz et al., 2011).

Blood pressure measurement

Diastolic and systolic blood pressures were measured using a digital blood pressure monitor – Omron HEM 705-IT (Omron Healthcare UK Ltd., Milton Keynes, UK). Three measurements were taken at two minute intervals; the average of the second and third measurements was used (Martin-Ruiz et al., 2011).

Lung function measurements

The spirometry and peak flow measurements were collected using MicroLab Spirometer and Spida V.5 software (Micro Medical, Rochester, UK). The aim was to obtain three technically satisfactory maximal effort 'blows' to generate reproducible FEV1, FVC and peak expiratory flow measurements (PEF); the blows were repeated until this was achieved or maximum effort reached (Fisher et al., 2016).

Statistical analysis

The 25(OH)D concentration was not normally distributed. The 25(OH)D concentration were categorised by widely used cut-off points according to the SACN Dietary Reference Intake report for vitamin D: <25 nmol/L (low), 25-50 nmol/L (moderate) and >50 nmol/l (high). To examine the association between 25(OH)D with baseline NT-proBNP, FEV1 and FVC, linear regression was used separately for each independent variable. The linearity and homoscedasticity assumptions were tested, with residuals normality versus predicted values plots. The multicollinearity between the confounders was also checked using the variance

inflation factor (VIF). The NT-proBNP was not normally distributed and transformed by log 10. Important confounders were selected based on their clinical and theoretical relevance, as well as univariate analysis with the NT-proBNP, FEV1, FVC, HbA1c and diastolic blood pressure. These confounders were then fitted, removed and refitted until the best possible (but parsimonious) model was achieved, while checking for model fit statistics throughout, using 10% of change-in-estimate. For each independent variable, Model 1 was the unadjusted model, Model 2 was further adjusted for sex and season, Model 3 was further adjusted for smoking, alcohol consumption and diastolic blood pressure and Model 4 was further adjusted for PA.

All analyses were performed using IBM's SPSS Statistics software, version 24 (IBM, New York) and $P < 0.05$ was considered statistically significant.

6.4. Results

Participant characteristics according to their 25(OH)D concentration

By using the 3 cut-offs of 25(OH)D concentration (low, moderate and high), there were significant differences between men and women, BMI categories, PA level, vitamin D containing medication and supplements usage, their general health rate, NT-proBNP level and HbA1c level as well as FEV1 and FVC measurements (Table 6.1). The majority of the participants were women, had normal weight, were moderately active, non-alcohol drinker, regular smokers and rated their health as good (Table 6.1).

Table 6.1: Participant characteristics by 25(OH)D concentration

	Low (n= 193)	Moderate (n= 302)	High (n=283)	All participants	p
Women % (n)	64.4 (123)	53.8 (162)	65.7 (186)	60.8 (471)	0.007
BMI % (n)					0.015
Underweight	22.2 (39)	30.0 (86)	35.5 (91)	30.0 (216)	
Normal	43.8 (77)	46.0 (132)	41.0 (105)	43.7 (314)	
Overweight	19.9 (35)	14.6 (42)	17.2 (44)	16.8 (121)	
Obese	14.2 (25)	14.2 (25)	6.3 (16)	9.5 (68)	
PA % (n)					0.001
Low	28.6 (54)	15.4 (46)	24.8 (70)	22.1 (170)	
Moderate	48.1 (91)	131.0 (44)	38.3 (108)	42.9 (330)	
High	23.3 (44)	40.6 (121)	36.9 (104)	35.0 (269)	
Alcohol drinkers % (n)					0.056
Never	42.9 (81)	40.1 (120)	40.7 (113)	41.0 (314)	
Moderate	30.2 (57)	39.5 (118)	41.0 (114)	37.7 (289)	
Heavy	11.6 (22)	10.7 (32)	9.4 (26)	10.4 (80)	
Smoking % (n)					0.447
Never	36.6 (70)	33.3 (100)	36.2 (102)	35.2 (272)	
Occasional	4.2 (8)	6.3 (19)	4.6 (13)	5.1 (40)	
Regular	59.2 (113)	60.3 (181)	59.2 (167)	59.6 (461)	
Vitamin D containing medication % (n)	0.0 (1)	6.0 (17)	38 (108)	16.5 (126)	<0.001
Supplements users % (n)	4.7 (9)	16.9 (51)	32.5 (92)	19.5 (152)	<0.001
Self-rated health % (n)					0.006
Very good	37.7 (72)	40.5 (122)	41.7 (118)	40.3 (312)	
Good	53.9 (103)	56.1 (169)	55.1 (156)	55.2 (428)	
Poor	6.3 (12)	2.1 (6)	2.1 (6)	3.0 (23)	
Disease count					0.675
mean (SD)	4.9 (1.8)	4.7 (1.6)	4.8 (1.9)	4.8 (1.8)	
NT-proBNP					0.039
median (IQ)	406.0 (746)	780.7 (594)	737.7 (585)	346.0 (592)	
HbA1c (%) mean (SD)	6.1 (1.1)	6.0 (0.7)	5.8 (0.6)	5.9 (0.7)	0.025
FEV1 mean (SD)	136.3 (53.6)	153.1 (53.5)	141.9 (53.8)	154.1 (53.8)	0.002
FVC mean (SD)	199.4 (73.7)	255.1 (74.5)	211.6 (74.7)	214.3 (74.7)	0.002
Blood pressure mean (SD)	73.5 (11.7)	74.1 (11.2)	73.9 (12.5)	73.9 (11.8)	0.779

BMI: body mass index. FEV1: Forced expiratory volume in one second. FVC: forced volume vital capacity. HbA1c: glycated hemoglobin. NT-proBNP, N-terminal pro b-type natriuretic peptide p, p-value: Mann-Whitney U test for continuous non-normally distributed variables or $\chi 2$ test for categorical variables. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate), >50 nmol/l (high).

Association between 25(OH)D concentration and NT-proBNP level

A significant positive association was found between low 25(OH)D concentration and NT-proBNP level in the unadjusted model (95% CI=34.6, 699.2, B=366.9 ±169.2, p=0.031) and even after adjusting for sex, season, smoking, alcohol consumption and diastolic BP (95% CI=23.9, 697.5 , B= 360.7±171.5 p= 0.036). However, this association disappeared after adjusting for PA (95% CI=-69.6, 528.7, B=229.5±171.0, p=0.132). No significant association was found between high 25(OH)D concentration and NT-proBNP level (Table 6.2).

At follow-up phases, no significant association was found between 25(OH)D concentration and NT-proBNP level at 18 and 36 months in both unadjusted and adjusted models.

When stratifying the analysis by sex, only men showed a significant positive association between low 25(OH)D and NT-proBNP level in the unadjusted model, even after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA (95% CI= 52.2, 1294.0, B=673.1±316.3, p=0.0034) (Table C in Appendix)

Association between 25(OH)D concentration and HbA1c level

A significant negative association was found between high 25(OH)D concentration and HbA1c level in the unadjusted model (95% CI=-0.2, -0.0, B=-0.1±0.0, p=0.014). This association remained even after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA (95% CI=-0.2, -0.0 , B=-0.1±0.0, p=0.023). No significant association was found between low 25(OH)D concentration and HbA1c level (Table 6.5).

At follow-up phases, no significant association was found between 25(OH)D concentration and HbA1c level at 18 and 36 months in both unadjusted and adjusted models.

Stratifying the analysis by sex, only women in the unadjusted model showed a significant negative association between high 25(OH)D and HbA1c level (95% CI= 0.3, -0.0, B= -0.1±0.0, p= 0.040). This association disappeared after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA (Table D in Appendix).

Association between 25(OH)D concentration and FEV1

A significant negative association was found between low and high 25(OH)D concentration and FEV1 measurement in the unadjusted model (95% CI= -26.6, -6.8, B=-16.7±5.0, p=<0.001) and (95% CI=-20.1, -2.3, B=-11.2±4.5, p= 0.014), respectively. The association remained between low 25(OH)D concentration and FEV1 measurement after adjusting for sex, season, smoking, alcohol consumption and diastolic BP (95% CI=-21.2, -3.8, B=-12.5±4.4, p= 0.005). However, this association disappeared after adjusting for PA (95% CI=-15.4, 1.3, B=-7.0±4.3, p=0.072). The association also disappeared between high 25(OH)D and FEV1 measurement in the adjusted model.

At 18 months, a significant negative association was found between low 25(OH)D concentration and FEV1 measurement in the unadjusted model (95% CI=-24.1, -1.2, B=-12.7±5.8, p=0.030). This association disappeared after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA. No significant association was found at 36 months.

Stratifying the analysis by sex, only women in the unadjusted model showed a significant negative association between low 25(OH)D and FEV1 measurement in the unadjusted model, even after adjusting for sex, season, smoking, alcohol consumption and diastolic BP (95% CI= -20.3, -2.0, B= -11.1±4.6, p= 0.017). This association disappeared after adjusting for PA (Table E in Appendix)

Association between 25(OH)D concentration and FVC

A significant negative association was found between low and high 25(OH)D concentration and FVC measurement in the unadjusted model (95% CI=-39.4, -11.9, B=-25.6±6.9, p=<0.001) and model (95% CI=-25.8, -1.1, B=-13.5±6.2, p=0.032), respectively. This association remained between low 25(OH)D concentration and FVC measurement even after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA (95% CI=-22.1, -0.5, B=-11.3±5.5, p=0.040). The association disappeared between high 25(OH)D and FVC measurement in the adjusted model (Table 6.4).

At 18 months, a significant negative association was found between low 25(OH)D concentration and FVC measurement in the unadjusted model (95% CI=-36.7, -5.1, B=-20.9±8.0, p=0.009). This association continued after adjusting for sex, season, smoking, alcohol consumption, diastolic BP (95% CI=-25.9, -0.1, B=-13.0±6.5, p=0.048), but it

disappeared after further adjustments for PA. No significant association was found at 36 months.

When stratifying the analysis by sex, both men and women showed a significant negative association between 25(OH)D concentration and FVC measurement in the unadjusted models (95% CI= -43.2, -1.7, B= -22.4±10.5, p=0.0034) and (95% CI= 27.3, -3.9, B= -15.6±5.9, p= 0.009), for men and women respectively. After adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA, the association disappeared for men. However, for women, the association continued after adjusting for sex, season, smoking, alcohol consumption and diastolic BP (95% CI= 28.2, -4.3, B= -16.2±6.0, p= 0.008), but it disappeared after further adjustments for PA (Table F in Appendix).

Association between 25(OH)D concentration and diastolic blood pressure

No significant association was found between low and high 25(OH)D concentration and diastolic BP in the unadjusted model (95% CI=-2.8, 1.4, B=-0.6±1.0, p=0.542) and (95% CI=-2.1, 1.7, B=-0.2±0.9, p=0.830), respectively, even after adjusting for sex, season, smoking, alcohol consumption, diastolic BP and PA (95% CI=-2.2, 2.1, B=-0.0±1.1, p=0.974) and (95% CI=-1.5, 2.5, B=0.4±1.0, p=0.631), respectively at baseline (Table 6.6). However, data were not available for follow-up phases.

When stratifying the analysis by sex, both men and women showed no significant association between low and high 25(OH)D concentration and diastolic BP in the unadjusted mode and even after adjusting for the related cofounders (Table G in Appendix).

Table 6.2: Association between 25(OH)D concentration and NT-proBNP level

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Baseline	Model 1	Low	366.9	0.006	34.6- 699.2	0.031
		Moderate	(ref)		(ref)	(ref)
		High	-43.0		-344.7- 258.6	0.779
	Model 2	Low	389.2	0.012	54.6- 723.9	0.023
		Moderate	(ref)		(ref)	(ref)
		High	26.4		-278.9- 331.9	0.865
	Model 3	Low	360.7	0.016	23.9- 697.5	0.036
		Moderate	(ref)		(ref)	(ref)
		High	-4.9		-313.9- 304.0	0.975
	Model 4	Low	229.5	0.044	-69.6- 528.7	0.132
		Moderate	(ref)		(ref)	(ref)
		High	22.5		-248.9- 294.0	0.871
18 months	Model 1	Low	146.2	-0.003	-317.5- 610.0	0.535
		Moderate	(ref)		(ref)	(ref)
		High	-97.3		-542.4- 347.6	0.667
	Model 2	Low	174.8	0.016	-311.3- 661.0	0.480
		Moderate	(ref)		(ref)	(ref)
		High	28.0		-441.6- 497.6	0.907
	Model 3	Low	167.0	0.019	-323.3- 657.4	0.503
		Moderate	(ref)		(ref)	(ref)
		High	4.3		-472.9- 481.6	0.986
	Model 4	Low	132.5	0.023	-358.9- 624.1	0.596
		Moderate	(ref)		(ref)	(ref)
		High	8.4		-467.8- 484.7	0.972
36 months	Model 1	Low	337.6	-0.007	-284.2- 959.6	0.285
		Moderate	(ref)		(ref)	(ref)
		High	16.3		-357.3- 390.0	0.931
	Model 2	Low	342.9	-0.022	-290.5- 976.5	0.286
		Moderate	(ref)		(ref)	(ref)
		High	24.3		-356.0- 404.8	0.899
	Model 3	Low	355.1	-0.014	-288.6- 998.9	0.277
		Moderate	(ref)		(ref)	(ref)
		High	45.1		-348.0- 438.3	0.821
	Model 4	Low	369.0	-0.023	-294.2- 1032.3	0.273
		Moderate	(ref)		(ref)	(ref)
		High	42.1		-357.2- 441.4	0.835

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high) NT-proBNP: N-terminal pro b-type natriuretic peptide. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex and season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

Table 6.3: Association between 25(OH)D concentration and HbA1c level

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Baseline	Model 1	Low	0.0	0.012	-0.0- 0.2	0.330
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.2- -0.0	0.014
	Model 2	Low	0.0	0.012	-0.0- 0.1	0.479
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.2- -0.0	0.023
	Model 3	Low	0.0	0.020	-0.0-0.1	0.522
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.2- -0.0	0.031
	Model 4	Low	0.0	0.027	-0.1- 0.1	0.811
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.2- -0.0	0.023
18 months	Model 1	Low	-0.042	-0.003	-0.1- 0.0	0.538
		Moderate	(ref)		(ref)	(ref)
		High	0.003		-0.1- 0.1	0.966
	Model 2	Low	-0.0	0.001	-0.2- 0.0	0.404
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.1- 0.1	0.955
	Model 3	Low	-0.0	0.009	-0.2- 0.0	0.287
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.1- 0.1	0.901
	Model 4	Low	-0.0	0.012	-0.2- 0.0	0.193
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.1- 0.1	0.840
36 months	Model 1	Low	0.01	-.002	-0.1- 0.1	0.783
		Moderate	(ref)		(ref)	(ref)
		High	-0.04		-0.1- 0.0	0.458
	Model 2	Low	0.0	0.003	-0.1- 0.1	0.769
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.1- 0.0	0.472
	Model 3	Low	0.0	0.017	-0.1- 0.1	0.783
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.1- 0.0	0.457
	Model 4	Low	0.0	0.018	-0.1- 0.1	0.919
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-0.0- 0.1	0.462

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). HbA1c: glycosylated haemoglobin. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex and season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

Table 6.4: Association between 25(OH)D concentration and FEV1 measurement

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Baseline	Model 1	Low	-16.7	0.014	-26.6- -6.8	<0.001
		Moderate	(ref)		(ref)	(ref)
		High	-11.2		-20.1 -2.3	0.014
	Model 2	Low	-12.2	0.284	-20.9- -3.5	0.006
		Moderate	(ref)		(ref)	(ref)
		High	-3.8		-11.7- 3.9	0.335
	Model 3	Low	-12.5	0.295	-21.2- -3.8	0.005
		Moderate	(ref)		(ref)	(ref)
		High	-3.5		-11.4- 4.3	0.377
	Model 4	Low	-7.0	0.355	-15.4- 1.3	0.072
		Moderate	(ref)		(ref)	(ref)
		High	-1.7		-9.2 -5.8	0.659
18 months	Model 1	Low	-12.7	0.005	-24.1- -1.2	0.030
		Moderate	(ref)		(ref)	(ref)
		High	-7.7		-17.9- 2.5	0.139
	Model 2	Low	-8.4	0.310	-18.5- 1.5	0.097
		Moderate	(ref)		(ref)	(ref)
		High	-1.7		-10.6- 7.1	0.701
	Model 3	Low	-8.1	0.316	-18.2- 1.9	0.115
		Moderate	(ref)		(ref)	(ref)
		High	-1.5		-10.5- 7.5	0.739
	Model 4	Low	-1.9	0.375	-11.7- 7.9	0.701
		Moderate	(ref)		(ref)	(ref)
		High	0.3		-8.3- 8.9	0.943
36 months	Model 1	Low	-7.7	0.000	-20.7- 5.2	0.244
		Moderate	(ref)		(ref)	(ref)
		High	-6.9		-18.3- 4.4	0.233
	Model 2	Low	-4.1	0.292	-15.5- 7.3	0.478
		Moderate	(ref)		(ref)	(ref)
		High	-1.3		-11.2- 8.6	0.795
	Model 3	Low	-5.1	0.297	-16.6- 6.2	0.373
		Moderate	(ref)		(ref)	(ref)
		High	-2.4		-12.4- 7.4	0.628
	Model 4	Low	-1.4	0.327	-12.7- 9.8	0.806
		Moderate	(ref)		(ref)	(ref)
		High	-1.2		-11.0- 8.4	0.794

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). FEV1: forced expiratory volume in one second. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex and season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

Table 6.5: Association between 25(OH)D concentration and FVC measurement

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Baseline	Model 1	Low	-25.6	0.016	-39.4- -11.9	<0.001
		Moderate	(ref)		(ref)	(ref)
		High	-13.5		-25.8- -1.1	0.032
	Model 2	Low	-17.9	0.389	-29.0- -6.8	0.002
		Moderate	(ref)		(ref)	(ref)
		High	-1.9		-11.9- 8.0	0.699
	Model 3	Low	-18.3	0.393	-29.5- -7.2	0.001
		Moderate	(ref)		(ref)	(ref)
		High	-1.8		-11.9- 8.2	0.721
	Model 4	Low	-11.3	0.444	-22.1- -0.5	0.040
		Moderate	(ref)		(ref)	(ref)
		High	0.5		-9.1- 10.2	0.915
18 months	Model 1	Low	-20.9	0.008	-36.7- -5.1	0.009
		Moderate	(ref)		(ref)	(ref)
		High	-9.1		-23.2- 4.9	0.204
	Model 2	Low	-13.6	0.408	-26.4- -0.8	0.036
		Moderate	(ref)		(ref)	(ref)
		High	-0.6		-12.0- 10.7	0.908
	Model 3	Low	-13.0	0.409	-25.9- -0.1	0.048
		Moderate	(ref)		(ref)	(ref)
		High	0.0		-11.4- 11.6	0.987
	Model 4	Low	-4.4	0.471	-16.8- 8.0	0.486
		Moderate	(ref)		(ref)	(ref)
		High	2.6		-8.3- 13.6	0.638
36 months	Model 1	Low	-12.1	0.000	-30.7- 6.4	0.200
		Moderate	(ref)		(ref)	(ref)
		High	-7.8		-24.1- 8.4	0.346
	Model 2	Low	-2.7	0.412	-17.9- 12.4	0.721
		Moderate	(ref)		(ref)	(ref)
		High	0.2		-12.8- 13.3	0.972
	Model 3	Low	-7.6	0.385	-22.9- 7.6	0.326
		Moderate	(ref)		(ref)	(ref)
		High	-1.1		-14.4- 12.2	0.867
	Model 4	Low	-5.8	0.379	-21.1- 9.4	0.455
		Moderate	(ref)		(ref)	(ref)
		High	-0.0		-13.3- 13.2	0.998

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). FVC: forced vital capacity. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex and season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

Table 6.6: Association between 25(OH)D concentration and diastolic blood pressure

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Baseline	Model 1	Low	-0.6	-0.002	-2.8- 1.4	0.542
		Moderate	(ref)		(ref)	(ref)
		High	-0.2		-2.1- 1.7	0.830
	Model 2	Low	-05	-0.001	-2.7- 1.6	0.636
		Moderate	(ref)		(ref)	(ref)
		High	0.3		-1.6- 2.3	0.740
	Model 3	Low	-0.6	-0.001	-2.8- 1.6	0.590
		Moderate	(ref)		(ref)	(ref)
		High	0.3		-1.7- 2.3	0.767
	Model 4	Low	-0.0	0.011	-2.2- 2.1	0.974
		Moderate	(ref)		(ref)	(ref)
		High	0.4		-1.5- 2.5	0.631

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for sex and season. Model 3 is further adjusted for smoking and alcohol consumption. Model 4 is further adjusted for PA.

6.5. Discussion

Main findings

We showed a positive association between low 25(OH)D concentration and NT-proBNP level among people aged 85 years and older in a limited adjusted model. That said, when 25(OH)D is <25 nmol/l, NT-proBNP is more likely to be decreased. The current study also showed the inverse association between high 25(OH)D concentration and HbA1c level, which means that HbA1c is reduced at concentration >50 nmol/l. Moreover, both low and high 25(OH)D concentration is negatively associated with both FEV1 and FVC measurements in the limited adjusted model in the very-old adults. That said, the participants with concentration between 25-50 nmol/l were more likely to have better FEV1 and FVC measurements. This study could not confirm the protective effect of the 25(OH)D on the metabolic and cardiopulmonary health biomarkers in the fully adjusted models or at 18 and 36 months.

Evidence from other studies

25(OH)D and NT-proBNP in the heart

BNP is a hormone that is synthesized by the cardiomyocytes (ventricles). The main stimulus responsible for increasing its synthesis and secretion is myocardial wall stress, which causes strong vascular relaxation and stimulates natriuresis (Nazemiyeh et al., 2015).

The current study found a positive association between the concentration of 25(OH)D and NT-proBNP level. This association could be explained by a direct and indirect mechanism. The direct mechanism is via the promoter region of the BNP gene that contains various transcription factors binding sites, including VDR in the heart (Santhekadur et al., 2017). Chen and colleagues demonstrated that VDR has the capacity to bind directly to the BNP gene promoter. They also found that the activation of myocyte hypertrophy is associated with a significant increase in VDR expression, implying the activation of a counter-regulatory mechanism to control growth in the hypertrophied heart (Chen et al., 2008). The indirect mechanism is via PTH, calcium or the inflammatory effect of 25(OH)D on the heart. As a result of the low concentration of 25(OH)D and VDR, heart failure occurs and the blood BNP increases.

The findings of the current study are inconsistent with that of an observational study on young CHD patients that failed to detect an association between 25(OH)D concentration and NT-proBNP level (Wetmore et al., 2011). These conflicting findings may be due to age-related changes. BNP is dependent on renal function for clearance, so it seems reasonable that age-dependent BNP elevation, as well as 25(OH)D reduction, may be partly due to the decline of GFR with ageing (Wiley et al., 2010). Besides, it is well documented that the BNP level increases with ageing (Keyzer et al., 2014), while VDR expression decreases with ageing (Bischoff-Ferrari et al., 2004a).

In contrast, other studies do not support the association between the concentration of 25(OH)D and CVD. For instance, older men showed no significant association between 25(OH)D concentration or vitamin D intake and CVD outcomes (Messenger et al., 2012). The Women's Health Initiative (WHI) also failed to identify any link between vitamin D and calcium supplements with CVD mortality and morbidity (Scragg et al., 2016). Similarly, a study by Hsia et al. (2007) argued that vitamin D and calcium supplements neither increased nor decreased the risk of CHD or strokes in postmenopausal women, who were generally deemed healthy throughout the 7-year randomized trial. Possible explanations of this null finding could be background calcium use, low doses of vitamin D, poor adherence to the study medication, or concurrent postmenopausal hormone therapy that may have interfered with the treatment effects.

25(OH)D and NT-proBNP in the lungs

The findings of the current study also showed a negative association between 25(OH)D and FEV1and FVC measurements, confirming the association between 25(OH)D concentration and the lungs found in previous studies. For instance, a positive association was found between the concentration of 25(OH)D deficiency and the severity of COPD (Finklea et al., 2011). Likewise, Janssens et al. (2010) demonstrated that a low 25(OH)D concentration was common in patients with COPD and associated with the severity of the disease, as measured by FEV1 and FVC.

There are several mechanisms that may explain this relationship. The first is the role that vitamin D plays in airway remodelling, by increasing the airway's smooth muscle mass, fibrosis by alteration in the extracellular matrix composition and subepithelial membrane thickening (Berraies et al., 2014). The second is the role that vitamin D plays in influencing

the development and modulation of the immune system by inhibiting the activation and proliferation of T lymphocytes (Aranow, 2011). Moreover, VDR in fetal type II cells influences lung development, growth, maturation and surfactant secretion. Therefore, 25(OH)D inhibits apoptosis and increases alveolar type II cell proliferation (Hornikx et al., 2012, Herr et al., 2011).

The association between 25(OH)D concentration and BNP level could be an indirect one due to the influence of low 25(OH)D on the lungs. Consequently, BNP is increased for vasodilation and antiproliferative actions (Leuchte et al., 2006). Previous observational studies reported three major connections between BNP and lung disease. First, NT-proBNP increases significantly with the severity of COPD (Chi et al., 2012). Second, blood NT-proBNP level are associated significantly with chronic respiratory failure (Leuchte et al., 2006) and third, blood NT-proBNP level are associated significantly with systolic pulmonary artery pressure (Nazemiyeh et al., 2015). However, there is a suggestion that pulmonary dysfunction is related to the severity of heart failure. Therefore, the increase in NT-proBNP level may cause a significant decrease in pulmonary volume (Nazemiyeh et al., 2015).

25(OH)D and metabolic syndromes

The results of the study were consistent with the previous finding that HbA1c was reduced significantly as vitamin D status increased in 1,000 diabetic patients aged 43 years-old (Buhary et al., 2017). In contrast, a total of 128 patients with diabetes mellitus, aged 57, were enrolled in one study. The mean HbA1c values of the patients were 9.18±2.52, and 98.4% of the diabetic patients had insufficient vitamin D status. However, no association between vitamin D status and glycaemic control was found in the study (Olt, 2015). However, the association between 25(OH)D concentration and HbA1c level could be mediated through VDR and DBP, which are present in the pancreatic tissue. As a result, 1,25(OH)D enhances insulin production and secretion via its action on the VDR (Kumar et al., 2018). Moreover, 1,25(OH)D improves insulin sensitivity by preventing the excessive synthesis of inflammatory cytokines (Szymczak-Pajor and Śliwińska, 2019).

Our results were inconsistent with the many observational studies that found an inverse association between vitamin D status and hypertension (for example, (Norman and Powell, 2014). In addition, a study by Pekkanen et al. (2015) proposed that a low 25(OH)D concentration may indicate an increased risk of systolic and diastolic dysfunction. This

variation between the results could be due to the participants' different ages. Very-old adults tend to have higher BP generally. Besides, the cross-sectional measurement of BP does not truly reflect the presence of the disease.

Strengths and limitations

This study has several strengths, including its prospective design and representativeness, as well as its robust statistical method. Furthermore, it assesses the association between 25(OH)D concentration and metabolic and cardiopulmonary health using different biomarkers. Finally, it controls for numerous potential confounders associated with MCPD and 25(OH)D concentration.

The findings reported here should be interpreted with caution due to the following limitations. First, this is a cross-sectional analysis and the results may not be representative of the prospective association between 25(OH)D concentration and biomarkers. Another limitation is that the number of the participants who used vitamin D containing medication is very low in the study. Therefore, the models did not include the vitamin D containing medication usage.

6.6. Summary

This study could not confirm the protective effect of the 25(OH)D on the metabolic and cardiopulmonary biomarkers in the fully adjusted model at baseline, as well and at the follow-up phases (18 and 36 months). Even though the limited adjusted model at baseline showed a significant association between 25(OH)D and the metabolic and cardiopulmonary biomarkers, given the wide 95% CI, I am inclined to say that 25(OH)D may not have the protective effect for metabolic and cardiopulmonary biomarkers.

Chapter 7: The association between 25-hydroxyvitamin D concentration and Telomere Length in the very-old: The Newcastle 85+ Study.

Key words: ageing; vitamin D; telomere length

7.1. Abstract

Introduction: Vitamin D may maintain Telomere Length, either directly or via its effects on mechanisms, including inflammation and/or the rate of cell proliferation. Whilst results from cross-sectional studies investigating the association between 25(OH)D concentration and Telomere Length have been mixed, there is a dearth of data from prospective studies which have assessed these associations

Aims*:* This study aimed to examine the association between 25(OH)D concentration and Telomere Length in very-old adults (>85 years-old) at baseline, 18 months and 36 months by controlling for related lifestyle factors.

Methodology*:* Our prospective cohort study comprised 775 participants from the Newcastle 85+ Study who had 25(OH)D measurements at baseline. Concentration of 25(OH)D was stratified as <25 nmol/l (low), 25-50 nmol/l (moderate) and >50 nmol/l (high). Peripheral blood mononuclear cell Telomere Length was measured by quantitative real-time PCR with modifications at baseline, 18 and 36 months from baseline.

Results*:* A positive significant association was found between 25(OH)D concentration and Telomere Length amongst very-old participants at baseline (95%CI= 12.0-110.3, B= 61.2$\pm$25.0, p=0.015). This association was negative at 18 months (95%CI=-59.9- -7.5, B= -33.7$\pm$13.3, p=0.012). but was non-significant at 36 months.

Conclusion*:* Circulating 25(OH)D concentration shows inconsistent relationships with Telomere Length over time in very-old (85+ years-old) adults.

7.2. Introduction

Telomeres, the specific DNA protein structures, are the cap at both ends of each chromosome. They protect the genome from nucleolytic degradation, unnecessary recombination, repair, and interchromosomal fusion (Pusceddu et al., 2015). Each DNA replication causes Telomere shortening and, when the Telomere Length reaches a critical limit, the cell undergoes senescence and/or apoptosis (Shammas, 2011). This situation can be reversed by the enzyme named Telomerase, which is responsible for Telomere Length maintenance (Muzumdar and Atzmon, 2012). Age is a well-established factor associated with Telomere shortening (Beilfuss et al., 2017). In humans, Telomere Length decreases at a rate of 24.8–27.7 base pairs per year (Shammas, 2011). Whilst age associated attrition in Telomeres is linked to many diseases of ageing and their complications, Telomere attrition with aging has been demonstrated in numerous studies, independent of the presence of any age related diseases (Muzumdar and Atzmon, 2012). This supports the view that shortened Telomere Length, observed in chronologic ageing may serve as a biomarker of age-related diseases (Muzumdar and Atzmon, 2012). On the other hand, it is purported that the cellular and tissue defects associated with Telomere dysfunction are mediated in part by oxidative stress and chronic inflammation mechanisms (Xu et al., 2009). Accelerated Telomere shortening is associated with mortality and many age-associated diseases, such as cardiovascular diseases, type 2 diabetes mellitus (T2DM), Alzheimer's disease (Pusceddu et al., 2015), as well as immune and infectious diseases (Cawthon et al., 2003) [Figure 7.1].

Evidence from animal models as well as human studies have demonstrated that various nutrients (e.g. folate, niacin, vitamin C, magnesium, zinc and omega-3 fatty acids), bioactives (e.g. polyphenols) and whole foods (e.g. tea) may influence Telomere Length and Telomere attrition through mechanisms related to cellular functions including DNA repair and chromosome maintenance, DNA methylation, inflammation, oxidative stress and Telomerase activity [For review see 7]. Vitamin D may influence Telomere Length through its biologically active hormone 1α,25 dihydroxyvitamin D3 (calcitriol) (Zarei et al., 2020a). Calcitriol is a potent immunosuppressant and has strong anti-inflammatory and anti-proliferative properties mediated in part by its ability to reduce gene expression of inflammatory mediators interleukin- 2 and interferon gamma (Paul, 2011). The anti-inflammatory and anti-proliferative properties of calcitriol may reduce cell turnover, thus potentially reducing their Telomere Length attrition (Paul, 2011).

Limited population studies have assessed the association between circulating 25(OH)D concentration [the most commonly used nutritional biomarker of vitamin D] and Telomere Length. A recent studyZarei et al. (2020a) demonstrated that only at insufficient concentration of 25(OH)D (<50 nmol/l), Telomerase activity is associated with survival times in CHD patients (mean age 59.9 years-old). In addition, the findings by Richards et al. (2007) and Liu et al. (2013) demonstrated a positive association between 25(OH)D concentration and Leukocyte Telomere Length (LTL) in women. On the other hand, a cross-sectional study of 2483 men aged 40-75 years did not observe a positive association between vitamin D biomarkers (25(OH)D and 1,25(OH)2D) and LTL (Julin et al., 2017). Liu et al. (2016) also found no association between absolute 25(OH)D concentration and long LTL, for the overall population or the subgroups in the study (men, women, black and white separately) although maintaining a 25(OH)D concentration ≥30 nmol/l was significantly associated with longer LTL in white participants only. Yet, Zhu et al. (2012) showed an increase in Telomerase activity in 19 overweight African-American women after 16 weeks of vitamin D supplementation.

These conflicting results may be attributed to the differences between sex, race and ages of study participants. For instance, the age range in one study was 48–93 years-old (a mean age of 62·8 years-old) (Liu et al., 2016), whereas Richards et al. (2007) studied younger women aged 18 –79 years-old (a mean age of 49·4 years-old). Besides, other studies failed to prove the association between 25(OH)D and Telomere Length in younger participants (mean age 31-39 years-old (Williams et al., 2016) (Hoffecker et al., 2013)). Generally, these studies included a wide age range of participants, participants younger than 70 years-old, or participants of only one sex, which limited the generalizability for those older than 80 years-old. Therefore, this study aimed to use the large dataset on both sexes from the Newcastle 85+ Study to examine the association between 25(OH)D concentration and Telomere Length in very-old adults (>85 years-old) group at baseline, at 18 months and at 36 months from baseline. We hypothesize that, by controlling related lifestyle factors, concentration of 25(OH)D >25 nmol/l will be positively associated with shorter Telomere Length in very-old adults.

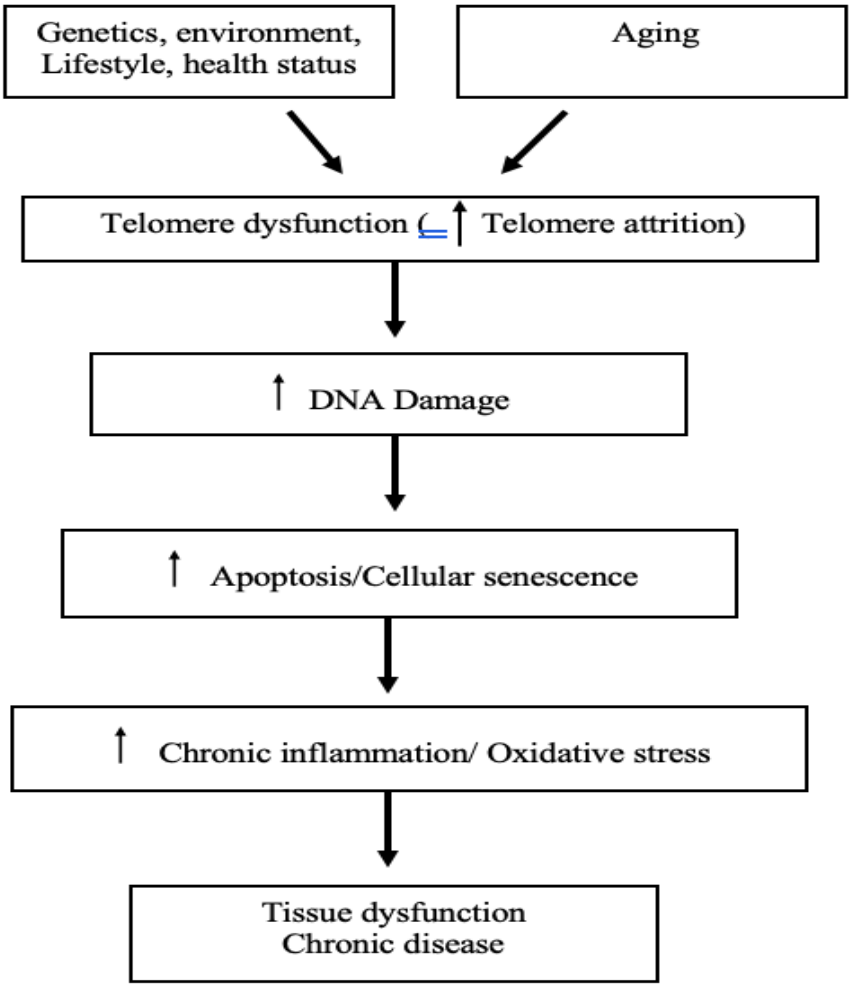


Figure 7. 1:**Schematic overview of the role of Telomeres in tissue dysfunction and chronic disease**

7.3. Methodology

Population Sample

The participants were taken from the Newcastle 85+ Study, which included both community-dwelling and institutionalised older adults aged 85 years-old at recruitment and living in Newcastle-upon-Tyne and North Tyneside. Both health assessments, which comprised questionnaires, measurements, function tests and a fasting blood sample as well as general practice medical records from which to extract data on diagnosed diseases and prescribed medication, were available for the 851 participants. This analysis included all of the Newcastle 85+ Study participants (N=775) whose data on health assessment, general practice records and 25(OH)D concentration were available at baseline (more details were provided in Chapter 2) (Collerton et al., 2009).

Telomere length

Telomere Length was measured as an abundance of telomeric template versus a single housekeeping gene by quantitative real-time PCR. The intra-assay coefficient of variation was 2.7% while the inter-assay coefficient of variation was 5.1%. Four internal control DNA samples were run within each plate to correct for plate–to-plate variation. The measurements were performed in quadruplicate. All PCRs were carried out on an Applied Biosystems 7900HT Fast Real Time PCR machine with a 384-well plate capacity (Martin-Ruiz et al., 2011).

Aviv et al. (2006b) proposes guidance for epidemiological research on the minimum numbers of subjects required for a given age range to determine whether the extrapolated Telomere attrition rate of two groups are significantly different. For longitudinal studies assessing Telomere Length in older adults (> 60 years) over time, the sample size required in each test group being compared for 80% power and $P<0.05$ using a two sided t-test is 104 participants (Aviv et al., 2006b). However, given the limited data on Telomere attrition in those aged 85-88 years (the age range of the participants in this analysis), any power calculations for very-old population are purely speculative.

Statistical analysis

The concentration of 25(OH)D was not normally distributed (and could not be normalized by transformation, as previously mentioned (Hill et al., 2016)). In addition, the Telomere Length was not normally distributed (and could not be normalized by transformation either). The 25(OH)D concentration was categorized by SACN cut-offs points for vitamin D: <25 nmol/L (low), 25-50 nmol/l (moderate) and ≥50 nmol/L (high). To examine the association between 25(OH)D and prospective Telomere Length at baseline, 18 months and 36 months, linear regression was used. The linearity and homoscedasticity assumptions were tested with residual normality versus predicted values plots. Important confounders were selected based on their clinical and theoretical relevance to the Telomere Length. These variables were then fitted, removed, and refitted until the best possible but parsimonious model was achieved while checking for model fit statistics throughout. Model 1 is an unadjusted model, Model 2 is adjusted for smoking and alcohol consumption, Model 3 is further adjusted for BMI and PA, and Model 4 is further adjusted for HbA1c%. The models were stratified by sex. A Dunn–Bonferroni post-hoc test was used if the null hypothesis was rejected.

All analyses were performed using IBM's SPSS Statistics software, version 24 (IBM, New York), and $P < 0.05$ was considered statistically significant.

7.4. Results

Participants' characteristics

By using the 3 cut-off of 25(OH)D concentration (low, moderate and high), there were significant differences between between men and women, BMI categories, PA level, vitamin D containing medication and supplements usage, their general health rate, Telomere Length HbA1c measurement (Table 7.1). The majority of the participants were women (n= 471), who had an normal BMI, were moderately active, moderate alcohol drinkers, regular smokers, their self-rated health was classed as good, and they had high concentration of 25(OH)D.

Table 7. 1: Participant characteristics by 25(OH)D cut-offs in the Newcastle 85+ Study

	Men (n= 304)	Women (n= 471)	All participants (n=778)	*p*
BMI				0.161
Underweight % (n)	26.3 (77)	32.4 (139)	29.9 (216)	
Normal weight % (n)	48.1 (141)	40.3 (173)	43.5 (314)	
Overweight % (n)	17.7 (50)	17.0 (73)	17.0 (123)	
Obese % (n)	8.5 (25)	10.3 (44)	9.6 (69)	
PA				<0.001
Low % (n)	19.9 (60)	23.6 (111)	22.2 (171)	
Moderate % (n)	32.8 (99)	49.6 (233)	43.0 (332)	
High % (n)	47.4 (143)	26.8 (126)	34.8 (269)	
Alcohol drink				<0.001
Never % (n)	4.0 (12)	15.5 (71)	10.8 (83)	
Moderate % (n)	67.0 (201)	68.6 (322)	68.0 (523)	
Heavy % (n)	19.0 (57)	4.9 (23)	10.4 (80)	
Smoking				<0.001
Never % (n)	25.4 (77)	41.2 (195)	35.1 (272)	
Regular % (n)	71.3 (216)	52.5 (248)	59.8 (464)	
Occasional % (n)	3.3 (10)	6.3 (30)	5.2 (40)	
Vitamin D containing medication % (n)	7.6 (23)	22.2 (105)	16.5 (128)	<0.001
Supplements users % (n)	17.4 (53)	21.0 (99)	19.5 (152)	0.220
Concentration of 25(OH)D nmol/l median (IQR)	42.8 (22.7)	47.0 (28.2)	45.3 (26.2)	0.030
25(OH)D				0.005
<25 nmol/l % (n)	22.4 (68)	25.9 (123)	24.6 (191)	
25-50 nmol/l % (n)	45.7 (139)	34.2 (162)	38.7 (301)	
≥50 nmol/l % (n)	31.9 (97)	39.9 (189)	36.8 (286)	
Self-rated health				0.277
Very good % (n)	43.1 (131)	38.4 (182)	40.2 (313)	
Good % (n)	53.6 (163)	56.3 (267)	55.3 (430)	
Poor % (n)	2.0 (6)	3.6 (17)	3.0 (23)	
Disease count mean (SD)	4.4 (1.57)	4.9 (1.81)	4.7 (1.74)	0.005
Telomere Length (kb) mean (SD)	3.8 (6.4)	3.7 (7.2)	3.7 (6.9)	0.002
HbA1c (mmol/mol) mean (SD)	5.8 (0.5)	6.0 (0.7)	5.9 (0.7)	0.155

BMI: body mass index. HbA1c: glycated haemoglobin. kb: kilo-base. P: p-value. Mann-Whitney U test for continuous non-normally distributed variables or $\chi 2$ test for categorical variables. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate), ≥50 nmol/l (high).

Predictors of Telomere Length

No significant association was found between Telomere Length and the relative confounders from the literature, such as smoking, alcohol consumption, PA, BMI, vitamin D containing medication usage, supplement, disease count and HbA1c% amongst all the participants. The only significant association was between Telomere Length and sex (95% CI= 0.000-0.001, p= <0.001). In addition, no significant association was found between Telomere Length and the confounders when the participants were stratified by sex, except for the BMI among the women (95% CI= 0.0034- 0.044, p= 0.039).

25(OH)D concentration and Telomere Length among the very-old adults at baseline

A positive association was found between 25(OH)D and Telomere Length in very-old adults (See Figure 7.1 for the distribution of 25(OH)D concentration by Telomere Length at baseline). Participants with 25(OH)D concentration >50 nmol/l had longer Telomere Length compared to those with concentration <50 nmol/l in the unadjusted model (95%CI= 17.8-109.9.16, B= 63.9$\pm$23.4, p= 0.007), and even after adjusting for relevant confounders, such as smoking, alcohol consumption, BMI, PA and HbA1C (95%CI= 12.0-110.3, B= 61.2$\pm$25.0, p=0.015) (Table 7.2).

25(OH)D concentration and Telomere Length by sex

Since sex was the only predictor of Telomere Length that was found for the current participants, the participants were stratified using this factor. When the participants were stratified by sex (Table 7.3), the very-old men with concentration between <25 nmol/l were more likely to have longer Telomere Length compared to those with concentration 25-50 nmol/l in the unadjusted model (95%CI= 1.9- 473.4, B= 237.7$\pm$119.7, p= 0.048) and even after adjusting for relevant confounders (95%CI= 14.9- 521.6, B= 268.3$\pm$128.6, p= 0.038).

In contrast, the very-old women with 25(OH)D concentration >50 nmol/l were more likely to have longer Telomere Length compared to those with concentration 25-50 nmol/l in the unadjusted model (95%CI= 15.6- 137.3, B= 76.4$\pm$30.9, p= 0.014). This association continued after further adjustments were made for smoking, alcohol consumption, BMI, and PA (95%CI= 6.8- 138.1, B= 72.4$\pm$33.3, p= 0.030) but it disappeared after adjusting for HbA1c (95%CI= -0.7- 131.1, B= 65.1$\pm$33.5, p= 0.053).

25(OH)D concentration and Telomere Length among the very-old adults at 18 months

A negative significant association was found between 25(OH)D concentration and Telomere Length at 18 months (See Figure 7.2 for the distribution of 25(OH)D concentration by Telomere Length at 18 months). Very-old participants with 25(OH)D concentration >50 nmol/l were more likely to have shorter Telomere Length compared to those with concentration 25-50 nmol/l in the unadjusted model (95%CI= -55.9- -5.8, B= -30.9±12.7, p= 0.016), and after adjusting for relevant confounders, such as smoking, alcohol consumption, BMI, PA and HbA1C (95%CI=-59.9- -7.5, B= -33.7±13.3, p=0.012) (Table 7.4).

25(OH)D concentration and Telomere Length by sex at 18 months

When the participants were stratified by sex (Table 7.5), a negative association was found between 25(OH)D and Telomere Length in the very-old men. Very-old men with concentration >50 nmol/l were more likely to have shorter Telomere Length compared to those with concentration <50 nmol/l in the unadjusted model (95%CI= -107.3- -25.5, B= -66.2±20.8, p= 0.002) and even after adjusting for relevant confounders (95%CI= -107.0- -23.3, B= -65.2±21.2, p= 0.002).

However, no significant association was found between 25(OH)D and Telomere Length in the very-old women at 18 months in both unadjusted and adjusted models (see Table 7.5).

25(OH)D concentration and Telomere Length among the very-old adults at 36 months

No significant association was found between 25(OH)D concentration and Telomere Length after 36 months in the unadjusted model, or even after adjusting for relevant confounders smoking, alcohol consumption, BMI, PA and HbA1C% in all participants and in men and women separately (see Figure 7.3 and Tables 7.6 and 7.7).

Table 7. 2: Association between different 25(OH)D cut-offs and Telomere Length at baseline

Model	25(OH)D	β coefficient	Adj. R Square	95% CI	p
Model 1	Low	84.8		-67.7, 237.5	0.275
	Moderate	(ref)	0.007	(ref)	(ref)
	High	63.9		17.8, 109.9	0.007
Model 2	Low	89.8		-63.9, 243.6	0.252
	Moderate	(ref)	0.007	(ref)	(ref)
	High	67.8		21.6, 114.1	0.004
Model 3	Low	88.5		-76.0, 253.2	0.291
	Moderate	(ref)	0.004	(ref)	(ref)
	High	64.4		15.5, 113.2	0.010
Model 4	Low	77.2		-88.3, 242.8	0.360
	Moderate	(ref)	0.004	(ref)	(ref)
	High	61.2		12.0, 110.3	0.015

CI: confidence interval. P: p-value. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c%.

Table 7. 3: Association between 25(OH)D cut-offs and Telomere Length by sex at baseline

Sex	Model	25(OH)D	β coefficient	Adj. R Square	95% CI	p
Men (n= 304)	Model 1	Low	237.7		1.9, 473.4	0.048
		Moderate	(ref)	0.009	(ref)	(ref)
		High	56.6		-13.6, 126.9	0.114
	Model 2	Low	267.5		29.2, 505.7	0.028
		Moderate	(ref)	0.007	(ref)	(ref)
		High	63.4		-7.6, 134.6	0.080
	Model 3	Low	262.2		10.8, 513.5	0.041
		Moderate	(ref)	0.004	(ref)	(ref)
		High	68.4		-5.8, 142.7	0.071
	Model 4	Low	268.3		14.9, 521.6	0.038
		Moderate	(ref)	0.004	(ref)	(ref)
		High	71.4		-3.6, 146.4	0.062
Women (n= 471)	Model 1	Low	28.8		-172.1, 229.7	0.778
		Moderate	(ref)	0.011	(ref)	(ref)
		High	76.4		15.6, 137.3	0.014
	Model 2	Low	20.8		-181.2, 223	0.839
		Moderate	(ref)	0.009	(ref)	(ref)
		High	79.9		18.8, 141.0	0.010
	Model 3	Low	-5.8		-225.2, 213.6	0.958
		Moderate	(ref)	0.010	(ref)	(ref)
		High	72.4		6.8, 138.1	0.030
	Model 4	Low	-23.3		-243.0, 196.3	0.835
		Moderate	(ref)	0.011	(ref)	(ref)
		High	65.1		-0.7, 131.1	0.053

CI: confidence interval. P: p-value. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c%.

Table 7. 4: Association between 25(OH)D cut-offs and Telomere Length at 18 months

Model	25(OH)D	β coefficient	Adj. R Square	95% CI	p
Model 1	Low	-1.1		-86.3, 84.1	0.979
	Moderate	(ref)	0.012	(ref)	(ref)
	High	-30.9		-55.9, -5.8	0.016
Model 2	Low	-2.2		-87.1, 82.7	0.959
	Moderate	(ref)	0.022	(ref)	(ref)
	High	-32.2		-57.2, -7.3	0.011
Model 3	Low	-8.5		-97.9, 80.8	0.851
	Moderate	(ref)	0.022	(ref)	(ref)
	High	-34.2		-60.0, -8.3	0.010
Model 4	Low	-8.1		-98.4, 82.0	0.859
	Moderate	(ref)	0.020	(ref)	(ref)
	High	-33.7		-59.9, -7.5	0.012

CI: confidence interval. BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. p: p-value. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c%.

Table 7. 5: Association between 25(OH)D cut-offs and Telomere Length by sex at 18 months

Sex	Model	25(OH)D	β coefficient	Adj. R Square	95% CI	p
Men (n= 304)	Model 1	Low	-3.9		-147.2, 139.3	0.957
		Moderate	(ref)	0.041	(ref)	(ref)
		High	-66.2		-107.3, -25.5	0.002
	Model 2	Low	-11.5		-156.8, 133.6	0.875
		Moderate	(ref)	0.036	(ref)	(ref)
		High	-69.0		-110.4, -27.7	0.001
	Model 3	Low	-16.7		-167.8, 134	0.827
		Moderate	(ref)	0.037	(ref)	(ref)
		High	-70.3		-112.3, -28.3	0.001
	Model 4	Low	-17.6		-167.6, 132.4	0.817
		Moderate	(ref)	0.038	(ref)	(ref)
		High	-65.2		-107.0, -23.3	0.002
Women (n= 471)	Model 1	Low	33.3		-73.2, 139.8	0.539
		Moderate	(ref)	-0.004	(ref)	(ref)
		High	-2.3		-34.0, 29.3	0.883
	Model 2	Low	38.2		-67.4, 143.8	0.477
		Moderate	(ref)	0.011	(ref)	(ref)
		High	-2.8		-34.4, 28.6	0.857
	Model 3	Low	21.8		-90.9, 134.6	0.704
		Moderate	(ref)	0.004	(ref)	(ref)
		High	-4.9		-38.2, 28.3	0.770
	Model 4	Low	24.7		-90.2, 139.6	0.672
		Moderate	(ref)	0.001	(ref)	(ref)
		High	-6.5		-40.6, 27.5	0.707

CI: confidence interval. BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. p: p-value. 25(OH)D cut-offs: <25 nmol/l, 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c%.

Table 7. 6: Association between different 25(OH)D cut-offs and Telomere Length at 36 months

Model	25(OH)D	β coefficient	Adj. R Square	95% CI	p
Model 1	Low	-12.6		-205.4- 180.2	0.898
	Moderate	(ref)	-0.002	(ref)	(ref)
	High	-28.6		-85.3- 28.1	0.322
Model 2	Low	-18.4		-213.3- 176.4	0.853
	Moderate	(ref)	-0.007	(ref)	(ref)
	High	-29.6		-86.8- 27.6	0.310
Model 3	Low	-5.7		-209.5- 198.1	0.956
	Moderate	(ref)	-0.003	(ref)	(ref)
	High	-38.1		-96.5- 20.1	0.199
Model 4	Low	16.2		-189.8- 222.3	0.877
	Moderate	(ref)	-0.004	(ref)	(ref)
	High	-36.8		-96.3- 22.6	0.225

CI: confidence interval. BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. p: p-value. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c.

Table 7. 7: Association between different 25(OH)D cut-offs and Telomere Length by sex at 36 months

Sex	Model	25(OH)D	B coefficient	Adj. R Square	95% CI	p
Men (n= 304)	Model 1	Low	12.7		-295.0- 320.5	0.935
		Moderate	(ref)	-0.013	(ref)	(ref)
		High	-1.1		-89.7- 87.4	0.980
	Model 2	Low	-2.1		-315.1- 310.8	0.989
		Moderate	(ref)	-0.025	(ref)	(ref)
		High	-4.7		-94.5- 84.9	0.916
	Model 3	Low	-10.9		-33.9- 317.1	0.948
		Moderate	(ref)	-0.018	(ref)	(ref)
		High	-11.9		-103.3- 79.4	0.797
	Model 4	Low	-12.1		-103.3- 82.7	0.827
		Moderate	(ref)	-0.015	(ref)	(ref)
		High	-10.2		-173.3- 89.5	0.530
Women (n= 471)	Model 1	Low	-34.0		-286.9- 218.9	0.791
		Moderate	(ref)	-0.002	(ref)	(ref)
		High	-45.1		-120.1- 29.8	0.237
	Model 2	Low	-33.5		-288.1- 221.0	0.795
		Moderate	(ref)	-0.010	(ref)	(ref)
		High	-44.5		-120.2- 31.0	0.247
	Model 3	Low	-32.3		-300.0- 235.4	0.812
		Moderate	(ref)	-0.009	(ref)	(ref)
		High	-53.3		-131.0- 24.2	0.177
	Model 4	Low	-0.8		-271.3- 269.6	0.995
		Moderate	(ref)	-0.008	(ref)	(ref)
		High	-50.5		-129.8- 28.8	0.211

CI: confidence interval. BMI: body mass index. PA: physical activity. HbA1c: glycated haemoglobin. p: p-value. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and PA. Model 4 is further adjusted for HbA1c.

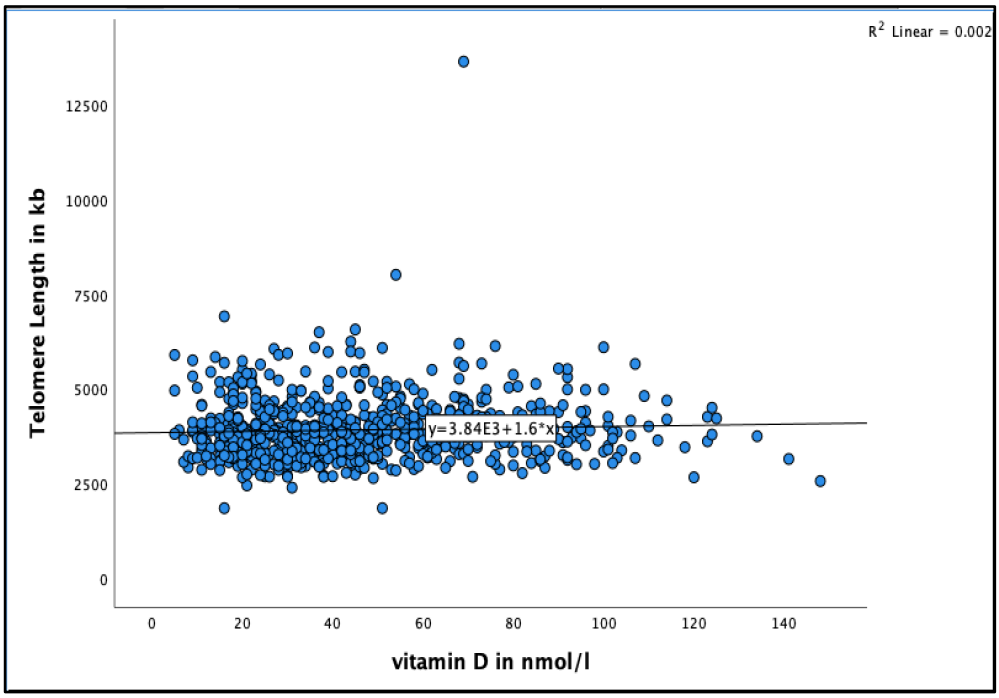


Figure 7. 2**: The association between 25(OH)D concentration and Telomere Length at baseline in the Newcastle 85+ Study**

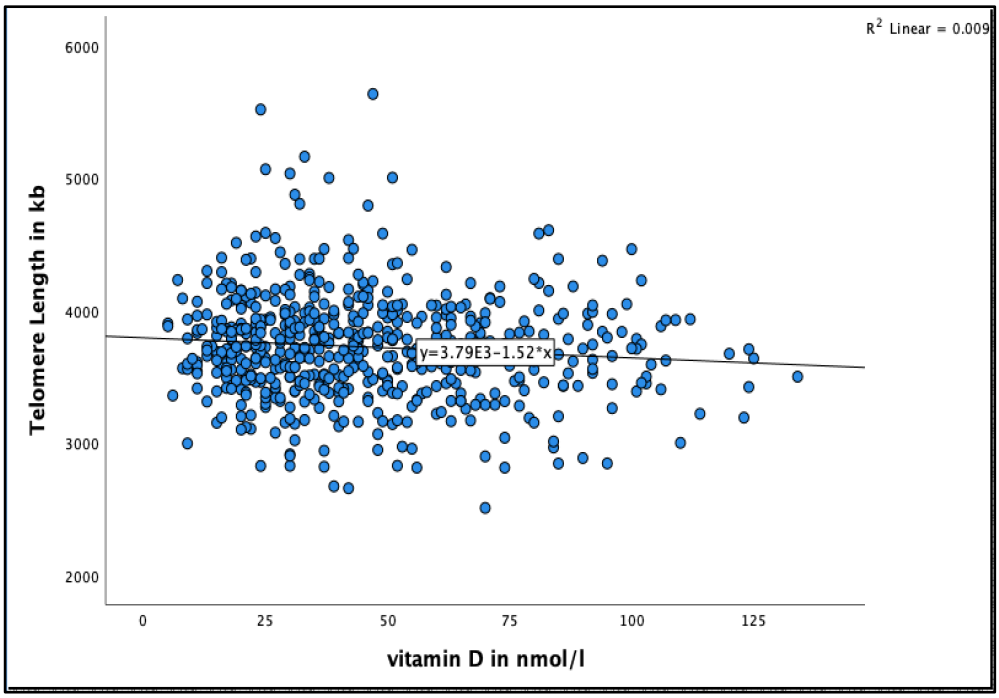


Figure 7. 3: **The association between 25(OH)D concentration and Telomere Length at 18 months in the Newcastle 85+ Study**

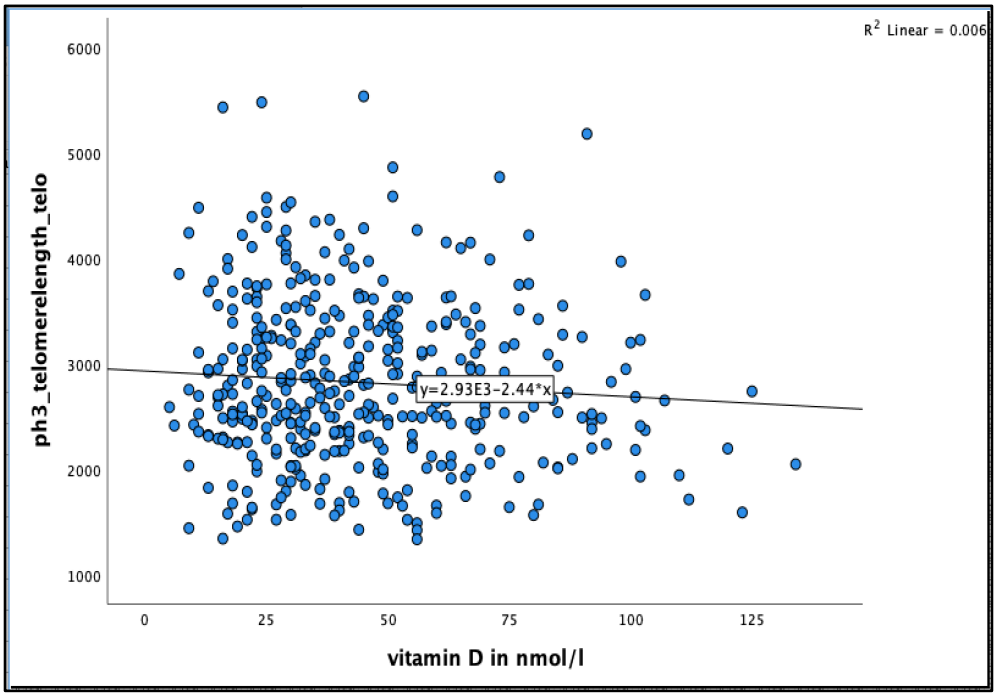


Figure 7. 4*:* **The association between 25(OH)D concentration and Telomere Length at 36 months in the Newcastle 85+ Study**

7.5. Discussion

Main findings

To our knowledge, this is the first study that examined the prospective association between 25(OH)D concentration and Telomere Length in very-old adults. Our results show that in fully adjusted models, whilst there was a significant positive association between 25(OH)D (>50 nmol/l) and Telomere Length at baseline, the direction of association was reversed after 18 months and absent at 36 months. However, it should be noted that the strength of the positive and negative association between 25(OH)D concentration and Telomere Length at baseline and 18, respectively were weak [adjusted R square 0.004 at baseline and 0.020 at 18 months].

Evidence from other studies

Telomeres, the specific DNA protein structures, are found at both ends of each chromosome. Their function is to protect the genome from nucleolytic degradation, unnecessary recombination, repair, and interchromosomal fusion (Pusceddu et al., 2015). Each DNA replication causes Telomere shortening, and when the Telomere Length reaches a critical limit, the cell undergoes senescence and/or apoptosis (Shammas, 2011). The rate attrition differs between individuals and tissues, and is influenced by multiple factors. Inflammation and oxidative stress are the key determinants of Telomere Length, and even though some of the factors that heighten oxidative stress and inflammation are genetic, others are clearly environmental in nature, such as smoking, obesity and a sedentary lifestyle. Moreover, several dietary factors, such as high energy consumption and the intake of high sugar foods, are also highly associated with inflammation (Julin et al., 2017, Beilfuss et al., 2017, Mazidi et al., 2017). While these lifestyle habits may be difficult to change, vitamin D concentration was easily modifiable through nutritional supplementation or sunlight exposure. Taking all of this into account, we sought to explore and study the association between 25(OH)D concentration and Telomere Length.

Our findings were in agreement with previous findings from two large studies on women by Richards et al. (2007) and Liu et al. (2013) (n=2160 and n=4604 participants, respectively). Both studies found that a higher 25(OH)D concentration was associated with longer LTL (Richards et al., 2007, Liu et al., 2013). Also, a recent study by Zarei et al. (2020a) found an interaction between vitamin D and telomerase with regards to their

relationship with the survival among 404 CVD patients. On the other hand, the findings from a large community-dwelling study conducted by Liu et al. (2016), failed to find any association between continuous 25(OH)D concentration and longer LTL not only for their entire population (n= 1154) but also in the white (n= 503), black (n=651), women (711), men (447) or race–sex subgroups. However, they found that concentration of vitamin D ≥30 nmol/l were significantly associated with longer LTL in whites only. That said, the participant in the current study were mostly (99.6% of the participants) white.

In a prospective study of 59 African-American systemic lupus erythematosus patients and their counterpart control subjects shorter Telomeres were seen among all subjects with a 25(OH)D concentration <50 nmol/L (Hoffecker et al., 2013). Interestingly, the patients who remained vitamin D deficient after three months of follow-up [n 29], tended to have shorter Telomeres than those patients whose 25(OH)D concentration were replete (Hoffecker et al., 2013), suggesting a protective role of 25(OH)D in maintaining Telomere Length. Moreover, a large community-dwelling study conducted by Mazidi et al. (2017), examined the association between 25(OH)D concentration and Telomere Length across a broad age range (age: 18-80-years-old). The participants were free of any history of diabetes, coronary heart disease, angina, myocardial infarction, stroke or congestive heart failure, in both men (n=2319) and women (n=2668) (Mazidi et al., 2017). A positive association was demonstrated between 25(OH)D concentration and Telomere Length in the limited-adjusted models. Both studies highlited the possible role of 25(OH)D concentration in the maintenance of Telomere Length (Mazidi et al., 2017).

Our study also demonstrated an association between 25(OH)D and Telomere Length in men, which is inconsistent with a cross-sectional study in white men (n=2483), which failed to observe an association between any of the vitamin D biomarkers (25(OH)D and 1,25(OH)D) and LTL (Julin et al., 2017). However, the participants in the study by Julin et al. (2017) were younger than our participants (the mean age was 64.1 years-old). Furthermore, they defined 25(OH)D concentration by four quartiles with higher cut-off (<50 noml/l was the lowest quartile) while the current study c showed that a 25(OH)D concentration >50 nmol/l was positively associated with Telomere Length at baseline.

Regarding the contribution of sex to the association between 25(OH)D concentration and Telomere Length, several biologically plausible explanations for a difference between men and women have been suggested, such as men, in general, having shorter Telomeres than women (Barrett and Richardson, 2011). In addition, estrogen can stimulate the

production of telomerase ans is a potent antioxidant and regulator of antioxidant genes while testosterone can reduce the presence of reactive oxygen species, has no antioxidant properties and is linked to increased susceptibility to oxidative stress (Barrett and Richardson, 2011, Aviv et al., 2006a). Therefore, it should be noted that the differences in sex could contribute toward the association between vitamin D status and Telomere Length.

Regarding the negative association between 25(OH)D concentration and Telomere Length at 18 months, the plausible explanations could be that 25(OH)D concentration was only measured at baseline and not at follow-up phases. Another explanation could be that concentration >50 nmol/l might not have protective effect on Telomere Length at very-old age. However, it should be considered that the model is not explaining much of the variation (Adj R2 considered very low). Besides, the 95% Cis were wide even when the relationship was positively significant at baseline indicates a less precise estimate of the relationship. That said, we could not ascertain the protective association of high concentration of 25(OH)D on Telomere Length in very-old adults in the current population.

There are several potential mechanisms that may explain the association between Telomere Length and 25(OH)D concentration. Generally, an activated form of vitamin D has autocrine and paracrine roles, including reducing Telomere shortening through both anti-inflammatory and antiproliferative mechanisms. First, the active form of vitamin D decreases the mediators of systemic inflammation, such as interleukin-2 and tumor necrosis factor. This was also confirmed by the negative association between 25(OH)D concentration and concentration of c-reactive protein. Furthermore, vitamin D receptor is expressed in the T and B lymphocytes, natural killer cells, and monocytes, which promote the down-regulation of cytokines and other proinflammatory factors. Thus, it follows that vitamin D would attenuate the rate of Telomere Length attrition (Mazidi et al., 2017, Zarei et al., 2020b). Conversely, retinoid x receptor (which is found widely distributed in cells and tissues and act as the major contributor to vitamin D dependent transcription) has other roles in the cell that are independent of the vitamin D pathway. Therefore, the association between one common variant and a long Telomere Length does not necessarily imply a link between 25(OH)D and Telomere Length (Julin et al., 2017).

Strengths and Limitations

The study has several strengths, including its unique design, as well as the facts that the analysis is concentrated on broadly representative age category of 85 years-olds; and that the statistical assumptions were met. Another key strength is that the study was adjusted for major potential confounders associated with Telomere Length (e.g. BMI, physical activity, smoking).

It should also be noted however, that the findings reported here should be interpreted with caution due to the following limitations: firstly, its epidemiological design restricts any inference about causal relationship. Secondly, we did not include wider dietary factors as covariate in our models as we had no a priori knowledge from our dataset that these factors could associate with Telomere Length. Therefore, the findings may be confounded by unmeasured or uncontrolled factors increasing the chance of Type I error. On the other hand, adding more confounders to the fully adjusted model may have resulted in non-significant (bias) result and reduced power to detect significant associations. Thirdly, even though we had longitudinal data on Telomere Length over 36 months, serum 25(OH)D data were only measured at baseline.

7.6. Summary

Among the very-old in the Newcastle 85+ cohort study, 25(OH)D concentration was positively associated with Telomere Length at baseline. However, given the wide 95% CI and the conflicting directions of the association at 18 months inclined to say that high concentration of 25(OH)D (>50 nmol/l) did not show protective effect on Telomere Length in very-old adults. In conclusion, high 25(OH)D concentration is positively associated with Telomere Length but does not have protective effect over time.

Chapter 8: Discussion and Conclusion

8.1. General Findings

Little is known about the vitamin D status of very-old adults and its association with health trajectory and ageing biomarkers. This thesis successfully used a unique cohort of more than 700 very-old adults to reach its objective, which was to provide an accurate snapshot of the association between the vitamin D status and functional health and ageing biomarkers of very-old adults from the Newcastle 85+ Study. This objective was broadly achieved and these are the general findings: (the specific results will be discussed later in this Chapter.)

1. Based on the systematic review, 25(OH)D concentration differed significantly between very-old adults across the globe and were heavily influenced by latitude and living conditions.
2. The highest 25(OH)D concentration was found among the community-dwelling very-old adults from the USA, while the lowest 25(OH)D was found among institutionalised very-old adults from Austria.
3. Using the Newcastle 85+ Study data, vitamin D deficiency [as defined by a 25(OH)D concentration <25 nmol/L] is highly prevalent (>26% of the participants) among very-old adults from North-East England.
4. The season of the blood sampling, using vitamin D containing medication and supplements and PA were strong predictors of 25(OH)D concentration in very-old people.
5. Cross-sectional analysis revealed a U-shaped association between 25(OH)D concentration and disability using the SACN cut-offs.
6. In the limited adjusted models, participants with a moderate concentration of 25(OH)D (25-50 nmol/l) were less likely to have a mild-to-moderate or moderate-to-severe disability trajectory over five years, compared to participants with low (<25 nmol/L) or high (>50 nmol/L) concentrations. However, after adjustment for PA, the association between vitamin D status and disability trajectory in very-old adults disappeared.
7. The analysis of this thesis could not confirm the protective effect of the 25(OH)D on the metabolic and cardiopulmonary biomarkers (NT-proBNP, HbA1c, FEV1, FVC and diastolic BP) in the fully adjusted model at baseline as well as at the follow-up phases (18 and 36 months).
8. In cross sectional analysis, high 25(OH)D concentration was positively associated with Telomere Length, a candidate biomarker of ageing.

9. Using 18 months data analysis, high 25(OH)D concentration is positively associated with Telomere Length but does not have protective effect.
10. Age and sex were the main predictors of Telomere Length in the very-old adults.

8.2. General Discussion and Public Health Implications

Besides, a healthy lifestyle, disease prevention and access to medicine during the last century, have all contributed towards a shift in the type of health problems that humans endure – from infectious diseases to complex chronic long-term diseases (Marengoni et al., 2011). The ageing phenomenon is characterized by multiple alterations and damage within the molecular pathways (Wagner et al., 2016). Therefore, living well for longer has become the key goal in achieving lower level of morbidity, fewer years of disability and a greater quality of life among the older population (Baugreet et al., 2017).

Ageing is the most profound risk factor for almost all non-communicable diseases (Kennedy et al., 2014); examples of this include disability, cardiovascular and respiratory diseases and diabetes (Canudas-Romo et al., 2016). Age-related changes in the bones cause frailty, fractures, falls and disability. For instance, approximately 40% of those aged 80 and older have experienced frailty (Mosele et al., 2013), which leads to disability, falls, hospitalization, institutionalization, mortality and increased medical costs (Al Snih et al., 2009). Another aspect that affects very-old adults is muscle mass and muscle strength; this also declines with age for both sexes. At age 40, progressive muscle mass loss begins, and approximately 8% of it is estimated to be lost per decade until the age of 70, at which point this then increases to 15% per decade (Keller and Engelhardt, 2013). Moreover, about 50% of all heart failure is diagnosed, and 90% of all heart failure deaths occur, among those aged 70 years and over (Strait and Lakatta, 2012). Ageing is also associated with a reduction in chest wall compliance and respiratory muscle strength, while air trapping and airspace size subsequently increases (Sharma and Goodwin, 2006). Lastly, ageing can be associated with alterations in glucose metabolism, including both relative insulin resistance and islet cell dysfunction, which in turn can lead to abnormal glucose metabolism (Kalyani and Egan, 2013).

Biomarkers are indicators of normal biological or pathogenic processes. Therefore, they are used to monitor or predict the state of health of the population as well as identify those susceptible to a particular health problem (Crimmins et al., 2008). The biomarkers of

ageing can be used to predict physiological age, which reflects the state of health of an individual (Xia et al., 2017). However, it is unlikely that a single biomarker is able measure biological aging. Therefore, a panel of biomarkers was proposed based on evidence to validate their association with ageing phenotypes, but there is no standard reference for biomarkers that provides a valid assessment for healthy ageing (Wagner et al., 2016).

Different biomarkers are used to assess different health aspects (Wagner et al., 2016). For example, physical capability is assessed by numerous activities, such as standing balance, grip strength, chair stand, timed-up-and-go, walking speed, the pegboard test and ADL. Furthermore, physiological function, such as cardiovascular function, is measured by blood lipid, BP and BNP level, while lung function is measured by FEV1, glucose metabolism is measured by fasting glucose, HbA1c level, and, lastly, body composition is measured by BMI, waist circumference, muscle mass, and BMD. In addition, cognitive function is evaluated using the NIH toolbox picture sequence memory test, California verbal learning test, Rey auditory verbal learning test, digit symbol coding test and verbal fluency. As for endocrine function, this is measured by sex hormone, growth hormone, HPA-axis, and PTH. Immune function can be evaluated by inflammatory factors, such as IL-6, TNF-a, CRP. Lastly, molecular/DNA-based markers, such as DNA/165hromosomal damage, Telomere Length and DNA repair, are also used to predict physiological age (Wagner et al., 2016).

The existing research has usually focused on the role of vitamin D status in relation to skeletal health. However, following the discovery of VDR throughout the body, its role in the prevention and treatment of chronic diseases has been widely studied, as discussed previously in the Introduction to this thesis (Høyer-Hansen et al., 2010) (see Chapter 1). Vitamin D deficiency has therefore been linked to various health problems, including cardiovascular disease, respiratory disease, diabetes, cognitive problems and immunity. However, the role of vitamin D in the prevention and treatment of diseases among very-old adults has not been sufficiently studied yet. Thus, understanding the association between vitamin D and chronic diseases among older adults, and whether the treatment of vitamin D deficiency can prevent or amend these disorders is important. This thesis sought to highlight the role that vitamin D may play in diseases associated with aging, such as disability and metabolic cardiopulmonary, and also addresses the association between vitamin D status and Telomere Length.

First, this thesis proved the vitamin D deficiency (as defined by a 25(OH)D concentration <25 nmol/L) is high among the very-old adults. Moreover, the season of blood

sampling, using vitamin D containing medication and supplements and PA were strong predictors of 25(OH)D concentration in both the very-old men and women as noted in Chapters 3 and 4.

Second, ADL was used to predict the disability trajectory among the very-old adults (in relation to their 25(OH)D) over five years. The ability to perform ADLs is achieved by combining the cognitive (e.g., reasoning, planning), motor (e.g., balance, dexterity) and perceptual (including sensory) abilities. It also shows the individual's ability to complete a task (physical and/or cognitive ability) against the ability to recognize that the task needs to be done without prompting (cognitive ability) (Mlinac and Feng, 2016). Our results were consistent with other studies, even those that adopted different disability measurements methods. For instance, Kotlarczyk et al. (2017) found that a slower gait speed and poorer IADL scores were associated with a low 25(OH)D concentration. Moreover, a positive association between the '8-foot walk' test and the 'sit-to-stand' test, with a concentration of 25(OH)D, was also found (Bischoff-Ferrari et al., 2004c). These results confirm the role of vitamin D status in maintaining musculoskeletal health and predicting disability.

Additionally, different physical and blood biomarkers were used to evaluate the role of vitamin D in physiological health among very-old adults. For example, we used NT-proBNP and BP as biomarkers for cardiovascular function, FEV1 and FVC as biomarkers for lung function, and HbA1c as a biomarker for glucose metabolism. Our results could not confirmed the role of vitamin D status in improving cardiovascular function, lung function and glucose metabolism. However, previous studies have suggested a positive association between 25(OH)D concentration and MCPD using other biomarkers. For example, the results of the linear regression showed an association between 25(OH)D concentration and HDL cholesterol (Sarmiento-Rubiano et al., 2018). In another community- dwelling sample of elderly participants, a higher 25(OH)D concentration was associated with less insulin resistance (Danziger et al., 2013). Moreover, in a small group of old adult patients with heart failure, a 25(OH)D concentration was found to be associated with peak VO2 (Boxer et al., 2010).

Finally, this thesis used Telomere Length as a biomarker for ageing and studied the role of vitamin D status in maintaining Telomere Length. Telomere Length is a genetic indicator that is used as either an indicator of the risk of aging, or as a biological marker for the ageing process *per se*. Research findings have consistently related a decreased Telomere Length to increased age (Crimmins, 2008). In terms of our findings, we confirm the role of

vitamin D status in maintaining Telomere Length. However, we failed to confirm the protective role of vitamin D status in maintaining Telomere Length over 18 and 36 months (i.e. (Richards et al., 2007, Liu et al., 2013)).

In summary, this PhD thesis confirms the positive role of vitamin D in improving the physical and physiological health of very-old adults. Using different functional and blood biomarkers, we suggest that maintaining moderate 25(OH)D concentration (25-50 nmol/l) among very-old adults is beneficial but we could not confirm it's protective effect in terms of sustaining metabolic and cardiopulmonary health, and preserving Telomere Length in the very-old adults. However, we indicate that physical activity rather than vitamin D status predicted the disability trajectory in very-old adults.

8.3. Recommendations for Future Research

There is a lack of nutritional data on this age group and, given the expected increase in the number of very-old adults, filling this evidence gap should be a high priority.

First, in Chapter 3, following a systematic review of global vitamin D status in very-old adults, we could not provide an accurate value for vitamin D status because of the heterogeneity existing between the included studies. Hence, a standardised method to determine the bioavailability of vitamin D from all sources, including sun exposure and supplement, as well as a standardised assay to measure vitamin D biomarkers, would be extremely useful, as using different measurement methods would cause inconsistency between the results due to over- or under-estimations.

Therefore, a new nutritional regulation for this age group is vital, as most of the nutritional recommendations and requirements (i.e. IoM, Endocrine Society, Australian NHMRC, WHO) place very-old adults in the same category as >70 years-old, whereas the nutritional needs of those who are over 80 years-old may be significantly different. For instance, the findings of this PhD thesis did not observe the protective effect of high concentration of 25(OH)D on metabolic cardiopulmonary diseases and Telomere Length. Additionally, men and women did not showed the same association between 25(OH)D concentration and disability trajectory and Telomere Length.

However, as the concentration of 25(OH)D in the Newcastle 85+ Study was only measured at baseline, we are unable to confirm the longitudinal association between 25(OH)D concentration and the functional and blood biomarkers in this PhD thesis.

Therefore, a cohort study of this age group with a follow-up measurement of 25(OH)D concentration, as well as biomarkers, would be beneficial to determine the prospective association between 25(OH)D and various health aspects.

Finally, RCTs – with enough follow-up time and sufficiently powered to detect the influence of 25(OH)D concentration of the very-old adults on their functional and blood biomarkers, especially those related to disability trajectory, metabolic cardiopulmonary disease and Telomere Length – are needed to confirm the findings of this PhD thesis.

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Appendices

A. Overview of variables collected in the Newcastle 85+ Study from Baseline to Phase 4

Variables Questionnaires	Ph1	Ph2	Ph3	Ph4	Comment
Aids/appliances and household modifications	Yes	No	No	No	
Alcohol	Yes	No	No	No	
Chest pain (cardiac)	Yes	Yes	Yes	No	Phase 2 cardiac subset only
Cough, sputum, wheeze	Yes	Yes	Yes	No	Questions on recent chest infection, use of antibiotics, use of steroids in Phase 2 and 3 only
Dietary assessment	Yes	No	No	No	
Disability and help receive	Yes	Yes	Yes	Yes	In Phase 1 also coded up to 4 causes of difficulty for each activity participants could not do
Education and work	Yes	No	No	No	
Ethnic origin	Yes	No	No	No	
Exhaustion	No	No	No	Yes	
Eyesight	Yes	Yes	Yes	Yes	
Falls	Yes	Yes	Yes	Yes	In Phase 1 extended questions for falls and izziness
Family data	Yes	No	No	No	
Finances	Yes	No	No	Yes	
Formal health and social care	Yes	Yes	Yes	Yes	
Fractures	Yes	No	No	No	
Geriatric depression scale	Yes	Yes	Yes	Yes	
Hearing	Yes	Yes	Yes	Yes	
How are you feeling today?	Yes	Yes	Yes	Yes	

Variables	Ph1	Ph2	Ph3	Ph4	Comment
Questionnaires					
Incontinence	Yes	Yes	Yes	Yes	
Join pain	Yes	No	No	No	
Key events since previous Phase	No	Yes	Yes	Yes	
Living arrangements	Yes	Yes	Yes	Yes	
Loneliness	Yes	Yes	Yes	Yes	
Long standing illness/disability/infirmity	Yes	Yes	Yes	Yes	
Medication, non-prescribed	Yes	No	No	No	
Medication, prescribed	No	Yes	No	No	Phase 2 cardiac subset only from GP records at Phase 1 and Phase 3
Occupational exposure to lung toxins	Yes	Yes	Yes	No	
Oral health	Yes	No	Yes	No	
Pain	Yes	Yes	Yes	Yes	Phase 1 included body map for pain locations
Physical activity	Yes	Yes	Yes	Yes	
Self-rated health	Yes	Yes	Yes	Yes	
Shortness of breath	Yes	Yes	Yes	Yes	Phase 2 cardiac subset only
Sleep	No	No	Yes	No	
Smoking	Yes	No	No	No	
Social participation	Yes	Yes	Yes	Yes	
Social support	Yes	Yes	Yes	Yes	
Measurements and function tests					
Demi-span	Yes	No	No	No	
Weigh	Yes	Yes	Yes	Yes	
Bioimpedance	Yes	No	Yes	No	
Waist-hip circumference	Yes	No	No	No	
CDR (Computerised cognitive assessment)	Yes	Yes	Yes	No	
SMMSE (Standardised mini-mental state examination)	Yes	No	Yes	Yes	

Variables	Ph1	Ph2	Ph3	Ph4	Comment
Questionnaires					
Hand-grip strength	Yes	Yes	Yes	Yes	
Chair stand test	No	Yes	Yes	No	
Timed up and go test	Yes	Yes	Yes	Yes	
7 day activity monitoring (accelerometer)	No	Yes	Yes	No	
Blood pressure	Yes	Yes	Yes	No	
Spirometry	Yes	Yes	Yes	No	
Oximetry	Yes	Yes	Yes	No	
ECG (Electrocardiogram)	Yes	Yes	Yes	No	Phase 2 cardiac subset only
Tooth count	Yes	No	Yes	No	
Cardiac echocardiogram	No	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Carotid intima media thickness	No	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Vascular resistance (sphygmocor and vicorder)	No	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Blood assays (no blood in Phase 4)					
Creatinine, urea, electrolytes	Yes	Yes	Yes	No	
Urate	Yes	No	No	No	
Liver Function test/bone panel	Yes	No	Yes	No	
Full blood count test	Yes	Yes	Yes	No	
HbA1c	Yes	Yes	Yes	No	
Fasting glucose	Yes	No	Yes	No	
Fasting lipid profile (cholesterol, Triglycerides, HDL, LDL)	Yes	No	Yes	No	
Apolipoproteins	Yes	No	Yes	No	
C-reactive protein (hs-CRP)	Yes	Yes	Yes	No	
Cortisol	Yes	No	Yes	No	
Thyroid function: TSH, FT4, T3 and ATPO	Yes (rev T3)	No	Yes (not ATPO)		

Variables	Ph1	Ph2	Ph3	Ph4	Comment
Questionnaires					
Rheumatoid factor	Yes	No	No	No	Cardiac subset only in Phase 2 or Phase 3
N-terminal pro-brain natriuretic peptide (NT-PRO BNP)	Yes	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Neuregulin (NRGB-1)	Yes	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Growth differentiation factor 15 (GDF-15)	Yes	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
ST-2	Yes	Yes	Yes	No	Cardiac subset only in Phase 2 or Phase 3
Endoglin	No	Yes	Yes	No	
DNA damage and repair	Yes	Yes	Yes	No	
Telomer length	Yes	Yes	Yes	No	
Telomerase	Yes	No	No	No	
Isoprostanes	Yes	Yes	Yes	No	
4-colour flow cytometry analysis of lymphocyte subpoplation	Yes	Yes	Yes	No	Fresh samples
10-colour flow cytometry analysis of lymphocyte subpoplation	No	Yes	No	No	Frozen samples
LPS-stimulated cytokine (IL-6 and TNF-alpha) production	Yes	Yes	Yes	No	
CMV (Cytomegalovirus)	Yes	Yes	No	No	Measured on all participants in Phase 2 and those not measured in Phase 2 were measured in Phase 1
Vitamin B12	Yes	No	No	No	
Red blood cell folate	Yes	No	No	No	
Ferritin	Yes	No	No	No	
Total homocysteine	Yes	No	Yes	No	
Vitamin B2 (EGRac)	Yes	No	No	No	

Variables	Ph1	Ph2	Ph3	Ph4	Comment
Questionnaires					
Vitamin B6 (PLP and PA)	Yes	No	No	No	
Vitamin C	No	Yes	No	Yes	
Vitamin D	Yes	No	No	No	
Mitochondrial haplotype	Yes	No	No	No	
Mitochondrial DNA sequencing	Yes	No	No	No	
Reactive oxygen production by mitochondria	No	No	Yes	No	
Genotyping	Yes	No	No	No	
GP record review (not done in Phase 2)					
Disease-ever diagnoses, pre-specified list of conditions of each category					
Cardiovascular/cerebrovascular	Yes	No	Yes	Yes	
Cancer	Yes	No	Yes	Yes	
Endocrine	Yes	No	Yes	Yes	
Eye	Yes	No	Yes	Yes	
Liver	Yes	No	Yes	Yes	
Musculoskeletal	Yes	No	Yes	Yes	
Neurological	Yes	No	Yes	Yes	
Psychiatric	Yes	No	Yes	Yes	
Respiratory	Yes	No	Yes	Yes	
Key event in last 66-12 months					
Blood pressure check (12 m)	Yes	No	Yes	Yes	
Influenza vaccination (12 m)	Yes	No	Yes	Yes	
Depression (12 m)	Yes	No	Yes	Yes	
Infections (12 m)	Yes	No	Yes	Yes	
Medication check (6 months)	Yes	No	Yes	Yes	
Prescribed medication	Yes	No	Yes	Yes	
Consultations with primary care team members in previous 12 months	Yes	No	Yes	yes (GP/Other)	
Hospital admissions	No	No	Yes	No	

B. Association between 25(OH)D concentration and disability trajectories (sensitivity analysis)

	Traj	25(OH)D	Model 1			Model 2			Model 3			Model 4		
			OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*	OR	95%CI	*p*
With PA	DT1	(ref)		(ref)			(ref)			(ref)			(ref)	
		<25 nmol/l	1.48	0.88-2.47	0.131	1.03	0.98-1.07	0.185	1.62	0.83-3.16	0.155	1.61	0.95-2.74	0.074
	DT2	25-50 nmol/l		(ref)			(ref)			(ref)				
		>50 nmol/l	1.12	0.72-1.75	0.594	1.44	0.86-2.42	0.165	1.27	0.74-2.17	0.376	1.08	0.69-1.68	0.735
		<25 nmol/l	1.73	0.85-3.52	0.130	1.70	8.83-3.47	0.144	1.89	0.77-4.60	0.159	1.95	0.94-4.06	0.071
	DT3	25-50 nmol/l		(ref)			(ref)			(ref)				
		>50 nmol/l	1.03	0.49-2.13	0.935	0.98	0.47-2.03	0.970	1.11	0.48-2.57	0.799	1.04	0.75-2.42	0.909
Without PA	DT1	(ref)		(ref)			(ref)			(ref)			(ref)	
		<25 nmol/l	1.82	1.14-2.89	0.011	1.77	1.11-2.83	0.016	2.05	1.14-1.51	0.021	1.97	1.22-3.17	0.005
	DT2	25-50 nmol/l		(ref)			(ref)			(ref)				
		>50 nmol/l	0.94	0.63-1.41	0.787	0.93	0.62-1.40	0.757	1.16	1.11-3.79	0.551	0.922	0.61-1.38	0.695
		<25 nmol/l	2.71	1.47-4.99	0.001	2.70	1.46-4.97	0.001	2.75	1.25-6.04	0.012	3.12	1.67-5.85	0.001
	DT3	25-50 nmol/l		(ref)			(ref)			(ref)				
		>50 nmol/l	0.83	0.45-1.55	0.571	0.84	0.45-1.56	0.588	0.93	0.44-1.96	0.868	0.83	0.45-1.56	0.579

DT1, very low disability; DT2, moderate disability; DT3 sever disability. 25(OH)D: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref), >50 nmol/l (high). PA: physical activity. OR, odd ratio. CI, confidence interval. *p,* p-value. Model 1 is further adjusted for grip strength. Model 2 is further adjusted for FFM. Model 3 is further adjusted for disease count. Model 4 is further adjusted for season.

C. Association between 25(OH)D concentration and NT-proBNP concentration by sex

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Men (n=304)	Model 1	Low	668.6	0.015	93.0- 1244.2	0.023
		Moderate	(ref)		(ref)	(ref)
		High	-41.4		-563.6- 480.6	0.876
	Model 2	Low	753.6	0.025	149.3- 1357.8	0.015
		Moderate	(ref)		(ref)	(ref)
		High	55.5		-503.3- 614.4	0.845
	Model 3	Low	761.2	0.017	150.5- 1372.0	0.015
		Moderate	(ref)		(ref)	(ref)
		High	-8.5		-576.9- 559.8	0.976
	Model 4	Low	673.1	0.021	52.2- 1294.0	0.034
		Moderate	(ref)		(ref)	(ref)
		High	-24.3		-544.5- 593.2	0.933
Women (n=474)	Model 1	Low	116.5	-0.003	-269.8- 502.9	0.554
		Moderate	(ref)		(ref)	(ref)
		High	-29.3		-379.4- 320.8	0.869
	Model 2	Low	170.8	-0.003	-219.8- 561.6	0.390
		Moderate	(ref)		(ref)	(ref)
		High	5.9		-348.7- 360.6	0.974
	Model 3	Low	135.0	0.021	-257.1- 527.1	0.499
		Moderate	(ref)		(ref)	(ref)
		High	-10.1		-368.7- 348.3	0.956
	Model 4	Low	-17.8	0.085	-400.9- 365.3	0.927
		Moderate	(ref)		(ref)	(ref)
		High	-138.3		-488.0- 211.3	0.437

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high) NT-proBNP: N-terminal pro b-type natriuretic peptide. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

D. Association between 25(OH)D concentration and HbA1c level by sex

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Men (n=304)	Model 1	Low	0.0	0.008	-0.1- 0.2	0.529
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.098
	Model 2	Low	0.0	0.007	-0.1- 0.2	0.743
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.114
	Model 3	Low	0.0	0.019	-0.1- 0.2	0.901
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.172
	Model 4	Low	-0.0	0.043	-0.2- 0.1	0.730
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.2- 0.0	0.272
Women (n=474)	Model 1	Low	0.0	0.012	-0.1- 0.2	0.525
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- -0.0	0.040
	Model 2	Low	0.0	0.005	-0.1- 0.2	0.526
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.102
	Model 3	Low	0.0	0.013	-0.1- 0.2	0.586
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.089
	Model 4	Low	0.0	0.015	-0.1- 0.2	0.750
		Moderate	(ref)		(ref)	(ref)
		High	-0.1		-0.3- 0.0	0.061

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). HbA1c: glycosylated haemoglobin. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

E. Association between 25(OH)D concentration and FEV1 measurement by sex

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Men (n=304)	Model 1	Low	-14.3	0.004	-30.6- 1.9	0.083
		Moderate	(ref)		(ref)	(ref)
		High	-7.1		-21.8- 7.6	0.059
	Model 2	Low	-13.4	-0.002	-30.3- 3.3	0.116
		Moderate	(ref)		(ref)	(ref)
		High	-5.4		-21.9- 9.9	0.487
	Model 3	Low	-14.3	-0.003	-31.4- 2.7	0.099
		Moderate	(ref)		(ref)	(ref)
		High	-4.6		-20.3- 11.1	0.564
	Model 4	Low	-4.9	0.115	-21.2- 11.3	0.551
		Moderate	(ref)		(ref)	(ref)
		High	-8.2		-23.0- 6.6	0.277
Women (n=474)	Model 1	Low	-10.5	0.007	-19.6- -1.4	0.024
		Moderate	(ref)		(ref)	(ref)
		High	-2.7		-10.9- 5.3	0.504
	Model 2	Low	-11.3	0.008	-20.5- -2.0	0.016
		Moderate	(ref)		(ref)	(ref)
		High	-2.7		-11.0- 5.5	0.522
	Model 3	Low	-11.1	0.020	-20.3- -2.0	0.017
		Moderate	(ref)		(ref)	(ref)
		High	-2.2		-10.5- 5.9	0.585
	Model 4	Low	-7.4	0.080	-16.3- 1.5	0.105
		Moderate	(ref)		(ref)	(ref)
		High	1.0		-7.0- 9.1	0.806

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). FEV1: forced expiratory volume in one second. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

F. Association between 25(OH)D concentration and FVC measurement by sex

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Men (n=304)	Model 1	Low	-22.4	0.011	-43.2- -1.7	0.034
		Moderate	(ref)		(ref)	(ref)
		High	-0.5		-19.3- 18.2	0.953
	Model 2	Low	-20.6	0.006	-42.0- 0.7	0.058
		Moderate	(ref)		(ref)	(ref)
		High	2.8		-16.7- 22.4	0.777
	Model 3	Low	-21.2	0.002	-42.9- 0.3	0.054
		Moderate	(ref)		(ref)	(ref)
		High	2.5		-17.4- 22.4	0.803
	Model 4	Low	-9.7	0.112	-30.4- 11.0	0.359
		Moderate	(ref)		(ref)	(ref)
		High	-1.8		-20.7- 17.0	0.846
Women (n=474)	Model 1	Low	-15.6	0.012	-27.3- -3.9	0.009
		Moderate	(ref)		(ref)	(ref)
		High	-3.8		-14.3- 6.6	0.470
	Model 2	Low	-16.2	0.012	-28.1- -4.3	0.007
		Moderate	(ref)		(ref)	(ref)
		High	-4.5		-15.1- 6.1	0.406
	Model 3	Low	-16.2	0.010	-28.2- -4.3	0.008
		Moderate	(ref)		(ref)	(ref)
		High	-3.9		-14.7- 6.8	0.469
	Model 4	Low	-11.5	0.067	-23.2- 0.2	0.054
		Moderate	(ref)		(ref)	(ref)
		High	0.2		-10.3- 10.8	0.968

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). FVC: forced vital capacity. PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for season. Model 3 is further adjusted for smoking and alcohol consumption and diastolic blood pressure. Model 4 is further adjusted for PA.

G. Association between 25(OH)D concentration and diastolic blood pressure by sex

Phase	Model	25(OH)D concentration	B coefficient	Adj. R Square	95% CI	*p*
Men (n=304)	Model 1	Low	-0.0	-0.001	-3.5- 3.4	0.976
		Moderate	(ref)		(ref)	(ref)
		High	1.8		-1.2- 4.9	0.240
	Model 2	Low	0.1	0.001	-3.4- 3.6	0.949
		Moderate	(ref)		(ref)	(ref)
		High	2.3		-0.8- 5.6	0.150
	Model 3	Low	-0.2	0.013	-3.8- 3.2	0.875
		Moderate	(ref)		(ref)	(ref)
		High	2.3		-0.9- 5.6	0.160
	Model 4	Low	0.6	0.033	-2.9- 4.2	0.738
		Moderate	(ref)		(ref)	(ref)
		High	1.9		-1.2- 5.2	0.234
Women (n=474)	Model 1	Low	-1.8	0.001	-4.5- 0.9	0.195
		Moderate	(ref)		(ref)	(ref)
		High	-1.7		-4.2- 0.7	0.170
	Model 2	Low	-1.0	-.003	-3.90 1.7	0.460
		Moderate	(ref)		(ref)	(ref)
		High	0.9		-3.5- 1.6	0.475
	Model 3	Low	-1.0	-0.006	-3.9- 1.7	0.455
		Moderate	(ref)		(ref)	(ref)
		High	-0.9		-3.5- 1.6	0.456
	Model 4	Low	-0.7	-0.003	-3.6- 2.1	0.616
		Moderate	(ref)		(ref)	(ref)
		High	-0.6		-3.3- 1.9	0.609

CI: confidence interval. 25(OH)D cut-offs: <25 nmol/l (low), 25-50 nmol/l (moderate) (ref) and >50 nmol/l (high). PA: physical activity. P: p-value. Model 1 is the unadjusted model. Model 2 is further adjusted for season. Model 3 is further adjusted for smoking and alcohol consumption. Model 4 is further adjusted for PA.

H. Conferences, Workshops and training courses attended

Conference/ Training	Date	Location
Skills in the Hills session	19th&20th Oct 2016	Newcastle University
HNRC Research Day & Annual Lecture	26th Oct 2016	Newcastle University
EndNote Workshop	8th Nov 2016	Newcastle University
Health & Safety Workshop	23rd Nov 2016	Newcastle University
Good Practice in Your Research Workshop	30th Nov 2016	Newcastle University
PGR Research Meeting	30th Nov 2016	Newcastle University
Voice Coaching & Body Language Workshop	5th Dec 2016	Newcastle University
HNRC Seminar - Portuguese Elderly Nutritional Status Surveillance System (PEN-3S) - Preliminary Results	8th Dec 2016	Newcastle University
Getting Started with IBM SPSS Statistics for beginners Workshop	9th Dec 2016	Newcastle University
Systematic Review	11th Jan 2017	Newcastle University
Practical Statistical 2 Workshop	23rd & 24th Jan 2017	Newcastle University
Writing your L.R Workshop	25th Jan 2017	Newcastle University
Writing Class	1st Feb- 20th Feb 2017	Newcastle University
Dealing with unknown vocabulary	22nd Feb 2017	Newcastle University
Introduction to Databases	27th Feb 2017	Newcastle University
Scientific Writing	28th Feb 2017	Newcastle University
AFRD Research Students Conference	23rd Apr 2017	Newcastle University
Speaking and listening class	13th & 25th Oct 2017	Newcastle University
Thesis writing class	17th Oct- 7th Nov 2017	Newcastle University
Data management workshop	31st Oct 2017	Newcastle University
Academic integrity & plagiarism workshop	9th Nov 2017	Newcastle University
SPSS beginners workshop	17th Nov 2017	Newcastle University
Medline workshop	23rd Nov 2017	Newcastle University
SPSS advance workshop	5th Nov 2017	Newcastle University
Recording your research workshop	18th Jan 2018	Newcastle University
Basic Stats workshop	23rd Jan 2018	Newcastle University
Document management workshop	25th Jan 2018	Newcastle University
Building robust search strategies workshop	30th Jan 2018	Newcastle University
Advanced SR workshop	21st Mar 2018	Newcastle University
Introduction to learning & teaching workshop	20th Apr 2018	Newcastle University
Academic writing workshop	8th May 2018	Newcastle University
Advanced power point workshop	24th May 2018	Newcastle University
Writing for publication workshop	25th May 2018	Newcastle University
Nutrition Future Conference	10th Sep 218	Nutrition Society-Newcastle
Statistics for Nutrition Research	17th Sep 2018	Nutrition Society-London

Conference/ Training	Date	Location
Introduction to critical appraisal workshop	27th Sep 2018	Newcastle University
Public Speaking workshop	24th Oct 2018	Newcastle University
Writing for publication workshop	9th Nov 2018	Newcastle University
Systematic Review course	12th-16th Nov 2018	Online course
Epidemiology course	16th Nov-14th Dec 2018	Online course
Power calculation &sample size overview lecture	3rd Dec 2108	Newcastle University
Nutrition and Ageing immune System	12th Feb 2019	Nutrition Society-online
Statistics for Nutrition Research (advance)-	2nd May 2019	Nutrition Society-London
Nutrition to Support Exercise	26th Jun 2019	Nutrition Society-online
GDPR course	1st Jul 2019	Online course
Level 2 Food Hygiene and Safety for Carteing	8th Jul 2019	Online course
Personalised Nutrition	18th Jul 2019	Nutrition Society-online
The Optimal Diet	29th Jul 2019	Nutrition Society-online
Carbohydrate quality and health	14th Oct 2019	Nutrition Society-online
Nutrition type and timing for optimal exercise	29th Oct 2019	Nutrition Society-online

I. Peer reviewed papers

Conference	Title	Date	Link
Nutrients Journal	The Association between 25-Hydroxyvitamin D Concentration and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study	September 2020	https://www.mdpi.com/2072-6643/12/9/2742/htm
Nutrients Journal	The association between 25-hydroxyvitamin D concentration and Telomere length in the very-old: The Newcastle 85+ Study	November 2021	https://www.mdpi.com/2072-6643/13/12/4341

nutrients

MDPI

Article

The Association between 25-Hydroxyvitamin D Concentration and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study

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Abstract: *Background*: Low vitamin D status is common in very old adults which may have adverse consequences for muscle function, a major predictor of disability. *Aims*: To explore the association between 25-hydroxyvitamin D [25(OH)D] concentrations and disability trajectories in very old adults and to determine whether there is an 'adequate' 25(OH)D concentration which might protect against a faster disability trajectory. *Methodology*: A total of 775 participants from the Newcastle 85+ Study for who 25(OH)D concentration at baseline was available. Serum 25(OH)D concentrations of <25 nmol/L, 25–50 nmol/L and >50 nmol/L were used as cut-offs to define low, moderate and high vitamin D status, respectively. Disability was defined as difficulty in performing 17 activities of daily living, at baseline, after 18, 36 and 60 months. *Results*: A three-trajectory model was derived (low-to-mild, mild-to-moderate and moderate-to-severe). In partially adjusted models, participants with 25(OH)D concentrations <25 nmol/L were more likely to have moderate and severe disability trajectories, even after adjusting for sex, living in an institution, season, cognitive status, BMI and vitamin D supplement use. However, this association disappeared after further adjustment for physical activity. *Conclusions*: Vitamin D status does not appear to influence the trajectories of disability in very old adults.

Keywords: vitamin D status; disability; very old adults

1. Introduction

Life expectancy is increasing worldwide. By 2050, it is predicted that there will be 379 million people aged 80 and above, and almost 10% of the population of developed countries will be aged ≥ 80 (OECD, 2013). Disability is defined as experiencing difficulty in performing activities that are essential

for independent living. Such activities comprise the basic activities of daily living (BADL), such as getting up and washing hands, and the instrumental activities of daily living (IADL), such as shopping for groceries and doing housework [1]. The frequency of ADL disability is higher among very old adults (those aged 80 and older) [2]. Difficulty with performing ADL is a predictor of longer hospital stays and of additional general practice (GP) visits [3]. Furthermore, disability increases the risk of mortality 2–3 fold among very old adults [4]. Generally, disability raises the amount of benefits paid for assistance programs and care facilities in developed countries; for example, it increases the cost of care by 22% in the United Kingdom alone [5].

Very old adults are more likely to have lower circulating concentrations of 25-hydroxyvitamin D [25(OH)D]. This is due to many reasons, including decreased production of vitamin D by skin, low exposure to sunlight, and low vitamin D intake as well as catabolism factors such as medication and disease [6]. Following hydroxylation of 25(OH)D in the kidney, 1,25(OH)D binds to its nuclear receptor (VDR) which is expressed in multiple tissues, including muscle. It then influences protein synthesis in the muscle, muscle calcium uptake and type 2 muscle's fibre size and number [7]. Low concentrations of 25(OH)D are associated with poor muscle strength [8]. After controlling for potential confounders, 25(OH)D deficiency prevalence rates were 31 and 43% higher among men and women with muscle weakness than those with normal strength, respectively [9]. Two potential mechanisms have been suggested to explain the association between 25(OH)D and muscle function. First, age-related reduction of 1,25(OH)D reduces the stimulation of VDR expression by muscle. Second, the decline of VDR expression upon aging leads to impaired muscle response to 1,25(OH)D [10].

Maintaining adequate concentrations of 25(OH)D may protect against disability in terms of both musculoskeletal and cognitive function; the few studies that hav ation have found inverse associations between 25(OH)D concentration and –13]. However, these studies have several limitations including: use of different definitions of low 25(OH)D concentration; being cross-sectional rather than longitudinal; recruited those aged 65 and over with few studies of the very old; being unrepresentative because they recruited women only [8,13], targeted at a specific ethnic group [12] or involved patients with a specific disease [11]. Consequently there is a need for longitudinal studies of associations between 25(OH)D concentration and disability trajectory and which focus on very-old adults, including those living in institutions.

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We have previously reported vitamin D status in participants from the Newcastle 85+ study and found that 33% of the participants had vitamin D concentration < 30 nmol/L [14]. Therefore, this study aims to explore the association between 25(OH)D concentration and disability trajectory over five years in the Newcastle 85+ study participants. It also aims to investigate whether there is a threshold concentration of 25(OH)D above which the disability trajectory among the very old adults is slowed. In line with our previous work on vitamin D status and cognitive decline [15] and all-cause mortality [16] which showed that both lower and higher vitamin D status was associated with adverse biological outcomes, in the current analysis we hypothesized that lower and higher 25(OH)D concentrations are associated with a faster disability trajectory in very old adults.

2. Materials and Methods

2.1. Study Population and Design (The Newcastle 85+ Study)

The participants were from the Newcastle 85+ Study, which is socio-demographically representative study of the general UK population. It included both population-based and institutionalised older adults born in 1921 (age 85 years at recruitment) and living in Newcastle-upon-Tyne and North Tyneside (northeast England). All those who met these inclusion criteria were invited to participate (n = 1459). Only those individuals with end-stage terminal illness (n = 11) were excluded. The recruitment and baseline assessment took place over a 17-month period in 2006–2007. The follow-up phases took place at 18 (Phase 2), 36 (Phase 3) and 60 months (Phase 4) from baseline [17]. A health assessment, comprising questionnaires, measurements, function tests

and a fasting blood sample, was carried out in the participants' usual place of residence. In addition, general practice medical records were reviewed to extract data on diagnosed diseases and prescribed medication. Both the health assessment and data extraction were conducted by trained research nurses following a standard protocol. In the UK, patients are registered with a single general practice that acts as a gatekeeper to secondary care and receives details of all hospital admissions and outpatient attendance. The review of the general practice records included hospital correspondence to ensure that all recorded disease diagnoses were extracted, irrespective of where and when the diagnosis was made. Our analysis included all Newcastle 85+ Study participants (n = 775) for which data on health assessment, general practice records and serum 25(OH)D concentration were available at baseline (Supplemental Figure S1). From these initial group, data on health assessment and general practice records were available for 631, 484 and 344 participants at phases 2, 3, and 4, respectively.

2.2. Ethical Approval

The research complied with the requirements of the Declaration of Helsinki. Ethical approval was obtained from the Newcastle and North Tyneside 1 Research Ethics Committee (reference number 06/Q0905/2). Written informed consent was obtained from the participants. Where individuals lacked the capacity to give consent, for example because of cognitive impairment, a formal written opinion was sought from a relative or carer. Participants could decline to take part in any element of the study protocol.

2.3. Measurement of Serum 25(OH)D Concentration

Serum 25(OH)D concentration was measured at baseline from blood samples collected between June 2006 and August 2007. After an overnight fast, 40 mL blood was drawn from the antecubital vein between 7:00 and 10:30 a.m. Ninety-five per-cent of the samples were received for processing within 1 h of venepuncture. The total 25(OH)D concentration was determined by the DiaSorin radioimmunoassay (RIA) kit (DiaSorin Corporation, Stillwater, MN, USA) according to the manufacturer's recommendations, using 25(OH)D-specific antibodies and 125I-labelled 25(OH)D (DiaSorin Corporation) as a tracer. The minimum detectable concentration of 25(OH)D was 5 nmol/L, and the inter-assay coefficients of variation ranged from 8.4% to 12.6% [18].

2.4. Disability Measures and Scores

At baseline and at each follow-up assessment, participants were asked about their ability to perform 17 activities comprising the basic and instrumental activities of daily living (BADLs and IADLs) and mobility items (Supplemental Table S1); these activities were taken predominantly from the Groningen Activity Restriction Scale [19]. The ability to perform the ADLs was self-reported by the participants. Each question was framed as 'can you' rather than 'do you', to assess the participants' maximum capability to perform the activities, accounting for situational responses. Each item reported as performed without difficulty scored 0 and each item performed with difficulty scored 1 (for a maximum score of 17). A disability score was calculated based on the total number of ADLs performed with difficulty or requiring an aid/appliance or personal help [17]. Participants were classified as having a disability if they had difficulty with at least one item.

2.5. Other Measures/Confounders

In addition, the following variables, which are known to influence 25(OH)D concentration, were taken into account in the analysis: demographic factors [sex, living arrangements, housing type, years of full-time education], anthropometry [weight, body composition, fat-free mass, BMI] health and morbidity [disease count, cognitive status via SMMSE] and lifestyle factors [smoking, alcohol consumption, physical activity]; details on these have previously been published [20] as well as information on use of supplements containing vitamin D (yes/no) obtained from the interviewer-administrated questionnaire and prescribed vitamin D medication from the GP records [14].

However, apart from the vitamin D-containing supplements, no other supplements (including calcium, magnesium or B-vitamins) were included in the analysis owing to the very modest differences to micronutrient intakes when including supplements [21], and the inherent limitations in supplement frequency data. The date on which the blood sample was drawn was recorded and used to derive the season of collection defined as spring (March–May), summer (June–August), autumn (September–November) and winter (December–February).

2.6. Statistical Analysis

Normality was assessed using the Shapiro-Wilk test and confirmed using Q-Q plots and histograms. Summary statistics of normally distributed continuous values are presented as means and standard deviations (SD), and non-Gaussian distributed variables as medians and interquartile ranges (IQR). Categorical data are presented as percentages (with corresponding sample sizes).

Group-based trajectory models (GBTM) were used to derive distinct clusters of participants' disability trajectories from baseline over the subsequent 60 months. Bayesian information criteria (BIC) were used to assess the best number of trajectories within the model. The model was then further assessed by the posterior probability of group membership >75%. Differences between disability trajectory groups were tested using the Kruskal-Wallis test for ordered non-normally distributed continuous variables (weight, BMI, fat-free mass, serum 25(OH)D, chronic disease count) and $\chi 2$ test for categorical variables (sex, physical activity, alcohol drinker, smoker, 25(OH)D, impaired cognitive status, living in an institution).

Multinomial regression was used to determine the association between disability and 25(OH)D concentration in both cross-sectional and longitudinal analysis. The concentration of 25(OH)D was not normally distributed, therefore, non-parametric analysis was used. The following cut-offs were used in the analysis: <25 nmol/L (low), 25 to 50 nmol/L (moderate) and >50 nmol/L (high) [22]. Important confounders were selected based on their clinical and theoretical relevance as well as univariate analysis with the disability trajectory. These confounders were then fitted, removed and refitted until the best possible but parsimonious model was achieved while checking for model fit statistics throughout, using 10% of change-in-estimate. The multi-collinearity between the confounders was assessed using VIF. Model 1 was an unadjusted model. Model 2 was adjusted for sex, living in an institution and season of blood collection. Model 3 was adjusted further for cognitive status, BMI and vitamin D containing medication. Model 4 was adjusted further for physical activity. The models were stratified by sex. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics software version 24 (IBM, Armonk, NY, USA) except for the disability trajectory that was derived using STATA v15.0 (package traj).

2.7. Sensitivity Analysis

To investigate the effects of grip strength, FFM (fat-free mass) and disease count, the models were further adjusted for each of these variables. Models were rerun, excluding those participants with evidence of cognitive impairment (SMMSE score < 26). The models were also stratified by season of blood collection using the same categories as [14].

3. Results

3.1. 25(OH)D Concentration and Disability at Baseline

A cross-sectional analysis of the association between 25(OH)D cut-offs and disability baseline data reveals a U-shaped association between 25(OH)D and disability. A significant association was found between 25(OH)D concentration and disability score at baseline, ($p = < 0.001$) and ($p = 0.002$) for low (<25 nmol/L) and high (>50 nmol/L) concentrations, respectively.

3.2. Disability Trajectories

The disability trajectories (DT; one linear (low to mild trajectory) and two quadratic (mild to moderate and moderate to severe trajectories)) from age 85 to 90 years were best presented by a triple-group model. The trajectories are plotted in Figure 1 and the characteristics of the participants with each of these trajectories are described in Table 1. DT1 represents a low-to-mild disability trajectory (group size: n = 249–33.4%), DT2 represents a mild-to-moderate disability trajectory (group size: n = 351–44%) and DT3 represents a moderate-to-severe disability trajectory (group size: n = 175–22.5%). The participants with a low-to-mild disability trajectory had a slightly increased disability trajectory over five years, while the participants with a mild-to-moderate or moderate-to-severe disability trajectory showed serious trajectories with advancing age, with the score (number of activities that the participants were unable to undertake, unaided) increasing from four to 9.5 and from 11 to 15, respectively.

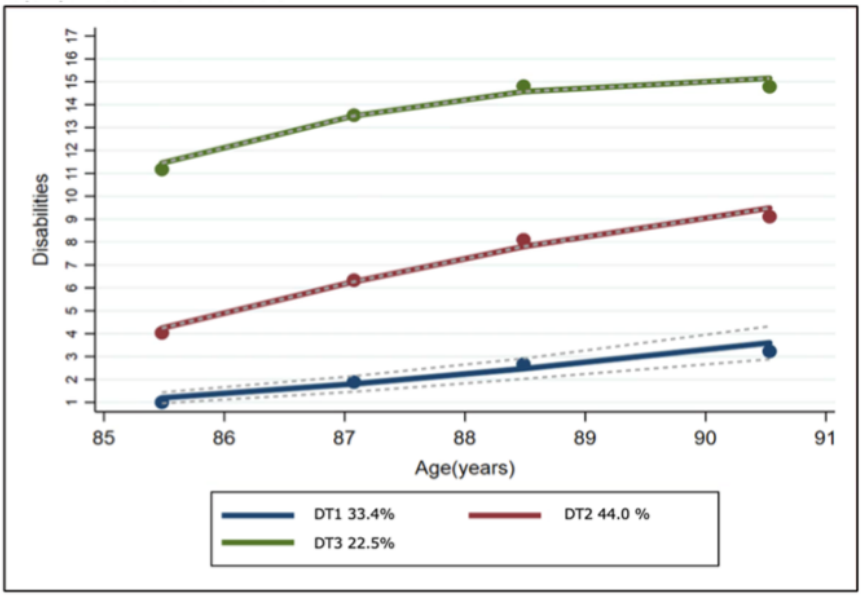

Figure 1. Disability trajectories with 95% confidence intervals in participants who had a serum 25(OH)D measurement available. DT1: Low to mild disability trajectory; DT2: Mild to moderate disability trajectory; DT3: Moderate to severe disability trajectory. Percentages represent group size. Disabilities resulted from calculating ADLs, IADLs and mobility. The grey dotted lines represent the 95% confidence intervals of the disability trajectories. ADL: activities of daily living, IADL: instrumental activities of daily living.

3.3. The Differences in Socioeconomic, Lifestyle and Health Factors between Disability Trajectories

Body weight, total number of years in education, fat-free mass and smoking did not differ significantly between participants in each of the three disability trajectories in. The participants in the three groups showed significant differences regarding their BMI, physical activity level, alcohol intake, vitamin D containing medication use, number of chronic diseases, cognitive status and living in an institution. However, the moderate-to-severe DT group was characterised by a higher percentage of women, a lower proportion of alcohol drinkers, living in an institution, being less physically active, having a higher number of chronic diseases and being cognitively impaired (Table 1). Although there were no significant differences in median serum 25(OH)D concentration between DT groups, the distribution of participants across the three categories of vitamin D adequacy based on 25(OH)D concentration (low, moderate and high) differed significantly across these three trajectories (Table 1).

Table 1. Participant characteristics by the three disability trajectories identified at baseline.

	Low-to-Mild (*n* = 249)	Mild-to-Moderate (*n* = 351)	Moderate-to-Severe (*n* = 175)	*p*
Women % (*n*)	48.4 (121)	56.6 (231)	69.3 (122)	<0.001
Weight (kg) mean (SD)	63.9 (11.8)	63.5 (13.4)	63.9 (14.3)	0.732
BMI mean (SD)	23.8 (3.8)	24.7 (4.4)	24.9 (5.2)	0.029
Fat-free mass (kg) mean (SD)	46.5 (9.2)	44.4 (9.1)	45 (8.9)	0.151
Total number of years in education % (*n*)				
0–9 years	61.9 (153)	62.1 (213)	70.3 (111)	0.241
10–11 years	23.9 (59)	24.2 (83)	22.2 (35)	
12–20 years	14.2 (35)	13.7 (47)	7.6 (12)	
Physical activity % (*n*)				
Low	2.4 (6)	27.3 (68)	70.3 (175)	<0.001
Moderate	15.8 (55)	58.7 (205)	25.5 (89)	
High	63.2 (110)	33.9 (59)	2.9 (5)	
Alcohol drinkers % (*n*)	80 (156)	72.4 (168)	55.3 (52)	<0.001
Smoking % (*n*)	3.6 (9)	8 (28)	4.5 (8)	0.124
Vitamin D containing medication % (*n*)	10 (25)	13.4 (47)	31.8 (56)	<0.001
Supplement users % (*n*)	23.3 (58)	20.8 (73)	12 (21)	0.012
Serum 25(OH)D nmol/L median (IQR)	42 (29–59)	36 (23–58)	39 (21–70)	0.178
25(OH)D				
<25 nmol/L (low) % (*n*)	26.4 (66)	36 (90)	37.6 (94)	0.02
25–50 nmol/L (moderate) % (*n*)	34.7 (122)	31.8 (112)	33.5 (118)	
>50 nmol/L (high) % (*n*)	38.1 (67)	19.9 (35)	42 (74)	
Chronic disease count mean (SD)	4.1 (1.5)	4.93 (1.75)	5.6 (1.9)	<0.001
Impaired cognitive status % (*n*)	12 (30)	23.3 (82)	57.5 (100)	<0.001
Living in institution % (*n*)	0.4 (1)	3.4 (12)	30.7 (54)	<0.001

BMI: body mass index. SD: standard deviations. IQR: medians and interquartile ranges. *p*, *p*-value: Kruskal-Wallis test for continuous non-normally distributed variables or $\chi 2$ test for categorical variables 25(OH)D: <25 nmol/L (low), 25–50 nmol/L (moderate), >50 nmol/L (high).

3.4. 25(OH)D Concentration and Disability Trajectory

The results of the analysis show that participants with low concentrations of 25(OH)D (<25 nmol/L) were more likely to have a mild-to-moderate disability trajectory (OR = 2.01, 95% CI = 1.29–3.14, $p = 0.002$) or a moderate-to-severe disability trajectory (OR = 3.39, 95% CI = 1.99–5.76, $p = 0.001$) than a low-to-mild disability trajectory compared to those with higher 25(OH)D concentrations in the unadjusted model, after adjusting for sex, living in an institution and season (OR = 2.01, 95% CI = 1.27–3.19, $p = 0.003$) and (OR = 3.02, 95% CI = 1.70–5.38, $p = 0.001$) and after further adjustment for cognitive status, BMI and vitamin D containing medication (OR = 1.97, 95% CI = 1.22–3.17, $p = 0.005$) and (OR = 3.12, 95% CI = 1.67–5.85, $p = 0.001$), respectively. However, this association disappeared after adjustment for physical activity (Table 2). The results also show that participants with high 25(OH)D concentrations were more likely to have a moderate-to-severe disability trajectory compared to those with moderate concentrations over five years but only in the unadjusted model (OR = 1.94, 95% CI = 1.23–3.06, $p = 0.004$). However, in the adjusted models, no association was found between high concentration of 25(OH)D and disability trajectory.

Table 2. Association between 25(OH)D concentration and disability trajectories.

Trajectories	25(OH)D	Model 1			Model 2			Model 3			Model 4		
		OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
DT1: Low-to-mild	(ref)		(ref)			(ref)			(ref)			(ref)	
	<25 nmol/L	2.01	1.29–3.14	0.002	2.01	1.27–3.19	0.003	1.97	1.22–3.17	0.005	1.61	0.95–2.74	0.074
DT2: Mild-to-moderate	25–50 nmol/L		(ref)			(ref)			(ref)			(ref)	
	>50 nmol/L	1.05	0.73–1.52	0.774	0.94	0.64–1.38	0.771	0.92	0.61–1.38	0.707	1.07	0.69–1.67	0.749
	<25 nmol/L	3.39	1.99–5.76	0.001	3.02	1.70–5.38	0.001	3.12	1.67–5.85	0.001	1.95	0.94–4.06	0.071
DT3: Moderate-to-severe	25–50 nmol/L		(ref)			(ref)			(ref)			(ref)	
	>50 nmol/L	1.94	1.23–3.06	0.004	1.34	0.80–2.22	0.254	0.83	0.45–1.55	0.577	1.02	0.49–2.12	0.945

CI: confidence interval. BMI: body mass index. OR: odd ratio. ref: reference. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) and >50 nmol/L (high); Number of participants with low, moderate and high 25(OH)D for DT1 is 66, 122, 67; DT2 is 89, 111, 34; DT3 is 94, 118, 74; Model 1 is the unadjusted model. Model 2 is further adjusted for sex, living in an institution and season. Model 3 is further adjusted for cognitive status, BMI and vitamin D containing medication. Model 4 is further adjusted for physical activity.

3.5. 25(OH)D Concentration and Disability Trajectory by Sex

Men with low concentrations of 25(OH)D were more likely to have a moderate-to-severe disability trajectory (OR = 3.55, 95% CI = 1.56–8.09, $p = 0.003$) than a low-to-mild disability trajectory compared to those with moderate concentrations in the unadjusted model, even after adjusting for living in an institution and season (OR = 4.42, 95% CI = 1.79–10.90, $p = 0.001$) and after further adjustment for cognitive status, BMI and vitamin D containing medication (OR = 3.83, 95% CI = 1.44–10.17, $p = 0.007$). However, this association disappeared after future adjustment for physical activity (Supplemental Table S2).

Women with low concentrations were more likely to have mild-to-moderate and moderate-to-severe disability trajectories than a low-to-mild disability trajectory compared to those with moderate concentrations in the unadjusted model (OR = 1.87, 95% CI = 1.03–3.39, $p = 0.039$) and (OR = 3.03, 95% CI = 1.50–6.13, $p = 0.002$), respectively. This association was maintained even after adjusting for sex, living in an institution and season (OR = 2.06, 95% CI = 1.12–3.83, $p = 0.020$) and (OR = 2.58, 95% CI = 1.21–5.50, $p = 0.014$), respectively. It also continued after further adjustment for cognitive status, BMI and vitamin D containing medication (OR = 1.95, 95%CI = 1.02–3.72, $p = 0.041$) and (OR = 2.70, 95% CI = 1.16–6.27, $p = 0.020$), respectively, but it disappeared after future adjustment for physical activity. Women showed a U-shaped association between 25(OH)D and a moderate-to-severe disability trajectory but only in the unadjusted model (OR = 2.29, 95% CI = 1.25–4.17, $p = 0.007$).

3.6. Sensitivity Analysis

Using the same models with further adjustment for grip strength, fat-free mass and disease count separately, no association was found between 25(OH)D concentration and disability trajectory. However, when physical activity was removed from the model, after adjusting for grip strength, fat-free mass and disease count, participants with a low concentration were more likely to have mild-to-moderate and moderate-to-severe disability trajectories.

The models were also rerun excluding individuals with cognitive impairment (SMMSE < 26). Participants with normal cognitive status ($n = 561$) who had a low concentration were more likely to have a moderate-to-severe disability trajectory (OR = 2.30, 95% CI = 1.15–4.58, $p = 0.017$) than a low-to-mild disability trajectory in the unadjusted model. This was also maintained in the adjusted models for the same confounders: sex, living in an institution and season (OR = 2.14, 95% CI = 1.04–4.39, $p = 0.038$) and even after adjustment for BMI and vitamin D containing medication (OR = 2.44, 95% CI = 1.13–5.27, $p = 0.022$) (Supplemental Table S3). This association disappeared after further adjustment for physical activity.

Participants' characteristics by season have been described previously. Stratifying the analysis by season, no association was found between 25(OH)D and disability trajectory in Spring ($n = 121$). Participants with low concentrations were more likely to have a moderate-to-severe disability trajectory compared to those with moderate concentrations in the unadjusted model for Summer ($n = 309$) and Autumn ($n = 180$) (OR = 2.96, 95% CI = 1.17–7.48, $p = 0.021$) and (OR = 6.03, 95% CI = 1.47–24.77, $p = 0.013$), respectively. However, the association disappeared in the adjusted models. For Winter ($n = 168$), participants with low concentrations were more likely to have a moderate-to-severe disability trajectory (OR = 5.83, 95% CI = 1.81–18.74, $p = 0.003$) compared to normal concentrations in the unadjusted model and after adjustment for sex and living in an institution (OR = 6.44, 95% CI = 1.79–23.12, $p = 0.004$) and after further adjustment for cognitive status, BMI and vitamin D containing medication (OR = 5.10, 95% CI = 1.28–20.37, $p = 0.021$). This association disappeared after adjustment for physical activity. On the other hand, participants with high concentrations were more likely to have a moderate-to-severe disability trajectory (OR = 6.92, 95% CI = 2.23–21.43, $p = 0.001$) compared to normal concentrations in the unadjusted model and after adjustment for sex, living in an institution and season (OR = 4.51, 95% CI = 1.24–16.37, $p = 0.022$). After adjustment for cognitive status, BMI and vitamin D containing medication, the association was attenuated although

it became stronger after adjustment for physical activity (OR = 6.11, 95% CI = 1.01–36.75, $p = 0.048$) (Supplemental Table S4).

Serum 25(OH)D was used as a continuous variable in the analysis, but no association was found between 25(OH)D and disability (baseline data) or disability trajectory (follow up phase data) in the unadjusted or adjusted models.

4. Discussion

4.1. Main Findings

For the current analysis, the disability trajectories model was best presented by the triple-group model. These trajectories differed from two previously derived disability trajectories in different samples of the Newcastle 85+ Study [23,24]. We showed that, in partially adjusted models, people aged 85+ years with a 25(OH)D concentration (25–50 nmol/L) were more likely to have less disability at baseline and a slower disability trajectory over the following five years. However, in fully adjusted models, our results did not show a protective effect of any 25(OH)D concentration on trajectories of disability over five years.

4.2. Evidence from Other Studies

The lack of an association between 25(OH)D concentration and disability trajectories in our study is inconsistent with the findings of prospective cohort studies that investigate the association between vitamin D status and disability in those age 65 years and over. For example, two studies found that a 25(OH)D < 50 nmol/L increased the risk of disability in arthritis and multiple sclerosis patients, respectively [11,12]. Likewise, Semba, et al. [13] found that a 25(OH)D < 50 nmol/L was associated with a higher possibility of disability in women aged over 65 years and living in the community. The higher risk of a disability trajectory amongst participants with a concentration higher than 50 nmol/L in the cross-sectional analysis and in unadjusted models in the prospective analyses could be driven largely by those with cognitive impairment or those taking vitamin D containing medication or prescribed medication. First, our overall analysis showed that the association between a high concentration and disability trajectory disappeared after adjusting for these variables. Moreover, excluding participants with low SMMSE scores supports this finding. On the other hand, participants with a normal cognitive status did not show an association between high concentration and disability trajectories. In addition, there is no general agreement amongst researchers regarding the optimal concentration of 25(OH)D in relation to disability trajectory. The Institute of Medicine (IoM) defines vitamin D deficiency as a concentration of 25(OH)D < 30 nmol/L, and vitamin D adequacy as a concentration of >50 nmol/L for all age groups based on integrating data from several health outcomes and PTH [25]. In contrast, the UK Scientific Advisory Committee on Nutrition (SACN) defines vitamin D deficiency at a 25(OH)D concentration < 25 nmol/L [26]. However, our previous findings from the Newcastle 85+ study have documented a U-shaped association between 25(OH)D and muscle strength and performance [27].

Generally, the main role of vitamin D is to support musculoskeletal health. Therefore, maintaining an moderate 25(OH)D concentration is essential in order to slow the effect of ageing on the bones and muscles. Ageing is accompanied by a redistribution of the cortical and trabecular bone [28]. Moreover, a low 25(OH)D concentration increases osteoblastic activity and bone turnover [29,30]. A significant positive association has also been documented between 25(OH)D concentration, BMD [31] and type II muscle fibre [32] in older people. In addition, the VDR expression is reduced in the muscles as part of ageing [33]. A positive association between 25(OH)D concentration and muscle strength has been reported [32]. Therefore, a lack of VDR, which is expected in very-old adults, leads to reduced muscle mass and strength, as explained previously. Furthermore, studies in rats have demonstrated that a high PTH, due to a low concentration of 25(OH)D, induces muscle catabolism and reduces calcium transport in the skeletal muscle [32], thereby leading to low muscle strength. Combined, this can explain the effect of a low concentration of 25(OH)D on the onset and progression of disability.

In addition, the association between moderate 25(OH)D concentration and physical performance and strength has been confirmed previously [10]. Kotlarczyk, et al. [34] found that slower gait speed and lower IADL scores were associated with low 25(OH)D concentration. Moreover, a positive association between the 8-foot walk test and the sit-to-stand test, with a concentration of 25(OH)D, was also found [35]. These results indicate that a low concentration of 25(OH)D was associated with low muscle strength, which is a predictor of disability. Consistent with our results, Granic, et al. [27] demonstrated that a 25(OH)D concentration of > 30 nmol/L maintains muscle strength, but a concentration of > 50 nmol/L did not have further muscular or musculoskeletal benefits in the very old adults. However, whilst we appreciate that muscle function and disability per se are different parameters, a comparison nonetheless is relevant because of the role of muscle function in contributing to disability.

Physical activity is clearly a predictor of disability [11], although the association between PA and 25(OH)D was conflicted between the studies. First, a high concentration of 25(OH)D can positively influence the intensity of PA [36]. However, a converse association is also suggested [37]. In the same vein, a study analysing the data from NHANES reported that PA is generally associated with a high concentration of 25(OH)D, whether this activity occurs indoors or outdoors [38]. Therefore, restricted PA, which is associated with disability, can have an adverse effect on 25(OH)D concentration, possibly due either to a defect in metabolism or limited exposure to sunlight. Besides, PA is accompanied by improved health, stronger muscles and a lower BMI, which are all associated with 25(OH)D concentration [13,39,40]. Furthermore, the progression of disability is accompanied by a greater risk of feeding disability onset [41]; this contributes to the risk of nutrient deficiency, including vitamin D. Our results show that the association between 25(OH)D and the disability trajectory disappeared after adjusting for PA. This suggests that the association between 25(OH)D concentration and disability could be due the effect of PA rather than 25(OH)D concentration. This means those with a higher PA have a better vitamin D status and, obviously, those with a high PA have less disability.

Age-related changes also result in body composition changes. For that reason, a lean body mass is significantly lower in older adults compared to younger ones—a change that accelerates after the age of 60 [42]. The univariate analysis of our data showed an association between fat-free mass and disability trajectories. However, the evidence demonstrated that the amount of fat mass but not fat-free mass was associated with muscle function and disability. For instance, Sternfeld, et al. [43] and Visser, et al. [44] agreed that there was no association between physical disability and total body skeletal muscle mass, while a high percentage of fat mass was associated with physical disability.

Even though a higher lean-to-fat ratio was associated with a faster walking speed, this suggests that the impact of a lean mass is important in relation to the amount of body fat. Indeed, fat-free mass was not a significant predictor of mobility-related disability in the regression model [45]. The association between 25(OH)D concentration and fat and lean mass could be explained by the escalation in fat mass, which may enhance the storage of vitamin D and, consequently, lower the circulating 25(OH)D [40]. However, the adjustment for BMI in the model, and for FFM in the sensitivity analysis, did not affect the association between 25(OH)D concentration and disability trajectory in the current study.

Our results also suggest that in partially adjusted models, men with a low concentration of 25(OH)D were more likely to develop only a severe disability trajectory, while women with a low concentration were more likely to develop either a moderate or a severe disability trajectory. This could be explained by the findings of Granic, et al. [46], who demonstrated that men had better muscle strength and physical performance (measured by grip strength and timed-up-and-go), but a steeper decline in both grip strength and timed-up-to-go over five years. Similarly, Millán-Calenti et al. [3]. reported that older men and women (80+ years) have a higher risk of being dependent (OR = 1.10) using ADL and IADL compared to younger adults (65+), but the risk among women is even higher (OR = 2.48). Conversely, our results are inconsistent with the findings of Semba, et al. [13], who demonstrated that only women with a low 25(OH)D concentration were at risk of having a disability. This difference could be due to the smaller number of men included in the studies compared to women.

The association between 25(OH)D concentration and disability trajectories in the current study varies by season. In the spring, no association was found, whereas a significant adverse association was found between a 25(OH)D concentration lower than 25 nmol/L and disability trajectory, but only in the unadjusted model. However, in Winter, a U-shaped association was found between 25(OH)D concentration and disability trajectory. This conflicting results between the seasons could be explained by the differences between the participants' cognitive status, PA and vitamin D containing medication usage. The total number of participants in the Spring was the lowest (n = 121); consequently, Spring was associated with the lowest percentage of participants who had normal cognitive status (14.5%), were physically active (14.5%) and who took vitamin D containing medication (15.5%) when compared to the other seasons. This may explain the failure to detect the association. On the contrary, the data showed that, in the Winter, of the 165 participants, 14 were cognitively impaired and took vitamin D containing medication; nine of the cognitively impaired participants were physically active compared to the 52 participants who had a normal cognitive status and were physically active. Therefore, a potential negative effect of the highest 25(OH)D tertile on disability trajectory could be partly driven by those who have an impaired cognitive status, that influences their PA, and by those who have reached a higher concentration through taking vitamin D containing medication shortly before the baseline assessments.

4.3. Strengths and Limitations

Our study has several strengths, including its prospective design, its broad representativeness of the population in England and Wales, large number of participants, the five-year follow up to measure disability, the robustness of the clustering technique (GBTM) used to derive disability trajectory, and the adjustment for several potential confounders associated with disability and 25(OH)D concentration. Physical activity and season, which could reflect UV exposure, were also considered in the models. Determining the disability by using 17 ADLs that compromise BADL, IADL and mobility items is also a strength of this study. Moreover, our study used prevalent cut-offs of serum 25(OH)D to determine the concentration required to predict the onset and progress of disability trajectory.

However, the findings reported here should be interpreted with caution due to the following limitations. First, the concentration of 25(OH)D was only measured at baseline, so it might change during the subsequent five years depending on sun exposure, season, supplement intake, physical activity and disease. However, the changes in these variables are unlikely across the follow-up phases, if only disease/disability may increase but not the others. Another limitation was that the frequency or dose of supplements used as well as UV exposure were not measured. Finally, it is possible that some disability transitions were not fully captured during the follow up phases, as these were 18 or 24 months apart.

5. Conclusions

We found a U-shaped association between 25(OH)D and disability at baseline with both low (<25 nmol/L) and high (>50 nmol/L) 25(OH)D concentrations associated with faster disability. However these findings should be interpreted with caution as residual confounding is very likely driving these associations. In fully adjusted models, we failed to find a significant association between any 25(OH)D concentration and disability trajectories over 5 years. However, it should be noted that sample size limitations may have precluded the detection of statistically significant findings and that larger studies are needed for this research question.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/9/2742/s1, Supplementary Figure S1: The Newcastle 85+ Study recruitment. Supplementary Table S1. Self-reported activities of daily living. Supplementary Table S2. Association between different 25(OH)D cut-offs and disability trajectories by sex. Supplementary Table S3. Association between 25(OH)D concentration and disability trajectories of people with normal cognitive status. Supplementary Table S4. Association between different 25(OH)D cut-offs and disability trajectories by season.

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The Association between 25-Hydroxyvitamin D Concentration and Telomere Length in the Very-Old: The Newcastle 85+ Study

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Abstract: (1) Introduction: vitamin D may maintain the telomere length, either directly or via the inflammation effect and/or modulating the rate of cell proliferation. Whilst results from cross-sectional studies investigating the association between 25(OH)D concentration and telomere length have been mixed, there is a dearth of data from prospective studies which have assessed these associations. This study aimed to examine the association between 25(OH)D concentration in plasma and telomere length in blood cells in very-old adults (≥85 years old) at baseline, 18 months and 36 months by controlling for related lifestyle factors. (2) Methodology: our prospective cohort study comprised 775 participants from the Newcastle 85+ Study who had 25(OH)D measurements at baseline. Plasma 25(OH)D was stratified as <25 nmol/L (low), 25–50 nmol/L (moderate) and >50 nmol/L (high). Peripheral blood mononuclear cell telomere length was measured by quantitative real-time polymerase chain reaction at baseline, 18 and 36 months from baseline. (3) Results: a positive significant association was found between 25(OH)D concentration and telomere length amongst very-old participants at baseline (95% CI = 12.0–110.3, B = 61.2 ± 5.0, $p = 0.015$). This association was negative at 18 months (95% CI = −59.9–−7.5, B = −33.7 ± 13.3, $p = 0.012$) but was non-significant at 36 months. (4) Conclusion: Circulating 25(OH)D concentration shows inconsistent relationships with telomere length over time in very-old (85+ year old) adults.

Keywords: ageing; vitamin D; telomere length

1. Introduction

Telomeres, the specific deoxyribonucleic acid (DNA) protein structures, are the cap at both ends of each chromosome. Telomeres play an essential role in protecting the genome against nucleolytic destruction, recombination, repair, and interchromosomal fusion [1]. Each DNA replication causes telomere shortening and at the point when telomeres reach a critical limit, the cell undergoes senescence and/or apoptosis [2]. The enzyme telomerase plays a key role in the maintenance of telomere length [3]. Age is a well-established factor associated with telomere shortening [3,4]. In humans, telomere length decreases at

a rate of 24.8–27.7 base pairs per year [2]. While telomere attrition with age is linked to a variety of age-related diseases and their complications, telomere attrition with ageing has been documented in multiple investigations regardless of the existence of any age-related disorders [3]. These observations support the view that shortened telomeres may serve as a potential candidate biomarker of ageing [3]. Telomere length is also affected by a combination of factors, including sex, genetics and lifestyle [2]. On the other hand, it is purported that the cellular and tissue defects associated with telomere dysfunction are mediated in part by oxidative stress and chronic inflammation mechanisms [5]. Accelerated telomere shortening is associated with mortality and many age-associated diseases, such as cardiovascular diseases, type 2 diabetes mellitus (T2DM), Alzheimer's disease [1], as well as immune and infectious diseases [6] (Figure 1).

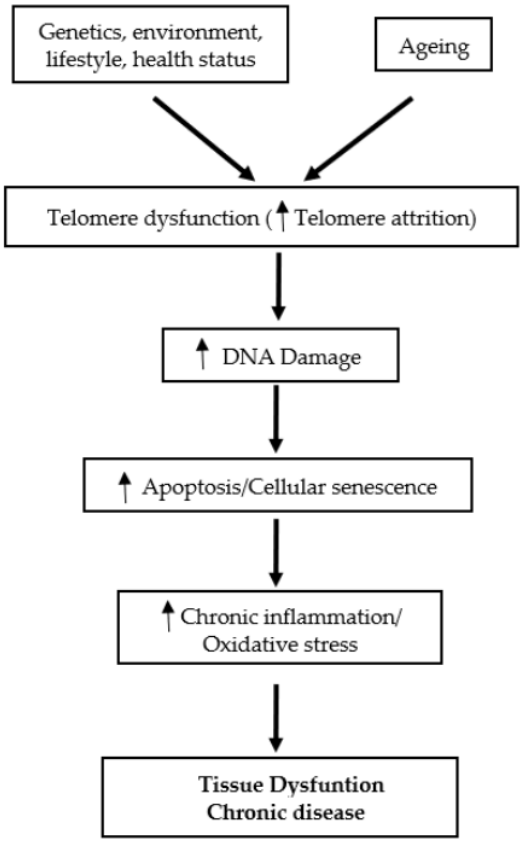

Figure 1. Schematic overview of the role of telomeres in tissue dysfunction and chronic disease.

Evidence from animal models as well as human studies have demonstrated that various nutrients (e.g., folate, niacin, vitamin C, magnesium, zinc and omega-3 fatty acids), bioactives (e.g., polyphenols) and whole foods (e.g., tea) may influence telomere length and telomere attrition through mechanisms related to cellular functions including DNA repair and chromosome maintenance, DNA methylation, inflammation, oxidative stress and telomerase activity I (For review see [7]). Vitamin D may influence telomere length through its biologically active hormone 1α,25 dihydroxyvitamin D3 (calcitriol) [8]. Calcitriol is a potent immunosuppressant and has strong anti-inflammatory and anti-proliferative properties mediated in part by its ability to reduce gene expression of inflammatory

mediators interleukin- 2 and interferon gamma [7]. The anti-inflammatory and anti-proliferative properties of calcitriol may reduce cell turnover, thus potentially reducing their telomere length attrition [7].

Limited population studies have assessed the association between circulating 25(OH)D concentration [the most commonly used nutritional biomarker of vitamin D] and telomere length [4,8–16]. A recent study [9] demonstrated that only at insufficient concentrations of 25(OH)D (50–75 nmol/L), telomerase activity is associated with survival times in CHD patients (mean age 59.9 years-old). The findings by Richards et al. [10] and Liu et al. [11] demonstrated a positive association between 25(OH)D concentration and leukocyte telomere length (LTL) in women. On the other hand, a cross-sectional study of 2483 men aged 40–75 years did not observe a positive association between vitamin D biomarkers (25(OH)D and 1,25(OH)2D) and LTL [12]. Liu et al. [13] also found no association between absolute 25(OH)D concentrations and long LTL, for the overall population or the subgroups in the study (men, women, black and white separately) although maintaining a 25(OH)D concentration $\geq$30 nmol/L was significantly associated with longer LTL in white participants only. Yet, Zhu et al. [14] showed an increase in telomerase activity in 19 overweight African-American women after 16 weeks of vitamin D supplementation.

These conflicting results may be attributed to the differences between gender, race and ages of study participants. For instance, the age range in one study was 48–93 years-old (a mean age of 62.8 years-old) [13], whereas Richards et al. [10] studied younger women aged 18–79 years-old (a mean age of 49.4 years-old). Besides, other studies failed to prove the association between 25(OH)D and telomere length in younger participants (mean age 31–39 years-old [15] [16]). Generally, these studies included a wide age range of participants with limited number of either very-old adults or participants younger than 70 years-old, or participants of only one sex, which limited the generalizability of the findings for those older than 80 years-old. Therefore, this study aimed to use the large dataset on both sexes from the Newcastle 85+ Study to examine the association between 25(OH)D concentration and telomere length in very-old adults (>85 years-old) [17] at baseline and after 18 and 36 months. We hypothesize that, by controlling related lifestyle factors, a concentration of 25(OH)D <25 nmol/L (used to define vitamin D deficiency) will be associated with shorter telomere length in very-old adults.

2. Materials and Methods

2.1. Population Sample

The participants were taken from the Newcastle 85+ Study, which included both community-dwelling and institutionalised older adults aged 85 years-old at recruitment and living in Newcastle-upon-Tyne and North Tyneside. Commencing in 2006, the study recruited a birth-cohort of more than 1000 adults aged 85 years from North-East England to understand in great detail, the biological, clinical and social determinants of health as the cohort ages. The study seeks to provide extensive data on the relevance of various social, molecular and cellular indicators in contributing to health of very old people [18]. Health assessments, which comprised questionnaires, measurements, function tests and a fasting blood sample as well as general practice (GP) medical records from which to extract data on diagnosed diseases and prescribed medication, were available for the 851 participants. The current paper includes those Newcastle 85+ Study participants (N = 775) whose data on health assessment, general practice records and 25(OH)D concentrations were available [18].

2.2. Ethical Approval

The study conformed with requirements set in the Declaration of Helsinki. The Newcastle and North Tyneside Research Ethics Committee granted ethical approval, and the participants provided signed informed consent (research project reference number: 06/Q0905/2).

2.3. Circulating 25(OH)D Assay and Definition of Vitamin D Status

Following an overnight fast, 40mL of blood was drawn from the participants via the antecubital vein between 7:00 am and 10:30 am. The majority of whole blood samples (95%) were received at the laboratory for processing within 1 h of venepuncture. The total 25(OH)D concentration in serum was estimated by the DiaSorin radio-immunoassay (RIA) kit (DiaSorin Corporation, Stillwater, MN) as described previously [19]. Circulating 25(OH)D measurements were only available at baseline sampling [19].

United Kingdom Scientific Advisory Committee on Nutrition (SACN) cut-offs, which are used for the dietary recommendations for the UK population, are used [20]. A circulating concentration of 25(OH)D < 25 nmol/L was used to indicate the risk of vitamin D deficiency [20].

2.4. Telomere Length

Telomere length of peripheral blood mononuclear cell (PBMCs) was measured as an abundance of telomeric template versus a single gene by quantitative real-time PCR. The intra-assay coefficient of variation was 2.7% while the inter-assay coefficient of variation was 5.1% [19]. Four internal control DNA samples were run within each plate to correct for plate–to-plate variation. The measurements were performed in quadruplicate. All PCRs were carried out on an Applied Biosystems 7900HT Fast Real Time PCR machine (Applied Biosystems, Foster City, CA, USA) with a 384-well plate capacity [19].

Aviv et al. 2006 [21] proposes guidance for epidemiological research on the minimum numbers of subjects required for a given age range to determine whether the extrapolated telomere attrition rate of two groups are significantly different. For longitudinal studies assessing telomere length in older adults (>60 years) over time, the sample size required in each test group being compared for 80% power and $p < 0.05$ using a two sided *t*-test is 104 participants [21]. However, given the limited data on telomere attrition in those aged 85–88 years (the age range of the participants in this analysis), any power calculations for very old populations are purely speculative.

2.5. HbA1c Measurement

The glycosylated haemoglobin % (HbA1c) was measured using a Tosoh Eurogenetics automated HLC-723G7 HPLC analyser (Tosoh Bioscience, Tokyo, Japan) [19].

2.6. Other Health and Lifestyle Variables

2.6.1. Health and Morbidity

Information on health and morbidity was collected from GP medical records by a trained nurse. Diseases were recorded via a predetermined list of key diseases. All diagnoses of listed diseases were scored as present (score 1) or absent (score 0), together with the date of first diagnosis. A simple disease count was used (maximum score 18) from selected chronic diseases (See Supplementary Box S1). The participants were included only if all of the variables were scored as present or absent [18].

2.6.2. Lifestyle

The multidimensional health questionnaire included gender (men or women) and lifestyle factors (smoking, alcohol consumption and physical activity). Using data from the Newcastle 85+ pilot study, a physical activity questionnaire (PAQ) was developed and tested in this age range before being implemented. The participants were divided into three groups: low (scores 0–1), moderate (scores 2–6), and high (scores 7–18) on the PAQ. Physical activity levels are classified based on the frequency and intensity of physical activity performed each week (supplementary data Box S1, available in Age and Ageing online) [22]. For smoking, participants were categorized as non-smokers and occasional and regular smoker. In regard to alcohol consumption, participants were categorized as non-drinkers, moderate drinkers and heavy drinkers. Nutritional status was assessed by

calculating body mass index (kg/m^2) (BMI) from recorded height and weight and included in the models owing to its influence on telomere length [23].

Vitamin D supplement use was divided into two categories: no supplement users and supplement users. Because the only information available on supplement use was the brand and type, micronutrient-containing supplements were assumed to be taken according to the manufacturer's instructions. The use of vitamin D supplements (yes/no) was collected via the interviewer-administered questionnaire, and prescriptions for vitamin D medicine were retrieved from GP records [24].

2.7. Statistical Analysis

The normality of the distributions was assessed by reviewing the histograms and Q-Q plots, and the Shapiro-Wilk test was applied. Normally-distributed, continuous variables are presented as means and standard deviations (SD), while non-Gaussian distributed variables are presented as medians and interquartile ranges (IQR). The categorical data are presented as percentages (with the corresponding sample size). Mann-Whitney and Kruskal-Wallis tests were used for ordered and non-normally distributed continuous variables, and a χ^2 test for categorical variables.

The concentration of 25(OH)D was not normally distributed (and could not be normalized by transformation, as described previously [25]). In addition, telomere length was not normally distributed (and could not be normalized by transformation either). The 25(OH)D concentration was categorized by SACN cut-offs points for vitamin D [19]: <25 nmol/L (as low). In order to have equal participants distributed between the groups, we used 25–50 nmol/L (as moderate) (used as reference in the analysis) and >50 nmol/L (as high). To examine the association between 25(OH)D and telomere length at baseline, 18 months and 36 months, linear regression was used. The linearity and homoscedasticity assumptions were tested with residual normality versus predicted values plots. Important confounders were selected based on their clinical and theoretical relevance to the telomere length. These variables were then fitted, removed, and refitted until the best possible but parsimonious model was achieved while checking for model fit statistics throughout. Model 1 is an unadjusted model, Model 2 is adjusted for smoking and alcohol consumption, Model 3 is further adjusted for BMI and physical activity, and Model 4 is further adjusted for HbA1c. The models were stratified by sex.

All analyses were performed using IBM's SPSS Statistics software, version 24 (IBM, New York, NY, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participants' Characteristics

By using the 3 cut-off of 25(OH)D concentration (low, moderate and high), there are significant differences between men and women, BMI categories, PA level, vitamin D containing medication and supplements usage, their general health rate, telomere length and HbA1c measurement (Table 1). The majority of the participants were women, have normal weight, moderately active, non-alcohol drinker, regular smokers and rate their health as good.

3.2. Predictors of Telomere Length

No significant association was found between telomere length and the relative confounders from the literature, such as smoking, alcohol consumption, PA, BMI, vitamin D containing medication usage, supplement, disease count and HbA1c% amongst all the participants. The only significant association was between telomere length and sex (95% CI = 0.000–0.001, $p < 0.001$). In addition, no significant association was found between telomere length and the confounders when the participants were stratified by sex, except for the BMI among the women (95% CI = 0.0034–0.044, $p = 0.039$).

Table 1. Participant characteristics by circulating 25(OH)D cut-offs in the Newcastle 85+ Study at baseline.

	Low (*n* = 193)	**Moderate (*n* = 302)**	**High (*n* = 283)**	**All (*n* = 778)**	***p***
Women % (*n*)	64.4 (123)	53.8 (162)	65.7 (186)	60.8 (471)	0.007
BMI					
Underweight % (*n*)	22.2 (39)	30.0 (86)	35.5 (91)	30.0 (216)	
Normal weight % (*n*)	43.8 (77)	46.0 (132)	41.0 (105)	43.7 (314)	0.015
Overweight % (*n*)	19.9 (35)	14.6 (42)	17.2 (44)	16.8 (121)	
Obese % (*n*)	14.2 (25)	14.2 (25)	6.3 (16)	9.5 (68)	
PA					
Low % (*n*)	28.6 (54)	15.4 (46)	24.8 (70)	22.1 (170)	0.001
Moderate % (*n*)	48.1 (91)	131 (44)	38.3 (108)	42.9 (330)	
High % (*n*)	23.3 (44)	40.6 (121)	36.9 (104)	35.0 (269)	
Alcohol drinkers					
Never % (*n*)	42.9 (81)	40.1 (120)	40.7 (113)	41.0 (314)	0.056
Moderate % (*n*)	30.2 (57)	39.5 (118)	41.0 (114)	37.7 (289)	
Heavy % (*n*)	11.6 (22)	10.7 (32)	9.4 (26)	10.4 (80)	
Smoking					
Never % (*n*)	36.6 (70)	33.3 (100)	36.2 (102)	35.2 (272)	0.447
Occasional % (*n*)	4.2 (8)	6.3 (19)	4.6 (13)	5.1 (40)	
Regular % (*n*)	59.2 (113)	60.3 (181)	59.2 (167)	59.6 (461)	
Vitamin D containing medication % (*n*)	0.0 (1)	6 (17)	38 (108)	16.5 (126)	<0.001
Supplement users % (*n*)	4.7 (9)	16.9 (51)	32.5 (92)	19.5 (152)	<0.001
Self-rated health					
Very good % (*n*)	37.7 (72)	40.5 (122)	41.7 (118)	40.3 (312)	0.006
Good % (*n*)	53.9 (103)	56.1 (169)	55.1 (156)	55.2 (428)	
Poor % (*n*)	6.3 (12)	2.1 (6)	2.1 (6)	3.0 (23)	
Disease count mean (SD)	4.9 (1.8)	4.7 (1.6)	4.8 (1.9)	4.8 (1.8)	0.675
Telomere length sample % (*n*)					
At baseline	44.7 (190)	42.3 (291)	42.3 (271)	42.9 (752)	0.678
At 18 months	32.2 (137)	32.6 (224)	33.7 (216)	32.9 (577)	
At 36 months	23.0 (98)	25.0 (172)	23.9 (153)	24.1 (423)	
Telomere length (kb) Median (IQR)					
At baseline	3827.1 (1641)	3721.0 (1094)	4009.5 (1021)	4034.6 (800.1)	0.006
At 18 months	3809.2 (236)	3811.8 (542)	3678.5 (487)	3785.2 (415.5)	
At 36 months	2702.1 (1184)	2718.9 (1142)	2781.3 (842)	2832.7 (741.2)	
HbA1c (%) mean (SD)	6.1 (1.1)	6.0 (0.7)	5.8 (0.6)	5.9 (0.7)	0.025

BMI: body mass index. HbA1c: glycated haemoglobin. Kb: kilo-base pair. *p*: *p*-value. Mann-Whitney U test for continuous non-normally distributed variables or $\chi 2$ test for categorical variables. 25(OH)D: <25 nmol/L (low), 25–50 nmol/L (moderate), >50 nmol/L (high).

3.3. Circulating 25(OH)D Concentration and Telomere Length among the Very-Old Adults at Baseline

A positive association was found between 25(OH)D and telomere length in very-old adults (See Figure 2 for the distribution of 25(OH)D concentrations by telomere length at baseline). Participants with 25(OH)D concentration >50 nmol/L had longer telomere length compared to those with concentration <50 nmol/L in the unadjusted model (95% CI = 17.8–109.9, B = 63.9 ± 23.4, $p = 0.007$), and even after adjusting for relevant confounders, such as smoking, alcohol consumption, BMI, physical activity and HbA1C (95% CI = 12.0–110.3, B = 61.29 ± 25.0, $p = 0.015$) (Table 2).

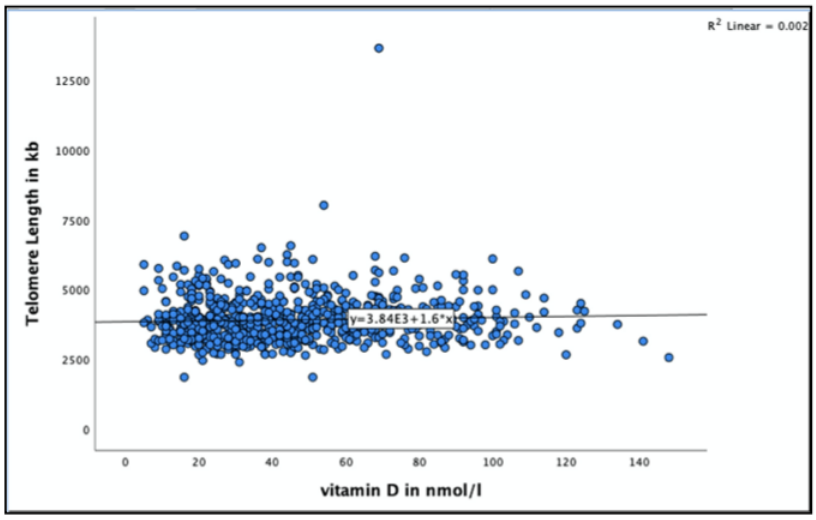

Figure 2. The association between 25(OH)D concentration and telomere length at baseline in the Newcastle 85+ Study.

Table 2. Association between 25(OH)D cut-offs and telomere length at baseline.

Model	25(OH)D	β Coefficient	Adj. R Square	95% CI	*p*
Model 1	Low	84.8	0.007	−67.7, 237.5	0.275
	Moderate	(ref)		(ref)	(ref)
	High	63.9		17.8, 109.9	0.007
Model 2	Low	89.8	0.007	−63.9, 243.6	0.252
	Moderate	(ref)		(ref)	(ref)
	High	67.8		21.6, 114.1	0.004
Model 3	Low	88.5	0.004	−76.0, 253.2	0.291
	Moderate	(ref)		(ref)	(ref)
	High	64.4		15.5, 113.2	0.010
Model 4	Low	77.2	0.004	−88.3, 242.8	0.360
	Moderate	(ref)		(ref)	(ref)
	High	61.2		12.0, 110.3	0.015

CI: confidence interval. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Ref: reference group. BMI: body mass index. HbA1c: glycated haemoglobin. Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c%.

3.4. Circulating 25(OH)D Concentration and Telomere Length by Sex

Since sex was the only predictor of telomere length that was found for the current participants, the participants were stratified using this factor. When the participants were stratified by sex (Table 3), the very-old men with concentration between <25 nmol/L were more likely to have shorter telomere length compared to those with concentration 25–50 nmol/L in the unadjusted model (95% CI = 1.9–473.4, B = 237.7 ± 119.7, $p = 0.048$) and even after adjusting for relevant confounders (95% CI = 14.9–521.6, B = 268.3 ± 128.6, $p = 0.038$).

Table 3. Association between 25(OH)D cut-offs and telomere length by sex at baseline.

Sex	Model	25(OH)D	β Coefficient	Adj. R Square	95% CI	*p*
Men (n = 304)	Model 1	Low	237.7	0.009	1.9, 473.4	0.048
		Moderate	(ref)		(ref)	(ref)
		High	56.6		−13.6, 126.9	0.114
	Model 2	Low	267.5	0.007	29.2, 505.7	0.028
		Moderate	(ref)		(ref)	(ref)
		High	63.4		−7.6, 134.6	0.080
	Model 3	Low	262.2	0.004	10.8, 513.5	0.041
		Moderate	(ref)		(ref)	(ref)
		High	68.4		−5.8, 142.7	0.071
	Model 4	Low	268.3	0.004	14.9, 521.6	0.038
		Moderate	(ref)		(ref)	(ref)
		High	71.4		−3.6, 146.4	0.062
Women (n = 471)	Model 1	Low	28.8	0.011	−172.1, 229.7	0.778
		Moderate	(ref)		(ref)	(ref)
		High	76.4		15.6, 137.3	0.014
	Model 2	Low	20.8	0.009	−181.2, 223	0.839
		Moderate	(ref)		(ref)	(ref)
		High	79.9		18.8, 141.0	0.010
	Model 3	Low	−5.8	0.010	−225.2, 213.6	0.958
		Moderate	(ref)		(ref)	(ref)
		High	72.4		6.8, 138.1	0.030
	Model 4	Low	−23.3	0.011	−243.0, 196.3	0.835
		Moderate	(ref)		(ref)	(ref)
		High	65.1		−0.7, 131.1	0.053

CI: confidence interval. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Ref: reference group. BMI: body mass index. HbA1c: glycated haemoglobin. Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c%.

In contrast, the very-old women with 25(OH)D concentration >50 nmol/L were more likely to have longer telomere length compared to those with concentration 25–50 nmol/L in the unadjusted model (95% CI = 15.6–137.3, B = 76.4 ± 30.9, p = 0.014). This association continued after further adjustments were made for smoking, alcohol consumption, BMI, and physical activity (95% CI = 6.8–138.1, B = 72.4 ± 33.3, p = 0.030) but it disappeared after adjusting for HbA1c (95% CI = −0.7–131.1, B = 65.1 ± 33.5, p = 0.053).

3.5. Circulating 25(OH)D Concentration and Telomere Length among the Very-Old Adults at 18 Months

A negative significant association was found between 25(OH)D concentration and telomere length at 18 months (See Figure 3 for the distribution of 25(OH)D concentrations by telomere length at 18 months). Very-old participants with 25(OH)D concentration >50 nmol/L were more likely to have shorter telomere length compared to those with concentration 25–50 nmol/L in the unadjusted model (95% CI = −55.9–−5.8, B = −30.9 ± 12.7, p = 0.016), and after adjusting for relevant confounders, such as smoking, alcohol consumption, BMI, physical activity and HbA1C HbA1C (95% CI = −59.9–−7.5, B = −33.7 ± 13.3, p = 0.012) (Table 4).

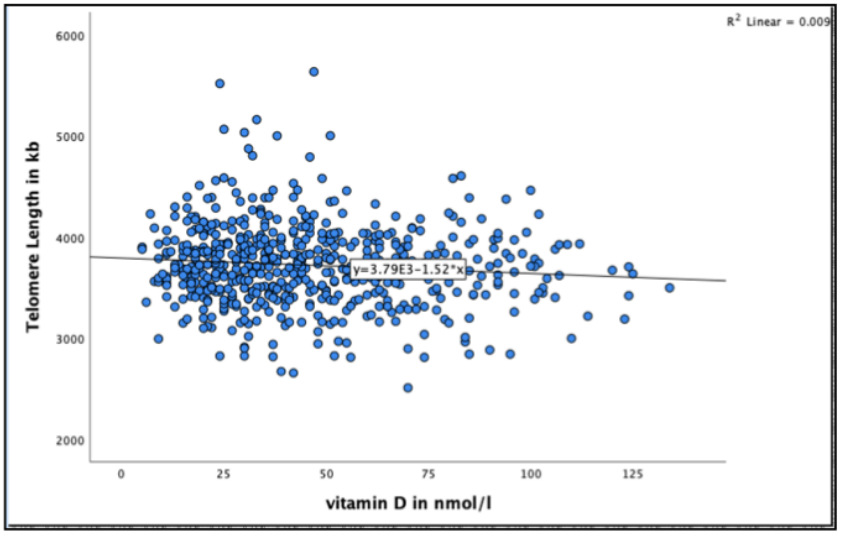

Figure 3. The association between 25(OH)D concentration and telomere length at 18 months in the Newcastle 85+ Study.

Table 4. Association between 25(OH)D cut-offs and telomere length at 18 months.

Model	25(OH)D	β Coefficient	Adj. R Square	95% CI	*p*
Model 1	Low	−1.1	0.012	−86.3, 84.1	0.979
	Moderate	(ref)		(ref)	(ref)
	High	−30.9		−55.9, −5.8	0.016
Model 2	Low	−2.2	0.022	−87.1, 82.7	0.959
	Moderate	(ref)		(ref)	(ref)
	High	−32.2		−57.2, −7.3	0.011
Model 3	Low	−8.5	0.022	−97.9, 80.8	0.851
	Moderate	(ref)		(ref)	(ref)
	High	−34.2		−60.0, −8.3	0.010
Model 4	Low	−8.1	0.020	−98.4, 82.0	0.859
	Moderate	(ref)		(ref)	(ref)
	High	−33.7		−59.9, −7.5	0.012

CI: confidence interval. BMI: body mass index. HbA1c: glycated haemoglobin. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c%.

3.6. Circulating 25(OH)D Concentration and Telomere Length by Sex at 18 Months

When the participants were stratified by sex (Table 5), a negative association was found between 25(OH)D and Telomere Length in the very-old men. Very-old men with concentration >50 nmol/L were more likely to have shorter telomere length compared to those with concentration <50 nmol/L in the unadjusted model (95% CI = −107.3–−25.5, B = −66.2 ± 20.8, p = 0.002) and even after adjusting for relevant confounders (95% CI = −107.0–−23.3, B = −65.2 ± 21.2, p = 0.002).

Table 5. Association between 25(OH)D cut-offs and telomere length by sex at 18 months.

Sex	Model	25(OH)D	β Coefficient	Adj. R Square	95% CI	*p*
Men (n = 304)	Model 1	Low	−3.9	0.041	−147.2, 139.3	0.957
		Moderate	(ref)		(ref)	(ref)
		High	−66.2		−107.3, −25.5	0.002
	Model 2	Low	−11.5	0.036	−156.8, 133.6	0.875
		Moderate	(ref)		(ref)	(ref)
		High	−69.0		−110.4, −27.7	0.001
	Model 3	Low	−16.7	0.037	−167.8, 134	0.827
		Moderate	(ref)		(ref)	(ref)
		High	−70.3		−112.3, −28.3	0.001
	Model 4	Low	−17.6	0.038	−167.6, 132.4	0.817
		Moderate	(ref)		(ref)	(ref)
		High	−65.2		−107.0, −23.3	0.002
Women (n = 471)	Model 1	Low	33.3	−0.004	−73.2, 139.8	0.539
		Moderate	(ref)		(ref)	(ref)
		High	−2.3		−34.0, 29.3	0.883
	Model 2	Low	38.2	0.011	−67.4, 143.8	0.477
		Moderate	(ref)		(ref)	(ref)
		High	−2.8		−34.4, 28.6	0.857
	Model 3	Low	21.8	0.004	−90.9, 134.6	0.704
		Moderate	(ref)		(ref)	(ref)
		High	−4.9		−38.2, 28.3	0.770
	Model 4	Low	24.7	0.001	−90.2, 139.6	0.672
		Moderate	(ref)		(ref)	(ref)
		High	−6.5		−40.6, 27.5	0.707

CI: confidence interval. BMI: body mass index. HbA1c: glycated haemoglobin. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L, 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c%.

However, no significant association was found between 25(OH)D and telomere length in the very-old women at 18 months in both unadjusted and adjusted models (see Table 5).

3.7. Circulating 25(OH)D Concentration and Telomere Length among the Very-Old Adults at 36 Months

No significant association was found between 25(OH)D concentration and telomere length after 36 months in the unadjusted model, or even after adjusting for relevant confounder smoking, alcohol consumption, BMI, physical activity and HbA1C% in all participants and in men and women separately (see Figure 4 and Tables 6 and 7)

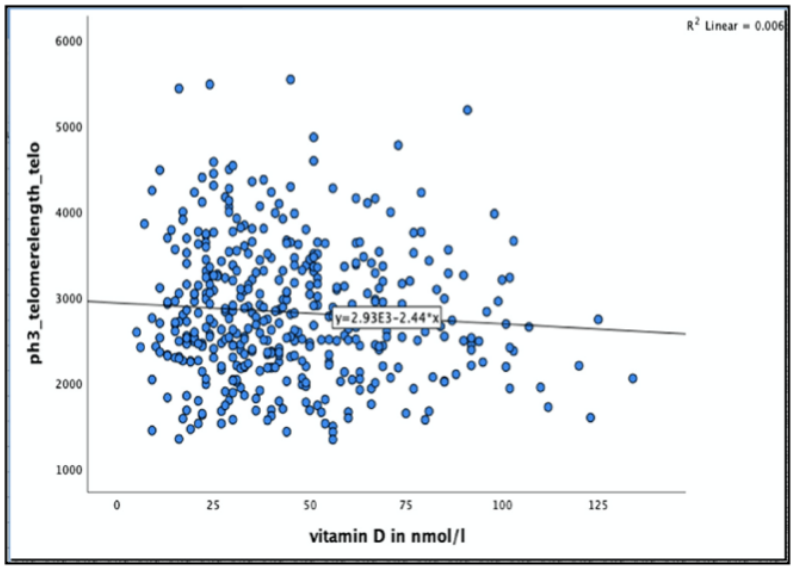

Figure 4. The association between 25(OH)D concentration and Telomere length at 36 months in the Newcastle 85+ Study.

Table 6. Association between different 25(OH)D cut-offs and Telomere length at 36 months.

Model	25(OH)D	β Coefficient	Adj. R Square	95% CI	*p*
Model 1	Low	−12.6	−0.002	−205.4–180.2	0.898
	Moderate	(ref)		(ref)	(ref)
	High	−28.6		-85.3–28.1	0.322
Model 2	Low	−18.4	−0.007	−213.3–176.4	0.853
	Moderate	(ref)		(ref)	(ref)
	High	−29.6		−86.8–27.6	0.310
Model 3	Low	−5.7	−0.003	−209.5–198.1	0.956
	Moderate	(ref)		(ref)	(ref)
	High	−38.1		−96.5–20.1	0.199
Model 4	Low	16.2	−0.004	−189.8–222.3	0.877
	Moderate	(ref)		(ref)	(ref)
	High	−36.8		−96.3–22.6	0.225

CI: confidence interval. BMI: body mass index. HbA1c: glycated haemoglobin. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c.

Table 7. Association between different 25(OH)D cut-offs and Telomere length by sex at 36 months.

Sex	Model	25(OH)D	B Coefficient	Adj. R Square	95% CI	*p*
Men (*n* = 304)	Model 1	Low	12.7	−0.013	−295.0–320.5	0.935
		Moderate	(ref)		(ref)	(ref)
		High	−1.1		−89.7–87.4	0.980
	Model 2	Low	−2.1	−0.025	−315.1–310.8	0.989
		Moderate	(ref)		(ref)	(ref)
		High	−4.7		−94.5–84.9	0.916
	Model 3	Low	−10.9	−0.018	−33.9–317.1	0.948
		Moderate	(ref)		(ref)	(ref)
		High	−11.9		−103.3–79.4	0.797
	Model 4	Low	−12.1	−0.015	−103.3–82.7	0.827
		Moderate	(ref)		(ref)	(ref)
		High	−10.2		−173.3–89.5	0.530
Women (*n* = 471)	Model 1	Low	−34.0	−0.002	−286.9–218.9	0.791
		Moderate	(ref)		(ref)	(ref)
		High	−45.1		−120.1–29.8	0.237
	Model 2	Low	−33.5	−0.010	−288.1–221.0	0.795
		Moderate	(ref)		(ref)	(ref)
		High	−44.5		−120.2–31.0	0.247
	Model 3	Low	−32.3	−0.009	−300.0–235.4	0.812
		Moderate	(ref)		(ref)	(ref)
		High	−53.3		−131.0–24.2	0.177
	Model 4	Low	−0.8	−0.008	−271.3–269.6	0.995
		Moderate	(ref)		(ref)	(ref)
		High	−50.5		−129.8–28.8	0.211

CI: confidence interval. BMI: body mass index. HbA1c: glycated haemoglobin. *p*: *p*-value. 25(OH)D cut-offs: <25 nmol/L (low), 25–50 nmol/L (moderate) (ref) and >50 nmol/L (high). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HbA1c.

4. Discussion

4.1. Main Findings

To our knowledge, this is the first study which has examined the prospective association between circulating 25(OH)D concentration and telomere length in very-old adults (≥85 years). Our results show that in fully adjusted models, whilst there was a significant positive association between 25(OH)D (>50 nmol/L) and telomere length at baseline, the direction of association was reversed after 18 months and absent at 36 months. However, it should be noted that the strength of the positive and negative associations between 25(OH)D concentration and telomere length at baseline and 18, respectively were weak [adjusted R square 0.004 at baseline and 0.020 at 18 months].

4.2. Evidence from Other Studies

Telomeres, the specific DNA protein structures, are found at both ends of each chromosome. Their function is to protect the genome from nucleolytic degradation, unnecessary recombination, repair, and interchromosomal fusion [1]. Each DNA replication causes telomere shortening, and when the telomere length reaches a critical limit, the cell undergoes senescence and/or apoptosis [2]. The rate attrition differs between individuals and tissues, and is influenced by multiple factors. Inflammation and oxidative stress are the key determinants of telomere length, and even though some of the factors that heighten oxidative stress and inflammation are genetic, others are environmental in nature, such as smoking, alcohol consumption, obesity and a sedentary lifestyle. Moreover, several dietary factors, such as high energy consumption and the intake of high sugar foods, are also highly associated with inflammation [4,26]. While these lifestyle habits may be difficult to change, vitamin D concentration was easily modifiable through nutritional supplementation or

sunlight exposure. Taking all of this into account, we sought to explore and study the association between 25(OH)D concentration and telomere length.

Our findings were in agreement with previous findings from two large studies on women by Richards et al. [10] and Liu et al. [11]] (n = 2160 and n = 4604 participants, respectively). Both studies found that a higher 25(OH)D concentration was associated with longer LTL [10,11]. Also, a recent study by Zarei et al. [8] found an interaction between vitamin D and telomerase with regards to their relationship with the survival among 404 CVD patients. On the other hand, the findings from a large community-dwelling study conducted by Liu and colleagues [13], failed to find any association between continuous 25(OH)D concentrations and longer LTL not only for their entire population (n = 1154) but also in the white (n = 503), black (n = 651), female (711), male (447) or race–sex subgroups. However, they found that concentrations of vitamin D $\geq$30 nmol/L were significantly associated with longer LTL in whites only [13].

In a prospective study of 59 African-American systemic lupus erythematosus patients and their counterpart control subjects shorter telomeres were seen among all subjects with a 25(OH)D concentration <50 nmol/L [16]. Interestingly, the patients who remained vitamin D deficient after three months of follow up (n = 29), tended to have shorter telomeres than those patients whose 25-hydroxyvitamin D levels were replete [16], suggestive of a protective role of 25(OH)D in maintaining telomere length. Moreover, a large community-dwelling study conducted by Mazidi and colleagues [7], examined the association between 25(OH)D concentration and telomere length across a broad age range (age: 18–80 years old). The participants were free of any history of diabetes, coronary heart disease, angina, myocardial infarction, stroke or congestive heart failure, in both men (n = 2319) and women (n = 2668) [7]. A positive association was demonstrated between 25(OH)D concentration and telomere length in the limited-adjusted models. Both studies highlighted the possible role of 25(OH)D concentration in the maintenance of telomere length [7,16].

Our study also demonstrated an association between 25(OH)D and telomere length in men, which is inconsistent with a cross-sectional study in white men (n = 2483), which failed to observe an association between any of the vitamin D biomarkers (25(OH)D and 1,25(OH)D) and LTL [12]. However, the participants in the study by Julin et al. [12] were younger than our participants (the mean age was 64.1 years old). Furthermore, they defined 25(OH)D concentration by four quartiles with higher cut-offs (<50 nmol/L was the lowest quartile) while the current study showed that a 25(OH)D concentration >50 nmol/L, was positively associated with telomere length at baseline.

Regarding the contribution of sex to the association between 25(OH)D concentration and telomere length, several biologically plausible explanations for a difference between men and women have been suggested, such as men, in general, having shorter telomeres than women [27]. In addition, estrogen can stimulate the production of telomerase and is a potent antioxidant and regulator of antioxidant genes [28]. Therefore, it should be noted that the differences in sex could contribute toward the association between vitamin D status and telomere length.

Regarding the negative association between 25(OH)D concentration and telomere length at 18 months, the plausible explanations could be that 25(OH)D concentration was only measured at baseline and not at follow up phases. Another explanation could be that concentration >50 nmol/L might not have protective effect on telomere length at very-old age. However, it should be considered that the model is not explaining much of the variation (Adj R2 considered very low). Besides, the 95% CIs were wide even when the relationship was positively significant at baseline indicates a less precise estimate of the relationship. That said, we could not ascertain the protective association of high concentration of 25(OH)D on telomere length in very-old adults in the current population.

There are several potential mechanisms that may explain the association between telomere length and 25(OH)D concentration. Generally, an activated form of vitamin D has autocrine and paracrine roles, including reducing telomere shortening through both anti-inflammatory and antiproliferative mechanisms. First, the active form of vitamin D

decreases the mediators of systemic inflammation, such as interleukin-2 and tumor necrosis factor [9]. Furthermore, vitamin D receptor is expressed in the T and B lymphocytes, natural killer cells, and monocytes, which promote the down-regulation of cytokines and other proinflammatory factors. Thus, it follows that vitamin D would attenuate the rate of telomere length attrition [7,29]. In addition, the retinoid x receptor (which is found widely distributed in cells and tissues and acts as the major contributor to vitamin D dependent transcription) may attenuate the relationship between vitamin D and telomere length has other roles in the cell that are independent of the vitamin D pathway. Therefore, the association between one common variant and a long telomere length does not necessarily imply a link between 25(OH)D and telomere length [12].

4.3. Strengths and Limitations

The study has several strengths, including its unique design, as well as the fact that the analysis is concentrated on a broadly representative age category of 85 years old; and that the statistical assumptions were met. Another key strength is that the study was adjusted for major potential confounders associated with telomere length (e.g., BMI, physical activity, smoking). It should also be noted however, that the findings reported here should be interpreted with caution due to the following limitations: firstly, its epidemiological design restricts any inference about causal relationships. Secondly, we did not include wider dietary factors as covariates in our models as we had no a priori knowledge from our dataset that these factors could associate with telomere length. As a result, unmeasured or uncontrolled factors may confound the findings, raising the risk of Type I error. Adding more confounders to the fully adjusted model, on the other hand, may have resulted in non-significant (bias) results and decreased power to detect significant relationships. Third, despite having longitudinal telomere length data spanning 36 months, serum 25(OH)D data was only collected at baseline.

5. Conclusions

Among the very-old in the Newcastle 85+ cohort study, 25(OH)D concentration was positively associated with telomere length at baseline. However, given the wide 95% CI and the conflicting directions of the associations at 18 months inclined to say that high concentration of 25(OH)D (>50 nmol/L) did not show protective effect on telomere length in very-old adults. In conclusion, high 25(OH)D concentration is positively associated with telomere length but does not have protective effect over time.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13124341/s1. Supplemental Box S1: List of diseases collected from GP medical records.

Author Contributions: S.H., T.R.H., T.A. and N.M. were responsible for conception and designed the manuscript; S.H. was responsible for statistical analyses; S.H. wrote the paper, and had primary responsibility for the final content of the manuscript; T.R.H., T.A., N.M., A.K., C.M.-R. and L.R. critically reviewed and revised the manuscript for scientific content, and approved the final version. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study conformed with requirements set in the Declaration of Helsinki. The Newcastle and North Tyneside Research Ethics Committee granted ethical approval (research project reference number: 06/Q0905/2).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: Data from the Newcastle 85+ Study is available through a formal application process to the study team. For details please visit https://research.ncl.ac.uk/85plus/datarequests/.

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Authors rebuttal Nutrients manuscript of Telomere Length

REVIEWER 1:

This is an interesting study conducted on a sample of 851 subjects from the cohort of the Newcastle 85+ Study. The authors aimed to examine the association between 25(OH)D concentration in plasma and telomere length in blood cells at baseline, 18 months, and 36 months, by controlling for related lifestyle factors. They showed that a high 25(OH)D concentration is positively associated with telomere length but does not have a protective effect over time.

Author Response

We believe that there are no specific comments for us to address from reviewer 1.

REVIEWER 2:

1. **Line 23:** It would be very helpful to explain the association between the 25(OH)D and the impact on telomere length as it is not clear.

Author Response

We have rephrased the sentence as follows to highlight the variability in outcomes from cross sectional studies and highlight the lack of data from prospective studies.

"*Whilst results from cross-sectional studies investigating the association between 25(OH)D concentration and telomere length have been mixed, there is a dearth of data from prospective studies which have assessed these associations*".

2. Line 25: You introduce the term very old : please explain why this term is used and why you are investigating persons over the age 85 years of age? Has the use of Very old been ratified within the literature and can you please quantify the term?

Author Response

We thank the reviewer for this important question. Terms like 'very old', 'oldest old' and 'super old' exist in the literature to describe people who have lived beyond life expectancy and whilst there is no official universally accepted definition of each term, it is relatively common (in the UK) to ascribe 'very old' to those people aged ≥ 85 years. Indeed, this description is used in research studies of those who reach 85 years and beyond (Hill et al. 2016).

*Hill TR, Mendonca N, Granic A, Siervo M, Jagger C, Seal CJ, Kerse N, Wham C, Adamson AJ, Mathers JC. What do we know about the nutritional status of the very old? Insights from three cohorts of advanced age from the UK and New Zealand. Proceedings of the Nutrition Society 2016, **75**(3), 420-430.*

In the UK, there are now more than 1·5 million very old people (2·5 % of total population) and the number is projected to rise to 3·3 million or 5 % over the next 20 years. This demographic represents the fastest growing segment of the UK population and many other European populations. Research studies involving very old people have been lacking until fairly recently and studies like the Newcastle 85+ Study (https://research.ncl.ac.uk/85plus/) were designed to address some of the knowledge gaps in health and wellbeing of this important age group.

In the revised introduction [last paragraph] text we have added the Hill et al. 2016 reference to the term ‘very old’ in support of its use and definition.

3. With a general reference of 25-80 ng/ml within in total serum for 25 Hydrocyvitamin D why do you believe you have seen the result and have you been able to quantify the levels that have given you the results indicated?

Author Response

We suspect that the reviewer means a general reference of 25-80 nmol/L (not ng/ml) for 25-hydroxyvitamin D as these were the units we used in our analysis?

Our decision to stratify 25(OH)D in to low (<25 nmol/L), moderate and high (>50 nmol/L) was based on UK public health guidance on what constitutes the risk of deficiency (i.e < 25 nmol/L) whilst other international advice, namely from the USA suggest 50 nmol/L to define nutritional adequacy. The baseline outcomes support a positive association between higher 25(OH)D (> 50 nmol/L) but this association was not seen in the prospective data, most likely because 25(OH)D was available in baseline samples only.

4. Line 40: What does They refer to please?

Author Response

The reference is to ‘telomeres’ so we have changed this wording in the text to make it clearer.

5. Line 44: Why is age a well-established factor, this is not clear as you may wish to explain this further.

Author Response

We agree with the reviewer that the influence of age on telomere length should be clearer. We have modified the relevant part of the text as follows:

"*Age is a well-established factor associated with telomere shortening (Beilfuss et al., 2017). In humans, telomere length decreases at a rate of 24.8–27.7 base pairs per year (Shammas, 2011). Whilst age associated attrition in telomeres is linked to many diseases of ageing and their complications, telomere attrition with aging has been demonstrated in numerous studies, independent of the presence of any age related diseases [3]. This supports the view that shortened telomere length, observed in chronologic ageing may serve as a biomarker of age independent of age-related diseases [3]*".

6. Line 48-50: Why is oxidative stress and chronic inflammation are considered to be the major drivers of telomere attrition? How does Oxidative stress impact and at what level would vitamin D impact change?

Author Response

We believe that we have addressed the first part of the reviewers comments related to telomere attrition and chronic inflammation/oxidative stress by including a figure on how telomere attrition may modulate tissue dysfunction and chronic disease as well as strengthening our arguments in the same paragraph on the role of ageing in these processes. (See Point 2 from Reviewer 3 below).

The question of what level of vitamin D would impact change of oxidative stress markers is a good one. Our interpretation of the (mainly epidemiological) literature is that the protective effect of 25OHD level depends on the definition of adequacy of 25OHD with studies using thresholds from >25 nmol/L up to 80 nmol/L.

7. Please note throughout the manuscript you have used two different reference style, will you please indicate which you wish to use, either author name or simple number referencing.

Author Response

We apologies for the inconsistent reference style in the manuscript and we have now used simple numbering throughout the revised manuscript.

8. Line 62 you say “they found”, please explain who they are?

Author Response

This sentence was merged with the preceding sentence as follows:

“*Liu et al. (2016) also found no association between absolute 25(OH)D concentrations and long LTL, for the overall population or the subgroups in the study (men, women, black and white separately) although maintaining a 25(OH)D concentration ≥30 nmol/l was significantly associated with longer LTL in white participants only”*.

Material and Methods

9. Why the number of participants, there is no G power to indicate assessment of a confidence level to accept the hypothesis and this needs to be addressed.

Author Response

The reviewer raises and excellent point. We have added a number of points (below and in the revised manuscript under section 2.4) related to sample sizes and power considerations based on an important review [Aviv et al 2006] highlighting several principles that should be considered in conducting epidemiological telomere research. However, these findings and recommendations apply to 60+ year old adults and are very unlikely to be applicable to 85+ year olds. Given the limited data on telomere attrition in those aged 85-88 years (the age range of the participants in this analysis), any power calculations are purely speculative.

Aviv et al, 2006. Human Telomere Biology: Pitfalls of moving from the laboratory to epidemiology

“*Aviv et al. 2006 proposes guidance for epidemiological research on the minimum numbers of subjects required for a given age range to determine whether the extrapolated telomere attrition rate of two groups are significantly different. For longitudinal studies assessing telomere length in older adults (> 60 years) over time, the sample size required in each test group being compared for 80% power and P<0.05 using a two sided t-test is 104”. However, given the limited data on telomere*

attrition in those aged 85-88 years (the age range of the participants in this analysis), any power calculations are purely speculative".

10. Line 98: Please explain why fasting overnight was necessary and how the assessment was made to indicate that you have the correct data to evaluate the outcomes.

Author Response

Whilst fasting per se does not appear to influence circulating 25(OH)D concentrations, the Newcastle 85+ study had a wider aim to assess a large panel of candidate biomarkers of ageing (total 74 biomarkers) many of which are best undertaken in a fasting blood sample (Carmen Martin-Ruiz et al. 2009- reference 17).

11. Line 100: Please explain "as quickly as possible" was there a set time frame to accept the blood samples, were the blood samples required to be spun down at the site of collection or was whole blood collected and sent to the laboratory?

Author Response

We have deleted "as quickly as possible" from the sentence to make it clearer and more succinct:

"*The majority of whole blood samples (95%) were received at the laboratory for processing within 1 hour of venepuncture*".

12. Line 125: You stated, "Diseases were recorded via a predetermined list of key diseases" please explain why these key diseases had been identified and what is the impact associated with these and the research being conducted?

Author Response

We defined the presence of common diseases in older age from health assessment data by standard cut-off points. Eighteen diseases were recorded on the participants General Practice Review Records hence these were included in the study. We knew *a priori* that disease status can impact telomere length and therefore included disease count as a covariate in the statistical models.

13. Line 142: You state" Vitamin D supplement use was divided into two categories: no supplement users and supplement users" With the Supplements, what were they and was the concentration of Vitamin D and how did this relate to the 25(OH) D identified within the blood samples?

Author Response

In the Newcastle 85+ study, only qualitative data on supplement use were collected. Information on supplement use was limited to type and brand so the frequency of supplement use had to be estimated on the basis of the manufacturer's recommendations. The information collected on use of supplements was limited to a simple yes or no consumption of one of four supplement types:

- **those taking fish and n-3 oil preparations**
- **single mineral/ vitamin preparations**
- **multivitamin and/or multimineral preparations**
- **other supplements.**

Cod liver oil and multivitamins were the most commonly consumed vitamin D supplements and these preparations typically contain 5 µg per tablet whereas a typical serving of liquid cod liver oil contains 10µg per serving. We have previously shown using multivariate ordinal regression models that non-use of vitamin D containing supplements predicted vitamin D deficiency in the Newcastle 85+ study participants (Hill et al. 2016- reference number 20), hence the rationale for including supplement use in the statistical analysis.

14. Figure 1: Can you please explain how the outliers were assessed and how did they impact your results?

Author Response

We used Tukey's method in SPSS to identify outliers and one sample was identified (with a very high telomere length > 12500 kb). However, removing this outlier did not change the relationship between 25(OH)D and telomere length. Therefore, we retained this sample in the final figure (Figure 1).

15. Line 273- 278: Please explain why this was the case at it is not clear in the discussion?

Author Response

In light of a similar comment from the third reviewer on this section, we have clarified the first paragraph of the discussion as follows:

"*To our knowledge, this is the first study which has examined the prospective association between circulating 25(OH)D concentration and telomere length in very-old adults (≥85 years). Our results show that in fully adjusted models, whilst there was a significant positive association between 25(OH)D (>50 nmol/l) and telomere length at baseline, the direction of association was reversed after 18 months and absent at 36 months. However, it should be noted that the strength of the positive and negative associations between 25(OH)D concentration and telomere length at baseline and 18, respectively were weak [adjusted R square 0.004 at baseline and 0.020 at 18 months]".*

16. Line 306-310: Why is this important in the essence of this discussion, please explain??

Author Response

We agree that these sentences need to be reworded so that they fit the essence of the discussion. The text has been amended as follows:

"***In a prospective study of 59 African-American systemic lupus erythematosus patients and their counterpart control subjects shorter telomeres were seen among all subjects with a 25(OH)D concentration <50 nmol/L [15]. Interestingly, the patients who remained vitamin D deficient after three months of follow up [n 29], tended to have shorter telomeres than those patients whose 25-hydroxyvitamin D levels were replete [15], suggestive of a protective role of 25(OH)D in maintaining telomere length***."

REVIEWER 3:

1. The abstract is very reasonable in answering the questions of what was done, what was found and concluded; however, the final statements about what was concluded (i.e. inconsistent relationship) is not necessarily giving a take-home message. Please elaborate/clarify.

   Author Response

   We agree with the reviewer that the conclusion could be clearer. Therefore we have revised our conclusion slightly as follows:

   "*Circulating 25(OH)D concentration shows a weak association with telomere length in very-old (85+ year old) adults*".

2. Lines 39-51 refers to one of the foundations of Ageing. Please clarify this by also referring to other key theories of ageing. I strongly recommend strengthening this paragraph by adding a schematic/figure to clarify the importance of telomere shortage.

   Author Response

   We thank the reviewer for this recommendation. As advised by the reviewer, we have included the figure below on the role of telomeres in tissue dysfunction/chronic disease in the revised introduction section. We have also clarified the impact of age in telomeres as recommended by Reviewer 2 (point 5) above which has significantly strengthened our arguments in this paragraph.

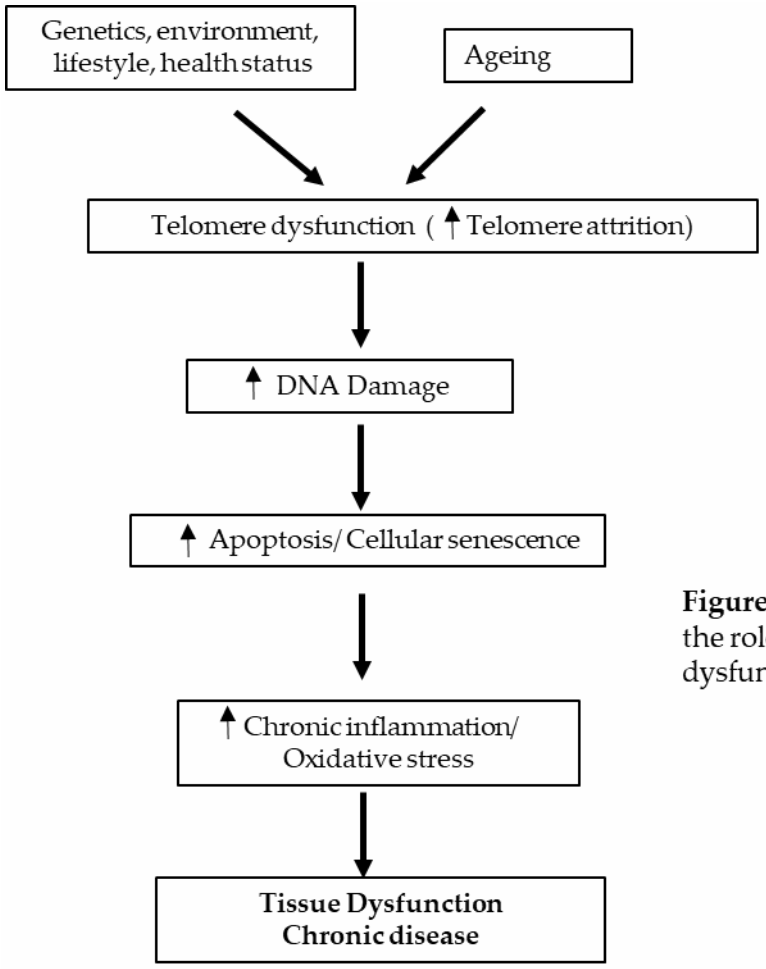

Figure 1: Schematic overview of the role of telomeres in tissue dysfunction and chronic disease

3. 52-3, important statement about the vitamin D and inflammation and telomere shortage, please elaborate and clarify as this is the basis and rationale of the study.

Author Response

We thank the reviewer for this comment and we have clarified the mechanistic basis for a role of vitamin D in telomere biology in the revised manuscript:

"*Vitamin D may influence telomere length through its biologically active hormone 1α,25 dihydroxyvitamin D3 (calcitriol). Calcitriol is a potent immunosuppressant and has strong anti-inflammatory and anti-proliferative properties mediated in part by its ability to reduce gene expression of inflammatory mediators interleukin-2 and interferon gamma [Ligi Paul REF]. The anti-inflammatory and anti-*

proliferative properties of calcitriol may reduce cell turnover, thus potentially reducing their telomere length attrition **[Paul, 2011]".**

4. Please consider referencing and in-text citation throughout as they seem to not follow the Nutrients specific authors and date.

Author Response

We apologise for the inconsistent reference style in the manuscript and we have now used simple numbering throughout.

5. Lines 52-66, before further progressing with vitamin D, please provide an introduction on the association between nutrition and telomere health including telomere regulation by nutrition, consumption of specific foods and nutrition, as these are important for further consideration of the relationship between vitamin D and telomere shortage.

Author Response

We thank the reviewer for this excellent suggestion. The following paragraph has been added to the relevant part of the introduction before the vitamin D aspects.

"Evidence from animal models as well as human studies have demonstrated that various nutrients (e.g. folate, niacin, vitamin C, magnesium, zinc and omega-3 fatty acids), bioactives (e.g. polyphenols) and whole foods (e.g. tea) may influence telomere length and telomere attrition through mechanisms related to cellular functions including DNA repair and chromosome maintenance, DNA methylation, inflammation, oxidative stress and telomerase activity [For review see Paul, 2010]"

6. Please provide an introduction to Newcastle 85+ study, to explain to establish why this study provides a good setting for investigation of the current associations.

Author Response

We thank the reviewer for this useful suggestion and we have added the following paragraph to the population sample section of the methods.

***"Commencing in 2006, the Newcastle 85+ Study recruited a birth cohort of more than one thousand 85 year olds from North East England to understand in great detail, the biological, clinical and social determinants of health as the cohort ages. The study aims to open up a wealth of knowledge teasing out the complex factors contributing to health in old age and aims to provide extensive data on the value of multiple molecular and cellular biomarkers in predicting and explaining individual differences in the health of very old people*".**

7. Lines 131-141, please clarify how nutritional status as an important correlate was assessed and taken into consideration for this study?

Author Response

We used BMI as a surrogate of nutritional status for this study as previous research has demonstrated a strong negative association between BMI and telomere length in a cross-sectional meta-analysis of 87 observational studies [Gielan et al. 2018- *American Journal of Clinical Nutrition*, Volume 108, Issue 3, September 2018].

We have modified slightly the relevant sentence in section 2.6 of the revised methods section as follows:.

"*Nutritional status was assessed by calculating body mass index (kg/m^2) (BMI) from recorded height and weight and included in the models owing to its influence on telomere length [Gielan et al. 2018*]".

8. Table 2 and 3, what does ref mean? please clarify.

Author Response

We have clarified in the table footnotes that "ref" denotes the reference group (moderate 25OHD concentrations) for the statistical analysis.

9. Lines 271-280, please reword the paragraph with shorter and clearer sentences.

Author Response

We thank the reviewer for this constructive suggestion. We have reworded the relevant paragraph in the revised manuscript.

10. Section 4.3, major improvement needed as there is no appreciation of a range of dietary and environmental factors that could be associated with telomere shortening. There are strong disease determinants of telomere shortening that have all been simplistically considered altogether, inadequate attention to environmental factors and oxidative stress, and an obvious major limitation regarding diet that needs to be fully considered in this section.

Author Response

We acknowledge these important limitations of our study and we have revised section 4.3 accordingly to account for these:

"The study has several strengths, including its unique design, as well as the fact that the analysis is concentrated on a broadly representative age category of 85 year olds; and that the statistical assumptions were met. Another key strength is that the study was adjusted for major potential confounders associated with telomere length

***(e.g. BMI, physical activity, smoking). It should also be noted however, that the findings reported here should be interpreted with caution due to the following limitations: firstly, its epidemiological design restricts any inference about causal relationships. Secondly, we did not include wider dietary factors as covariates in our models as we had no a priori knowledge from our dataset that these factors could associate with telomere length. Therefore, the findings may be confounded by unmeasured or uncontrolled factors increasing the chance of Type I error. On the other hand, adding more confounders to the fully adjusted model may have resulted in non-significant (bias) result and reduced power to detect significant associations. Thirdly, even though we had longitudinal data on telomere length over 36 months, serum 25(OH)D data was only measured at baseline*".**

J. Conferences presentation and Posters

Conference. location	Title	Date
NEPG conference, Newcastle Council	The Association between 25-Hydroxyvitamin D Concentration and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study	9th Nov 2018
HNRC Conference, Newcastle University	The Association between 25-Hydroxyvitamin D Concentration and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study	17th Oct 2018
NS Postgraduate Conference, Portrush	The relationship between 25-hydroxyvitamin D concentration and disability trajectories in very-old adults: The Newcastle 85+ Study	15th Feb 2019
Osteoporosis Conference 2020	The relationship between 25-hydroxyvitamin D concentration and disability trajectories in very-old adults: The Newcastle 85+ Study	September 2020
Nutrition Futures Live 2020, Online	The relationship between 25-hydroxyvitamin D concentration and Telomere Length in the very-old: The Newcastle 85+ Study	September 2020
Nutrition Futures Live 2020, Online	The relationship between 25-hydroxyvitamin D concentration and metabolic and cardiopulmonary health in the very-old: The Newcastle 85+ Study.	September 2020

The prospective relationship between 25(OH)D concentration and disability trajectories in very-old people

Abstract

Introduction: Very-old adults are more likely to develop disability and drop 25(OH)D concentration. Evidence confirmed the association between 25(OH)D concentration and muscusekletal health but limited research determine the potential adequate 25(OH)D concentration to slower the disability trajectories especially in the very-old.

Methodology: A 775 participants of the Newcastle 85+ Study, who have serum 25(OH)D available at baseline, were included. Concentration of 25(OH)D was consider as low and high using three different cut-offs. Disability was measured as difficulty to perform 17 activities of daily living at baselines and follow up phases. Group-based trajectory modelling was used to drive the trajectories. multinomial logistic regression was used to examine the relationship between 25(OH)D cut-offs and disability trajectories.

Results: Three disability trajectories model was derived (low, moderate and sever disability trajectories). Both IoM 3 cut-offs and SACN cut-offs show that participants with low concentration were more likely to have sever disability trajectories (OR: 0.49, 95%CI: 0.30-0.81, p=0.005) and (OR: 2.06, 95%CI: 1.32-3.21, p=0.001), respectively. After adjusted for sex, living in institute, supplement intake, and cognitive status, participants with low concentration were more likely to develop moderate and sever disability trajectories compared to low disability trajectory (OR: 1.62, 95%CI: 1.04-2.50, p=0.030 and OR: 2.67, 95%CI: 1.46-4.89, p=0.001) and (OR: 1.59, 95%CI: 1.06-2.39, p=0.023 and OR: 2.70, 95%CI: 1.60-4.55, p=0.001) for both IoM 3 cut-offs and SACN cut-offs, respectively. However, this relationship was disappeared after further adjustment for fat-free mass in the moderate disability but it continued in the sever disability (OR: 2.60, 95%CI: 1.38-4.90, p=0.003) and (OR: 2.68, 95%CI: 1.01-1.09, p=0.001) for both cut-offs, respectively.

Conclusion: An adequate concentration of 25(OH)D (>25 nmol/l and <50 nmol/l) could delay the onset and slower the progression of disability.

The relationship between 25-hydroxyvitamin D concentration and disability trajectories in very-old people: The Newcastle 85+ Study.

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Abstract

Introduction: We have previously reported that poor vitamin D is common in the very old (Hill et al., 2016) and that this poor vitamin D status has adverse consequences for muscle function (Granic et al., 2017). However, there is a dearth of data which has explored whether vitamin D status can delay the onset and slow down the trajectories of disability, especially in the very-old.

Aims: This study aims to explore the association between of 25-hydroxyvitamin D (25OHD) concentration and disability trajectories in the very-old. It also aims to define whether there is an 'adequate' 25(OH)D concentration which might protect against poorer disability trajectory.

Methodology: A total of 775 participants of the Newcastle 85+ Study, who had serum 25(OH)D available at baseline, were included. Concentration <25 nmol/L, 25-50 nmol/L and >50 nmol/L was used as a cut-offs to define vitamin D low, adequacy, and high, respectively. Disability was defined as difficulty performing 17 activities of daily living at baseline and after 18,36, and 60 months. Trajectories were derived by group-based trajectory modelling. The relationship between the 25(OH)D cut-offs and disability trajectories was examined by multinomial logistic regression.

Results: A three disability trajectories model was derived (low, moderate and severe). Participants with 25(OH)D concentrations lower than 25 nmol/l were more likely to have moderate and severe disability trajectories compared to those with adequate concentration (30-50 nmol/L), even after adjusting for sex, living in an institution, season, cognitive status, BMI and supplement use. However, this relationship disappeared after further adjusting for physical activity. High concentration (>50 nmol/l) was not found to be protective against a DT. Indeed, to the contrary, a high concentration was found to be related to the inception of disability.

Conclusion: Maintaining an adequate 25(OH)D concentration (between 25 nmol/l and 50 nmol/l) could delay the onset and slow the progression of disability.

Please include an outline of the work you will present.
(*This is not an abstract, rather a brief summary to help facilitate organizers when grouping oral communications into sessions).*

Very-old people are defined as those aged older than 80 or 85 years old, who are the fastest growing sector worldwide (Collerton et al., 2007). They are expected to have low concentrations of 25-hydroxyvitamin D [25(OH)D] for many reasons including the reduced ability of the skin to synthesis vitamin D by age (Gallagher, 2013). However, limited studies have evaluated 25(OH)D concentration among the very-0ld people and Since vitamin D plays an important role in the prevention and treatment of skeletal and non-skeletal diseases, such as cancer, cardiovascular disease, infections, and autoimmune disease (Bikle, 2014), it is necessary to evaluate this group's concentration of 25(OH)D. Therefore, this review aims to assess the distribution of serum 25OHD concentration and to consider the geographical and living conditions variation among very-old.

A total of 18 studies were included in the meta-analysis. The majority of the studies included both sexes, only one studies focused on men alone and two studies included women alone. Only two studies collected participants from community and institutionalized, four studies included participants from institutionalized only and the remaining studies included only community based participants. Of those who included community based participants, one study were from Asia, one from Australia, six from Europe, two from New Zealand, and four from the United States. Of those who included institutionalized participant, one study were from Asia and three from Europe. The two studies found from Asia were from Japan and China, no studies were found from other part of Asia, such as India and Middle East countries. No studies were found from Africa. Using the IoM cut-offs, participants from the community were reported to have an adequate concentration of 25OHD, especially those from the United States, New Zealand, and Austrila (Mean= 69.20nmol/l, 64nmol/l and 63.20 nmol/l, respectivly). Participants from Asia were reported the lowest 25OHD concentration but they still within the suffient rang (Mean = 37.20 nmol/l). However, it is only one study that included in the analysis. Male participants showed the highest concentration especially those from from the United States, New Zealand and Italy (Mean= 82nmol/, 80.50mol/l and 79.29nmol/l, respectivly.

A systematic review of observational studies reporting vitamin D status in very-old adults across the world

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Background: To date, the majority of studies assessing vitamin D status in older adults have focused on the 'young old' or those aged less than 80 years of age. As more people reach very old age it is imperative that vitamin D data and its determinants become available so that public health nutrition policy can devise age-appropriate guidance for this age group.

Objective: The study aimed to assess the 25(OH)D concentrations in very-old adults (i.e. those >80 years of age) across the globe, and to explore any associations with living status and geographical locations.

Method: Four databases (Medline, ProQuest, PubMed and Web of Science) were searched for community-dwelling and institutionalised participants aged 80 years-old and older with reported serum 25(OH)D from cross-sectional studies were included. The methodology of the systematic review is based on the published protocol on PROSPERO (ID: CRD42018117158).

Results: A total of 18 studies were included in this systematic review. Four studies were from the USA, nine from Europe, two from China and Japan, and three were from Australia and New Zealand. Among the European included studies, the range (and seasonal reporting) of 25(OH)D varied between the countries. The lowest concentration of 25(OH)D reported was 17.8 (16.6-19.1) nmol/l in Austria, while the highest concertation reported were 79.2 (74.4-84.0) nmol/l in Italy and 81.7 (52.1-111.3) nmol/l is Spain. Overall, the highest 25(OH)D concentration was reported in community-dwelling participants from the USA 82 (74.6-89.4) nmol/l which is located at a latitude of 42°N. The lowest concentration was reported in institutionalized from Austria at a latitude 48°N. Using the 25 nmol/l as threshold to define vitamin D deficiency (8 of the 18 studies), the highest prevalence of deficiency (33%) was found in Newcastle (UK), which is located at latitude 55°N, while the lowest prevalence of deficiency (2%) was found in New Zealand at latitude 38°S.

Discussion: Substantial proportions of very old adults across the globe have poor vitamin D status which is exacerbated by extremes of latitude and institutionalization.

Conclusion: 25(OH)D concentrations in very old adults vary significantly by latitude and living condition.

The relationship between 25-hydroxyvitamin D concentration and Telomere Length in the very-old: The Newcastle 85+ Study. By Sarah Hakeem[1,2]. [1]*Human Nutrition Research Centre, Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne NE2 4HH, UK.* [2]*College of Nursing, Umm Al-Quraa University, Makkah, Saudi Arabia.*

Telomeres, the specific DNA protein structures, are the cap at both ends of each chromosome to protect the genome from nucleolytic degradation, unnecessary recombination, repair, and interchromosomal fusion (Pusceddu et al., 2015) (Shammas, 2011). Vitamin D could positively maintain the Telomere Length, either directly or via its effects on mechanisms, including inflammation and/or the rate of cell proliferation (Mazidi et al., 2017). However, only a limited amount of studies have assessed the relationship between 25(OH)D concentration and Telomere Length. This study aimed to examine the relationship between 25(OH)D and Telomere Length in the very-old adults (>85 years-old).

The Newcastle 85+ Study is a socio-demographically representative study of the general UK population. It comprises both community-dwelling and institutionalized older adults if they aged 85 years-old at recruitment and living in Newcastle-upon-Tyne and North Tyneside. A cross-sectional analysis of 775 participants (with available 25(OH)D measurements) was conducted. Both health assessment and medical record data extraction were conducted by a trained research nurse (Collerton et al., 2009). A concentration of 25(OH)D <25 nmol/l was considered low and >25 nmol/l was considered sufficient. Telomere Length was measured as abundance of telomeric template vs. a single gene by quantitative real-time PCR with modifications. To examine the association between 25(OH)D with prospective Telomere Length, linear regression was used.

No significant association was found between 25(OH)D concentration and Telomere Length amongst all the participants or for men only in both the unadjusted model, and even after adjusted for relevant cofounders. However, 25(OH)D was positively associated with Telomere Length in women in the unadjusted model. This relationship also continued after adjustments were made for smoking, alcohol drink, BMI, PA and HAb1C%.

Association between different 25(OH)D cut-offs and Telomere Length by sex

Sex	Model	B coefficient	Adj. R Square	95% CI	*p*
Men (n= 304)	Model 1	-17.41	-0.003	-143.16-108.33	0.785
	Model 2	-20.26	-0.010	-147.02-106.49	0.753
	Model 3	4.58	-0.008	-131.03-140.40	0.946
	Model 4	5.79	-0.009	-131.65- 143.23	0.934
Women (n= 474)	Model 1	108.63	0.008	11.24-206.02	0.029
	Model 2	118.10	0.006	19.98-216.23	0.018
	Model 3	119.47	0.009	12.45-226.48	0.029
	Model 4	115.72	0.011	8.33-223.10	0.035

CI: confidence interval. p: p-value. 25(OH)D cut-offs: <25 nmol/l (low) (ref), 25-50 nmol/l (sufficient) and ≥50 nmol/l (optimal). Model 1 is the unadjusted model. Model 2 is further adjusted for smoking and alcohol. Model 3 is further adjusted for BMI and physical activity. Model 4 is further adjusted for HAb1c%.

Although the men did not show an association between 25(OH)D concentration and Telomere Length, maintaining adequate concentration of 25(OH)D (>25 nmol/l) could positively relate to Telomere Length only in women.

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The relationship between 25-hydroxyvitamin D concentration and metabolic and cardiopulmonary health in the *very-old*: The Newcastle 85+ Study. By Sarah Hakeem[1,2]. [1]*Human Nutrition Research Centre, Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne NE2 4HH, UK.* [2]*College of Nursing, Umm Al-Quraa University, Makkah, Saudi Arabia.*

Ageing is accompanied by multiple alterations and damage within the molecular pathways (Wagner et al., 2016), which results in an increased risk of developing chronic diseases. A positive association was found between 25(OH)D concentrations and cardiac and pulmonary diseases, which could explain by locally synthesized 25(OH)D in the heart and lungs, and its receptors, that work in an autocrine/paracrine manner in these tissues (Norman and Powell, 2014). However, no study has yet examined the effect of 25(OH)D on MCPD in very-old adults. Besides, it is unlikely that a single biomarker is able measure biological aging (Wagner et al., 2016). Therefore, this study aims to use metabolic markers [Glycated haemoglobin (HbA1c) (Kumar et al., 2018)], cardiac markers [N-terminal brain natriuretic peptide (NT-proBNP) (Passeri et al., 2016) and diastolic blood pressure)] and respiratory markers [Forced expiratory volume in one second (FEV1) and Forced vital capacity (FVC) (Finklea et al., 2011)] to examine the effect of 25(OH)D on heart and lungs.

A cross-sectional data of 775 participants (with available 25(OH)D measurements) were included from the Newcastle 85+ Study. The Newcastle 85+ Study is a socio-demographically representative study of the general UK population. It comprises both community-dwelling and institutionalized older adults. The participants were eligible to be included in the cohort study if they were born in 1921 and registered with one of the general practitioners in Newcastle upon Tyne. Both health assessment and medical record data extraction were conducted by a trained research nurse (Collerton et al., 2009). A concentration of 25(OH)D <25 nmol/l was considered low and ≥25 nmol/l was considered sufficient. To examine the association between 25(OH)D with baseline NT-proBNP, FEV1 and FVC, linear regression was used separately for each independent variable.

A linear regression showed an inverse relationship between 25(OH)D and NT-proBNP, even after adjusting for sex, BMI, smoking, alcohol and month. The analysis also showed that participants with a sufficient 25(OH)D concentration (<25 nmol/l) were more likely to have >400 pg/ml. However, our results did not prove the protective effect of 25(OH)D concentration >50 nmol/l. The analysis also showed a significant relationship between 25(OH)D and FEV1, FVC and HbA1c in both unadjusted and adjusted models. However, no relationship was found between 25(OH)D and diastolic.

The association between 25(OH)D concentration and biomarkers of MCPD

	B coefficient	Adj. R^2	p	95% CI
NT-proBNP	-0.11	0.021	0.012	-0.19- -0.02
HbA1c	0.068	0.051	0.055	-0.26- 0.01
FEV1	13.93	0.284	0.001	5.73- 22.31
FVC	17.96	0.383	0.001	7.68- 28.24
Diastolic	1.02	0.009	0.918	-1.90- 2.11

CI: confidence interval. p: p-value. 25(OH)D cut-offs: <25 nmol/l (low) (ref) and ≥25 nmol/l (sufficient). The model was adjusted for sex, BMI, smoking and alcohol and season.

In conclusion, sufficient 25(OH)D (>25 nmol/l) may improve the metabolic and cardiopulmonary health in very-old adults, as measured by NT-proBNP, FEV1, FVC and HbA1c.

Acknowledgments: The Newcastle 85+ Study has been funded by the Medical Research Council, Biotechnology and Biological Sciences Research Council, the Dunhill Medical Trust, Newcastle University, and the North of England Commissioning Support Unit (formerly NHS North of Tyne).

K. Gantt Chart