Vitamin D supplementation and vitamin D biomarkers in relation to sunlight exposure and musculoskeletal health in older adults using two different study designs

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Abstract

Vitamin D plays a role in musculoskeletal (MSK) health through genomic and non-genomic pathways. In the UK, SACN used a 25(OH)D concentration of 25 nmol/L as the basis for setting the (Recommended Nutrient Intake) RNI of 10 µg/day based on MSK outcomes (rickets, osteomalacia, falls, muscle strength and function). In setting vitamin D recommendations for North Americans, the IOM used a 25(OH)D concentration of 50 nmol/L as the basis for setting the (Recommended Dietary Allowances) RDA of 15 µg/day, based on the relationship between 25(OH)D and bone outcomes (rickets, osteomalacia, bone mineral density and calcium absorption). The differing thresholds used to define "vitamin D adequacy" may be due to (1) different MSK outcomes and approaches used to underpin the DRVs (2) geographical differences in vitamin D status and response to vitamin D supplementation and exposure to sunlight. Since vitamin D biomarkers and vitamin D supply required for optimum MSK function are not agreed universally, this thesis is set out to determine the effect of vitamin D supply on muscle function and to determine the association between commonly-used vitamin D biomarkers [25(OH)D and PTH] and muscle function [Grip Strength (GS), Timed-up and Go (TUG)] and bone health [quantitative ultrasound] in older adults using two different studies from the North East of England (55° North). In addition, because sunlight exposure is known to influence vitamin D biomarkers and response to vitamin D supplementation, this thesis also attempted to quantify the impact of sun exposure on vitamin D status in both studies. The first study was a Randomized Control Trial (RCT) of monthly vitamin D supplementation (12000 IU, 24000 IU and 48000 IU vitamin D_3) for 1 year on MSK health [Vitamin D in Older People study- VDOP (n=379; age >70 years)]. The second study was a follow-up study of older adults at moderate risk of colon cancer [the Biomarkers of Risk of Colon Cancer Follow-Up study - BFU (n=47, age >49 years)]. Baseline findings from the VDOP study showed that plasma 25(OH)D concentration, <25 nmol/L was associated with lower odds [OR 0.34, 95% CI, 0.166 - 0.691] of higher muscle function, compared with plasma 25(OH)D concentrations ≥ 25 nmol/L, after adjusting for the season of blood sampling and relevant confounders. Serum PTH was not associated with any MSK parameter at baseline. Vitamin D supplementation with 12000IU, 24000IU or 48000 IU monthly for one year increased 25(OH)D concentrations in a dose dependent manner, but had no effect on GS or TUG. Using a detailed sun exposure questionnaire to derive a composite sunshine exposure score, personal UV exposure failed to predict post-intervention 25(OH)D concentration in any of the vitamin D treatment groups. Findings from the BFU study showed that serum 25(OH)D concentration was not associated with GS, TUG or QUS in older adults. Serum PTH concentration was associated with QUS but not GS or TUG. Holiday visits was the only sun exposure variable which was associated with serum 25(OH)D concentration (p=0.042) at baseline in VDOP study. Sixteen participants were vitamin D deficient (25 – 50 nmol/L) at follow-up which increased from 7 participants at baseline over the 12 year follow-up period since the initial phase of data collection. In summary, low dose vitamin D supplementation improved vitamin D status in older adults such that vitamin D deficiency was prevented but it failed to improve muscle function. In line with SACN recommendations it may be prudent to maintain serum 25(OH)D > 25 nmol/L throughout the year to maintain healthy musculoskeletal functioning.

This PhD thesis is dedicated with love and affection to my loving parents, husband, son Saneth and daughter Dahamsa who contributed to the happiness and success of my life.

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List of Publications

Published articles

1. Ranathunga R, Hill TR, Mathers JC, Francis RM, Prentice A, Schoenmakers I and Aspray TJ (2019) 'No effect of monthly supplementation with 12000 IU, 24000 IU or 48000 IU vitamin D_3 for one year on muscle function: The vitamin D in older people study', *Journal of Steroid Biochemistry of Molecular Biology*, 190, pp 256 - 262.

2. Ranathunga RMTK, Hill TR, Aspray TJ (2017) "Can we evaluate the contribution of sunlight to 25(OH)D status?", *Osteoporosis Reviews*, Autumn Issue, 25 (4), pp. 16 – 20.

3. F.C. Malcomson, S.P. Breininger K. ElGendy, A. Joel, T. Ranathunga , T. Hill, D. Michael Bradburn1, D.M. Turnbull, L.C. Greaves J.C. Mathers, (2019) Design and Baseline Characteristics of the BORICC Follow-Up (BFU) Study: a 12+ years follow-up of the Biomarkers Of Risk of Colorectal Cancer Study. *Journal of Nutrition, Health and Aging*. 25(3), pp 231-238.

Conference abstracts

1. RMTK Ranathunga, FC Malcomson, TR Hill and JC Mathers (2018) Relationship between sun exposure behaviors and muscle function and bone strength of older adults in North East England. Nutrition Society Nutrition Futures Conference, 10 – 11th September, The Assembly rooms, Newcastle, UK. p. 286.

2. RMTK Ranathunga, TR Hill, JC Mathers, I Schoenmakers and TA Aspray (2018) "The association between circulating 25-hydroxyvitamin D concentration and Grip Strength and Timed-Up and Go test in 70+ year old adults: Baseline findings from the Vitamin D Supplementation in Older People (VDOP) study", Nutrition Society Summer Conference, 10-12th July, University of Leeds, UK, p 288.

3. RMTK Ranathunga, TR Hill, JC Mathers, I Schoenmakers and TA Aspray (2018) "No effect of monthly supplementation with 12000IU, 24000IU and 48000IU vitamin D3 for 1 year on muscle function: The vitamin D supplementation in older people (VDOP) study", 21st Workshop on Vitamin D, 16th - 19th May, Barcelona, Spain, p. 20.

4. RMTK Ranathunga, TR Hill, JC Mathers, I Schoenmakers and TA Aspray (2017) "Sunshine exposure and serum 25OHD concentrations during a 12 month randomized control trial with high dose vitamin D supplementation: results from the Vitamin D Supplementation in Older People (VDOP) study, Nutrition Society Summer Conference 2017 : Improving Nutrition in Metropolitan Areas, 10th - 12th July , King's College, London, UK.p 114.

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List of Abbreviations

A	
AE	Adverse Events
ALP	Alkaline Phosphate
AI	Adequate Intake
B	
BMI	Body Mass Index
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BFU	BORICC Follow-Up
BORICC	Biomarkers of Risk of Colon Cancer
BUA	Broadband Attenuation
BMR	Basal Metabolic Rate
C	
CaBP	Calcium Bonding Proteins
CaSR	Calcium Sensing Receptors
COMA	Committee on Medical Aspects
CARU	Clinical Aging Research Unit
CRF	Case Report Forms
D	
DBP	Vitamin D Binding Proteins
DEXA	Dual Energy X ray Absorptiometry
DEQAS	Vitamin D External Quality Assurance Scheme
DMEC	Data Monitoring and Ethics Committee
CTU	Clinical Trial Unit
CRC	Colorectal Cancer
E	
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Link Immunosorbent Assay
ECaC	Epithelial Calcium Channels
ESFA	European Food Safety Authority
F	
FM	Fat Mass
FFM	Fat Free Mass
FFQ	Food Frequency Questionnaire
G	
GP	General Practitioner
GFR	Glomerular Filtration Rate
GS	Grip Strength
GCP	Good Clinical Practice
GRP	Global Research Partnership
GI	Gastrointestinal Tract
H	
HC	Hip Circumference
HDL	High Density Lipoproteins
I	
IU	International Unit
IOM	Institute of Medicine
IGF	Insulin Growth Factor
IL-6	Interleukin – 6
IDM	Index of Multiple deprivation
L	
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
M	
MSK	Musculoskeletal
MED	Medium Erythema Dose
MRC	Medical Research Council
N	
NDNS	National Diet and Nutrition Survey
NOS	National Osteoporosis Society
NHS	National Health Services
NTGH	North Tyneside General Hospital

P

PTH	Parathyroid Hormone

Q

QOL	Quality of Life
QCT	Quantitative Computed Tomography
QUS	Quantitative Ultrasound

R

RCT	Randomized Control Trials
RDA	Reference Dietary Allowance
RNI	Recommended Nutrient Intake
RXR	Retinoid X receptor
REC	Research Ethics Committee
SI	Stiffness Index
SR	Sarcoplasmic Reticulum
RXR	Retinoid X receptor

S

SI	Stiffness Index
SACN	Scientific Advisory Committee on Nutrition
SED	Standard Erythemal Dose
SOS	Speed Of Sound
SPF	Sun Protection Factor
SHPT	Secondary Hyperparathyroidism
SOP	Standard Of Procedures
SR	Systematic Review

T

TUG	Timed-Up and Go test
TUIL	Tolerable Upper Intake Level
TBW	Total Body Water
TSC	Trial Steering Committee

U

UVB	Ultraviolet B Radiation
UVA	Ultraviolet A Radiation
UVI	Ultraviolet Index

V

VDOP	Vitamin D in Older People
VDR	Vitamin D Receptors
VDD	Vitamin D Deficiency

W

WC	Waist Circumference
WHO	World Health Organization

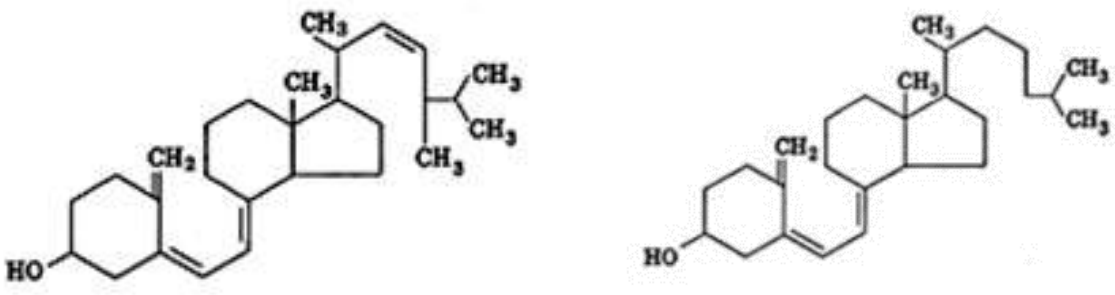
Other

25(OH)D	25-hydroxyvitamin D
$25(OH)_2D$	25 - hydroxycholecalciferol
$25(OH)_3D$	25-hydroxyvitamin D_3
7-DHC	7-dehydrocholesterol
USAE	Unexpected Serious Adverse Reactions

Chapter 1 Literature Review

1.1 Introduction

Vitamin D is classified as a secosteroid compound and it is a fat-soluble vitamin. The two major forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_2 ($C_{28}H_{44}O$) differs from vitamin D_3 ($C_{27}H_{44}O$) in the side chain attached to the secosteroid skeleton and it has an additional methyl group on the 24th carbon atom and a double bond between 22nd and 23rd carbon atoms (Norman, 2008) (Figure 1.1). Vitamin D_2 is produced by plants, while vitamin D_3 is synthesized in the skin upon Ultraviolet B radiation (UVB). Studies show that D_3 is the predominant source of vitamin D in most humans and, therefore, makes a bigger contribution to vitamin D status (SACN, 2016; and reviewed later in this chapter). Rickets, the bone disease caused by inadequate vitamin D, was first described in details by F. Glisson in 1650 (Wolf, 2004).



Vitamin D_2 ***Vitamin D_3***

Figure 1.1 : Chemical structures of vitamin D_2 and D_3 (Source : SACN, 2016)

The aims of this literature review section is to describe,

1. The physiological and nutritional aspects of vitamin D including the biomarkers of vitamin D status.
2. The determinants of vitamin D status including the role of sun exposure.
3. The evidence base for the role of vitamin D biomarkers (sun exposure and different metabolites of vitamin D) and vitamin D supplementation in musculoskeletal (MSK) health.

1.2 Vitamin D Synthesis and Metabolism

Vitamin D can be synthesized in the skin upon UVB (wavelength 290 – 320 nm) radiation. Exposure to sufficient sunlight is important for maintaining optimum vitamin D status, because there are few vitamin D rich foods (Holick, 2007) and for most people, these are eaten in insufficient amounts to meet vitamin D needs. Most of the UVB radiation from the sun is absorbed by the ozone layer and only 1% reaches the earth at noon time in the areas near the equator. The energy content of the UV rays and wave length of UV rays are inversely related. Thus, UVB has more energy compared with Ultraviolet A Radiation (UVA) (wave length 320 – 400 nm). Therefore UVB rays can penetrate more deeply into the skin than can UVA (Holick, 2016).

The first step of vitamin D synthesis takes place in the epidermis of the skin. First, UVB irradiation converts 7 – dehydrocholesterol (7-DHC) to pre-vitamin D_3. This previtamin D_3 is thermodynamically unstable and undergoes spontaneous isomerization into cholecalciferol in the plasma membrane of epidermal and dermal cells within 3 days (Holick *et al*, 1980). Then vitamin D_3 is transported bound to Vitamin D Binding Protein (DBP) in the circulation and hydroxylated to 25-hydroxyvitamin D (25(OH)D) in the liver. This hydroxylation process is catalyzed by cytochrome *P450 2R1* (encoded by the gene *CYP2R1*) (Henry, 2011) and the produced 25(OH)D circulates in the blood circulation bound to DBP. This 25(OH)D is taken up by the kidney by endocytosis via the endocytic receptor megalin in renal proximal tubular cells (Dusso, 2011) in preparation for the second hydroxylation step in which 25(OH)D is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). This $1,25(OH)_2D$ is considered as the active form of vitamin D which responsible for various function of vitamin D (Nykjaer *et al*., 2001). This 2^{nd} hydroxylation process is catalyzed by the mitochondrial *P450* enzyme 1α-hydroxylase (encoded by the gene *CYP27B1*) (Prentice *et al.*, 2008) (Figure 1.2). Production of $1,25(OH)_2D$ also occurs in other tissues including placenta, breast, colon, prostate, endothelial cells, pancreatic islets, parathyroid glands and macrophages for paracrine and autocrine functions in these tissues (Norman, 2008). The large majority of 25(OH)D and $1,25(OH)_2D$ (85 – 90%) in the blood is bound to DBP, most of the remainder is bound to albumin and less than 1% is free in the circulation (Bikle *et al*., 1986). The metabolic fate of 25(OH)D depends on the calcium status of the body. When more calcium is required, a greater proportion of 25(OH)D undergoes 1α-hydroxylation to produce $1,25(OH)_2D$ (Jones, 1998).

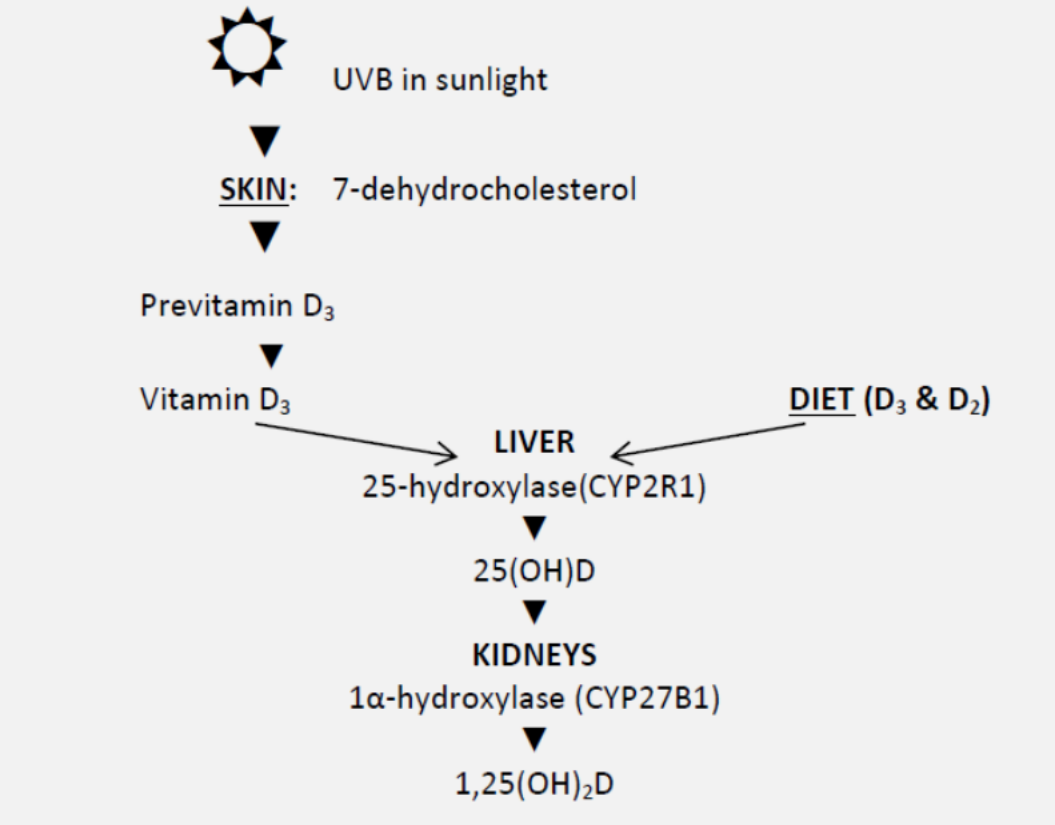


Figure 1.2 : Process of vitamin D synthesis in the skin (Source : SACN, 2016)

Vitamin D_2 and D_3 are absorbed by unsaturable passive diffusion process in the small intestine. However at high concentration of vitamin D in the intestinal lumen, vitamin D is absorbed by passive diffusion in jejunum and ileum (Reboul *et al*., 2011). Vitamin D_3 synthesized in the skin enters the extracellular fluid and then diffuses into dermal capillaries (Holick, 2011). Various factors such as the form of vitamin D (D_2 or D_3), dietary fiber content of the diet, amount of triglycerides in the diet, presence of other micronutrients (vitamin A, K and E), genetic factors, obesity, vitamin D status of the person and age affect the bioavailability of vitamin D (Maurya and Aggarwal, 2017) in the diet. After entering the circulation 25(OH)D is transported to the liver bound to DBP, where the active form of vitamin D ($1,25(OH)_2D$) is produced. Dietary vitamin D_2 and D_3 are transported in chylomicrons via the lymph and blood plasma to the liver. The vitamin D metabolites are transported in blood bound primarily to DBP and albumin. Approximately 85 – 90% of vitamin D metabolites are bound to DBP while 12 – 15% of vitamin D metabolites are bound to albumin (Bokle, 2017). Adipose tissue is considered as the major storage site for vitamin D. However, some evidence says that muscle also is a storage tissue for 25(OH)D (Girgis *et al*., 2014).

1.3 Regulation of 25(OH)D and 1,25(OH)$_2$D Production

Plasma 25(OH)D concentration is not subjected to feedback regulation, but appears to reflect vitamin D supply from cutaneous synthesis and the diet. Plasma 25(OH)D concentration depends on the amount of vitamin D delivered to the liver, the amount produced by the liver and its half-life in the plasma (Prentice *et al*., 2008). These are affected by a number of factors such as the amount of vitamin D entering the body, the amount of body fat and muscle mass, rate of 25(OH)D uptake and the rate of conversion to other metabolites (such as 1,25(OH)$_2$D, 24,25(OH)$_2$D). Other factors affecting plasma 25(OH)D concentration include the volume of extracellular fluid and DBP concentration (Bolland *et al*., 2007). The serum concentration of 25(OH)D has also been reported to decrease during the acute-phase response to inflammation (Silva and Furlanetto, 2015).

Synthesis of 1,25(OH)$_2$D in the kidney is tightly regulated. Up-regulation is through the action of parathyroid hormone (PTH) and down-regulation is through fibroblast growth factor 23 *(FGF23)* and direct negative feedback by 1,25(OH)$_2$D itself (Henry, 2011). Calcium-sensing proteins in the parathyroid gland stimulate PTH secretion in response to a fall in serum ionized calcium concentration. PTH stimulates the production of the *CYP27B1* enzyme in the proximal cells of the kidney (Bajwa *et al*., 2008) which increases the renal synthesis of 1,25(OH)$_2$D. Plasma 1,25(OH)$_2$D exerts a direct negative feedback by down-regulating the expression of the gene for *CYP27B1*, the enzyme required for its synthesis (Henry, 2011). It also exerts indirect negative feedback by reducing secretion of PTH. Additionally, 1,25(OH)$_2$D induces its own degradation by stimulating the production of the *CYP24A1* enzyme, a 24-hydroxylase that converts 1,25(OH)$_2$D and 25(OH)D to water soluble compounds which are excreted through bile (Holick, 2011). Wat *et al*., 2007 reported that an optimal level of 25(OH)D that suppress serum PTH concentration was 75 nmol/L. PTH began to rise when 25(OH)D levels were below 75 nmol/L whereas PTH levels did not change when 25(OH)D levels were above 75 nmol/L. Lu *et al*., 2012 showed that the levels of procollagen 1 N-terminal peptide (*P1NP*) and beta C-telopeptide of collagen (*β-CTX*) started to increase when serum 25(OH)D levels were less than 75 nmol/L (Lu *et al*., 2012).

1.4 Biological Functions of Vitamin D

Mechanisms of the biological function of vitamin D can be categorized as genomic and non-genomic action of 1,25 (OH)$_2$D (Ceglia and Harris, 2013). Mainly it is believed that vitamin D metabolites can affect muscle cell metabolism through gene transcription and variation in the Vitamin D Receptor (VDR) allele (Barr *et al*., 2010). VDRs are found in muscle tissues

and are involvedin the activation of muscle protein synthesis. Once VDR is transported to the nucleus by an intracellular binding protein called, calcitriol, it binds to the nuclear receptor which results in gene transcription for de novo protein synthesis. At the nuclear level, the activation of VDR induces hetero-dimerization between the active VDR and the retinoic receptor. This leads to the activation of the vitamin D response element, a complex of genes coding for the "Genomic effects" of vitamin D (Boland, 2011).

Nongenomic action is occurring via a nonnuclear or membrane-associated VDR. Nonnuclear VDR is thought to be associated with proteins which are stimulated by *IGF-1* and result in activation of mitogenaci-activated protein kinase (*MAPK*) and phosphoinositol-3 kinase (*PI3K)/Akt* cascades pathways that regulate cell differentiation and growth. Nongenomic mechanisms also mediate the actions of $1,25(OH)_2D$ on calcium influx and muscle contractility (Ceglia and Harris, 2013).

Vitamin D shows its genomic effects by activating the gene transcription via intranuclear VDR. It results in the synthesis of specific proteins that influence muscle calcium handling, phosphate transport across the cell membrane, and muscle cell differentiation and proliferation. Vitamin D regulates muscle calcium uptake by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma. Modifications in intracellular calcium level controls contraction and relaxation of the muscle. Further, $1,25(OH)_2D$ modulates intracellular calcium levels by stimulating the expression of a calcium-binding protein called calbindinD9K. One of these $1,25(OH)_2D$-dependent proteins is a calmodulin-binding component of the myoblast cytoskeleton. Calmodulin is a calcium binding protein (CaBP) that regulates several cellular processes including muscle contraction. Vitamin D appears to play a role in the regulation of phosphate metabolism in myoblasts as well. Exposure to $1,25(OH)_2D$ stimulates accelerated phosphate uptake and accumulation in cells. These effects may occur through the effects on the expression of *FGF-23*, which is a key hormone regulate phosphate homeostasis and effects on phosphate transporters. The study by Ceglia and Harris (2013) *et al*. found that administration of $1,25(OH)_2D$ to myoblasts up-regulated gene pathways responsible for myogenic differentiation including insulin growth factor 2 (*IGF-2*) expression (Figure 1.3) .

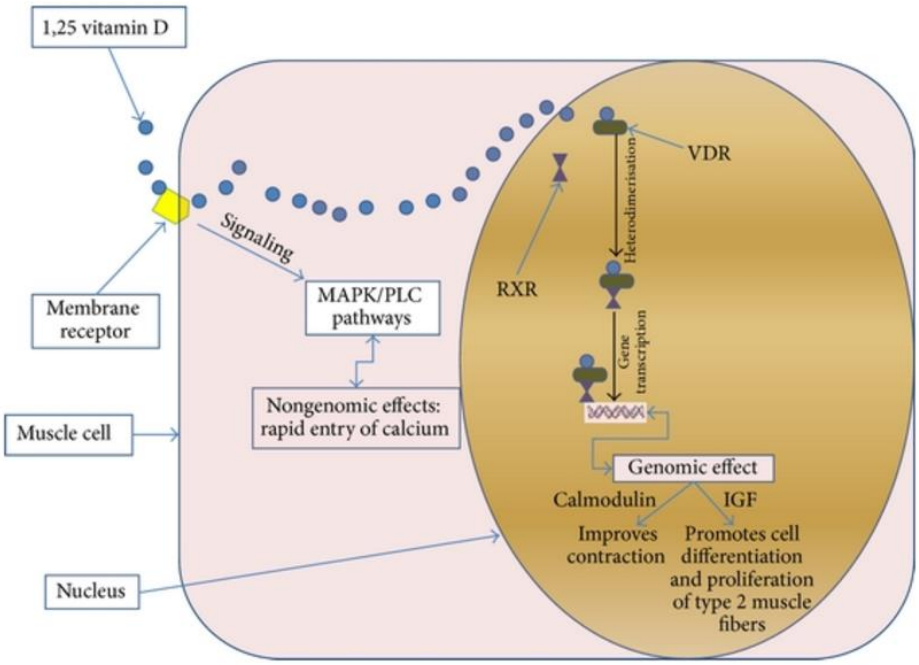


Figure 1.3 : Effect of 1,25(OH)$_2$D on muscle cells : Genomic and non genomic pathways (Source : Ceglia and Harris, 2013)

(MAPK : Mitogenaci Activated Protein Kinase, VDR : Vitamin D Receptors, RXR : Retinoid X receptors, IGF : Insulin Like Growth Factors)

1.5 Biomarkers of Vitamin D Status and Vitamin D Metabolites

1.5.1 Serum or plasma total 25(OH)D concentration and free 25(OH)D concentration

The plasma total 25(OH)D concentration which is the summation of 25(OH)D$_2$ and 25(OH)D$_3$ is a useful vitamin D biomarker. It is widely used to diagnose Vitamin D Deficiency (VDD) in both clinical and non-clinical settings. Serum/plasma 25(OH)D has a comparatively long half-life of 2-3 weeks and its concentration is not under tight homeostatic regulation. Therefore 25(OH)D concentration reflects the vitamin D supply and usage over a period of time (Ross *et al*., 2011). Serum 25(OH)D is commonly bound to DBP and albumin which can be converted into hormonally active 1,25(OH)$_2$D in the kidney, colon and several other tissues. Most circulating 25(OH)D is bound to either DBP (88%) or albumin (12%) and only a small fraction (less than 1%) circulates in a free form. The albumin-bound fraction of 25(OH)D plus free fraction are therefore called as the bioavailable fraction of 25(OH)D. Therefore total plasma/serum 25(OH)D include both free and bound forms of 25(OH)D. The total plasma concentration of 25(OH)D depends on many factors, including the amount of vitamin D entering the body from the skin and diet, the amount of body fat and muscle, the activity of 25-hydroxylase, DBP production in the liver, the volume of the extracellular compartment, the expression and affinity of VDR in target tissue, the efficiency of cellular uptake of 25(OH)D and its rate of conversion to 1,25(OH)$_2$D or 24,25(OH)$_2$D, the amount of

25(OH)D produced by the liver and the half-life of 25(OH)D in the plasma (Prentice *et al.,* 2008).

1.5.2 Total serum/plasma 1,25(OH)$_2$D and free 1,25(OH)$_2$D concentrations

Serum/plasma 1,25(OH)$_2$D is the active form of vitamin D which is responsible for all biological functions in the body and it is the summation of both 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$. The majority of 25(OH)D and 1,25(OH)$_2$D is primarily bound to DBP and approximately 10–15% of 25(OH)D and 1,25(OH)$_2$D to albumin. Some of the researchers use 1,25(OH)$_2$D as one of the functional biomarkers of vitamin D under normal physiological conditions. In contrast, 1,25(OH)$_2$D is not considered as a valuable nutritional biomarker for vitamin D status as circulating 1,25(OH)$_2$D concentration is under homoeostatic control and it has a short half-life of a few hours (Ross *et al*., 2011). In addition, 1,25(OH)$_2$D concentration is not considered as an ideal biomarker of vitamin D, as it is not an accurate predictor of the cellular 1,25(OH)$_2$D concentration in all target cells. The free fraction of 1,25(OH)$_2$D in plasma may be an index of the amount available for cellular uptake. The half-life of 1,25(OH)$_2$D in plasma provides information about the balance between supply and metabolism at the tissue level (Prentice *et al*., 2012)

1.5.3 Plasma PTH concentration

PTH has been proposed as a functional marker of vitamin D and it is considered as elevated plasma PTH concentration is a risk factor for osteoporosis. PTH secretion is stimulated when plasma ionized calcium decreases as the result of low dietary calcium intake or poor calcium absorption. High dietary phosphorus intake and high plasma phosphate concentration can also induce PTH secretion. An inverse relation between plasma concentrations of PTH and 25(OH)D has been reported (Bates *et al.,* 2003). However, the plasma PTH concentration varies widely within and among individuals because the plasma PTH concentration depends on many factors other than vitamin D status, such as stage of life, ethnic background, dietary calcium and phosphorus intakes, time of day, kidney function, physical inactivity, drug use and the laboratory method used to analyse the PTH concentration (Prentice *et al.,* 2008).

1.5.4 Serum / plasma 24,25(OH)$_2$D concentration

In addition to the 1,25(OH)$_2$D, in the kidney, 25(OH)D is further hydroxylated to 24,25 dihydroxyvitamin D. Production of 24,25(OH)$_2$D is usually the first step in the metabolic pathway to inactivate 25(OH)D, which prevents vitamin D intoxication (Norman, 2008). Plasma concentration of 24,25(OH)$_2$D is directly related to 25(OH)D concentration. Its concentration is about 50-fold higher than that of 1,25(OH)$_2$D.The ratio of serum 24,25(OH)$_2$D to 25(OH)D concentration has also been suggested as an indicator of VDD

(Cashman *et al*., 2015). The role of $24,25(OH)_2D$ is not very clear. However, some researchers suggest that $24,25(OH)_2D$ plays a role in calcium and phosphorus homeostasis in cartilage and bone (Edouard *et al*., 2012).

1.6 Dietary Sources of Vitamin D

Only a small proportion of commonly consumed foods are significant sources of vitamin D (Holick 2004). Oily fish such as salmon, tuna and mackerel, meat, egg yolk, sundried mushrooms and fortified foods are the most common vitamin D rich foods (Food Standard Agency, 2002). Seamans & Cashman, 2009, reported that for each additional 1 µg (40 IU) of vitamin D consumed, serum 25(OH)D concentrations increased approximately by 0.58 nmol/L. In addition, a recent meta-analysis showed that vitamin D_3 is more effective in raising serum 25(OH)D concentrations than vitamin D_2 (Tripkovic *et al.*, 2012). Since most foods are poor sources of vitamin D (see Table 1.1), it is difficult to obtain large amounts of vitamin D from the diet (O'Mahony *et al*., 2011).

Table 1:1 Vitamin D content of dietary sources of vitamin D

Foods	**Mean vitamin D content (Per 100g or 100mL)**	
	µg	**IU**
Herring (grilled)	16.1	644
Salmon (formed, grilled)	7.8	312
Mackerel	8.5	340
Sardine (grilled)	5.1	204
Tuna (cooked)	3.1	124
Eggs (whole, boiled)	3.2	128
Fortified bran flakes	4.2	168
Fortified cornflakes	4.2	168
Fortified rice cereals	4.2	168
Fat spread	7.5	300
Milk (per 100 mL)	1.0	40
Yogurt	1.2	48
Liver (lamb, fried)	0.9	36
Beef	0.7	28

(Source: McCance and Widdowson's Food Composition Tables, 2019)

1.7 Nutritional Requirements for Vitamin D and Scientific Basis

Using the risk-assessment framework commonly used to set Upper Levels for nutrients, the Institute of Medicine (IOM) in their recent Dietary Reference Intake (DRI) report (Ross *et al.,* 2010) set a 25(OH)D concentration of 30 nmol/L as indicative of vitamin D deficiency based on integrating a number of key bone health outcomes, including rickets, osteomalacia, impaired calcium absorption and lower BMD. The nature of the relationship between 25(OH)D concentration and bone health outcomes will be discussed in detail later in this review. It is noteworthy that the IOM committee concluded that there was insufficient evidence to define vitamin D deficiency based on non-skeletal outcomes. Based on the relationship between 25(OH)D status and those aforementioned bone health outcomes, and using both data from epidemiological and intervention studies, the IOM established a population 25(OH)D concentration of 40 nmol/L and 50 nmol/L as the basis for setting an Estimated Average Requirement (EAR) of 10 µg/day and a Recommended Daily Allowance (RDA) of 15 µg/day, respectively in people aged 1-70 years. The IOM set an RDA of 20 µg/day for individuals aged >70 years, while it could only establish an Adequate Intake (AI) of 5µg/day for infants < 1 year (Ross *et al.,* 2010). The EAR is the amount of a nutrient which meets the needs of half (50%) the population while the RDA is the amount of a nutrient which will meet the needs of practically all (97.5%) healthy persons in a population. The AI is an estimation of the observed dietary intake of an asymptomatic population. The approach and conclusions of the recent IOM report (Ross *et al.,* 2010) was a significant deviation from those of the previous IOM DRI report of 1997 (Institute of medicine, 1997) in that for the first time an EAR and RDA was established for children and adults. In the past only an AI of 5 µg/day could be derived for persons up to 70 years (Institute of Medicine, 1997). Two of the caveats of the IOM report are that the vitamin D recommendations (1) assume an adequate dietary calcium intake and (2) assume a negligible contribution from sunlight to 25(OH)D concentrations. It is also noteworthy that in terms of adverse effects, the Tolerable Upper Intake Level (UL) for vitamin D which is the highest level of daily consumption that current data have shown to cause no side effects is 100 µg/day (Ross *et al.,* 2010) while in the older DRI report (Institute of Medicine, 1997) it was set at 50 µg/day. In 1998, the UK Committee on Medical Aspects of Food and Nutrition Policy (COMA) concluded that a prudent public health approach to safeguard against vitamin D deficiency and its adverse effect on bone health would be to retain the Reference Nutrient Intake set in 1991 (10 µg/day for those aged >65 years).

The Scientific Advisory Committee for Nutrition (SACN) in the UK re-evaluated nutritional requirements for vitamin D for the British population in 2016 (SACN, 2016). The findings of the report suggests a Reference Nutrient Intake (RNI) of 10 µg/d (400 IU/d), throughout the year, for everyone in the general UK population aged 4y and above. The approach used in deriving the new RNIs involved determining the dietary input of vitamin D required to keep the circulating 25(OH)D above 25 nmol/L (the population protective cut-off to protect musculoskeletal health) (SACN, 2016). The RNI of 10 µg/d (400 IU/d) for the general UK population includes pregnant and lactating women and population groups at increased risk of VDD. Since, there were insufficient data to set RNIs for children aged under 4y, Safe Intakes were recommended for this age group (8.5-10 µg/340-400 IU per day for all infants aged under 1y and 10 µg/400 IU per day for ages 1 up to 4y). SACN were unable to quantify and take account of sunlight exposure in setting the DRVs because of the number of factors that affect endogenous vitamin D synthesis. The EFSA and the Endocrine Society also set targets for vitamin D intake and these together with the IOM and SACN recommendations are tabulated in Table 1.2.

Table : 1.2 : Different criteria of defining vitamin D adequacy in adults

Organizations which set the cut-off value	Target population	25(OH)D cut-off (nmol/L)	Basis for the cut-off	Reference
IOM	North Americans	>50 : sufficient 30 - 50 : insufficient < 30 : deficient	Establishing RDA of 15 µg/day for vitamin D based on bone outcomes (Presence of rickets and osteomalacia, BMD and calcium absorption)	Ross *et al*., 2011
SACN	UK population	> 25 : adequate	Basis for establishing RNI of 10 µg/day based on MSK outcomes (Presence of rickets, osteomalacia, falls, muscle strength and function)	SACN, 2016
EFSA	Europeans	>50	AI of 15 µg/day	EFSA *et al.*, 2016
Endocrine Society	Patients who are at risk of VDD	>50	Patient management	Holick *et al.*, 2011

Some previous studies have suggested the required serum 25(OH)D concentration for different health outcomes. Heaney and Holick, 2011 suggested that serum concentration of 25(OH)D higher than 70 – 80 nmol/L is the optimal level for children, adolescents, adults for the both skeletal and non-skeletal outcomes. According to a systematic review, serum 25(OH)D concentrations between 22.5 – 94 nmol/L are associated with higher BMD in all life stages. Therefore they concluded that serum 25(OH)D concentrations between 90 and 100 nmol/L are desirable for the prevention of hip and any non-vertebral fractures in older adults. In the same systematic review they reported that for lower-extremity strength, serum 25(OH)D concentrations >40 nmol/L is desirable, but a concentration between 90 to 100 nmol/L would optimal (Bischoff-Ferrari *et al.*, 2006).

Vitamin D intake is expressed in International Units (IU) or in micrograms (μg). One IU of vitamin D is defined by the World Health Organization (WHO) as the activity produced by 0.025 μg of crystalline vitamin D_3 (1 μg = 40 IU). According to the past studies of older persons, 25(OH)D concentrations can be increased by 10–40 nmol/L with an intake of 400 IU vitamin D/day (Heaney *et al*., 2003) by 31 nmol/L with the intake of 600 IU/day (Vieth *et al*., 2004), and by 50–65 nmol/L, with the intake of 800 IU vitamin D/day (Dawson-Hughes *et al*., 1997) Mean concentrations of 75 to 100 nmol/L can be achieved with the intake of 700 to 1000 IU/day among young and older adults. In young men and women, 4000 IU vitamin D/day (100 μg/day) may increase 25(OH)D concentrations by 56 nmol/L (Bischoff-Ferrari *et al.,* 2006). However, it is believed that the amount required for children is 8.5 – 10 μg/day (SACN, 2015). IOM suggests that individuals over 70 years should have 800 IU/day of dietary intake of vitamin D (Ross *et al.,* 2011) which is equivalent to 20 μg/day. Recommended Nutrient Intake (RNI) values of vitamin D, according to the SACN, for different life stages are listed in Table 1.3.

Table 1.3: Vitamin D requirement for different life stages according to the SACN recommendations

Life stages	UK RNI values (μg)
0 – 6 months infants*	8.5
7 – months – 3 years*	7.5
4+ years**	10
Pregnant women	10
Lactating women	10

** Safe Intake* *Source: SACN, 2016*
*** Includes all at risk groups in the population*

1.8 Dietary Vitamin D Intake of Older Adults

Though SACN recommends to have 10 μg/day of dietary vitamin D, most research shows that the consumption of dietary vitamin D is below this level among older adults. A RCT of vitamin D supplementation conducted in Cork, which found the dose related increase in serum 25(OH)D in older adult reported that average habitual intake of vitamin D was 4·4 μg/day among elderly subjects. They further stated that the vitamin D intake that maintained serum 25(OH)D concentrations >25 nmol/L in 97.5% of the population was 8.6 μg/day (Cashman *et al.,* 2009). In 2014, National Adult Nutrition Survey (NANS) in Ireland predicted that the median intake of vitamin D_2 ranged from 1.7 to 2.3 μg/day on serum 25(OH)D concentrations (Cashman *et al*., 2014). According to a study conducted in Newcastle, UK median dietary vitamin D intake of older adults aged above 85 years was 2.9 μg/day (Granic *et al*., 2017). Among Korean adults aged above than 20 years the mean vitamin D intake was 4.0 ± 0.17 μg/day and 2.6 ± 0.1 μg/day for men and women, respectively (Touvier *et al.,* 2015). The National Diet and Nutrition Survey, UK in 2016-17, reported that vitamin D intake in all age groups, for both genders and for all income groups was below the RNI for vitamin D in the UK (NDNS, 2019).

1.9 Deficiency Symptoms of Vitamin D

Rickets and Osteomalaca are the main deficiency disease of VDD. Rickets is the VDD disease in children, while Osteomalacia is the deficiency disease in adults. Serum 25(OH)D concentration < 25 nmol/L, is usually defined as VDD rickets among children (SACN, 2015). In rickets , bone formation rate is accelerated and mineralization of growth plates get reduced (Parfitt, 2003). This leads to inappropriate shape and low bone strength which lead to low BMD and weak weight bearing bones (Bishop, 1999). Metaphyseal bone sites are naturally

extended in patients with rickets, therefore they have difficulty in walking (Agarwal *et al*., 2009), suffer comorbidities such as respiratory infections (Karatekin *et al*., 2009) and muscle weakness (Cangussu *et al*., 2015). Osteomalacia is the adult form of rickets, which results from a chronic VDD affecting both bones and muscles. Increased risk of fractures, muscle or bone pain, and advanced deformities in bones are some of the common symptoms of Osteomalacia (Narchi *et al*., 2001).

1.10 Vitamin D Status in the UK and European Population

In the past few years, prevalence of VDD has increased in nonequatorial locations in the world (Cashman *et al*., 2016). A recent study conducted using a representative sample of the European population found that 13.0% of individuals had serum 25(OH)D concentrations <30 nmol/L on average in a year. Among them, 17.7% and 8.3% of people had serum 25(OH)D concentration below 30 nmol/L during the winter and summer, respectively. In the same study, the prevalence of VDD was 40.4% according to the cut-off of < 50 nmol/L (Cashman *et al*., 2016). Another recent study conducted in Mexico recruiting older adults revealed that, 37.3% of them were vitamin D deficient and majority were women (Contreras-Manzano *et al*., 2017). Another survey conducted recruiting Irish adults showed that the year-round prevalence rates for serum 25(OH)D concentration < 30, < 40, < 50 and < 75 nmol/L were 6.7, 21.9, 40.1 and 75.6 %, respectively (Cahman *et al*., 2013). During winter, the prevalence for the same serum 25(OH)D concentrations were 11.1, 31.1, 55.0 and 84.0 %, respectively. A cohort study conducted in England recruiting 711 788 children aged 0-17 years found that the crude rate of VDD increased from 3.14 per 100 000 person-years to 261 per 100000 person-years during the period of 14 years (from 2000 – 2014) (Basatemur *et al*., 2017).

According to the National Diet and Nutrition Survey, 2017 – 19, UK, during January-March, 29% of adults aged 19 to 64 years (28% of women, 30% of men) and 27% of adults aged 65 years and over (24% of women, 32% of men) had a serum 25(OH)D concentration below 25 nmol/L. During April-June this prevalence decreased to 19% for adults aged 19 to 64 years (16% of women, 22% of men) and 9% for adults aged 65 years and over (9% of women, 9% of men). In contrast, during July-September, 4% of adults aged 19 to 64 years (5% of women, 4% of men) and 4% of adults aged 65 years and over (6% of women, 1% of men) had a 25(OH)D concentration below 25(OH)D nmol/L. The percentages were increased in October-December showing 15% of adults aged 19 to 64 years (10% of women, 20% of men) and 15% of adults aged 65 years and over (15% of women, 14% of men) had a serum 25(OH)D concentration below 25 nmol/L (NDNS, 2019).

1.11 Laboratory Assessment of Vitamin D Metabolites and PTH

Several methodologies have been developed to measure vitamin D metabolites in plasma or serum samples such as high Performance Liquid Chromatography (HPLC), Radioimmunoassay (RIA), Automated Immunoassays, Enzyme-linked Immunosorbent Assay (ELISA) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Currently, RIAs and LC-MS/MS are the commonly used methods to assess 1,25$(OH)_2$D and 24, 25$(OH)_2$D. LC- MS/MS detection method has been established as the gold standard method for testing vitamin-D metabolites (Galior *et al.*, 2018).

1.11.1 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

This technique is based on the dissociation of 1,25$(OH)_2$D and 25(OH)D from its binding sites by ionization and absorption of excess energy. After the separation of interfering substances 1,25$(OH)_2$D and 25(OH)D are identified in the chromatographic media. LC-MS/MS has high specificity, sensitivity and wider dynamic range compared to immunoassay methods (El-Khoury *et al*., 2011). This method provides better accuracy at medical decision levels to correctly classify patients as vitamin-D deficient and sufficient. Different LC-MS/MS methods are available to measure several metabolites of vitamin D including 25(OH)D, 1,25$(OH)_2$D, 24,25$(OH)_2$D and vitamin D_3 epimer separately (Ketha *et al.*, 2016). Although LC-MS/MS is considered the gold standard method for 25(OH)D testing, initial capital investment, the labor and time intensive nature of development and implementation, slower turnaround time, need of high sample volume, the requirement of calibration to standardize the measurements are the some of the limitations of this method. Because of the high cost, this method is not commonly used (Grebe and Singh, 2011).

1.11.2 High Performance Liquid Chromatography (HPLC)

This technique is based on the isolation of serum or plasma 25(OH)D in chromatographic media of spectral characteristics of the compounds. The main strength of this method is that it can be used to measure both 25(OH)D and 1,25$(OH)_2$D with good recoveries, convenience and high throughput. Potential interfering substances such as proteins and lipid are removed before adding into HPLC which is also considered as one of the advantages of this method. Further, reliability, low batch to batch variation and low limit of detection is considered as the other advantages. However, there is a lack of consistency in standardization procedures. Therefore most HPLC extraction and procedural losses are corrected by the inclusion of internal standard. Poor specificity, matrix interference and lack of common standard, highly skilled operation requirements, expensive nature, large sample volume and slow sample

throughput are the main disadvantages of this method. Therefore this method is not suitable for routine use (Martin and Newton 1998).

1.11.3 Enzyme-linked Immunosorbent Assay (ELISA)

The assay utilizes a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH)D. For a reliable determination of 25(OH)D, it is necessary to release 25(OH)D from the 25(OH)D-VDBP-complex. Standards, controls and test samples which needed to be assayed for 25(OH)D are prediluted with the releasing reagent and transferred to the microplate coated with 25(OH)D. After an incubation an anti-25(OH)D antibody is added to release 25(OH)D. During an incubation step, 25(OH)D in the sample and a fixed amount of 25(OH)D bound to the microtiter well compete for the binding of the antibody. Then a peroxidase-conjugated antibody is added into each microplate well. A complex of 25(OH)D – anti-25(OH)D antibody – peroxidase conjugate is formed. Tetramethylbenzidine is used as a peroxidase substrate. Finally, an acidic solution is added to terminate the reaction, whereby the color changes from blue to yellow. The intensity of the yellow color is inversely proportional to the concentration of 25(OH)D. A dose response curve of the absorbance unit (optical density, at 450 nm) vs. concentration is generated and the standard 25(OH)D in the samples can be determined from this curve. Long laboratory procedures, necessity of centralized laboratory equipment and large sample volume requirements are the limitations of this method (Arneson and Arneson, 2013).

1.11.4 Radioimmunoassay (RIA)

RIA method is based on a competitive principle with a goat antibody against 25(OH)D, an iodinated (125I) $25(OH)D_3$ tracer, and donkey anti-goat precipitating complex as the secondary antibody. The first part of the assay involves a rapid extraction of 25(OH)D and other hydroxylated metabolites from serum or plasma with acetonitrile. Following extraction, the sample, antibody, and tracer are incubated for 90 min at 20–25°C. Separation is accomplished after a 20 minutes incubation at 20–25°C with the secondary antibody. A buffer is then added prior to centrifugation to reduce non-specific binding. Radioactivity is measured by a gamma counter and is inversely proportional to the concentration of 25(OH)D in the sample (Wagner *et al.*, 2009). This method is suitable for the laboratories with large testing volume. Also it is reliable, accurate, convenience and fast. However, $1,25(OH)_2D$ and 25(OH)D cannot be measured separately by this method. Multiple phase separation steps and requirement special instruments are the main limitations of this method (Wagner *et al.*, 2009).

1.11.5 Chemiluminescence Immunoassays

This assay uses magnetic particles (solid phase) coated with antibody against 25(OH)D and 25(OH)D conjugated to an isoluminol derivative (tracer). During the first incubation phase, 25(OH)D is dissociated from binding protein by buffer containing 10% ethanol and then binds to the anti 25(OH)D antibody on the solid phase. After a second incubation with the tracer, the unbound material is washed off and starter reagents are added to generate a flash chemiluminescent signal which is measured by a photomultiplier and is inversely related to 25(OH)D concentration. This method is more practical and an easier method compared to other methods. High throughput capacity and lower sample volume requirement are also the advantage of this method. However, the concentrations of $1,25(OH)_2D$ and 25(OH)D cannot be measured separately by this method (Wagner *et al*., 2009).

1.11.6 Roche Electrochemiluminescence Immunoassay (ECLIA)

Roche modular 25(OH)D ECLIA assay is a direct competitive electrochemiluminescence immunoassay for human serum or plasma. The assay employs microparticles coated with streptavidin and a polyclonal sheep antibody against 25(OH)D, which is labelled with ruthenium. In the first incubation, $25(OH)D_3$ in the sample competes with biotin labelled 25(OH)D for binding with the anti-25(OH)D antibody. In the second incubation, the biotin 25(OH)D/anti-25(OH)D antibody immunocomplex becomes bound to the microparticles via interaction of biotin and streptavidin. The microparticles are then magnetically captured onto the surface of an electrode. A voltage is applied to the electrode to produce a chemiluminescent emission, which is measured by a photomultiplier and is inversely proportional to 25(OH)D concentration (Wagner *et al.*, 2009).

1.11.7 Electrochemiluminesence assay of PTH analysis

PTH was analyzed using the sandwich test principle of electrochemiluminescence technology. The analysis was carried-out at Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne. In this assay a biotinylated monoclonal antibody reacts with the N-terminal fragment and a monoclonal antibody labelled with a ruthenium complex reacts with the C-terminal fragment. In the second incubation, after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the micro-particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell molecular. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined

via a calibration curve generated by 2-point calibration and a master curve provided via the reagent barcode.

1.12 Quality Assurence of Vitamin D Analysis in Laboratories

The College of American Pathologists (CAP) and Vitamin-D External Quality Assessment Scheme (DEQAS) surveys are used to monitor the performance of laboratories using various methods for testing vitamin D metabolite (Burdette *et al*., 2017). Lack of standardization has been recognized as a challenge in steroid hormone testing. The Center for Disease Control and Prevention has established a Vitamin-D Standardization Certification Program (VDSCP) focused on providing reference measurements for 25(OH)D, to assess the accuracy and precision of vitamin D tests, to monitor their performance over time and provide technical support to external quality assurance programs, proficiency testing programs and research studies. A recent study by VDSCP established core performance criteria, namely CV ≤ 10% and mean bias ≤ 5% for 25(OH)D testing. Inter-laboratory performance of 25(OH)D measurement was compared by providing a set of 50 individual donor samples to 15 laboratories representing national nutrition survey laboratories, assay manufacturers, and clinical or research laboratories. Samples were analyzed using immunoassays and LC-MS/MS. All LC-MS/MS results achieved VDSCP criteria, whereas only 50% of immunoassays met the criterion for ≤ 10% CV and only three of eight immunoassays achieved the ≤ 5% bias (Wise *et al.,* 2017).

1.13 Determinants of Vitamin D Status

Sun exposure related variables, dietary intake and supplemental vitamin D are mainly associated with vitamin D status of a person. In addition some personal factors such as age, gender, BMI, alcohol intake and smoking status, are also associated with a person's vitamin D status.

1.13.1 Sunlight exposure factors

It is believed that about 90% of total serum 25(OH)D concentration is accounted by cutaneous synthesis (Fraser and Milan 2013). Several studies also confirmed that the UVB exposure is the main determinant of the serum 25(OH)D concentration in the skin (Hedlund *et al.,* 2013, Freedman *et al.,* 2013, Berger *et al.,* 2012). Time spent outside during sunny weather, frequent outdoor activities, time of the day of sunlight exposure, clothing habits, use of sunblock and skin exposure area are some of the sunlight exposure related factors that determine the 25(OH)D concentration (Touvier *et al*., 2015, Voipio *et al*., 2015). Blinkley *et al*., (2009) reported that the efficacy of the vitamin D synthesis in the skin is inversely related

to vitamin D status of the individual (Nair and Maseeh, 2012). Liver 25-hydroxylase enzyme is inhibited by 25(OH)D, which result in hindering the hydroxylation of vitamin D_3 to 25(OH)D (Binkley *et al*., 2009). Various personal and environmental factors are associated with vitamin D synthesis in the skin upon UVB exposure.

Zenith angle, season and latitudes

Zenith angle is the angle between the vertical local line and line of straight to the sun. The intensity of UV radiation reduces with increasing zenith angle. This is caused by two effects. First, the intensity of down-welling solar irradiance is proportional to the cosine of the solar zenith angle. Second, the length of the atmosphere that irradiance has to pass through increases with increasing zenith angle, which causes a high level of absorption of irradiance by atmospheric gases and aerosols. For a particular site at the earth's surface, the solar zenith angle depends on latitude, time of day and the season (Fioletov *et al*., 2010).

The seasonal variation in serum 25(OH)D concentration is documented in many studies (Brembeck *et al*., 2016, Jolloffe *et al*., 2015, Touvier *et al*., 2015). According to a study conducted among older adults live in care homes in the East London found that, there was 10.3 nmol/L increase in serum 25(OH)D concentration during summer compared to winter (Jolliffe *et al*., 2015). The seasonal difference in serum 25(OH)D concentration has also been reported in UK dwelling South Asian women. It is reported that the prevalence of VDD in older adults was 2% higher during winter season compared to autumn season (Darling *et al*., 2013). People with large seasonal changes in 25(OH)D concentration may have suboptimal vitamin D concentration for most of the time of the year (Christensen *et al*., 2010). In addition to the variation in UVB exposure, seasonal changes in 25(OH)D concentration is also caused by the disparity in the activity of the hydroxylase enzyme which controls vitamin D metabolism. These enzymes include 1-hydroxylase (*CYP 27B1*) and 24-hydroxylase (*CYP24A1*) (Christensen *et al*., 2010).

Latitude is a well-known factor affecting sun exposure of the population. The conversion of 7-DHC to previtamin D does not occur in the winter months among the people who live in the areas located in the latitudes above 40^0N. Also when altitude rises, even summer synthesis of vitamin D is reduced. Due to the lower solar zenith angle, the intensity of UV rays is higher at the countries of low latitudes than that of the countries in the high latitudes. However, the effect of latitudes may vary depending on the cloud cover and the level of air pollution (Fioletov *et al*., 2010). In contrast, it is believed that the people who live in higher latitudes evolved to synthesize a high amount of vitamin D effectively (Mei *et al*., 2007).

UV index and solar spectrum

UV index (UVI) gives an indication of the amount of UV rays that reach to the earth at solar noon time to produce erythema, which reflects as a single number. Erythema is a condition that skin turns to light pink in color upon sunlight exposure. It is the main sunlight exposure related factor that affects vitamin D synthesis. The UVI can be computed by multiplying the erythemal irradiance, in watts per m^{-2}, by 40. This index was introduced in Canada in 1992 in response to growing concern about the increased availability of UV radiation due to the reduction of the ozone layer. The World Meteorological Organization and the World Health Organization in 1994 adopted UVI as a standard indicator of UV levels (Fioletov *et al*., 2010).

Atmospheric gases, particles and clouds

The wide varieties of aerosol types are available in the atmosphere can be categorized as man-made and natural. Volcanoes, forest fires (Fioletov *et al*., 2001) and deserts (Sarra *et al*., 2003) are some of the natural sources of aerosols while power plants, factories, biomass burning automobiles and aircraft are some of the artificial sources. Absorption of UV by aerosols in urban areas reduces UV rays that reach the earth by 10%-15%. As a result of the ozone layer, only less than 3% of radiation reaches the earth's surface (Fioletov *et al*., 2003). Sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) also can absorb UVB radiation. SO_2 causes significant absorption up to 50% of erythemal UV. Clouds can reduce UV and visible solar radiation reaching the earth surface but not by the process of absorption by the process of scattering (Fioletov *et al*., 2010). A study that was conducted in Boston to compare previtamin D_3 production on a cloudless day and cloudy day, found that vitamin D_3 production efficiency was reduced by ~20% on a cloudy day compared to cloudless day (Holick *et al*., 2007).

Altitude

Surface UV radiation increases with increasing altitude. UVI increases with decreasing pressure and as a result, areas in higher elevation receive more UV radiation compared atsea level. In addition, the UV absorbing aerosols are located at low elevation and reduce the UV level at the ground level. Data showed that erythemal UV increases between 7% - 15% per km of altitude (Zaratti *et al*., 2003). A study conducted in Australia using young and middle aged adults in three locations proved that VDD decrease with increasing altitudes (Engelsen, 2010). In addition another study reported that the chance of synthesising vitamin D on the mountain Everest at 5350 m height was 400% compared to the sea level (Holick *et al*., 2007).

Time of the day

Early morning and late afternoon sunlight is inefficient to synthesise vitamin D as a result of increasing solar angle (Holick 2007). To identify the effect of time of the day on vitamin D synthesis Mei *et al*.,2017 carried out a research in Australia. A sealed 7-dehydrocholesterol in ethanol under argon in borosilicate ampoules was exposed to sunlight for one hour, in the early morning, noon and late afternoon. Samples were then analysed by HPLC for the conversion of 7-DHC to previtamin D_3. Amount of previtamin D_3 was high in the samples placed under the noon sunlight. Production of vitamin D between the hours 8 – 10 am and 4-6 pm was 20% less than that produced at mid-day. Also they examined the vitamin D synthesis at noon time in June and October. About 0.8% of 7-DHC was converted to previtamin D_3 after 5 minutes exposure in June, at 35 minutes exposure, 3.3% of 7-DHC was converted to previtamin D_3 in October. The efficiency of converting 7-DHC to previtamin D_3 was linear as a function of time over a period of 30 minutes. At early morning and late afternoon the zenith angle is more oblique resulting in a long distance for the solar UVB photons to pass through, which increase the possibility of absorbing UVB by the ozone layer and reduce the UVB reach to the earth surface (Fioletov, 2010).

Skin exposure area and duration of sun exposure

Several studies have indicated that serum 25(OH)D concentration is positively associated with the time duration of sunlight exposure (Patwardhan *et al*., 2015, Touvier *et al*., 2015, Wakayo *et al*., 2015). High duration of sun exposure might be associated with a high physical activity which itself have a positive effect on vitamin D status (Brock *et al*., 2010). Patwardhan *et al*., (2015) stated that the association between serum 25(OH)D concentration and sun exposure depends on the HDL concentration of the person.. A research conducted using Muslim students who had traditional clothing which covers the most parts of the body showed lower serum 25(OH)D concentration than in the students who had normal clothing style (Wakayo *et al*., 2015).

1.13.2 Diet and vitamin D supplements

Dietary sources of vitamin D comes from plant sources, D_2 called ergocalciferol or animal sources, D_3 called cholecalciferol. It is believed that the increase in serum 25(OH)D concentration upon dietary intake of vitamin D rich food is low compared to sun exposure (Fraser and Milan, 2013). It is reported that D_3 is more effective than D_2 in increasing serum 25(OH)D concentration (Tripkovic *et al*., 2012). It was suggested that efficient absorption of vitamin D is dependent upon the presence of fat in the intestinal lumen (Maurya and Aggarwal, 2017). Some physiological factors may also affect the response of dietary vitamin

D intake to the serum 25(OH)D concentration. For example, the larger the BMI, the smaller the rise in serum 25(OH)D concentration. Further, the higher the baseline serum 25(OH)D concentration, the smaller the reached concentration of 25(OH)D in response to a dietary vitamin D (Maurya and Aggarwal, 2017).

Vitamin D supplementation is a safe and inexpensive method to overcome VDD (Hathcock *et al*., 2008). Both D_2 and D_3 are available as dietary supplements. The relative efficacy of D_2 vs D_3 in humans is not well understood. However, it is believed that both appear to be effective in preventing and treating VDD. A single dose of 50,000 IU of D_2 or D_3 produced a similar increase in the total 25(OH)D concentration, but the obvious longer half-life of D_3 suggests that less frequent dosing is needed for D_3 (Armas *et al*., 2004). A daily dosing study of 1000 IU of D_2 vs D_3 showed no difference in concentration of 25(OH)D_2, 25(OH)D_3, or total 25(OH)D concentration after the supplementation (Holick *et al*., 2008). Another study which compared 1600 IU of D_2 once daily vs 1600 IU of D_3 once daily vs 50,000 IU of D_2 once monthly vs 50,000 IU of D_3 once monthly suggested that D_3 is more effective as it showed higher levels of 25(OH)D_3 at the end of 1 year supplementation period (Binkley *et al*., 2009). It is recommended that both D_2 and D_3 be taken with a meal containing fat, ensure the maximum absorption of vitamin D_2 and D_3. Since 1997, the Food and Nutrition Board has advised an Adequate Intake (AI) of vitamin D of 200 to 600 IU/day to treat vitamin D deficiency (IOM, 1997).

How much vitamin D is needed to correct severe VDD (25(OH)D nmol/L) is questionable. Although it is not validated by clinical trials, a commonly applied strategy is to prescribe a "loading dose" (eg, 50,000 IU of vitamin D orally once weekly for 2-3 months, or 3 times weekly for 1 month) to correct severe VDD. A review paper suggested that a minimum total dose of 600,000 IU is the best dose to achieve endpoint serum 25(OH)D concentration greater than 75 nmol/L (Pepper *et al*., 2009). Although many different strategies may be used in treating VDD, a common mistake is to stop treatment or provide inadequate vitamin D maintenance dosing once the 25(OH)D level reaches the optimal range. Regardless of initial vitamin D therapy, and assuming no change in lifestyle or diet, a maintenance daily dose of 800 to 2000 IU or more is needed to avoid recurrent deficiency (Heaney, 2005). A maintenance average dose of 2000 IU/day meets the current safe upper limit guidelines and is below the safe upper limits reported by Hathcock *et al*., 2008.

1.13.3 Personal factors

Age and gender

It is well-known fact that the ability of vitamin D synthesis in the skin is reduced with aging. Among older people whose age is higher than 65 years, the skin 7-dehydrocholesterol concentrations are about 25 % those for a person of 20–30 years old (McLaughlin *et al*., 1985). Low levels of 7-DHC, poor function in liver and kidney function, exposure to sunlight, low physical activity and increased use of medication are the possible reasons for low vitamin D synthesis in older people (Cashman *et al*., 2014, Freedman *et al.,* 2013, Berger *et al*., 2012). On the other hand, there is evidence of low serum 25(OH)D concentration among females compared to males (Chailurkit *et al*., 2011, Lu *et al*., 2009). Females have more Fat Mass (FM) compared to males, therefore more vitamin D is trapped inside the FM and may not present in the serum to be detected by the laboratory method of assessing vitamin D status. In addition, a survey conducted in China revealed that females use more clothe cover and sun cream than males, which leads to low vitamin D status compared to males. Further they reported that a higher percentage of women did not like to go out compared to men, which may also cause lower vitamin D status compared to men (Kotta *et al.,* 2015).

Use of sun protection cream and clothing

Sun protection cream with Sun Protection Factors (SPF) of 8 absorb 92 – 95% of UVB photons and reduce the cutaneous vitamin D synthesis to the same degree (Matsuoka *et al*., 1987). The use of sun protection cream is the major factor that reduces vitamin D synthesis in the skin in the people who live in the countries located near the equator (Nimitphong and Holick, 2013). However, the different practices of sun cream application may have different impacts on vitamin D synthesis (Engelsen, 2010). Use of sunblock is recommended for the prevention of sunburn and skin cancer, which has raised concerns that its application may inhibit or prevent vitamin D synthesis (Springbett *et al*., 2010). A review of studies reported that the majority of people did not use sunblock at the recommended concentration and therefore, use of sunblock did not significantly reduce the production of vitamin D (Norval and Wulf, 2009). Overall, the data suggested that vitamin D synthesis is still possible even when sunscreens are used at the application density used for SPF testing (SACN, 2016).

During winter time people wear more clothes to cover most parts of the body which inhibit the penetration of UV radiation through to the skin (Parisi *et al*., 2003). A study done in Istanbul recruiting female undergraduate students found that the Islamic dressing style considerably associated with low level of serum 25(OH)D concentration. They stated that

VDD was 55% in those with covered dressing style whilst it was 20% in those with uncovered dressing style among the school children (Buyuruslu *et al.*, 2014).

Skin color

Skin color also plays a role in vitamin D status in a population. Dark-skinned ethnic subgroups had 3 to 71 fold prevalence of serum 25(OH)D <30 nmol/L compared to white populations (Cashman *et al*., 2016). Vitamin D synthesis is highly dependent on the concentration of melanin in the skin as melanin absorbs and scatters UVB radiation, resulting in less efficient conversion of 7-dehydrocholesterol to previtamin D_3 (Jablonski *et al.*, 2004). Therefore, dark-skinned individuals experience slower vitamin D synthesis than light-skinned ones. This is especially important at higher latitudes where the intensity and duration of sunlight are low. However, although individuals with light skin are more efficient in producing vitamin D, they are affected by an high level of sunburn, a lower tanning ability (Wagner *et al*., 2002) and a greater susceptibility to skin cancers (Sturm *et al.*, 2002).

Physical activity

Some studies reported that vitamin D status is high among the people who are more physically active (Touveir *et al.*, 2015, Freeman *et al*., 2013, Berger *et al.*, 2012) independent of time spent for outdoor activities (Scott *et al.*, 2010). However, indoor exercises also result in improved 25(OH)D concentration as physical activity has the direct effect on serum 25(OH)D concentration by changing the calciotropic hormones and increase the concentration of *IGF* (Maiimoun and Sultan, 2009, Gomez *et al*., 2004). High-intensity physical activity may also contribute to higher vitamin D status in older adults through increasing and maintaining muscle mass. Increased muscle mass can uptake and store 25(OH)D in muscles. The magnitude of the relationship between physical activity and vitamin D status depends on sex and the intensity of the physical activity (Scott *et al*., 2010).

Body composition and BMI

A systematic review with meta analysis of 34 cross sectional studies of adults reported a significant inverse, but weak, association between BMI and serum 25(OH)D levels. This inverse associations were significant in both genders (Saneei *et al*., 2013). There are studies that investigated the association between BMI and vitamin D status in older adults. A recent cross sectional study of older adults >65 years (n=1842) found that BMI was inversely associated with serum 25(OH)D levels after adjustment for confounders. They reported that this association was most prominent in individuals with a BMI in the 'overweight' and 'obesity' range and fat percentage in the last two upper quartiles (Araghi *et al*., 2015). Another cross sectional study of large number of older adults in Mexico found that

overweight/obesity was significantly associated with low concentrations of serum 25(OH)D concentration (Rontoyanni *et al.*, 2017).

Smoking and alcohol intake

Smoking and drinking alcohol are associated with a lower concentration of 25(OH)D in the serum (Voipio *et al.*, 2015, Jungert and Neuhauser – Berthold, 2015). Smoking may interfere with the synthesis of 25(OH)D by inhibiting the expression of cytochorome *P450 2RI* in the liver. The benzopyrene produced by cigarette smoke may increase the degradation of $25(OH)D_3$ and $1,25\ (OH)_2D_3$ by stimulating the $1,25(OH)_2D_3$-dependent induction of cytochrome *p450* (Matsunawa *et al*., 2009). According to a recent review paper, alcohol intake had a positive association with 25(OH)D concentration. However the authors elaborated that further research is needed to identify the association between alcohol intake and serum 25(OH)D concentration, considering the health status of alcohol users and with a more standardized methodology to confirm this finding.

1.14 Quantifying Cutaneous Vitamin D Synthesis and Recommendations for Sun Exposure

Human exposure to UVR can be measured by Standard Erythema Dose (SED) and Medium Erythema Dose (MED). The SED refers to erythemal effective radiant exposure from natural and artificial sources of UVR. One SED is equal to an erythemal effective radiant exposure of 100 J/m^2. One MED is the dose of UVR required to cause sunburn or slight pinkness in the skin (SACN 2016). Thus, it is a measure of the variable nature of individual sensitivity to UVR (Diffey, 2002). Full body UV exposure causing slight pinkness in skin is similar to the oral intake of 250 – 625 µg (10,000IU – 25,000 IU) of vitamin D. It is considered that 1000 IU vitamin D is generated by exposing 25% of the body area to sunlight which is equivalent to 0.25 MED. Therefore, exposure of 6% of the body to 1 MED is equivalent to taking about 600 – 1000 IU of vitamin D. The "Holick Rule" says the exposure of face, arms and legs for a period equal to 25% of the time of one 1 MED for 2-3 times a week can satisfy the body's vitamin D requirement. It is estimated that for elderly people exposure of hands, face and arm to sub-erythemal dose of radiation, 10-15 min between 11.00 am - 2.00 pm for 2-3 times a week in the summer can satisfy the requirement of 400 IU/day of vitamin D (Holick, 2004). Therefore, it is said that, exposure of arms and legs for 5 to 30 minutes (depending on the time of day, season, latitude, and skin pigmentation) between the hours of 10 am - 3 pm twice a week is often adequate for optimum vitamin D status (Holick, 2004). Dark-skinned individuals require a greater duration of sunlight exposure than light-skinned individuals to synthesize the same amount of vitamin D_3 with the same amount of MED (Webb and

Engelsen, 2006). Webb *et al*., 2010, carried out a study using artificial UVB and they concluded that when wearing knee -length shorts and T-shirt with sleeves to mid upper arms at latitude of 30^0N – 60^0N, midsummer noontime sun exposure for approximately 30 minutes for 3 times a week could be enough for archiving 25(OH)D levels above ~ 63 nmol/L. Similarly, Barger – Lux and Heaney, 2002 reported that to optimize dermal production of vitamin D, staying in the direct sunlight only for 15 minutes, while maximizing the skin exposure area might be sufficient without prolonging sun exposure.

However, many research scientists advise getting 1000–4000 IU of vitamin D per day. These amounts are usually not available from usual daily sun exposure. However, for normal summer clothing, the exposed skin (at least 25% of the body area) needs to be sunburnt in order to produce 4 000 IU. The skin would then receive a UV dose associated with clearly elevated skin cancer risk (Webb and Engelesenl, 2008). Cancer Research UK has established the following guidelines to advise people on safe sun exposure, which is called Sun SMART. This concept says 5 facts about safe sun exposure, i) Spend time in the shade between 11.00 am and 3.00 pm ii) Make sure you never burn iii) Aim to cover up with a T-shirt, hat and sunglasses iv) Remember to take extra care with children v) Then use UPF 15^+ sunscreen e (Cancer Research UK, 2003).

1.15 Methods of Assessing Sun Exposure

Factors associated with UV exposure can be categorized into environmental and personal factors. Latitude, season, time of the day, ground reflectivity, and cloud or tree cover are some of the environmental factors, while sun-protection behaviors, amount of time spent outdoors, clothing pattern, duration of sun exposure and holiday visits are some of the personal factors. The anatomical distribution of UV also depends on standing or sitting postures and body site (McCarty, 2008). Assessing sun exposure is often used as a proxy indicator to serum 25(OH)D concentrations in most vitamin D studies based on large populations. Several tools have been identified to assess sun exposure that is categorized as direct and indirect methods (McCarty, 2008).

1.15.1 Self-reported questionnaire and diaries

Self-reported questionnaires are the most widely used indirect method of assessing sun exposure. However, no standard questionnaire has yet been developed, which may be used in all circumstances. Typical questions included in such questionnaires are sunlight exposure, time of the day, skin exposure area, sun protection practices, season, latitudes/place of living, cloud cover/tree cover, occupational behaviours and details of sunny holidays (Detert *et al*.,

2015). In some studies, each question was given a score to calculate composite scores for the sun exposure questionnaires (Humayun *et al.*, 2012). Sun exposure questionnaires and diaries have some limitations. The main limitation is the large individual variation in serum 25(OH)D concentration cannot be explained by self-reported sun exposure. In addition, recall bias may be a source of confounding when using such questionnaires and they may be a burden to the participants, as they need at least 30 minutes to complete the questionnaire. Some questionnaires need further improvements and tools to validate, while others are suitable only for specific durations such as during the summer (Yu *et al*., 2009).

1.15.2 Dosimeters / Daysimeter

Use of Dosimeters is a direct method of determining UVR that measured using either physical, chemical or biological methods. Physical dosimeters comprise a small UV detector electrically connected to a data logger, which is worn on a belt, in a pocket or the outer surface of the garments. Chemical dosimeters measure a chemical change in response to UV exposure, mostly using a thermoplastic polysulfone film, while a biological dosimeter measures the biological effects of UV exposure on the body, typically reflected by changes in biomarkers such as scoring of chromosomes abnormalities or circulating lymphocytes. The use of a Daysimeter is also similar to dosimeter. It measures circadian light exposure and incorporates an activity meter to estimate the circadian light/dark exposure and activity/rest patterns. It consists of two optical sensors. The first sensor detects optical radiation and a second sensor has an intrinsic long-wavelength response cut-off at approximately 580 nm together with a UV blocking filter creating a spectral response peaking at approximately 460 nm. An accelerometer within the Daysimeter is used to detect the subject's activity. Measures obtained from the Daysimeters can be summarized by calculating hourly averages of the 30 seconds data points of light exposure for each day (Bajaj *et al*., 2011).

1.15.3 Weather stations and equations

UVR can be estimated using the data from satellite, spectrophotometers and computers located in weather stations. The estimate of UVR is mainly based on the measurements of total ozone, ground albedo (reflection) and cloud transmittance. These techniques have some limitations including short-term variation in the cloud cover changes can have a large effect on UV radiance. Also UVR measurements may vary with the frequency of the measurements. UV data from satellites provide UVR measurement for a large geographical area, thus the actual UV value at the ground level may vary from the obtained value. Further, there are lots of other factors affecting the ground level UVR, which are not easy to estimate, such as water

vapor, temperature, albedo, zenith angle and snow cover. Despite these limitations, such weather parameters may help to estimate long-term sun exposure (Fioletov *et al*., 2010).

There is a body of literature on the derivation of equations to calculate UVI, using the measurements obtained by spectrophotometers and satellite from weather stations. Most of these equations consider solar zenith angle and total ozone. However, equations do not account for many factors that can affect UVI, especially the cloud cover, haze, other gases, surface elevation and surface albedo, which are main limitations of this method (O'Sullivan *et al*., 2017).

1.16 Role of Vitamin D in Bone Health: Mechanism of Action

The definition of the optimal 25(OH)D concentration has been the concentration that maximally suppresses PTH concentration. This 25(OH)D is related to bone metabolism, because elevated PTH is associated with increased bone loss (Bischoff-Ferrari *et al*., 2006). The active form of vitamin D ($1,25(OH)_2D$) plays a role in bone by increasing the absorption of calcium in the small intestine. In addition, vitamin D increases the absorption of other essential minerals, such as P, Mg, Zn and Mn and enhances the renal reabsorption of calcium and phosphorus. Thus, $1,25(OH)_2D$ is the major regulator of calcium homoeostasis, which helps in bone mineralization (Wintermeyer *et al.,* 2016). Furthermore, $1,25(OH)_2D$ regulates the phosphate and calcium balance by supporting the intestinal absorption via epithelial calcium channels and CaBP. To obtain its biological activity, $1,25(OH)_2D$ needs to be bound to the VDR whose property it is to form heterodimers with related receptors (e.g., the retinoid X receptor, (RXR))and bind to vitamin D response elements to initiate intracellular signaling cascades (Wacker *et al*., 2013). As one possible response, 1, 25(OH)D enhances the expression of the receptor activator of NFKB ligand (*RANKL*) in osteoblasts. *RANKL* associates with the receptor activator of NFKB (*RANK*) on immune cells to induce differentiation into osteoclasts to release calcium and phosphorus from the skeleton into the blood. This role of vitamin D on bones is illustrated in figure 1.4 (Takahashi *et al*., 2014).

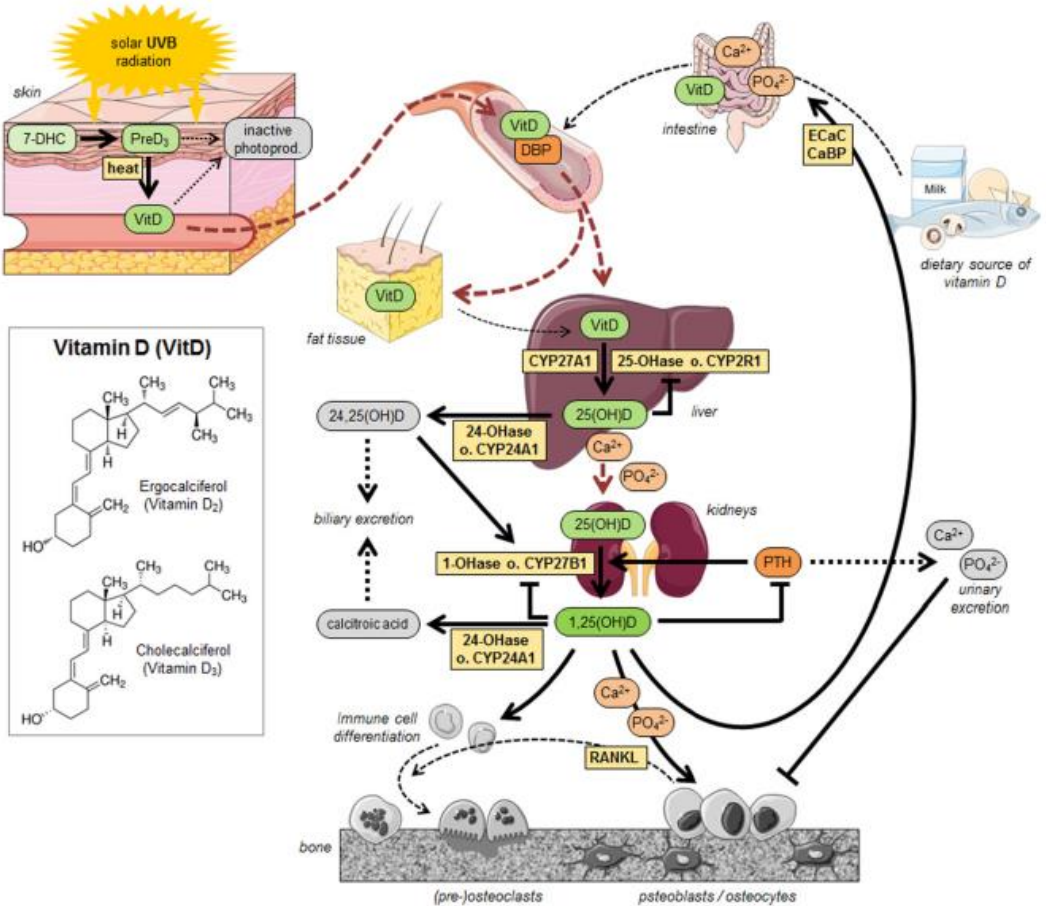


Figure 1:4 Role of vitamin D in bone health (Source : Wintermeyer *et al.*, 2016)

(VitD : Vitamin D, 7DHC : 7-Dehydrocholesterol, DBP : Vitamin D Binding Proteins, PTH : Parathyroid Hormones)

1.17 Evidence of Vitamin D on Bone Health From RCT and Epidemiological Studies

Recent cross sectional studies (Meng *et al.*, 2017, Molsele *et al*., 2013), a longitudinal study (Kuchuk *et al.*, 2009), a systematic review (Zhao *et al.*, 2017) and a RCT (Jackson *et al*., 2006) have reported the positive association of serum 25(OH)D and bone health. There is epidemiological evidence that higher serum 25(OH)D levels are associated with a greater BMD in both young and old populations, with a linear relationship maintained up to a serum 25(OH)D concentration of approximately 75 nmol/L. However, it is reported that this association differs according to ethnic group (Bischoff-Ferrari *et al*., 2004).

A number of placebo-controlled intervention trials have examined the effect of vitamin D supplementation on BMD, factures and falls. Doses of vitamin D supplement used in those studies ranged from 300 IU/day to 7,000 IU/day (Aspray *et al*., 2018, Reid *et al.*, 2014, Macdonald *et al*., 2013, Sanders *et al*., 2010). According to the above studies consistent relationship between vitamin D supplementation and BMD was not reported across all

anatomical sites. As an example Reid *et al*, (2014) reported the improvement in BMD in the femoral neck, while Macdonald *et al*., 2017 reported the improvement in hip BMD in older adults. Conversely a recent meta-analysis of 33 randomized clinical trials which included 51 145 older participants (Zhao *et al*., 2017), concluded that the use of calcium, vitamin D, or combined calcium and vitamin D supplements was not associated with a lower risk of fractures among community-dwelling older adults. Hip fracture was defined as the primary outcome while secondary outcomes were nonvertebral fractures, vertebral fractures, and total fractures (Zhao *et al*., 2017).

It has been suggested that vitamin D supplementation in daily dose of at least 800–1000 IU is required to decrease the incidence of both falls and fractures in older adults (Bischoff-Ferrari *et al.,* 2012) while a Cochrane review reported that no evidence that vitamin D supplementation on fracture prevention (Avenell *et al*., 2014). Whereas another study using 300,000 IU of vitamin D_2 given intramuscularly to care home residents for 10 months had no effect on hip fracture incidence (Low *et al*., 2016). Another study of the yearly oral dose of 500,000 IU vitamin D supplementation for the women aged over 70 years was associated with an increased risk of both fractures and falls during the first 3 months after the administration. (Sanders *et al*., 2010). Most recently, a study looking at monthly bolus doses of three regimens (60,000 IU D_3 versus 24,000 IU plus 300 μg calcifediol versus 24,000 IU D_3) observed significantly more falls in the 60,000 IU and the 24,000 IU plus calcifediol groups compared with the 24,000 IU group (Bischoff-Ferrari *et al.*, 2005). It has been suggested that the effect of vitamin D supplementation seen in some studies is due to the prevalence of hypovitaminosis D and the treatment of mineralization disorder in associated osteomalacia, rather than an effect of the supplement on the prevention of osteoporosis (Macdonald *et al*., 2013).

1.18 Methods of Assessing Bone Health

1.18.1 Dual Energy Extra Absorptiometry (DEXA)

Dual Energy Extra Absorptiometry (DEXA) is the most widely used gold standard method of assessing BMC and BMD (Punda and Grazio, 2014). It uses two energy beams, low and high, the low energy beam is attenuated by the soft tissue and the high-energy beam is attenuated only by the bone tissue. The degree of attenuation of the two beams is used to estimate BMC and BMD (Kanis, 2002). DEXA requires less time for the examination and it is widely used to diagnose osteoporosis and to predict the fracture risk. The limitations of DEXA scans are the two-dimensional projection images that measure BMD (i.e as grams of bone per unit area)

and it does not separate the effects of true bone density (i.e. grams of bone per unit volume) (Blake and Fogelman, 2009).

1.18.2 Heel Ultrasounds Scanning (US)

Ultra Sound Scanning devices are easy to use, portable, and low in cost. Ultrasound scanners are based on the principle that the intensity and Speed Of Sound (SOS) waves that are altered when passing through a substance and this change can be measured. In the heel bone ultrasound scanner, high frequency sound waves are allowed to send to the heel bone (Os Calcis). In this technique, the person's heel is surrounded by warm water encapsulated between two membranes. From one side of the transducer convert an electrical signal into a sound wave which passes through the water and person's heel. Then at the opposite side of the machine again converts sound waves back to electrical signals. The machine measures SOS and the frequency dependent attenuation called, Broadband Ultrasound Attenuation (BUA). The machine provides an index called Stiffness Index (SI) combing BUA and SOS (Chin and Ima-nirwana, 2013).

1.18.3 Quantitative Computer Tomography

Quantitative Computed Tomography (QCT) is advantageous compared to other techniques as it can measure the true volumetric density (expressed in mg/cm^3). Trabecular bone located in the lumbar spine can be measured independently of the surrounding cortical bone by QCT. This technique has low precision as a result of longer scanning time and high radiation dose compared to DXA measurements. Usually, the spine QCT is used as a primary diagnostic method in patients with severe degenerative disease of the spine, scoliosis, lumbar compression fractures or obesity. Recently, automated scanning and analysis software with new CTs have been developed with the high-resolution, high-quality image to assess the microstructure of the trabecular bones (Schreiber *et al*., 2014).

1.18.4 Fractures

Beyond changes in BMD, the important clinical outcome is osteoporotic fractures. It is considered as an important clinical outcome that reflects the bone quality. A Fracture Risk Assessment Tool for evaluating fracture risk has been developed. A tool called FRAX was developed to evaluate a person's 10-year probability of hip fracture and major osteoporotic fractures at spine, forearm, hip, or shoulder. This tool is applicable to people aged 30–85 years (IOM, 2011).

1.18.5 Bone resorption and bone formation markers

Molecular markers of bone metabolism are novel tools which detect the dynamics of bone remodeling with respect to bone formation and resorption. This method is wider available, reliable, cost-effective and sensitive. Different assays for bone turnover markers would complement the measurement of BMD in the management of osteoporosis, especially in the follow-up of the patients who are on antiresorptive or bone formation therapies. Propeptides of type 1 collagen, Alkaline phosphatase (ALP), Osteocalcin (OC) are the commonly used bone formation markers, while Telopeptides of type 1 collagen, Hydroxyproline, Pyridinium crosslinks, Bone sialoprotein, Osteoprotegerin, Cathepsin K are some of bone resorption markers (Shetty *et al*., 2016)

1.19 Role of Vitamin D in Muscle Function in Older Adults : Mechanism of Action

The loss of muscle strength and muscle mass are main changes occur during aging process. Loss of muscle strength and muscle mass cause an increased risk of falls, mobility disabilities and bone fractures (Cangussu *et al*., 2015). Loss of muscle mass and function in the elderly is called sarcopenia (Fielding *et al*., 2011) which leads to functional impairments such as falls, mobility, disability, fractures and poor Quality Of Life (QOL) (Yu *et al.*, 2014). Reasons for sarcopenia is multifactorial. Genetics, environmental and hormonal factors play a role in the occurrence of sarcopenia (Fielding *et al*., 2011). Older people naturally have low vitamin D status as a results of low dietary intake, less sunlight exposure, low skin thickness, low amount of 7-DHCavailability in the skin, poor absorption of vitamin D in the intestine, reduction in expressions of VDR gene in muscles, excessive use of multiple drugs and poor function of kidney and liver cause for this situation (Bruyere *et al*., 2014, Holick, 2006, Bischoff-Ferrari *et al*., 2004). Therefore vitamin D is a very important critical nutrient for older adults.

There is also strong evidence for the direct effect on muscle function with the localization of the VDR in skeletal muscle cells (Ceglia and Harris 2013). Effect of vitamin D ($1,25(OH)_2$Dthe active form of vitamin D) on muscle is by increasing calcium accumulation in the sarcoplasmic reticulum by increasing the number of calcium-binding sites or altering the efficiency of these sites for calcium uptake (Ceglia and Harris, 2013). Based on a vitamin D supplementation study conducted in rats, it was suggested that vitamin D can increase ATP and leucine in skeletal muscles (Endo *et al*., 2003). Another concern is brought up by a study conducted using rats which showed the increase in serum phosphorus level (Schubert and DeLuca, 2010). In addition to calcium, vitamin D handles phosphorus in muscle cells. Furthermore vitamin D involves in preventing sarcopenia by expression of the gene for

muscle contractile protein synthesis, muscle fiber differentiation and increase the oxidative capacity of mitochondria (Ceglia, 2008).

1.20 Role of Vitamin D in Muscle Function : Evidence from RCT and Epidemiological Studies

Several recent cross-sectional studies (Berners *et al*., 2018, Brech *et al.,* 2017, Bischoff-Ferrari, 2004), a longitudinal study (Granic *et al*., 2017) and RCTs (Cangussu *et al*., 2015, Broe *et al*., 2007, Sato *et al*., 2005) have demonstrated the positive associations between vitamin D and muscle function in older adults. In a study conducted, with nursing homes residents, with an average age of 89 years, who were randomized to receive one of four oral dose of vitamin D_3 supplements (200 IU, 400 IU, 600 IU and 800 IU) or placebo daily for 5 months it was shown showed that the highest dose receivers had the lowest number of falls compared to the other groups (Broe *et al.,* 2007). Positive effects of oral vitamin D_3 supplements on Grip Strength (GS) and chair rise test were reported in a study of postmenopausal women aged 50 – 65 years who received 1000 IU of oral vitamin D_3 daily for 9 months period (Cangussu *et al*., 2015). A study of ambulatory older adults with the history of falls and serum 25(OH)D < 30 nmol/L who received a single intramuscular injection of 60000 IU of ergocalciferol reported the beneficial effect on functional performance, reaction time and balance but not muscle strength (Dhesi *et al*., 2004). Another RCT of ambulatory older adults living in a nursing home with serum 25OHD concentration < 75 nmol/L, randomized to receive the oral or intramuscular injection of 600000 IU of cholecalciferol for 12 weeks demonstrated an improvement in muscle strength which was assessed using quadriceps and physical performance battery (Tellioglu *et al*., 2012). According to Zhu *et al*., 2010, among community-dwelling older adults aged 70 – 90 years with serum 25(OH)D concentration < 60 nmol/L (24 ng/mL) supplemented with 1000 IU of vitamin D_2 daily for 1 year period, improved TUG test only among the older adults who were the slowest and weakest at the baseline.

There are several epidemiological studies that showed the positive association of vitamin D status and muscle function. A large study of community dwelling older men and women in which was the muscle function was assessed by tandem test, 5 timed chair stands gait speed, 6-minute walking distance, GS, and quadriceps strength, showed that lower 25(OH)D levels were associated with worse coordination and weaker strength in women, a slower walking time and a lower upper limb strength in men, and a weaker aerobic capacity in both genders (Toffanello *et al*., 2012). A cross sectional study of 127 pre-frail and frail elderly people in Netherlands showed 25(OH)D concentration was low among the older adults who had low

muscle function which was assessed by appendicular lean mass, leg strength, handgrip strength and physical performance (short physical performance battery) (Tieland *et al*., 2009). In the Newcastle 85^{+} Study (n = 845), which is a longitudinal study of older adults showed that, low baseline 25(OH)D concentration contributed to muscle strength decline in the very old and particularly in men (Granic *et al*., 2017). Another cross sectional study performed in American and Swedish older adults who were examined for potential participation in a combined exercise and nutrition intervention trial showed that higher serum levels of 25(OH)D associated with better performance on the chair stand test (Berens *et al*., 2018).

1.21 Assessment of Muscle Function

1.21.1 Handgrip Strength Test (GS)

Grip Strength (GS) is a widely used low-cost method to measure upper body muscle strength. It is the simplest method for assessment of muscle function in clinical settings, specially to assess sarcopenia (Roberts, 2011). Low values are associated with falls (Sayer *et al*., 2006), disability, impaired health, poor QOL (Syddall *et al*., 2009), and increased mortality (Gale *et al*., 2007, Cooper *et al*., 2010). A hand grip dynamometer is the equipment used to measure GS (Cruz-Jentoft, 2010). The dominant hand is usually recorded, before taking the measurements (Hogrel, 2015). Subjects are advised to sit on an armchair and hold the hand grip dynamometer using one hand, while keeping the arm on the chair arm. Study participants are asked to squeeze the handle of the dynamometer using his or her maximum strength for a few seconds when measurements are taking. The value displayed in the dial is the value needs to be recorded. This should be performed for three times for one hand and then the same procedure should be repeated with the other hand. Recently published cutoff value for predicting sarcopenia is less than 20 kg for females and less than 30 kg for males (Filippin *et al*., 2017).

1.21.2 Timed-Up and Go test (TUG)

The ‘Timed-Up and Go’ (TUG) test is a simple and widely used clinical measure of lower body muscle strength (Weiss *et al*., 2010). To perform this test, participants are asked to sit on backed-chair. The participant should stand from the chair without any support, walk for a 2 m or 3 m distance, turn back, walk back to the chair and sit on the same chair. The total time spent on this activity is recorded. Usually, this test is performed three times and the mean value is recorded (Herman *et al*., 2011). A systematic review reported that TUG test value of 13.5 sec is considered as the cutoff for the risk of falling in older adults (Barry *et al*., 2014). But according to a study conducted in for Brazil recruiting older adults the predicted TUG test

value was 12.47 sec for the risk of falls (Alexandre *et al*., 2012). According to Filippin *et al*., 2014, cutoff values for predicting sarcopenia is 7.5 seconds.

1.21.3 Falls

Falls are a common event among older adults, and are the leading cause of severe injuries, and hip fractures, in older people (Edwards *et al*., 2013). A study of 2299 residents aged 65 years or older in Hertfordshire, UK, showed that a history of any type of fall from the age of 45 years onwards resulted in an unadjusted fracture hazard ratio of 7.31 and 8.56 in men and women, respectively (Watanabea *et al*., 2014). To maintain the balance contributions from vision, peripheral sensation, vestibular sense, muscle strength, neuromuscular control and reaction time should be coordinated properly. With increased age, there is a progressive loss of functioning of these systems and increase a risk of falls and thus details about falls are used as an indicator of muscle function. By using simple tests of vision, leg sensation, muscle strength, reaction time and standing balance, it is possible to identify accurately older people at risk of falls (Lord and Sturnieks, 2005).

1.21.4 Physical Performance Battery Score (SPPB)

In clinical practice, gait speed (timed to walk 4 m distance), sit-to-stand time, and standing balance are collectively measured within the context of the Short Physical Performance Battery (SPPB). This test has been validated in large-scale epidemiological studies and characterized lower extremity functional performance using timed measures of standing balance (side-by-side stand, tandem and semi-tandem positions), gait speed (timed 4-metre walk), and lower extremity strength (timed test of five chair rises). Score obtained on a 12-point summary scale indicates a gradient of functional decline that is predictive of mobility-related disability, institutionalization, and mortality. It is generally accepted that a total SPPB score ≤10 indicates functional impairment in older populations (each test is scored from 0 to 4) (Cooper *et al*., 2013).

1.22 Mechanism of Action of Vitamin D in Colorectal Cancer (CRC)

Some studies have linked polymorphisms of genes of the vitamin D system (*VDR, CYP27B1, CYP24A1, GC, DHCR7* and *CYP2R1*) to CRC prognosis or response to therapy (Jiang e*t al.*, 2018). Calcitriol inhibits proliferation, sensitizes to apoptosis, and promotes differentiation of colon carcinoma cells through the regulation of genes and the modulation of signalling pathways. (1) Proliferation : Calcitriol reduces cell proliferation by several mechanisms: downregulation of cyclin-dependent kinases (CDKs), induction of CDK, inhibiting proliferative signaling pathways generated by epidermal growth factor (*EGF*) and insulin-like growth factor II (*IGF–II*) (Ched *et al*., 2002). Non-genomic (transcription-independent)

effects of calcitriol are mediated by either extranuclear VDR or alternative receptors. In colon carcinoma cells, calcitriol induces a transcription-independent signalling pathway that involves a rapid increase in the intracellular concentration of Ca^{2+} and the subsequent activation of the small RhoA GTPase (Ordóñez-Morán *et al*., 2008). (2) Apoptosis : Calcitriol sensitizes colon carcinoma cells to the initiation of apoptosis by several agents through the upregulation of proapoptotic genes, the down-regulation of survival genes (survivin and thymidylate synthase) and via interference with IL-1β secretion by macrophages (Barbáchano *et al*., 2017). (3) Migration, invasiveness and angiogenesis : Calcitriol suppresses the expression of *DKK-4,* which promotes invasion, angiogenesis, and chemoresistance in colon carcinoma cells. In addition, calcitriol down regulates the angiogenic phenotype of colon carcinoma cells by controlling the expression of several genes (Ben-Shoshan *et al.,* 2007). (4) MicroRNAs :Calcitriol regulates the expression of several microRNAs (miRs) in human colon carcinoma cells. One of them is miR-22, which is induced by calcitriol which mediates the antiproliferative and antimigratory effects of calcitriol in colorectal cancer cells. Further, miR-22 inhibits proliferation, migration, invasion, and xenograft tumor growth in several cancer systems including colorectal cancer (Figure 1.5) (Wang *et al*., 2018).

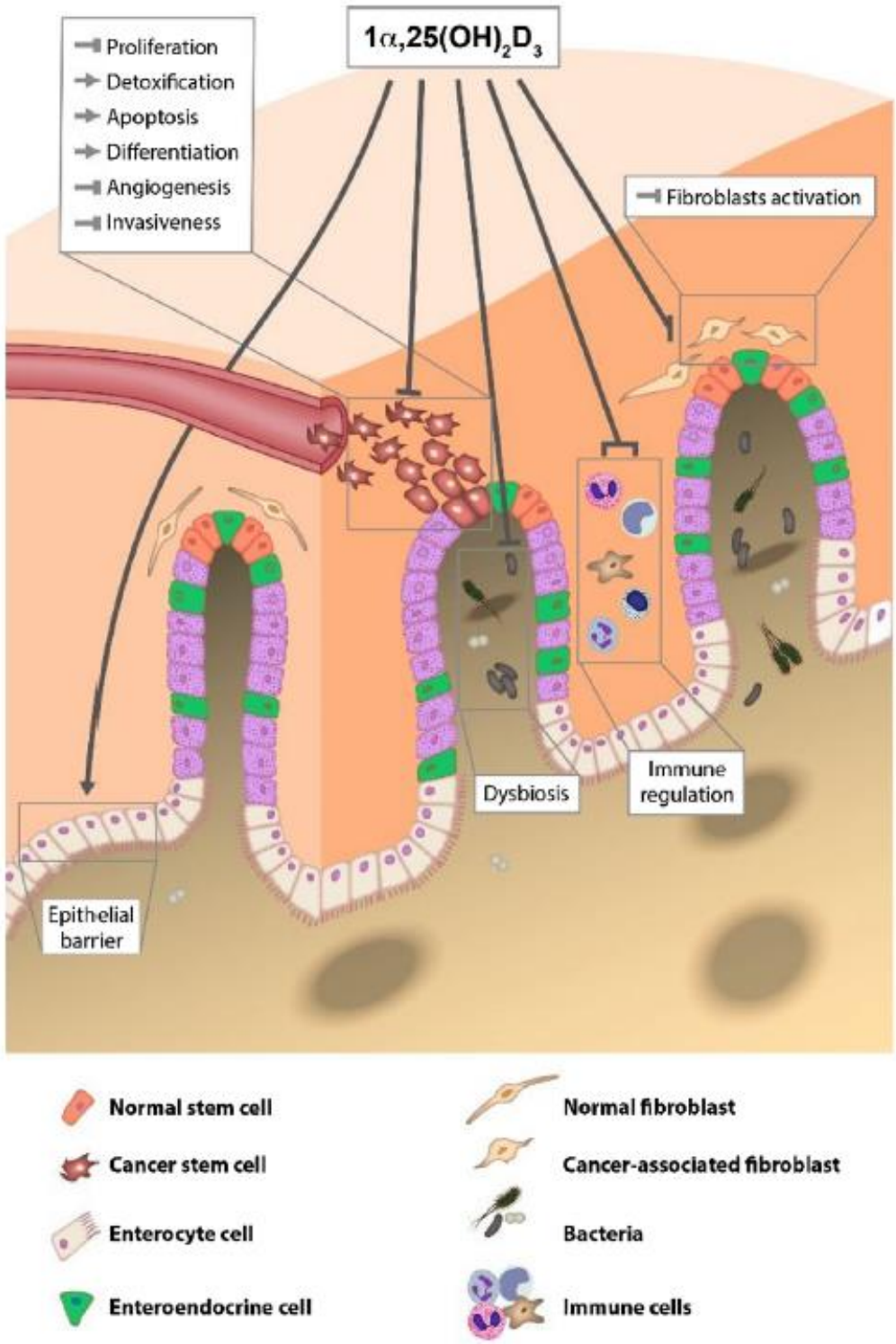


Figure 1.5 : Effect of Calcitrol on the cell types present in the colon to prevent colon cancer (Source : Ferrer-Mayorga *et al.*, 2019)

1.23 Evidence of association between vitamin D status and Colorectal Cancer (CRC) risk

Epidemiologic research showed that incidence and death rates for certain cancers were lower among individuals live in southern latitudes, than among those living at northern latitudes (Eide *et al*., 2005). Therefore researchers hypothesized that variation in vitamin D levels might account for this association (National Cancer Institute, 2013). Experimental evidence has suggested a possible association between vitamin D and cancer risk. The mechanistic studies showed that vitamin D has been found to have several activities that might slow or prevent the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth, stimulating apoptosis, and reducing the tumor blood vessel formation (angiogenesis) (Thorne *et al*., 2008). A systematic review with meta-analysis of 9 prospective cohort studies showed that vitamin D intake and blood 25(OH)D levels were inversely associated with the risk of CRC (Ma *et al*., 2011). A meta-analysis of five nested case control studies showed that maintaining a serum 25(OH)D concentration of 82 nmol/L compared with 30 nmol/L resulted in 50% lower risk of CRC (Gorham *et al*., 2007).In a recent case-control study with 1,248 cases of incident of CRC and 1,248 controls found that, those in the highest quintile of plasma 25(OH)D concentration had a 40% lower risk of CRC than did those in the lowest quintile after adjustment for potential confounders (Jenab *et al.,* 2010).

1.24 Parathyroid Hormone (PTH), its Functions, Synthesis and Regulation

PTH is an 84-amino acid peptide hormone that is responsible for maintaining calcium and phosphorus homeostasis in the human body. This hormone is secreted by chief cells of the parathyroid gland in response to hypocalcemia which is identified by "Calcium Sensing Receptors" (CaSR) in the thyroid cell membrane (Fraser, 2009). The signal for an increase in PTH production and secretion is a reduced extracellular ionized calcium concentration, while the signal for a reduction in PTH production and secretion is an increase in extracellular ionized calcium concentration. There is a bifunctionality of PTH - calcium interrelationship, which serum calcium concentration control PTH secretion, while PTH regulates the serum calcium secretion. When PTH is secreted by parathyroid cells as a result of low Ca^{2+} concentration, it acts on kidney cells to increase renal tubular reabsorption of calcium and conversion of 25(OH)D to 1,25 $(OH)_2D$ by activating of the renal hydroxylase enzyme (Taylor *et al*., 2008). PTH has been proposed as a functional marker of vitamin D status largely because an elevated plasma PTH concentration is known as a risk factor for osteoporosis. PTH is linked to 25(OH)D through the calcium-phosphate homeostatic system (Prentice, 2008).

PTH maintains serum calcium concentration through direct action on bone and kidney and indirectly, through the action on gastrointestinal tract. In addition, in bones, PTH controls Ca^{2+} release to the extracellular fluids with both the rapid release from the calcium pool and the slow mechanism with stimulation of increased bone turnover (Parfitt, 2003). In addition to calcium, PTH regulates phosphorus metabolism. It decreases the serum phosphorus level by inhibiting renal phosphate reabsorption. Hence, increased plasma PTH concentration leads to increased renal phosphate excretion with decreased plasma phosphate levels. As PTH directly influence Ca and phosphorus metabolism, PTH is involved in bone and muscle function (Berqwitz *et al.*, 2010).

The inverse association between PTH and serum 25(OH)D concentration has been identified in many studies (Chang *et al.,* 2017, Jungert and Neuhauser-Berthold, 2015). Therefore there is a seasonal variation in the PTH concentration of the body (Piak *et al*., 2010). In addition, dietary and plasma calcium level and short-term phosphorus intake and determine PTH concentration in the body. Low level of extracellular plasma calcium concentration and lower calcium intake elevate PTH levels. Higher plasma phosphate level directly stimulates PTH release from parathyroid tissue. Therefore high phosphorus intake increases the PTH concentration in serum. Body Mass Index (BMI), age, vitamin D status, stage of life, time of the day, ethnicity, dietary calcium and phosphorus intake, kidney function, physical activity, drug use, age and gender are some of the determinants of PTH concentration in the body (Fraser *et al*., 2009, Patel *et al*., 2007, Vieth *et al*, 2003). Individuals with a higher BMI had higher PTH levels compared to individuals with a lower BMI. This relationship might be due to lower serum 25(OH)D levels in obese individuals compared to non-obese individuals (Kamycheva *et al*., 2004). Acute ethanol administration in healthy volunteers (Laitinen *et al*., 1991) resulted in lower PTH levels while human studies of chronic alcohol consumption have shown elevated PTH levels (Feitelberg *et al.,* 1987).

Diurnal variation in PTH concentration was identified first in 1960s with the peak concentration reported in the early morning. This diurnal variation may have an important effect on bone remodeling which showed the increment of several markers of bone resorption in the early morning (Fraser *et al.,* 2004). It is believed that biological fluctuations in circulating levels of PTH may have an anabolic effect on bone. Some studies have documented a diurnal rhythm for markers of the bone formation such as osteocalcin and the propeptide of type I collagen both of which peaked in the early morning hours and, in the case of osteocalcin, level increases with the highest PTH concentration (Fuleihan *et al*., 1997).

1.25 Hyperparathyroidism and hypoparathyroidism

Hyperparathyroidism is a condition with elevated PTH. There are two types of hyperparathyroidism, primary hyperparathyroidism (PHPT) or secondary hyperparathyroidism. Primary hyperparathyroidism is the excessive release of PTH caused by an adenoma of one or more parathyroid glands or hyperplasia of all four glands when there is hypercalcemia or normal-high serum calcium levels. PHPT is diagnosed based upon levels of blood calcium and PTH. In most people with hyperparathyroidism both calcium and PTH levels are higher than the normal. Bone density testing is usually recommended for people with hyperparathyroidism. Secondary hyperparathyroidism, is caused by the deficiency in vitamin D or uremia (Fraser, 2009).

Hypoparathyroidism is a disorder of PTH deficiency caused by either autoimmune destruction of the parathyroid glands, failure in the connection with other endocrine glands or removal of parathyroid tissue. Sometimes hypoparathyroidism can occur as a congenital disorder in which the parathyroids and other derivatives of the 3rd and 4th pharyngeal pouches do not develop. Very rarely, intracellular processing defects or activating mutations of the calcium sensing receptor can also lead to hypoparathyroidism (Brown, 2009).

For the diagnosis of primary hyperparathyroidism the blood phosphate level, the vitamin D level, urine calcium levels (a urine test of calcium collected over a 24-hour period), and the blood creatinine level (a measure of kidney function) needto be tested. Vitamin D levels should be checked to diagnose secondary hyperparathyroidism. Patients with elevated calcium and/or parathyroid hormone levels should also have their bone density tested, by a DEXA-scan. Normal blood levels of PTH vary according to the lab that measures the hormone, but most laboratories use PTH concentration, 15 - 65 Pg/mL as the normal level (Pallan *et al.*, 2012).

1.26 Effect of PTH in Muscle Function

Previous studies have reported that hypoparathyroidism or hypocalcemia affect muscle function (Van *et al*., 2006, Syriou *et al*., 2005, Nora *et al*., 2004) as a result on low Ca^{2+} concentration in serum. But on the other hand, a high level of PTH have been implicated in sarcopenia and loss of muscle strength (Visser *et al*., 2003). Whether the effect of PTH on muscle is associated with low vitamin D level or independent is not clear (Houston *et al*., 2011). Recent evidence suggests that PTH concentration, independent of vitamin D levels, may be important for maintaining muscle integrity and physical function and thereby, preventing falls (Wat *et al*., 2007). A longitudinal study showed that a low 25(OH)D

concentration and high PTH concentration were the determinants of sarcopenia and loss of muscle strength (Visser *et al*., 2003). It is found that infusion of PTH at high concentration increases protein catabolism and decreased the number of type 2 muscle fibres, intracellular energy-rich phosphate compounds, and mitochondrial oxygen uptake (Mosekilde *et al*., 2005). On the other hand, it has been found that high PTH levels result in increased skin pigmentation and thereby reduction in vitamin D production in the skin (Cosman *et al*., 2007). The presence of *FGF-2* in muscle fibers along the muscle - bone interface suggests that PTH regulation of *FGF-2* maintain coordinately the growth and development of bone and muscle. Further it is believed that PTH plays a role in muscle and bone together via the activation of *IGF-1* (Datta, 2014). It is proposed that PTH enhances the breakdown of DBP-actin complex. If the effects of PTH were to both enhance DBP uptake and to facilitate breakdown of DBP-actin complexes, it would increase binding sites for $25(OH)D_3$ within the muscle cell. It is suggested that the presence of small elevations in PTH enhance uptake of DBP into muscle, and increase the breakdown of DBP-actin complexes, then there are more binding sites for $25(OH)D_3$ in muscle, thus protecting $25(OH)D_3$ from degradation (Abboud *et al*., 2017). PTH stimulates synthesis of interleukin – 6 (*IL-6*) in the liver and the higher *IL-6* concentration was associated with faster muscle loss (Schaap *et al*., 2009). The role of PTH on muscle function is illustrated in a hypothetical model in Figure 1.6.

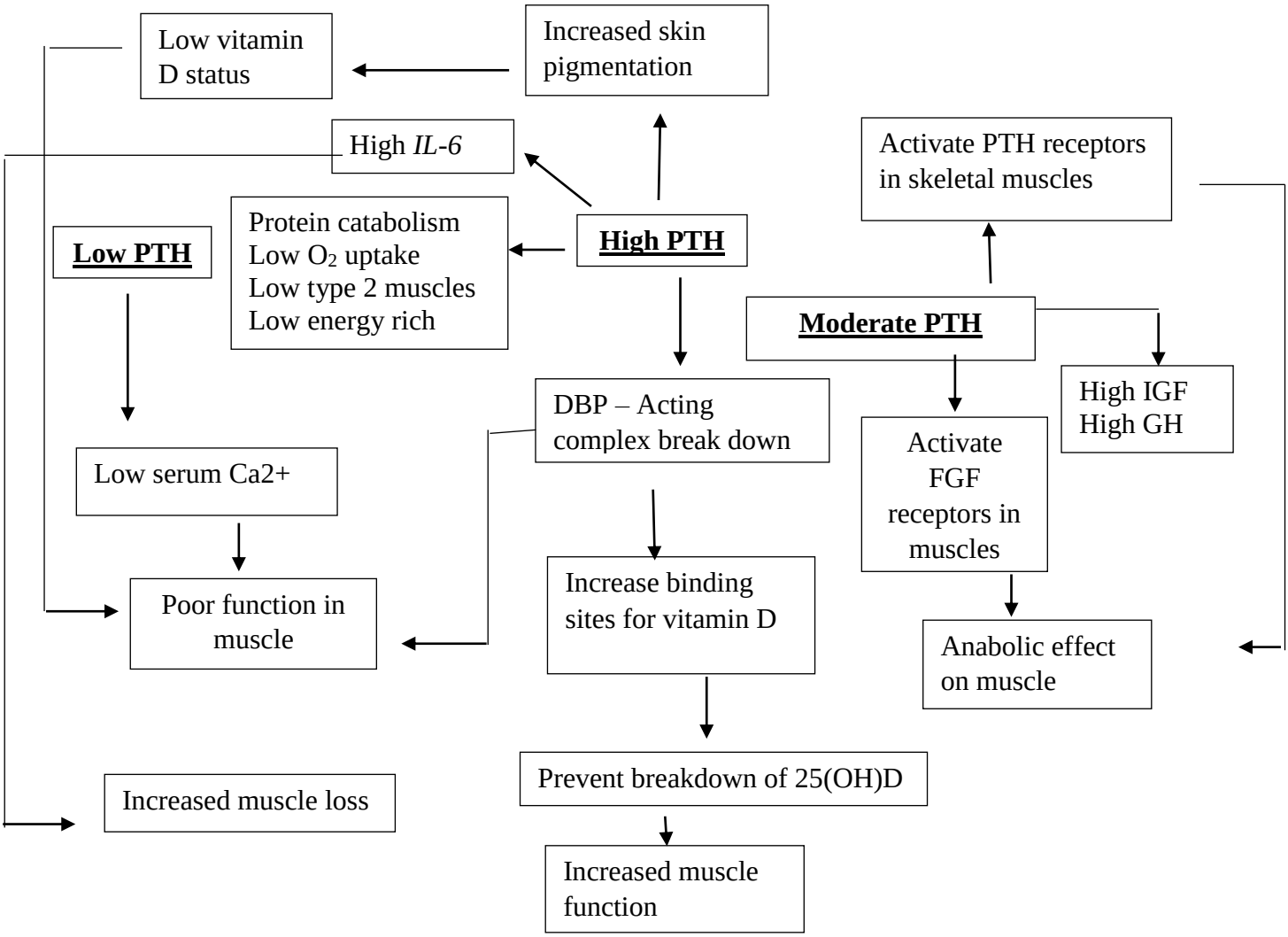


Figure 1.6 Conceptual diagram of the effect of PTH on muscle function (Adapted from Silva and Bilezikian, 2015)

1.27 Role of PTH in Bone Health

PTH induced bone resorption is mediated by increased activity of bone-resorbing cells called osteoclasts However, some studies indicated that PTH does not directly activate osteoclast, but PTH enhances bone resorption through its actions on osteoblasts and osteocytes (Xiong *et al*., 2012). The regulation of the remodeling process of bone is primarily through the osteoblasts, which express receptors for both PTH and $1,25(OH)_2D$. It is believed that PTH mainly has an indirect stimulation on osteoclasts by binding to neighboring osteoblasts. Elevated PTH levels induce an increased release of receptor activator of nuclear factor-kb ligand (*RANKL*), which binds to its receptor (*RANK*) on osteoclast precursor cells and thereby activate the formation of osteoclasts.

In younger and healthy individuals, increased activation of osteoclasts is normally followed by a balanced formation of new bone under the process of bone remodeling. However, this increased resorption of bone is seen in both trabecular and cortical bone in hyperparathyroidism and causes a temporary bone loss. But it is reversible if the remodeling cavities are refilled with new bone (Rolighed *et al*., 2014). The PTH-induced increase in bone turnover is probably the main reason for reduced BMD (Favus *et al.*, 2006). However, if the remodelling process is not balanced, the increased bone resorption leads to a catabolic state with a subsequent loss of bone mass (Heaney *et al*., 2009). Apart from that, PTH acts on bone cells to increase expression of *FGF- 23* (Silver *et al*., 2013). Thought it is believed that catabolic effect of PTH on the bone can be seen in hyperparathyroidism, PTH stimulates both bone resorption and bone formation, and the final outcome on bone mass, either catabolic or anabolic. This outcome is depending on the dose and periodicity of the PTH signals on bone tissues. Rolighed *et al*., 2014 mentioned that, continuous exposure to PTH resulted in catabolic effects on the skeleton, while intermitted low dose of PTH results in osteoanabolic effects (Silva and Bilezikian, 2015). Therefore treatment with PTH, in low dose has osteoanabolic effect and therefore used as a treatment for osteoporosis (Greenspan *et al*., 2007). The role of PTH in bone tissue is illustrated in Figure 1.5.

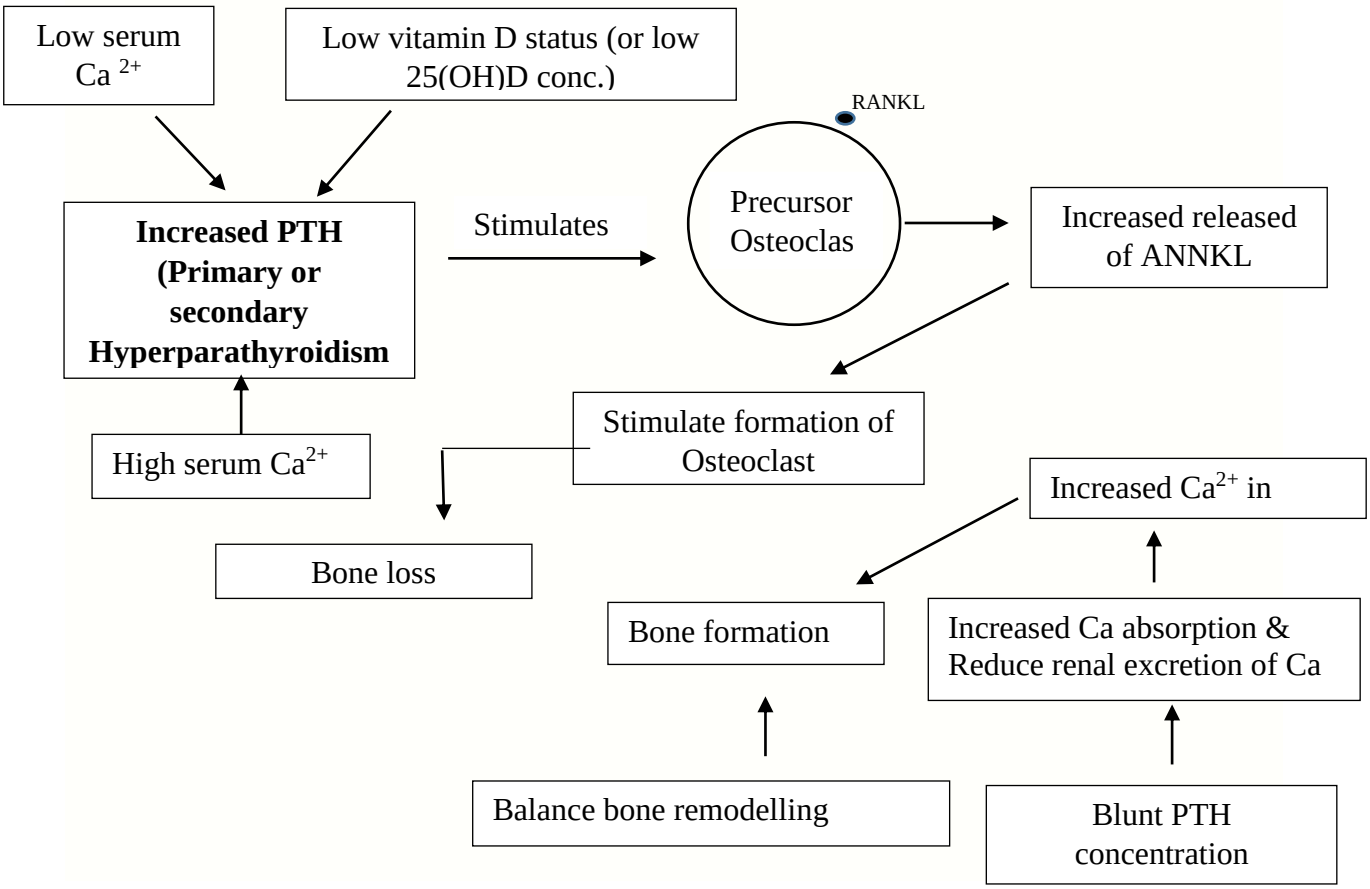


Figure 1.7 : Conceptual model illustrating the functional role of PTH concentration on bone health (Figure adapted from Silva and Bilezikian, 2015)

1.28 Hypotheses, Aims and Objectives

The association between serum 25(OH)D concentration and muscle function in older adults has been reported in previous studies with no agreement on what constitutes optimal vitamin D status for muscle health. The inconsistent findings between studies might be attributed to the different cut-off values used to define VDD and the insufficient control of potential confounders such as seasonality, dietary and/or supplemental intake of vitamin D, subject numbers, laboratory assay for assessing vitamin D status, age, body weight, body composition and PTH concentration. In this PhD study, I will explore the association between serum 25(OH)D concentration and MSK function in older adults living in the North East of England (an area with a high prevalence of VDD) in two different study designs whilst adjusting for several notable confounders. In terms of randomized trial evidence of vitamin D supplementation and muscle health there are conflicting findings in the literature regarding the efficacy of supplementation in improving outcomes. One of the major limitations of

many RCTs of vitamin D supplementation on MSK health is the general 'adequacy' of the recruited sample in terms of vitamin D status at baseline. Most trials to date have also focused on daily vitamin D supplementation where compliance has been problematic. In this PhD study, I aim to determine the effect of monthly oral supplemental vitamin D_3 (12,000 IU, 24,000 IU or 48,000 IU of vitamin D_3) for 12 months on muscle function in a large sample of 70+ years old adults (n = 379) from the North East of England.

Understanding the nature of the impact of sunshine exposure on vitamin D status is important not only because it influences dietary vitamin D requirements (Cashman *et al*. 2008; 2009) but also because achieved serum 25(OH)D concentrations in RCTs of vitamin D supplementation will be influenced by sunshine exposure as well as the vitamin D administered as part of trials. However, assessing personal sunshine exposure in studies is notoriously challenging due to the many factors influencing personal UV exposure. In this PhD I will attempt to estimate the influence of sunshine exposure on vitamin D status in both the cross sectional and RCT studies using sunshine exposure questionnaires adapted from previously published literature.

Whilst the role of PTH (as a biomarker of vitamin D status) in bone metabolism is well known, PTH might also influence pathways in muscle metabolism (as outlined in Figure 1.6 above) and may therefore influence muscle function outcomes including strength and function. Whether the putative relationship between PTH and MSK health is independent of vitamin D status remains unclear. Therefore, in this PhD I will attempt to shed some light on the relationship between PTH and muscle function in the two aforementioned studies. I will approach this question using cross sectional and intervention data.

Finally, given the established link between vitamin D and CRC (IARC, 2008), I will attempt to evaluate the association between vitamin D biomarkers and MSK function in older adults who are at moderate risk for CRC as well as examine the impact of sun exposure on vitamin D biomarkers in this population.

The hypotheses of this PhD study are:

1. Lower serum 25(OH)D concentrations will be associated with poorer MSK outcomes
2. Vitamin D supplementation at doses equivalent to 400 IU/day, 800 IU/day and 1600 IU/day for 12 months will result in improvements in muscle function in dose dependent manner.
3. Sunshine exposure assessed by newly adapted questionnaires will predict vitamin D status in the cross sectional analyses and will modulate the response to vitamin D supplementation in the RCT analysis.

4. PTH concentration will influence MSK outcomes in both cross sectional and RCT analyses.

The two study designs used in this PhD and discussed in more detail in Chapter 2 are:

i) A RCT of three doses of supplemental vitamin D in older adults (Vitamin D in Older People Study (VDOP) Study).

ii) A cross sectional study of older adults who participated in the BORICC Follow-Up (BFU) Study.

Objectives

The specific objectives of this project were:

1. to investigate the effects of three doses of supplemental vitamin D_3 (12000 IU, 24000 IU, 48000 IU) monthly for 12 months on markers of muscle function in older adults from VDOP study.
2. to quantify the contribution of cumulative sun exposure to serum 25(OH)D concentration among older adults supplemented with vitamin D_3 monthly for one year from VDOP study.
3. to investigate relationships between i) sun exposure behaviours and serum 25(OH)D concentration and ii) serum 25(OH)D concentration and MSK outcomes in participants in the BFU study.
4. to investigate relationships between PTH concentration, 25(OH)D concentration and MSK outcomes of older adults in both the VDOP and BFU studies.
5. to identify the change in serum 25(OH)D concentration and dietary vitamin D intake of older adults who are at moderate risk of CRC during the 12 year period from BFU study.

Chapter 2 Materials and Methods

Overview of research studies used in this PhD study and associated statement of my personal contribution

This PhD study, utilizes data collected from two studies

1) A single centered, double-blind, parallel RCT of three study groups that was designed to test the effect of three doses (12000 IU, 24000 IU and 48000 IU) of oral vitamin D_3 supplement on bone health in older adults (The Vitamin D in Older People Study; VDOP).
2) A 12 year follow-up study of people at moderate risk of colon cancer called the Biomarkers of Risk of Colon Cancer Follow-up Study (BFU study).

Further details of both studies are described in this chapter. In the VDOP study, I was involved in the data cleaning, coding and analysis of all data relating to muscle function. I led on the peer-reviewed publication arising from this thesis (Chapter 3; Ranathunga *et al*. 2019). The chief investigator of the VDOP study was Dr. Terry Aspray. Prof. Roger M. Francis, Prof. Inez Schoenmakers, Dr. Gail Goldberg, Prof. Elaine McColl and Dr. Ann Prentice were the co-investigators of the VDOP study. Christine Harle was the Chief Trial Manager, while Jennie Parker was the Assistant Trial Manager of the study. Tom Chadwick played the role of the statistician. Planning and implementing the study as well as applying for the ethical approval was done by Dr. Terry Aspray with the help of trial management team. The VDOP study was supported by Arthritis Research UK (Clinical Studies grant 19544) and also supported by the Medical Research Council, UK (MRC program no. U105960371).

In the BFU study I was centrally involved in participant recruitment, welcoming the participants, measuring muscle function, bone health parameters and anthropometric measurements, as well as contributed to obtaining ethical approval. I led on designing the sun exposure questionnaire. In addition to data entering and data analyzing I was also involved in conducting awareness events for the participants (called "showcase events"). Prof. John Mathers was the chief investigator of the BFU study. Dr. Mike Bradburn, Dr. Laura Greaves were the co-investigators. Post-doctoral fellow Dr. Fiona Malcomson was responsible in day-to-day management of the study with the help of four PhD students including me.

2.1 VDOP (Vitamin D in older People) Study

2.1.1 Study design

The VDOP study is a non-commercial, single centered, double-blind, parallel RCT of three study groups that was designed to test the effect of three doses (12000 IU, 24000 IU and 48000 IU) of oral vitamin D_3 supplement given each month on Bone Mineral Density (BMD) in men and women aged 70 years or older. Study visits took place at baseline and thereafter at 3-monthly intervals (5 in total) for one year period. Recruitment of participants began in November 2012 and was completed in May 2013 in Newcastle upon Tyne (55^0N). The first participant visit was on 8th November 2012 and the last participant visit was on the 6th June 2014. Findings on the effect of vitamin D supplementation on BMD, which was the primary aim of this study was published elsewhere (Aspray *et al*., 2018).

2.1.2 Participant recruitment

A total of 379 community-dwelling men and women aged 70 years or older, resident in Newcastle upon Tyne, were recruited. Potential participants were identified through General Practice (GP) registers at specific surgeries. At initial check, participants' age was checked to confirm that they were 70 years or older and the other inclusion criteria listed below under 2.1.3. The consort diagram of the VDOP study is illustrated in Figure 2.1. Invitation letters (total of 7726) were sent to potential participants with the Participants Information Sheet (PIS) and the contact details of the research team at the Clinical Aging Research Unit (CARU). When potential participants contacted the CARU, they were given an expression of interest slip to return to CARU if they interested in taking part in the study. In addition, posters were displayed in waiting areas of the North Tyneside General Hospital (NTGH). Patients who were interested in taking part in the study were asked to speak to their GP who provided them with the PIS and the expression of interest slip if they appeared to be eligible to take part in the study. Once the potential participant had returned their expression of interest slip, a member of the research team contacted the participant by telephone to inquire about the individual's medical conditions and to further check eligibility for the study. If the participant met the inclusion criteria, they were invited to attend the initial screening study visit at CARU. Out of 548 participants who expressed the interest in participating in the study, 522 participants were telephone screened and 433 of them were eligible for the study. Of the eligible participants, 392 participants provided the informed consent. These eligible participants were screened further to check the inclusion and exclusion criteria, however, 13 older adults failed the screening test as they did not meet the inclusion criteria. Therefore 379 participants were randomized to receive one of three doses of vitamin D_3 supplement, 12000

IU (n=126), 24000 IU (n=125) and 48000 IU (n=128) vitamin D_3, respectively, monthly for a 12 months period.

2.1.3 Inclusion criteria

Ambulatory community-dwelling men and women 70 years or older and the individuals who able to give written consent by their own and the individuals who willing to visit to the study centre for six times and the individuals who can be contacted by telephone at monthly intervals between study visits over twelve months were considered as the inclusion criteria for the study. The inclusion criteria were mainly focused on age and the ability to participate in the study. If any of the above conditions were not met, the participants were not eligible to participate in the study. If any of the participants was not eligible to take part in the study, "Participant Withdrawal Form" was completed, mentioning the reason for the exclusion.

2.1.4 Exclusion criteria

Exclusion criteria comprised, taking vitamin D supplements at a dose greater than 400 IU/day or calcium at a dose greater than 500 mg/day, a fragility fracture within the previous 6 months, treatment with an anti-resorptive or anabolic treatment for osteoporosis in the previous three years, a history of renal stones, previous hip replacement or primary hyperparathyroidism, hypercalcaemia (albumin adjusted plasma calcium >2.60 mmol/L), hypocalcaemia (albumin-adjusted plasma calcium < 2.15 mmol/L) or an estimated glomerular filtration rate (eGFR) less than 30 ml/min/1.73 m^2). Participants who had multivitamin or supplement containing vitamin D (<400 IU/day) or calcium (<500 mg/day) were not asked to discontinue using it.

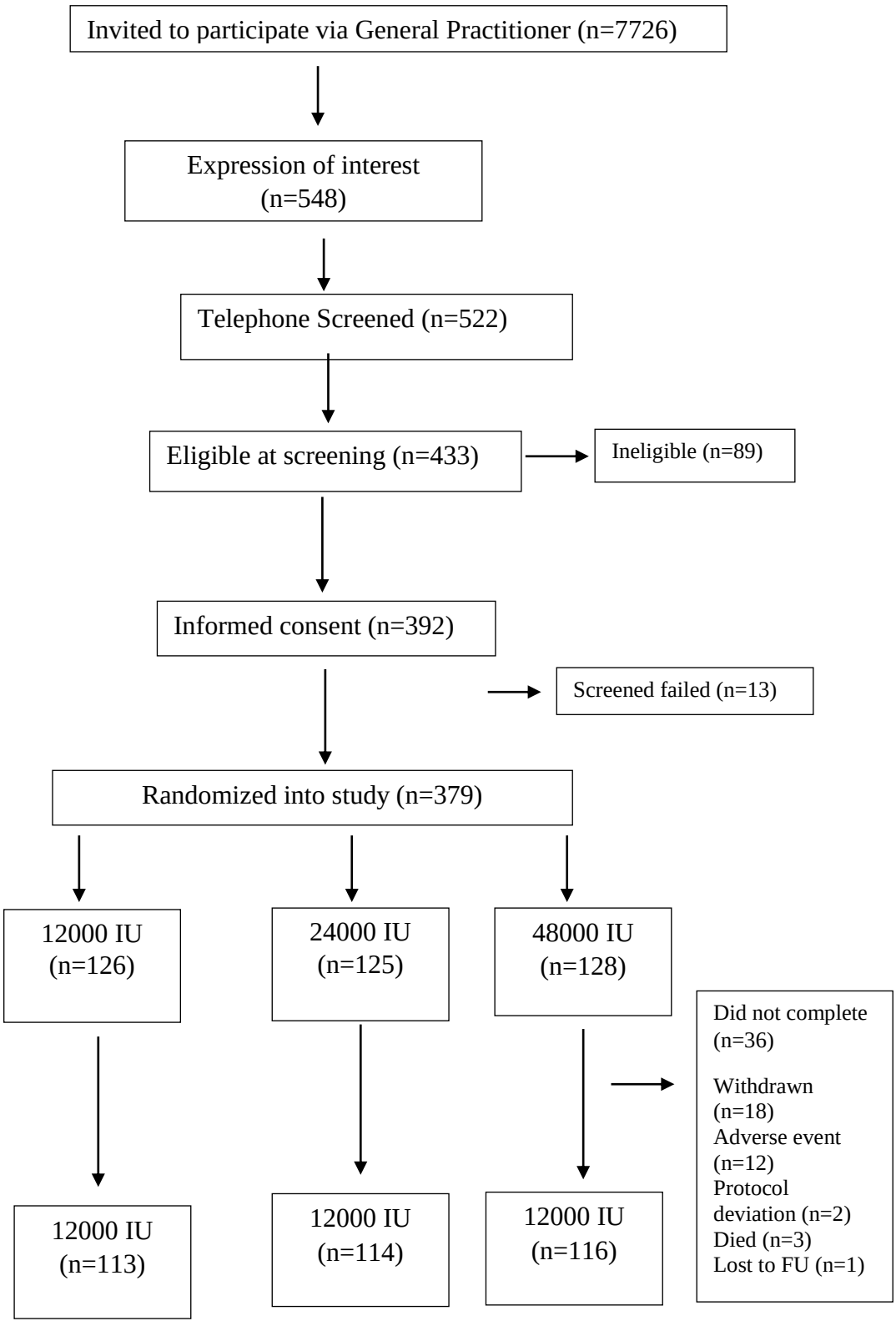


Figure 2.1 : Consort diagram of the VDOP study

2.1.5 Ethical consideration

The favorable opinion was obtained from Tyne and Wear South Research Ethics Committee (REC, 12/NE/0050) and the Research and Development approval was obtained from the sponsor, Newcastle upon Tyne Hospitals NHS Foundation Trust. All study procedures were carried out according to the recommendation which was adopted by 18th World Medical Assembly, Helsinki 1964 and later revisions. Prior to the enrolment into the study, informed consent was obtained from each participant from their legally acceptable representative with the date of receiving the consent. For the participants who could not consent for themselves, an appropriate independent witness provided the written consent. Favorable ethical opinion and Clinical Trial Authorization from relevant Competent Authorities were taken prior to the commencement of the study. Local approval was taken before recruitment of the study participants. A written copy of local approval documentation was provided to the Newcastle Clinical Trials Unit before initiating the study. Personal data was regarded as strictly confidential. To preserve the anonymity, all data including the biological samples were identified by a unique study identification number. The study complied with the Data Protection Act, 1998. All study records and Investigator Site Files were kept at the site in a locked filing cabinet with restricted access.

2.1.6 Consent procedures and confidentially

A discussion about the informed consent took place with the trained staff member with the participants. The participants were given an opportunity to ask questions about the study. After providing the information about the study, participants were given a minimum of 24 hours to decide whether or not to participate in the study. Those wishing to take part in the study were provided a written signed and dated informed consent, which was witnessed and dated by a member of the research team. Written informed consent was obtained from the participant prior to the randomization and the study procedures. The original signed consent form was retained in the Investigator Site File, with a copy in the clinical notes and another copy provided to the participants. The participants provided the consent to their GP being informed of their participation in the study. The right to refuse to participate in the study without giving reasons was respected and the health care that they received was not altered for not being participated in the study.

2.1.7 Vitamin D supplementation

Three doses of vitamin D supplementations were 12000 IU once monthly (equivalent to 400 IU/day), 24000 IU once monthly (equivalent to 800 IU/day) and 48000 IU once monthly (equivalent to 1600 IU/day) for one year period. The lowest dose was chosen to be equivalent

to the Recommended Nutrient Intake (RNI) defined by the Scientific Advisory Committee on Nutrition (SACN), 400 IU (10µg) per day (SACN, 2015), the second dose corresponds to the North American RDA for adults aged 70+ years, defined by Institute of Medicine (IOM), 800IU/day and the highest dose was twice the RDA defined by IOM (and four times the UK RNI), which was below the tolerable upper intake of 4000IU/day, declared by IOM (IOM, 2011). These monthly doses of the supplement were given for a 12 month period. Vitamin D supplementation was administered orally under the direct supervision of the nurse at baseline, second (after 3 months of supplementation), third (after 6 months of supplementation) and fourth (after 9 months supplementation) study visits at the study center. At the end of each study visit, 2 bottles of the vitamin D liquid for the next two months to take at home were given along with written instructions. The administration of vitamin D at home was unsupervised, but the participants were contacted by telephone each month to remind them to take the vitamin D supplements, as well as to check whether the participants had missed any vitamin D supplements and to know the date of the supplements taken and to report any adverse events.

All vitamin D doses were filled in 10 mL amber glass bottles (target fill volume of 3 mL including 0.6 mL overage). The formulation, manufacture and release of the vitamin D were undertaken by Swedish clinical supplies company licensed by for the manufacture and release of vitamin D for clinical trial use in the EU (MODE Pharma, UK). The vitamin D_3 preparation was a liquid, containing an appropriate dose of vitamin D_3 (Vigantol) solution in a concentration of 20,000 IU/mL mixed with Miglyol oil to a total volume of 3.0 mL, Miglyol oil contains no vitamin D and is indistinguishable from Vigantol. Both Vigantol and Miglyol were provided by MODE Pharma, UK, a clinical trials supply company, who sourced the product and provided from the third party. Randomization group 1 received 0.6 mL Vigantol and 1.8 mL Miglyol oil, group 2 received a mixture of 1.2 mL Vigantol and 1.2 mL Miglyol oil, group 3 received 2.4 mL Vigantol oil. Side effects were documented by the participant throughout the course of the trial and assessed by the clinical team at each telephone conversation and at the study center. Patients returned all vitamin D bottles in their original packaging to the research team once they come to the study center. Documentation of prescribing, dispensing and return of vitamin D_3 was maintained for study records. Case Report Forms (CRFs) (Appendix A) were checked for missing data and the reason for the missing data was recorded. Concomitant medication and therapies were updated in CRFs including the medication, dosage and the reason for the use. When the individuals were withdrawn from the study, “Participants withdrawal form” was completed with the reason for withdrawal stated as “screen failure”, “withdrawn by the investigator”, “participant decided to

withdrawn" with the signature of chief investigator. First date and the last date of the treatment were recorded in the CRF. Once the trail is finished, "trial completion form" was completed, with the first and the last date of the treatment, whether the person completed the trial or not and the reason for the discontinuation of the trial or date of death.

2.1.8 Study safety and quality control

Adverse events (AE), serious adverse events (SAE) and suspected unexpected serious adverse reactions (SUSAR) were recorded throughout the study. Those adverse events were recorded with the severity that indicated by a code (1 mild, 2 moderate, 3 severe), the relationship to the treatment, start date, end date of the adverse event and the action taken for the adverse event. In addition, the safety of the supplementation was assessed by performing full blood count, plasma calcium, serum creatinine and liver function tests at each study visit. Information on clinical fractures during the study also was recorded as a safety measure. The doses used in this study had not previously been reported to be associated with the adverse events and even the highest dose of vitamin D_3 used in this study was less than half the tolerable upper limit of intake advised by the United States Institute of Medicine (Ross, 2011). As vitamin D was given without additional calcium supplementation, a significant risk to participants was even less likely. Quality control was maintained through adherence to the Newcastle upon Tyne Hospitals NHS Foundation Trust Standard Operating Procedures (SOPs), study protocol, the principles of Good Clinical Practice (GCP), research governance and clinical trial regulations. An independent data monitoring and ethics committee which consisted of 2 physicians (not connected to the trial) and one statistician undertook the independent review to monitor the efficacy and safety endpoints of the trial. Only the DMEC had access to un-blinded study data. A Trial Steering Committee (TSC) was established to provide overall supervision of the trial. The TSC consisted of the principal investigator, study collaborators, a lay member from the advisory group of National Osteoporosis Society, a clinician with the expertise of vitamin D therapy who acted as independent chair of the TSC. This committee provided the overall supervision of the study on behalf of the study sponsor and study funder to ensure that the study was rigorously conducted in accordance with the principles of GCP.

2.1.9 Randomization

Randomization was done at the baseline visit and the randomization number was recorded in the CRF. Patients were randomized in a 1:1:1 ratio to receive vitamin D_3 12000 IU, 24000 IU or 48000 IU. A statistician who was not involved in the study, produced a computer-generated allocation list that included a sequential study number that linked to a randomly allocated

treatment arm of the study. This number was kept blind from both the participants and investigators. Each bottle of vitamin D supplements had a unique identification number, and each patient was assigned one of these study numbers. Once a person enrolled in the study, a study number was allocated sequentially.

2.1.10 Screening study visit

A screening study visit was conducted within 7-28 days after the recruitment and 2 weeks prior to the baseline study visit. All the study visits were conducted at CARU at the CAV, Newcastle University. At the screening study visit, participants were instructed to provide informed consent and to provide a "screening safety blood samples" to confirm the eligibility and the safety to take part in the study. In addition to collecting fasted blood samples and urine samples, demographic information, details about QOL were collected. Further, physical examination that measured Waist Circumference (WC), Hip Circumference (HC), TUG, GS test and the body composition was carried-out. At the screening visit, inclusion criteria and exclusion criteria were further checked and all details were recorded. Individuals who did not meet the inclusion criteria for the study, were excluded and the reason for not recruiting was recorded in a separate form. Potential participants were allocated a screening number and they were identified using this number throughout the study.

2.1.11 Baseline study visit (study visit 1)

A baseline study visit was conducted approximately within 2 weeks after the screening visit. At this visit, sun exposure details of the last three months, demographic information, food consumption data and use of vitamin D supplements were gathered using questionnaires. Food Consumption data was gathered using CalQuest Food Frequency Questionnaire (FFQ) which was designed to assess habitual vitamin D and calcium intake only. The validity of this questionnaire was tested using two commonly used standards of dietary assessment methods, five-day duplicate diets and seven-day weighed dietary inventories (Nelson *et al*., 1988).This questionnaire consisted of 28 food groups including questions on the type of foods consumed eg milks, yoghurts, cheeses, breads, spreading fats and sources of water consumed. In addition to the weight and height measurements, TUG and GS were also measured the study visit. Further, fasted study blood samples and urine samples were collected from the participants at the study center in the baseline study visit (Figure 2.2). Administration of the first dose of vitamin D supplement was done at the study center with the direct supervision of the nurse.

2.1.12 Study visits 2, 3, 4 and 5

Details relating to study visits are presented in Figure 2. After three months of vitamin D supplementation, all participants were invited for the second study visit to gather information about sun exposure, dietary intake, to perform muscle function tests, to collect blood samples and check the medical condition along with the Adverse Events (AE) of vitamin D supplementation. After 6 months of vitamin D supplementation, all participants were invited for the third study visit to gather the above information including the body weight, Fat Mass (FM) and Fat Free-Mass (FFM). Similarly, after 9 months and 12 months of 1st vitamin D supplementation, participants were asked to visit the study center for the 4th and 5th study visit to gather the same information as previous study visits.

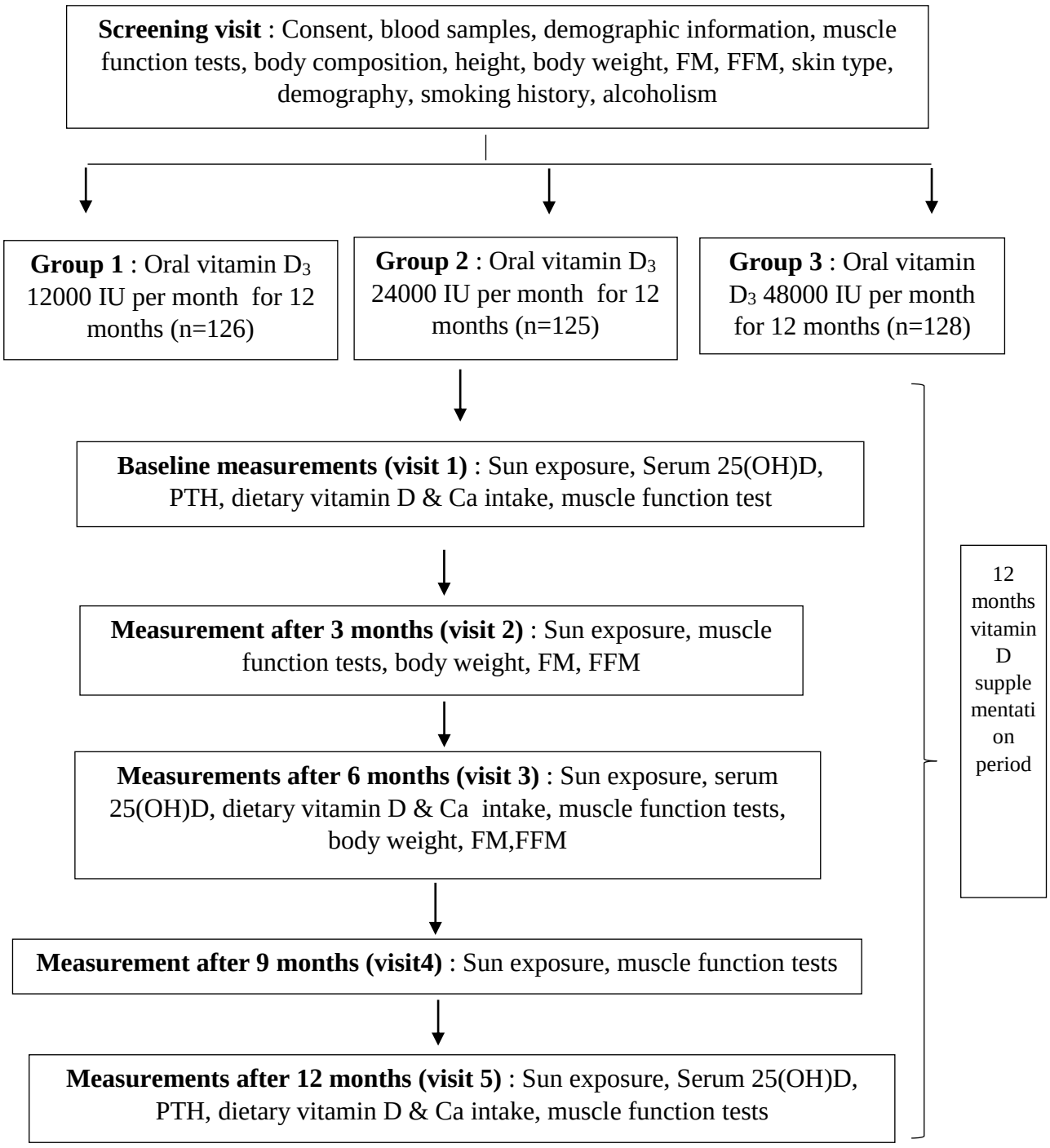


Figure 2.2 : Outcome measures at all five study visits in the VDOP study

2.1.13 Funding and sponsorship

This trial was supported by a Clinical Studies Grant (MP/ID19544) from Arthritis Research UK. This work was partly supported by Newcastle University and funding from the core programme of the MRC Nutrition and Bone Health Group at MRC Human Nutrition Research funded by the UK MRC, grant code U10596037. Arthritis Research UK was not involved in the analyses or in the decision to publish these results from the trial. The Newcastle upon Tyne Hospitals NHS Foundation Trust was the sponsor for this study. The sponsor was

responsible for the conduct of the trial according to guidelines declared by GCP, Global Research Partnership (GRP), the Data Protection Act and the Declaration of Helsinki. The sponsor was also responsible for Pharmacovigilance. These responsibilities were delegated to the Principal Investigator Dr T. Aspray and Newcastle Clinical Trial Unit (CTU). The sponsor did not play any role in the design, implementation, analysis or interpretation of the data of the study.

2.2 The Biomarkers of Risk of Colon Cancer (BORICC) Study

2.2.1 Study design

The Biomarkers of Risk of Colon Cancer (BORICC) study was a cross-sectional study conducted in 2006 with the objective of developing and validating novel biomarkers of bowel cancer risk and to determine their relationships with habitual diet and nutritional status.

2.2.2 Participant recruitment

Ethical approval for the BORICC study was obtained from the Northumberland Local Research Ethics Committee in 2005 (Project reference 04/Q0902/6). Community-dwelling men and women aged 17 – 78 years were recruited to this study in 2006 via the gastroenterology outpatient clinics at Wansbeck General Hospital, Ashington, Northumberland. All participants had been referred to the hospital for investigation of gastrointestinal symptoms and underwent screening by flexible sigmoidoscopy. Those in whom no gastrointestinal pathology including colorectal neoplasia, colonic inflammation, diverticulae or familial predisposition to colorectal CRC was found, were discharged as "nothing found" and were recruited to the BORICC 1 Study (healthy participants, n-268). In addition, a further group of participants with adenomatous polyps (premalignant lesion associated with increased CRC risk were recruited to the BORICC 2 Study (polyp patients, n=95). For both BORICC 1 and BORICC 2 studies, invitation letters were sent to patients in advance of their outpatients' visit for a flexible sigmoidoscopy and written informed consent was obtained before recruitment to the study.

2.3 The BORICC Follow-Up (BFU) Study

2.3.1 Participant recruitment

After approximately 12 years, participants in the original BORICC were invited to participate in the BORICC Follow-Up (BFU) Study. The primary objective of the BFU study was to identify the effect of aging on biomarkers of bowel cancer risk. The secondary objectives were to investigate the associations between lifestyle factors, particularly the diet, physical

activity and obesity, and a panel of mucosal molecular biomarkers linked with colorectal cancer risk. In addition, The BFU Study investigated i) relationships between vitamin D status and musculoskeletal function of older adults and ii) the effect of sun exposure on vitamin D status of older adults. Participant recruitment to the BFU study was undertaken between May 2017 and May 2018 at NTGH, North Shields, Northumberland. Potential participants from original BORICC Study 1 and 2 were identified by screening for inclusion using the Single View patient information database available via the NTGH. Invitation letters, including the study information sheet (Appendix B) were sent in batches of 20 letters, approximately 6 weeks prior to the proposed study visit. Invitation letters were re-sent to those BORICC Study participants who did not respond. Individuals who interested in taking part in the study were asked to contact the research team by telephone calls or by email. During the telephone calls, participants were given the opportunity to discuss the study and raise their queries if they had. Then one member of the research team completed the "BFU Participant Interest Form" (Appendix C), which included the date of calling, participant's name, contact details, dominant hand, along with the checklist of participants understanding about the study, presence or absence of dementia, use of medications (warfarin, rivaroxaban, apixaban, clopidogrel, dipyridamole) and participants ability of visiting the study center. If possible, the hospital study visit was arranged at least two weeks after the date of the telephone call or email, allowing time to send the study pack (Figure 2.3). Alternatively, a possible date was arranged and the participants were contacted later to inform the study visit day. If the participant requested further information about the study, they were invited to participate for the "show-case" event which was a one-hour session that described the all aspects of the study. After the "show-case event", subjects were given a date for the study visit, if they expressed their willingness to take part in the study at the "show-case" event. Otherwise, they were contacted by the research team later to inquire about the willingness to take part in the study.

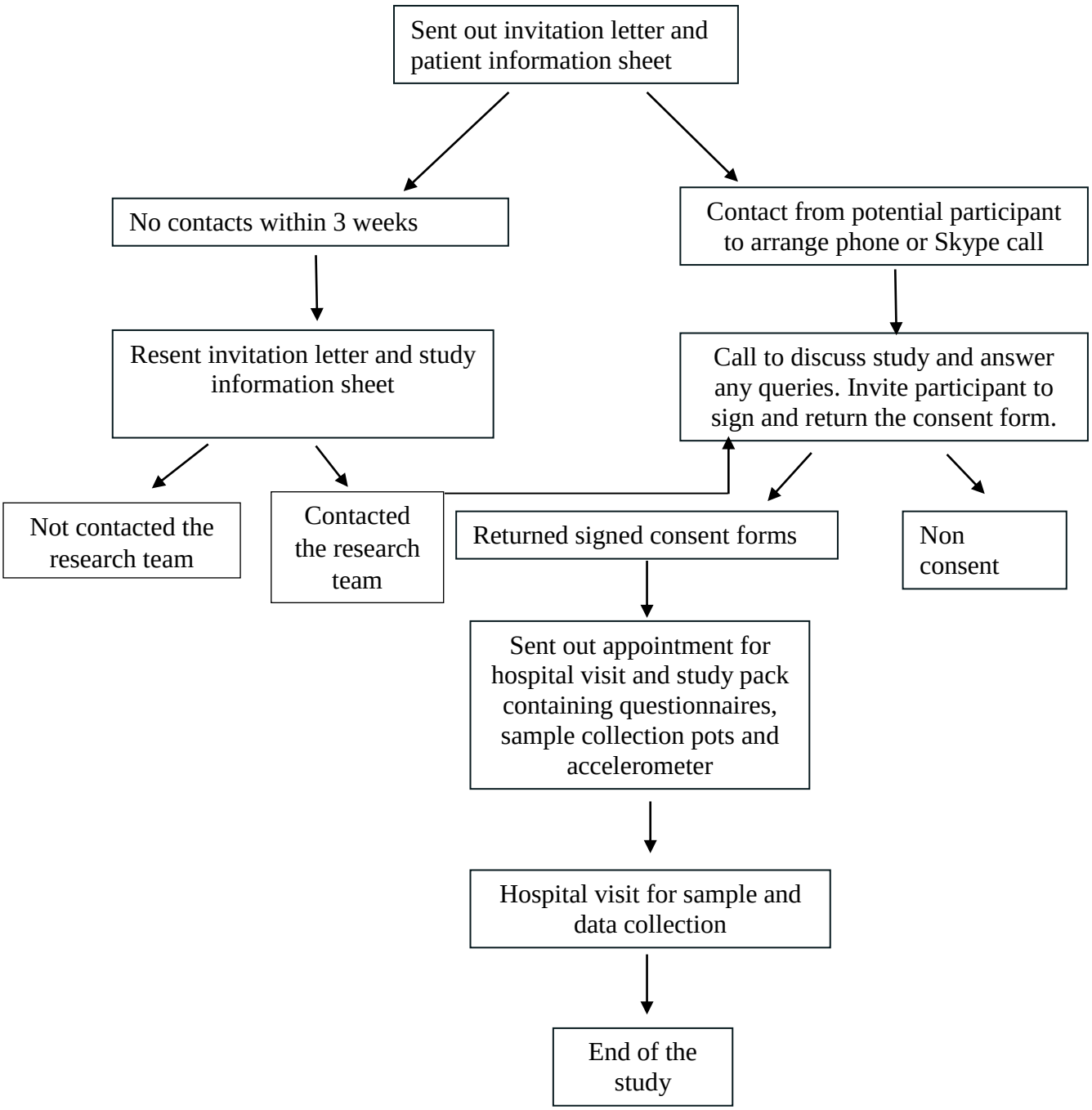


Figure 2.3: Participant recruitment procedure for the BFU Study

Out of total BORICC study participants (363), 48 the participants diet during 12 years follow-up period. In addition, 4 participants were excluded as they met the execution criteria of having Dementia. Therefore the total number of potential participants for the BFU study was 311. Invitation letters were sent to all potential participants. A total of 55 participants replied for the invitation letters expressing the interest to take part in the study, while 10 letters were not delivered because of wrong address. From the 55 who expressed interest, 47 participants were recruited to the BFU Study and all of them completed the study (Figure 2.4).

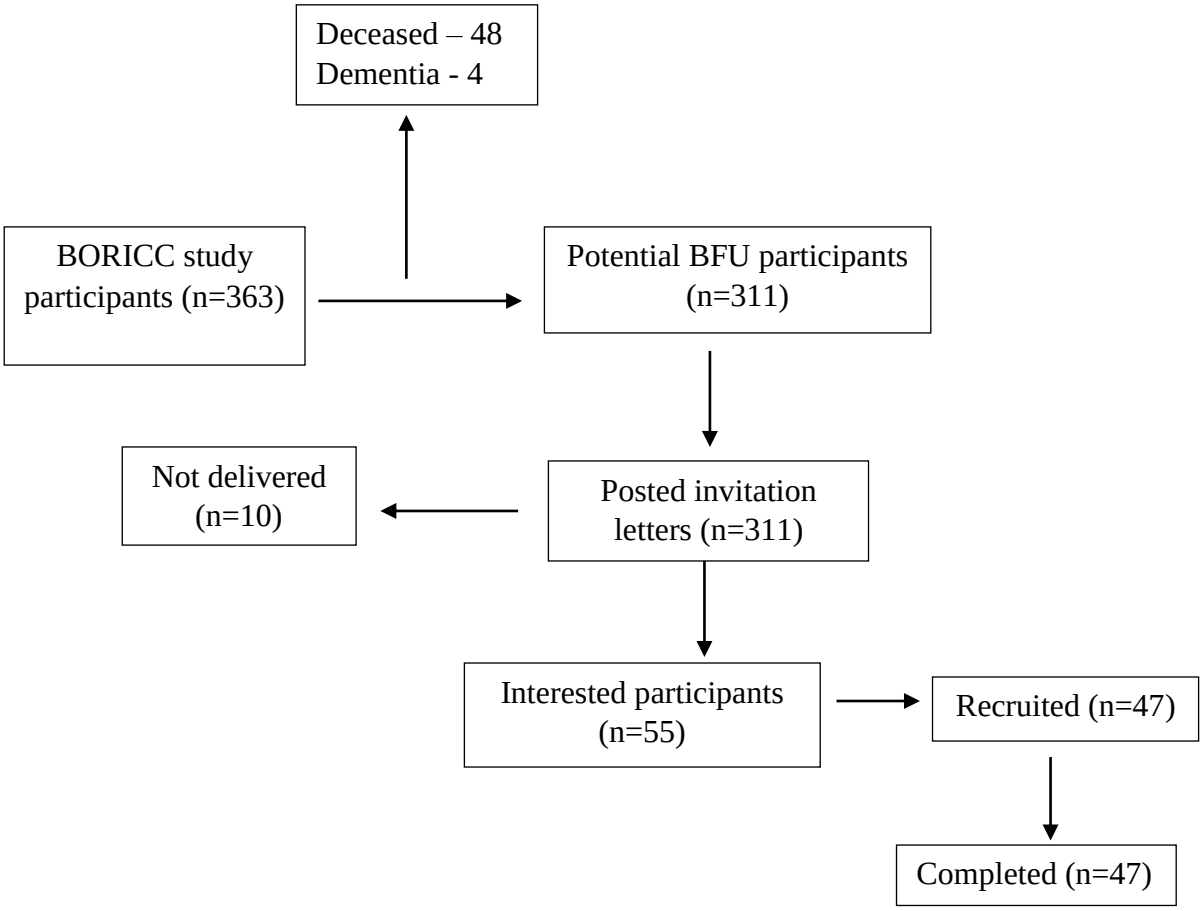


Figure 2.4 : Participant recruitment for the BFU Study

If the member of the research team was not available to answer the telephone call, "BFU potential participate call log" (Appendix D) form was filled, with the date, name of the caller, telephone number and the best time to contact them by a member of the research team. During the call, if the participant expressed willingness to take part in the study, they were asked to send the signed consent form to study center in the given stamped envelope. Once recruited, participants were given an identification number and all data were recorded under this number. Study data were stored anonymously in password-protected computer in a locked cabinet with the security pass-protected building in the Medical School, Newcastle University. The study pack consisted of study instructions, three copies of consent forms, hospital study visit instruction, direction to the study center, FFQ, lifestyle questionnaire, sunlight exposure questionnaire, accelerometer instructions, accelerometer record sheet, sleep log, stool collection instruction, urine collection instruction, Home Samples and Questionnaire Collection Record Sheet (checklist for all samples and questionnaire completion), accelerometer in bubble wrap bag, stool collection Fecotainer, cool bag for stool

collection, cool block for stool collection, two urine collection pots, urine collection tubes that separated into two sets of four. Before participants visit the study center they were asked to fill “at home sample and questionnaire collection record sheet” (Appendix E) to ensure that they have collected all samples and fill all the questionnaires.

Study packs were posted through the first class post in brown envelope. However, procedures of urine samples and stool samples collection and processing were not described in this particular PhD thesis as those details are out of the scope of this PhD study.

2.3.2 Ethical consideration

Ethical approval for the BFU Study was granted by the West Midlands - Coventry & Warwickshire Research Ethics Committee on 29th November 2016 (REC No. 16/WM/0424). Two amendments were made to the study; the first to include a response card and invite potential participants to Showcase events described below (approved May 2017) and the second to call any participants not returning their response cards to check whether they had received the study invitation letter (approved December 2017). Approval for the storage of data was provided by the Northumbria NHS Foundation Trust. All study procedures were conducted according to the guidelines laid down in the declaration of Helsinki.

2.3.3 Inclusion and Exclusion criteria

All individuals who participated in the original BORICC study 1 and BORICC study 2 were potentially eligible to participate in the BFU Study. Exclusion criteria included, actively undergoing chemotherapy or radiotherapy treatment, being unable to provide informed consent or being unable to attend the study center at NTGH.

2.3.4 Funds and sponsorships

The BFU Study (recruitment and sample collection) was funded by the MRC as part of the Centre for Ageing & Vitality (Ref: MR/M501700). Sponsorship for the BFU Study was provided by the Northumbria NHS Foundation Trust.

2.3.5 Study visits

Each participant was asked to visit the study centre only once at ward 1, NTGH. Two rooms were allocated to get body measurements and clinical measurements from the participants, separately. One member of the research team welcomed the participants at the entrance of the first room and introduced all members of the research team. At the first room all questionnaires that filled by the participants were checked for missing or incomplete details. If there were any incomplete or missing data, the member of the research team corrected those after having a discussion with the participant. Out of three copies of the consent form, one

copy was given to the participant after further confirming the willingness to take part in the study. Other two copies were kept in the file with participants' details and their questionnaires. In addition the cool bag with a stool sample, urine samples and accelerometer were collected from the participant in the first room. Then body weight, height, WC, HC, body fat percentage, muscle function tests were measured in the same room. After that a medical doctor asked the medical conditions and medication history from the participant and filled the "Patient Health History Questionnaire" (Appendix F). After giving the forms to claim the travelling expenses, the participant was sent to the second room. The second room was a room with a clinical bed that facilitate the venipuncture and rigid sigmoidoscopy procedure. Collection of 30 mL of blood samples, Buccal cells sample and ten biopsy samples were done by the practiced medical practitioner in the second room. Participants were given aftercare advice sheet after the whole procedures. All information and samples were collected during one study visit which lasted for approximately 1 hour. Participants were not given any payment for participating in the study except the travelling expenses.

2.4 Data collection

2.4.1 Demographic information

VDOP study : Date of birth, gender, ethnicity (white British, white other, black African, black other, other), smoking history including the average number of cigarettes and the age that they started the smoking and the alcohol consumption with the average number of units were recorded in a questionnaire (Appendix A).

BFU study : Data on age, gender, ethnicity and the date of birth were obtained using the questionnaires sent to participants in their study pack. Index of Multiple Deprivation (IMD) was used to identify the socioeconomic status.

2.4.2 Medical history

VDOP study : At the screening visit and at each study visit the physical examination was done. Details about the medication use, and the usage of vitamin D supplements were also identified monthly by telephone calls or by face to face discussion at the study center. During the intervention period, any changes to the participant's medication were recorded including the type of the medication, total dosage, frequency, route of administration, start date and the end date.

BFU study : Past medical history of the participants were collected by face to face discussion with the participants using a short "Patient Health History Questionnaire" (Appendix F) by the medical practitioner at the study center. Presence or absence of selected disease

conditions, especially the non-communicable diseases listed in this questionnaire (diabetes, heart diseases, osteoporosis, diabetes, blood pressure, cancer) was checked and recorded after discussing with the participant.

2.4.3 Sunlight Exposure

VDOP study : Interviewer administrated sunlight exposure questionnaire was used to gather the information about habitual sun exposure of previous three months (Appendix G). Sun exposure questionnaire was adopted from Macdonald *et al.*, 2013. According to Macdonald study, sun exposure was measured using sun exposure questionnaire and they obtained a significant correlation between sun exposure score and serum 25(OH)D concentration. Further, they have used multiplicative sun exposure score to assess the sun exposure which was adapted from Macdonald *et al*., 2008. However, the sun exposure questionnaire used in the present study was not validated. These details were gathered at all study visits (at baseline, at 3rd month, at 6th month, at 9th month and at 12th month). The questionnaire consisted of questions about the frequency of sun exposure, parts of the body exposed to sunlight, use of sunblock, part of the body that applied sunblock, use of sunbed, details about the holiday visits. Participants were asked these details separately for last month, the last 2 months and for last 3 months. Fitzpatrick skin type (Fitzpatrick, 1988) of the participants was recorded at the screening visit based on the following five skin types (Table 2.1).

Table 2.1 Fitzpatrick skin types classifications

Skin types	Description
Type I	Burns easily, never tans, white, very fair, red or blond hair, blue eyes, freckles
Type II	Burns easily, tans minimally, white, fair, red or blond hair, blue, hazel, or green eyes.
Type III	Burns moderately, tans gradually, cream white, fair with any eye or hair color, very common.
Type IV	Burns minimally, tans well, brown, typical Mediterranean Caucasian skin.
Type V	Rarely burns, tans profusely, dark brown, mid-eastern skin types

Source : Fitzpatrick, 1998

BFU Study: A Sun Exposure Questionnaire based on that developed by Macdonald, 2013 was used to gather the information about sun exposure habits during last year. This

questionnaire included the details Fitzpatrick skin type (I to VI), frequency of sun exposure, duration of sun exposure, sun avoidance habits, clothing style, frequency of wearing less clothes, use of sunblock, use of sunbeds, details about mountain climbing and skiing and details about holiday visits (Appendix H). This questionnaire was pre-tested using 10 university students and relevant amendments were done before sending it to the participants.

2.4.4 Dietary intake

VDOP study : CalQuest Food Frequency Questionnaire (FFQ) which was designed to assess only vitamin D and calcium intake was used to gather information on food intake over the previous year. The validity of this questionnaire have been tested using two commonly used standards of dietary assessment methods, five-day duplicate diets and seven-day weighed dietary inventories (Nelson *et al.*, 1988). This questionnaire consisted of 28 food groups and the questions on the type of milk, fat spread and sources of water consumed (Appendix I). Food and drinks belongs to several food groups such as beverages, milk and milk products, cereals and cereal products, nuts and seeds, beans and pulses, vegetables, eggs, meat and meat products, fruits and drinking water are the listed foods and drinks were listed in the questionnaires. Participants were asked to record the consumption frequency of listed foods in the questionnaire that were commonly consumed calcium and vitamin D rich foods in the UK. This FFQ was used to gather the information about dietary intake at the screening visit and subsequently during 3rd and 5th visits. Participants were given the questionnaires at screening visit to complete on their own at home and bring to the study centre. Incomplete data were filled at the study centre by a member of the research team.

BFU Study*:* Details about habitual diet were collected using a slight modification of the validated FFQ employed by the EPIC Study (Bingham *et al*., 1997). The same questionnaire was used to assess dietary intake of participants in the BORICC Study at the baseline (12 years ago) (Appendix J).

2.4.5 Muscle function tests

Hand Grip Strength Test (GS)

GS was used to measure upper body strength using a portable hydraulic hand dynamometer (Jamar, 12-0240, SN 04201084, USA) in both the BFU study and the VDOP study. At first, a member of the research team explained the procedure for assessing the hand grip strength to the study participants including the purpose of the measurement. Then the dominant hand of the participants was recorded in the "Physical Capability Record Sheet" (Appendix K). Further, details about any physical and clinical problems in hands that the participants reported were recorded in the record sheet before performing the test. Next participants were instructed to sit on an armchair resting forearm with the elbow bent at a 90-degree angle on the arm of the chair, holding the dynamometer (Figure 2.5).

Figure 2.5 : Use of portable hydraulic hand dynamometer during measuring the GS (Source : IPTC photo metadata)

Finally, the participant was asked to squeeze the handle of the dynamometer using his/her maximum strength following the command "3, 2,1 and squeeze". When the red needle of the dynamometer stopped moving, the participant was asked to stop squeezing. The value displayed on the dial was recorded to the nearest round number. This test was performed three times for each hand alternatively. Participants were given the opportunity to have a practice turn before taking the actual measurements. For most participants, the grip handle in the handgrip dynamometer was set to level 2 but if the participants had wide hands, the handle was set to the level 3.

Timed-Up-and-Go test (TUG)

TUG is a widely used measure of mobility in older people. Lower the total time to complete the TUG is associated with poor motor function and the variety of adverse health outcomes associated with ageing (Weiss *et al*., 2010). In both the VDOP and BFU studies TUG test was used to measure the lower body muscle strength. For this test, a chair with the standing height of 40-50 cm from the ground and a stopwatch were used. A line was marked on the cement floor with 3 m distance apart from the chair (Figure 2.6). First, the member of the research team explained the procedure of the test to the study participants including the purpose of the measurement. Then the participants were asked to sit on the chair with both feet on the ground and placing the arms on the thighs. After that, the participants were asked to stand from the chair without helping his/herself with the arms, jumping or leaping. Next the participants were asked to walk to the mark on the floor in normal day to day speed once the command was given as 3.2,1 and go. They were asked to stand once the researcher said "go". Then they were asked to turn around the mark and walk back to the chair and sit on the same chair without helping themselves (Figure 2.7). The stopwatch was started as soon as the person left the chair and stopped as soon as the person sitting on the chair. The total time spent on this activity in seconds was measured and recorded. This test was performed for three times and the mean value was calculated. At least 10-second reset was given in between each attempt. Any medical problems related to the walking was recorded. All measurements were recorded in "physical capability test record" sheet.

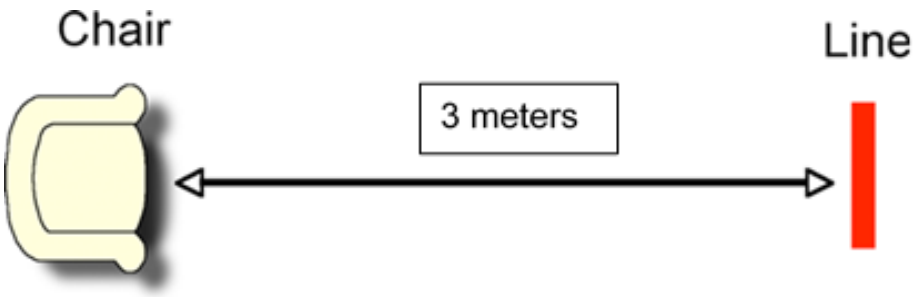


Figure 2.6 : Overview of Timed-Up and Go set-up

Figure 2.7 : Illustration of performance of Timed-Up and Go test (TUG)

2.4.6 Body weight and body composition

In both VDOP study and BFU study body weight and body composition (fat % in BFU study) were measured using TANITA TBF-300MA bio-impedance scale to nearest 0.1 kg (Figure 2.8). At first, the foot plate was cleaned using wet wipes for surface disinfection before getting the measurements. Participants' height, age, sex, type of body (Standard Male/ Female /Athletic Male and Female) were fed into the machine. The weight of 0.5 kg was reduced from each participant for the weight of the clothes. Then the participant was asked to empty their pockets, remove shoes, wrist watches, extra clothing such as jackets before getting the measurements. Next, participants were asked to step on the weighing scale when the "step on" symbol was flashed, placing feet wholly on both anterior and posterior electrodes and remain

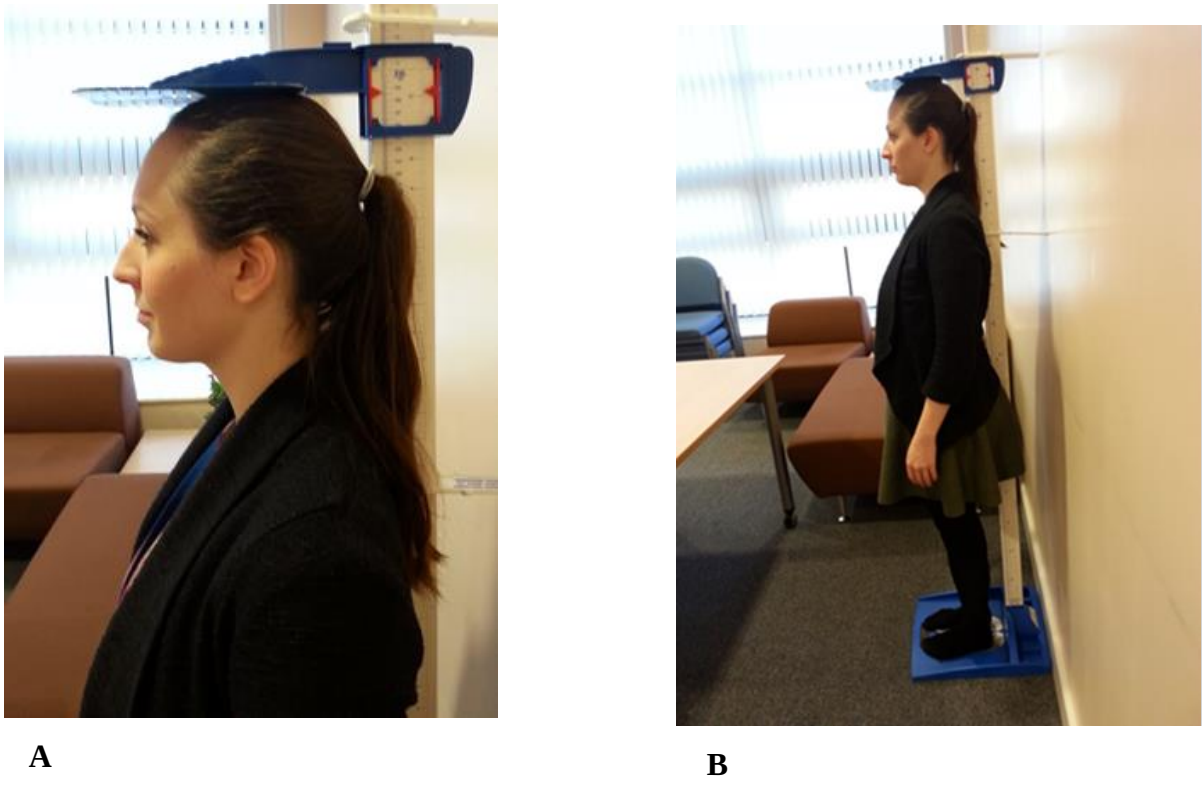
still until the measurement was completed. The participant was asked to get down from the scale once the measurement was finished. A print of the weight and body composition was obtained and it was given to participants after recording the measurement in the anthropometry and body fat recording sheet. Weight measurement was obtained only once. In the VDOP study, body weight and body composition were measured at the screening visit, the visits at 3rd month, 6th month, 9th month and after 12th months of intervention at the study center. In addition to the weight measurement, BMR, BMI, impedance, fat percentage, FM, FFM and Total Body Water (TBW) were recorded in VDOP study by the research nurse.

Figure 2.8 : TANITA TBF-300MA bio-impedance scale

2.4.7 Height

In both VDOP study and BFU study the height was measured at the study center. A Stadiometer (Seca) was used to measure the height in VDOP study while Leicester stadiometer was used in the BFU study. Before getting the measurements the stadiometer was kept on the firm cement floor and stabilizers rested against the vertical wall surface. All participants were given a short description of the way of measuring the height. Foot plate, head plate and skeletal scale were cleaned before getting the measurements using the wet tissues. Participants were asked to remove their socks and shoes and step onto the stadiometer, stand as straight as possible with feet together on the foot-plate, arms by their side hanging loosely, palms facing the thighs, both heels placed together touching the base of the vertical board of the stadiometer, buttocks and upper back contacted the back of the stadiometer, without leaning. Jawline was adjusted to 90° of the trunk. The participant's head was positioned so that the Frankfurt Plane was horizontal. It was checked whether the ear hole aligned with the bottom of the eye socket (Figure 2.9). Participants were asked to maintain normal breathing and gently lower the horizontal headboard to the most superior point of the

head, compressing the hair. Then height measurement was recorded on the anthropometry and body fat percentage recording sheet to nearest 0.1 centimetre (cm). The measurement was taken two times and the mean value was recorded. If the 2nd measurement was not within 1 cm of the previous recording, another measurement was taken and the closest two values were considered to calculate the mean.



A **B**

Figure 2.9 : Positioning the head (A), legs and back (B) against the stadiometer while taking the height measurement

2.4.8 Waist Circumference (WC)

WC was measured from all participants in both VDOP and BORICC studies using a stretchable tape. Participants were asked to remove heavy upper garments and then the lowest rib and suprailiac crest were identified. If the person had high adiposity, they were asked to identify those two points by themselves. The measurement was made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest (Figure 2.10) (WHO, 2008). Participants were asked to exhale during the measurements. Member of the research ensured that the tape was lying flat on the skin and horizontal. The measurement was recorded to the nearest 0.1 cm. The procedure was repeated and the mean value was recorded. If the second measurement was not within 1 cm of the first, the third measurement was measured.

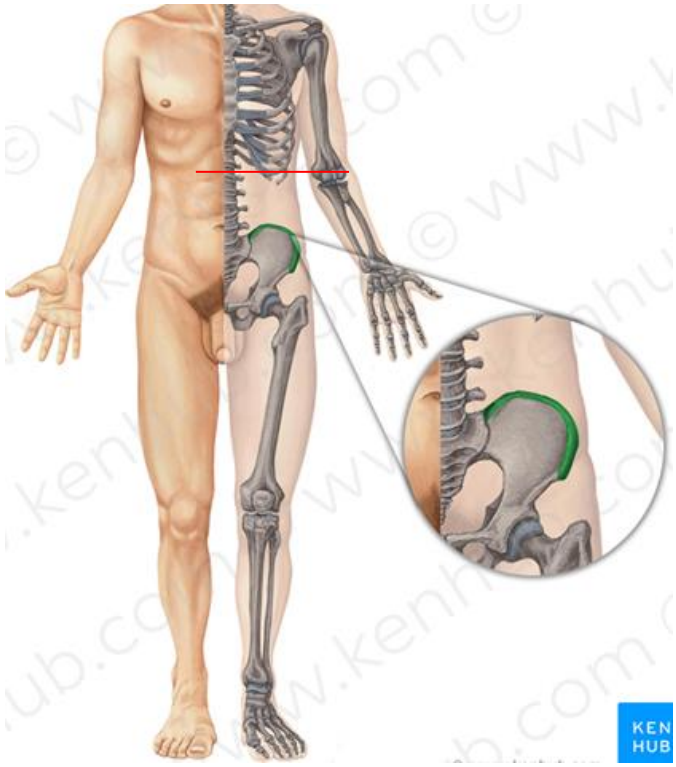


Figure 2.10: Measuring point at the waist to measure the Waist Circumference (Source :WHO, 2008)

2.4.9 Hip Circumference (HC)

HC was measured in both BORICC and VDOP studies using the stretchable measuring tape. The measure was taken at the widest portion of the buttocks with light clothing (Figure 2.11). A member of the research team had to make sure that the tape was lying flat on the skin and horizontal. Measurement was obtained to the nearest 0.1 cm and two measurements were obtained. If the second measurement was not within 1 cm of the first measurement, the third measurement was taken and the closest two measurements were considered to measure the mean value. Measurements from all participants were taken by the same investigator throughout the study to avoid the investigator's bias.

Figure 2.11 : Way of measuring Hip Circumference (Source : WHO, 2008)

2.4.10 Body composition

In VDOP study, FM and FFM were measured using Body Composition Analyzer (TANITA Corp, Tokyo, Japhan). The person was asked to step on the analyzer with bare foot. FM, FFM, fat percentage, impedance, BMI, Total Body Water (TBW) and Basal Metabolic Rate (BMR) that displayed on the scale were recorded. Body weight, height, BMI, body fat, waist and hip circumferences were recorded in the "Anthropometric and body fat percentage record sheet" (Appendix L) with the date, time participants ID and any problem related to measurements were recorded.

2.4.11 Bone QUS measures

Heel bone ultra-sound scanner (Achilles, UK) (Figure 2.12) was the equipment used to measure the bone mass of the heel bone of the participants. The Achilles EXPII is a bone ultra-sound scanner, that use high-frequency sound waves (ultrasounds) to examine the bone status in the heel. During the measurement procedure the heel is surrounded by warm water encapsulated between inflated membranes, which is the optimum medium for the transmission of the ultrasound. A transducer of the one side of the heel transmits an electrical signal into a sound wave, which passes through the water and person's heel. Transducer at a fixed distance on the opposite side of the heel receives the sound wave and converts it to an electrical signal that is analyzed. The machine measures the Speed Of Sound (SOS) and the frequency dependent attenuation of the sound waves and combines them to form a clinical measure called a "Stiffness Index" (SI). This value indicates the risk of osteoporosis fractures

and comparable to BMD, which expresses as t–score. The measurement is taken in Os Calcis bone at the heel, which is highly trabecular and weight bearing bone with high metabolic rate and turn over as hip and spin bones.

In the BFU study, this test was performed in the first room of the study center by the same investigator throughout the study. As the first step, the surface of the machine including the "calf support" and "footplate" were cleaned using wipes for surface disinfection tissues. The second step was to perform the quality assurance test. To perform this test, both membranes of the machine and both side of the QA cylinder were spared 70% ethanol and then QA cylinder was placed between deflated membranes. Next, the command was given to the machine to perform the test. If the test was not passed, membranes were filled with battery water or membranes were changed (if required) and the test was performed again. This quality assurance was performed each day before getting the measurement. As the thirds step, the reference population was set to "Europeans". Participants were given a short description of the measurement mentioning that this test was about the bone strength and the way of getting the measurement. Participants' age, ID number were fed to the machine and the gender and the foot (left or right) were selected in the LED screen before getting the measurements. Participants were asked to remove the socks and shoes and sit in front of the machine on a chair. Then ethyl alcohol was sprayed two times into both sides of the heel and the membranes of the machine. Next, participants were asked to lay the foot flatly on the "footplate" of the machine and the rest the calf slightly on the "calf support" (Figure 2.13 and 2.14). Percipients were asked to position the foot so that the white rib on the "footplate" was between the first two toes. Then the command was given to the machine to perform the test. Participants were asked to keep the foot still until the measurement was completed. All values displayed on the digital screen were printed off by the machine. Those values were recorded including the SI, T-score and Z-score in the "Bone Densitometry Record Sheet" (Appendix M). Two measurements from each foot were recorded and the mean value of each foot was calculated.

Figure 2.12 : Heel bone ultra-sound scanner

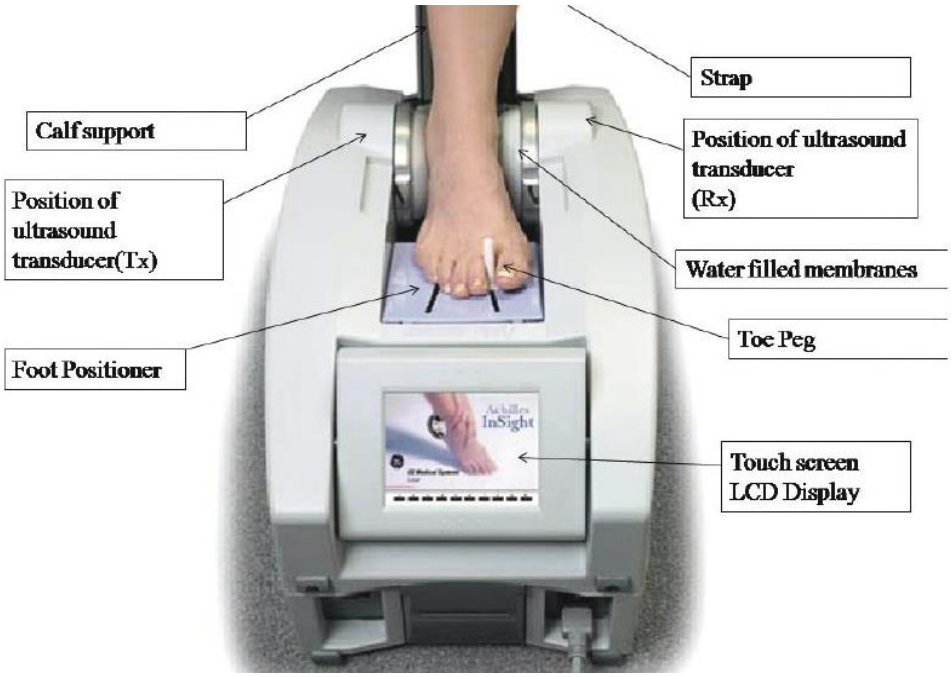


Figure 2.13 The working position and the components of the ultrasonometer device (Wishvanath *et al.*, 2011)

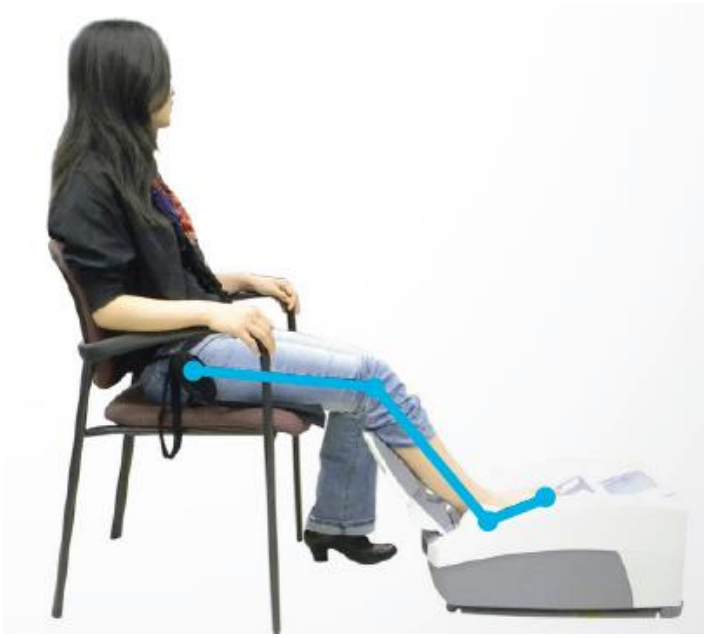


Figure 2.14 : Positioning the foot on the "footplate" of the bone scanner

2.4.12 Blood samples collection

VDOP study : Venous blood samples were collected from the over-night fasted participants at the initial screening visit and at last study visit after 12-month supplementation by venipuncture procedure. The time and date of the sample collection were recorded. Blood drawing of all study participants was done by a practiced medical practitioner. Vacutainers were used to centrifuge and store the samples in -80^{o}C freezer until shipped to central labs for analysis. All samples were collected at 8.30 – 11.30 am at the study center. Plasma samples were used to analyze the total 25(OH)D concentration. All samples were labelled with the study identification number, initials and date of birth of the participants. After venipuncture, breakfast was offered at the study center. Time of the samples stored in the freezer and the freezer temperature were recorded in record forms.

BFU study : A total of 30 mL of random non-fasted venous blood samples were collected by venipuncture procedure. A medical practitioner collected the blood samples from all study participants. A aliquot of 500 µL serum samples were allocated to Eppendorf tube for the analysis of total serum 25(OH)D while EDTA added 500 µL of plasma sample was allocated for the analysis of PTH. As soon as the blood was collected to vacutainers, it was gently inverted to mix well and then the samples were centrifuged in the NTH at room temperature 7000 rpm for 10 minutes. The serum samples were aliquetted into two 1.5 mL tubes (approx. 500µl per tube) and stored at -80 ^{o}C in the freezer in NTGH until transport to the laboratory in

Newcastle University for analysis. All blood samples were collected from all study participants from May 2017 to April 2018. Dates and time of the samples collected were recorded.

2.5 Laboratory analysis

2.5.1 Total serum 25(OH)D

VDOP study : Overnight fasting venous blood samples were collected from participants at each visit. Blood collection and processing protocols, biochemical measurement, methods and quality control of assay performance were strictly standardized, as previously described (Schoenmakers *et al*., 2003). The 25(OH)D_2 and 25(OH)D_3 concentrations in plasma were measured by Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) in duplicate samples as described in Schleicher *et al*., 2011. Lithium Heparin added plasma samples were used for the 25(OH)D analysis. Total 25(OH)D concentration was calculated by summing up the values for 25(OH)D_2 and 25(OH)D_3. The test was done in Immulite, Siemens Healthcare Diagnostics Ltd, Camberley, UK. Assay performance was monitored using a kit and in-house control and under strict standardization according to ISO 9001 : 2000. Quality assurance of 25(OH)D assay was performed as a part of the Vitamin D External Quality Assurance Scheme (DEQAS) (www. deqas.org) and National External Quality Assurance Scheme (www.ukneqas.org.uk). The Analysis was repeated if the coefficient of variation was more than 10%. Assay performance was monitored using chrome systems and in-house controls, and is traceable to NIST standards. The inter-assay variation was less than 10% for 25(OH)D_2 and less than 7% for 25(OH)D_3 and the limit of quantification was 6 nmol/L.

BFU study : Total serum 25(OH)D concentration was analyzed in the Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne, using the Roche method. EDTA added serum samples were used for the total serum 25(OH)D analysis in duplicate samples. Considering the time limitation, ease of sample analysis and transport as well as the restricted budget for the laboratory analysis, serum samples were sent to our local biochemistry hospital laboratory at the Freeman hospital which uses the Roche assay with DEQAS certification.

2.5.2 Parathyroid Hormones (PTH)

VDOP study : EDTA plasma was used for the analysis of PTH by immunoassay. The analysis of PTH was carried-out at Immulite, Siemens Healthcare Diagnostics Ltd, Camberley, UK. Similar to 25(OH)D, quality assurance of PTH analysis was also performed as a part of DEQAS and National External Quality Assurance Scheme. Inter and intra-assay

CV were consistently below 4% in this laboratory. For the PTH analysis, between-assay CV was 3.1%.

BFU study : PTH was analyzed using the sandwich test principle of electrochemiluminescence technology. The analysis was carried-out at Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne. Inter-assay CV was 1.4% while intra-assay CV was 2.9%".

2.6 Statistical analysis

Data were analyzed using SPSS statistical package for Windows version 24.0. Specific details of the statistical methods used in each study are provided in each of the experimental chapters which follow this one.

Chapter 3 : The Effect of Vitamin D_3 Supplementation for One Year on Muscle Function in Older Adults

3.1 Introduction

Loss of muscle mass and decreased muscle strength are features of ageing, with an annual loss of 0.5 – 1.0 % of muscle mass per year after 70 years of age (Mitchell *et al.,* 2012) and a 10 - 15% decline in muscle strength per decade in older people of aged 70-79 years (Goodpaster *et al*., 2006). Decreased muscle mass and strength results in sarcopenia, which is associated with poorer Quality Of Life (QOL), loss of independence and increased health care costs (Yu *et al.,* 2014). Assessment of GS and TUG are the widely used methods to test the muscle strength and to identify the presence of sarcopenia (Dodds *et. al.,* 2014).

Links between vitamin D status and muscle function have been reported based on mechanistic *in vitro* studies (Bischoff –Ferrari *et al.,* 2014), human observational studies (Berners *et al*., 2018, Brech *et al.*, 2017, Ceglia and Harris., 2013, Giris *et al*., 2013), longitudinal studies (Granic *et al*., 2017) and intervention studies (Muir and Montero-Odasso, 2011). Some observational (Tieland *et al*., 2013, Toffanello *et al*., 2012, Muir and Montero-Odasso, 2011) and longitudinal (Granic *et al*. 2017) studies have reported positive associations between serum 25(OH)D concentration and muscle function in older adults, whereas other studies did not find such association (Berners *et al*., 2018). These conflicting findings may be due to the differences in the characteristics of the population and differences in the vitamin D status of the participants. Current evidence suggests that vitamin D status is associated with muscle strength, function and physical performance in older adults (over 60 years of age) only when serum 25(OH)D concentration falls below 50 nmol/L (Rajnmark, 2011). The National Osteoporosis Society (NOS) (https://nos.org.uk/media/2073/vitamin-d-and-bone-health-adults.pdf) also suggests, that serum 25(OH)D concentration < 25 nmol/L is considered as deficient, 30 – 50 nmol/L might adequate for some people while > 50 nmol/L is adequate for almost all in the population based on optimum bone and muscle health outcomes (Francis *et al*., 2013).

The findings from RCTs of vitamin D are inconsistent, reflecting the variation in characteristics of the study population (e.g. age, gender, baseline vitamin D status), study design and the nature of the intervention (route, dose, frequency and form of vitamin D supplementation). Some studies showed the positive effect of vitamin D supplementation on muscle function only in older adults whose baseline serum 25(OH)D concentrations <30 nmol/L (Beaudart *et al*., 2014) or <50 nmol/L (Rajnmark, 2011). However, less number of

studies have been conducted recruiting large number of free-living older adults. In addition the effect of vitamin D supplementation doses correspondent to RNI for vitamin D (400 IU) defined by SACN, North American RDA for vitamin D (800 IU/day) defined by IOM and the tolerable upper intake level (TUIL) (4000 IU/day) of vitamin D have not been studied. Further the plasma concentration and vitamin D supply required for optimal muscle function in older adults is not well understood.

Therefore this study attempted to address the issues of past studies recruiting large number (379) of free living older adults and giving three different doses correspondent to the UK RNI for vitamin D, US RDA for vitamin D and the tolerable upper intake of vitamin D. We undertook a secondary analysis of a 1-year dose-ranging randomized vitamin D_3 supplementation trial evaluating its effects on muscle function (Aspray *et al*., 2018).

Therefore aims of this chapter were to;

i) determine the association between common vitamin D biomarkers (25(OH)D) on muscle function in older adults

determine the effect of vitamin D supplementation on muscle function in older adults. The objectives of this chapter were to;

i) investigate relationships between serum 25(OH)D concentration and muscle function in older adults from VDOP study
ii) investigate the effects of three doses of supplemental vitamin D_3 (12000 IU, 24000 IU, 48000 IU) monthly for 12 months on muscle function in older adults from VDOP study

3.2 Materials and Methods

Data from the Vitamin D in Older People (VDOP) study which was a randomized double-blind interventional trial of 379 male and female older adults aged 70 years or older, was used for the present study. In the original VDOP study to compare the absolute change in BMD at the hip, giving a 2-sided significance level of 0.05 and a power of 90%, 125 participants per arm were required. This resulted in a planned sample size of 375 participants to allow for 20% attrition by 12 months. This estimation was based on the variance in change in BMD over 12 months from a previous study (Macdonald *et al*., 2013) and enabling detection of a 0.006g/cm^2 difference between 2 arms. However in a retrospective power calculation considering GS, giving a 2-sided significance level of 0.05 and a power of 80% the detectable changes in GS over one year as 2.8 kg (Frederiksen *et al*., 2006) with an SD for that

difference of 11.6 kg the required sample size was 137 per arm. Allowing 20% attrition, the required sample size was 164 per arm.

3.2.1 Sunshine exposure, dietary intake, anthropometry and demographic variables

Data on GS, TUG test, anthropometry, including, height, weight, FM and FFM, dietary intake of vitamin D, serum 25(OH)D and PTH concentration at baseline and after the total supplementation was gathered for the present analysis. Further details about participants' recruitment, inclusion and exclusion criteria, vitamin D supplementation, outcome measures, and analysis of biological samples are described in subsection 2.1, 2.4 and 2.5, respectively in Chapter 2.

3.2.2 Data and statistical analysis

Baseline data were available for 379 older adults, while 343 older adults completed the intervention study. Thus, the total sample of 379 was used for the baseline data analysis, while the data from 343 older adults were used to investigate the intervention effects after 12 months. Older adults who used vitamin D supplements were omitted in this analysis. Older adults were sub-divided into two groups based on baseline plasma 25(OH)D concentration < 25 nmol/L, which is the cut-off of value of vitamin D inadequacy used in the UK (SACN, 2016) and plasma 25(OH)D concentration < 50 nmol/L, which is the cut-off for vitamin D inadequacy used in North America (Ross *et al*., 2011). Statistical analysis of the data was conducted using SPSS for Windows version 24.0. Kolmogorov-Smirnov test was used to evaluate the distribution of the variables and those that were not normally distributed were log transformed prior to the analysis and were near normally distributed after the conversion. Primary outcomes for the study were GS and TUG in response to supplementation with 12000 IU, 24000 IU and 48000 IU vitamin D_3 per month.

Baseline 25(OH)D, baseline muscle function variables, age, weight, height, FM, FFM and vitamin D intake were predetermined as potential confounders. Multinomial logistic regression analysis was used to investigate the association between plasma 25(OH)D concentration (based on two cut-offs, < 25 nmol/L and < 50 nmol/L) and muscle function at baseline, adjusting for confounders. The ANOVA test was used to test the effect of vitamin D supplementation on muscle function, plasma 25(OH)D and PTH concentration. The ANCOVA was used to test for the effect of the treatment on post-intervention variables after controlling for potential confounders (age, weight, height, FM, FFM and vitamin D intake). The Bonferroni test was used for post hoc comparisons. Stepwise linear regression analysis was used to identify the predictors of GS and TUG test before and after supplementation. Paired t test was used to identify changes in serum 25(OH)D, PTH, GS and TUG test within

each dose groups during the supplementation period. A P value <0.05 was considered as significant.

3.3 Results

Vitamin D was administered orally under direct supervision of a research nurse at baseline and at the third, sixth and ninth month study visits. At the end of each study visit, participants are given two bottles of vitamin D for the two subsequent months before their next scheduled study visit together with written instructions on how to take the supplement. On months when the participant was not attending the clinic, they were contacted by telephone, to remind them to take the supplement. Participants were directed to return all supplement bottles in their original packaging to the research team. Dropout rates of the participants in 12000 IU, 24000 IU and 48000 IU dose groups were 10.3%, 8.8% and 9.3%, respectively. Twelve (12) participants showed an Adverse Event (AEs) and withdrew from the study. The withdrawal was related to the vitamin D intervention only for one person. There was no evidence of a dose effect and no overall difference in the AEs were found between the vitamin D doses (Aspray *et al.*, 2018). Table 3.1 presents the participants' characteristics at baseline, stratified by vitamin D_3 supplementation dose. Baseline values for the main outcome measures, GS, TUG and plasma 25(OH)D concentration were similar across the intervention groups as were mean values for the main confounders including weight, height, BMI and age indicating that randomization was successful. The initial characteristics of the baseline sample (379 participants) and the sample of older adults who completed the intervention study (343 participants) were similar (data are not shown).

Table 3.1 : Participants' characteristics at baseline by the dose of vitamin D supplementation

Characteristics	12000 IU (n=126)			24000 IU (n=125)			48000 IU (n=128)			P value[1]
	n (%)	Mean	SD	n (%)	Mean	SD	n (%)	Mean	SD	
Age (years)		74.6	4.0		75.0	4.2		75.4	4.4	
Age (n, % ≥70 <71.5)	33 (26.2)			31 (24.8)			33 (25.8)			0.793
Age (n,% ≥ 71.5 < 74)	36 (28.6)			30 (24.6)			28 (21.9)			
Age (n,% ≥ 74 < 77)	29 (23.0)			33 (26.4)			29 (22.7)			
Age (n,% ≥ 77)	28 (22.2)			31 (24.8)			38 (29.7)			
Gender (n, % males)		126 (54.8)			125 (52.8)			128 (49.2)		0.669
Weight (kg)	126	73.9	11.8	125	77.1	14.0	128	76.1	14.2	0.150
Height (cm)	126	167.4	8.1	125	167.0	9.8	128	167.4	10.0	0.925
Waist (cm)	125	94.5	11.4	125	97.7	14.0	127	97.5	14.3	0.102
Hip (cm)	125	103.9	8.2	125	105.8	9.5	127	105.3	10.5	0.256
Body Mass Index (BMI) (kgm^{-2})	126	26.3	3.6	124	27.5	4.1	127	27.2	4.0	0.047
<18.5		0 (0.0)			1 (0.8)			0 (0.0)		0.115
18.5 – 24.9		50 (41.0)			31 (25.4)			41 (33.6)		
25.0 – 29.9		60 (32.3)			65 (34.9)			61(32.8)		
≥30.0		16 (33.2)			28 (33.0)			26 (33.8)		
Body fat %	124	31.9	8.6	125	32.9	7.7	127	32.5	7.8	0.633
Grip Strength (GS) (kg) (Reference range for sarcopenia; <20kg for female, < 30kg for male)	126	28.5	13.4	124	28.8	13.0	127	28.1	12.1	0.980

Table 3.1 continue

TUG[3] (s) (Reference range for sarcopenia: 10.85 s)	125	10.8	2.5	124	11.6	2.9	127	11.9	3.6	0.016
Plasma 25(OH)D[4] (nmol/L)	126	41.3	19.9	124	39.5	20.6	128	38.9	19.7	0.518
< 25 nmol/L	33 (26.2)			34 (27.4)			.35 (27.3)			0.745
25 – 50 nmol/L	52 (41.3)			56 (45.2)			61 (47.1)			
50 – 75 nmol/L	35 (27.8)			26 (21.0)			24 (18.8)			
> 75 nmol/L	6 (4.8)			7 (6.5)			8 (6.3)			
PTH (Pg/ml) (Ref range : 10.4 – 60.4 Pg/mL)	126	48.6	25.7	123	47.4	23.3	128	49.9	21.3	0.688
Dietary vitamin D intake (µg/day)	119	3.6	2.0	121	3.6	2.5	123	4.0	3.0	0.520

[1] *ANOVA test*

Table 3.2 : M**ultinomial logistic regression analysis[1] of relationships between plasma 25(OH)D concentration, categorized according to SACN and IOM cut-offs and muscle function at baseline**

	Total population (n=379)			**Males (n=198)**			**Females (n=181)**		
Classification of 25(OH)D	**OR**	**CI**	**p value[2]**	**OR**	**CI**	***p* value[3]**	**OR**	**CI**	***p* value[3]**
Grip Strength (kg)									
25(OH)D < 25 nmol/L[2]	0.339	0.166 – 0.691	0.003	0.333	0.137 – 0.810	0.015	0.251	0.063 – 1.001	0.050
25(OH)D ≥25 nmol/L	**Reference**			**Reference**			**Reference**		
25(OH)D <50 nmol/L[3]	0.990	0.510 – 1.922	0.976	0.588	0.216 – 1.443	0.229	1.870	0.520 – 6.719	0.338
25(OH)D ≥50 nmol/L	**Reference**			**Reference**			**Reference**		
Timed-Up and Go test (s)									
25(OH)D < 25 nmol/L	0.645	0.388 – 1.070	0.090	0.501	0.229 – 1.093	0.084	0.720	0.358 – 1.446	0.355
25(OH)D ≥25 nmol/L	**Reference**			**Reference**			**Reference**		
25(OH)DD < 50 nmol/L	0.697	0.424 – 1.147	0.155	0.775	0.386 – 1.543	0.468	0.584	0.273 – 1.250	0.166
25(OH)D ≥ 50 nmol/L	**Reference**			**Reference**			**Reference**		

[1]To be in the category of higher muscle function based on dichotomisation at the median value [2] SACN cut-off [3]IOM cut-off
[2] Adjusted for gender, age, body weight, height, season of blood collection, Fat Mass (FM) and Fat Free Mass (FFM)
[3] Adjusted for age, body weight, height, FM and FFM

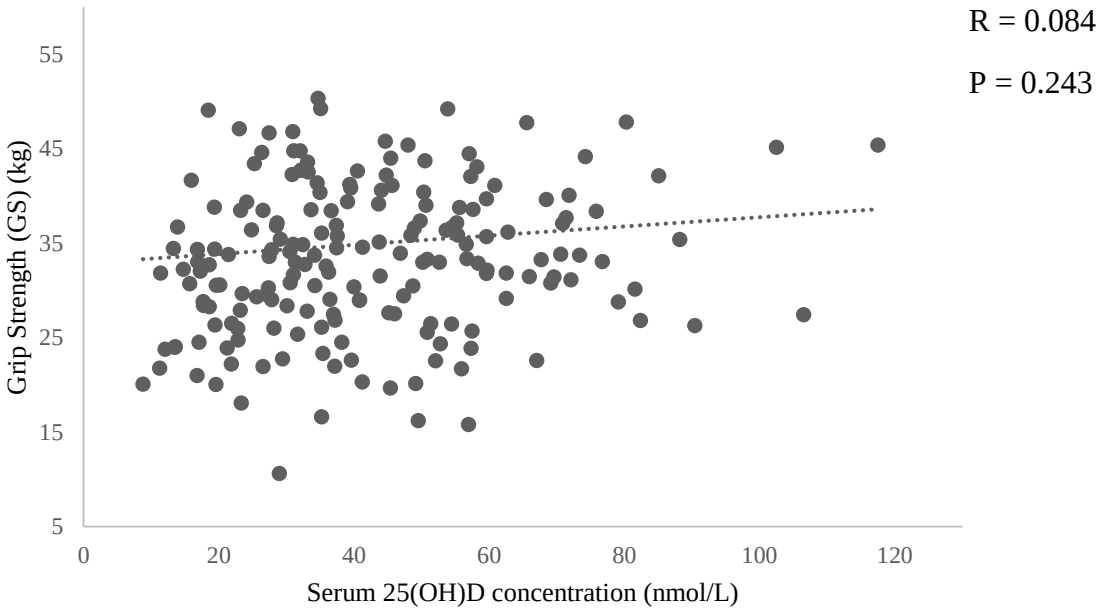


Figure 3.1 : Relationship between Grip Strength (GS) and serum 25(OH)D concentration at baseline in males

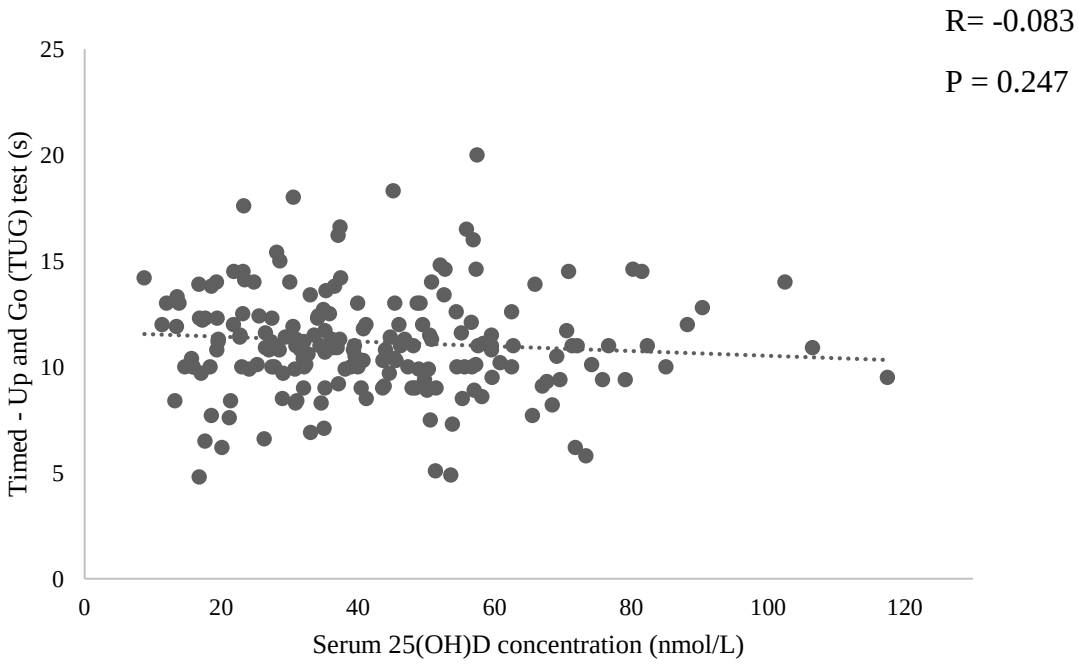


Figure 3.2 : Relationship between Timed-Up and Go test (TUG) and serum 25(OH)D concentration at baseline in males

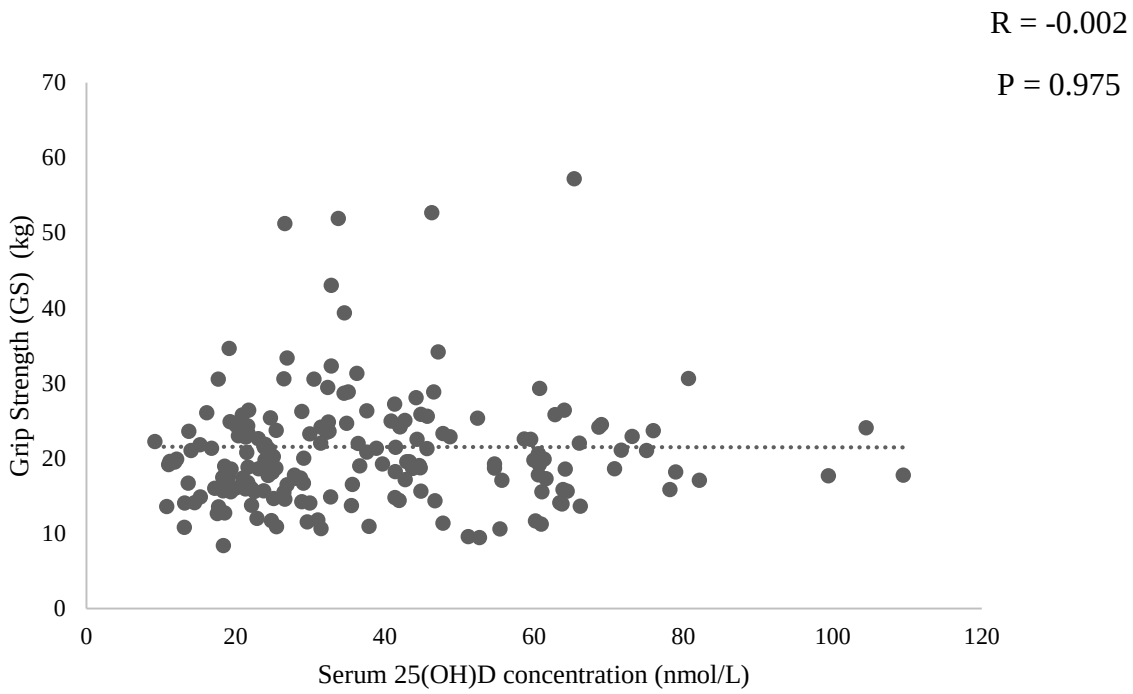


Figure 3.3 : Relationship between Grip Strength (GS) and serum 25(OH)D concentration at baseline in females

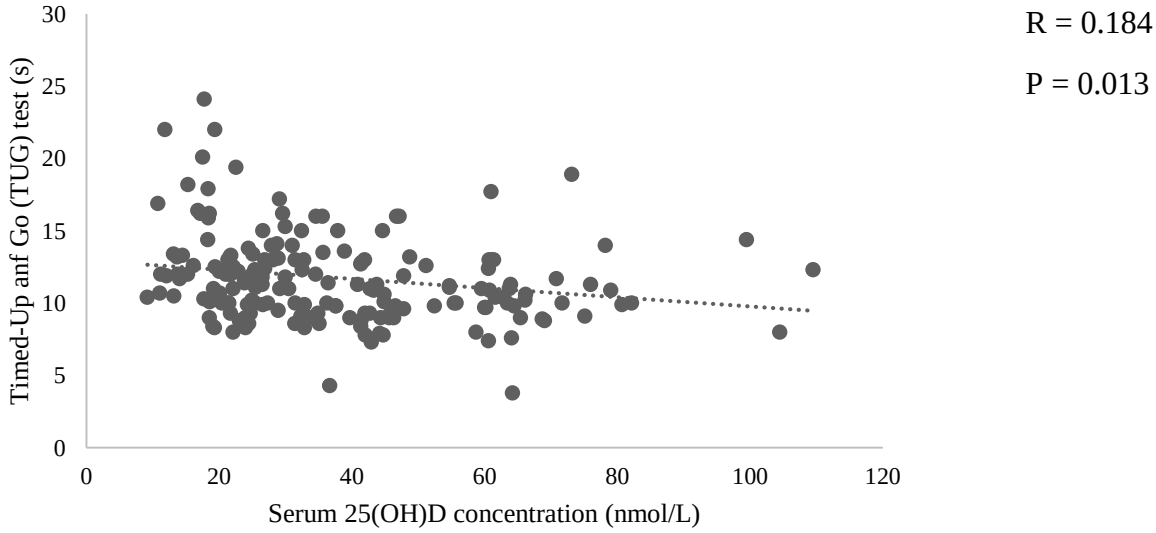


Figure 3.4: Relationship between Timed-Up and Go (TUG) test and serum 25(OH)D concentration at baseline in females

Table 3.2 shows the multinomial logistic regression analysis of the relationships between baseline plasma 25(OH)D concentration and muscle function variables according to the cut-offs values of plasma 25(OH)D concentrations from SACN, 2016 (25 nmol/L) and IOM, 2011 (50 nmol/L). After adjusting for age, body weight, height, FM, FFM, vitamin D intake and season blood collected, older adults who had plasma 25(OH)D concentration < 25 nmol/L at baseline were significantly (p=0.003) less likely to have GS above the median compared with individuals with plasma 25(OH)D concentration >25 nmol/L. This relationship was evident for GS for both males (p= 0.015) and females (p= 0.050). When using the cut-off value of 50 nmol/L, there were no relationships between vitamin D status and either GS or TUG for all participants and for both gender groups. Figures 3.1, 3.2, 3.3 and 3.4 further illustrate the association between serum 25(OH)D and TUG test and GS in males and females.

Table 3.3 shows the effect of vitamin D supplementation on post-interventional and change (Δ) in muscle function variables, plasma 25(OH)D concentration and PTH concentration by the dose of vitamin D supplementation. After one year of vitamin D supplementation, there were no differences between treatment doses for post-intervention GS or TUG. In addition, there were no significant changes in GS and TUG from between intervention arms, with and without adjustment for baseline values, age, gender, weight, height, FM, FFM and vitamin D intake. Further, subgroup analysis of those with a baseline 25(OH)D concentration < 50 and <25 nmol/L, did not show any effect differences between intervention arms in post-intervention GS and TUG, or for change in GS and TUG. As expected, there were significant differences between treatment arms in post-interventional plasma 25(OH)D and change in 25(OH)D concentration. This relation was the same for the sub-group analysis those with baseline plasma 25(OH)D concentration < 50 nmol/L and < 25 nmol/L. After the supplementation, the change in plasma 25(OH)D concentrations were 14.3 (SD 12.6), 25.3 (SD 18.0) and 40.9 (SD 19.8) nmol/L for the 12000 IU, 24000 IU and 48000 IU dose group, respectively. There was no significant difference in uncorrected post-interventional PTH in the total sample, although after correction for confounders this was significant. Subgroup analysis of those with a baseline 25(OH)D concentration < 50 nmol/L, change in PTH significantly differed between intervention arms before and after adjusting for confounders. Serum 25(OH)D concentration increased significantly after 12 month supplementation in all dose groups (p<0.001). PTH concentration decreased only in the high dose group (p<0.001) while GS was significantly decreased for all dose groups within 12 month period. TUG test values did not change significantly over time in any of the treatment groups.

Table 3.3 : Effect of vitamin D supplementation on post-interventional and change (Δ) in muscle function variables, plasma 25(OH)D concentration and PTH concentration by the dose of vitamin D supplementation

Parameters	12000 IU/month	24000 IU/month	48000IU/month	p^1	p^2
Total sample (n=343)					
Plasma 25(OH)D (nmol/L)	**(n=113)**	**(n=114)**	**(n=116)**		
Pre-intervention	41.2 (20.3)	39.4 (20.8)	38.5 (19.4)	0.495	
Post-intervention	55.9 (15.6)	64.6 (15.3)	79.0 (15.1)*	<0.001	<0.001
P value[3]	<0.001	<0.001	<0.001		
Change (Δ) in 25(OH)D	14.3 (12.6)	25.3 (18.0)	40.9 (19.8)*	<0.001	<0.001
PTH[4] (pg/mL)					
Pre-intervention	46.8 (23.5)	47.1 (23.9)	50.6 (21.6)	0.443	
Post-intervention	44.0 (21.3)	44.6 (24.5)	40.1 (18.4)	0.244	0.016
P value[3]	0.095	0.076	<0.001		
Change (Δ) in PTH	-2.9 (18.4)	-3.1 (18.2)	-10.6 (15.4)*	<0.001	<0.001
GS[5] (kg)					
Pre-intervention	27.5 (12.7)	29.4 (13.2)	28.1 (12.2)	0.641	
Post-intervention	24.7 (10.1)	26.2 (10.6)	25.7 (9.4)	0.692	0.449
P value[3]	0.012	<0.001	<0.001		
Change (Δ) in GS	-2.8 (11.6)	-3.2 (8.1)	-2.4 (7.7)	0.820	0.426
TUG[6] (s)					
Pre-intervention	10.9 (2.5)	11.5 (2.9)	11.8 (3.5)	0.187	
Post-intervention	11.5 (2.6)	12.0 (3.7)	11.9 (3.2)	0.437	0.713
P value[3]	0.656	0.084	0.519		
Change (Δ) in TUG	0.56 (2.32)	0.46 (2.77)	0.15 (2.5)	0.773	0.680
Older adults with baseline 25OHD < 50 nmol/L (n=242)					
Plasma 25(OH)D (nmol/L)	**(n=75)**	**(n=83)**	**(n=84)**		
Pre-intervention	30.2 (10.9)	29.1 (10.3)	29.6 (10.6)	0.862	
Post-intervention	49.7 (11.8)	60.5 (14.8)	76.8 (14.3)*	<0.001	<0.001
Change (Δ) in 25(OH)D	19.3 (10.0)	31.3 (15.0)	47.4 (15.3)*	<0.001	<0.001
PTH (pg/mL)					
Pre-intervention	49.9 (24.9)	52.9 (24.4)	54.2 (22.0)	0.210	
Post-intervention	45.7 (21.5)	48.9 (24.5)	41.0 (19.7)	0.137	0.101
Change (Δ) in PTH	-4.2 (21.1)	-4.0 (19.5)	-13.4 (15.3)*	<0.001	0.002
GS (kg)					
Pre-intervention	26.5 (11.8)	29.1 (13.6)	28.3 (12.8)	0.457	
Post-intervention	23.8 (10.3)	25.5 (10.3)	25.1 (9.3)	0.801	0.403
Change (Δ) in GS	-2.7 (12.5)	-3.6 (8.5)	-3.1 (8.4)	0.486	0.889
TUG (s)					
Pre-intervention	10.9 (2.3)	11.8 (2.9)	11.8 (3.2)	0.094	
Post-intervention	11.7 (2.8)	12.3 (3.9)	12.0 (3.5)	0.560	0.630
Change (Δ) in TUG	0.74 (2.4)	0.45 (2.8)	0.29 (2.3)	0.512	0.823
Older adults with baseline 25OHD < 25 nmol/L (n=93)					
Plasma 25(OH)D (nmol/L)	**(n=31)**	**(n=30)**	**(n=32)**		
Pre-intervention	19.3 (4.1)	18.2 (4.5)	18.8 (3.9)	0.582	
Post-intervention	41.9 (10.7)	55.5 (14.7)	73.7 (12.8)*	<0.001	<0.001
Change (Δ) in 25(OH)D	22.6 (9.3)	37.3 (14.0)	54.9 (12.7)*	<0.001	<0.001
PTH (pg/mL)					
Pre-intervention	61.9 (27.8)	59.5 (19.3)	58.8 (23.2)	0.930	
Post-intervention	50.4 (20.6)	47.7 (16.6)	41.5 (24.1)	0.102	0.143
Change (Δ) in PTH	-12.2 (25.9)	-11.9 (17.4)	-17.4 (14.7)	0.470	0.563
GS (kg)					
Pre-intervention	25.6 (13.8)	24.1 (9.1)	25.8 (14.2)	0.895	
Post-intervention	22.3 (11.2)	22.5 (9.0)	22.8 (7.9)	0.903	0.860
Change (Δ) in GS	-3.3 (16.1)	-1.6 (4.8)	-3.0 (9.1)	0.814	0.714
TUG (s)					
Pre-intervention	11.0 (2.4)	12.8 (3.7)	12.5 (4.1)	0.938	
Post-intervention	11.9 (2.8)	13.7 (4.1)	12.9 (4.1)	0.798	0.379
Change (Δ) in TUG	0.91 (2.6)	0.88 (2.7)	0.38 (2.5)	0.806	0.823

* *Significantly different from 12000 IU and 24000 IU groups* [1]*One-way ANOVA followed by Bonferroni test*

[2] *ANCOVA controlled for baseline values of the variables, age, gender, weight, height, Fat Mass (FM), Fat Free Mass (FFM) and vitamin D intake*

[3] *Within group comparison : paired t-test* [4]*Parathyroid Hormone* [5]*Grip Strength* [6]*Timed-Up and Go test*

Figure 3.5 depicts the proportion of older adults above and below various vitamin D cut-offs at baseline and after vitamin D supplementation. At baseline, the percentage of older adults with serum 25(OH)D concentration < 25 nmol/L were 26.2%, 27.2% and 27.3% for the 12000IU, 24000IU and 48000IU dose groups, respectively (p>0.05). The percentage of older adults with serum 25(OH)D <25 nmol/L after vitamin D supplementation, was 0.9% in the 12000IU dose group, whilst none of the participants who took the 24000 IU and 48000 IU dose of vitamin D had serum 25(OH)D < 25 nmol/L. The proportion of older adults with serum 25(OH)D > 75 nmol/L increased with increasing dose of vitamin D supplementation. Only those participants who took the 48000IU dose of vitamin D maintained 25(OH)D concentrations above 50 nmol/L after 12 months. So in summary, 12000IU monthly for 1 year was sufficient to keep nearly all participants above a 25(OH)D concentration of 25 nmol/L, whilst the 48000IU monthly dose was required to keep all participants above the 50 nmol/L threshold.

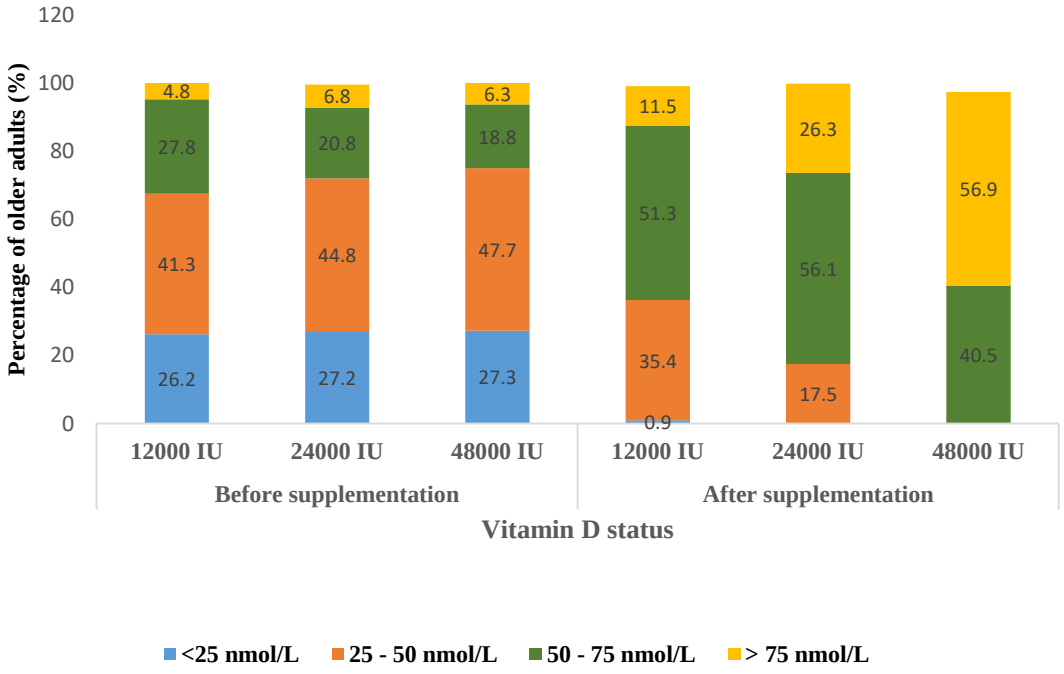


Figure 3.5 Vitamin D status of older adult before and after the supplementation

Table 3.4 shows the changes in dietary, anthropometric and demographic variables in all dose groups during 12 months supplementation period. Changes in dietary, anthropometric and demographic variables during the 12 months period was not significant in all dose groups.

Table 3.4 : Changes of dietary, anthropometric and demographic characteristic during supplementation period by the dose of vitamin D supplementation

Characteristics	Total sample	12000 IU	24000 IU	48000 IU
Fat Mass (kg)				
Before supplementation	24.9 (8.3)	23.8 (8.3)	26.0 (8.3)	24.7 (8.2)
After supplementation	24.8 (8.2)	23.8 (7.9)	26.0 (8.1)	24.7 (8.4)
P value	0.902	0.973	0.931	0.893
Fat Free Mass (kg)				
Before supplementation	50.8 (10.4)	49.5 (8.9)	51.9 (10.9)	50.9 (10.9)
After supplementation	50.6 (10.3)	49.4 (9.0)	51.9 (10.6)	51.0 (11.0)
P value	0.348	0.622	0.125	0.893
Body fat %				
Before supplementation	32.5 (8.0)	32.2 (8.5)	33.2 (7.8)	32.2 (7.6)
After supplementation	32.5 (8.1)	32.3 (8.2)	33.5 (7.9)	32.3 (7.7)
P value	0.631	0.619	0.239	0.773
Weight				
Before supplementation	75.9 (13.7)	73.3 (11.5)	77.9 (14.7)	76.4 (14.3)
After supplementation	75.5 (13.7)	73.1 (11.5)	77.3 (14.1)	76.1 (14.9)
P value	0.700	0.466	0.018	0.203
Body Mass index (kgm^{-2})				
Before supplementation	27.0 (3.9)	26.3 (3.6)	27.7 (4.20)	27.1 (3.4)
After supplementation	26.8 (3.9)	26.2 (3.55)	27.1 (4.08)	26.9 (4.1)
P value	0.088	0.409	0.022	0.196
Dietary vitamin D intake (µh/day)				
Before supplementation	3.8 (2.5)	3.6 (1.9)	3.7 (2.4)	4.0 (3.0)
After supplementation	3.9 (2.9)	3.6 (2.2)	3.8 (2.9)	4.3 (3.5)
P value	0.418	0.816	0.972	0.358
Dietary calcium intake (mg/day)				
Before supplementation	835.9 (390.5)	824.6 (358.0)	814.4 (400.2)	866.2 (412.4)
After supplementation	792.4 (377.9)	783.1 (343.6)	758.2 (358.1)	832.0 (423.6)
P value	0.021	0.222	0.079	0.312

Table 3.5 shows the predictors of GS and TUG test before and after vitamin D supplementation. Significant determinants of GS before supplementation were FFM, age, gender and height, while age, gender and height were the predictors of GS after the vitamin D supplementation. Similarly, age and fat percentage were the determinants of TUG test before supplementation while age, BMI and height were the determinants of the TUG test after the supplementation. The single biggest predictor of GS before and after supplementation was the gender while the single biggest predictor of TUG test before and after supplementation was age.

Table 3.5 : Multivariate models of predictors of Grip Strength and Timed-Up and Go test before and after the supplementation.

Predictors	**Constant**	**B**	**D**	**β**	**p**	**CI**
			Before supplementation			
Grip Strength						
R^2	0.363					
Constant	6.081					
Fat Free Mass (kg)		0.356	0.102	0.285	0.001	0.156 – 0.556
Age (years)		-0.458	0.133	-0.136	0.002	-0.687 – (-0.163)
Gender		-4.876	1.736	-0.190	0.005	-8.289 – (-1.462)
Height (cm)		0.230	0.102	0.166	0.025	0.029 – 0.431
Timed-Up and Go test						
R^2	0.130					
Constant	-7.839					
Age (years)		0.236	0.037	0.322	<0.001	0.164 – 0.308
Body fat %		0.073	0.019	0.193	<0.001	0.036 – 0.110
			After supplementation			
Grip strength						
R^2	0.564					
Constant	-13.02					
Gender		-9.27	1.052	-0.465	<0.001	-11.34 – (-7.201)
Height (cm)		0.358	0.055	0.341	<0.001	0.249 - 0.467
Age (years)		-0.223	0.085	-0.099	<0.001	-0.930 -0.056
Timed-Up and Go test						
R^2	0.159					
Content	3.141					
Age (years)		0.192	0.035	0.227	<0.001	0.123 – 0.261
Body Mass Index (kgm^{-2})		0.166	0.041	0.202	<0.001	0.084 – 0.247
Height (cm)		-0.061	0.017	-0.179	<0.001	-0.095 – (-0.027)

Independent variables were BMI, body weight, height, body fat percentage, Fat Free Mass, gender, age

3.4 Discussion

3.4.1 Main findings

This double-blind, randomized controlled study found that monthly vitamin D_3 supplementation with 12000 IU, 24000 IU and 48000 IU (which corresponds to 400 µg, 600 µg and 1200 µg of dietary vitamin D per day) for one year produced significant dose-related increases in plasma 25(OH)D concentration but had no effect on muscle function in older adults. However, at baseline, there was a negative association between plasma 25(OH)D concentration and GS for those with baseline plasma 25(OH)D concentration <25 nmol/L. After supplementation, there were no associations between plasma 25(OH)D concentration and muscle function. To our knowledge, this is the first dose-ranging RCT conducted in the UK, with a large number of free-living older adults, evaluating the effect of vitamin D supplementation on muscle function.

3.4.2 Comparison with other studies

Following table (Table 3.6) summarises the findings of past RCTs and systematic reviews (SR). According to all these studies, inconsistent findings are attributed to the differences in study design including the differences in cohort characteristics, duration, dosage, formulations, route of vitamin D supplementation and the functional outcomes measured. However in summary the improvement in muscle function can be seen only in older adults whose baseline vitamin D status < 60 nmol/L or older adults whose muscle functions were weak at baseline.

Table 3.6: Summary of the previous studies of vitamin D supplementation and muscle function in older adults

Study	Target group	Sample size	Dose and duration	Method of assessing muscle function	Conclusion
RCT, Kotlarczyk *et al*., 2017.	Female adults of long term care residence ; 65 + years	137	800 IU daily for 24 months	Gait speed and physical performance test	No effect of supplementation
RCT, Bischoff-Ferrari *et al*., 2016	Home dwelling men and women : 70 + years	200	24 000, 60 000 or 24 000 D_3 with 300 µg of calciferol monthly for 12 months	Short physical performance battery	No improved in lower extremity muscle function
RCT, Hansen *et al*., 2015	Postmenopausal women aged 75 years or younger with baseline 25(OH)D 35 – 67 nmol/L	230	800 IU and 50 000 vitamin D twice monthly for one year	Five sit - stand test and TUG test	No effect on muscle function
RCT; Broe *et al*., 2007.	Nursing home residents, average age of 89 years	124	One of 200 IU, 400 IU, 600 IU and 800 IU; daily for 5 months	Rate of falls	Reduced falls in high dose group
RCT, Cangussu *et al*., 2015	Postmenopausal women aged 50 – 65 years	160	Oral D_3 supplements; 1000 IU for 9 months	GS and chair rise test	Improvement in muscle strength in vitamin D supplemented people
RCT, Dhesi *et al*., 2004	Older adults with serum 25(OH)D < 30 nmol/L	139	Single dose of 60,000 IU ergocalciferol	Aggregate functional performance time, Choice reaction time, quadriceps strength,	Improvement in balance, reaction time, functional performance, not the muscle strength
RCT, Tellioglu *et al*., 2012	Older adults (65 +) in nursing homes with serum 25(OH)D concentration < 30 nmol/L	116	60,000 IU for 12 weeks	Quadriceps an physical performance battery	Improved the muscle strength.
RCT, Zhu *et al*., 2010	Community dwelling older adults, 70 – 90 years with serum 25(OH)D < 60 nmol/L	302	1000 IU daily D2 for 1 year	TUG	Improved the TUG only in the older adults who were slowest and weakest at baseline

Table 3.6 continue

Study	Target group	Sample size	Dose and duration	Method of assessing muscle function	Conclusion
SR, Stockton *et al*., 2011	Adults whose baseline serum 25(OH)D < 25 nmol/L.	17 studies	All forms and doses of vitamin D supplementation	Evaluation of muscle strength	Positive effect on muscle function in older adults whose baseline serum 25(OH)D less than 25 nmol/L
SR, Rosendahl-Riise *et al*., 2017	Community dwelling older adults age 65+ years	15 studies	Bolus injection or oral vitamin D dose ranging from 1000 IU – 600000 IU given daily or weekly for 16 weeks – 20 months	GS and TUG	No effect on muscle function

To support the finding of negative association between GS and plasma 25(OH)D concentration below 25 nmol/L at baseline, Wu *et al*., 2017, reported that the serum 25(OH)D concentration of 29 – 33 nmol/L may optimise MSK health in middle-aged women (36 - 57 years). Similar to our study Grimaldi *et al*., 2013, also reported a positive association between serum 25(OH)D concentration and GS, but not with other tests of muscle function and suggested that this might be related to anatomical site differences in the androgenic effect of vitamin D or to differences in vitamin D receptor expression between upper and lower body muscle and consequently muscle function.

GS loss in our study was much higher than the reported values in previous studies. The annual loss of GS among the older people aged 65 - 75 years reported in the previous studies ranged from 0.3 to 1.3 kg (Turusheva *et al*., 2017, Xue *et al*., 2010, Frederiksen *et al*., 2006). The higher decline in muscle function in our study might be due to the fact that the mean age of our participants was on average 75 years and they were in a phase of the rapid ageing process with rapid muscle degradation. Though the GS is the standard method to assess sarcopenia, differences in the equipment and methods used in various studies may have caused variation in the measurements, making it difficult to compare between studies (Roberts *et al*., 2011).

According to this study, plasma 25(OH)D concentration < 25 nmol/L was negatively associated with GS. This finding supports the recommendation of SACN, UK that for the protection of MSK health, serum 25(OH)D concentration should not fall below 25 nmol/L throughout the year (SACN, 2016). The US Institute of Medicine (IOM) defines the desired range of 25(OH)D as 30 - 50 nmol/L and ESFA (EFSA *et al*., 2016) advises a target value of 50 nmol/L for the general population. The US Endocrine Society advises a target range of > 50 nmol/L for patient management who are at risk of VDD (Holick *et al*., 2011). In addition, Kotlarczyk *et. al*., 2017 suggested that at least a concentration between 30-40 ng/ml (~ 75 – 100 nmol/L) is required for older adults for optimum muscle function (Kotlarczyk *et al*., 2017). With regards to vitamin D supplementation, the American Geriatrics Society (Broe *et al*., 2007) and the Endocrine Society (Holicket *et al*., 2011) recommend vitamin D supplementation of 600–1000 IU/day in older adults who are at risk of falling. A SR of vitamin D supplementation trials also reported a daily dose of 700–1000 IU for physical performance and to prevent falls (Bischoff-Ferrari *et al*., 2009). SACN, 2016 suggests that for the adults > 50 years, the beneficial effect of vitamin D supplementation on muscle strength and function can be seen among the adults at the mean baseline serum 25(OH)D concentrations ranging between 25 and 66 nmol/L. In this study, except the highest dose group, none of the other intervention groups reached a mean post-intervention plasma

25(OH)D concentration above this range. Accordingly, it can be speculated that the dosage used in this study may not have been high enough to reduce the negative effects of the ageing process. The absence of a detectable effect of supplementation in this study may also be attributed to the fact that only 30% of our participants were vitamin D deficient (<25 nmol/L) at baseline. However, as a result of lack of a placebo group, data on "natural decline" of muscle function of this group was not available. Thus the effect of vitamin D supplementation cannot be compared with that of non-supplemented individuals.

For tissues other than the kidney, total 25(OH)D may not fully reflect its availability for, local hydroxylation into $1,25(OH)_2D$ which is the active metabolite of vitamin D. Although $1,25(OH)_2D$ is responsible for the biological action of vitamin D, its systemic concentration does not reflect function at the target tissue level (Bike *et al*., 2017, Prentice *et al*., 2008). Many vitamin D target tissues, including the muscle tissue, are known to express the $1,25(OH)_2D$ producing enzyme *CYP27A1* for auto- and paracrine functions. Some reports suggest that muscle tissue may be capable of internalising vitamin D binding protein bound 25(OH)D, although it remains to be determined whether this is a significant route of cellular supply of 25(OH)D (Girigis *et al*., 2013). To date, no data are available to identify whether free 25(OH)D provides a better prediction of muscle function compared to total 25(OH)D.

3.4.3 Strengths and limitations

The lowest intervention dose (which corresponds to the current UK dietary recommendation) group was used as the reference group but did not include a placebo group in our study design as directed by the approving authorities. As a result, we could not establish the effect of three doses of vitamin D supplements compared to a placebo group. Further, plasma 25(OH)D concentration was measured at baseline and after 1-year supplementation, for both of which blood samples were collected early winter to late spring, during which in non-supplemented individuals, vitamin D status is lower than the year-round average. Therefore, vitamin D status of individuals at baseline and post-intervention may have been misclassified and not fully reflect the trajectory of vitamin D status throughout the year. Further, this population was not selected randomly from the community. They were invited on the basis of screening of pre-specified criteria in their electronic health record. Also, there may have been self-selection bias as those that expressed an interest to participate in the study may be more health conscious. In addition, the response to vitamin D supplementation and status may have been influenced by factors not measured in this study, such as the distribution of type I and type II of muscle fibres, genetic factors, habitual physical activity or exercise habits and hormonal factors. This study had several strengths. The large sample size compared to previous studies

and the number of available measurements that could potentially influence muscle force, i.e. those related to body composition and size. The use of three different vitamin D doses corresponding to the UK RNI (SACN, 2016) the US RDA (Ross, 2011) and the value below the Tolerable Upper Intake Level (TUIL) was a strength to this study.

3.4.4 Conclusions

At baseline, plasma 25(OH)D concentration < 25 nmol/L was negatively associated with GS for both male and females. Vitamin D supplementation significantly increased the plasma 25(OH)D concentration of older adults in all doses of supplementation. Vitamin D supplementation with 12000 IU, 24000 IU and 48000 IU for 12 months had no effect on muscle function in adults older than 70 year.

Chapter 4 :Relationship Between Sunlight Exposure and Serum 25(OH)D Concentration During a 12 Month Randomized Control Trial With High Dose Vitamin D_3 Supplementation: Results from the Vitamin D in Older People (VDOP) Study

4.1 Introduction

Cutaneous synthesis of vitamin D upon UVB radiation from sunlight is the main source of vitamin D for most humans (Holick, 1994). It has been assumed that 80 – 90% of the vitamin D requirement is provided by skin synthesis (Fraser and Milan, 2013), while diet makes a relatively small contribution (SACN, 2015). There is evidence that the prevalence of VDD has increased during the past decade in most countries (Bishoff-Ferrari *et al.,* 2006) which is attributed to insufficient cutaneous synthesis of vitamin D as a result of low sunlight exposure. Skin synthesis of vitamin D is affected by both personal and environmental factors. Age, clothing habits, skin color, frequency of outdoor activities, holiday visits and the use of sunblock are the important personal factors (Touvier *et al*., 2015, Darling *et al*., 2014, Tsiaras and Weinstock 2011) while, season, altitude, attitude, pollution and time of the day are important environmental factors (Touvier *et al*., 2015, Brembeck *et al.,* 2015) that influence the cutaneous synthesis of vitamin D. To optimize vitamin D status, vitamin D supplementation is recommended for individuals with low sunlight exposure or low cutaneous synthesis (O'Sullivan *et al*., 2019). However, excess exposure to sunlight increases the risk of skin cancer, cataract and skin aging (Health and Safety Environment, 2012). Therefore vitamin D synthesis upon UVB exposure has become an important concern in the area of vitamin D research.

Limited data are available about the relative contribution of sun exposure and vitamin D supplementation to plasma 25(OH)D which is the biomarker of vitamin D status (Ross *et al*, 2010). Current recommendations for the prevention of VDD focus on vitamin D supplementation and thus physicians are insecure about what advice to offer regarding sun exposure recommendations (Holick *et al*., 2011). It is believed that the effectiveness of vitamin D supplementation on plasma 25(OH)D concentration is altered by the dermal production of vitamin D (O'Sullivan *et al.,* 2019). The contribution of cumulative sun exposure behaviors over a period of time on plasma 25(OH)D concentration in free-living older adults in the UK has not been investigated. Furthermore, the relative contribution of cumulative sun exposure to plasma 25(OH)D concentration among older adults supplemented with vitamin D has not been studied. Therefore, the aim of the present study was to quantify

the impact of sun exposure on vitamin D status in older adults supplemented with twelve monthly doses of vitamin D_3 supplements (12000 IU, 24000 IU and 48000 IU) in the UK.

The objectives of this study were to,

1) investigate the relationships between sun exposure behaviours and serum 25(OH)D concentration in older adults.

2) quantify the contribution of cumulative sun exposure to serum 25(OH)D concentration among older adults supplemented with vitamin D_3 monthly for one year.

4.2 Methods

Data from VDOP (Vitamin D in Older Adults) study was used for the present study. The primary aim of the VDOP study was to test the effect of three doses of monthly vitamin D_3 supplements (12000 IU, 24000 IU and 48000 IU) on BMD in men and women aged 70 years or older. The aim of this chapter of the PhD thesis was to identify the relationship between the cumulative sun exposure and total plasma 25(OH)D concentration in older adults of the VDOP study.

4.2.1 Study design and participant recruitment

VDOP study is a randomized double-blind interventional trial of 379 male and female older adults aged 70 years or older, living in the North-East of England (55^0N), which conducted from November 2012 to May 2013. Details about the recruitment of participants, inclusion criteria and exclusion criteria are described in chapter 2 in this PhD thesis. Total of 379 older adults enrolled for the study and seven (7) older adults were excluded from the baseline data analysis due to the missing measurements of plasma 25(OH)D concentration and/or sun exposure. In addition, older adults who used dietary supplements of multivitamins, calcium, vitamin D and fish oil (n=33) were excluded from data analysis. Hence, total baseline sample consisted of 339 older adults. Further details about study design and participant recruitment and ethical approval are described in section 2.1 in Chapter 2.

4.2.2 Study visits and vitamin D supplementation

Participants were randomized to receive one of three doses of vitamin D_3, 12000 IU, 24000 IU or 48000 IU, monthly for one year. Study visits took place at baseline and thereafter at 3-monthly intervals (5 in total) for a one year period. Vitamin D_3 supplements were given at each study visit and the two bottles of vitamin D supplements were given to all participants to take at home for the next two months. Of the total participants, 62% of participants began the 12 months supplementation during the time of the year where there is no UVB radiation at the

study location (55^0N), defined as October to March. The rest of the participants began the 12 months supplementation during summer months defined as April to September.

4.2.3 Sunshine exposure, dietary intake, anthropometry and demographic variables

Assessment of sunlight exposure

Sun exposure questionnaire which was adopted from Macdonald *et al*., 2013 was used to gather the information about habitual sun exposure behaviors during the last three months (each month separately). However this questionnaire was not previously validated. The frequency of sun exposure, use of sunbed, skin exposure area, sunblock usage, details about holiday visits and the Fitzpatrick skin types (Fitzpatrick, 1988) were collected using this questionnaire (Appendix G). Except for the skin types, all other information was collected at all five study visits (at baseline, after 3 months, 6 months, 9 months and after 12 months).

Analysis of sun exposure data

The following factors were considered when analyzing the sun exposure data.

1. Sun exposure details of each month were approximately similar, therefore, sun exposure detail of the previous month was considered in the analysis.
2. Skin exposure area was calculated according to Petersen *et al*., 2013 which accounted for 6%, 2.5%, 12.5%, 38.5% and 28% for the head, both hands, both arms, both legs and stomach and back skin areas of the whole body, respectively.
3. Skin type I and II and skin type IV and V were combined in the data analyses due to the low number of participants in each skin type.
4. If the participants' previous month's sun exposure of each data collection point fell during April to September, it was considered as 'summer sun exposure' while previous month's sun exposure fell during October to March it was considered as 'winter sun exposure'.
5. Only about 6% of participants answered the question about skin area that applied sunblock at the baseline study visit. Therefore information about skin area of sunblock used was not considered in the data analysis.
6. Sun exposure questionnaire did not include a direct question about the sunblock use, therefore individuals who answered the question about the skin area of sunblock use, were considered as the sunblock uses.

Calculation of cumulative Sun Exposure Score (SES)

SES of each time point was calculated according to the scoring system described by Pilz *et al.,* 2012. Following equation was used to calculate the sun exposure score.

SES = [Score for the frequency of outdoor activity + score for the skin exposure area + score for the sunblock use + score for holiday visits + score for the season of holiday visit]

Questionnaires which were answered coving the sun exposure details only during the summer months (April to September) at each study visit were considered in the analysis. Each study participants had 2 or 3 study visits that covered the sun exposure details during summer months of the previous year. The mean SES of 3 or 2 study visits was used to get the SES of the whole year. Scores for the responses of each question in the questionnaire are displayed in Table 4.1.

Table 4.1 : Scoring system used in the analyses of sun exposure questionnaire

Question in the questionnaire	Responses	Score
Frequency of outdoor activities	Often	3
	Occasionally	2
	Seldom/never	1
Skin exposure area	Bathing suit	4
	Face, hand, arms and legs	3
	Face, hand and arms	2
	Face and hand	1
Sunblock use	No	1
	Yes	0
Holiday visits	Yes	1
	No	0
Duration of holiday visits	< 7 days	1
	5.– 14 days	2
	14– 21 days	3
	>21 days	4
Skin types	I	5
	II	4
	III	3
	IV	2
	V	1
Season of the holiday visits	During summer months	1
	During winter months	0

Dietary intake

The CalQuest dietary questionnaire, which was adapted from Calquest (Nelson *et al*., 1998) was used to gather the information about dietary intake at the screening visit and subsequently during 3rd and 5th study visits. Dietary intake of calcium and vitamin D were calculated. The mean values for dietary calcium and vitamin D intake across these 3 time points were used in subsequent data analysis.

Anthropometry and sociodemographic information

Height and weight were measured at 3 months intervals at the study center, WC and HC were also measured at the first study visit. BMI was calculated by dividing the weight in kg by square of height in meters. A questionnaire was used to collect information on medical history, vitamin D supplement use, lifestyle information and sociodemographic information.

Blood samples

Overnight fasting venous blood samples were collected from participants at each study visit. Concentrations of $25(OH)D_2$ and $25(OH)D_3$ concentrations in plasma were measured by LC-MSMS, before and after the intervention. Total plasma 25(OH)D concentration was calculated by summing-up $25(OH)D_2$ and $25(OH)D_3$ values. EDTA plasma was used for the analysis of PTH by immunoassay (Immulite, Siemens Healthcare Diagnostics Ltd, Camberley, UK).

4.2.4 Statistical analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS 24.0, Chicago). Older adults who used vitamin D supplements were omitted in the present analysis. Baseline characteristics of the study population were presented as mean $\pm$ SD and percentages. Data were checked for normality using the Kolmogorov-Smirnov statistic. Plasma 25(OH)D concentration at baseline was not normally distributed and, therefore was converted to natural logs values, prior to the analysis. Baseline and endpoint plasma 25(OH)D concentration that was stratified based on demographic, biochemical, sun exposure and anthropometric variables were compared using one-way ANOVA and Student's t-test. For the variables that had more than two categories, one-way ANOVA was used, while for the variables that had two categories Student's t-test was used. The Bonferroni test was used for post hoc comparisons between means. Stepwise linear regression analysis was executed to identify the predictors of baseline and endpoint plasma 25(OH)D concentration for each vitamin D dose group separately and for the total sample. SES was added to the models to represent the sun exposure behaviors of older adults. The dose of supplementation, baseline plasma 25(OH)D concentration, BMI, age, gender, dietary vitamin D intake, dietary calcium

intake, FFM and FM were the other predictor variables added into the regression models. These variables were selected on the basis of prior knowledge of the factors associated with plasma 25(OH)D concentration. Baseline 25(OH)D concentration, dietary vitamin D intake, calcium intake, BMI age and SES were the continuous variables. Gender and dose of supplementation (12000 IU, 24000 IU and 48000 IU) were the categorical variables. Sun exposure related variables were compiled to get the SES, described by Pilz *et al*., 2012. Variables that had more than two categories were entered into the model as dummy coded variables. Scatter plots were used to show the relationship between PTH and plasma 25(OH)D concentration for both males and females and for both before and after vitamin D supplementation. Simple linear regression analysis was performed to identify the relationship between PTH and 25(OH)D concentration for males and females and for both before and after the supplementation. A p value less than 0.05 was considered statistically significant.

4.3 Results

4.3.1 Basic characteristics and vitamin D intake

Table 4.2 shows the characteristics of the study participants at baseline. Mean plasma 25(OH)D and PTH concentrations were 38.2 (SD 19.2) nmol/L and 48.9 (23.4) Pg/mL, respectively. Mean age and BMI were 75.0 (SD 4.2) years and 27.0 (SD 4.0) kgm^{-2}, respectively. The percentage of males and females was approximately similar. Twenty four percent (24%) of males and 36.7% of females had plasma 25(OH)D concentration less than 25 nmol/L. Vitamin D status of males and females at baseline is depicted in Figure 4.1. Majority of males (98.1%) and females (98.7%) did not meet the RNI for vitamin D (10 µg/day) that is recommended by SACN (SACN, 2016).

Table 4.2: Characteristics of the study population at baseline (n=339)

Characteristics	Mean (SD)
Age (years)	75.1 (4.2)
Body Mass Index (kgm^{-2})	27.1 (4.0)
Serum 25(OH)D conc. (nmol/L)	38.2 (19.2)
< 25 nmol/L (n (%))	99 (29.2)
< 50 nmol/L (n (%))	256 (75.5)
Parathyroid Hormones conc (Pg/ml)	48.9 (23.4)
Mean dietary vitamin D intake (µg/day)	3.8 (2.6)
Mean dietary calcium intake (mg/day)	838.3 (392.5)
% who did not meet the RNI for vitamin D (n(%))	
Males	332 (98.1%)
Females	334 (98.7 %)
Gender (n (%))	
Males	175 (51.6)
Females	164 (48.4)

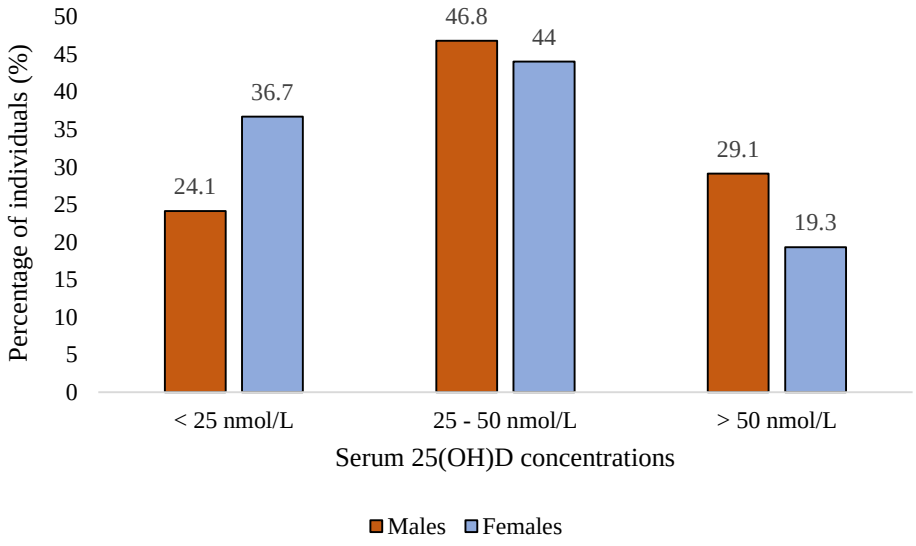


Figure 4.1 Vitamin D status of males and females at baseline

4.3.2 Relationships between plasma 25(OH)D concentration and sun exposure, demographic, dietary, and anthropometric variables at baseline

Table 4.3 summarizes the plasma 25(OH)D concentration by sun exposure, demographic, dietary and anthropometric variables at baseline. Participants who sampled during summer, had significantly high plasma 25(OH)D concentration compared to the participants sampled during winter (p=0.035). Similarly, participants who had holiday visits at the baseline study visit had significantly higher plasma 25(OH)D concentration compared to the older adults who did not have the holiday visits (p=0.003). Gender, age and vitamin D intake were

significantly associated with plasma 25(OH)D concentration (p = 0.003, p = 0.033, p = 0.046, respectively). Participants who had a normal BMI range and the overweight BMI range had significantly higher serum 25(OH)D concentration compared to obese individuals (p < 0.001).

Table 4.3 Plasma 25(OH)D, concentration by sun exposure, demographic, dietary and anthropometric variables at baseline (n=339)

Variables	n	Plasma 25(OH)D conc. Mean (SD)	P value[1]
Sun exposure variables			
Frequency of sun exposure			
Often	240	35.0 (18.8)	0.185
Occasionally	67	30.7 (16.9)	
Seldom/never	27	30.2 (19.2)	
Use of sunblock			
No	319	33.2 (16.2)	0.756
Yes	20	34.9 (18.3)	
Sun exposure season[2]			
Winter (Oct – Mar)	235	32.5 (15.3)	0.043
Summer (Apr – Sep)	104	36.6 (17.6)	
Season of blood sampling			
Winter (Oct – Mar)	238	36.7 (19.1)	*0.035*
Summer (Apr – Sep)	101	41.5 (19.4)	
Holiday visits			
No	244	32.0 (19.7)	0.003
Yes	95	38.5 (18.6)	
Length of holiday visits			
< 2 weeks	58	35.8 (16.7)	0.147
≥ 2 weeks	37	42.3 (19.9)	
Fitzpatrick skin type			
Type I & II	113	30.1 (15.9)	0.002
Type III	164	40.8[2] (19.4)	
Type IV & V	57	40.9[2] (22.9)	
Skin exposure area			
< Median (6% of total body area)	196	33.6 (15.6)	0.782
≥ Median	143	34.0 (16.3)	
Demographic, dietary and anthropometric variables			
Gender			
Male	175	36.5 (18.6)	0.003
Females	164	30.9 (15.4)	

Table 4.3 continue

Variables	n	Plasma 25(OH)D conc. Mean (SD)	P value[1]
Age categories			
1 st quartile (< 71.5 yrs)	84	34.3 (15.3)	0.033
2nd quartile (71.5 – 74.0 yrs)	86	36.2 (16.9)	
3rd quartile (74.1 – 77.1 yrs)	86	35.3 (16.3)	
4th quartile (>77.1 yrs)	83	29.3[3](15.2)	
Body Mass Index (kgm^{-2})			
Normal (18.50 – 24.99)	110	40.7[4] (19.4)	<0.001
Overweight (25.00 – 29.99)	165	40.5[4] (20.0)	
Obese (> 30.00)	64	27.7 (12.2)	
Dietary vitamin D intake (µg/day)			
< Median (3.22)	165	32.0 (17.9)	0.046
≥ Median	165	35.7 (19.7)	
Dietary calcium intake (mg/day)			
< Median (767.9)	165	34.2 (19.6)	0.762
≥ Median	165	33.5 (20.1)	

[1] One way ANOVA for the variables that had more than two categories and Student's t-test for the variables that had two categories with Bonferroni post-hoc comparison
[2] Significantly different from skin types 1& II
[3] Significantly different from1st, 2nd and 3rd quartiles
[4] Significanly different from obese individuals

Table 4.4 presents the total predictors (demographic, dietary and anthropometric variables) and sun exposure predictors of plasma 25(OH)D concentration at baseline. Only 59 individuals completed the sun exposure questionnaire covering the sun exposure details during summer months. BMI and gender, explained 6.5% of the variance in baseline plasma 25(OH)D concentration. Female gender was a negative predictor of baseline plasma 25(OH)D while BMI was a positive predictor of baseline plasma 25(OH)D concentration. However, in this multivariate model SES, dietary calcium intake, vitamin D intake, age, FFM and FM were not significant predictors of baseline serum 25(OH)D concentration.

Table 4.4 : Total predictors[1] of plasma 25(OH)D concentration in older adults at baseline[2] (n=59)

Total predictors		B	SE	β	p	CI
R²	**0.065**					
Constant	**123.76**					
Body Mass Index (kgm^{-2})		-1.105	0.701	-0.208	0.030	-2.769 – 0.163
Male		Reference				
Females		-6.790	0.500	-0.179	0.024	18.29 – 251.50
Dietary calcium intake (mg/day)		-0.001	0.003	-0.022	0.706	-0.012 – 0.013
Dietary vitamin D intake (µg/day)		0.667	0.719	0.129	0.358	-0.777 – 2.111)
Age		-0.649	0.679	-0.140	0.344	-2.015 – 0.716
Sun Exposure Score		-0.530	1.226	0.066	0.667	-1.933 – 2.993
Fat Free Mass (kg)		-0.248	0.526	-0.137	0.639	-1.306 – 0.810
Fat Mass (kg)		-0.097	0.826	-0.039	0.907	-1.757 – 1.562

[1] Sun exposure, dietary, sociodemographics and anthropometric variables,
[2]*Stepwise multiple linear regression.*
Independent variables were Body Mass Index,, Fat Mass Index,, Fat Free Mass, gender, age, dietary calcium intake, dietary vitamin D intake, Sun Exposure Score

4.3.3 Relationship between plasma 25(OH)D concentration, sun exposure, demographic, dietary and anthropometric variables after supplementation

Table 4.5 illustrates the mean plasma 25(OH)D concentration by cumulative SES, demographic, dietary and anthropometric variables by the dose of vitamin D supplementation at the end of total supplementation. Older adults who had high BMI value had significantly lower plasma 25(OH)D concentration than the other counterpart, irrespective to the dose of vitamin D supplementation. Older adults who had the highest vitamin D intake was associated with high serum 25(OH)D only in high dose group. SES was not associated with serum 25(OH)D concentration in any of the dose groups.

Figure 4.2 shows percentage of participants with serum 25(OH)D concentration below threshold of vitamin D status according to vitamin D supplementation groups. Plasma 25(OH)D concentration was significantly associated with the dose of supplementation ($p<0.001$), which showed the highest value for the high dose group. After the supplementation mean plasma 25(OH)D concentration was 66.1 (SD 17.8) nmol/L and only 1 person (0.03% of the population) was vitamin D deficient according to the UK cutoff of VDD (25 nmol/L) in low dose group. None of the older adults were vitamin D deficient according to UK cutoff of vitamin D deficiency in 24000 IU and 48000 IU dose groups. After total supplementation, 12.9 % of the population and 5.5% of the population were vitamin D deficient according to the US cutoff of VDD (50 nmol/L).

Table 4.5 : Relationship between cumulative sun exposure, demographic, dietary and anthropometric variables and plasma 25(OH)D concentration by the dose of supplementation after 12 month supplementation.

Variables	Post intervention plasma 25(OH)D concentration (Mean (SD))							
	n	Total sample	n	12000 IU	n	24000 IU	n	48000 IU
Mean 25(OH)D (SD)	308	66.2 (17.8)	102	54.7 (14.6)	104	65.0 (15.2)	102	78.8 (14.8)
P value						*< 0.001[1]*		
Sun Exposure Score								
< Median (10.5)	140	*66.2 (18.2)*	*43*	*53.6 (15.2)*	*49*	*64.2(14.5)*	*48*	*76.1 (15.2)*
	168	*65.9 (17.5)*	*59*	*55.3 (14.3)*	*55*	*65.4 (15.9)*	*54*	*77.9 (14.6)*
Age (years)								
1st quartile (< 72.5)	77	64.6 (21.5)	29	53.3 (13.7)	25	60.9 (19.9)	23	83.0 (20.0)
2nd quartile (72.5–75.0)	77	67.0 (17.6)	25	54.7 (16.0)	29	68.1 (12.7)	23	79.0 (16.0)
3rd quartile (75.1– 78.1)	77	65.6 (14.9)	27	57.6 (15.0)	27	64.8 (13.0)	23	75.6 (10.4)
4th quartile (>78.1)	76	67.1 (16.9)	20	51.7 (13.9)	23	65.1 (14.4)	33	77.9 (11.9)
P value[2]		*0.792*		*0.555*		*0.395*		*0.405*
Gender								
Males	158	65.4 (17.1)	52	54.1 (15.3)	55	65.2 (14.2)	51	77.1 (13.9)
Females	150	66.8 (18.5)	50	55.3 (14.1)	49	64.5 (15.3)	51	80.3 (15.7)
P value[2]		*0.251*		*0.667*		*0.805*		*0.282*
Vitamin D intake (µg/day)								
< Median (5.5)	154	66.0 (19.0)	54	53.7 (13.3)	54	64.6 (15.4)	46	82.2 (16.9)
> Median	150	66.4 (16.5)	45	56.2 (16.1)	49	65.1 (15.2)	56	75.8 (12.4)
P value[2]		*0.855*		*0.399*		*0.880*		*0.029*
Calcium intake (mg/day)								
< Median (768.9)	154	66.4 (19.4)	49	54.7 (15.6)	58	63.1 (15.9)	47	82.3 (16.6)
> Median	154	65.9 (16.1)	53	54.5 (13.9)	46	67.2 (14.1)	55	75.8 (12.7)
P value[2]		*0.825*		*0.955*		*0.184*		*0.024*
BMI (kgm^{-2})								
Normal (18.50 – 24.99)	109	70.0 (17.3)	41	58.2 (13.7)	30	70.3 (15.1)	38	82.5 (13.2)
Overweight (25.00 – 29.99)	143	65.8 (18.2)	47	52.9 (15.4)	51	65.7 (12.9)	45	79.3 (16.2)
Obese (> 30.00)	56	59.0 (15.3)	14	49.8 (13.1)	23	55.9 (16.4)	19	69.5 (10.6)
P value[3]		*0.001*		*0.102*		*0.002*		*0.006*

1 Significantly differ between all three groups [2]Student's t test [3]One way ANOVA

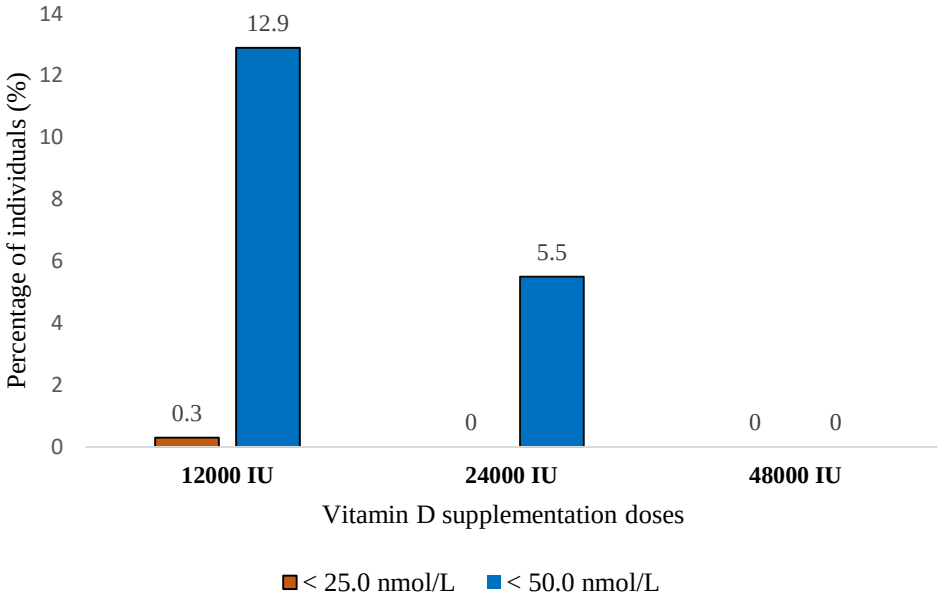


Figure 4.2 : Percentage of participants with serum 25(OH)D concentration below threshold of vitamin D status according to vitamin D supplementation groups

Table 4.6 shows the anthropometric, demographic, dietary and sun exposure predictors of the endpoint plasma 25(OH)D concentration. The contributors to the explained variance of the endpoint plasma 25(OH)D concentration of the total sample were the dose of supplementation, baseline plasma 25(OH)D, FFM and BMI which, collectively contributed 56.7% of the variance of the endpoint plasma 25(OH)D. Baseline plasma 25(OH)D concentration and FFM were the significant predictors for the endpoint plasma 25(OH)D concentration in low dose group which explained 59.9% of the variance of 25(OH)D concentration. Baseline plasma 25(OH)D concentration and BMI were the predictors of plasma 25(OH)D concentration in medium dose group. Those variables explained 38% of the variation in endpoint serum 25(OH)D concentration. Similarly,baseline plasma 25(OH)D concentration, BMI and vitamin D intake were the predictors of endpoint plasma 25(OH)D concentration in 48000 IU dose group which explained 24.9% of the variability of the endpoint plasma 25(OH)D concentration. The variance explained by the models were decreased with increasing dose of vitamin D supplementation. SES was not retained in the any of the models that explained the endpoint serum 25(OH)D concentrations.

Table 4.6 Predictors of plasma 25(OH)D concentration after 12 months supplementation by the dose of supplementation*

		B	SE	β	p	CI
Total predictors		**Plasma 25(OH)D**				
Total sample (n=308)						
R²	56.7					
Constant	71.31					
48000IU dose		26.04	1.688	0.688	<0.001	22.92 – 29.36
24000 IU dose		12.15	1.687	0.322	<0.001	8.881 – 15.47
12000 IU dose		**Reference**				
Baseline 25(OH)D (nmol/L)		0.397	0.036	0.433	<0.001	0.326 - 0.468
Fat Free Mass (kg)		-0.252	0.070	-0.145	<0.001	-0.390– (-0.114)
Body Mass Index (kgm^{-2})		-0.659	0.189	-0.146	<0.001	-1.02 – (-0.290)
12000IU dose group (n=102)						
R²	59.9					
Constant	48.91					
Baseline 25(OH)D (nmol/L)		0.567	0.05	0.736	<0.001	0.469 – 0.666
Fat Free Mass (kg)		-0.341	0.107	-0.206	0.002	-0.553 – (- 0.129)
24000 dose group (n=104)						
R²	38.0					
Constant	84.82					
Baseline 25(OH)D (nmol/L)		0.337	0.060	0.453	<0.001	0.218 – 0.456
Body Mass Index (kgm^{-2})		-1196	0.294	-0.328	<0.001	-1.779– (-0.612)
48000 dose group (n=102)						
R²	24.9					
Constant	96.5					
Baseline 25(OH)D (nmol/L)		0.239	0.071	0.304	<0.001	0.097 – 0.381
Body Mass Index (kgm^{-2})		-0.938	0.338	-0.256	0.006	-1.620 – (- 0.277)
Vitamin D intake (µg/day)		-1.191	0.554	-0.191	0.034	-2.291 – (-0.091)

**Stepwise multiple linear regression, Endpoint plasma 25(OH)D concentration was the independent variable while Body Mass Index, gender, age, dietary calcium intake, dietary vitamin D intake, cumulative SES were the dependent variables*

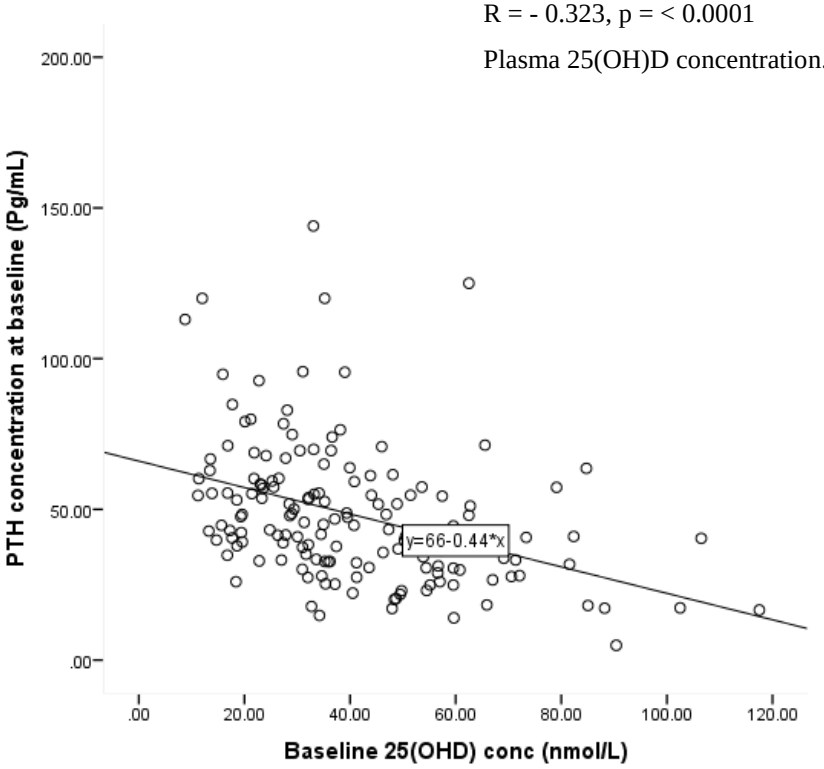
4.3.4 Relationships between plasma 25(OH)D concentration and PTH concentration

Figure 4.3 shows the relationships between plasma 25(OH)D and PTH concentration in males and female at baseline ($r = -0.323$, $p = <0.001$ and $r= -0.344$, $p <0.001$, respectively). In both cases, there was a clear significant negative relationship between plasma 25(OH)D concentration and the PTH concentration. Similarly, figure 4.4 shows the negative associations between 25(OH)D concentration and the PTH concentration for both males and females after the total supplementation.

A

R = - 0.323, p = < 0.0001

Plasma 25(OH)D concentration. = -0.291 (PTH) + 54.91



B

R = - 0.344, p <0.0001

25(OH)D concentration = -0.247 (PTH) + 47.32

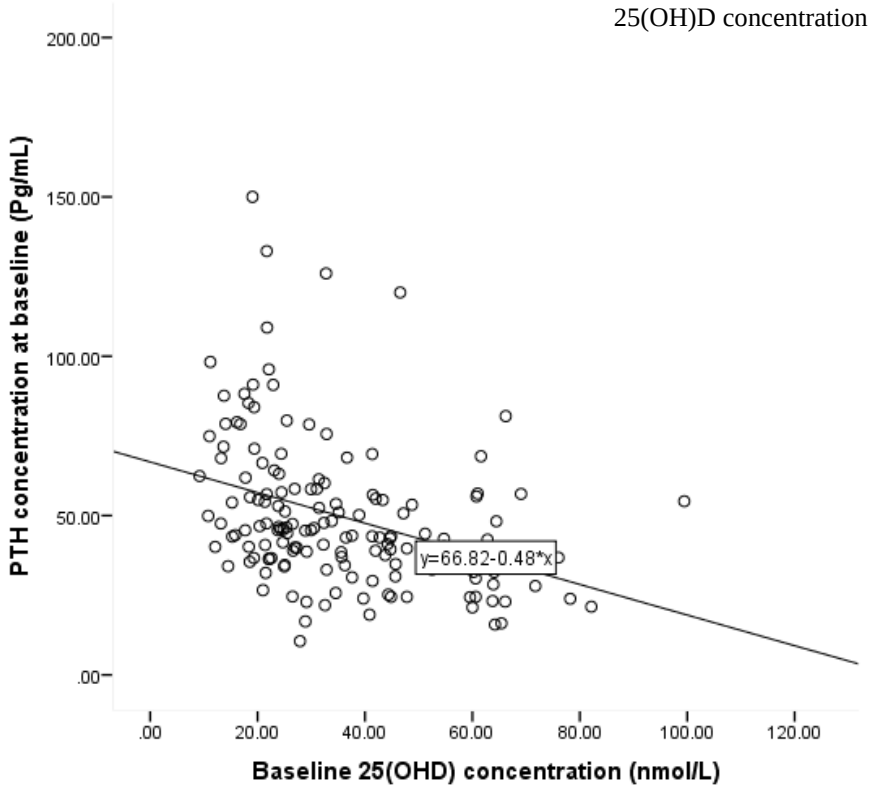
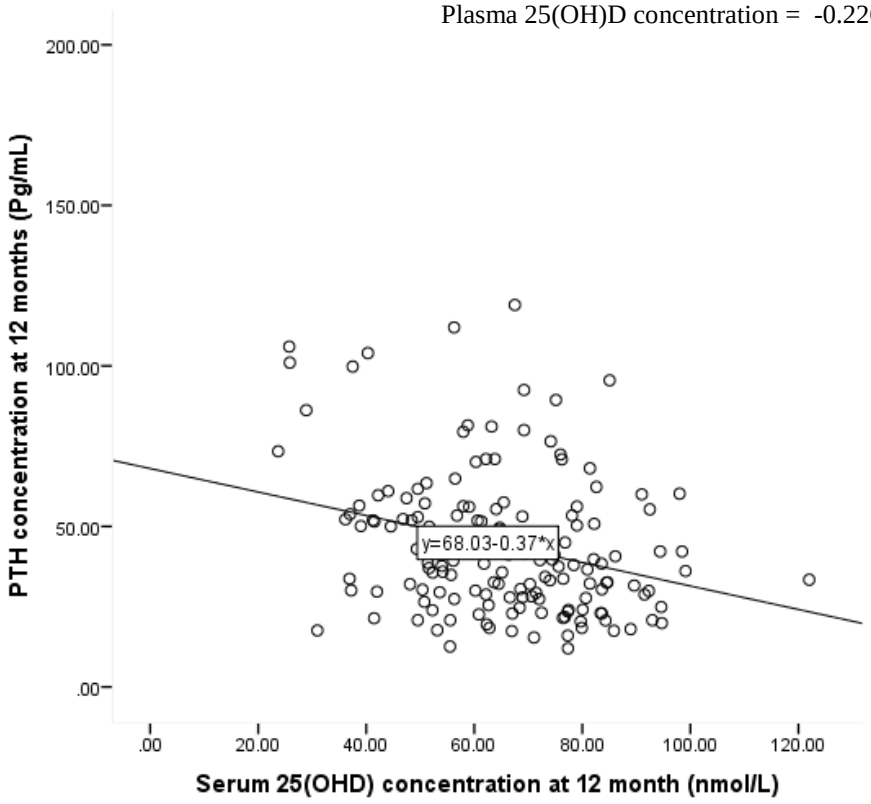


Figure 4.3 : Relationship between plasma 25(OH)D concentration and PTH concentration in males (A) females (B) at baseline

A

R = -0.288, p < 0.0001

Plasma 25(OH)D concentration = -0.226 (PTH) + 75.35



B

R = -0.281, p < 0.0001

Plasma 25(OH)D concentration = -0.247 (PTH) + 76.85

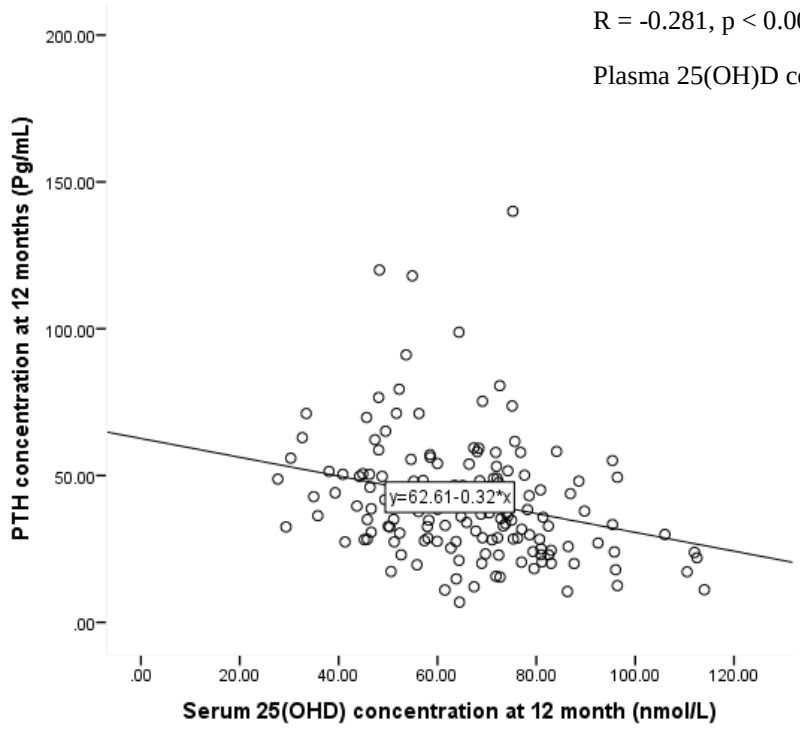


Figure 4.4 : Relationship between plasma 25(OH)D concentration PTH concentration in males (A) and females (B) after the supplementation

Table 4.7 : Linear regression analysis between plasma 25(OH)D and PTH concentration before and after the supplementation by gender

	Constant	Regression coefficient for PTH conc.	SE	P
At baseline				
Males	54.91	-0.291	3.23	<0.001
Females	47.32	-0.247	32.11	<0.001
After supplementation				
Males	75.35	-0.226	2.977	<0.001
Females	76.85	-0.247	3.221	<0.001

According to table 4.7, there was a negative significant relationship between plasma 25(OH)D and PTH concentration for both before and after the supplementation and for both males and females. The regression coefficient was approximately similar for both males and females.

4.4 Discussion

4.4.1 Main findings

The aim of this study was to quantify the impact of cumulative sun exposure on plasma 25(OH)D concentrations in older adults supplemented with monthly doses of 12000 IU, 24000 IU and 48000IU vitamin D_3 for one year. BMI and gender were the predictors of plasma 25(OH)D concentration before vitamin D supplementation while the dose of vitamin D supplement, baseline 25(OH)D concentration, FFM and BMI were the main predictors of plasma 25(OH)D concentration after the vitamin D supplementation in the total sample. None of the sun exposure variables predicted of plasma 25(OH)D concentration before and after the supplementation in all dose groups.

4.4.2 Interpretation of the main findings

Change in plasma 25(OH)D concentration

After 12 months supplementation, the plasma 25(OH)D concentrations in low, medium and high dose groups were increased in dose-dependent manner. The increase in plasma 25(OH)D concentration of 12000 IU, 24000 IU and 48000 IU dose groups were 14.3 (SD 12.6), 25.3 (SD 18.0) and 40.9 (SD 19.8) nmol/L, respectively which were significantly higher than the baseline values of each dose group. According to a past study of vitamin D supplementation in adult women aged 40-55 years whose the baseline serum 25(OH)D concentration was less

than 62 nmol/L (25 ng/mL) who were supplemented with 1000 IU of daily vitamin D for 12 weeks showed 25 nmol/L (10 ng/mL) increase in serum 25(OH)D concentration was seen. Approximately this was a 1 nmol/L increase with the intake of 1 µg supplemental vitamin D. This study too reported 1 nmol/L increase in plasma 25(OH)D concentration with 1 µg intake of vitamin D (Aspray *et al*., 2018). Similar increases in serum 25(OH)D concentrations are reported by some other studies of older adults (Vaes *et al*., 2018, Brooks and Greene-Finestone, 2017, Zhu *et al*., 2010). O'Sullivan *et al*., 2019, reported 0.6 nmol/L increase in plasma 25(OH)D concentration, with 1 µg of supplemented vitamin D in Irish adults whose median age was 44 years. Another study conducted using postmenarchal adolescents who were supplemented with 150000 IU every 3 months for a one year period showed the serum 25(OH)D concentration increased from 19 to 57 nmol/L after supplementation period. Approximately this increase is 1 nmol/L of serum 25(OH)D with the intake of 1 µg of vitamin D intake per day (Ward *et al*., 2010). The increase in plasma 25(OH)D concentration in this study participants was similar to the increase of plasma 25(OH)D with the younger participants who supplemented with the same amount of vitamin D. In 12000 IU and 24000 IU dose groups 16% and 5.5% of the participants were vitamin D deficient according to the US cutoffs of vitamin D deficiency. In 48000 IU dose group none of the study participants was vitamin D deficient for any of the cutoff values. Therefore this study revealed that 48000 IU dose is successful in improving plasma 25(OH)D concentration in older adults.

Mean plasma 25(OH)D concentration of older adults of this study at baseline was 38.2 (SD 19.2) nmol/L which was comparable with recent studies conducted in the UK recruiting older adults (Granic *et al*., 2015). This study further demonstrated that the increase in plasma 25(OH)D concentration among older adults whose baseline 25(OH)D concentration less than 25 nmol/L was higher than the older adults whose baseline 25(OH)D concentration less than 50 nmol/L, which was reported by some previous studies as well (Whiting *et al*., 2015, Ross *et al*., 2011). Low prevalence (only one person) of VDD in this study after the supplementation (according to SACN cutoff of < 25(OH)D nmol/L), implicated the success of the vitamin D supplementation programme in maintaining appropriate serum 25(OH)D concentration in older adults. According to the Public Health England Survey, conducted in 2016/2017, 27% of older adults aged 65 years and over had a serum 25(OH)D concentration below 25 nmol/L (NDNS, 2019) which is very similar to the value reported by our study (29%). Therefore sample of older adults in our study is approximately similar to the representative sample of older adults in the UK.

Association of sun exposure and plasma 25(OH)D concentration

Since the SES score was not associated with plasma 25(OH)D throughout the study, one could tentatively conclude that supplementation was more successful than sunshine exposure in improving vitamin D status of older adults. However, the use of this questionnaire like all others in the literature are crude measures of sun exposure and do not fully capture the complexity of personal sun exposure over time. This argument is further supported by the work of SACN who were unable to quantify the relative contribution of sunshine exposure to vitamin D status for the UK population in their DRV report. In chapter 3 (RCT) I have provided a detailed overview of the impact of vitamin D supplementation dose on 25(OH)D concentrations as well as maintaining adequate vitamin D status after 12 months using a number of commonly reported cut-offs. The results from this chapter appear to suggest that in addition to supplementation, BMI and gender also influence 25(OH)D concentrations at baseline and after 12 months. Making any direct comparisons of the relative contribution of these predictors to sunshine exposure should be made with caution for the aforementioned reasons.

In contrast to our findings, a study conducted in Dublin (53^0N) reported that daily ambient UVB dose was more effective in improving plasma 25(OH)D concentration compared to vitamin D supplementation in Irish adults who supplemented with 200 IU/day of vitamin D_3 for one year (O'Sullivan *et al*., 2019). Methods used to assess sun exposure and the dose of vitamin D supplements might be attributed to this inconsistent finding. UVB radiation dose of the area using the data from weather station was used by O'Sullivan *et al*., 2019, which is different from the personal ambient UVB radiation dose. In our study we used sun exposure questionnaire to gathered the data about personal sun exposure. The doses that used in our study were correspondent to 400 IU/day, 800 IU/day and 12000 IU/day, which was higher than the dose used by O'Sullivan *et al*., 2019 (200 IU/day). Because of these disparities, the findings of two studies cannot be compared.

Thought the holiday visits and dark skin types were associated with baseline plasma 25(OH)D concentration, SES did not associated with the serum 25(OH)D concentration. Low predictive ability of plasma 25(OH)D concentration by SES in this study might be attributed to the limitation of the sun exposure questionnaire which had vague questions about the sun exposure habits. In addition, some sun exposure related variables were not captured by this questionnaire such as time of the day of sun exposure, clothing, duration of sun exposure, the frequency of sunblock usage and SPF factor.

The positive association of dark skin and plasma 25(OH)D concentration at baseline reported in this study was unexpected. Literally, dark skin has more melanin that blocks the penetration of UVB radiations to the skin, thus reduce the synthesis of vitamin D (Chen *et al*., 2007). In the same study they stated that the dark-skinned individuals had the highest threshold for synthesizing detectable previtamin D_3 resulting in high vitamin D status compared to the fair-skinned individuals. Contradictory finding in this study might be attributed to the limitation of Fitzpatrick skin types in determining melanin density (Eiler *et al*., 2013). In this study the majority of the individuals who had dark skin types were males (55%) and they had better vitamin D status compared to females. Therefore, positive association of fair skin and plasma 25(OH)D concentration might be hidden by being males. On the other hand, dark-skinned people might stay in sunlight for a long time period without applying sunblock which facilitates vitamin D synthesis. Some previous studies too reported the positive association between dark skin types and the vitamin D status (Touvier *et al*., 2015 *et al*, Ginter *et al*., 2013, Bogh *et al*., 2010).

Wider determinants of plasma 25(OH)D concentration

Some previous studies showed the positive association of vitamin D intake and serum 25(OH)D concentration in older adults (Bertrand *et al*., 2010, McCullought *et al*., 2010). According to the ANOVA analysis vitamin D intake was positively associated with plasma 25(OH)D concentration before supplementation. However, this finding was not confirmed by the regression analysis, before and after the supplementation. Recent few studies on older adults and adults have also reported the similar null finding of the association of vitamin D intake and 25(OH)D concentration (Gungert and Berthold 2015, Freedman *et al*., 2013, Hedlund *et al*., 2013). Very low vitamin D intake (3.8 (SD 2.6) µg/day) by our study participants might be the reason for this null finding (Thuesen *et al*., 2012). In this study, similar to some previous studies, BMI, age and gender were also played the role in predicting baseline plasma 25(OH)D concentration (Buyukuslu *et al*., 2014, Freeman *et al*., 2013). The negative association of FFM and BMI plasma 25(OH)D concentration was not masked by the vitamin D supplements.

Relationship between 25(OH)D and PTH concentration

Many studies in older adults reported the negative association of serum 25(OH)D concentration and PTH concentration (Chang *et al*., 2017, Jungert and Neuhauser-Berthold, 2015, Arabi *et al*., 2012) similar to this study. Age, gender, ethnicity, sex hormone level, calcium status and kidney function are some of the factors that significantly associated with

PTH concentration in previous studies (Hill *et al*., 2010, Adami *et al*., 2008). However, a positive association of PTH concentration and gender before or after the supplementation was not reported in this study.

4.4.3 Strengths and limitations of the study

To our knowledge, this is the first study that determined the relationship between cumulative sun exposure and plasma 25(OH)D concentrations in a RCT of a large group of free-living older adults at a Northern latitude, where cutaneous production of vitamin D is not possible all year around. Standard intervention with relevant doses, which corresponds to 400, 600 and 1200 µg of dietary vitamin D per day, using randomized double-blind study design in both men and women are also strengths and novel aspects in this study. Determining the effect of one-year cumulative sun exposure added a novelty to this study. Use of a wide range of variables such as sun exposure, dietary, anthropometric, socio-demographic, biochemical that associated with 25(OH)D concentration was an added advantage to this study. The RCT design used in this study provide the strong design to test the impact of sun exposure behaviors on vitamin D status since the bias introduced by "as-needed" supplement taking is minimized.

This study had a few limitations. Estimation of UVB radiation exposure by self-reported sun exposure questionnaire was the main limitation of this study. True ambient UVB radiation of the older adults might be underestimated by the sun exposure questionnaires. Also this questionnaire did not include the questions about the duration of sun exposure, details about sunblock usage, time of the day of the sunlight exposure and sun protection habits. Also personal sun exposure measured during the past year at five time point might not reflect the true ambient UVB exposure of the whole year. In addition, older adults were selected for this study from the GP's registry, thus older adults who were more health conscious might participated in the study. Therefore, there was a healthy selection bias and selected older adults might not represent the general population in the UK. Further, the vitamin D status was measured only at baseline and at the end of the supplementation, which does not reflects the year-round vitamin D status of the older adults. Therefore it might not accurate to determine the association between plasma 25(OH)D concentration which measured at two-time points and cumulative sun exposure data of the whole year.

4.4.4 Public Health implications and future studies

This study highlights that vitamin D supplementation was powerful in improving vitamin D status in older adults compared to the sunlight exposure. This study opens a debate on the recommendation for sun exposure and vitamin D supplementation in older adults. The positive effect of vitamin D supplementation on plasma 25(OH)D concentration compared to sun exposure in older adults was an important finding in this study. This finding can be used in future studies of determining the required oral dose of vitamin D for older adults to optimize the vitamin D status. However, future studies are needed for accurate measurements of personal sun exposure the tools such as dosimeters. Further, recruiting the representative sample of older adults in the UK is necessary in order to establish accurate guidelines for sun exposure. Also as the sun exposure guidelines are widely differed according to the skin types. However the Fitzpatrick skin type classifications have some limitations in assessing melanin density, therefore futures studies are needed with accurate methods of determining the melanine density.

4.4.5 Conclusions

Gender and BMI were the negative predictors of plasma 25(OH)D concentration, while baseline plasma 25(OH)D concentration, the dose of vitamin D supplementation, FFM and BMI were the positive predictors of plasma 25(OH)D concentration after the total supplementation. The sun exposure score did not predict endpoint plasma 25(OH)D in any of the dose groups, before or after the supplementation. Therefore doses of vitamin D supplementation was more effective in improving vitamin D status of older adults compared to the sun exposure. In addition, baseline plasma 25(OH)D concentration, gender, FFM and BMI were more prominent factors that contributed to vitamin D status in older adults supplemented with vitamin D.

Chapter 5 : Serum 25(OH)D Concentrations in Relation to Sunlight Exposure, Lifestyle Factors and Musculoskeletal Outcomes in Biomarker of Risk of Colon Cancer Study (BORICC) Follow-up (BFU) study

5.1 Introduction

Muscles and bones are two major components of the MSK system that is essential for body structure, strength and movement. During aging, bone and muscle mass reduce which cause poor QOL (Atlantis *et al*., 2008). The balance between bone synthesis and bone resorption is altered with the relative increase in resorption during aging (Szule, 2006). Similarly, the changes in muscle mass and function get reduce during aging (Mitchell *et al.,* 2012). There is a substantial literature that links serum 25(OH)D concentration (the widely-accepted marker of vitamin D status) and MSK outcomes (Dawson-Hughes, 2008). However, older adults are at high risk of VDD as a result of the poor dermal capacity of vitamin D synthesis (Gennari, 2001) and low dietary intake of vitamin D (Bailey *et al*., 2010). Therefore vitamin D is a particularly important critical nutrient for older adults. In addition to vitamin D status, age, gender, body composition, physical activity, ethnicity and genetics affect the MSK outcomes in older adults (Assunção *et al.*, 2017, Tyrovolas *et al*., 2015).

Muscle depletion is one of the body composition changes which occurs in cancer patients. Muscle depletion of cancer patients is characterized by a reduction in muscle size and increased infiltration by inter and intramuscular fat, called myosteatosis (Fearon *et al*., 2011). It is reported that the incidence of muscle depletion varies from 15 to 70 % for patients treated for CRC (Martin *et al*., 2013). It has been identified that vitamin D plays a preventive role in CRC (Woolcott *et al*., 2010). A review of case-controlled studies has established that there is an inverse relationship between serum 25(OH)D concentration and the incidence of polyps and adenomas in the colon (Moon *et al*., 2013). Similarly, dietary vitamin D_3 intake and sunlight exposure were lower in CRC patients compared to controls in a case-control study reported by Robsahm *et al.,* (2004). Similarly, Ng *et al.,* (2008) reported that higher vitamin D_3 concentration was associated with lower CRC incidence, reduced polyp recurrence and better overall survival of CRC patients.

Similar to muscle health, nearly all cancers can have significant negative effects on the skeleton. Cancer is a major risk for both generalized and local bone loss, independent of cancer type (Reuss-Borst *et al*., 2012). Cancer-associated bone loss is the result of multiple, inter-related factors. These include both the direct effects of cancer cells and the effects of

therapies used in cancer treatment. Further, the skeleton is also the most common site of metastatic disease, as cancer cells grow within the bone, induce osteoblasts and osteoclasts to produce factors which stimulate further cancer growth. Accordingly, the optimal management of skeletal health is important for cancer patients, for their survival and longevity (Ng *et al.*, 2008).

Quantitative Ultrasound (QUS) is a convenient and widely used screening tool for osteoporosis (Hien *et al*., 2005). Two parameters generated by QUS are the Speed of Sound (SOS) and the Broadband Attenuation (BUA). The SOS is the division of transmission time of the sound waves by the bones and broadband attenuation of sound refers to the slope between attenuation of sound signals and its frequency. Attenuation occurs because the energy is absorbed by the soft tissue and bone when the sound waves travel through them. Stiffness Index (SI), is derived from the above basic measurements, by the most sophisticated QUS equipment (International Osteoporosis Society, 2001). Therefore SOS, BUA and SI are associated with BMD. Thus are used as the measures of fracture prediction (Chin and Ima-Nirwana, 2013). Heel bone qualitative ultrasound scanner is widely used equipment to measure SOS, BUA and SI in Os Calcis (Hien *et al*., 2005). Similarly, to assess muscle functions, many studies used TUG test and GS. Both these tests are recommended to use as the measures of muscle function in the elderly (Bohannon, 2001, Podsiadlo *et al*., 1991). GS is considered as a tool to assess the upper extremity muscle function (Bohannon, 2008) and presence of sarcopenia (Cruz-Jentoft *et al*., 2010), while TUG test is considered as a measure of the lower extremity muscle function (Bohannon, 2008).

Sun exposure habits such as frequency and duration of sun exposure, holiday visits, season, sunblock use and sun avoidance habits have been demonstrated as the factors that associated with the serum 25(OH)D concentration (Mei *et al*., 2017, Kimlin *et al*., 2014). In addition, demographic characteristics, BMI, body composition, and dietary intake of vitamin D are also associated with serum 25(OH)D concentration (Kimlin *et al.*, 2014, Freedman, 2013). The latest findings on the impact of those factors on serum 25(OH)D in older adults who are at risk of colon cancer in the UK are relatively limited.

Therefore, we hypothesised that there is a positive relationship between serum 25(OH)D concentration, MSK outcomes and sun exposure variables in older adults who are at moderate risk of CRC. Therefore, the aims of present study were;

1) to identify the association between serum vitamin D status and MSK outcomes in older adults
2) to identify the association between the sun exposure behavious, and vitamin D status in free-living older adults who are at moderate risk of CRC.

Objectives of this chapter of the PhD thesis were;

1) to investigate relationships between sun exposure behaviours and serum 25(OH)D concentration in participants in the BFU study.
2) to investigate the relationship between serum 25(OH)D concentration and muscle function in participants in BFU study.
3) to investigate the relationship between serum 25(OH)D concentration and bone health in older adults in BFU study.

5.2 Methods

5.2.1 Participants' selection

Participants were recruited from those who had taken part in the original Biomarkers of Risk of Colon Cancer (BORICC) Study 1 and 2 approximately 12 years ago. In the BORICC Follow-Up (BFU) Study, participants who met the inclusion criteria (section 5.2.2) were sent invitation letters and those individuals who were interested in taking part in the study were asked to contact the research team by telephone or by email. During the telephone calls, participants were given a time and date for the study visit at the North Tyneside General Hospital (NTGH). If the participant requested further information about the study, they were invited to participate in the "show-case" event which was a one-hour session that described all the aspects of the study. Study packs including a FFQ, sun exposure questionnaire and physical activity questionnaire, were sent to participants by post, 2 weeks prior to their study visits. According to the power calculation of BFU study in detecting an association between 25(OH)D and muscle function, considering 95% confidence interval, 5% margin error and 2.8 kg of GS difference, 385 participants would be needed. Further details about the recruitment of participants are described in 2.3 and 2.4 under Chapter 2.

5.2.2 Inclusion and exclusion criteria

All individuals who participated in the original BORICC study 1 and BORICC study 2 were eligible for this study. Exclusion criteria included: actively undergoing chemotherapy or radiotherapy treatment, being unable to provide consent or being unable to attend the study center at NTGH.

5.2.3 Ethical approval, funding and sponsorship

A favorable opinion for the study was obtained from the West Midlands – Coventry & Warwickshire Research Ethics Committee (REC reference 16/WM/0421). All study procedures were conducted according to the guidelines laid down in the declaration of Helsinki. Informed consent to review medical records, collect blood and other biological samples, obtain dietary, lifestyle, sun exposure, anthropometric and physical capability data was obtained from all participants. Sample collection and participant recruitment were funded by the MRC as part of the Centre for Ageing & Vitality (Ref: MR/M501700). Sponsorship of the study was provided by the Northumbria NHS Foundation Trust, Research Support Unit.

5.2.4 Sunshine exposure, dietary intake, anthropometry and demographic variables

All questionnaires were posted to the participants two weeks prior to the study visit. During the study visits in the NTGH, all questionnaires, including sun exposure questionnaire and FFQ were checked for the missing or incomplete details by a member of the research team and corrections were made, if necessary.

Demographic and medical information

Age, gender, ethnicity and date of birth were gathered using the questionnaires that used to gather information about sun exposure, medical and health history. Past medical history of the participants was collected by face to face discussion with the participants using a short "Patient Health History Questionnaire" (Annexure F) by a medical practitioner. Presence or absence of selected disease conditions, date of diagnosing, the stage of the diseases and the medications given were recorded in this questionnaire.

Body weight, height and body fat %

Body weight also measured using the TANITA TBF-300MA bio-impedance scale to the nearest 0.1kg and the measurement was taken only once. Body fat % was measured using the same scale to nearest 0.1 kg. Body height was measured using the Stadiometer (Seca) to the nearest 0.1 cm. Two height measurements were taken and the mean value was recorded.

Sun exposure

Sun Exposure Questionnaire (Annexure H) was used to gather information about sun exposure habits during the previous year. This questionnaire included the details about, gender, age, occupation (indoor or outdoor), Fitzpatrick skin type (I to V), frequency and duration of sun exposure, sun avoidance habits, clothing habits, use of sunblock, use of

sunbeds, details about mountain climbing/skiing, skin exposure area and details about holiday visits.

Muscle function

GS and TUG tests were used to assess the muscle function of the study participants. Portable hydraulic hand dynamometer (Jamar, 12-0240, SN 04201084, USA) was used to measure the GS. Three measures from each hand were taken and the mean value of all 6 values was used in the analysis. Time taken to walk 3 m distance in his/her usual speed was recorded as the TUG test. The test was performed three times and the mean value was considered for the analysis. Further details about muscle function tests were described in Chapter 2 under section 2.4.5.

Bone densitometry

Heel bone ultra-sound scanner (Achilles, EXPII, UK) was used to measure the bone mass at the heel bone (Os Calcis) of the participants. BUA, SOS (m/sec) and SI were recorded twice in both right and left Os Calcis. Mean SOS, BUA and SI values were calculated from those four measurements. Further details about bone densitometry measures were described in Chapter 2 under the section 2.4.11.

Dietary intake

Details about the habitual diet of the previous year were collected using the validated FFQ employed by the EPIC Study (Bingham *et al*., 1997) (Annexure J). This questionnaire consisted of commonly consumed 10 food groups and the participants were asked to record the frequency of consumption of those foods.

Blood samples and biochemical analysis

Random non-fasted venous blood samples were collected by venipuncture for the analysis of serum 25(OH)D and PTH concentration. All the blood samples were collected from study participants from May 2017 to April 2018. Total serum 25(OH)D and PTH concentration were analysed by Roche method and sandwich test principle of electrochemiluminescence technology, respectively in the Newcastle laboratories, Freeman Hospital, Newcastle upon Tyne. Further details of laboratory analysis of blood samples were described in chapter 2 under section 2.5.

5.2.5 Data and statistical analysis

SPSS statistical package for Windows version 24.0 was used for the data analysis. Older adults who used vitamin D supplements were also included in present analysis. Variables that

were not normally distributed were log transferred (natural log) to obtain normal or near normal distribution prior to statistical analysis. A *P* value less than 0.05 was considered statistically significant. Summary data were expressed as mean, SD, frequencies, percentages and bar graphs, as appropriate. The one-way ANOVA was used to determine the differences between the means of demographic, biochemical, sun exposure and anthropometric variables that had three or more independent groups. Similarly, the student's t - test was used to determine the differences between the means of demographic, biochemical, sun exposure and anthropometric variables that had two independent groups. The Bonferroni test was used for post hoc comparisons between means. Partial and simple correlation analysis were performed to identify the relationship between serum 25(OH)D concentration, MSK function with and without adjusting for BMI and gender, respectively. Student's t-test was used to compare measures of muscle function and bone health in those with serum 25(OH)D concentration above and below 50 nmol/L based on IOM classification of adequacy. ANCOVA was used to compare measures of muscle function and bone health in those with serum 25(OH)D concentration above and below 50 nmol/L based on IOM classification of adequacy, adjusting for the confounders.

5.3 Results

5.3.1 Basic Characteristics

Table 5.1 shows the basic characteristics of the study participants. The proportion of males and females was equal in this study. Mean age and serum 25(OH)D concentration of the participants were 66.8 (SD 8.3) years and 66.3 (SD 28.8) nmol/L, respectively. Their GS and TUG tests values were 27.6 (SD 9.2) kg and 9.1 (SD 4.0) s, respectively. The bone health-related parameters, BUA, SOS and SI at the Os Calcis were 106.8 (SD 20.7), 1564.9 (SD 40.7) m/sec and 91.0 (SD 18.9), respectively. Majority (48.9%) of the study participants had BMD according to the T score of the heel bone. Only 4% of older adults were in the category of risk for osteoporosis.The majority of the participants (61.7%) had a history of non-communicable diseases (either hypertension, diabetes, cardiovascular diseases, cancer or a combination of these conditions). Out of 20 supplement users, 9 individuals used multivitamins which contained vitamin D. However the amount of vitamin D present in those vitamin D supplements was unknown.

Table 5.1: Basic characteristics of BFU Study participants (n=47)

Characteristics	Mean (SD) / n (%)	
Age (years)	66.8 (8.3)	
Gender (n (%))		
Males	23 (48.9)	
Females	24 (51.1)	
Body Mass Index (kgm^{-2})	28.3 (4.7)	
Weight (kg)	81.5 (17.6)	
Height (cm)	169.2 (10.6)	
Grip Strength (kg)	27.6 (9.2)	
Timed – Up and Go test (s)	9.1 (4.05)	
Broad Band Attenuation	106.8 (20.7)	
Speed of Sound (m/s)	1564.9 (40.7)	
Stiffness Index	91.0 (18.9)	
T score at heel bone	-0.708 (1.43)	
>-1.0 (% (n)) : Normal BMD	18 (38.3)	
-1.0 – (-2.5) (% (n)) : Low BMD	23 (48.9)	
< 2.5 (% (n)) : Risk for osteoporosis	4 (8.5)	
Dietary vitamin D intake (µg/day)	3.0 (1.4)	
Dietary calcium intake (mg/day)	891.5 (404.8)	
Serum 25(OH)D conc. (nmol/L)	66.4 (28.8)	
Plasma Parathyroid Hormones (PTH) conc. (Pmol/L)	4.5 (1.43)	
Having a history of NCDs	**n (%)**	
Yes	29 (61.7)	
No	18 (38.2)	
Use of vitamin D supplements		
Yes	20 (42.5)	
No	27 (57.5)	
Having polyps		
Yes	10 (21.3)	
No	37 (78.7)	
	Males (Mean (SD))	**Females (Mean (SD))**
Body fat %	29.7 (7.9)	38.6 (5.4)
Waist Circumference (cm)	102.4 (15.0)	90.7 (11.1)
Hip Circumference (cm)	106.3 (9.4)	103.8 (9.2)

5.3.2 Factors associated with serum 25(OH)D concentration

Table 5.2 presents the association between sun exposure variables, demographic, anthropometric, biochemical parameters and serum 25(OH)D concentration of the study participants. Having the holiday visit was the only sun exposure variable that had a significant relationship between serum 25(OH)D concentration, which showed the highest serum 25(OH)D concentration among the holiday visitors (p=0.042). Older adults who used vitamin D containing supplements had the highest serum 25(OH)D concentration compared to non-users (p=0.003).

Table 5.2: Association between sun exposure, demographic, anthropometric, dietary, biochemical parameters and total serum 25(OH)D concentration study participants

Variables	n (%)	Serum 25(OH)D conc (nmol/L)	P value[1]
Blood collected season			
Winter	22 (46.8)	59.4 (30.4)	0.124
Summer	25 (53.2)	72.4 (26.5)	
Frequency of outdoor activities			
More than 2 times/week	13 (25.5)	60.1 (29.7)	0.370
Everyday	34 (74.5)	68.7 (28.5)	
Out-door duration at weekdays			
< 2 hours	24 (46.8)	65.6 (33.1)	0.860
≥ 2 hours	23 (48.9)	67.1 (24.2)	
Out-door time at weekends			
< 2 hours	14 (29.8)	77.1 (36.9)	0.163
≥ 2 hours	33 (70.2)	61.7 (23.8)	
Sun avoidance behaviours			
Try to avoid direct sun	7 (14.9)	64.0 (26.8)	0.780
Sometimes stay in the sun	26 (55.3)	69.0 (27.5)	
Enjoy staying in the sun	14 (29.8)	62.5 (33.4)	
Deliberately wear less clothes			
Never/rarely	11 (6.4)	61.3 (35.9)	0.372
Sometimes	10 (21.3)	78.3 (17.4)	
Usually	20 (42.6)	60.8 (22.1)	
Always	6 (12.8)	73.8 (46.2)	
Use of sun cream			
Yes	33 (25.5)	67.0 (26.9)	0.815
No	14 (74.5)	64.8 (33.9)	
Holiday visits			
Yes	25 (53.2)	75.3 (31.9)	0.042
No	22 (46.8)	58.4 (23.7)	
Number of days of holiday visits			
<15 days	17 (70.8)	73.2 (33.3)	0.700
≥15 days	7 (29.2)	78.7 (25.1)	
Outdoor frequency during holidays			
Often	19 (79.2)	75.3 (27.6)	0.184
Not often	5 (20.8)	55.0 (21.3)	
Skin type			
Type I & II	11 (23.4)	64.2 (26.9)	0.728
Type III, IV & V	36 (76.6)	66.9 (27.5)	
Gender			
Males	23 (48.9)	61.3 (29.7)	0.243
Females	24 (51.1)	71.2 (27.7)	
Body Mass Index (BMI)			
Normal weight	14 (29.8)	77.4 (32.0)	0.087
Overweight and obese	33 (70.2)	61.6 (26.5)	
Age			
<Median (67 years)	25 (53.2)	67.5 (27.4)	0.764
≥Median	22 (46.8)	64.9 (30.9)	
Vitamin D supplement use			
Yes	27 (57.4)	74.8 (30.1)	0.003
No	20 (42.6)	51.6 (23.6)	

Table 5.2 continue…..

Variables	n (%)	Serum 25(OH)D conc (nmol/L)	P value[1]
Vitamin D intake			
<Median (2.74 µg/day)	23 (48.9)	67.4 (29.9)	0.806
≥Median	24 (51.1)	65.3 (28.3)	
Parathyroid Hormones (PTH)			
<Median (4.30 Pmol/L)	22 (46.8)	74.6 (31.9)	0.098
≥Median	25 (53.2)	54.8 (24.5)	
History of NCDs			
Yes	29 (61.7)	67.8 (27.8)	0.654
No	18 (38.2)	63.9 (27.8)	
Presence of Polyps			
Yes	10 (21.27)	58.6 (35.7)	0.130
No	37 (78.73)	68.4 (26.8)	

[1]ANOVA for the variables that had more than two categories, Student's t test for the variables that had two categories

Table 5.3 describes difference in serum 25(OH)D concentration of holiday visitors and non-visitors in relation to socioeconomic status, dietary intake, supplement use and frequency of outdoor activities. This association remained the same after adjusting for the socioeconomic status, dietary intake, supplement use and frequency of outdoor activities. The association between serum 25(OH)D and holiday visits was not altered after adjusting for the socioeconomic status, dietary intake, supplement use and frequency of outdoor activities.

Table 5.3 : Differences between holiday visitors and non-visitors in relation to socioeconomic status, dietary intake, supplement use and frequency of outdoor activities

Characteristics	Holiday visitors	Non-holiday visitors	P value[1]	P value[2]
Serum 25(OH)D concentration	75.3 (31.9)	58.4 (23.7)	0.042	0.047
Index of Multiple Deprivation	6.9 (2.7)	6.6 (2.7)	0.667	
Dietary vitamin D intake	2.9 (1.3)	3.1 (1.5)	0.585	
Vitamin D supplement use				
Yes	10	10		
No	12	15	0.773	
Frequency of outdoor activities				
30 min – 2 hours	9	5		
>2 hours	13	20	0.201	

[1] ANOVA

[2]ANCOVA adjusted for socioeconomic status, dietary intake, supplement use and frequency of outdoor activities

5.3.3 Factors associated with MSK outcomes

Table 5.4 demonstrates the relationship between age, gender, PTH concentration, BMI, presence of polyps and MSK outcomes. GS and SI were significantly associated with age which showed the highest value for the young participants (p= 0.017 and 0.05, respectively). Except TUG test, all parameters of MSK outcomes were significantly associated with gender, which reported the highest value for the males. Older adults who had the lowest PTH concentration had the highest GS. Though the BMI was not significantly associated with any of the MSK functions, Body fat percentage was significantly associated with GS which showed the highest GS value for the adults with a low-fat percentage. Only 17.3% of males had low GS compared to the reference value of 30 kg, while 45.8% of females had GS below the reference value of 20 kg (Bohannon, 2008). Similarly percentage of older adults who had TUG value higher than 10.85 s which is the cut-off value for sarcopenia (Martinez *et al*., 2015) was 19.6%. Thirteen percent (n=3) of males and 25% (n=6) of females who had GS values below the reference range were older than 65 years and 70 years, respectively. Similarly, the relationships between musculoskeletal functions (GS, TUG and SI) and body fat percentage for males and females are illustrated in figure numbers 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 5.10 and 5.11.

Table 5.4 : Factors associated with musculoskeletal function in BFU Study participants

	GS	TUG	SI	SOS	BUA
Age (years)					
<Median (67.0)	30.5 (9.3)	8.7 (4.1)	96.1 (20.3)	1575.2 (44.2)	107.8 (25.6)
≥Median	24.1 (7.9)	9.5 (4.0)	85.1 (15.6)	1553.1 (33.5)	105.4 (11.5)
P value	0.017	0.341	0.050	0.069	0.193
Gender					
Male	34.6 (7.0)	8.6 (2.4)	100.8 (18.2)	1579.9 (42.8)	112.6 (26.2)
Female	21.3 (5.7)	9.6 (5.2)	81.5 (14.4)	1550.6 (33.4)	100.8 (9.0)
P value[1]	<0.001	0.640	<0.001	0.014	<0.001
PTH (Pmol/L)					
<Median (4.30)	31.2 (10.3)	9.2 (4.1)	90.0 (15.9)	1559.0 (32.1)	110.2 (10.9)
≥Median	24.5 (7.03)	8.9 (4.1)	91.9 (21.7)	1570.6 (47.5)	103.5 (25.9)
P value[1]	0.013	0.817	0.739	0.344	0.429
Body Mass Index (kgm^{-2})					
<Median (28.67)	26.9 (8.5)	8.9 (3.8)	89.2 (18.5)	1563.1 (40.1)	107.1 (12.3)
≥Median	28.0 (9.9)	9.2 (4.4)	92.8 (19.5)	1566.8 (42.1)	106.3 (26.2)
P value[1]	0.687	0.858	0.527	0.760	0.419
Body fat percentage (%)					
<Median (35.5)	30.8 (8.5)	8.3 (2.1)	96.5 (21.0)	1574.9 (47.9)	107.9 (27.2)
≥Median	24.8 (9.0)	9.8 (5.2)	86.2 (15.81)	1556.2 (31.6)	105.6 (11.1)
P value[1]	0.030	0.317	0.068	0.126	0.063
Having polyps					
Yes	29.1 (10.7)	9.4 (1.7)	91.4 (17.2)	1561.6 (33.4)	110.3 (13.6)
No	27.0 (8.7)	9.0 (4.5)	90.8 (19.6)	1565.8 (42.9)	105.7 (21.8)
P value[1]	0.525	0.816	0.934	0.775	0.536

[1]*Student's t test*

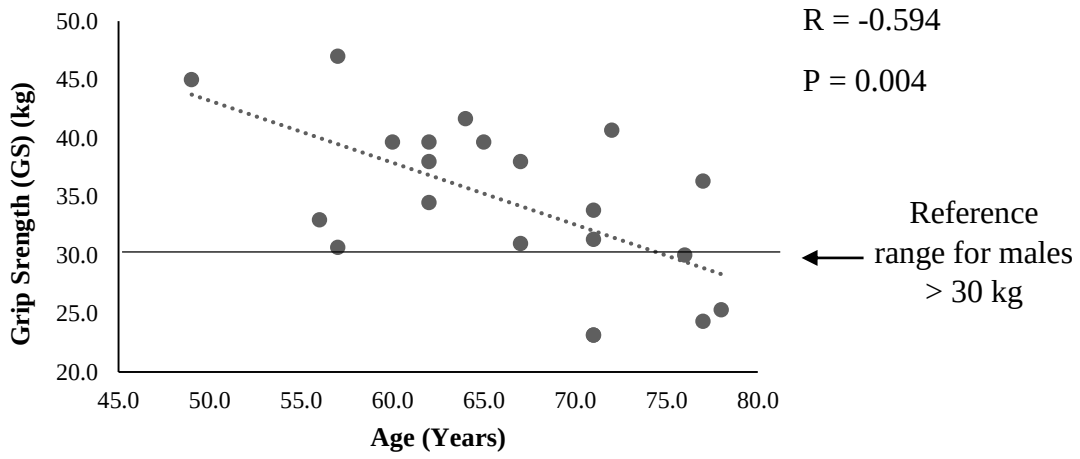


Figure 5.1 : Relationship between age and Grip Strength (GS) in males

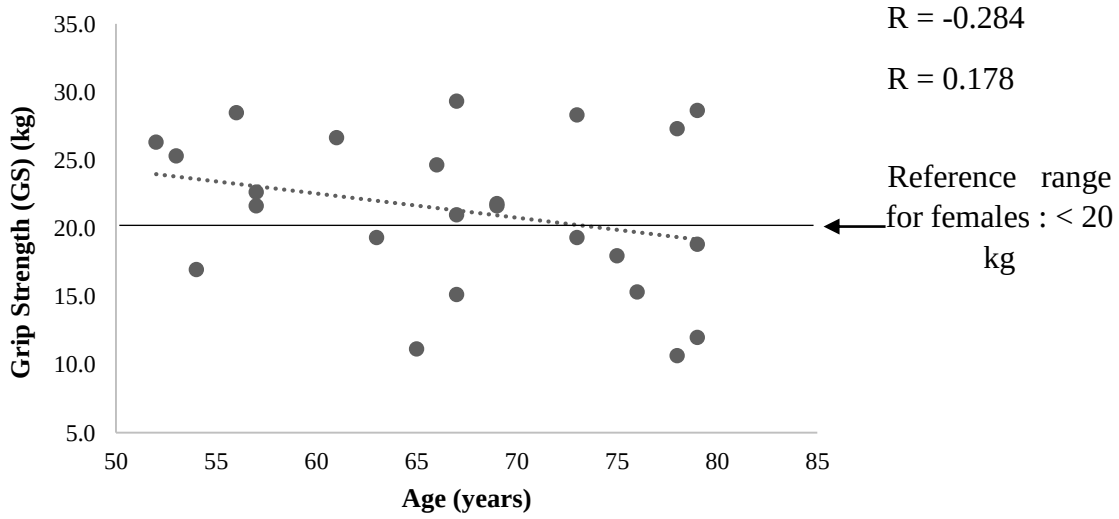


Figure 5.2 : Relationship between age and Grip Strength (GS) in females

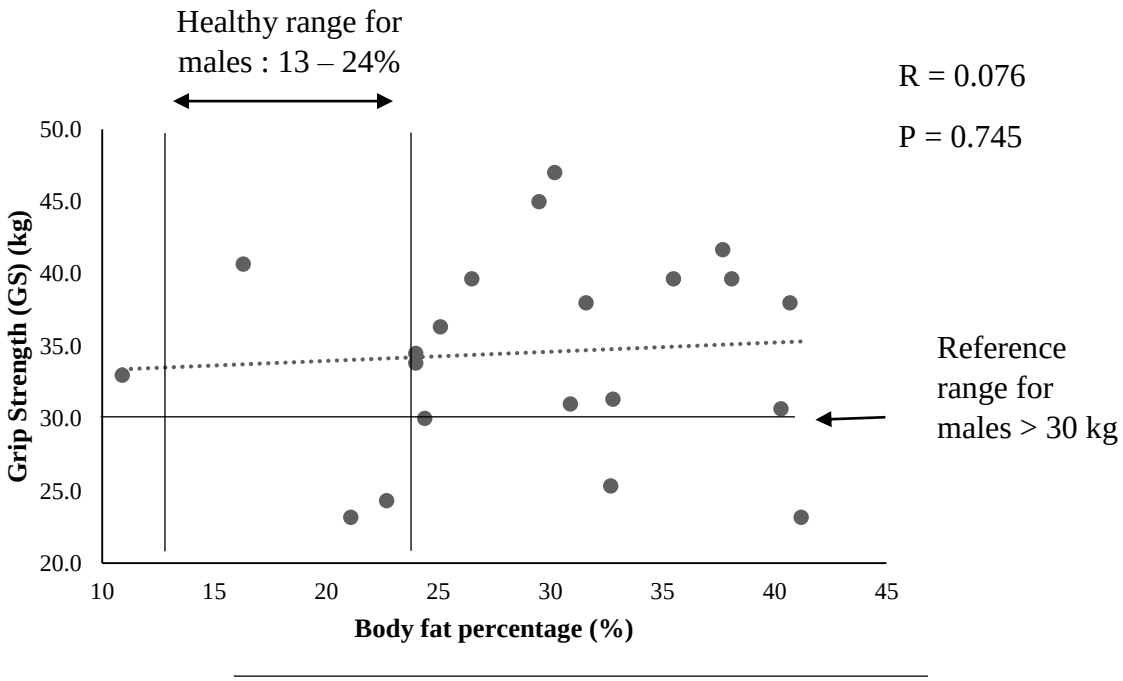


Figure 5.3 : Relationship between Grip Strength (GS) and body fat% in males

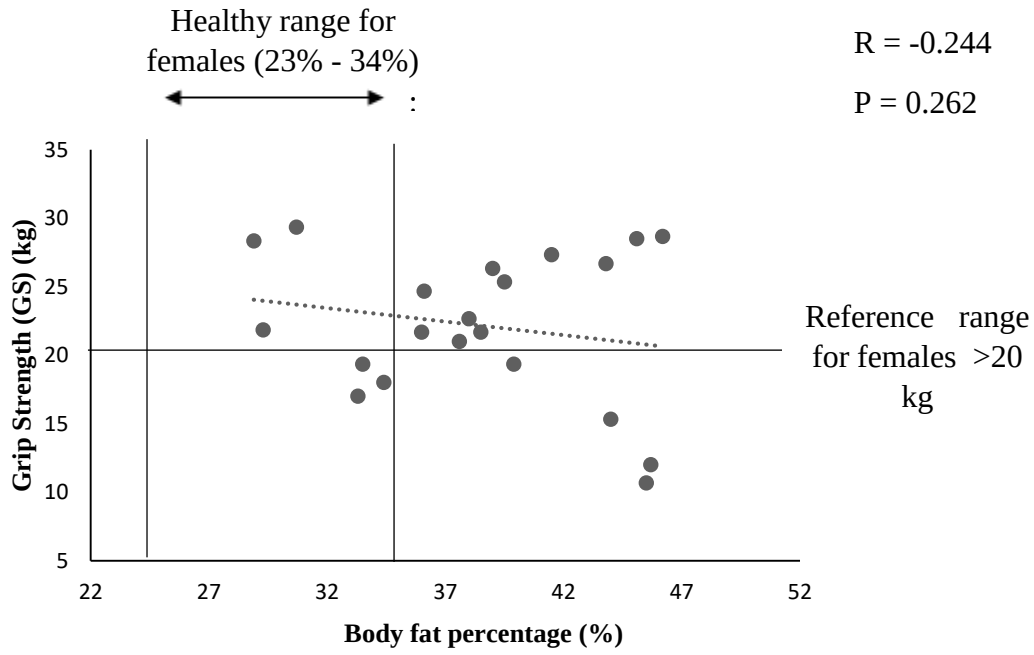


Figure 5.4 : Relationship between Grip Strength (GS) and body fat% in females

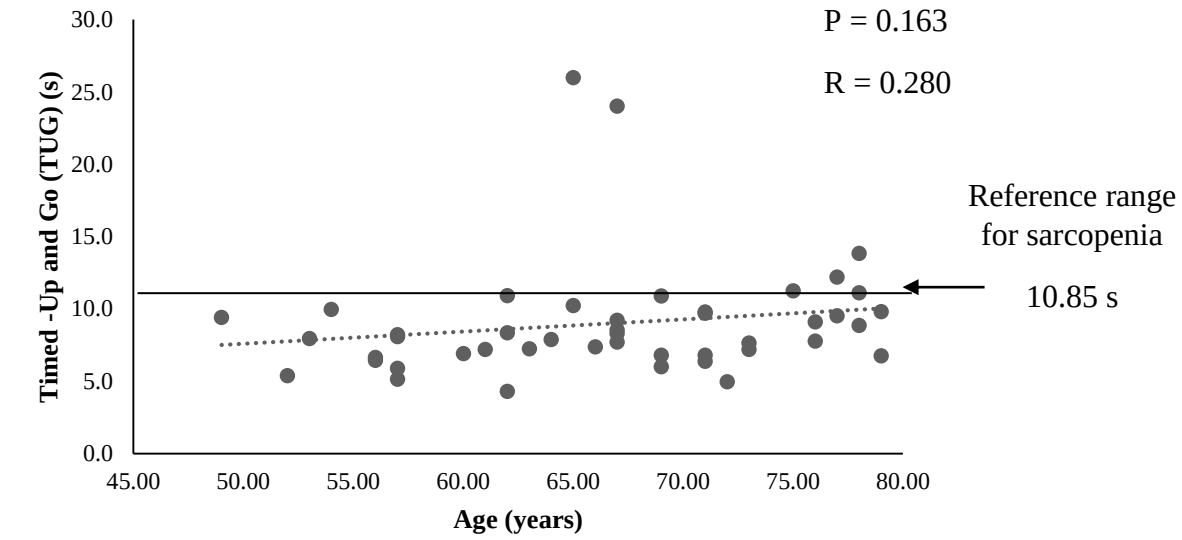


Figure 5.5 : Relationship between age and Timed-Up and Go test (TUG) test

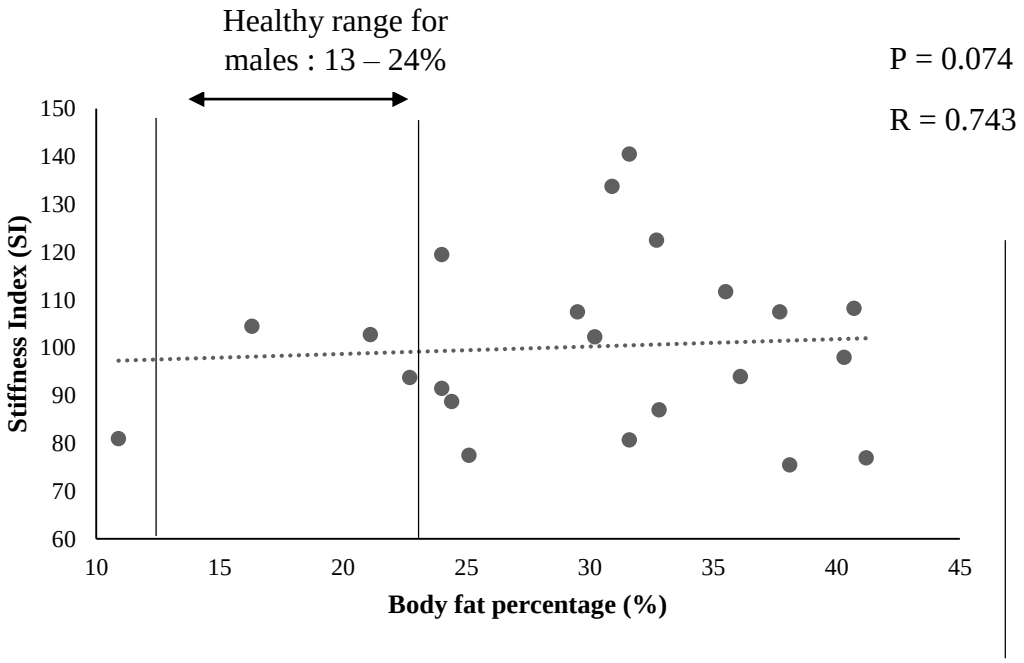


Figure 5.6 : Relationship between body fat% and Stiffness Index (SI) in males

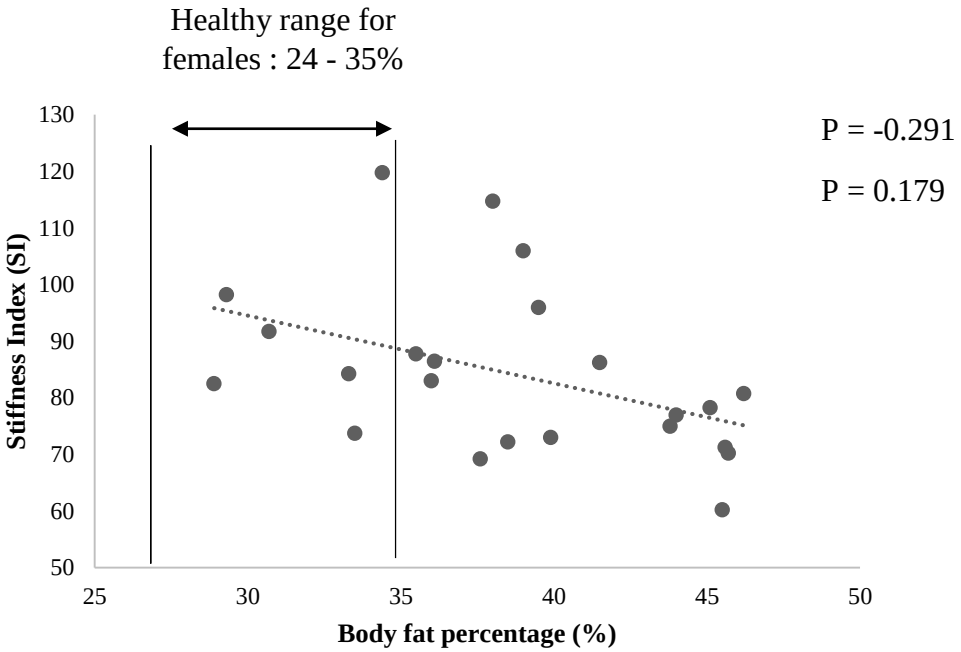


Figure 5.7 : Relationship between body fat% and Stiffness Index (SI) in females

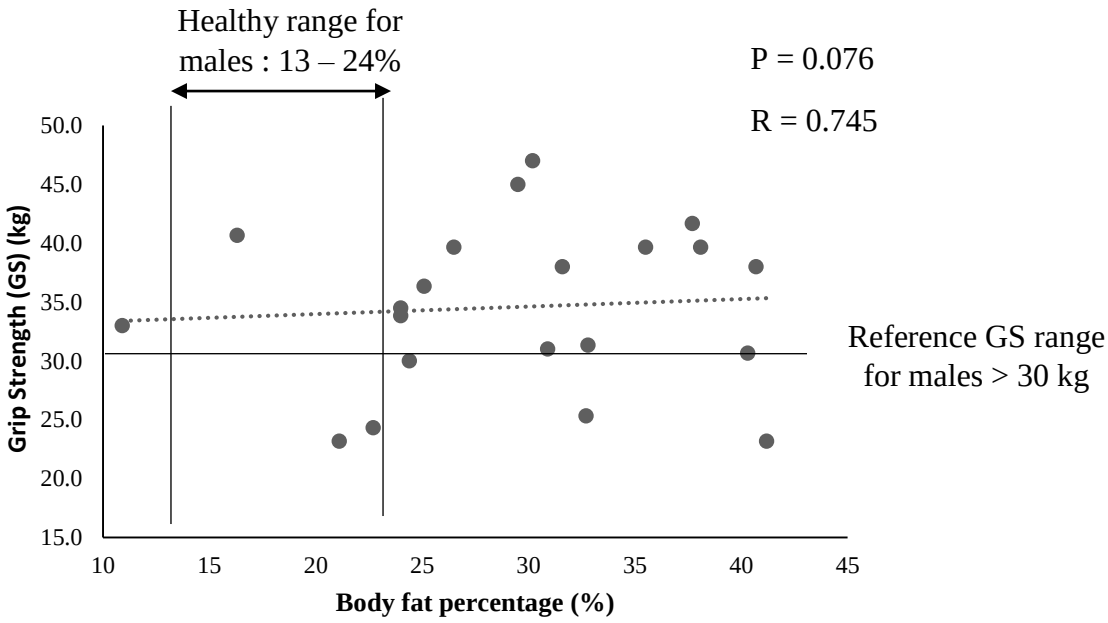


Figure 5.8 : Relationship between body fat % and Grip Strength (GS) for males

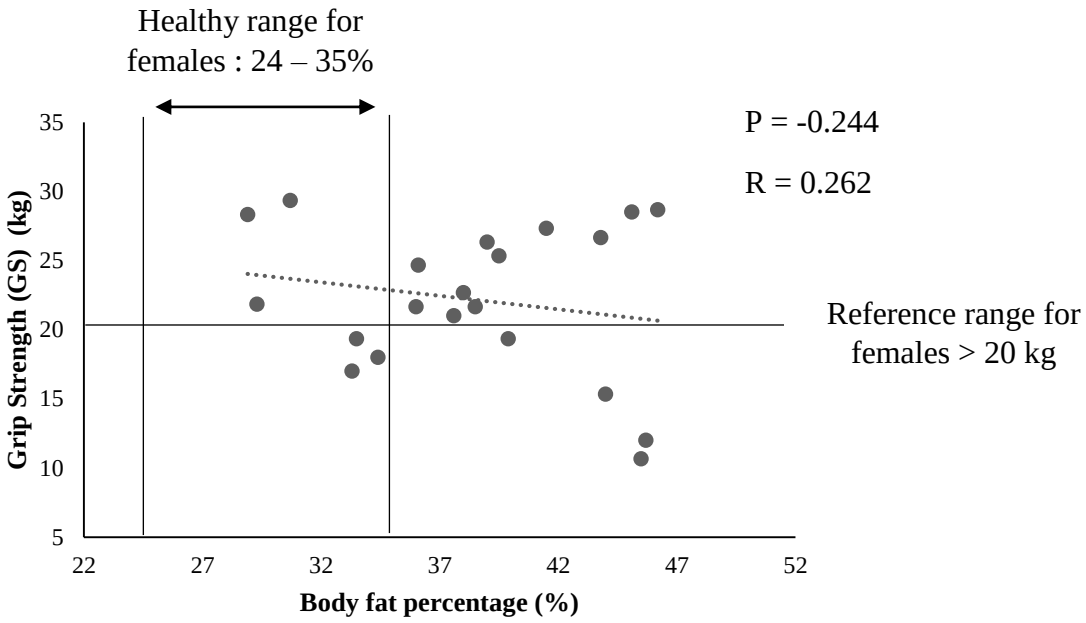


Figure 5.9 : Relationship between body fat % and GS for females

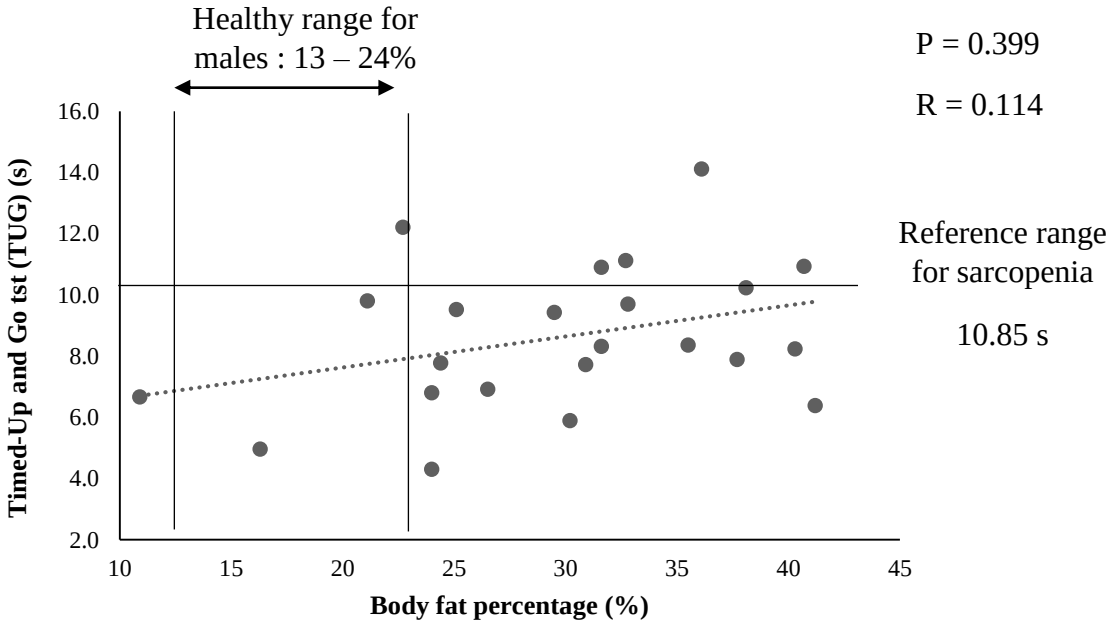


Figure 5.10: Relationship between body fat % and TUG test in males

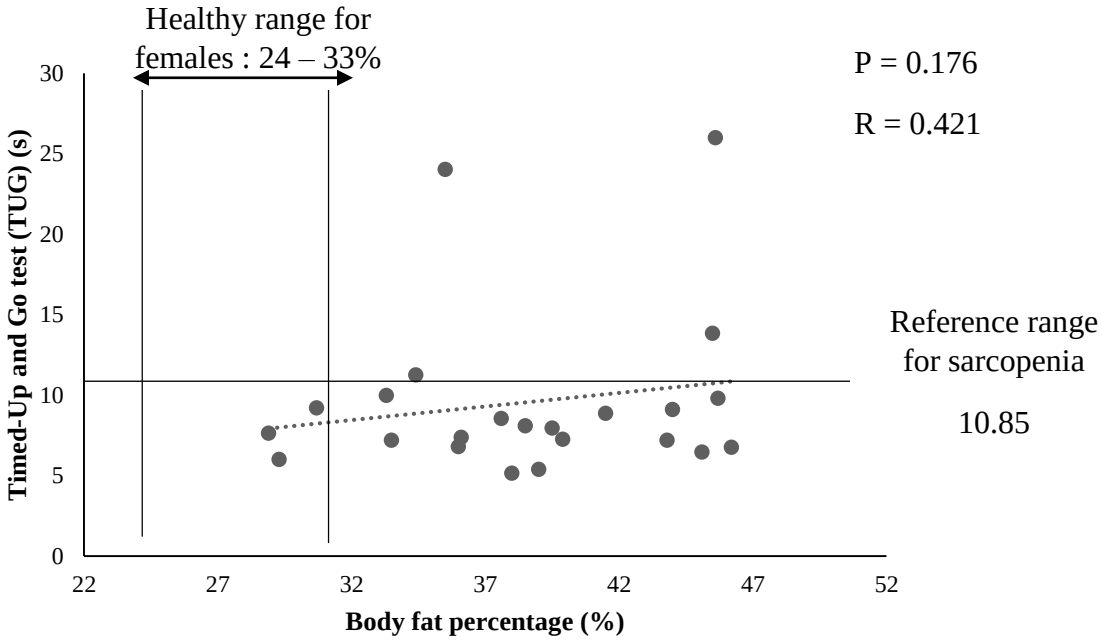


Figure 5.11 : Relationship between body fat % and Timed-Up and Go test in females

5.3.4 : Relationship between MSK function and serum 25(OH)D concentration

Table 5.5 illustrates the simple and partial correlations between MSK function and serum 25(OH)D concentration. Both muscle health and bone health was not significantly associated with serum 25(OH)D concentration with or without adjusting for BMI, gender.

Table 5.5 : Simple and partial correlations between serum 25(OH)D concentration and MSK function in older adults

Variables	Simple Correlations	P value[1]	Partial correlation	P value[2]
Muscle function				
Grip Strength (kg)	0.016	0.914	0.038	0.818
Timed Up and Go test (s)	0.193	0.200	0.119	0.465
Bone health				
Stiffness Index	-0.150	0.325	0.001	0.994
Speed of Sound (m/s)	-0.097	0.528	0.037	0.822
Broadband Ultrasound Attenuation	-0.075	0.618	-0.045	0.781

[1]Unadjusted *[2]Adjusted for age, BMI and gender*

When the serum 25(OH)D concentration was dichotomized at 50 nmol/L (IOM criterion of adequacy), there were no significant differences in MSK outcomes in those with low versus adequate serum 25(OH)D concentration with and without adjustment for BMI and gender. However, the mean age was higher in those with low compared with adequate serum 25(OH)D concentration (Table 5.6).

Table 5.6 : Comparison between Musculoskeletal function by low and adequate serum 25(OH)D concentration

Variables	Serum 25(OH)D < 50 nmol/L (n=18)	Serum 25(OH)D ≥ 50 nmol/L (n=29)	P value[1]	P value[2]
Serum 25(OH)D (nmol/L)	38.7 (7.7)	83.4 (23.1)	-	-
Parathyroid Hormones (Pmol/L)	4.7 (1.4)	4.5 (1.4)	0.613	0.289
Speed of Sound (m/s)	1569.5 (38.4)	1562.1 (42.4)	0.553	0.057
Broadband Attenuation	167.3 (20.8)	160.8 (17.7)	0.229	0.380
Stiffness Index	93.9 (19.0)	89.2 (19.0)	0.431	0.659
Grip Strength (kg)	28.6 (9.1)	26.8 (9.3)	0.543	0.274
Timed – Up and Go test (s)	9.2 (1.9)	9.0 (4.9)	0.861	0.156

[1] *Student's t test* [2]*ANCOVA test adjusted for BMI and gender*

5.4 Discussion

5.4.1 Main findings

The aims of this study were to identify the association between sun exposure behaviours and serum 25(OH)D concentration and to identify the relationship between MSK outcomes and serum 25(OH)D concentration in older adults who are at moderate risk of CRC. This study reported that serum 25(OH)D concentration did not significantly associate with MSK outcomes in older adults who are at moderate risk of CRC. Moreover, this study found that having holiday visits was the only positive significant factor associated with serum 25(OH)D concentration.

5.4.2 Interpretation of main findings

Vitamin D status

In this study, none of study participants was VDD based on the UK cut-off (25 nmol/L) of VDD. According to the US cut-offs of vitamin D insufficiency (50 nmol/L), only 38.3% the participants in this study were vitamin D insufficient. Many case control studies and SR report a lower serum 25(OH)D concentration in CRC patients compared to controls (Song *et al*., 2016, Chandler *et al.*, 2015, Ma *et al*., 2011, Woolcott *et al*., 2010). A case control study of patients whose age was 45 – 75 years, showed lower serum 25(OH)D concentration among CRC patients compared to the controls (Woolcott *et al*., 2010). The reported serum 25(OH)D

concentrations of cases and controls of this study were 57.9 and 62.4 nmol/L, respectively (Woolcott *et al*., 2010). Another case control study of adults whose mean age was 58 years also reported lower serum 25(OH)D concentration in cases (54.6 nmol/L) compared to the controls (59.6 nmol/L) (Chandler *et al*., 2015). Further this study reported that serum 25(OH)D concentration in CRC cases and controls were 21.9 (SD 8.3) and 23.9 (SD 9.8) nmol/L, respectively, which was lower than the value reported in this study. Whilst the lower 25(OH)D observed in CRC patients relative to controls might be due to differing vitamin D behaviours (sun exposure, diet, supplements) between these patients, it is also known that CRC itself might increase turnover of vitamin D metabolites via upregulation of macrophage and immune system activation through regulation by 1,25(OH)D (Gregory *et al*., (2010); Golden *et al.,* (2012).

Low prevalence of VDD in this study group indicated that this participants were comparatively healthy. Nearly half of the population had vitamin D containing supplementations and it might be the reason for this low prevalence of VDD in our study population. Alternatively, nearly half of the participants had holiday visits and it was a positive significant factor associated with the serum 25(OH)D concentration which might be attributed for the better vitamin D status in this population. Though the mean dietary vitamin D intake (3.0 $\pm$ 1.4 µg/day) was below the recommended intake of 10 µg/day, vitamin D containing dietary supplements use and the sun exposure through holiday visits contributed to the better vitamin D status.

Sun exposure variables and serum 25(OH)D concentration

A recent case control study in CRC patients aged 20 – 85 years old, reported that risk for colon cancer was low in the patients who had frequent sun exposure during the previous summer (Valles *et al*., 2018). Gorham *et al*., (2007) and Garland *et al*. (1990) compiled a number of epidemiological studies and noted that there was a strong negative correlation between latitude, sun exposure, and poor vitamin D status and the risk for developing many deadly cancers, including colon, breast, ovarian, and melanoma. According to the present study, having holiday visits was a significant factor that positively associated with the serum 25(OH)D concentration. Though it was not significant, this study showed the seasonal variation in serum 25(OH)D concentration similar to some of the previous studies (Hedlund *et al*., 2013, Freedman *et al*., 2013, Mie *et al.,* 2007). In addition, older adults who had habits of having fewer clothes and more frequent outdoor activities for a long time had higher serum 25(OH)D concentration compared to their respective counterparts. Therefore, the details about the sun holidays, clothing habits, duration and frequency of sun exposure are important

factors to be included in the sun exposure questionnaires to assess vitamin D status. The low sample size might be attributed to the non-significant relationship of sun exposure variables and serum 25(OH)D concentration.

Relationship between serum 25(OH)D concentration and muscle function

The cut-off values for GS for males and females for the sarcopenia were 30 and 20 kg, respectively (Filippin *et al*., 2017) which are lower than the mean values reported in the present study. According to Martinez *et al*., (2015), cut-off value for the TUG test was 10.85 s for sarcopenia, which was higher than the mean value of our study. Therefore we can postulate that the muscle health of this study participants was relatively satisfactory. In line with our findings, some cross-sectional studies indicated that serum 25(OH)D concentration was not positively associated with muscle function in older adults. A study carried out in the US recruiting, black, hispanic and white male participants whose age was 30 – 79 years stated that serum 25(OH)D concentration was not associated with lean body mass, muscle strength or physical function (Ceglia *et al*., 2011). In contrast, some cross-sectional studies showed the positive relationship between serum 25(OH)D concentration and muscle functions in older adults (Berners *et al*., 2018, Haaf *et al*., 2018). Berners *et al*., (2018) also reported a positive association between serum 25(OH)D and muscle function in a cross sectional study of mobility limited adults whose mean age was 77.6 years. A study of older adults aged 65 – 88 years, living in Netherlands, stated the positive relationship between serum 25(OH)D concentration and physical performance (Haaf *et al.,* 2018). A cross-sectional study of postmenopausal women stated that women with hypovitaminosis D had significantly lower GS, short physical performance battery and gait speed compared to the women with normal level of serum 25(OH)D concentration (Iolascon *et al*., 2015). Present study reported the differences in muscle function variables between the older adults who had polyps and older adults who did not have polyps. The relationship between vitamin D status and muscle function is inconsistent across studies. The different findings between studies might be attributed to the ethnicity of the participants, assay methods used in the analyzing serum 25(OH)D concentration, level of vitamin D status of the participants, SES, living status (free living or institutionalized), methods used in assessing muscle function and other factors which may influence muscle function which may not have been accounted for in the analyses.

Absence of the positive relationship between serum 25(OH)D concentration and muscle function in this study might be partly attributed to the low prevalence of VDD and narrow range of the serum 25(OH)D concentration. The association of serum 25(OH)D concentration and muscle function might be undetectable as a result of this narrow range between serum

25(OH)D concentration. According to our previous study of older adults in Newcastle upon Tyne, the association of serum 25(OH)D with muscle function can be seen only in older adults with serum 25(OH)D concentration below 25 nmol/L, which partly explained by previous finding (Ranathunga *et al.,* 2019). Relatively satisfactory muscle function of this study participants might be a one of the reasons for not existing the positive association between serum 25(OH)D concentration and muscle function in this study participants. Some SR and case control studies stated the depletion of muscle function and mass among cancer patients (Christensen *et al.,* 2014, Martin *et al*., 2013, Fearon *et al*., 2011). However, the majority of our study participants was healthy and only 21.2% of participants had polyps in present study. This might be the reason that we did not observe the poor muscle function in this group. Low statistical power resulted from low sample size in this study also might be another reason for the unexpected result regarding the association of serum 25(OH)D and muscle function.

Relationship between serum 25(OH)D concentration and bone health

A significant association between serum 25(OH)D and bone health was not reported in this study. Supporting to these findings, a cross sectional study conducted in Palestine recruiting postmenopausal osteoporotic and normal adult women reported that there was no direct correlation between BMD measured at the total hip and Femoral neck and serum 25(OH)D concentration in both the osteoporotic and normal women (Kharroubi *et al.,* 2017). There are published research that focused on bone health parameters in CRC patients. A follow-up study of 9.5 years in 1471 postmenopausal women of 60 years old, showed that low BMD increased the risk of CRC and they further reported that low BMD was associated with 20% of CRC (Ganry *et al.,* 2008).

A study conducted in Italy, recruiting 134 older adults showed that serum 25(OH)D concentration was significantly and positively associated with total bone and the cortical bone cross-sectional area in the tibia (Mosele *et al.,* 2013). Another cross-sectional study of older people whose age was 65 – 88 years conducted in Netherlands, reported a positive relationship between serum 25(OH)D concentration and BMD. They reported that heel bone BUA and SOS also were higher in high 25(OH)D groups. They explained that BMD and total BMC increased up to serum 25(OH)D concentration of 50 – 60 nmol/L (Kuchuk *et al*., 2009). A postmenopausal women's study whose age was 48 – 65 years in Italy, pointed out that the women with serum 25(OH)D concentration below 25 nmol/L had low BMC and high level of bone turn over markers (Napoli *et al*., 2014). In contrast, a prospective study of men and women aged 66 – 96 years conducted in Iceland revealed that though maintaining serum

25(OH)D concentration above 30 nmol/L would be advantageous for the bone health, having higher values than this did not show the additional benefit for the bone health (Steingrimsdottir *et al*., 2014), which partly explain the findings regarding the association between bone health and serum 25(OH)D concentration in present study. However, all these studies were varying in participants' characteristics, vitamin D status of the participants, the technology used to assess the bone health and the bone site assessed which resulted in difficulty in comparing our study findings with previous studies. Further, lack of studies in past literature which assessed bone quality using heel QUS measures made our results difficult to compare with previous studies. Low statistical power and the cross-sectional nature of the study, might have contributed to the unexpected finding of this study.

5.4.3 Strengths and limitations

This study is one in the limited number of studies that identified the relationship between serum 25(OH)D concentration and the heel bone parameters measured using QUS technique in older adults with moderate risk of CRC. Identifying the relationship between muscle function and serum 25(OH)D concentration in the same study was an advantage. In addition the use of a more comprehensive questionnaire to assess the sun exposure was a strength to this study. Questions on sunblock use, details about holiday visits, the time duration of the outdoor activities, clothing habits, sun avoidance habits and frequency of outdoor activities were included in the questionnaire in our study. But all these questions were not include in the same questionnaire that used in the previous studies. Our study had several limitations. The low sample size was the main limitation which caused low statistical power. Recruired sample size was 385 individuals. This number is several times higher than the numbers recruited to the current study. Participants of this study were the volunteers who took part in a study of 12 years ago and thus older adults were not a group of randomly selected older adults living in the UK. Therefore this findings may not be able to generalize for the total population in the UK.

5.4.4 Public health implications

This study emphasized the importance of sun holidays to improve vitamin D status in older adults who are at moderate risk of CRC. Based on our findings we can hypothesize that, information on sunny holidays, frequency and duration of sun exposure, are the important questions to be included in sun exposure questionnaires to use as a proxy indicator of vitamin D status of older adults. Lack of significant positive association of sun exposure details and serum 25(OH)D concentration highlighted the need for developing validated sun exposure questionnaire to capture sun exposure details more precisely.

5.4.5 Conclusions

In conclusion, serum 25(OH)D concentration was not associated with MSK outcomes of older adults who are at moderate risk of CRC. Having holiday visits was the only behavioural factor that positively associated with serum 25(OH)D concentration in free living older adults with moderate risk of colon cancer in Newcastle upon Tyne. Further studies are needed, recruiting a large sample of participants that represent older adults in the UK and accurate methods of assessing MSK functions will be needed in future studies.

Chapter 6: Relationship Between Plasma PTH Concentration and Musculoskeletal Outcomes in Older Adults

6.1 Introduction

Parathyroid Hormone (PTH) is a peptide hormone that is responsible for maintaining calcium and phosphorus homeostasis (Berqwitz *et al*., 2010). PTH plays a role in bone health (Datta *et al*., 2014) and muscle function (Houston *et al.,* 2008). At a mechanistic level, it has been observed that increased PTH concentration activates osteoclast cells in bone tissues and thereby increased the rate of bone loss which leads to lower bone mass (Lips, 2001). Further, comparatively low PTH concentration influences the bone mass by regulating *IGF – 1*, which has an anabolic effect on bones (Tahimic *et al*., 2013). PTH plays a role in muscle function by influencing muscle protein metabolism, modulating type II muscle fibres, controlling mitochondrial O_2 uptake and synthesis of ATP in muscle cells (Mosekilde *et al*., 2005). Some cross sectional studies (Bird *et al.,* 2018, Renoud *et al*., 2014) and longitudinal studies (Houston *et al*., 2008, Dhesi *et al.,* 2002) have reported that elevated PTH concentration was associated with greater muscle loss and poorer muscle function (Mosekilde *et al*., 2005). In contrast, a 5 years follow-up study indicated that serum PTH concentration was not associated with muscle functions or falls in older people (Faulkner *et al*., 2006). In the same way, the cross sectional studies in older people have reported the positive association between PTH concentration and bone mass (Kota *et al*., 2015, Samrook *et al*, 2004), while a follow-up study of older adults (Abiri *et al*., 2012) and a cross sectional study of adults (Fujiyoshi *et al*., 2013) showed a negative association between PTH concentration and BMD. Therefore, prior findings of the association between PTH and MSK function are divergent.

There is a negative relationship between serum 25(OH)D concentration and PTH concentration (Han *et al*., 2014). In addition various factors regulate PTH concentration, such as vitamin D status, stage of life, time of the day, ethnicity, dietary calcium and phosphorus intake, kidney function, physical activity, drug use, age and gender (Patel *et al*., 2007, Fraser *et al*., 2007, Vieth *et al*, 2003). PTH concentration increases with increasing age, as a result of lower serum 25(OH)D, impaired kidney function, low calcium intake and impaired calcium absorption (McKenna, 1992). Normal blood levels of PTH vary according to the laboratory that measured the PTH concentration. Most of the laboratories have stated the normal level of PTH as 15 - 65 Pg/mL (Pallan *et al.,* 2012). As muscle function, muscle mass and bone mass reduce with increasing age (Araujo *et al*., 2014), maintaining appropriate PTH concentration at older ages may be important to optimize MSK outcomes. Whether the association between PTH and MSK outcome is independent of serum 25(OH)D is questionable (Arabi *et al.,*

2012). Also prior findings of the association between PTH and MSK outcomes are not consistent. Relatively few studies investigating the relationship between PTH concentration and MSK outcomes in older adults have been conducted. We hypothesized that PTH concentration might influence the MSK outcomes in older adults.

The aim of this study was to determine the association between vitamin D biomarker (PTH) and MSK health in older adults using two study designs.

Therefore objectives of this chapter were,

1 to investigate relationships between PTH concentration, 25(OH)D concentration and muscle function of older adults in both the VDOP and BFU studies.
2. to investigate relationships between PTH concentration, 25(OH)D concentration and bone health of older adults in both the VDOP and BFU studies.

6.2 Methods

6.2.1 Study designs

Data from two studies, Vitamin D in Older People (VDOP) study and the Biomarkers of Risk of Colon Cancer (BORICC) Follow-Up (BFU) study were used in the present study. The VDOP study is a double-blind, RCT that designed to test the effect of three doses of monthly oral vitamin D_3 supplements (12000 IU, 24000 IU and 48000 IU) on BMD of older adults. The BFU Study is a long-term (12+ years) follow-up study of participants in BORICC study which was designed to discover and validate novel biomarkers of bowel cancer risk and to determine their relationships with habitual diet and nutritional status. Further details of the designs of these two studies were described in Chapter 2 of this thesis under the sections of 2.1.1 and 2.2.1.

6.2.2 Selection of the participants

The VDOP study consisted of a total of 379 community dwelling men and women aged 70+ years or older, resident in Newcastle upon Tyne. Out of 379 participants recruited, 343 older adults completed the trial, thus VDOP follow-up study consisted of 343 older adults. A total of 47 older adults from the BORICC study participated in the BFU study. Further details about the selection of participants, inclusion and exclusion criteria of both studies were described in the Chapter 2 under the sections of 2.1.2 and 2.2.2.

6.2.3 Sunshine exposure, dietary intake, anthropometry and demographic variables

Muscle function and bone health

GGS and TUG were used to measure the upper and lower body muscle strength, respectively in both studies. In the BFU study, heel bone ultra-sound scanner (Achilles, UK) was used to measure the bone outcomes at the heel bone (Os Calcis). The Speed of Sound (SOS), Stiffness Index (SI) and Broad Ultrasound Attenuation (BUA) were the bone outcomes that measured using heel bone ultrasound scanner. Further details about these measures were described in Chapter 2 under the subsections of 2.4.5 and 2.4.11.

Demographic information and dietary information

Demographic information was gathered using questionnaires in both studies. CalQuest dietary calcium and vitamin D intake questionnaire, which was adopted from Calquest (Nelson *et al*., 1998) was used to gather the information about the food intake of the in the VDOP study. The validated FFQ employed by the EPIC (European Prospective Investigation into Cancer and Nutrition) Study (Bingham *et al*., 1997) was used in the BFU study.

Anthropometry and body composition

In both studies, weight and height were measured. Body composition was measured in VDOP study, while the fat percentage was measured in the older adults of BFU study. Body weight and body composition (fat % in the BFU study) were measured using TANITA TBF-300MA bio-impedance scale in both studies. Height was measured using stadiometer. FM and FFM were also recorded in the VDOP study.

6.2.4 Laboratory Analysis

In VDOP study, plasma PTH concentration was measured by immunoassay (IMMULITE® 1000 Immunoassay System) at Siemens Healthcare Diagnostic, Ltd, Camberley, UK. In BFU study, plasma PTH concentration was analysed by the sandwich test principle of Electrochemiluminescence technology at Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne. The reference range for PTH concentration used by these laboratories was 10.4 - 60.4 Pg/mL (~1.1 – 6.4 Pmol/L) (with normal serum calcium concentration). In VDOP study, total plasma 25(OH)D concentration values were obtained by summing up the values for $25(OH)D_2$ and $25(OH)D_3$, which were measured using LC-MSMS in Siemens Healthcare Diagnostics Ltd, Camberley, UK. In BFU study, serum 25(OH)D concentration was measured using Roche Immunoassay method in Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne.

6.2.5 Statistical Analysis

Data of the total sample of 379 older adults recruited for the VDOP study was used in the baseline data analysis while data from all participants who completed the intervention trial (n=343) were used in the follow-up data analysis. Older adults who used vitamin D supplements were omitted in the data analysis related to VDOP study. Data from 47 participants of BFU study was used in the present study. SPSS statistical package for Windows version 24.0 was used for analysing the data. A p value <0.05 was considered as statistical significant. Summary data were expressed as mean, SD, frequency and percentages, as appropriate. Laboratory cut-off value of 10.4 - 60.4 Pg/mL was used to identify the older adults with hyperparathyroidism (> 60.4 Pg/mL), hypoparathyroidism (<10.4 Pg/mL) and normal (10.4 – 60.4 Pg/mL) which was defined by the Newcastle Laboratories, Freeman Hospital, Newcastle upon Tyne. Simple correlation analysis was used to identify the factors associated with PTH concentration for both studies. Partial correlation was used to identify the association between PTH concentrations, MSK outcomes adjusted for age, gender, BMI, FM and serum 25(OH)D concentration for both studies. ANCOVA was used to investigate the association between serum 25(OH)D, PTH concentrations, muscle function of VDOP study participants adjusted for age, gender, BMI, FM and serum 25(OH)D concentration by the dose of vitamin D supplementation. The same test was used to investigate the associations between PTH status (categorised as hyperparathyroidism and normal) and MSK outcome variables adjusting for age, gender and BMI. Stepwise linear regression was used to identify the significant predictors of MSK outcomes for both studies.

6.3 Results

6.3.1 Vitamin D in older people (VDOP) study

Table 6.1 presents the basic characteristics of participants' of the VDOP study at baseline. Mean age and BMI of the older adults were 75.0 (SD 4.21) years and 27.0 (SD 3.9) kgm^{-2}, respectively. Mean plasma 25(OH)D and PTH concentrations were 39.9 (SD 20.1) nmol/L and 48.7 (SD 23.5) Pg/mL, respectively. Mean GS and TUG tests were 28.6 (SD 13.21) kg and 11.4 (SD 3.01) s, respectively. Percentages of males and females in the study population were approximately similar. About 9% of the population used vitamin D containing dietary supplements at baseline. According to reference range (10.4 – 60.4 Pg/mL) stated by the laboratories, 21.5 % of the VDOP participants were in the stage of hyperparathyroidism at baseline.

Table 6.1 : Characteristics of VDOP study participants at baseline (n=379)

Characteristics	Mean (SD)
Age (years)	75.0 (4.21)
Gender (Male n (%))	198 (52.2)
Weight (kg)	75.7 (13.6)
Body Mass Index ($kgm^{-2)}$	27.0 (3.9)
Fat Mass (kg)	24.7 (8.3)
Fat Free Mass (kg)	50.8 (10.2)
Serum PTH concentration (Pg/mL)	48.7 (23.5)
Serum 25(OH)D concentration (nmol/L)	39.9 (20.1)
Grip Strength (kg)	28.6 (13.2)
Timed-Up and Go test (s)	11.4 (3.1)
Calcium intake (mg/day)	835.7 (388.1)
Vitamin D intake (µg/day)	3.8 (3.2)
Serum PTH status	
Hyperparathyroidism (n(%))	81 (21.5)
Hypoparathyroidism (n(%))	1 (0.3)
Normal (n(%))	295 (78.2)
Use of vitamin D supplements (n (%))	
Yes	34 (9.0)
No	343 (91.0)

Table 6.2 summarises the outcomes of the partial correlation analysis between PTH concentration, demographic, anthropometric, dietary, biochemical and muscle function variables before and after vitamin D supplementation. Plasma 25(OH)D concentrations, BMI and body weight were significantly correlated with PTH concentration before and after the supplementation. GS and TUG tests were not significantly associated with PTH concentration either at baseline or at the endpoint after adjusting for age, gender, BMI, FM and serum 25(OH)D concentration. Based on the GS cut-off values for sarcopenia for males (30 kg) and for females (20 kg), 32.3% and 53.6% of males and females were sarcopenic, respectively. Based on the TUG test cut-off value of 10.85 s for sarcopenia, 54.4% of the older people was sarcopenic.

Table 6.2 : Simple and partial correlation between PTH concentration, demographic, anthropometric, dietary, biochemical and muscle function variables before and after 12 months vitamin D supplementation

Variables (Dependent variable : PTH concentration Pg/mL)	Before supplementation		After supplementation[1]	
	Correlation Coefficient (r)	P value	Correlation Coefficient (r)	P value
Simple correlation				
Age (years)	0.160	0.002	0.077	0.159
BMI (kgm^{-2})	0.208	<0.001	0.113	0.039
Weight (kg)	0.164	0.001	0.148	0.006
Height (m)	0.002	0.967	0.103	0.059
Fat Mass (kg)	0.214	<0.001	0.098	0.074
Fat Free Mass (kg)	0.024	0.644	0.114	0.037
Serum 25(OH)D concentration (nmol/L)	-0.307	<0.001	-0.270	<0.001
Calcium intake (mg/day)	-0.042	0.427	0.067	0.230
Vitamin D intake (µg/day)	-0.052	0.325	-0.071	0.205
Partial correlation				
Grip Strength (kg)	0.030	0.559[2]	0.015	0.791[2]
Timed-Up and Go test (s)	-0.027	0.601[2]	-0.039	0.483[2]

[1] *Pooled data from three treatment groups.*
[2]*Adjusted for age, gender, BMI, FM and plasma 25(OH)D concentration*

Table 6.3 shows PTH, 25(OH)D concentrations, muscle function and bone health parameters by the dose of vitamin D supplementation, adjusting for age, gender, BMI, FM. PTH concentration reduced after vitamin D supplementation with no significant differences according to vitamin D dose. As expected, serum 25(OH)D concentration was increased in a dose dependent manner following vitamin D supplementation. GS was reduced after the supplementation in all dose groups with the lowest reduction in high dose group. Similarly, the time taken to walk 3 m distance was increased in all three dose groups with the lowest increase in the high dose group. But those relations were not statistically significant.

Table 6.3 : PTH concentration and MSK outcomes at baseline and after 12 months by dose of supplementation

Variables	Total sample (n=379)	12000 IU (n=113)	24000 IU (n=114)	48000 IU (n=116)	P value[1]
PTH[2] at baseline (Pg/mL)	48.7 (23.5)	48.6 (25.5)	47.4 (23.3)	50.1 (21.3)	0.547
PTH at 12 month (Pg/mL)	42.9 (21.6)	43.9 (21.5)	44.8 (24.5)	39.6 (18.0)	0.410
Δ PTH (Pg/mL)	-5.5 (17.6)	-3.0 (18.5)	-3.1 (18.1)	-10.4 (15.4)	0.068
Serum 25(OH)D at baseline (nmol/L)	40.0 (20.1)	41.6 (19.9)	39.5 (20.6)	38.8 (19.7)	0.914
Serum 25(OH)D at 12 month (nmol/L)	66.5 (18.0)	55.9 (15.6)	64.6 (15.3)	79.0 (15.1)	< 0.001
Δ Serum 25(OH)D (nmol/L)	26.7 (20.2)	14.3 (12.6)	25.3 (18.0)	40.6 (19.9)	< 0.001
GS[3] at baseline (kg)	28.6 (12.2)	28.5 (13.4)	28.8 (12.9)	28.2 (12.1)	0.878
GS at 12 month (kg)	23.2 (12.1)	24.7 (10.1)	26.2 (12.6)	25.6 (9.4)	0.188
ΔGS (kg)	-5.4 (12.8)	-2.7 (11.7)	-3.1 (7.9)	-2.4 (7.7)	0.898
TUG[4] at baseline (s)	11.4 (3.1)	10.8 (2.5)	11.6 (2.9)	11.8 (3.5)	0.098
TUG at 12 month (s)	11.8 (3.2)	11.4 (2.6)	11.9 (3.7)	11.9 (3.2)	0.833
ΔTUG (s)	0.4 (2.5)	0.6 (2.2)	0.4 (2.8)	0.1 (2.5)	0.490

[1]ANCOVA, between three dose group, adjusted for age, gender, BMI, FM, plasma 25(OH)D
[2] Parathyroid Hormones [3]Grip Strength [4]Timed-up and go test

According to Table 6.3, TUG and GS were not associated with PTH concentration before or after vitamin D supplementation.

Table 6.4 : Relationship between PTH status and MSK function before and after total vitamin D supplementation

	Before supplementation (Mean (SD))		
	Hyperparathyroidism[1] (n=72)	**Normal (n=303)**	**P value[2]**
Grip Strength (kg)	28.0 (15.2)	28.5 (12.6)	0.752
Timed-Up and Go test (s)	11.5 (3.3)	11.4 (3.1)	0.960
	After supplementation (Mean (SD))		
	Hyperparathyroidism[1] (n=44)	**Normal (n=293)**	**P value[2]**
Grip Strength (kg)	25.3 (10.8)	25.7 (9.8)	0.975
Timed-Up and Go test (s)	12.4 (3.7)	11.7 (3.1)	0.201

[1]PTH concentration > 60.4 Pg/mL
[2]ANCOVA, adjusted for age, gender, BMI, plasma 25(OH)D concentration

Table 6.4 shows the PTH concentration at baseline, after 12 months supplementation and changes of PTH concentration and its association with muscle function variables at baseline and after the supplementation in VDOP study. PTH concentration at baseline, 12th month and change in PTH concentration were not significantly associated with TUG test and GS for any of the dose groups.

Table 6.5 : Simple correlation between PTH concentration and muscle function variables at baseline, at 12 month and the (Δ) change of muscle function variables in VDOP study

	Baseline total sample	PTH After 12 months			Change in PTH concentration			
		Total sample	**12000 IU**	**24000 IU**	**48000 IU**	**Total sample**	**12000 IU**	**24000 IU**	**48000 IU**
GS[1] Baseline	R =0.049 P= 0.347								
GS at follow-up		R= 0.013 P = 0.814	R = 0.045 P=0.649	R= 0.011 P= 0.908	R = 0.026 P = 0.791				
GS change						R=0.038 P = 0.493	R=0.056 P = 0.567	R = -0.057 P = 0.560	R = 0.139 P = 0.158
TUG[2] baseline	R=0.052 P = 0.318								
TUG at follow-up		R=0.027 P = 0.628	R = -0.009 P = 0.930	R=0.060 P = 0.539	R = 0.060 P = 0.538				
TUG change						R = -0.006 P = 0.913	R = -0.154 P = 0.121	R = 0.150 P = 0.125	R = -0.090 P= 0.363

[1] *Grip Strength* [2]*Timed-Up and Go test*

Table 6.6 shows the predictors of muscle function variables before and after the supplementation. Age and female gender were negative predictors of GS at both baseline and after the supplementation. FFM and height were the significant positive predictors for GS at baseline and after the supplementation**.** Serum 25(OH)D concentration was retained as a positive predictor for GS after the supplementation. Percentage of the variance of GS, explained by the predictors were 36% and 56% before and after the supplementation, respectively. Age was a positive predictor of TUG test at both baseline and after the supplementation, while height was a negative predictor of TUG test at both baseline and after the supplementation. FM was a positive product of TUG test at baseline while BMI was a positive predictor for the endpoint TUG test. PTH concentration was not retained in any of the models.

Table 6.6 : Linear regression analysis[1] predicting GS and TUG before and after the vitamin D supplementation.

Muscle function variables	Constant	β	B	SE	CI	P value
Before supplementation						
Grip Strength (R^2 = 36.0)						
Fat Free Mass (kg)	-0.566	0.266	0.344	0.105	0.137 – 0.551	<0.001
Height (cm)		0.193	0.276	0.106	0.069 – 0.484	0.009
Age (years)		-0.133	-0.429	0.138	-0.701 – (-0.157)	0.002
Gender (Females vs males)		-0.179	-4.758	1.779	-8.296 – (-1.221)	0.009
Timed-Up and Go test (R^2 = 15.5)						
Age (years)	5.49	0.306	0.225	0.036	0.153 -0.296	<0.001
Fat Mass (kg)		0.218	0.079	0.019	0.043 – 0.116	<0.001
Height (cm)		-0.229	-0.075	0.023	-0.120 – (-0.030)	<0.001
Gender (Females vs males)		-0.146	-0.881	0.434	-1.734 – (-0.028)	0.043
After the supplementation						
Grip Strength (R^2= 56.4)	-1.737					
Gender (Females vs males)		-0.410	-8.051	1.158	-10.329 – (-5.774)	<0.001
Height (cm)		0.232	0.256	0.067	0.123 – 0.388	<0.001
Age (years)		-0.125	-0.279	0.088	-0.471 – (-0.124)	0.002
Fat Free Mass (kg)		0.191	0.163	0.067	0.031- 0.296	0.014
Timed –Up and Go test (R^2 = 14.0)	0.506					
Age (years)		0.257	0.196	0.040	0.118 – 0.274	<0.001
Body Mass Index (kgm^{-2})		0.197	0.156	0.041	0.075 – 0.236	<0.001
Height (cm)		-0.132	-0.044	0.017	-0.078 – (-0.010)	0.012

[1] *Stepwise Linear Regression analysis adjusted for serum 25(OH)D concentration*

6.3.2 BFU Study

Table 6.7 presents the basic characteristics of the participants in the BFU Study. Mean age and BMI were 66.8 (SD 8.3) and 28.3 (SD 4.7) kgm^{-2}, respectively. The mean PTH concentration was 42.9 (SD18.7) Pg/mL. The proportions of males and females were approximately equal.

Table 6.7 : Basic characteristics of participants in the BFU Study (n=47)

Characteristic	Mean (SD)
Age (years)	66.8 (8.3)
Males (n (%))	23 (49)
Body Mass Index (kgm^{-2})	28.3 (4.7)
Weight (kg)	81.5 (17.6)
Height (m)	169.2 (10.6)
Waist Circumference (cm)	96.5 (14.3)
Hip Circumference (cm)	105.0 (9.3)
Fat percentage (%)	34.2 (8.1)
Current serum 25(OH)D nmol/L	66.3 (28.8)
Plasma Parathyroid Hormones (Pg/mL)	42.9 (18.7)
Grip Strength (kg)	27.6 (9.2)
Timed Up and Go test (seconds)	9.1 (4.05)
Broadband Ultrasound Attenuation	106.7 (20.3)
Speed of Sound (m/s)	1564.9 (40.6)
Stiffness Index	90.9 (18.9)
Dietary vitamin D intake (µg/day)	3.0 (1.4)
Dietary calcium intake (mg/day)	891.4 (404.8)
Having a history of NCDs (n (%))	
Yes	29 (61.7)
No	18 (38.2)
Use of vitamin D supplements	
Yes	20 (42.6)
No	27 (57.4)
Serum PTH states	
Hyperparathyroidism[1]	6 (12.8)
Normal[2]	41 (87.2)

[1]PTH concentration > 60.4 Pg/mL [2]PTH concentration, 10.4 – 60.4 pg/mL

Table 6.8 demonstrates the correlation coefficient between PTH concentration, demographic, anthropometric, biochemical parameters and MSK outcomes in older adults. None of the anthropometric, dietary and muscle function variables was significantly correlated with PTH concentration. Bone health outcome measures, SOS, SI and BUA were significantly associated with PTH concentration after adjusting for age, gender and BMI.

Table 6.8 : Correlation between PTH concentration, demographic, anthropometric and biochemical parameters in older adults

Variables (Dependent variable: PTH conc (Pg/mL)	Correlation coefficient	P value
Simple correlation (n=47)		
Age (years)	0.143	0.338
Body Mass Index (kgm^{-2})	-0.024	0.875
Fat percentage (%)	0.051	0.738
Serum 25(OH)D concentration (nmol/L)	-0.122	0.413
Vitamin D intake (µg/day)	-0.044	0.770
Calcium intake (mg/day)	-0.017	0.911
Weight (kg)	-0.158	0.290
Height (height)	-0.252	0.088
Waist Circumference (cm)	-0.146	0.328
Hip Circumference (cm)	-0.078	0.603
Partial correlations (n=47)		
Muscle function variables		
Grip Strength (kg)	-0.037	0.820[1]
Timed-Up and Go test (s)	-0.042	0.792[1]
Bone health variables		
Speed of Sounds (m/s)	0.377	0.014[1]
Stiffness Index	0.402	0.008[1]
Broadband Ultrasound Attenuation	0.382	0.012[1]

[1]*Adjusted for age, gender, Body Mass Index and serum 25(OH)D concentration*

Table 6.9 demonstrates the predictors of muscle function and bone health outcomes selected by stepwise linear regression analysis. Age and gender were the only significant predictors of GS which were the negative predictors of GS. This model explained 62% of the variability of the GS. None of the variables was retained for the model for the TUG test. Female gender was a significant negative predictor of MSK outcomes, while PTH concentration was a significant positive predictor of SOS and SI. Variance explained by the models for SOS, BUA and SI were 22.8%, 8.6% and 43.9%, respectively.

Table 6.9 : Linear regression[1] analysis of predictors of MSK outcomes in BFU Study participants (n=47)

Variables	Constant	β	B	SE	CI	P value
Grip Strength (R^2 = 62.0)	55.75					
Gender (Females vs males)		-0.715	-13.000	1.732	-16.496 – (-9.505)	<0.001
Age (years)		-0.294	-0.319	0.103	-0.528 – (-0.111)	0.004
Timed-Up and Go test			No variables were retained			
Speed of Sound (R^2 = 22.8)	1541.29					
Gender (Females vs males)		-0.452	-36.346	11.341	-59.234 – (-13.459)	0.003
Parathyroid Hormones (Pg/mL)		0.322	9.336	4.090	1.083 - 17.590	0.028
Broad Band Attenuation (R^2 = 8.6)	112.60					
Gender (Females vs males)		-0.293	-11.753	5.781	-23.404 – (-0.103)	0.048
Stiffness Index (R^2 = 43.9)	127.88					
Gender (Females vs males)		-0.608	-22.783	4.556	-31.984 – (-13.582)	<0.001
PTH (Pg/mL)		0.346	4.666	1.660	1.314 - 8.019	0.008
Age (years)		-0.302	-0.695	0.272	-1.245 – (-0.146)	0.014

[1]*Stepwise linear regression adjusted for serum 25(OH)D concentration*

6.4 Discussion

The aim of this study was to investigate the association between PTH concentration and MSK outcomes in older adults, adjusting for serum 25(OH)D concentration. In the cross-sectional (BFU) study, PTH concentration was positively associated with bone health outcomes in older adults, independent of serum 25(OH)D concentration, but not with the muscle function. In RCT (VDOP study) PTH concentration was not associated with muscle function at both before and after vitamin D supplementation.

6.4.1 Interpretation of findings

Relationship between PTH and bone health

In agreement with our study, some prior studies have reported the positive associations between PTH concentration and bone health outcomes. A study conducted in the UK recruiting osteoporotic postmenopausal women showed that patients with blunted PTH concentration showed the protection against PTH mediated bone loss (Sahota *et al*., 2004). Another two previous RCTs involving postmenopausal women showed that, injection of PTH reduced the risk of vertebral fractures (Neer, 2010, Greenspan *et al*., 2007). The mechanism underlines the positive association of PTH concentration and bone mass is well established. PTH increases calcium absorption in the small intestine by up-regulating the production of the active metabolite of vitamin D ($1,25(OH)_2D$) in the kidneys which facilitate the increase in BMD (Abel *et al*., 2005). It is believed that continuous exposure to the high concentration of PTH results in catabolic effects on the skeleton, while exposure of intermitted low dose of

PTH results in an anabolic effect on bone. The low dose of PTH stimulates *IGF – 1* thereby facilitate bone formation (Silva and Bilezikian, 2015). In addition, it is reported that intermittent PTH administration directly acts on osteoblasts to promote osteoblastogenesis, reduce osteoblast apoptosis, and reactivates inactive lining cells of the bones which ultimately supports bone formation. Also, the low dose of PTH stimulates the expression of genes that signals bone formation, such as the osteoblast-specific transcription factor Runx2, Osteocalcin, Alkaline Phosphatase, Collagen type I alpha 1 (COL1A1) (Silva and Bilezikian, 2015). The reason for the positive association of PTH concentration and bone health outcomes in our study might be due to the comparatively low PTH concentration of the study participants that did not reach the level of hyperparathyroidism. Only 19% of our participants were in the category of hyperparathyroidism in cross sectional study. In addition mean PTH concentration of older adults was 42.9 Pg/mL which was lower than the value reported Kota *et al*., 2013 (53.1 Pg/mL) in a study of adults whose mean age was 62.5 (SD 6.5) years. This particular study showed the negative association between PTH concentration and BMD. However hyperparathyroidism can be categorized as, primary and secondary hyperparathyroidism. Primary hyperparathyroidism is characterized by the independent high production of PTH, with the presence of hypercalcemia or normal-high serum calcium levels (Cordellat, 2012). The available data of this study was not sufficient to decide whether the participants had secondary or primary hyperparathyroidism.

In contrast to this study the negative associations between high PTH concentration and bone mass have been reported by some studies (Kota *et al*., 2013, Zhae *et al*., 2007). One of the mechanisms identified so far, to support this negative association is the stimulation of osteoclast cells by PTH through the action of *RANK* receptors located in osteoblast, and subsequently increase the bone resorption process (Rolighed *et al*., 2014).

Relationship between PTH and muscle function

The association between PTH concentration and muscle function was not found in either study. Some previous studies (Renound *et al*., 2014, Houston *et al*., 2008, Cosman *et al*., 2007) have reported the negative association between elevated PTH concentration and muscle function. The mechanisms underline this negative association between hyperparathyroidism and muscle function are increased protein catabolism, decreased number of type 2 muscle fibres, increased intracellular energy-rich phosphate compounds, and mitochondrial oxygen uptake (Mosekilde *et al*., 2005). Further higher, PTH levels are primarily the result of increased skin pigmentation and thereby reduce vitamin D production in the skin (Cosman *et al*., 2007). In contrast, Abboud *et al*., 2017 stated that PTH has an anabolic effect on muscle

function as a result of increased the uptake and retention of $25(OH)_3D$ in the skeletal muscle cells. The reason for this null finding in this study might be attributed to the low range of PTH concentration in BFU study (21.7 – 71.7 Pg/mL) and a low statistical power resulted by low sample size. I reported that female gender and age were negatively associated with MSK outcomes in both studies, which is a universally agreed phenomenon. Bone mass reduces with increasing age as a result of the hormonal and body compositional changes, activity patterns and psychological influences (Neer, 2010).

6.4.2 Strengths and Limitations

Determining the relationship between MSK outcomes and PTH concentration in two study designs, large sample size in a RCT and cross sectional study is a novel approach in this study. Association of PTH and MSK outcome before and after high doses of vitamin D supplementation is also an advantage of this study. Adjusting the statistical models developed for MSK outcomes for the various factors such as demographic, anthropometric and biochemical variables were added advantage to this study. Limitation of this study includes a low sample size in the cross sectional study. Therefore, the findings cannot be generalized for the general population in the UK. In addition, the insufficient data to identify older adults with primary and secondary hyperparathyroidism in this study is a limitation. The method that I used to measure MSK outcomes is not the gold stand methods of assessing bone mass, which also a limitation in this study.

6.4.3 Public health implication

A positive association between PTH concentration and bone health outcome reported in this study highlights the importance of maintaining appropriate PTH concentration below hyperparathyroisms to optimize bone health. According to our study PTH concentration between 21.7 – 71.7 Pg/mL would be beneficial for better bone health outcomes. As PTH concentration did not show an association with muscle function, future studies are required with a large representative sample of the general public and with the combination of different methods of assessing muscle function and bone health outcomes.

6.4.4 Conclusions

In conclusion, serum PTH concentration had a positive association with bone health in older adults but not with muscle function. Advancing age and female gender were significant predictors for bone parameters at the heel bone while age, gender, FM, FFM, BMI and height were the predictors of muscle function. Further studies are needed to confirm the findings with large sample size and with the slandered methods of assessing muscle function and bone health.

Chapter 7 : The 12 Years Change in 25(OH)D Concentration in a Group of People at Moderate Risk of Colorectal Cancer

7.1 Introduction

Colorectal Cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer death in the world, accounting for about 1.4 million new cases each year (Ferlay *et al.,* 2013). CRC is the 4th most common cancer in the UK, accounting for 12% of all new cancer cases. Research reports stated that there are around 41,700 new CRC cases in the UK every year (Cancer Research UK, 2015). CRC develops from malignant transformation of the epithelium of the large intestine (Ferrer-Mayorga *et al.,* 2019). Many epidemiological and observational studies have reported that VDD is associated with high incidence of CRC and mortality from CRC (Chandler *et al*., 2015, Mohr *et al*., 2015, Ma *et al*., 2011). Inverse associations between sun exposure and mortality from CRC also have been reported (Mondul *et al.,* 2017). Studies have proved that large intestine is the organ that consisted of the highest expression of VDR (Ferrer-Mayorga *et al*., 2019). *In vitro* studies reported that calcitriol inhibits proliferation, sensitizes to apoptosis, and promotes differentiation of colon carcinoma cells, through the regulation of genes and the modulation of signalling pathways in tumour cells (Krishnan *et al*., 2010).

Sunlight exposure is the main source for vitamin D, while diet provides minor contribution to vitamin D status (Fraser and Milan 2013). Older people are believed to be at risk of developing VDD as a result of low cutaneous synthesis, poor dietary intake and low physical activity (Cashman *et al*., 2014, Freedman *et al.,* 2013). Among older people whose age is higher than 65 years, the skin 7-DHC concentrations is about 25 % compared to a person of 20–30 years old (McLaughlin *et al*., 1985). Seasonal variation in the serum 25(OH)D concentration was reported in many studies of older adults (Patwardhan *et al*., 2015, Touvier *et al*., 2015, Freedman *et al.,* 2013). Therefore, older adults live in higher latitudes are believed to have sub optimum vitamin D status during winter months as a result of low sunlight availability (Holick *et al*., 2011). Though vitamin D rich food items are limited, SACN, 2016, stated that RNI for vitamin D is 10µg/day for older adults (SACN, 2016). Many studies reported that older adults are not meeting the RNI for vitamin D (Granic *et al*., 2015, Hill *et al*., 2005).

Understanding vitamin D status, its determinants both cross-sectionally and over time might provide strategies to optimize vitamin D status in people at risk of CRC. From a bone health perspective, these patients should maintain an ideal vitamin D status. As discussed later in

this chapter, the potential benefit to CRC risk of maintaining higher 25(OH)D remains speculative but based on the existing evidence, it can be concluded that maintaining a concentration of 25(OH)D > 25 nmol/L would have tangible benefits to health.

Therefore, the aim of this study was to identify the change in vitamin D status and vitamin D intake during a 12 year period in older adults who are at moderate risk of CRC. Therefore the objectives of this chapter were,

i) to identify the change in serum 25(OH)D concentration of older adults who are at moderate risk of CRC during the 12 year period from BFU study.
ii) to identify the change in dietary vitamin D intake of older adults who are at moderate risk of CRC during the 12 year period from BFU study.

7.2 Methods

7.2.1 Recruitment of participants

After approximately 12 years, participants in the BORICC (Biomarker of Risk of Colon Cancer) study were invited to participate in the present study called BORICC Follow-Up (BFU) Study. The original BORICC study was conducted in 2006 with the objective of developing and validating novel biomarkers of bowel cancer risk and to determine their relationships with habitual diet and nutritional status. A total of 47 older adults from the original BORICC study agreed to participate in the BFU study. Further details about the recruitment of participants, inclusion and exclusion criteria of original BORICC study and BFU study were described in Chapter 2 under the subsections of 2.3 and 2.4.

7.2.2 Anthropometry, socio-demographic information, dietary intake

Anthropometry (weight, height, FM, FFM, WC, HC), socio-demographic information, dietary intake, history of NCD dietary supplement use and serum 25(OH)D concentration were measured at follow-up. Serum 25(OH)D concentration of follow-up study was measured using the Roche method in Newcastle Laboratories, Freeman Hospital. Socio-demographic information was gathered using a questionnaire. The FFQ used in this study is based on the EPIC study and can be found in Appendix J of the thesis. In-house software based on MaCance and Widdowson's 7th Edition Food Composition tables was used to quantify the vitamin D intake. Dietary vitamin D intake which gathered using FFQ of the same participants 12 years ago was obtained from the past records. However this value was obtained only for 37 older adults. The baseline serum 25(OH)D which measured using IDS Radioimmunoassay in 12 years ago also was gathered from the past records. BMI and the use of vitamin D supplements 12 years ago were also gathered from the past records.

7.2.3 Statistical analysis

Characteristics of the older adults at baseline and follow-up study were presented as mean an SD. To evaluate the seasonal changes of serum 25(OH)D, older adults were grouped into two, based on the time of blood sampling. Older adults sampled during April – September and October to March were categorized as Summer and Winter sampling, respectively. Seasonal change of serum 25(OH)D concentration at follow-up study as well as vitamin D status at baseline and follow-up were presented in a bar graph. Vitamin D status was presented in a bar graph. To identify the vitamin D status of older adults at baseline and follow-up studies, older adults were grouped into two based on the both SACN cut-off of VDD (25 nmol/L) (SACN, 2016) and IOM cut-off of vitamin D insufficiency (50 nmol/L) (IOM, 2011). Parallel plots were used to present the changes in vitamin D intake and vitamin D status during 12 year period. The supplement uses were also included in these parallel plots. However, the details of the amount of vitamin D intake from supplements were not available in this study. Baseline and follow-up serum 25(OH)D concentration and dietary intake were compared using paired Student's t test. ANOVA test was used to compare the serum 25(OH)D concentration between the seasons.

7.3 Results

Table 7.1 shows the characteristics of the participants at baseline and at follow-up. The mean age of the participants was 66.8 (SD 8.3) years at follow-up. Percentage of males and females and the BMI of the participants were approximately equal at baseline and at follow-up. Serum 25(OH)D concentrations at baseline and at follow-up were 79.2 (SD 38.3) nmol/L and 66.4 (28.8) nmol/L, respectively. Similarly, dietary vitamin D intake at baseline and at follow-up were 3.9 (SD 1.7), 3.0 (SD 1.4) µg/day, respectively. Percentage of older adults who used vitamin D containing dietary supplements at baseline and follow-up were 25.5% and 46.2%, respectively.

Table 7.1 : Characteristics of older adults at baseline and follow-up studies

Characteristics	Follow-up study (n=47)	Baseline study (n=47)	P value
	Mean (SD) / n (%)		
Body Mass Index (kgm^{-2})	28.3 (4.7)	28.2 (4.8)	0.688[1]
Serum 25(OH)D (nmol/L)	66.4 (28.8)	79.2 (38.3)[2]	0.215[1]
Dietary vitamin D intake (µg/day)	3.0 (1.4)	3.9 (1.7)	0.057[1]
Use of vitamin D supplements			
Yes	20 (42.5)	12 (25.5)	
No	27 (57.5)	34 (72.3)	0.192[3]
Season of sampling			
Summer	25 (53.2)	16 (51.6)	
Winter	22 (46.8)	15 (48.3)	0.106
Age (years)	66.8 (8.3)		
Gender (n (%))			
Males	23 (48.9)		
Females	24 (51.1)		
Weight (kg)	81.5 (17.6)		
Height (m)	169.2 (10.6)		
Waist Circumference (WC) (cm)	96.6 (14.3)		
Hip Circumference (HC) (cm)	105.0 (9.3)		
Fat percentage (%)	34.3 (8.1)		
Plasma PTH (Pmol/L)	4.6 (1.4)		
Dietary calcium intake (mg/day)	891.5 (404.8)		
Gender (n (%))			
Males	23 (48.9)		
Females	24 (51.1)		
Having a history of NCDs (n(%))			
Yes	29 (61.7)		
No	18 (38.2)		
Had polyps at baseline screening			
No	37 (78.7)		
Yes	10 (21.3)		

[1] *Paired t test* [2] *Out of 47 older adults, serum 25(OH)D data were available only for 37 individuals*
[3] *Chi-squared test*

Figure 7.1 summarises the vitamin D status of the participants at follow-up and baseline (12 years ago). At follow-up, 16 participants were in the D status group of 25 – 50 nmol/L, compared only 7 people at baseline, 12+ years ago. The percentage of older adults in the serum 25(OH)D group > 75 nmol/L at baseline and follow-up was equal. None of the older adults at follow-up study had serum 25(OH)D concentration less than 25 nmol/L. Only one participant had serum 25(OH)D concentration less than 25 nmol/L at baseline. Though it was not significant, mean serum 25(OH)D concentration at follow-up (66.4 SD 28.8) nmol/L was lower than the baseline serum 25(OH)D concentration (79.2 SD 38.3) nmol/L (p=0.215). Figure 7.2 further illustrates the individual change in the vitamin D status of older adults during 12 year follow-up period.

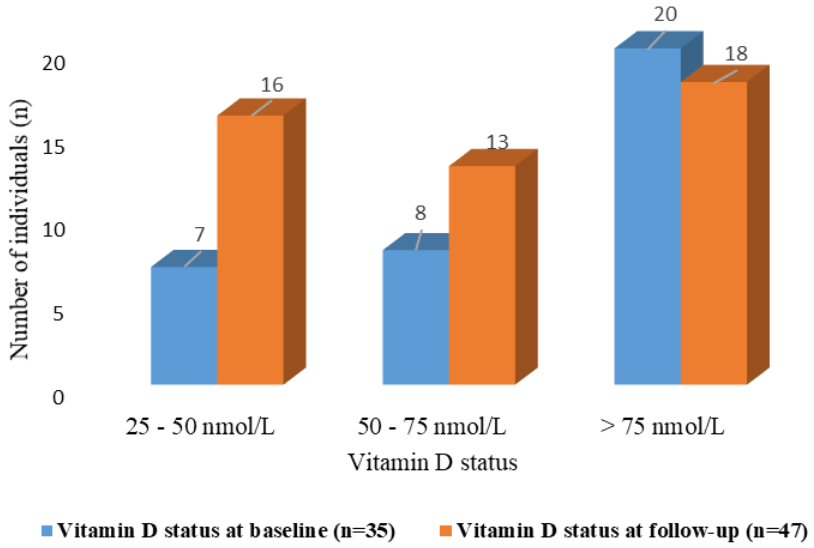


Figure 7.1 : Vitamin D status of the participants at baseline and at follow-up studies

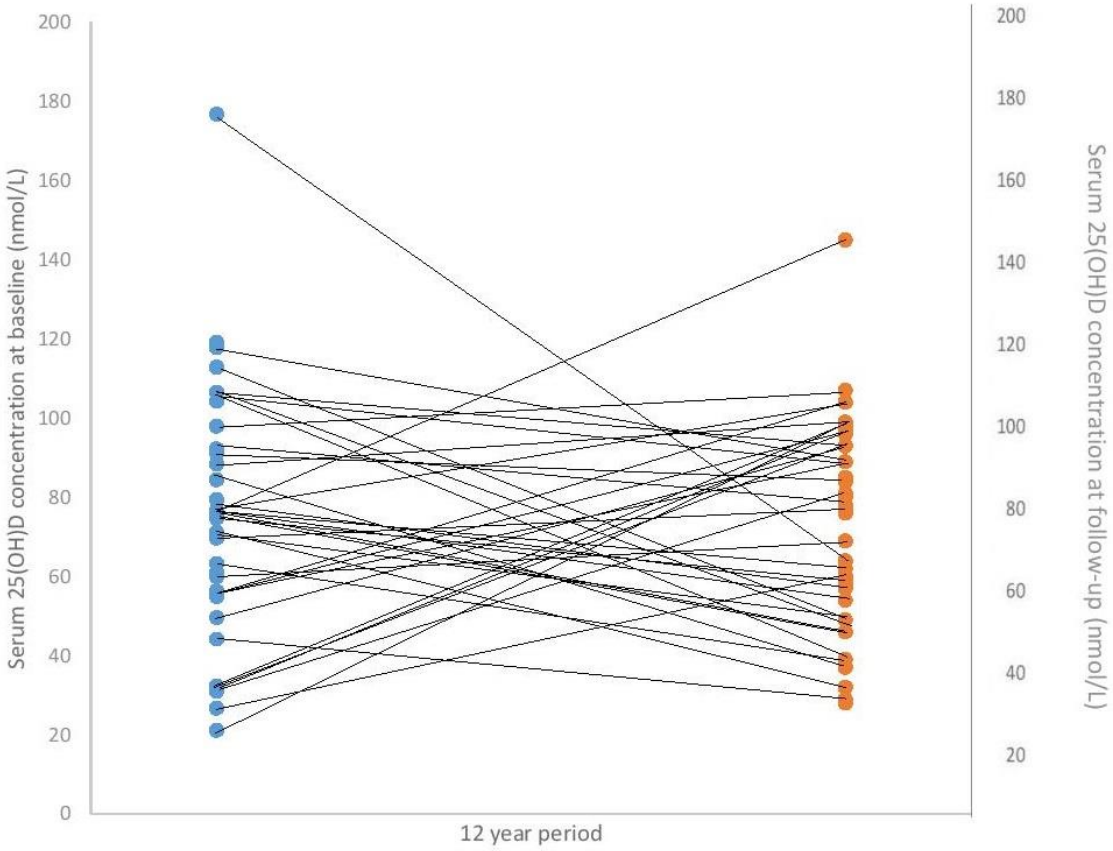


Figure 7.2 : Change of individual vitamin D status during 12 years period among older adults (n=47)

Figure 7.3 depicts the seasonal differences of serum 25(OH)D concentration at follow-up. Mean serum 25(OH)D concentration in winter sample (n=22) was 59.4 (SD 30.4) nmol/L while mean serum 25(OH)D concentration in summer sample (n=25) was 72.4 (SD 72.4) nmol/L. Serum 25(OH)D concentration was not associated with age (p=0.764) and gender (p=0.243).

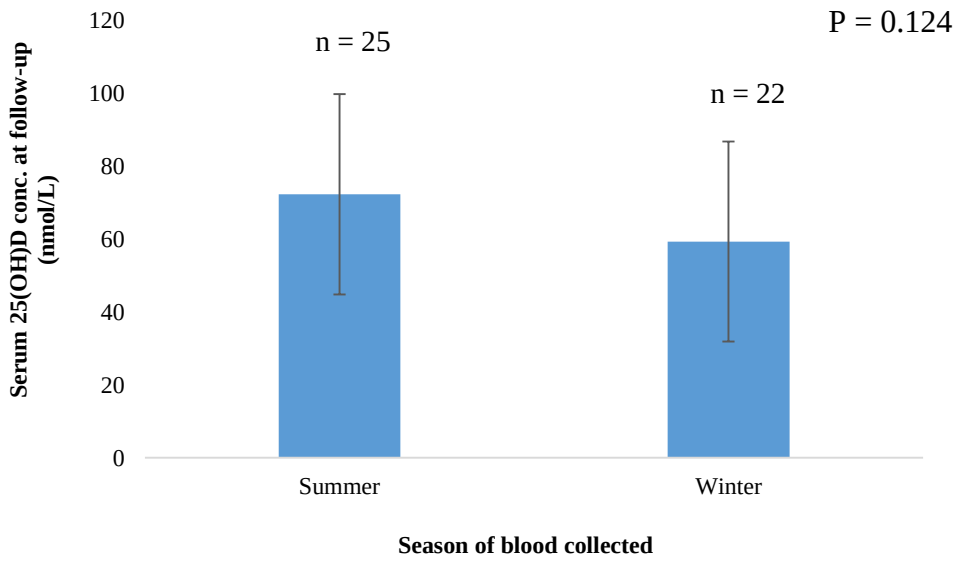


Figure 7.3 Serum 25(OH)D concentration during summer and winter

Figure 7.4 shows the change of dietary vitamin D intake of individual older adults during the 12 year period. Mean vitamin D intake of older adults at follow-up (3.0 SD 1.4) µg/day was not significantly different to mean vitamin D intake at baseline (3.9 SD 1.7) µg/day. Participants who took vitamin D supplements were included on figure 7.4. The two participants who had vitamin D intakes > 8 µg/day were supplement users and from the FFQ data appeared to have fish and meat intake (the two greatest sources of vitamin D in the UK diet based on NDNS data in older adults).

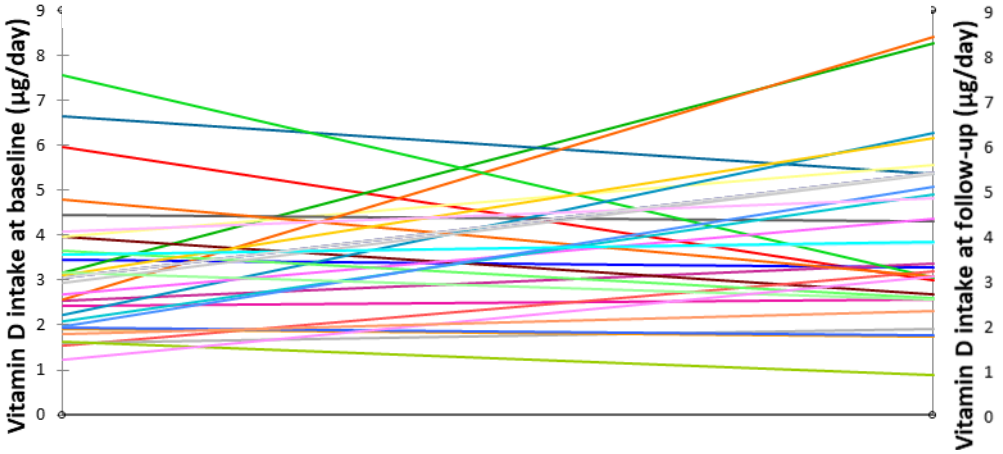


Figure 7.4 : Change of individual dietary vitamin D intake during 12 years period among older adults (n=37)

7.4 Discussion

7.4.1 Main findings

According to SACN cut-off of VDD (25 nmol/L), only one participant was vitamin D deficient at baseline, while none of the older adults were vitamin D deficient at follow-up. Sixteen older adults were in the vitamin D status group of 25 – 50 nmol/L at follow-up which increased from 7 participants at baseline during the 12 year follow-up period. Overall the vitamin D status of the older adults was quite satisfactory at both follow-up and baseline. One reason for the marginally worse [but not statistically significant] vitamin D status at follow up might be attributed to the method used in assessing serum 25(OH)D concentration. At the baseline assessment, the IDS EIA method was used to assess vitamin D status and this method has been shown to over-report the serum 25(OH)D concentration (Enko *et al.*, 2014).

7.4.2 Discussion of main findings

Compared to the previous studies conducted in older adults in the UK, mean serum 25(OH)D concentration of our study participants at both baseline and follow-up studies were comparatively satisfactory. According to a study conducted in Newcastle, UK in older adults, mean serum 25(OH)D concentrations were 27, 45, 43 and 33 nmol/L during spring, summer, autumn and winter, respectively (Hill *et al.*, 2015). Further, the serum 25(OH)D concentration of BFU study participants at both baseline and follow up were higher than the baseline value reported in the VDOP study (38.2 nmol/L) (Aspray *et al.*, 2018). According to another study conducted in London, serum 25(OH)D concentration of older adults was 42.7 (SD 22.0) nmol/L (Jolliffe *et al.*, 2016). A study of Irish older adults' serum 25(OH)D concentration ranged from 38.5 – 69.6 nmol/L (Forstyhe *et al.*, 2012). Therefore, the serum 25(OH)D

concentration values reported in our studies were comparably high compared to the values reported for the older adults in the UK in previous studies. None of the participants was vitamin D deficient according to the UK cut-off of VDD (25 nmol/L) at follow-up. According to US cut-off, 17 older adults were vitamin D inadequate at follow-up. One reason for the better vitamin D status in this population might be attributed to the method used in assessing serum 25(OH)D concentration. At the follow-up Roche method was used, which might over-reported the serum 25(OH)D concentration (Enko *et al*., 2014). Also a considerable number of people consumed vitamin D containing supplements which might cause for higher serum 25(OH)D concentration. In this study reduction in serum 25(OH)D was not significant. In contrast to our study, Elstgeest *et al*., 2018 showed that during a 13 year follow-up periods, serum 25(OH)D concentrations increased in 32.4% of the older cohort of 65 – 88 years, while there was a 69.8% increase 25(OH)D in the younger cohort of 55 – 65 years age during 6 year follow-up period.

Mean vitamin D intake at baseline and at follow-up of our study were also comparative to the values reported by recent studies of older adults in the UK (Granic *et al*., 2015, Hill *et al*., 2005), which ranged from 2.9 to 5.7 µg/day. At baseline and at follow-up time points, none of the participants met the RNI of vitamin D (10 µg/day) (SCAN, 2016).

Several studies have demonstrated the seasonal changes in serum 25(OH)D in older adults which showed the highest value during summer (Touvier *et al*., 2015, Freedman *et al*., 2013, Hedlund *et al*., 2013, Darling *et al*., 2012). However, the seasonal variation showed in our study was unexpected. We reported the highest serum 25(OH)D concentration during winter and the lowest value during spring. Some previous studies showed that, vitamin D_3 produced in the skin takes some time to show as 25(OH)D in the serum which varying from 2 weeks to 6 months (Lawson *et al*., 1979). This concept partly explains our unexpected finding.

Several mechanisms of the vitamin D in CRC prevention have been proposed. Mainly, the vitamin D and its metabolites reduce the incidence of several types of cancer by inhibiting tumor angiogenesis, stimulating mutual adherence of cells, enhancing intercellular communication through gap junctions and thereby strengthening the inhibition of proliferation. Vitamin D metabolites also help in maintaining the normal calcium gradient in the colon epithelial crypts and high serum concentration of 25(OH)D are associated with noticeably decreased proliferation of high-risk epithelial cells in the colon (Garland *et al.*, 2006). However study findings on the association of vitamin D and CRC are not consistent. An ecological study conducted in US during 1970 – 1994 reported that solar UVB radiation dose was associated with reduced risk of cancer of the breast, colon, ovary, and prostate. They

further reported that the annual number of premature deaths from cancer due to lower UVB exposure was 21,700. Therefore this particular study concluded that increased UVB exposure or supplementary vitamin D consumption, could be helpful to reduce most of cancer incidences (Grant 2002). They highlighted that 1,25$(OH)_2$D was the form of vitamin D that played a role in cancer prevention (Grant, 2002).

One of the study similar to this study, which was a 9 year follow-up study of postmenopausal women who were at risk of colon cancer, reported that those in the highest intake of total calcium and vitamin D were at a 45% reduced risk of colorectal cancer (RR, 0.55; 95% CI, 0.32-0.93) compared with the women who consumed low levels of both nutrients (Zheng *et al.*, 1998). Another prospective cohort study of 35,216 women in US aged 55-69 years who did not have the history of cancer reported that intakes of calcium and vitamin D were significantly and inversely associated with the risk of CRC after adjusting for the age (Bostick *et al.*,1993). A case control study (age, race and sex matched) of 8 year follow-up period conducted recruiting 25620 volunteers age higher than 35 years in Washington reported that risk of getting colon cancer decreased by three-fold in people with a serum 25(OH)D concentration of 20 ng/ml or more. They further found that the risk of getting colon cancer was reduced by 80% in the individuals whose serum 25(OH)D concentration between 52.4 – 117.3 nmol/L (21 – 47 ng/mL). However, they reported that the highest 25(OH)D quintile had a non-significantly higher risk of colon cancer than the other groups from 49.9 – 102.3 nmol/L (20 - 41 ng/mL). They explained that these unexpected findings might be due to the fact that, at the very high 25(OH)D concentrations, some unnecessary metabolites are unable to be removed from the plasma which possibly increases the risk of CRC (Garland *et al.*, 1989). A study conducted in Canada examined the association between sulfur dioxide and ultraviolet-light-blocking aerosols in 20 Canadian cities, and age-adjusted breast and colon cancer mortality rates in the surrounding cities. Further, statistically significant positive associations were found between these two measures of air pollution and age-adjusted mortality rates for CRC. Therefore they highlighted that low vitamin D synthesis in the skin at the high level of pollution might cause for the increased incidence of CRC (Gorham *et al*., 1989).

A pooled analysis of a vitamin D RCT and prospective studies of vitamin D status and cancer risk [n 2,304] suggests those participants with a 25(OH)D concentration > 100 nmol/L had a substantial reduction in risk of all invasive cancers (including colorectal cancer) suggesting that maintaining a 25(OH)D concentration above 100 nmol/L might be protective against cancer risk (McDonnell *et al*., 2016). It is noteworthy that only 7 out of the 47 participants in

the BFU study achieved this concentration of 25(OH)D and so it remains unclear as to whether these patients are truly at risk of CRC based on their vitamin D status.

There is a debate among scientist regarding the effect of vitamin D on CRC. The active hormonal form of vitamin D [1,25(OH)D)] exhibits cancer-preventative effects by inhibiting proliferation and promoting differentiation through a genomic pathway, but also has non-genomic actions through altering serum calcium concentration (Feskanich *et al.* 2004). A case control study conducted in Los Angeles, reported that vitamin D intake was not associated with the development of CRC (Peters *et al.,* 1992). A follow-up study (13.5 years) of women (n=61463) with CRC reported, 572 new cases of CRC which did not associate with vitamin D intake (Terry *et al*., 2002). A case control study of adults men and women (cases were with colorectal cancer) showed that dietary vitamin D intake was not associated with CRC (RR = 1.04, 95% CI 0.65-1.67) (Kampman *et al.,* 1994).

7.4.3 Strengths and limitations

To best of our knowledge this is the first follow-up study conducted in the UK recruiting older adults who are at moderate risk of CRC to evaluate the change in serum 25(OH)D concentration over 12 year period. Assessing dietary vitamin D intake in the same group using the same tool is an advantage to this study. However the methods used to assess the serum 25(OH)D in two studies are different. The baseline serum 25(OH)D was measured using IDS Radioimmunoassay, while Roche immune assay was used in follow-up study. Change in the vitamin D status in two studies cannot be compared accurately. The very low sample size might be the main limitation of this study. Baseline serum 25(OH)D concentration was available only for 37 older adults, which we cannot compare the baseline and follow-up values accurately. In addition there was a selection bias in the BORICC study. The participants of BORICC study were the patients who referred for the investigation of gastrointestinal symptoms and underwent screening by flexible sigmoidoscopy. Therefore the participants were not the representative sample of the general population.

7.4.4 Public health implications

This study showed that whilst there was no significant change in vitamin D status over a 12 year period in older adults at moderate risk of CRC, there was an apparent increase in the % of those with 25(OH)D concentrations < 50 nmol/L at follow up (7 v's 16 individuals respectively). Whilst the true implication of this finding for overall CRC risk remains unknown, it would be prudent for these patients to maintain serum 25(OH)D concentrations > 50 nmol/L. Such a recommendation might also have benefits to individual bone health as this threshold underpins the population RDA for vitamin D (Ross *et al.* 2010).

7.4.5 Conclusions

This study concluded that the number of older adults with VDD increased from 7 to 16 during 12 years period in older adults who were at moderate risk of CRC. Though the serum 25(OH)D and vitamin D intakes of older adults were reduced during 12 years period, these reductions were not statistically significant. Future studies are required to confirm this finding recruiting large representative sample of older adults of CRC risk.

Chapter 8: General Discussion and Conclusions

8.1 Overview of the PhD study

A reduction in MSK function is one of the characteristics of aging which impacts on QOL (Carson *et al.,* 2018). Vitamin D plays a role in MSK health through genomic and non genomic pathways (Ceglia and Harris, 2013). However, the vitamin D supply required for the optimum MSK function is not universally agreed. Different thresholds are used to define "vitamin D adequacy" by SACN and IOM for the UK and for the US populations, respectively. The SACN uses 25(OH)D concentration 25 nmol/L as the basis for setting the RNI for vitamin D based on the MSK outcomes (SACN, 2016), while IOM uses 25(OH)D concentration 50 nmol/L as the basis for setting the RDA for vitamin D based on the bone outcomes (Ross *et al.*, 2011). These different thresholds used by the SACN and IOM are attributed to the different outcomes used in setting DRVs, geographical variation in vitamin D status, different response to vitamin D supplementation and differences in sunlight exposure. Sun exposure is considered as the marker of vitamin D supply and it is believed that about 90% of the vitamin D is supplied by the skin synthesis upon UVB radiation exposure (Fraser and Milan, 2013). However, assessing the relative contribution of sun exposure to vitamin D status in older adults is challenging due to the complexities associated with personal UV exposure in people.

Previous studies have demonstrated both anabolic (Iwaniec *et al.*, 2007) and catabolic (Visser *et al*., 2003) effects of PTH (a functional biomarker of vitamin D) on MSK outcomes. However, whether the association between PTH concentration and MSK function is independent of serum 25(OH)D concentration is uncertain. VDD is widespread worldwide, especially in countries located in higher latitudes where sunlight availability is low at certain times of the year. Older people have less capacity for dermal synthesis of vitamin D due to lower availability of 7-dehydrocholesterol, poor kidney and liver function and low outdoor activities which reduces sunlight exposure (Gennari, 2001).

This PhD study attempted to determine the effect of vitamin D supply on muscle function and to determine the association between commonly-used vitamin D biomarkers [25(OH)D and PTH] and muscle function and bone health in older adults using two different studies from the North East of England (55° North). In addition, this PhD study attempted to quantify the impact of sun exposure on vitamin D status in older adults using the same two studies.

The hypotheses of this PhD study are:

1. Lower serum 25(OH)D concentrations will be associated with poorer MSK outcomes
2. Vitamin D supplementation at doses equivalent to 400 IU/day, 800 IU/day and 1600 IU/day for 12 months will result in improvements in muscle function in dose dependent manner.
3. Sunshine exposure assessed by newly adapted questionnaires will predict vitamin D status in the cross sectional analyses and will modulate the response to vitamin D supplementation in the RCT analysis.
4. PTH concentration will influence MSK outcomes in both cross sectional and RCT analyses.

The two study designs used in this PhD and discussed in more detail in Chapter 2 are:

i) A RCT of three doses of supplemental vitamin D in older adults (Vitamin D in Older People Study (VDOP) Study).

ii) A cross sectional study of older adults who participated in the BORICC Follow-Up (BFU) Study.

Objectives

The specific objectives of this project were:

1. to investigate the effects of three doses of supplemental vitamin D_3 (12000 IU, 24000 IU, 48000 IU) monthly for 12 months on markers of muscle function in older adults from VDOP study.
2. to quantify the contribution of cumulative sun exposure to serum 25(OH)D concentration among older adults supplemented with vitamin D_3 monthly for one year from VDOP study.
3. to investigate relationships between i) sun exposure behaviours and serum 25(OH)D concentration and ii) serum 25(OH)D concentration and MSK outcomes in participants in the BFU study.
4. to investigate relationships between PTH concentration, 25(OH)D concentration and MSK outcomes of older adults in both the VDOP and BFU studies.
5. to identify the change in serum 25(OH)D concentration and dietary vitamin D intake of older adults who are at moderate risk of CRC during the 12 year period from BFU study.

8.2 Discussion of Main Findings

8.2.1 Effect of vitamin D supplementation on muscle function

Analysis of data from the VDOP Study showed that vitamin D_3 supplementation at the doses of 12000 IU, 24 000 IU and 48 000 IU monthly for 12 months had no effect on muscle function in older adults aged > 70 years. Therefore our study did not generate the evidence to support 2nd hypothesis. A positive effect of vitamin D supplementation on muscle function was not observed even in older adults whose baseline serum 25(OH)D concentration below 25 nmol/L. However, only 27% of the participants in VDOP Study had serum 25(OH)D concentration below 25 nmol/L at baseline. If having low vitamin D status initially is a determinant of response to vitamin D supplementation (Stockton *et al*., 2011) then the fact that almost three quarters of the participants had adequate vitamin D status at baseline might be the reason for the absence of a positive effect of vitamin D supplementation on muscle function. Alternatively, or in addition, it may be necessary to raise serum 25(OH)D concentration above the highest concentration achieved in this intervention study (i.e. 79.0 nmol/L for the 48000 IU group) to demonstrate a benefit for MSK health but this suggestion is very speculative. Except only 0.9% of the participants in the low dose group, all of the participants who had 25(OH)D concentrations < 25 nmol/L at baseline became vitamin D sufficient after the intervention, demonstrating the efficacy in supplementation in improvement in 25(OH)D concentrations. However, there was no improvement in the GS or TUG test based on the improvement in the vitamin D status.

This PhD study also attempted to identify the circulating 25(OH)D concentration required for the optimum MSK function in older adults. The IOM recommends serum 25(OH)D > 50 nmol/ for optimum bone health. Furthermore, Holick *et al*., 2011 suggested that serum 25(OH)D concentration higher than 75 nmol/L might be sufficient for most of the health outcomes. There has been some support for the idea that achieving serum 25(OH)D concentration > 90 - 100 nmol/L following vitamin D supplementation would optimize MSK outcomes (Kotlarczyk *et al*., 2017; Bischoff-Ferrari *et al*., 2006). However, data from this PhD thesis does not lend support for the use of higher 25(OH)D thresholds above 90-100 nmol/L for optimal muscle function.

8.2.2 Relationship between serum 25(OH)D concentration and MSK function

The relationships between serum 25(OH)D concentration and MSK outcomes was investigated in both the BFU Study and in the RCT (VDOP Study) under this PhD study. Baseline plasma 25(OH)D concentration less than 25 nmol/L had a negative association between upper GS in the VDOP study. This finding supported the recommendation from

SACN that "For the protection of MSK health, serum 25(OH)D concentration should not fall below 25 nmol/L throughout the year" (SACN, 2016). However in the BFU Study, serum 25(OH)D concentration was not associated with either muscle function (GS and TUG) or bone health (heel bone density measured by ultrasound) in older adults. Therefore second hypothesis was partly accepted. In the BFU Study, mean serum 25(OH)D concentration was 79.2 (SD 38.3) nmol/L and none of the participants had serum 25(OH)D concentration below 25 nmol/L. The better vitamin D status, narrow range in serum 25(OH)D concentration (27 - 149 nmol/L) and low sample size in the cross sectional study might be attributed to this null finding. It is also likely that muscle function was already adequate in these older participants.

8.2.3 Determinants of vitamin D status including sun exposure

The determinants (sun exposure, demographic, dietary and anthropometric) of serum 25(OH)D concentration in BFU study and baseline plasma 25(OH)D concentration of the VDOP study were identified in this PhD study. In addition, the association between mean SES of one year period and serum 25 (OH)D was identified in VDOP study after 12 months supplementation. In RCT before vitamin D supplementation, dark skin types and holiday visits predicted plasma 25(OH)D concentration along with age, female gender and BMI. However, SES was not associated with serum 25(OH)D concentration at baseline in VDOP study. Though SES was not associated with serum 25(OH)D concentration, questions about holiday visits, sun exposure season and skin type predicted baseline serum 25(OH)D concentration. SES did not predict serum 25(OH)D concentration after total supplementation in any of the dose groups. Baseline plasma 25(OH)D concentration and the dose of vitamin D supplements were the noticeable predictors of endpoint plasma 25(OH)D concentration in all dose groups.

In BFU study holiday visit was the only significant sun exposure predictor of serum 25(OH)D concentration which was also a predictor of baseline plasma 25(OH)D concentration of the VDOP Study. Findings of both studies showed good agreement of using the details about holiday visits to use as a proxy indicator of vitamin D status in older adults. The general limitation of a sun exposure questionnaires might be attributed to the non-significant associations between SES and serum 25(OH)D concentration. According to these findings, 3rd hypothesis of this PhD study can partly be accepted, but only for the older adults who do not use vitamin D supplements. In VDOP study, the sun exposure questionnaire consisted of four broad questions on skin types, frequency of sun exposure, skin exposure area and the area of sunblock use at home and at holiday visits. The sun exposure questionnaire used in the BFU Study was more comprehensive than the one that used in VDOP Study. Because it

consisted of 14 questions which gathered details about skin types, duration of outdoor activities at weekends and in weekdays, sun avoidance habits, clothing style, sunblock use and the details about sun holidays. Aggregating the answers of sun exposure questions to drive SES might lead for a crude measure of sun exposure, which resulted in null finding regarding the association of serum 25(OH)D concentration and sun exposure after vitamin D supplementation.

Dietary vitamin D did not associate with serum 25(OH)D concentration in either studies. The influence of sun exposure variables and diet on plasma 25(OH)D concentration is minimum compared to vitamin D supplementation, anthropometric and demographic variables. Therefore vitamin D supplementation is more effective in improving vitamin D status compared to sun exposure and dietary vitamin D intake in older adults.

8.2.4 Relationship between PTH concentration and MSK function

The relationship between PTH and MSK function of older adults in both studies were explored in this PhD study. A significant association between PTH concentration and muscle function in either study was not reported. However, there was a significant positive association between PTH concentration and bone health parameters in the cross sectional study. Therefore our findings partly supported 4th hypothesis of this PhD study. This finding should be interpreted with caution. Because a past research described that PTH has an anabolic effect on bone tissues at a low concentration while the catabolic effect at the higher concentration (Silva and Bilezikian, 2015). The positive association of PTH concentration and bone health outcomes reported in this study might be attributed to the low range of PTH concentration that did not reach the level of hyperparathyroidism. Therefore the identified PTH concentration range between 21.7 – 71.7 Pg/mL in this study would be advantageous to have a positive association between bone health outcomes in older adults.

8.2.5 Change in serum 25(OH)D concentration during 12 years period

Change in serum 25(OH)D concentration during a 12 year period in older adults who are at moderate risk of CRC was investigated in this study. Prevalence of VDD (serum 25(OH)D concentration < 50 nmol/L) of older adults was increased from 14.9% to 38.3% during a 12 year period. The reduction in vitamin D intake and serum 25(OH)D concentration during 12 years period were not statistically significant. However, serum 25(OH)D concentration values at baseline and at follow-up studies cannot be entirely compared, as the methods used assess the vitamin D concentrations are different. At baseline serum 25(OH)D concentration was measured by IDS immunoassay method while at follow-up, Roche immunoassay was used. Whilst there have been a number of excellent literature sources attempting to harmonize

vitamin D status data in other studies, mainly national surveys through the use of calibration equations, to my knowledge most of these calibration efforts have focused on recent mainstream assay platforms such as LC-MS/MS, Diasorin Liaison Total and Roche assays. These three assays alone are used by 58% of the 926 laboratories in the 2017 Vitamin D DEQAS and represent just 10% of the total number of analytical platforms used in the scheme (total = 30). The manual IDS ELIA kit used to assess 25(OH)D at baseline in the CRC risk participants was a popular assay from 2002-2010 but has now been replaced by automated platforms. The Roche assay used to assess 25(OH)D in the 12 year follow up study is now a commonly used automated clinical assay which was launched in 2009. Thus, the lack of calibration data between the manual IDS EIA and the automated Roche assay makes any meaningful adjustment difficult in this study and for these reasons no attempt was made at trying to harmonize the two sets of 25(OH)D data.

8.3 Strengths and Limitations

One of the main strengths of this PhD study was the use of two study designs, RCT and a cross sectional study to test the hypothesis. Prior studies reported the contradictory finding on the association between plasma 25(OH)D and MSK outcomes in older adults depending on the study designs. Therefore the hypothesis could be tested by overcoming the limitations of each study design. The cross sectional study showed the snapshot of the association of serum 25(OH)D, PTH concentration and MSK outcomes in older adults. In a cross sectional study design, the true association of serum 25(OH)D concentration and MSK outcomes might be masked by other factors such as socioeconomic, demographic, environmental and biological factors.

Determining the association of serum 25(OH)D concentration and MSK outcomes in a large sample of free living older adults in the RCT added strength to this PhD study. This is the first RCT conducted in the UK to identify this association in older adults. Assessment of several variables that potentially influence muscle function such as body composition, anthropometric variables, PTH concentration and dietary intake was an advantage to this PhD study. There is a disagreement among scientist regarding the optimum serum 25(OH)D concentration required to optimize the health outcomes and for the cutoff value of the serum 25(OH)D concentration for the VDD. Therefore use of three different doses that correspondent to the RNI value for the vitamin D in the UK, RDA value for the vitamin D in the US and the value below the Tolerable Upper Intake Level to investigate the effect of vitamin D supplementation on muscle function added a novelty to this PhD study. Investigation of the relative contribution of cumulative sun exposure of a whole one year period to plasma

25(OH)D concentration, compared to the vitamin D_3 supplementation was also a novel approach in this study. In BFU study we followed up the older adults of moderate risk of CRC for 12 years period and determined the association between serum 25(OH)D concentration and MSK outcomes which also added a novelty to this study. In addition this is the first study conducted in the UK to identify the changes of serum 25(OH)D concentration of older adults who are at moderate risk of CRC.

However, this PhD study had some limitations. Not having a placebo group in the RCT was the main limitation of VDOP study. In addition, in the RCT, determining the plasma 25(OH)D concentration before and after the supplementation, might not reflect the year around vitamin D status of older adults. Further the population of both studies were not randomly selected from the community, thus we cannot generalize our findings for the general population of the UK. The low sample size of the cross sectional study was another limitation. On the other hand use of only GS and TUG test to evaluate muscle function might not reflect the true muscle function of the older adults. Similarly, the use of Ultra Sound technique might not accurate in assessing bone quality and it is not an ideal tool to diagnose fracture risk. The use of a questionnaire to assess the sun exposure might also lead to underestimation of actual personal sun exposure of older adults.

8.4 Public Health Implications

Without doubt, the most important finding in this PhD study regarding the determinants of vitamin D status was the clearly evident dose response relationship between supplemental vitamin D and improvement in vitamin D status over 12 months i.e. throughout a whole year and during a full winter in Newcastle. A dose of vitamin D equivalent to 10 µg/day (i.e. 12,000IU monthly) was sufficient to keep all participants above a 25(OH)D concentration of 25 nmol/L. These findings reinforce the advice from SACN regarding the population DRVs for vitamin D in the UK where the RNI for all people > 4 years of age including those in at risk groups is 10 µg/day. As this study failed to find positive associations between dietary sources of vitamin D and serum 25(OH)D, the PhD findings lend support to the use of supplements in meeting vitamin D requirements in older adults. The weaker associations between holiday visits, being female, age, BMI and having darker pigmented skin and 25(OH)D concentrations in VDOP participants at baseline suggests that these factors may also have relevance in informing public health advice in terms of reducing VDD although the evidence for this is not as robust as for the RCT evidence on the benefits of vitamin D supplementation.

8.5 Future Studies

Past research findings reported that, incorporating physical activity intervention along with the vitamin D supplementation would help to improve the muscle function compared to vitamin D supplementation along (Antoniak and Greig, 2017). Future studies are recommended, incorporating the physical activity along with the vitamin D supplementation trial to test the effect of vitamin D supplementation on muscle function in older adults. Similarly, a prior study revealed that the effect of vitamin D supplementation can be seen only in the older adults whose baseline serum 25(OH)D concentration less than 25 nmol/L (Stockton *et al.,* 2011). Therefore future studies are required to carry out recruiting the older adults whose baseline 25(OH)D concentration less than 25 nmol/L. New studies are also needed using the combination of several methods of assessing MSK outcomes such as use of the falls data, Short Physical Performance Battery Score, gait speed, computer tomography and use of DEXA. In addition improving RCT design with a placebo group would be an advantage in future studies to evaluate the effect of vitamin D supplementation compared to the vitamin D supplement non-users. Use of sun exposure questionnaire is an indirect method of assessing sun exposure, which is less accurate and underestimates the personal sunlight exposure (Diffey, 2007). Therefore it is worth to conduct future studies using direct methods of assessing personal sun exposure such as the use of dosimeters. Future studies are needed to carry out recruiting a large representative sample of older adults in the UK to confirm these findings.

8.6 Conclusions

From this PhD study I can conclude that older adults with serum 25(OH)D concentration < 25 nmol/L may have poor muscle function than those with serum 25(OH)D concentration higher than 25 nmol/L. PTH concentration did not associate with muscle function but showed the evidence for an association with heel bone QUS. Whilst vitamin D supplementation at doses of 12000 IU, 24000 IU and 48000 IU monthly for 12 months increased 25(OH)D concentration in a dose dependent manner, it had no effect on GS and TUG performance in older adults. As observed in the RCT, vitamin D supplementation appeared to overwhelm the effect of sun exposure on 25(OH)D concentration. In the cross sectional study, sunny holidays were positively associated with serum 25(OH)D concentration in older adults. In conclusion, vitamin D supplementation does not improve muscle function in older adults with a low prevalence of VDD [25(OH)D < 25 nmol/L)]. In line with SACN recommendations it may be prudent to maintain serum 25(OH)D > 25 nmol/L throughout the year to maintain MSK health.

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Appendixes

Appendix A : Case Report form of VDOP study

Optimising Vitamin D Status in Older People:
A Randomised Controlled Trial of Vitamin D Supplementation

Screening visit

Subject ID		Subject Initials		Visit Date	Day / Month / Year

(A) INCLUSION CRITERIA

Please record the correct answers to the following questions: 0 = No 1 = Yes 2 = N/A

1. Ambulant, community dwelling men and women aged 70 years and above. ☐
2. Individuals capable of giving informed consent on their own behalf. ☐
3. Individuals willing to attend the Study Centre (CARU) on six occasions and to be contacted by telephone at monthly intervals between study visits over twelve months. ☐

The inclusion criteria are mainly restricted to age and ability to participate in the study. The age cut-off used is the same as the threshold for older age used in the most recently published

*NOTE: *If any of the above questions are answered "NO", the subject is not eligible to participate in the study*

(B) EXCLUSION CRITERIA

Please record the correct answers to the following questions: 0 = No 1 = Yes 2 = N/A

1. Current antiresorptive or anabolic treatment for osteoporosis. ☐
2. Treatment with bisphosphonates for osteoporosis in past two years ☐
3. Current supplement use of vitamin D (>400 IU/day) or calcium (>500mg/day) (including use of over-the-counter preparations). ☐
4. Fragility fracture in the previous six months. ☐
5. Known primary hyperparathyroidism. ☐
6. Hypercalcaemia (albumin-adjusted plasma calcium > 2.60 mmol/l) ☐
7. Renal impairment (stage 4-5 chronic kidney disease: GFR <30 ml/min/1.73m^2) ☐
8. History of renal stones ☐

The exclusion criteria include the presence of a known health condition or treatment which is likely to independently affect the outcome measures (criteria: 1, 2, 3, 4, 8, 7, 8) or increase the risk of developing adverse effects with vitamin D supplementation (criteria 6, 9).

*NOTE: *If any of the above questions are answered "YES", the subject is not eligible to participate in the study*

Optimising Vitamin D Status in Older People:
A Randomised Controlled Trial of Vitamin D Supplementation

Screening visi

Subject ID | Subject Initials | Visit Date (Day / Month / Year)

(C) CONFIRMATION OF ELIGIBILITY

1. In the investigator's opinion, on the basis of the screening assessments, is the subject still eligible and fit to participate in this study?

☐ 0 = No *(withdraw subject from study)* 1 = Yes

2. CI or delegate

Print Name

Signature

Date: (Day / Month / Year)

(D) INFORMED CONSENT

1. Has informed consent been obtained ☐ Yes ☐ No

2. Date of consent (Day / Month / Year)

(E) ANTHROPOMETRY

1. Height ___ • ___ cm

2. Weight ___ • ___ kg

3. Demispan ___ • ___ cm

4. Temperature ___ • ___ °C

Estimating height from demispan

Measure the distance from the middle of the sternal notch to the tip of the middle finger (left arm if possible). Check that patient's arm is horizontal and in line with shoulders. Calculate stature (in cm) from the formula below:

Females
Height in cm = (1.35 x demispan (cm)) + 60.1
Males
Height in cm = (1.40 x demispan (cm)) + 57.8

5. Blood pressure ___ / ___ (Systolic / Diastolic)

6. Pulse ___ per minute

7. Waist/Hip ratio ___ / ___ = ___ (*waist* / *hip*)

Optimising Vitamin D Status in Older People:
A Randomised Controlled Trial of Vitamin D Supplementation

Screening

Subject ID | Subject Initials | Visit Date (Day / Month / Year)

(F) BODY COMPOSITION ANALYSER

1. BMI •
2. Basal Metabolic Rate (BMR) kJ
3. Impedance (Ω)
4. Fat • %
5. Fat Mass (FM) • kg
6. Fat Free Mass (FFM) • kg
7. Total Body Water (TBW) • kg

(G) DYNAMOMETER

1. Hand Grip

	Test 1		Test 2		Test 3		Mean	
	Left	Right	Left	Right	Left	Right	Left	Right

H) TIMED UP AND GO TEST

1. Please indicate the number of seconds the test took

(I) SKIN TYPE

1. Please indicate Fitzpatrick skin type in box below

Skin Type	Description	Skin colour	Please tick
I	Burns easily, never tans	White; very fair; red or blond hair; blue eyes; freckles	
II	Burns easily, tans minimally	White; fair; red or blond hair; blue, hazel, or green eyes	
III	Burns moderately, tans gradually	Cream white; fair with any eye or hair colour; very common	
IV	Burns minimally, tans well	Brown; typical Mediterranean caucasian skin	
V	Rarely burns, tans profusely	Dark Brown; mid-eastern skin types	
VI	Never burns, deeply pigmented	Black	

(J) DEMOGRAPHICS

1. Date of birth (Day / Month / Year)
2. Gender male female
3. Ethnicity *(see below)* If other, specify

ETHNICITY CODES:

Please write in the box the code number from the list below that applies to the patient.

1. White British 2. White other 3. Black African 4. Black other 5. Other

4. Smoking history 1. Current 2. Ex 3. Never
5. Average number smoked per day
6. Age when first started smoking

(ex = stopped >6 months ago, otherwise class as current)

7. Alcohol 1. Yes 2. No U/wk

Optimising Vitamin D Status in Older People:
A Randomised Controlled Trial of Vitamin D Supplementation

Screening visi

Subject ID | Subject Initials | Visit Date (Day / Month / Year)

(K) BLOOD COLLECTION AND PROCESSING

1. Has the patient had anything to eat or drink today? ☐ Yes ☐ No

2. Blood collection date/time: *(patient must be sitting)* (Day / Month / Year) (hrs : mins)

3. Biochemistry — *Please record whether blood was collected at this visit* 1=Yes 2=No

	Collected (see above)	Results		Reference Ranges	Comments
Sodium			mmol/L	133 - 146	
Potassium			mmol/L	3.5 - 5.3	
Urea			mmol/L		
Creatinine			umol/L	75-140 Males 62-120 Females	
MDMR eGFR			ml/min		
Total Protein			g/L		
Albumin			g/L		
Serum calcium			mmol/L		
Adjusted calcium			mmol/L		
Magnesium			mmol/L		
Phosphate			mmol/L		
Total bilirubin			umol/L		
ALP			U/L		
ALT			U/L		
4. Full Blood Count					
Hb			g/dL		
Haematocrit			1/1		
RBC			$x10^{12}$/L		

Optimising Vitamin D Status in Older People:
A Randomised Controlled Trial of Vitamin D Supplementation

Screening visi

Subject ID | Subject Initials | Visit Date (Day / Month / Year)

(K) BLOOD COLLECTION AND PROCESSING *continued*

4. Full Blood Count *cont* — *Please record whether blood was collected at this visit* 1=Yes 2=No

	Collected (see above)	Results		Reference Ranges	Comments
MCV			fl		
MCH			pg/cell		
Platelets			$x10^9/L$		
WBC			$x10^9/L$		
Neutrophils			$x10^9/L$		
Lymphocytes			$x10^9/L$		
Monocytes			$x10^9/L$		
Eosinophils			$x10^9/L$		
Basophils			$x10^9/L$		

5. Blood volumes collected for HNR analysis — *Please record whether blood was collected at this visit* 1=Yes 2=No

		Collected (see above)	Comments
EDTA	4ml		
LH	9ml		
Serum	2.5ml		

6. Plasma aliquots — *Please record each aliquot that was collected at this visit* 1=Yes 2=No

LH1: | LH2: | LH3: | LH4: | Serum:

EDTA1: | EDTA2: | EDTA3: | EDTA4:

Comments

7. Time aliquots stored in freezer ____ hrs : ____ mins

8. Freezer temp ____ °C

9. Name of person processing the blood: ____ Signature: ____ Date: ____

Subject ID | Subject Initials | Visit Date (Day / Month / Year)

(L) SPOT URINE COLLECTION AND PROCESSING

1. Time of last urine production: *within last 24 hrs* — hrs : mins
2. Was urine collected? Yes / No
3. Time of spot urine: hrs : mins
4. Total volume of urine collected: ml

Appendix B : Invitations letter and patient information sheet : BFU study

Northumbria Healthcare NHS
NHS Foundation Trust

BFU

Dear ……

Thank you very much for your participation in the BORICC Study several years ago. We are writing to inform you that we are conducting a follow-up study to investigate the effects of ageing (12+ years) and lifestyle factors on changes in biomarkers (a biological marker) of bowel cancer risk.

We have organised three showcase events at North Tyneside General Hospital for participants from the original BORICC Study to provide you with information on the findings from the study 12+ years ago and on what we plan to do in the follow-up study. It will also be an opportunity for you to see the equipment that we will be using, to try out some of our tests, such as hand grip strength, and to ask questions about the new project. Light refreshments will be provided.

We would like to invite you to participate in one of these showcase events which have been organised at different times of the week to try to accommodate everyone.
The following are the event dates:

- 11am on Thursday 31st August
- 4pm on Thursday 31st August
- 11am Saturday 2nd September

If you would like to attend, please confirm your attendance by returning the enclosed card or by contacting the research team (details below). In addition, please let us know if these dates are unsuitable for you and you are interested in attending a future event. If you are interested in taking part in the study but would prefer not to attend a showcase event, please indicate this on the card.

Dr. Fiona Malcomson
Human Nutrition Research Centre
Newcastle University
1st Floor Biomedical Research Building
Campus for Ageing and Vitality
Newcastle upon Tyne
NE4 5PL
Telephone: 0191 2081141 (please ask for Fiona or another member of the BFU Study team)
Mobile: 07791642754
Email: fiona.malcomson@newcastle.ac.uk

Thank you for your cooperation which is very much appreciated.

Yours sincerely

Mike Bradburn, Consultant Surgeon, Wansbeck Hospital

1. Invitation

You are being invited to take part in a research follow-up study conducted by Northumbria NHS Foundation Trust and Newcastle University. Before you decide whether you would like to take part, please take time to read the following information carefully. You may wish to discuss the study with relatives, friends and your GP. Please contact the research team if there is anything that is not clear to you, if you have any questions or would like more information.

Your decision will not affect any other aspect of the care that you may be receiving, or may receive in the future, at the hospital. If you do decide to participate, we will ask for your permission to inform your GP, so that they are aware of your participation in the study. Thank you very much for taking the time to read this.

2. Why is this study being performed?

Bowel problems are common and lifestyle factors, such as diet and physical activity, are known to be important in the development of certain diseases of the large bowel (colon). Ageing is also a strong contributor to changes in the large bowel and disease development. In the original BORICC Study, we found that diet affects certain proteins and genes in the cells of the large bowel. In this follow-up study, we would like to investigate the effects of ageing (over a period of about 10 years) on cells in the large bowel and to what extent these changes are affected by lifestyle factors. We hope that this study will give us a better understanding of the relationship between ageing, diet and physical activity and the health of the bowel.

3. Why have I been asked to participate in the study?

You have been invited to participate in this follow-up study because you took part in the original BORICC Study about 10 years ago. As we are taking follow-up measurements, we will recruit the original participants from the BORICC Study so that we can compare the data from 10 years ago with data collected in this later study.

4. Do I have to take part?

It is for you to decide whether you wish to take part. If you decide to participate in this study, you will be asked to sign a consent form. Even if you do decide to take part in this study, you can withdraw at any time. If you decide to withdraw from the study, you will not have to tell us why and it will not affect your treatment in any way.

5. What will I have to do as a volunteer?

As mentioned above, if you decide that you wish to volunteer, then you will be invited to sign a consent form.

We would like to collect the following information and samples during a clinic appointment at North Tyneside General Hospital, which will last around 1 hour:

- Medical history
- Height, weight, waist and hip measurements
- Body fat percentage measured using bioimpedance weighing scales with footplates
- Blood samples (seven 5ml tubes, equating to no more than 35ml)
- Sigmoidoscopy and collection of 10 rectal biopsies (please see below)
- One inner cheek swab

- Musculoskeletal function tests (timed up and go, hand grip strength and heel ultrasound tests)

We would also like you to do the following at home:

- Complete a food frequency questionnaire (this will take approximately 30 minutes)
- Complete a lifestyle questionnaire (this will take approximately 5 minutes)
- Complete a sunlight exposure questionnaire (this will take approximately 10 minutes)
- Collect two urine samples and one stool sample - we will provide you with instructions and pots for sample collection
- Wear a physical activity monitor (a small wrist-worn device) for one week

The above questionnaires, physical activity monitor and sample collection pots will be posted to you in advance. We will then ask you bring these along with you to your hospital appointment.

6. Does the study involve any invasive tests?

To obtain small samples of tissue (biopsies) from the bowel wall we will need to perform a short camera examination of your bowel called a sigmoidoscopy. We plan to take ten pinch biopsies from the bowel wall at a distance of 10cm (4 inches) from the back passage. This will be performed by a clinician trained in this endoscopic examination who will examine for any conditions which may cause pain, such as fissures or haemorrhoids, before proceeding.

The procedure is associated with some discomfort but this is usually mild. The procedure should not be painful and is normally carried out without the need for anaesthetic or sedation. If you were to find the procedure too uncomfortable then you would, of course, be able to ask the clinician to stop. In our previous study, we completed 81 similar procedures and did not encounter anybody who found the procedure too uncomfortable to ask the doctor to stop. You should not experience any pain during the taking of biopsies from the bowel wall.

7. How does the follow-up study differ from the original BORICC Study?

The sigmoidoscopy used to collect the bowel wall samples is a less invasive procedure than that used in the original BORICC study. In the original study, bowel wall biopsies were collected during colonoscopy or flexible sigmoidoscopy procedures which require bowel preparations (such as enemas, suppositories or strong laxatives). In contrast, a sigmoidoscopy does not require you to take any bowel preparation and the camera does not have to go through the bends in the bowel. The procedure should take only approximately 15 minutes.

We will also be taking some additional information from you in this follow-up study which we did not collect in the original BORICC Study:

- Physical activity data collected by a physical activity monitor worn on your wrist for one week
- Sunlight exposure data (to measure vitamin D) collected from a questionnaire
- Physical capability data collected from two muscle function tests (the ‘timed stand up and go test’ the ‘hand grip strength’ test and the heel ultrasound test). The ‘timed stand up and go’ test involves sitting on a chair, standing up, walking to a cone and returning to sit back down on the chair. The handgrip strength involves sitting and resting your arm on an armchair, then gripping and squeezing a dynamometer (a small device that measures force) as hard as you can. The heel ultrasound test involves

placing your foot in the heel ultrasound machine's footwell and sitting still for approximately 10 seconds whilst the device makes the measurements.

- Buccal (inner cheek) samples collected with a swab by rubbing the inner cheek of the mouth.

8. What are the risks of sigmoidoscopy examination and biopsies?

Some people may experience some bloating, cramping, abdominal discomfort and excess wind after the procedure. This is usually from the air that we puff in to the bowel in order to expand it during the examination, which we try to minimise. If these side effects do occur, they are unlikely to last for more than 24 hours and usually resolve without the need for any treatment.

The sigmoidoscopy examination is generally considered a safe procedure, but it does carry a very small risk of complications. It is important that you understand this before deciding whether or not to participate. There is a very small chance (1 in 65,000) of a perforation (a puncture) of the bowel. Although very rare, a perforation will usually require surgery. Significant bleeding is also a rare (1 in 10,000) potential complication. The risk of bleeding or perforation is higher with each biopsy that is being taken. There are no other known lasting adverse effects of this test. If you were to suffer any of the following symptoms within 48 hours after a sigmoidoscopy, you are advised to consult a doctor immediately:

- Severe abdominal pain
- Significant rectal bleeding
- Fever

9. What happens if anything goes wrong?

If, in the very unlikely event, taking part in this study causes any harm to you, there are no special compensation arrangements, but you will still be entitled to complain through the usual hospital procedures. If you are harmed due to someone's negligence or wrongdoing then you may have grounds for legal action, but you may have to pay for it. You may withdraw from the study at any time without explaining why and this will not affect any future care that you may receive.

10. Will my information be confidential?

Yes, all of the information we will collect from you during the study will be strictly confidential. This information will be kept securely while the study is taking place. Only the research team will have access to the study data. Furthermore, your information and data will be anonymised and you will be given a specific study ID number. Specific details, which could identify you, will only be available to the research team. Your GP will be informed that you are taking part in this study.

11. What will happen to the samples collected in this study?

The samples that are collected will be examined at laboratories in Newcastle University. All samples will be stored securely. We will perform tests to look for various markers of cell and metabolic activity. We will try to link these changes with your habitual diet, based on the information you gave us in the food frequency questionnaire, and physical activity levels, based on your lifestyle questionnaire and physical activity monitor data.

After the study has finished, the samples will be stored in our laboratory freezers in accordance with government regulations. Your name and details will not be recorded on the samples. All of the samples will be anonymised, meaning that the data resulting from your samples cannot be traced to yourself.

We will keep the samples so that we can do further testing if new techniques or markers are discovered, without having to collect any new samples. We may send samples to collaborating research institutes for additional analyses but this will not be for financial gain.

12. How often will I need to visit the hospital if I decide to volunteer for the study?

You will be asked to attend the hospital for approximately 1 hour on one occasion only. We will reimburse any travel expenses incurred.

13. What benefits will I get from the study?

You will not directly benefit from volunteering to participate in this study. However, we will give you feedback on your diet and lifestyle which you may find useful. The research may well help us understand how ageing and lifestyle factors, such as diet and physical activity, affect large bowel health and consequently the development of diseases of the large bowel, such as bowel cancer. This may lead to the development of prevention and/or treatment strategies. Once the study is completed, a summary of the findings can be made available to you upon request.

14. Do I need to take a day off work?

We understand that people lead busy lives. If appointments during usual working hours do not suit your lifestyle, then we may be able to work flexibly in order to accommodate you. You can discuss this in more detail with one of the members of the research team.

15. Who has reviewed the study?

The West Midlands – Coventry & Warwickshire Research Ethics Committee has reviewed the study (REC reference 16/WM/0424).

16. Who is performing the research?

- Professor John Mathers is Professor of Human Nutrition and Director of the Human Nutrition Research Centre (HNRC) at Newcastle University
- Mr. Mike Bradburn is a consultant surgeon at Wansbeck General Hospital
- Dr. Laura Greaves is a Research Fellow at Newcastle University
- Dr. Fiona Malcomson is a Research Associate at Newcastle University
- Mr. Abraham Joel is a Teaching and Research Fellow at Northumbria NHS Foundation Trust and will be conducting this research as part of his PhD project
- Mrs. Stella Breininger is a PhD student at Newcastle University and will be conducting this research as part of her PhD project
- Mrs. Thilanka Ranathunga is a PhD student at Newcastle University and will be conducting this research as part of her PhD project
- Mr. Khalil El Gendy is a MD student at Newcastle University and will be conducting this research as part of his PhD project

17. Who should I contact if I have questions or would like additional information?

Dr. Fiona Malcomson
BFU Study Research Team
Human Nutrition Research Centre
Newcastle University
1st Floor Biomedical Research Building
Campus for Ageing and Vitality
Newcastle upon Tyne
NE4 5PL
Telephone: 0191 2081141 (please ask for Fiona or another member of the BFU Study research team)
Mobile: 07791642754
Email: fiona.malcomson@newcastle.ac.uk

In addition, you may contact the Patient Advice Liaison Service (PALS) for confidential advice, support and information on health-related matters.

Patient advice and liaison services: Wansbeck General Hospital
Woodhorn Lane
Ashington
Northumberland
NE63 9JJ
Telephone: 0800 0320202
Email: northoftynepals@nhct.nhs.uk

18. What should I do next if I would like to volunteer?

If you think that you would like to volunteer in this study, we invite you to contact the research team in writing or by telephone, when we will provide more information about the study and answer any further questions. If you are willing to participate, we will arrange your study visit at the hospital and send you a consent form (to be signed and brought to the hospital study visit), the questionnaires to be completed, together with the sample collection pots and a physical activity monitor.

Please contact us if you have any questions or would like any additional information. Study coordinator, Dr. Fiona Malcomson, Research Associate, Human Nutrition Research Centre Newcastle University

Appendix C : Participants Study Interest form : BFU study

BFU Participant Study Interest Form

Participant ID: Date:

Recorded by:

Participant Name: _____

Address: _____

_____

_____

Telephone Number: _____

Mobile Number: _____

Email: _____

Dominant Hand (for accelerometer):

Right □ Left □

Checklist:

- Ensure participant understands study information □
- Answered any additional questions □
- Checked participant does not have dementia □
- Checked participant is not on the following medication: warfarin, rivaroxaban, apixaban, clopidogrel, dipyridamole □
- Described study pack □
- Checked participant is able to travel to attend hospital visit □
- Arranged participant study visit for:

  Time:_____ Date: _____

  N.B. Ensure there are at least 2 weeks in between phone call and study visit to allow time for posting of study pack and wearing of accelerometer.
  Or
  Asked for later suitable study visit dates (e.g. if participant is going on holiday):

  _____

Appendix D : Potential Participants call logs : BFU study

BFU Potential Participants Call Log

Please record the details of any calls made to the BFU (BORICC Follow Up) team (Fiona, AJ, Stella and Khalil) in the table below.

Please ask the caller for their name, telephone number and the best day and time to call them, and let them know that a member of the research team will return their call as soon as possible.

In the meantime, we are contactable by email at: Fiona.malcomson@ncl.ac.uk or by mobile telephone: 07791642754

Thank you very much.

Date	Name	Telephone number (home and/ or mobile)	Best time to contact

Appendix E : At-Home Sample and Questionnaire Collection Record Sheet

Participant ID :

At-Home Sample and Questionnaire Collection Record Sheet

Thank you very much for taking the time to complete the BFU Study questionnaires and sample collection. Please make sure you bring these to your study visit at the hospital. Please make sure you bring your accelerometer and sleep log with you to the study visit also.

To help you, please find below checklists that you can tick when you have completed the questionnaires and collected the samples.

Questionnaires:

Questionnaire	Completed (√)
Food Frequency (FFQ)	
Lifestyle	
Sunlight exposure	

Sample collection:

For the urine and stool samples, please also write down the date and the time that you collected each sample.

Sample	Collected (√)	Date	Time
Weekday urine sample			
Weekend day urine sample			
Stool sample			

Physical activity:

Item	Completed (√)
Accelerometer (worn 7 days)	
Accelerometer record sheet	
Sleep Log	

Appendix F : Patient Health History

Participant ID:
Date:

Patient Health History

DOB: **Age:**

Ethnicity: **Bristol Stool Chart Type:**

Past Medical History (√)

Cancer	☐	IBD	☐
Asthma	☐	Mental health disease	☐
COPD	☐	Type 1 diabetes	☐
CVD	☐	Type 2 diabetes	☐
Hypertension	☐	*Liver disease:*	
Osteoporosis	☐	Alcohol-related	☐
Osteoarthritis	☐	NAFLD	☐
Adenomatous Polyps	☐	Hepatitis	☐
Stroke	☐	Primary biliary cirrhosis	☐
Chronic kidney disease	☐		

Details of conditions (e.g. type, date of diagnosis, stage):

_____

_____

_____

Other conditions (and details):

_____

_____

CRC family history

Number of 1st degree relatives with CRC or adenomatous polyps: _____

For female participants

Number of children: _____

Menopausal status:

Pre-menopausal ☐ Peri-menopausal ☐ Post-menopausal ☐

Drug History

Please include details such as drug name, dose, date taken, treatment duration (acute or chronic)

Check not on warfarin, apixaban, clopidogrel, dipyrimadole, rivaroxaban

Current medication

Previous medication (over last 10 years)

Appendix G : VDOP Sunshine Exposure

Study number ___ ___ ___ **Date of Birth** _____/_____/_____ **Initials** ___ ___ ___

VDOP Sunshine Exposure Questionnaire

(Member of research team to ask participant following questions and record answers)

- Whilst living at your home address, over the last three months, have you been outdoors in sunny weather often, occasionally, rarely or never?
- When you went outside in sunny weather which parts of the body's skin do you usually expose: the head, hands, arms, legs, stomach or back?
- Do you use sun block? If so, which parts of the body do you use it on?

Please tick the relevant boxes below for the last three months

	Outdoors			Body/skin exposure					Sunblock			
	Often	Occasionally	Seldom/ never	Head	Hands	Arms	Legs	Stomach/ back	Face	Arms	Legs	Stomach/ back
3 months ago												
2 months ago												
Last month												

How often do you use a sunbed?

_____Times a year

Please list any holidays/visits abroad or to the South of England which you have taken in the last 3 months

Please tick the relevant boxes below for every holiday/visit separately [code 1=local; 2=UK<150miles south; 3=UK>150miles South]

Please tick the relevant boxes below for every holiday/visit separately [code 1=local; 2=UK<150miles south; 3=UK>150miles South; 4=Abroad]

- Whilst away from home, over the last three months, have you been outdoors in sunny weather often, occasionally, rarely or never?
- When you went outside in sunny weather which parts of the body's skin do you usually expose: the head, hands, arms, legs, stomach or back?
- Do you use sun block? If so, which parts of the body do you use it on?

Holiday/ visit 1

Where did you visit …… [ ] (add distance code)

In which month did you visit ……

Length of stay ……

	Outdoors			Body/skin exposure					Sunblock			
	Often	Occasionally	Seldom/ never	head	hands	arms	legs	Stomach/ back	face	arms	legs	Stomach/ back
3 months ago												
2 months ago												
Last month												

Holiday/ visit 2

Where did you visit …… [ ] (add distance code)

In which month did you visit ……

Length of stay ……

	Outdoors			Body/skin exposure					Sunblock			
	Often	Occasionally	Seldom/ never	head	hands	arms	legs	Stomach/ back	face	arms	legs	Stomach/ back
3 months ago												
2 months ago												
Last month												

(if you need more space, please use an additional form)

Appendix H : BFU Sun exposure questionnaire

Sun Exposure Questionnaire

Name: ……

Participant ID number:

This is a questionnaire about your habitual sun exposure. When you answer the questions, please think about sun exposure in your day-to-day life and on any holidays.

Gender : Male □ Female □

2. Age : _____ years
3. Occupation : Indoors□ Outdoors □ Both □

4. Please tick in the last column that describe your skin type.

Skin type	**Description**	**Skin colour**	**Please tick**
i	Burns easily, never tans	White, very fair	
ii	Burns easily, tans minimally	White, fair	
iii	Burns moderately, tans gradually	Cream white, fair	
iv	Burns minimally, tans well	Brown, typical Mediterranean Caucasian skin	
v	Rarely burns, tans profusely	Dark Brown, mid-eastern skin types	
vi	Never burns, deeply pigmented	Black	

5 (a) How often are you outdoors for at least half an hour between 10am – 3pm during the sunny months (April-September)?

Less than once a week □

1-2 times a week □

More than 2 times a week □

Every day □

5 (b) How much time would you spend outdoors on a week days (Monday to Friday) during the sunny months (April-September)?

Less than 15 minutes □
Between 15 and 30 minutes □
Between 30 minutes and 2 hours □

More than 2 hours □

5 (c) How much time would you spend outdoors on a weekend (Saturday - Sunday) during the sunny months (April-September)?

Less than 15 minutes □
Between 15 and 30 minutes □
Between 30 minutes and 2 hours □
More than 2 hours □

6. When you are outdoors during the sunny months (April – September), do you stay in the sun or do you seek the shed?

I try to avoid staying in direct sunshine □

I sometimes stay in the sunshine □
I enjoy staying in the sunshine (e.g. to suntan) □

7. How would you deliberately wear less clothing so as to get direct sunlight on your skin (e.g sleeveless tops shorts)?

Never	Rarely	Sometimes	Usually	Always
□	□	□	□	□

8. Please rank (by putting a number from 1- 4 in the box) in order what you most wear when you are outdoors during the sunny months (April to September).

	Please rank with 1 being most likely and 4 being least likely
Long sleeves and **long** trousers or tights	☐
Short sleeves and **long** trousers or skirt	☐
Short Sleeves and **Short** skirt or shorts	☐
Swimwear or light beach clothes	☐

9. When you are outdoor during the sunny months (April – September), how often would you wear maximum protection sun screen (**SPF 15 to 30**)?

Never	Rarely	Sometimes	Usually	Always	Other
□	□	□	□	□	□

10. Do you use a sun lamp or sun-bed regularly (at least once per week)?
Yes □ No □

11 (a) Have you been in the mountain or on a skiing holiday within last 3 months?
Yes □ No □

11 (b) If yes, for how long? Please include total number of days of all holidays.

…….. days

12 (a) Have you been on holidays to a sunny places (including sunny weather in England) since last 3 months?
Yes □ No □

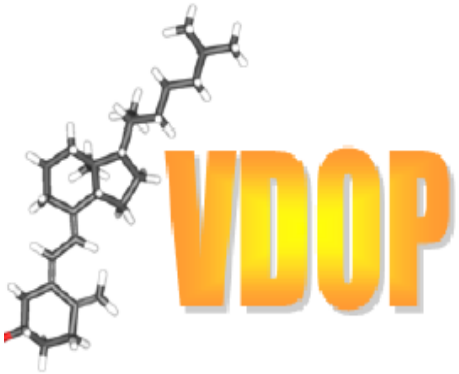
12 (b) If yes, for how long? Please include the total number of days of all holidays.…….. days

13. Please complete the following table with information on any holiday(s) (including in the UK and broad) you have had in the last 3 months.

Holiday	**Location**	**Month(s)**	**Length (days)**	**How often were you outdoors?**			**Please tick the parts of the body that were usually exposed to the sun**					**Please tick which parts of the body you applied sun block to**			
				Often	Occasionally	Seldom/ Never	Head/ face	Hands	Arms	Legs	Stomach and/or back	Face	Arms	Legs	Stomach and/or back
1															
2															
3															
4															
5															

- Please mention any other relevant comments.

Appendix I : Food Frequency Questionnaire VDOP study



Dietary Calcium and Vitamin D Questionnaire

Date of completion ...../...../.....

Please circle which type of food you eat: **Omnivore** (eats all types of food) **/ Vegetarian / Vegan**

Think about your eating habits over the last year. Please go through the questionnaire and circle the appropriate answers please note you may circle more than one answer. We would like to know the following:

- Whether or not you eat the food listed
- How often you have it if you do eat it
- How much you have on the days when you eat the food

Type of milk usually used in all circumstances (Please circle)	1. Whole milk, semi-skimmed, skimmed or channel island 2. Long-life milk (UHT), sterilised 3. Powdered milk: please specify which brand 4. Soya milk: please specify which brand 5. Sheep milk 6. Goats milk 7. Calcium fortified milk please specify: 8. Vitamin D fortified milk please specify: 9. I don't use milk	

Types of fat spread usually used in all circumstances (please circle)	1. Butter 2. Hard margarine (specify brand) 3. Soft margarine (specify brand) 4. Low fat spread (specify brand) 5. I don't use any fat spreads	Thick, medium or thinly spread Thick, medium or thinly spread Thick, medium or thinly spread Thick, medium or thinly spread

Type of Food/Beverage	Not eaten	Once every 3-4 weeks	Once every 1-2 weeks	1-2 days per week	3-5 days per week	6-7 days per week	Amount per day	
Tea	1	2	3	4	5	6	……cups ……mugs	With milk? No Yes Type of milk used?
Coffee	1	2	3	4	5	6	……cups ……mugs	With milk? No Yes Type of milk used?
Hot milky drinks: Horlicks Ovaltine Hot chocolate Very milky coffee Complan	1	2	3	4	5	6	……cups ……mugs	With milk? No Yes Type of milk used? Type of hot milky drink?

Type of Food/Beverage	Not eaten	Once every 3-4 weeks	Once every 1-2 weeks	1-2 days per week	3-5 days per week	6-7 days per week	Amount per day		
Milk based drink i.e. milk alone, Smoothies, Milkshakes	1	2	3	4	5	6	.....small glasses .....large glasses .....cups .....mugs	Amount of milk used?	
Breakfast cereal	1	2	3	4	5	6	Number of portions..... Brands most usually eaten	Small Medium Large	Milk? Yes No
Bread, toast	1	2	3	4	5	6	Number of slices per day.....	Type of bread: White Brown	Granary Wholewheat/Whole meal Multiseeded Other please Specify

Eggs	1	2	3	4	5	6	Number of eggs	Ways cooked/eaten: Boiled (hot or cold) Poached Fried Scrambled Omelette
Nut and seed spreads	1	2	3	4	5	6	Number of slices spread	Peanut/Hazelnut/Sunflower Sesame spread (tahini) Other nut or seed spread please specify:
Beans and pulses (baked beans, chick peas, kidney beans, lentils)	1	2	3	4	5	6	Number of portions.....	Small Medium Large
Vegetables other than potatoes	1	2	3	4	5	6	Number of portions... ..... Portion Size: Small Medium Large	Dark green leafy vegetables Other green vegetables Root vegetables Salad vegetables
Cakes , Scones	1	2	3	4	5	6	Number of portions.....	Small Medium Large

Trout Tuna								
Liver – cooked e.g. with onions, as pate/spreads	1	2	3	4	5	6	Number of portions	Type usually eaten: Chicken Duck Pork Beef
Whole nuts or seeds added to cereal or eaten as a snack: e.g. Almonds Brazil Hazel Sesame seeds Walnuts	1	2	3	4	5	6	Number of portions	Type of nuts regularly eaten
Dried fruits: e.g. Apricots Figs Currants	1	2	3	4	5	6	Amount eaten per day	Type of dried fruit regularly eaten?
Lasagne	1	2	3	4	5	6	Number of portions	Small Medium Large
Quiche /flan	1	2	3	4	5	6	Number of portions	Small Medium large

Macaroni cheese	1	2	3	4	5	6	Number of portions	Small Medium large
Creamed soup	1	2	3	4	5	6	Number of portions	Small Medium large
Bottled water	1	2	3	4	5	6	Amount drunk per day .....bottles	Brand?
Tap water from Newcastle area please state of you have consumed water from any other area	1	2	3	4	5	6	......small glasses .....large glasses	

Appendix J : Food Frequency questionnaire – BFU study

Food Frequency Questionnaire

CODE:_____

Date:_____

Thank you for agreeing to take part in the study. This is a simple questionnaire designed to help us understand what kinds of food you normally eat. It is not a test, so there are no right or wrong answers.

It is your usual diet we are interested in.

All information will be treated in the strictest confidence.

Thank you for your time

Please turn over and read the instructions for answering questions before completing the questionnaire.

FFQ Questionnaire

About the food you eat

The following questions are about the food you usually eat and how often you eat certain foods. Please read the following instructions before answering the questions.

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year.**

EXAMPLE:

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
BREAD & SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✓	

EXAMPLE:

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
POTATOES, RICE & PASTA (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

It is your turn to answer now!

Please put a tick in each box to indicate how often, **on average**, you have eaten each food **during the past year.**

Please estimate your average food use as best you can, and please answer every question - do not leave **ANY** lines blank. **Please put a tick on every line.**

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
19. MEAT & FISH *(medium serving)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew casserole, curry or bolognese	1	2	3	4	5	6	7	8	9
Beefburgers	1	2	3	4	5	6	7	8	9
Pork: roast, chops, stew, slice or curry	1	2	3	4	5	6	7	8	9
Lamb: roast, chops, stew or curry	1	2	3	4	5	6	7	8	9
Chicken, turkey or other poultry: including fried, casseroles or curry	1	2	3	4	5	6	7	8	9
Bacon	1	2	3	4	5	6	7	8	9
Ham	1	2	3	4	5	6	7	8	9
Corned beef, Spam, luncheon meats	1	2	3	4	5	6	7	8	9
Sausages	1	2	3	4	5	6	7	8	9
Savoury pies, e.g. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls, scotch egg	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
PLEASE PUT A TICK ON EVERY LINE.									

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
19. MEAT & FISH, (continued) *(medium serving)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Liver, liver pate, liver sausage	1	2	3	4	5	6	7	8	9
Fried fish in batter, as in fish and chips	1	2	3	4	5	6	7	8	9
Fish fingers, fish cakes	1	2	3	4	5	6	7	8	9
Other white fish, fresh or frozen, e.g. cod, haddock, plaice, sole, halibut	1	2	3	4	5	6	7	8	9
Oily fish, fresh or canned, e.g. mackerel, kippers, tuna, salmon, sardines, herring	1	2	3	4	5	6	7	8	9
Shellfish, e.g. crab, prawns, mussels	1	2	3	4	5	6	7	8	9
20. BREAD & SAVOURY BISCUITS *(one slice or biscuit)*									
White bread and rolls	1	2	3	4	5	6	7	8	9
Scones, teacakes, crumpets, muffins or croissants	1	2	3	4	5	6	7	8	9
Brown bread and rolls	1	2	3	4	5	6	7	8	9
Wholemeal bread and rolls	1	2	3	4	5	6	7	8	9
Cream crackers, cheese biscuits	1	2	3	4	5	6	7	8	9
Pitta bread, naan bread, chapati	1	2	3	4	5	6	7	8	9
Garlic bread	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
PLEASE PUT A TICK ON EVERY LINE.									

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
21. CEREALS *(one bowl)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Porridge, Readybrek	1	2	3	4	5	6	7	8	9
Sugar coated cereals e.g. Sugar Puffs, Cocoa Pops, Frosties	1	2	3	4	5	6	7	8	9
Non-sugar coated cereals e.g. Cornflakes, Rice Crispies	1	2	3	4	5	6	7	8	9
All Bran, Bran Flakes, Muesli	1	2	3	4	5	6	7	8	9
Wholegrain cereals e.g. Cheerios, Weetabix, Shredded Wheat	1	2	3	4	5	6	7	8	9
22. POTATOES, RICE & PASTA *(medium serving)*									
Boiled, mashed, instant or jacket potatoes	1	2	3	4	5	6	7	8	9
Chips, potato waffles	1	2	3	4	5	6	7	8	9
Roast potatoes	1	2	3	4	5	6	7	8	9
Yorkshire pudding, pancakes, dumpling	1	2	3	4	5	6	7	8	9
Potato salad	1	2	3	4	5	6	7	8	9
White rice	1	2	3	4	5	6	7	8	9
Brown rice	1	2	3	4	5	6	7	8	9
White or green pasta, e.g. spaghetti, macaroni, noodles	1	2	3	4	5	6	7	8	9
Tinned pasta, e.g. spaghetti, ravioli, macaroni	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
22. POTATOES, RICE & PASTA (continued) *(medium serving)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Super noodles, pot noodles, pot savouries	1	2	3	4	5	6	7	8	9
Wholemeal pasta	1	2	3	4	5	6	7	8	9
Lasagne, moussaka, cannelloni	1	2	3	4	5	6	7	8	9
Pizza	1	2	3	4	5	6	7	8	9
23. DAIRY PRODUCTS & FATS									
Single or sour cream *(tablespoon)*	1	2	3	4	5	6	7	8	9
Double or clotted cream *(tablespoon)*	1	2	3	4	5	6	7	8	9
Low fat yoghurt, fromage frais *(125g carton)*	1	2	3	4	5	6	7	8	9
Full fat or Greek yoghurt *(125g carton)*	1	2	3	4	5	6	7	8	9
Dairy desserts *(125g carton)*, e.g. mousse	1	2	3	4	5	6	7	8	9
Cheese, e.g. Cheddar, Brie, Edam *(medium serving)*	1	2	3	4	5	6	7	8	9
Cottage cheese, low fat soft cheese *(medium serving)*	1	2	3	4	5	6	7	8	9
Eggs as boiled, fried, scrambled, omelette etc. *(one)*	1	2	3	4	5	6	7	8	9
Quiche *(medium serving)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
23.(b) The following on bread or vegetables *(teaspoon)*	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Butter	1	2	3	4	5	6	7	8	9
Block margarine, e.g. Stork, Krona	1	2	3	4	5	6	7	8	9
Polyunsaturated margarine, e.g. Flora sunflower	1	2	3	4	5	6	7	8	9
Other soft margarine, dairy spreads, e.g. Blue Band, Clover	1	2	3	4	5	6	7	8	9
Low fat spread, e.g. Gold	1	2	3	4	5	6	7	8	9
24. SWEETS & SNACKS									
Sweet biscuits, chocolate, e.g. digestive *(one)*	1	2	3	4	5	6	7	8	9
Sweet biscuits, plain, e.g. Nice, ginger *(one)*	1	2	3	4	5	6	7	8	9
Cakes e.g. fruit, sponge, sponge pudding *(medium serving)*	1	2	3	4	5	6	7	8	9
Sweet buns & pastries e.g. flapjacks, doughnuts, Danish pastries, cream cakes *(medium serving)*	1	2	3	4	5	6	7	8	9
Fruit pies, tarts, crumbles *(medium serving)*	1	2	3	4	5	6	7	8	9
Milk puddings, e.g. rice, custard, trifle *(medium serving)*	1	2	3	4	5	6	7	8	9
Chocolates *(small bar or $\frac{1}{4}$ pound of chocolates)*	1	2	3	4	5	6	7	8	9
Ice cream, choc ices *(one)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
24. SWEETS & SNACKS (continued)	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Sweets, toffees, mints *(one packet)*	1	2	3	4	5	6	7	8	9
Sugar added to tea, coffee, cereal *(teaspoon)*	1	2	3	4	5	6	7	8	9
Crisps or other packet snacks e.g. Wotsits *(one packet)*	1	2	3	4	5	6	7	8	9
Peanuts or other nuts *(one packet)*	1	2	3	4	5	6	7	8	9
25. SOUPS, SAUCES AND SPREADS									
Vegetable soups *(bowl)*	1	2	3	4	5	6	7	8	9
Meat soups *(bowl)*	1	2	3	4	5	6	7	8	9
Sauces, e.g. white sauce, cheese sauce, gravy *(medium serving)*	1	2	3	4	5	6	7	8	9
Tomato based sauces, e.g. pasta sauces *(medium serving)*	1	2	3	4	5	6	7	8	9
Tomato ketchup, brown sauce *(tablespoon)*	1	2	3	4	5	6	7	8	9
Relishes, e. g. pickles, chutney, mustard *(tablespoon)*	1	2	3	4	5	6	7	8	9
Low calorie, low fat salad cream or mayonnaise *(tablespoon)*	1	2	3	4	5	6	7	8	9
Salad cream, mayonnaise *(tablespoon)*	1	2	3	4	5	6	7	8	9
French dressing *(tablespoon)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
25. SOUPS, SAUCES AND SPREADS (continued)	Never or less than once/ month	1-3 per month	Once A Week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Other salad dressing *(tablespoon)*	1	2	3	4	5	6	7	8	9
Marmite, Bovril *(teaspoon)*	1	2	3	4	5	6	7	8	9
Jam, marmalade, honey, syrup *(teaspoon)*	1	2	3	4	5	6	7	8	9
Peanut butter *(teaspoon)*	1	2	3	4	5	6	7	8	9
Chocolate spread, chocolate nut spread *(teaspoon)*	1	2	3	4	5	6	7	8	9
Dips, e.g. houmous, cheese and chive *(tablespoon)*	1	2	3	4	5	6	7	8	9
26. DRINKS									
Tea *(cup)*	1	2	3	4	5	6	7	8	9
Coffee, instant or ground *(cup)*	1	2	3	4	5	6	7	8	9
Coffee whitener, e.g. Coffee-mate *(teaspoon)*	1	2	3	4	5	6	7	8	9
Cocoa, hot chocolate *(cup)*	1	2	3	4	5	6	7	8	9
Horlicks, Ovaltine *(cup)*	1	2	3	4	5	6	7	8	9
Wine *(glass)*	1	2	3	4	5	6	7	8	9
Beer, lager or cider *(half pint)*	1	2	3	4	5	6	7	8	9
Port, sherry, vermouth, liqueurs *(glass)*	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
26. DRINKS (continued)	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Spirits, e.g. gin, brandy, whisky, vodka *(single)*	1	2	3	4	5	6	7	8	9
Low calorie or diet fizzy soft drinks *(glass)*	1	2	3	4	5	6	7	8	9
Fizzy soft drinks, e.g. Coca cola, lemonade *(glass)*	1	2	3	4	5	6	7	8	9
Pure fruit juice (100%), e.g. orange, apple juice *(glass)*	1	2	3	4	5	6	7	8	9
Fruit squash or cordial *(glass)*	1	2	3	4	5	6	7	8	9
27. FRUIT *(1 fruit or medium serving)* *For very seasonal fruits such as strawberries, please estimate your average use when the fruit is in season									
Apples	1	2	3	4	5	6	7	8	9
Pears	1	2	3	4	5	6	7	8	9
Oranges, satsumas, mandarins, tangerines, clementines	1	2	3	4	5	6	7	8	9
Grapefruit	1	2	3	4	5	6	7	8	9
Bananas	1	2	3	4	5	6	7	8	9
Grapes	1	2	3	4	5	6	7	8	9
Melon	1	2	3	4	5	6	7	8	9
*Peaches, plums, apricots, nectarines	1	2	3	4	5	6	7	8	9
*Strawberries, raspberries, kiwi fruit	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
27. FRUIT (continued) ***(1 fruit or medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tinned fruit	1	2	3	4	5	6	7	8	9
Dried fruit, e.g. raisins, prunes, figs	1	2	3	4	5	6	7	8	9
28. VEGETABLES Fresh, frozen or tinned ***(medium serving)***									
Carrots	1	2	3	4	5	6	7	8	9
Spinach	1	2	3	4	5	6	7	8	9
Broccoli	1	2	3	4	5	6	7	8	9
Brussels sprouts	1	2	3	4	5	6	7	8	9
Cabbage	1	2	3	4	5	6	7	8	9
Peas	1	2	3	4	5	6	7	8	9
Green beans, broad beans, runner beans	1	2	3	4	5	6	7	8	9
Marrow, courgettes	1	2	3	4	5	6	7	8	9
Cauliflower	1	2	3	4	5	6	7	8	9
Parsnips, turnips, swedes	1	2	3	4	5	6	7	8	9
Leeks	1	2	3	4	5	6	7	8	9
Onions	1	2	3	4	5	6	7	8	9
Garlic	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

PLEASE PUT A TICK ON EVERY LINE.

FOODS & AMOUNTS	AVERAGE USE LAST YEAR								
28. VEGETABLES Fresh, frozen or tinned (continued) ***(medium serving)***	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Mushrooms	1	2	3	4	5	6	7	8	9
Sweet peppers	1	2	3	4	5	6	7	8	9
Beansprouts	1	2	3	4	5	6	7	8	9
Green salad, lettuce, cucumber, celery	1	2	3	4	5	6	7	8	9
Mixed vegetables (frozen or tinned)	1	2	3	4	5	6	7	8	9
Watercress	1	2	3	4	5	6	7	8	9
Tomatoes	1	2	3	4	5	6	7	8	9
Sweetcorn	1	2	3	4	5	6	7	8	9
Beetroot, radishes	1	2	3	4	5	6	7	8	9
Coleslaw	1	2	3	4	5	6	7	8	9
Avocado	1	2	3	4	5	6	7	8	9
Baked Beans	1	2	3	4	5	6	7	8	9
Dried lentils, beans, peas	1	2	3	4	5	6	7	8	9
Tofu, soya meat (TVP), Vegeburger	1	2	3	4	5	6	7	8	9
	Never or less than once/ month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick on EVERY line

29. (a) What type of milk did you most often use?

Select one only

Full cream……❒1
Channel Islands……❒2
Dried milk……❒3
Semi-skimmed……❒4
Skimmed……❒5
Soya……❒6
Other……❒7
None……❒8

4/52

29. (b) Approximately, how much milk did you drink each day, including milk with tea, coffee, cereals etc?

None……❒1
Quarter of a pint (roughly 125mls)……❒2
Half a pint (roughly 250mls) ……❒3
Three quarters of a pint (roughly 375mls) ……❒4
One pint (roughly 500mls) ……❒5
More than one pint (more than 500mls) ……❒6

4/53

30. What kind of fat did you most often use for frying, roasting, grilling etc?

Select one only

Butter……❒1
Lard/dripping……❒2
Solid vegetable fat……❒3
Margarine……❒4
Vegetable oil……❒5
Olive oil……❒6
None……❒7

4/54

31. How often did you eat food that was fried at home?

Select one only

Daily……❒1
1-3 times a week……❒2
4-6 times a week……❒3
Less than once a week……❒4
Never……❒5

4/55

32. How often did you eat fried food *away* from home?

Select one only

Daily……❒1
1-3 times a week……❒2
4-6 times a week……❒3
Less than once a week……❒4
Never……❒5

4/56

33. (a) How often did you add salt to food while cooking?

Select one only

Always……❒1
Usually……❒2
Sometimes……❒3
Rarely……❒4
Never……❒5

4/57

33. (b) How often did you add salt to any food at the table?

Select one only

Always……❒1
Usually……❒2
Sometimes……❒3
Rarely……❒4
Never……❒5

4/58

34. Do you follow a special diet?
Please tick all that apply.

No……❒1
Yes, due to a medical condition/allergy……❒2
Yes, to lose weight……❒3
Yes, because of personal beliefs ……❒4
Yes other……❒5

4/63

35. Over the last year, how often have you eaten organic foods?

Select one only.

Most days……❒1
Once or twice a week……❒2
Once a month……❒3
Never/hardly ever……❒4

4/64

36. Have you taken any of the following during the past year?

a) Vitamins (e.g. multivitamins, vitamin B, vitamin C, folic acid)

Yes……❒1
No……❒2

If YES, what type and when _____

4/65

b) Minerals (e.g. iron, calcium, zinc, magnesium)

Yes……❒1
No……❒2

If YES, what type and when _____

4/66

c) Fish oils (e.g. cod liver oil, omega-3)

Yes……❒1
No……❒2

If YES, what type and when _____

4/67

d) Other food supplements (e.g. oil of evening primose, starflower oil, royal jelly, ginseng)

Yes……❒1
No……❒2

If so, what type and when _____

4/68

37. During the course of last year, on average, how many times did you eat the following foods?

Food Type	Times/week	Portion size
Vegetables (not including potatoes)		medium serving 5/41
Salads		medium serving 5/43
Fruit and fruit products (not including fruit juice)		medium serving or 1 fruit 5/45
Fish and fish products		medium serving 5/47
Meat, meat products and meat dishes (including bacon, ham and chicken)		medium serving 5/49

38. How often do you eat fruit or vegetables from a garden or allotment?

Select one only.

Most days……❒1
Once or twice a week……❒2
Once a month……❒3
Never/hardly ever……❒4

5/50

39. How would you most commonly eat the following vegetables?
If you have not eaten the vegetable listed within the last year, please tick the 'Not eaten ' box.

Select one box only for each vegetable;

	Raw	Boiled < 10mins	Boiled >10mins	Steamed	Fried	Not eaten
Asparagus	❒	❒	❒	❒	❒	❒
Artichoke	❒	❒	❒	❒	❒	❒
Beansprouts	❒	❒	❒	❒	❒	❒
Beetroot	❒	❒	❒	❒	❒	❒
Broad beans	❒	❒	❒	❒	❒	❒
Broccoli	❒	❒	❒	❒	❒	❒
Brussel Sprouts	❒	❒	❒	❒	❒	❒
Cauliflower	❒	❒	❒	❒	❒	❒

	Raw	Boiled < 10mins	Boiled >10mins	Steamed	Fried	Not eaten
Cabbage	❒	❒	❒	❒	❒	❒
Chick Peas	❒	❒	❒	❒	❒	❒
Courgette	❒	❒	❒	❒	❒	❒
Curly Kale	❒	❒	❒	❒	❒	❒
Green Beans	❒	❒	❒	❒	❒	❒
Leeks	❒	❒	❒	❒	❒	❒
Lentils	❒	❒	❒	❒	❒	❒
Lettuce	❒	❒	❒	❒	❒	❒
Mixed veg frozen	❒	❒	❒	❒	❒	❒
Mixed veg canned	❒	❒	❒	❒	❒	❒
Parsnip	❒	❒	❒	❒	❒	❒
Peas	❒	❒	❒	❒	❒	❒
Red Kidney Beans	❒	❒	❒	❒	❒	❒
Runner Beans	❒	❒	❒	❒	❒	❒
Spinach fresh	❒	❒	❒	❒	❒	❒
Spinach frozen	❒	❒	❒	❒	❒	❒
Sweetcorn fresh	❒	❒	❒	❒	❒	❒
Sweetcorn canned	❒	❒	❒	❒	❒	❒

40. For the following foods, please list the top three makes and/or brands you most commonly consume.
If you do not eat this type of food please tick the 'not eaten' box.

Bread: 1:_____

2:_____

3:_____ Not eaten ❒

Breakfast Cereal

1:_____

2:_____

3:_____ Not eaten ❒

About you and your health

41. For your age, would you say that your health was:

Please tick one box on the scale of 1 to 5:

	1	2	3	4	5	
very good	❒	❒	❒	❒	❒	very poor

5/51

42. Which of the following best describes your daily work or other daytime activity that you usually do?

Please tick one box only.

I am usually sitting and do not walk about much……❒1

I stand or walk about quite a lot, but do not have to carry or lift things very often ……❒2

I usually lift or carry light loads or have to climb stairs or hills often ……❒3

I do heavy work or carry heavy loads often ……❒4

5/52

43. Please give the average number of hours per week you spend doing sports and other activities.

Please write in the amount for each; if none write "0"

a) Mildly energetic

(e.g. walking, gardening, playing darts, general housework) _____ hour/s

b) Moderately energetic

(e.g. heavy housework or gardening, dancing, golf, cycling, leisurely swimming) _____ hour/s

c) Vigorous

(e.g. running, competitive swimming or cycling, tennis, football, squash, aerobics) _____ hour/s

5/64

44. Do you smoke?

Yes, I smoke daily ……❒1
Yes, I smoke occasionally ……❒2
No, I used to smoke ……❒3
No, I have never smoked ……❒4

5/65

44. If yes or you used to smoke, how much, on average, do you (or did you) smoke a day?
Please write in the amount for each; if none write "0"

Cigarettes ____
Cigars ____
Ounces tobacco ____

5/74

45. In the past 12 months have you taken an alcoholic drink:
Please tick one box.

Twice a day or more ……❒1
Almost daily ……❒2
Once or twice a week ……❒3
Once or twice a month ……❒4
Special occasions only ……❒5
Not at all ……❒6

5/75

46. In a typical 7-day week, including the weekend, how many standard drinks of alcohol do you drink? *(see the table below)*
Please write the number in the box below.

I usually drink ____ standard drinks of alcohol per week

5/76

ONE STANDARD DRINK = ½ pint of beer or ½ pint cider or ½ pint lager or 1 glass of wine, martini, or cinzano or 1 small glass of Sherry or Port or 1 measure of Spirits (gin, whiskey, vodka etc.) or 1 measure liquor **A PINT OF BEER, CIDER, OR LAGER COUNTS AS TWO STANDARD DRINKS** A DOUBLE MEASURE OF SPIRITS COUNTS AS TWO STANDARD DRINKS

47. (a) Do you have any long-term illness, physical or mental health problem or handicap?

Yes……❒1
No……❒2

5/91

48. (b) If yes, does this limit your daily activity in any way?

Yes……❒1
No……❒2

5/92

THANK YOU FOR TAKING TIME TO COMPLETE THIS QUESTIONNAIRE

Appendix K : Physical Capability Tests Record Sheet

Participant ID: **Date:**

HAND GRIP STRENGTH

Dominant hand:

Test	Left hand force (kg)	Right hand force (kg)
1		
2		
3		

Any comments (e.g. pain, problems):

TIMED UP AND GO

Test	Time (seconds)
1	
2	
3	

Any comments (e.g. pain, problems):

Appendix L : Anthropometrics and Body Fat Percentage Record Sheet

Participant ID : **Date and time:**

Anthropometrics and Body Fat Percentage Record Sheet

Measurement	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body fat (%)	Waist (cm)	Hip (cm)
1						
2						
Additional repeat (if required)						

Any comments (e.g. problems, clothing worn):

Appendix M : Bone Densitometry Record Sheet

Participant ID:

Date:

Bone Densitometry Record Sheet

Test	Right heel 1	Right heel 2	Left heel 1	Left heel 2
Stiffness index				
BUA				
SOS				
% Young adult				
T score				
% Age Matched				
Z score				

Any comments (e.g. fractures, pain, problems):

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No effect of monthly supplementation with 12000 IU, 24000 IU or 48000 IU vitamin D3 for one year on muscle function: The vitamin D in older people study

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ABSTRACT

Vitamin D plays a role in muscle function through genomic and non-genomic processes. The objective of this RCT was to determine the effect of monthly supplemental vitamin D_3 onmuscle function in 70+ years old adults. Participants (n = 379) were randomized to receive, 12,000 IU, 24,000 IU or 48,000 IU of vitamin D_3 monthly for 12 months. Standardized Hand Grip Strength (GS) and Timed-Up and Go (TUG) were measured before and after vitamin D_3 supplementation. Fasting total plasma 25 hydroxyvitamin D (25OHD) and Parathyroid Hormone (PTH) concentrations were measured by Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) and immunoassay, respectively. Baseline plasma 25OHD concentrations were 41.3 (SD 19.9), 39.5 (SD 20.6), 38.9 (SD 19.7) nmol/L; GS values were 28.5 (SD 13.4), 28.8 (SD 13.0) and 28.1 (SD 12.1) kg and TUG test values were 10.8 (SD 2.5), 11.6 (SD 2.9) and 11.9 (SD 3.6) s for the 12,000 IU, 24,000 IU and 48,000 IU dose groups, respectively. Baseline plasma 25OHD concentration < 25 nmol/L was associated with lower GS ($P = 0.003$). Post-interventional plasma 25OHD concentrations increased to 55.9 (SD 15.6), 64.6 (SD15.3) and 79.0 (SD 15.1) nmol/L in the 12,000 IU, 24,000 IU and 48,000 IU dose groups, respectively and there was a significant dose-related response in post-interventional plasma 25OHD concentration ($p < 0.0001$). Post-interventional GS values were 24.1 (SD 10.1), 26.2 (SD10.6) and 25.7 (SD 9.4) kg and TUG test values were 11.5 (SD 2.6), 12.0 (SD 3.7) and 11.9 (SD 3.2) s for 12,000 IU, 24,000 IU and 48,000 IU dose groups, respectively. The change (Δ) in GS and TUG from pre to post-intervention was not different between treatment groups before and after the adjustment for confounders, suggesting no effect of the intervention. Plasma 25OHD concentration was not associated with GS and TUG test after supplementation. In conclusion, plasma 25OHD concentration < 25 nmol/L was associated with lower GS at baseline. However, monthly vitamin D_3 supplementation with 12,000 IU, 24,000 IU and 48,000 IU, for 12 months had no effect on muscle function in older adults aged 70+ years.

Trial Registration : EudraCT 2011-004890-10 and ISRCTN35648481.

1. Introduction

Loss of muscle mass and decreased muscle strength are features of ageing, with an annual loss of muscle mass of 0.5–1.0% per year after 70 years of age [1] and a 10–15% decline in muscle strength per decade in older people aged 70–79 years [2]. Decreased muscle mass and strength can

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result in sarcopenia, which is associated with poorer quality of life, loss of independence and increased health care costs [3]. Assessment of Hand Grip Strength (GS) and Timed-Up and Go (TUG) are the widely used methods to test the muscle strength and identify the presence of sarcopenia [4].

Links between vitamin D status and muscle function have been reported based on mechanistic *in vitro* studies [5], human observational [6–11], longitudinal [12] and intervention studies [13,14]. Some observational [6,9,13] and longitudinal [12] studies have reported positive associations between serum 25-hydroxyvitamin D (25OHD) concentration and muscle function in older adults, whereas other studies did not find an association [11]. These conflicting findings may be due to the differences in the characteristics of the population and differences in the vitamin D status of the participants. Current evidence suggests that vitamin D status is associated with reduced muscle strength, function and physical performance in older adults (over 60 years of age) only when serum 25OHD concentration falls below 50 nmol/L [15]. The scientific advisory committee on nutrition (SACN) recommended that serum 25OHD concentration should be at least 25 nmol/L all year round for optimal bone and muscle health [16].

The findings from vitamin D intervention studies are inconsistent, reflecting the variation in characteristics of the study population (e.g. age, gender, baseline vitamin D status), study design and nature of the intervention (route, dose, frequency and form of vitamin D supplementation). Some studies show the positive effect of vitamin D supplementation on muscle function only in older adults whose baseline serum 25OHD concentrations < 30 nmol/L [17] or < 50 nmol/L [15].

Since the plasma concentration and vitamin D supply required for optimal muscle function in older adults are not well understood, we undertook a secondary analysis of a 1-year dose-ranging randomised vitamin D_3 supplementation trial, to evaluate its effects on muscle function [18].

2. Materials and methods

Vitamin D in older people (VDOP) study was a randomized double-blind interventional trial in 379 male and female older adults aged 70 years or older, living in the North-East of England (55 °N), which recruited from November 2012 and May 2013. The primary aim of this study was to assess the effect of monthly dose of 12,000 IU, 24,000 IU or 48,000 IU (equivalent to 400 IU, 800 IU or 1600 IU per day) vitamin D_3 (Vigantol, Merck Sereno GmbH, Darmstadt Germany) on bone mineral density [18].

Potential participants were identified through screening of the electronic records of 25 GP practices. Exclusion criteria comprised: taking vitamin D supplements at a dose greater than 400 IU/day or calcium at a dose greater than 500 mg/day, a fragility fracture within the previous 6 months, treatment with an anti-resorptive or anabolic treatment for osteoporosis in the previous three years, a history of renal stones, previous hip replacement or primary hyperparathyroidism, hypercalcaemia (albumin adjusted plasma calcium > 2.60 mmol/L), hypocalcaemia (albumin adjusted plasma calcium < 2.15 mmol/L) or an estimated glomerular filtration rate (eGFR) less than 30 ml/min/1.73 m^2). Ethical permission was given by the Tyne and Wear Research Ethics Committee (REC,12/NE/0050). All participants provided written informed consent. The sponsor, Newcastle upon Tyne NHS Foundation Trust, provided the Research and Development approval for the study (Trial registration: EudraCT 2011 – 004890-10 and ISRCTN 35648481). Further details about participant recruitment are published elsewhere [19].

2.1. Intervention and study visits

Participants were randomized to receive one of three doses of vitamin D_3, 12,000 IU, 24,000 IU or 48,000 IU, monthly for one year. Both participants and investigators were blinded to the treatment received. Study visits took place at baseline and thereafter at 3-monthly intervals (5 in total). Participants were provided with 3-monthly supplies of vitamin D_3 at each study visit.

2.2. Outcome measures

GS (kg) in both the right and left hand was measured using a Jamar hand-grip dynamometer (Jamar, Bollington, USA). Three measurements were taken, and the mean value was used for analysis. The TUG test was performed once and recorded as the time taken in seconds (s) to stand from a sitting position in an arm chair and walk 3 m distance [20]. GS and TUG were measured before and after 1 year of supplementation. Anthropometry, including, height, weight, Fat Mass (FM) and Fat-Free Mass (FFM) were measured at three-month intervals. Height was measured using a stadiometer and weight, FM and FFM were measured using a bioelectrical impedance analyser (Tanita Crop, Tokyo, Japan). Habitual dietary vitamin D intake was assessed using a food frequency questionnaire (FFQ) at the screening visit, the 3rd and 5th visits. The vitamin D intake was calculated as the mean value of FFQ data of screening visit, the 3rd and the 5th visits.

2.3. Biochemical analysis

Overnight fasting venous blood samples were collected from participants at each visit. The $25OHD_2$ and $25OHD_3$ concentrations in plasma were measured by Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS), before and after the intervention. Total 25OHD concentration was calculated by summing $25OHD_2$ and $25OHD_3$ values. EDTA plasma was used for the analysis of PTH by immunoassay (Immulite, Siemens Healthcare Diagnostics Ltd, Camberley, UK). Quality assurance of 25OHD and PTH assay were performed as the part of vitamin D Quality Assessment Scheme (http://www.deqas.org/) and the National External Quality Assessment Scheme (https://ukneqas.org.uk/). Inter assay variations were < 10% and < 7% for $25OHD_2$ and $25OHD_3$, respectively.

2.4. Data and statistical analysis

Baseline data were available for 379 older adults, while 343 older adults completed the intervention study. Thus, the total sample of 379 was used for the baseline data analysis, while the data from 343 older adults were used to investigate the intervention effects after 12 months. Older adults were sub-divided into two groups based on baseline plasma 25OHD concentration < 25 nmol/L, which is the cut-off of value of vitamin D used in the UK to indicate an increased risk of deficiency [16] and plasma 25OHD concentration < 50 nmol/L, which is the cut-off for vitamin D inadequacy used in North America [21], both of which have recently been incorporated in to the National Osteoporosis guidelines in the UK [22]. Statistical analysis of the data was conducted using SPSS for Windows version 13.0. Kolmogorov-Smirnov test was used to evaluate the distribution of the variables and those that were not normally distributed were log transformed prior to the analysis and were near normally distributed after the conversion. Primary outcomes for the study were GS and TUG in response to supplementation with 12,000 IU, 24,000 IU and 48,000 IU vitamin D_3 per month.

Baseline 25OHD, baseline muscle function variables, age, weight, height, FM, FFM and vitamin D intake were predetermined as potential confounders. Multinomial logistic regression analysis was used to investigate the association between muscle function at baseline and plasma 25OHD concentration (based on whether plasma level was above or below two cut-offs: 25 nmol/L and 50 nmol/L), adjusting for confounders. The ANOVA test was used to test the effect of vitamin D supplementation on muscle function, plasma 25OHD and PTH concentrations. ANCOVA was used to test for the effect of the treatment on post-intervention variables after controlling for potential confounders (age, weight, height, Fat Mass (FM), Fat Free Mass (FFM) and vitamin D intake). The Bonferroni test was used for *post hoc* comparisons. Multiple linear regression was used to test potential effect of plasma 25OHD concentration on muscle function after supplementation. A P value < 0.05 was considered as significant.

Table 1
Participants' characteristics at baseline by the dose of vitamin D supplementation.

Characteristics	12,000 IU			24,000 IU			48,000 IU		
	n (%)	Mean	SD	n (%)	Mean	SD	n (%)	Mean	SD
Age (years)	126	74.6	4.0	125	75.0	4.2	128	75.4	4.4
Age (n, % ≥ 70 < 71.5)	33 (26.2)			31 (24.8)			33 (25.8)		
Age (n,% ≥ 71.5 < 74)	36 (28.6)			30 (24.6)			28 (21.9)		
Age (n,% ≥ 74 < 77)	29 (23.0)			33 (26.4)			29 (22.7)		
Age (n,% ≥ 77)	28 (22.2)			31 (24.8)			38 (29.7)		
Gender (n, % males)	126 (54.8)			125 (52.8)			128 (49.2)		
Weight (kg)	126	73.9	11.8	125	77.1	14.0	128	76.1	14.2
Height (cm)	126	167.4	8.1	125	167.0	9.8	128	167.4	10.0
Waist (cm)	125	94.5	11.4	125	97.7	14.0	127	97.5	14.3
Hip (cm)	125	103.9	8.2	125	105.8	9.5	127	105.3	10.5
BMI[1] (kgm^{-2})	126	26.3	3.6	124	27.5	4.1	127	27.2	4.0
< 18.5	0 (0.0)			1 (0.8)			0 (0.0)		
18.5 – 24.9	50 (41.0)			31 (25.4)			41 (33.6)		
25.0 – 29.9	60 (32.3)			65 (34.9)			61(32.8)		
≥ 30.0	16 (33.2)			28 (33.0)			26 (33.8)		
Body fat %	124	31.9	8.6	125	32.9	7.7	127	32.5	7.8
GS[2] (kg)	126	28.5	13.4	124	28.8	13.0	127	28.1	12.1
TUG[3] (s)	125	10.8	2.5	124	11.6	2.9	127	11.9	3.6
Plasma 25OHD[4] (nmol/L)	126	41.3	19.9	124	39.5	20.6	128	38.9	19.7
PTH[5] (Pg/ml)	126	48.6	25.7	123	47.4	23.3	128	49.9	21.3
Dietary vitamin D intake (μg/day)	119	3.6	2.0	121	3.6	2.5	123	4.0	3.0

1Body Mass Index 2 Grip Strength 3 Timed-Up and-Go4 25-hydroxy vitamin D 5Parathyroid Hormone.

3. Results

Table 1 presents the participants' characteristics at baseline, stratified by vitamin D_3 supplementation dose. Baseline values for the main outcome measures, GS, TUG and plasma 25OHD concentration were similar across the intervention groups as were mean values for the main confounders including weight, height, BMI and age indicating that randomization was successful. The initial characteristics of the baseline sample (379 participants) and the sample of older adults who completed the intervention study (343 participants) were similar (data are not shown).

Table 2 shows the multinomial logistic regression analysis of the relationships between baseline plasma 25OHD concentration and muscle function variables according to the cut-offs values of plasma 25OHD concentrations from SACN, 2016 (25 nmol/L) and North American Institute of Medicien (IOM), 2011 (50 nmol/L). After adjusting for age, body weight, height, FM, FFM and vitamin D intake, older adults who had plasma 25OHD concentration < 25 nmol/L at baseline were significantly ($p = 0.003$) less likely to have GS above the median compared with individuals with plasma 25OHD concentration > 25 nmol/L. This relationship was evident for GS for both males ($p = 0.015$) and females ($p = 0.050$). When using the cut-off value of 50 nmol/L, there was no relationship between vitamin D status and either GS or TUG for all participants and for both gender groups.

After one year of vitamin D supplementation, there were no differences between treatment doses for post-intervention GS or TUG. In addition, there were no significant changes in GS and TUG from baseline between intervention arms, with and without adjustment for baseline values, age, gender, weight, height, FM, FFM and vitamin D intake. Further, subgroup analysis of those with a baseline 25OHD concentration < 50 and < 25 nmol/L, did not show any significant differences between intervention arms in post-intervention GS and TUG, or for change in GS and TUG. As expected, there were significant differences between treatment arms in post-interventional plasma 25OHD and change in 25OHD concentration. This relation was the same for the sub-group analysis in those with baseline plasma 25OHD concentration < 50 nmol/L and < 25 nmol/L. After the supplementation, the mean change in plasma 25OHD concentration was 14.3 (SD 12.6), 25.3 (SD 18.0) and 40.9 (SD 19.8) nmol/L for the 12,000 IU, 24,000 IU and 48,000 IU dose group, respectively. There was no significant difference between the groups in unadjusted PTH post-intervention, although the decrease in PTH was significant larger at the

Table 2
Multinomial logistic regression analysis1 of relationships between plasma 25OHD concentration, categorized according to SACN and IOM cut-offs, and muscle function at baseline.

	Total population (n = 379)			Males (n = 198)			Females (n = 181)		
Classification	OR	CI	P value*	OR	CI	p value**	OR	CI	P value**
GS (kg)									
25OHD < 25 nmol/L[2]	0.339	0.166 – 0.691	0.003	0.333	0.137 – 0.810	0.015	0.251	0.063 – 1.001	0.050
25OHD ≥ 25 nmol/L	**Reference**			**Reference**			**Reference**		
25OHD < 50 nmol/L[3]	0.990	0.510 – 1.922	0.976	0.588	0.216 – 1.443	0.229	1.870	0.520 – 6.719	0.338
25OHD ≥ 50 nmol/L	**Reference**			**Reference**			**Reference**		
TUG (s)									
25OHD < 25 nmol/L	0.645	0.388 – 1.070	0.090	0.501	0.229 – 1.093	0.084	0.720	0.358 – 1.446	0.355
25OHD ≥ 25 nmol/L	**Reference**			**Reference**			**Reference**		
25OHD < 50 nmol/L	0.697	0.424 – 1.147	0.155	0.775	0.386 – 1.543	0.468	0.584	0.273 – 1.250	0.166
25OHD ≥ 50 nmol/L	**Reference**			**Reference**			**Reference**		

1To be in the category of higher muscle function based on dichotomisation at the median value [2] SACN cut-off [3]IOM cut-off.
* Adjusted for gender, age, body weight, height, fat mass (FM), fat free mass (FFM) and dietary vitamin D intake.
** Adjusted for age, body weight, height, FM, FFM and dietary vitamin D intake.

Table 3
Effect of vitamin D supplementation on post-interventional and change (Δ) in muscle function variables, plasma 25OHD concentration and PTH concentration by the dose of vitamin D supplementation.

Parameters Total sample (n = 343)	12,000 IU/month	24,000 IU/month	48,000 IU/month	*p*1	*p*2
Plasma 25OHD[3] (nmol/L)	**(n = 113)**	**(n = 114)**	**(n = 116)**		
Pre-intervention	41.2 (20.3)	39.4 (20.8)	38.5 (19.4)	0.495	
Post-intervention	55.9 (15.6)	64.6 (15.3)	79.0 (15.1)*	< 0.0001	< 0.0001
Change (Δ) in 25OHD	14.3 (12.6)	25.3 (18.0)	40.9 (19.8)*	< 0.0001	< 0.0001
PTH[4] (pg/mL)					
Pre-intervention	46.8 (23.5)	47.1 (23.9)	50.6 (21.6)	0.443	
Post-intervention	44.0 (21.3)	44.6 (24.5)	40.1 (18.4)	0.244	0.016
Change (Δ) in PTH	−2.9 (18.4)	−3.1 (18.2)	−10.6 (15.4)*	< 0.0001	0.001
GS[5] (kg)					
Pre-intervention	27.5 (12.7)	29.4 (13.2)	28.1 (12.2)	0.641	
Post-intervention	24.7 (10.1)	26.2 (10.6)	25.7 (9.4)	0.692	0.449
Change (Δ) in GS	−2.8 (11.6)	−3.2 (8.1)	−2.4 (7.7)	0.820	0.426
TUG[6] (s)					
Pre-intervention	10.9 (2.5)	11.5 (2.9)	11.8 (3.5)	0.187	
Post-intervention	11.5 (2.6)	12.0 (3.7)	11.9 (3.2)	0.437	0.713
Change (Δ) in TUG	0.56 (2.32)	0.46 (2.77)	0.15 (2.5)	0.773	0.680
Older adults with baseline 25OHD < 50 nmol/L (n = 242)					
Plasma 25OHD (nmol/L)	**(n = 75)**	**(n = 83)**	**(n = 84)**		
Pre-intervention	30.2 (10.9)	29.1 (10.3)	29.6 (10.6)	0.862	
Post-intervention	49.7 (11.8)	60.5 (14.8)	76.8 (14.3)*	< 0.0001	< 0.0001
Change (Δ) in 25OHD	19.3 (10.0)	31.3 (15.0)	47.4 (15.3)*	< 0.0001	< 0.0001
PTH (pg/mL)					
Pre-intervention	49.9 (24.9)	52.9 (24.4)	54.2 (22.0)	0.210	
Post-intervention	45.7 (21.5)	48.9 (24.5)	41.0 (19.7)	0.137	0.101
Change (Δ) in PTH	−4.2 (21.1)	−4.0 (19.5)	−13.4 (15.3)*	0.001	0.002
GS (kg)					
Pre-intervention	26.5 (11.8)	29.1 (13.6)	28.3 (12.8)	0.457	
Post-intervention	23.8 (10.3)	25.5 (10.3)	25.1 (9.3)	0.801	0.403
Change (Δ) in GS	−2.7 (12.5)	−3.6 (8.5)	−3.1 (8.4)	0.486	0.889
TUG (s)					
Pre-intervention	10.9 (2.3)	11.8 (2.9)	11.8 (3.2)	0.094	
Post-intervention	11.7 (2.8)	12.3 (3.9)	12.0 (3.5)	0.560	0.630
Change (Δ) in TUG	0.74 (2.4)	0.45 (2.8)	0.29 (2.3)	0.512	0.823
Older adults with baseline 25OHD < 25 nmol/L (n = 93)					
Plasma 25OHD (nmol/L)	**(n = 31)**	**(n = 30)**	**(n = 32)**		
Pre-intervention	19.3 (4.1)	18.2 (4.5)	18.8 (3.9)	0.582	
Post-intervention	41.9 (10.7)	55.5 (14.7)	73.7 (12.8)*	< 0.0001	< 0.0001
Change (Δ) in 25OHD	22.6 (9.3)	37.3 (14.0)	54.9 (12.7)*	< 0.0001	< 0.0001
PTH (pg/mL)					
Pre-intervention	61.9 (27.8)	59.5 (19.3)	58.8 (23.2)	0.930	
Post-intervention	50.4 (20.6)	47.7 (16.6)	41.5 (24.1)	0.102	0.143
Change (Δ) in PTH	−12.2 (25.9)	−11.9 (17.4)	−17.4 (14.7)	0.470	0.563
GS (kg)					
Pre-intervention	25.6 (13.8)	24.1 (9.1)	25.8 (14.2)	0.895	
Post-intervention	22.3 (11.2)	22.5 (9.0)	22.8 (7.9)	0.903	0.860
Change (Δ) in GS	−3.3 (16.1)	−1.6 (4.8)	−3.0 (9.1)	0.814	0.714
TUG (s)					
Pre-intervention	11.0 (2.4)	12.8 (3.7)	12.5 (4.1)	0.938	
Post-intervention	11.9 (2.8)	13.7 (4.1)	12.9 (4.1)	0.798	0.379
Change (Δ) in TUG	0.91 (2.6)	0.88 (2.7)	0.38 (2.5)	0.806	0.823

1One-way ANOVA followed by Bonferroni test.
2ANCOVA controlled for baseline values of the variables, age, gender, weight, height, Fat Mass (FM), Fat Free Mass (FFM) and vitamin D intake.
325-hydroxyvitamin D 4 Parathyroid Hormone5Grip Strength 6Timed-Up and Go test.
* Significantly different from 12,000 IU and 24,000 IU groups.

highest dose after correction for confounders. In subgroup analyses, change in PTH was significantly different between intervention arms before and after adjusting for confounders comparing those with baseline 25OHD concentrations equal to or above with those below 50 nmol/L at baseline but this was not the case for the 25 nmol/L cut point (Table 3).

After supplementation, plasma 25OHD concentration was not significantly associated with either GS or TUG. Significant determinants for GS after supplementation were height ($p < 0.0001$), gender ($p = < 0.0001$), age ($p = 0.002$), concurrent body weight ($p = 0.040$) and FM ($p = 0.040$). Similarly, the determinants of TUG were age ($p < 0.0001$), height ($p < 0.0001$), fat mass ($p = < 0.0001$), gender ($p = 0.018$) and vitamin D intake ($p = 0.019$) (data are not shown).

4. Discussion

4.1. Main findings

This double-blind, randomized controlled study found that monthly vitamin D_3 supplementation with 12,000 IU, 24,000 IU and 48,000 IU (which corresponds to 400, 600 and 1200 μg of dietary vitamin D per day) for one year produced significant dose-related increases in plasma 25OHD concentration but had no effect on muscle function in older adults. However, at baseline, there was an association between plasma 25OHD concentration and GS, with significantly lower GS for those with baseline plasma 25OHD concentration < 25 nmol/L in both males and females. After supplementation, there were no associations

between plasma 25OHD concentration and muscle function. To our knowledge, this is the first dose-ranging RCT conducted in the UK, with a large number of free-living older adults, evaluating the effect of vitamin D supplementation on muscle function.

4.2. Comparison with other studies

In line with our findings, previous RCTs reported that vitamin D supplementation had no beneficial effect on muscle function in older adults, irrespective of the dose of vitamin D supplements given. A recent study of female adults of long-term care residence aged 65 + years, supplemented with the oral dose of 800 IU vitamin D_3 daily for 24 months, reported no effect of the supplementation on muscle function measured by gait speed and physical performance test [23]. Another study that conducted recruiting home-dwelling men and women aged > 70 years who were randomized to receive one of the oral doses of 24,000 IU, 60,000 IU or 24,000 IU vitamin D_3 with 300 μg of calcifediol monthly for 12 months, reported no improvement in lower extremity function measured by short physical performance battery [24]. Hansen et al., 2015 reported that among postmenopausal women aged 75 years or younger with baseline 25OHD concentration 14–27 ng/mL, (~35–67.5 nmol/L) and supplemented with an oral dose of 800 IU or 50,000 IU vitamin D_3 twice monthly for one year had no effect on muscle function, assessed by the 'five sit-stand' test and TUG test [25]. Further, a recent systematic review of community-dwelling older adults aged 65 + years showed that bolus injection or oral vitamin D supplementation of dose ranging from 1000 IU – 600,000 IU, given daily or weekly for duration ranging from 16 weeks to 20 months, had no effect on GS and TUG test [26].

In contrast, some RCTs have shown improvements in muscle function following vitamin D supplementation in older adults. A study conducted among residents of nursing homes of average age of 89 years, who had been randomized to receive one of four oral dose of vitamin D_3 supplements (200 IU, 400 IU, 600 IU and 800 IU) or placebo daily for 5 months, showed that those receiving the highest dose had the lowest number of falls compared to the other groups [27]. Positive effects of oral vitamin D_3 supplements on GS and chair rise test were reported in a study of postmenopausal women aged 50–65 years who received 1000 IU of oral vitamin D_3 daily for 9 months [28]. A study of ambulatory older adults with the history of falls and serum 25OHD < 12 μg/L (~ 30 nmol/L) who received a single intramuscular injection of 60,000 IU of ergocalciferol reported a beneficial effect on functional performance, reaction time and balance but not on muscle strength [29]. Another RCT of ambulatory older adults live in a nursing home with a serum 25OHD concentration < 30 ng/mL (~ 75 nmol/L), randomized to receive the oral or intramuscular injection of 600000 IU of cholecalciferol for 12 weeks, demonstrated an improvement in muscle strength assessed using quadriceps and physical performance battery [30]. According to Zhu et al., 2010, among community-dwelling older adults aged 70–90 years with serum 25OHD concentration < 24 ng/ml (~ 60 nmol/L) supplemented with 1000 IU of vitamin D_2 daily for 1 year, improved TUG test only among the older adults who were the slowest and weakest at the baseline [31]. Similarly, a systematic review with a meta-analysis reported that vitamin D supplementation had a positive effect on muscle function in older adults whose baseline serum 25OHD concentration was < 25 nmol/L [32]. These inconsistent findings are likely attributed to differences in study design including cohort characteristics, duration, dosage, formulations, route of vitamin D supplementation and the functional outcomes measured.

To support our finding of an association between GS and plasma 25OHD concentration below 25 nmol/L at baseline, Wu et al., 2017, reported that the serum 25OHD concentration of 29–33 nmol/L may optimise musculoskeletal health in middle-aged women (36–57 years) [33]. Similar to our study Grimaldi et al., 2013, also reported a positive association between serum 25OHD concentration and GS, but not with other tests of muscle function and suggested that this might be related to anatomical site differences in the androgenic effect of vitamin D or to differences in vitamin D receptor expression between upper and lower body muscle and consequently muscle function [34].

GS loss in our study was much higher than the reported values in previous studies. The annual loss of GS among the older people aged 65–75 years reported in previous studies ranged from 0.3 to 1.3 kg [35–37]. Though the GS is the standard method to assess sarcopenia, differences in the equipment and methods used in various studies may have caused variation in the measurements, making it difficult to compare between studies [38].

In this study we found that plasma 25OHD concentration < 25 nmol/L was associated with a lower GS. This finding supports the recommendation of SACN, UK that for the protection of musculoskeletal health, serum 25OHD concentration should not fall below 25 nmol/L throughout the year [16]. The US Institute of Medicine (IOM) defines the desired range of 25OHD as 30–50 nmol/L and ESFA (European Food Safety Authority [39];) advises a target value of 50 nmol/L for the general population. The US Endocrine Society advises a target range of > 50 nmol/L for patient management who are at risk of vitamin D deficiency [40]. In addition, Kotlarczyk et al., 2017 suggested that at least a concentration between 30–40 ng/ml (~75–100 nmol/L) is required for older adults for optimum muscle function [23]. With regards to vitamin D supplementation, the American Geriatrics Society [41] and the Endocrine Society [40] recommend vitamin D supplementation of 600–1000 IU/day in older adults who are at risk of falling. A systematic review of vitamin D supplementation trials also reported a daily dose of 700–1000 IU for physical performance and to prevent falls [42]. SACN, 2016 suggests that for the adults > 50 years, the beneficial effect of vitamin D supplementation on muscle strength and function can be seen among the adults at the mean baseline serum 25OHD concentrations ranging between 25 and 66 nmol/L. In our study, none of the intervention groups reached a mean post-intervention plasma 25OHD concentration above this range. Accordingly, it can be speculated that the dosages used in this study may not have been high enough to reduce the negative effects of the ageing process. The absence of a detectable effect of supplementation in this study may also be attributed to the fact that only 30% of our participants had a 25OHD < 25 nmol/l at baseline. However, as a result of lack of a placebo group we did not have data on "natural decline" of muscle function of this group, thus we could not compare the effect of vitamin D supplementation with that in non-supplemented individuals.

For tissues other than the kidney, total 25OHD may not fully reflect its availability for local hydroxylation into $1,25(OH)_2D$, which is the active metabolite of vitamin D. Although $1,25(OH)_2D$ is responsible for the biological action of vitamin D, its systemic concentration does not reflect function at the target tissue level [43,44]. Many vitamin D target tissues, including the muscle tissue, are known to express the $1,25(OH)_2D$-producing enzyme CYP27A1 for auto- and paracrine functions. Some reports suggest that muscle tissue may be capable of internalising vitamin D binding protein bound 25OHD, although it remains to be determined whether this is a significant route of cellular supply of 25OHD [8]. To date, no data are available to identify whether free 25OHD provides a better prediction of muscle function compared to total 25OHD.

4.3. Limitations, strengths and future studies

We used the lowest intervention dose (which corresponds to the current UK dietary recommendation) as the reference group but did not include a placebo group in our study design as directed by the approving authorities. As a result, we could not establish the effect of three doses of vitamin D supplements compared to a placebo group. Further, we only measured plasma 25OHD concentration at baseline and after 1-year supplementation. These samples were collected early winter to late spring, during which vitamin D status is lower than the year-round average in non-supplemented individuals. Therefore, the

vitamin D status of individuals at baseline and post-intervention may have been misclassified as it may not have fully reflected the trajectory of vitamin D status throughout the year. Further, this population was not selected randomly from the community. They were invited on the basis of screening of pre-specified criteria in their electronic health record. Also, there may have been self-selection bias as those that expressed an interest to participate in the study may have been more health conscious. In addition, the response to vitamin D supplementation and status may have been influenced by factors not measured in this study, such as the distribution of type I and type II of muscle fibres, genetic factors, habitual physical activity or exercise habits and hormonal factors.

This study had several strengths. Its large sample size and the number of available measurements that could potentially influence muscle force, i.e. those related to body composition and size. The use of three different vitamin D doses corresponding to the UK Recommended Nutrient Intake (RNI) [16], the US Recommended Dietary Allowances (RDA) [21] and the value below the Tolerable Upper Intake Level (TUIL) was a strength of this study. We considered the effect of vitamin D supplementation, without an exercise intervention, on muscle. Few trials have looked at the potential interaction between exercise and vitamin D, although a recent systematic review presented evidence of an additive effect of resistance exercise and vitamin D3 supplementation for the improvement of muscle strength in older adults, and we would support suggestions that this is an important area for future research [45].

5. Conclusions

At baseline, plasma 25OHD was associated with GS in both male and females, but only below the cut-off level of 25 nmol/L. Vitamin D supplementation significantly increased the plasma 25OHD concentration of older adults in all doses of supplementation. Vitamin D supplementation with 12,000 IU, 24,000 IU and 48,000 IU for 12 months had no effect on muscle function in adults older than 70 years.

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Role of funders

The study design was internationally peer-reviewed for the Arthritis Research UK as part of the funding decision process. Reviewers' recommendations were taken into account in designing the trial. Arthritis Research UK were not involved in the analyses or in the decision to publish these results from the trial.

Role of sponsors

The sponsor was responsible for the conduct of the trial according to guidelines declared by GCP, GRP, the Data Protection Act and the Declaration of Helsinki. The sponsor was also responsible for Pharmacovigilance. These responsibilities were delegated to the Principal Investigator Dr T. Aspray and Newcastle CTU. The sponsor did not play any role in design, execution, analysis or interpretation of the data,

Conflict of interests

Authors declare that they have no competing interests.

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IN THIS ISSUE...

Sarcopenia: Epidemiology and treatment

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Sarcopenia is an age-related syndrome characterised by progressive decline in skeletal muscle mass concomitant with decreased strength and/or function[1,2]. It is a major contributor to the risk of physical frailty, functional impairment in older people, poor health-related quality of life and premature death[3]. Sarcopenia is responsible for considerable healthcare expenditure. The USA has estimated the economic costs associated with sarcopenia to be approximately $18.5 billion, accounting for 1.5% of the country's total health expenditure[2]. Sarcopenia is characterised by many features, which include the loss of muscle mass, altered muscle composition, infiltration with fat and fibrous tissue and alterations in innervation[2] A better understanding of these factors might help us to develop strategies that target these effects. However, research into sarcopenia has perhaps been hampered by uncertainty regarding how best to define the condition. In this review we briefly summarise the current definitional approaches to sarcopenia and discuss the epidemiology, causes of the condition, as well as possible therapeutic advances in treatment.

Can we evaluate the contribution of sunlight to 25(OH)D status?

Can we evaluate the contribution of sunlight to 25(OH)D status?

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Sunlight exposure is the main source of vitamin D synthesis in the skin. The actual contribution of sun exposure to overall vitamin D status is still questionable. This article provides brief overview of different methods of assessing sun exposure and the extent to which those methods accurate in quantifying the sun exposure to use as a proxy indicator of serum 25(OH)D concentration which reflects the vitamin D status. Knowing the accurate method to measure the sun exposure would help to set the dietary recommendation for vitamin D and recommendation for sun exposure for optimum health.

Introduction

Exposure to solar ultraviolet (UV) radiation at a wavelength of 290 – 315 nm (UVB) is the main source of vitamin D synthesis in the human body. Natural food sources of vitamin D include oily fish, eggs and meat, although the vitamin D content of these foods can be variable and dependent mainly on animal feeding practices. Fortified foods such as enriched dairy foods and cereals are also important dietary sources of the vitamin D but fortification strategies vary significantly within and between countries. Circulating levels of 25-hydroxyvitamin D (25(OH)D) in the blood are recognised to be the best estimate of vitamin D status, because this biomarker is believed to provide a good estimate of vitamin D exposure from sunlight exposure and diet. However, the relative contribution of sunlight to serum 25(OH)D is difficult to measure. It is postulated that 90% of circulating 25(OH)D is explained by cutaneous synthesis in response to UVB exposure with the rest coming from dietary sources, but it remains difficult to make robust and generalizable estimates[1]. Seasonal variations in the vitamin D status of populations at northern and southern latitudes also provides evidence to support the observation that sun exposure is the major contributor to vitamin D status[2].

Sunlight exposure varies widely among individuals all over the world. Normally, we use UV index (UVI) to measure the intensity of UV radiation (UVR). UVI of a particular geographical region is mainly determined by the solar zenith angle, defined as the angle between the horizontal local line and a straight line to the sun. Therefore, UVI depends on the climatic season, latitude and time of day. In addition, UVI is influenced by the ozone layer, cloud cover, temperature, altitude, surface reflection, vapour pressure and the level of pollution[2]. Individual UV exposure in the same geographical setting will also vary due to personal factors. Sun protection behaviours, frequency of outdoor activities, holiday visits, clothing pattern, skin exposure area to sunlight, age and skin colour are some of the factors which are recognised to influence this[3]. Huge inter-individual and geographical variations of sun exposure are considerable barriers to the precise estimation of sun exposure.

Diffey observed a huge seasonal variation of UV radiation availability, with summer UVB 20 times greater than that seen in winter. However, the UV exposure of an individual living at a specific location fluctuates based on the time spent outdoors and different days throughout the year. The UV dose absorbed by the skin is further modified by sun protection factors such as the use of hats, sunscreens and clothing. Further the time spent outside during weekdays and weekends, and the holiday visits are also varied the individual UV exposure. To evaluate this,

Diffey developed a numerical model to estimate the variability of population daily solar UV exposure throughout the year by using ambient UV, time spent outdoors and exposure of the face relative to ambient[4].

Identifying the level of required sun exposure for optimum vitamin D status must be balanced against the risk of exposure to UVR, which is also an important environmental factor predicting the risk of skin cancer. Failure to resolve some of these issues resulted in the Scientific Advisory Committee on Nutrition (SACN) in the UK and the Institute of Medicine in North America being unable to define an appropriate level of sun exposure to optimise vitamin D status in their respective reports on dietary vitamin D requirements [2,5].

Measures of sun exposure

Human exposure to UVR can be measured by standard erythema dose (SED) and medium erythema dose (MED). The SED refers to erythemal effective radiant exposure from natural and artificial sources of UV radiation. One SED is equal to an erythemal effective radiant exposure of 100 J/m2[6]. One MED is the dose of UVR required to cause sunburn or slight pinkness in the skin, which will differ according to the skin colour, time of the year, behaviours and age[2]. Thus, it is a measure of the variable nature of individual sensitivity to UV radiation[7].

Studies often divide estimates of UV exposure into environmental and personal factors. Environmental exposure to UV varies according to latitude, season, time of the day, ground reflectivity, and cloud or tree cover, while personal UV exposure depends on sun-protection behaviours, amount of time spent outdoors, clothing pattern, holiday visits and many more factors. The anatomical distribution of UV also depends on standing or sitting postures and body site[3].

Tools for assessing sun exposure

Determining serum 25(OH)D concentrations in large population-based studies can be impractical and expensive. Therefore attempts to try and validate suitable sunshine exposure tools as a surrogate of vitamin D status may provide an attractive alternative. Several such tools can be used to assess sun exposure, as a proxy measure of vitamin D status. These methods can be categorised as direct and indirect methods and each method has its own limitations and strengths[3].

Self-reported questionnaire and diaries

Self-reported questionnaires are the most widely used indirect method of assessing sun exposure. However, no standard questionnaire has yet been developed, which may be used in all circumstances. Typical questions included aiming to evaluate the duration of sunlight exposure, time of the day, skin exposure area, sun protection practices, season, latitudes/place of living, cloud cover/tree cover, occupational behaviours and details of sunny holidays [8-10]. In some studies, each question was given a score to calculate composite scores for the sun exposure questionnaires[11,12]. For such studies it is important to validate the methodology and a number of such studies are summarised in *Table 1*. Typically, sun exposure questionnaires and diaries are compared with actual values for UVR availability/exposure but most questionnaires have some limitations.

Can we evaluate the contribution of sunlight to 25(OH)D status?

Table 1 : Summary of past research findings that used sun exposure questionnaires and diaries as a proxy indicator of vitamin D status

Reference	Participants	Place	Method	Finding
Detert et al. (2015)[8]	University students and primary health care patients (n=150)	Sweden & Australia	Questionnaire on sun habit including sun protection practice was used. It was used again after 1 month for the same people to check the reliability	The questionnaire had acceptable validity and reliability. It can be used for populations of varying UVR exposure.
Yu et al. (2009)[10]	All ages.	North California, Atlanta, Georgia	Activity based and time based sun exposure questionnaires were used.	The activity-based questionnaire was the best tool to measure sun exposure. Validity of this questionnaire was uncertain but the reliability was high.
O'Sullivan et al. (2017)[21]	60 years or older	Ireland	Questionnaires on sun enjoyment, life-style and vitamin D supplement was used. Tropospheric Emission Monitoring Internet Service data was used to estimate actual UVB dose. Serum 25(OH)D also was measured.	Sun enjoyment was strongly and positively associated with serum 25(OH)D. But accurate UVB measurement may require further improvement to the questionnaire.
Rosso et al.,(2002)[22]	Patients with skin cancer and population controls	Italy, Spain and France	Skin characteristics, sunburns, skin exposure history was gathered using previously used questionnaire on the same people.	Reproducibility of the questionnaire was poor, and The education level of participants was an important predictor.
Humayun et al., (2012)[12]	A group of university students and staff members	Pakistan	Long and short versions of sun exposure questionnaires were used. Dosimeters were used to validate the questionnaire.	Correlation between time spent outdoor and dosimeter values were R=0.36 (p=0.01) for long version and 0.43 (p=0.01) for short version in relation to 25(OH) D. They appeared to be valid tools in epidemiological studies
van der Maei et al., (2006)[14]	Case control study of patients with Multiple Sclerosis	Australia	Self-reported questionnaire on sun exposure was used. Serum 25(OH) D was measured.	Correlation of sun exposure score and 25(OH)D was 0.39 (p<0.01). Large variation in 25(OH)D was not explained by the questionnaire.
Brot et al. (2001)[13]	Perimenopausal women	Danish women	Self-reported questionnaire was used and serum 25(OH)D was measured	Low correlation of sun exposure score with vitamin D level (R=0.29, p=<0.01) and large variation in 25(OH)D was not explained by the questionnaire.
Sullivan et al. (2003) [23]	Girls of 9-11 years old	Orono, Maine (44° 53' N)	Diaries with time spent outdoor were used. Adjusted self-reported minutes of outdoors for the time of the day was calculated.	Correlation between UVB exposure and self-reported minutes outdoors adjusted for time of the day was 0.64. After adjusting for the ambient UVB, the tool can be used to assess sun exposure.

Observed measures of UVR, estimates obtained by personal UV dosimeters and serum 25(OH) D concentrations are generally used to validate the questionnaires. Although most research has found a significant correlation with self-reported and objective measures of UV exposure, the correlation coefficient values showed that the large proportion of the variance in objective UV measures are not explained by the self-reported measures[13 14].

Unfortunately, much of the variation in serum 25(OH)D concentration was unexplained by self-reported sun exposure. In addition, recall bias may be a source of confounding when using such questionnaires and they may be a burden to the participants, as they can need at least 30 minutes to complete. Some questionnaires need further improvements and tools to validate, while others are suitable only for specific durations[10] such as during the summer. In defence of the questionnaire methodology, in principle, it is cost-effective and easy to administer (even by telephone interview) and questionnaires can also estimate historical sun exposure and used repeatedly throughout a period of study.

McCarthy has extensively reviewed this topic[3], recommending that questionnaires could be improved by including details about sunscreen use in terms of coverage, SPF, water resistance, reapplication, clothing pattern, age, skin color.

Karagas et al. highlighted, the importance of identifying new techniques to validate the questionnaires, such as incorporating a molecular genetic marker of UVR damage. In addition, gathering ambient UVR collected from different sources also might helpful for accurate validation of questionnaires[9].

Diaries can also be used to assist in the estimation of UVR. In this method, participants need to record their outdoor activities with sun exposure time. Each minute outdoors during each hour can be tallied and multiply by peak hour average broadband UVB to get the total minutes outdoors adjusted to a standard of peak UVB for further accurate estimates. However, the use of a diary can alter the activity patterns of the participants and lead to inaccurate estimates of sun exposure[15].

Dosimeters /Daysimeter

Dosimeters can use physical, chemical or biological methods used to estimate UVR exposure by direct measurement for an individual. Physical dosimeters comprise a small UV detector electrically connected to a data logger, which is worn on a belt, in a pocket or the outer surface of the garments. Chemical dosimeters measure a chemical change in response to UV exposure, mostly using a thermoplastic polysulfone film, while a biological dosimeter measures the biological effects of UV exposure on the body, typically reflected by changes in biomarkers such as scoring of chromosomes abnormalities or circulating lymphocytes[16].

The use of a Daysimeter is also similar to dosimeter. It measures circadian light exposure and incorporates an activity meter to estimate the circadian light/dark exposure and activity/ rest patterns. It consists of two optical sensors. The first sensor detects optical radiation and a second sensor has an intrinsic long-wavelength response cut-off at approximately 580 nm together with a UV blocking filter creating a spectral response peaking at approximately 460 nm. An accelerometer within the Daysimeter is used to detect the subject's activity. Measures obtained from the Daysimeters can be summarized by calculating hourly averages of the 30 seconds data points of light exposure for each day[17].

Weather stations

UVR can be estimated using the data from satellite, spectrophotometers and computers located in weather stations. The estimate of UVR is mainly based on the measurements of total ozone, ground albedo (reflection) and cloud transmittance. Using modern technology,

irradiance estimates from satellites can be further improved to account for the effect of UV absorbing aerosols. However, these techniques have limitations. Short-term variation in the cloud cover changes can have a large effect on UV radiance. Also, UVR measurements may vary with the frequency of the measurements. UV data from satellites provide UVR measurement for a large geographical area, thus the actual UV value at the ground level may vary from the obtained value. Further, there are lots of other factors affecting the ground level UVR, which are not easy to estimate, such as water vapour, temperature, albedo, zenith angle and snow cover[18]. Despite these limitations, such weather parameters may help to estimate long-term sun exposure rather than solely sun exposure over a short time period.

Equations

There is a body of literature on the derivation of equations to calculate UVI, using the measurements obtained by spectrophotometers and satellite in weather stations. Most of these equations consider solar zenith angle and total ozone. However, equations do not account for many factors (already mentioned) that can affect UVI, especially the cloud cover, haze, other gases, surface elevation and surface albedo, which present the main limitation to this method [19, 20].

Conclusion

Assessing the contribution of sun exposure to vitamin D status presents difficulties, as a number of environmental and personal factors need to be taken into account. Assessing sun exposure using questionnaires is the easiest and lowest cost method to determine the impact on vitamin D status in a community setting. However, the questionnaires themselves have limitations. Further development of more precise questionnaires, which account for all the variables that affect sun exposure, is important but validation remains a challenge. In addition to the typical questions on sun exposure, detail about sunblock use, occupation, skin type, place of living and age are vital to consider. Further research is needed to develop tools and to validate them. So far, the contribution of estimates of vitamin D synthesis to dietary guidelines for vitamin D intake has been disappointing and recommendations are also complicated in view of the need to evaluate safe levels of sun exposure.

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Protocol

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Design and baseline characteristics of the Biomarkers Of Risk In Colorectal Cancer (BORICC) Follow-Up study: A 12+ years follow-up

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Abstract

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Age is the strongest non-modifiable risk factor but it is estimated that over half of CRC cases are linked with lifestyle factors such as diet. The Biomarkers Of RIsk of Colorectal Cancer (BORICC) Study recruited 363 participants in 2005 to investigate the effects of lifestyle factors on biomarkers of CRC risk. **Aim:** In the present BORICC Follow-Up (BFU) Study, we are using a longitudinal study design to investigate the effects of ageing (12+ years) and lifestyle factors on biomarkers of CRC risk and on healthy ageing. **Methods:** BFU Study participants attended a study visit at North Tyneside General Hospital (UK) for collection of biological samples, including blood and rectal biopsies, and information collected included anthropometric measurements, a Health & Medications Questionnaire, physical activity and sedentary behaviour, and habitual diet. Furthermore, musculoskeletal function was assessed by heel bone densitometry, timed up and go and hand grip strength as markers of healthy ageing. The BFU Study outcomes will be similar to those measured at baseline in the BORICC Study, such as DNA methylation and mitochondrial function, with additional measurements including the gut microbiome, faecal short-chain fatty acid concentrations and expression of genes associated with CRC. **Results:** Participants' recruitment to BFU Study and all sample and data collection have been completed. Forty-seven of the original BORICC participants were re-recruited to the BFU Study (mean age 67 years, 51% female). The recruits included 37 initially healthy participants and 10 participants who had adenomatous polyps at baseline. Approximately 70% of participants were over-weight or obese. **Conclusion:** Ultimately, identifying lifestyle factors that can reduce CRC risk, and understanding the underlying mechanisms for the effects of lifestyle and ageing on CRC risk, could lead to early prevention strategies.

Keywords

Colorectal cancer risk, biomarkers, longitudinal study, ageing

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and fourth most common cause of cancer-related death. Older age is the strongest risk factor for CRC and risk increases exponentially after the age of 50. In the UK, between 2013 and 2015, 44% of CRC cases were diagnosed in people aged ≥75 years and the highest incidence rates were in individuals aged 85–89 years. Up to 15% of CRCs are inherited with the majority (>85%) occurring sporadically. CRC risk is also modified by environmental and lifestyle factors, notably diet and physical activity, and it is estimated that 54% of CRC cases in the UK are preventable (Brown et al., 2018). It is therefore important to determine which lifestyle factors have chemoprotective

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properties or are associated with an increased risk of CRC and to understand the mechanisms by which they modulate CRC risk. For example, obesity, high intake of red and processed meat and high levels of sedentary behaviour increase CRC risk, whereas higher intakes of dietary fibre and high levels of physical activity are protective (World Cancer Research Fund/American Institute for Cancer, 2018). The mechanisms through which lifestyle factors modulate CRC risk include genomic damage caused by inflammation, oxidative stress and metabolic stress which may result in increased cell proliferation and reduced apoptosis. In particular, adverse dietary factors may dysregulate gene expression (leading to increased expression of oncogenes and reduced expression of tumour suppressor genes) through epigenetic mechanisms including DNA methylation, histone modifications, changes in chromatin structure and altered patterns of expression of non-coding RNA, for example, microRNA (miRNA).

The Biomarkers of RIsk of Colon Cancer (BORICC) Study recruited 363 patients attending Wansbeck General Hospital, Northumberland, UK for diagnostic endoscopy (flexible sigmoidoscopy or colonoscopy) in 2005. Of these, 262 patients with no detectable large bowel pathology were recruited to the 'healthy' arm of the study (BORICC 1) whilst 101 patients with adenomatous polyps were recruited to the 'polyp' arm (BORICC 2). The aim of the study was to identify and validate potential biomarkers of CRC risk. The BORICC Study investigated effects of lifestyle factors, particularly diet and adiposity, and age on biomarkers of CRC risk, with a focus on DNA methylation (Tapp et al., 2013). At baseline, age was a major determinant of gene-specific methylation levels (Tapp et al., 2013). In addition folate status (plasma folate and red cell folate concentrations), plasma 25-hydroxyvitamin D and plasma selenium and waist and hip circumferences were associated with both gene-specific, for example, *WIF-1* and *SFRP1*, and global (*LINE-1*) DNA methylation levels (Tapp et al., 2013). These findings suggested that these nutrition-related factors modulate methylation patterns in the colorectal mucosa of healthy individuals and that effects on DNA methylation may be a potential mechanism for the modulation of CRC risk by lifestyle factors. In addition, low selenium status was associated with aberrant gene expression detectable at mRNA and protein levels in the colorectal mucosa, including genes implicated in cancer, cell growth and proliferation, cell death and the inflammatory response (Meplan et al., 2016). Using BORICC Study samples, we also observed that clonal expansion of mitochondrial DNA mutations is the main mechanism causing age-related mitochondrial dysfunction in the apparently-healthy colorectal epithelium (Greaves et al., 2014).

The aim of the BORICC Follow-Up (BFU) Study is to investigate the effects of increasing age, as well as lifestyle factors – notably diet, adiposity, physical activity and sedentary behaviour – on biomarkers of large bowel health and CRC risk. In addition, we aim to investigate the effects of change in lifestyle factors over 12+ years on markers of healthy ageing, large bowel health and CRC risk.

We hypothesise that (i) biomarkers of CRC risk worsen with age; and (ii) higher risk lifestyles, including unhealthy dietary patterns, higher adiposity and low physical activity, exacerbate this age-related increase in risk. This project aims to test these hypotheses by examining differences in biomarkers of CRC risk cross-sectionally and by determining changes in these biomarkers longitudinally (after 12+ years) in BFU Study participants.

Methods

Study design

The BFU Study is a longitudinal 12+ years follow-up of participants in the BORICC Study (recruited in 2005) that investigated the effects of lifestyle on biomarkers of CRC risk. The BFU Study will investigate the effects of ageing (12+ years) on these biomarkers. Longitudinally, we will investigate how lifestyle factors at baseline impact on biomarkers of CRC risk 12+ years later, and how change in lifestyle factors (e.g. increased adiposity or change in diet) influence these biomarkers. Cross-sectionally, we will investigate associations between current lifestyle factors and CRC-related biomarkers. The trial is registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT04005742).

Study size

To provide an estimate of the numbers of participants that we would need to re-recruit, we used data for differences in our primary outcome, faecal calprotectin (FCP) concentrations, between participants who differed in mean age by 10 years at baseline for a power calculation. Using an alpha of 0.05 for 2-sided tests, mean FCP concentrations of 11.0 and 16.7 mg/kg for the younger and older groups (differing in mean age by 10 years), respectively, and a standard deviation for the whole population of 10.4, showed that we required 53 participants (power of test = 0.8).

Ethical approval

Ethical approval for the BFU Study was granted by the West Midlands – Coventry and Warwickshire Research Ethics Committee on 29th November 2016 (REC No. 16/WM/0424). Two amendments were made to the study; the first to include a response card and invite potential participants to Showcase events described below (approved May 2017) and the second to call any participants not returning their response cards to check whether they had received the study invitation letter (approved December 2017). Caldicott approval for the storage of data was provided by the Northumbria National Health Service (NHS) Foundation Trust.

Participant recruitment

Participants who took part in the BORICC Study at baseline were invited to participate in the BFU Study. Exclusion criteria included not being able to provide informed written

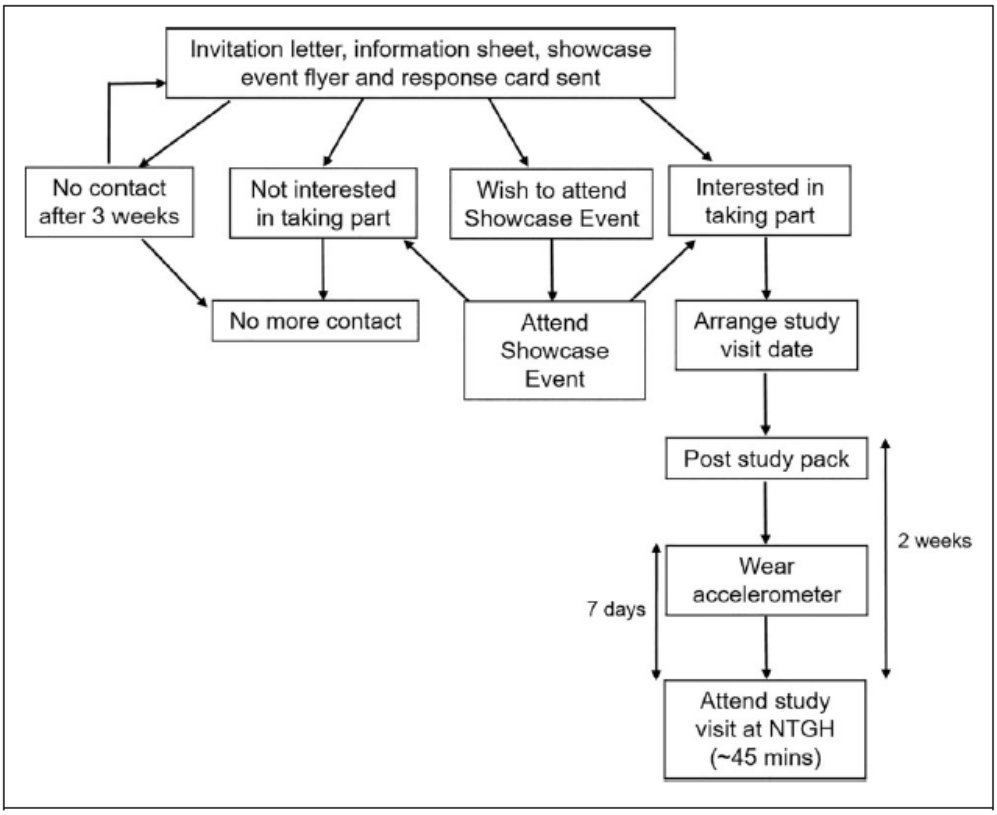

Figure 1. Schematic of participant recruitment to the Biomarkers Of RIsk of Colorectal Cancer Follow-Up (BFU) Study.

consent or not being able to travel to attend the hospital study visit. Participants on anticoagulant medication that may increase their risk of bleeding during rigid sigmoidoscopy were invited to participate in all aspects of the study except the collection of rectal mucosal biopsies. Participant recruitment started in March 2017 and ended in June 2018. The steps taken to recruit participants to the BFU Study are summarised in Figure 1.

Invitation letter

An invitation letter, information leaflet explaining the study, a flyer advertising BFU Study Showcase events and a response card with a stamped and addressed envelope were sent to potential participants in batches of 60 invitations. If no response was received within 3 weeks, a second invitation letter was posted. Participants were asked to return their response cards to the research team by ticking one of four possible responses:

(1) Yes, I am interested in attending the BFU Study Showcase Event on ___.
(2) Yes, I am interested in attending the BFU Study Showcase Event however these dates are not suitable for me.
(3) Yes, I am interested in taking part in the BFU Study but I prefer not to attend a Showcase event.
(4) No, I am not interested in taking part in the BFU Study and I do not want to be contacted again by the research team.

Those interested in taking part in the study were asked to contact the research team by telephone, email or post. If a participant responded saying that they would like to attend a Showcase event, a suitable date and time was arranged and a letter was posted to them with directions to the North Tyneside General Hospital (NTGH) Education Centre.

Study pack

Study packs were posted to participants approximately two weeks before their arranged study visit date. This was to allow participants sufficient time to wear the accelerometer for a week before the visit, to complete the questionnaires and to collect urine and stool samples.

The study packs contained a folder including:

(1) Study pack instructions
(2) Consent forms
(3) Hospital study visit instructions and directions

(4) Food frequency questionnaire (FFQ)
(5) Lifestyle questionnaire
(6) Sunlight exposure questionnaire
(7) Accelerometer instructions, record sheet and sleep log
(8) Stool collection instructions
(9) Urine collection instructions
(10) At-home sample and questionnaire collection record sheet

The following were also included in the study pack:

(1) Accelerometer (GENEActiv™)
(2) Stool collection pot (Fecotainer®)
(3) Cool bag and block for transportation of samples
(4) Urine collection pots × 2
(5) Urine vacutainers × 8 separated into two zip lock bags

FFQ. Participants completed a FFQ that was adapted from that used in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study (Bingham et al., 1997; Kroke et al., 1999). Details of adaptations are provided in the Supplemental material.

Physical activity and sedentary behaviour. Physical activity and sedentary behaviour were assessed subjectively via a self-reported lifestyle questionnaire used previously in the EPIC Study (Cust et al., 2008) and used in the BORICC Study at baseline. This was extended to collect information on sedentary behaviour using the Longitudinal Aging Study Amsterdam sedentary behaviour questionnaire (Visser and Koster, 2013).

Physical activity and sedentary behaviour were also assessed objectively using the GENEActiv™ accelerometer. Participants were asked to wear the accelerometer on their non-dominant hand continuously for 7 days prior to their study visit. Sleep logs were used to determine sleep and non-sleep times for participants and to identify shift workers. An accelerometer record sheet was also completed to record any periods of time when the accelerometer was removed.

Sunlight exposure questionnaire. Habitual sunlight exposure, including day-to-day sun exposure and sun exposure during holidays, was assessed using an adapted questionnaire (Cashman et al., 2008). This sunlight exposure questionnaire has been used previously to determine the effect of sunlight exposure on nutritional requirements for vitamin D with good predictability.

Urine and stool sample collection. Equipment for the collection of urine and stool at home were included in the study packs posted to participants. Participants were asked to collect two urine samples, one on a weekend day and one on a weekday. Mid-stream urine specimens were collected in a sample collection container and transferred to four vacutainer tubes which were stored in the fridge. Stool samples were collected in a Fecotainer® (AT Medical, The Netherlands). Participants were asked to collect their stool samples on the day of, or the day before, their study visit. Participants recorded the date and time the urine and faeces samples were collected and brought these to their study visit in the cool bags provided. The stool samples were homogenised by mashing in a strong, zip-lock bag and aliquoted using sterile plastic spatulas into five bijou tubes. Urine and stool samples were stored at −80°C.

Study visit

Study visits, lasting approximately 45 minutes (30 minutes if rectal mucosal biopsies were not collected), were carried out at North Tyneside General Hospital (UK). Consent forms were counter-signed by a member of the research team, questionnaires and paperwork were checked to ensure completion and the accelerometers were collected. Anthropometric measures (described below) and three musculoskeletal function tests (heel bone densitometry, hand grip strength and timed up and go test) were performed. A clinical member of the research team (AJ or KEG) completed the Health & Medications Questionnaire and described the clinical procedures (buccal cell swabs, blood samples and collection of rectal biopsies) to be made. In the clinical sample room, samples were collected in the order blood samples, buccal cell sample and rectal mucosal biopsies.

Anthropometric measurements. Height was measured using a Leicester stadiometer (Seca) in the Frankfurt Plane and Tanita digital scales (Tanita Europe B.V., The Netherlands) were used to measure body mass, body fat percentage and body mass index (BMI). Waist and hip circumferences were measured as described by Marfell-Jones et al. (2006). All measurements were performed twice or repeated until within 0.1 cm for height, 0.1 kg for weight and 1 cm for waist and hip circumferences. The mean of the two closest measurements was used for further analyses.

Heel bone densitometry. Heel bone density was measured using the Achilles' heel ultrasound device (GE AchillesTM EXPII ultrasonometer) in compliance with the product manual guidelines. Participants were asked to remove their shoes and socks and sit on a chair. Participants placed one foot in the footplace of the instrument making sure the heel was as far back as possible and the calf was resting on the calf rest. Participant details (participant identification, age, and gender) were entered into the instrument. Membranes of the heel ultrasound device and either side of the heel were sprayed with 70% ethanol solution and the measurement was made. The measurement was made twice for both heels and an average calculated for each heel. Calibration and quality control tests were completed prior to each participant using a phantom.

Hand grip strength. Hand grip strength was measured with a Jamar dynamometer. The participant's dominant hand was

recorded as well as any problems or pain in their arms or hands. Participants sat on a chair with an armrest and rested one forearm on the armrest, with the elbow bent at a 90° angle. Three measurements were made per arm, following a practice measurement.

Timed up and go test. Prior to starting, participants were asked if they suffered from any lower body problems or pain. Participants were asked to sit on a chair and, when instructed, to stand up, without help from arms or leaping, and to walk three metres to a floor marker at a comfortable speed without running, turning, walking back to the chair and sitting down, again without jumping or helping with their arms. The timer was started as soon as the participants were instructed to 'go' and stopped as soon as the participant sat down. The test was repeated three times with a 30 second break between tests.

Biological sample collection

Buccal cell swabs. Participants were asked to swab up and down the inside of their cheeks firmly with the buccal swab (SK-2 S DNA buccal swabs (Isohelix™, UK)) for one minute. Swabs were placed immediately in the 2 ml collection tube and 500 µl BuccalFix stabilisation buffer (Isohelix™, UK) were added.

Blood sample collection. Blood samples were collected in four 5 ml BD Vacutainer® SST™ II Advance tubes with gold hemogard closure (Becton Dickinson, UK), two 4 ml BD Vacutainer® K_3EDTA tubes (Becton Dickinson, UK) and one 5 ml fluoride/oxalate tube. An aliquot of whole blood from a K_3EDTA tube was separated to be sent for analysis of haemoglobin A1c (HbA1c) concentration. Vacutainers were centrifuged at 7000 rpm for 10 minutes immediately after collection. Serum and plasma were aspirated and divided into 500 µl aliquots in 1.5 ml centrifuge tubes. After aspiration of serum from the BD Vacutainer® SST™ II Advance tube with gold hemogard closure tubes, white blood cells were removed using a Pasteur pipette and aliquoted into four separate 1.5 ml centrifuge tubes. Aliquots of whole blood, plasma and serum were sent immediately to North Tyneside General Hospital laboratories for analysis of HbA1c, glucose and lipids, respectively. Serum aliquots were sent to the Newcastle Laboratories (Royal Victoria Infirmary and Freeman Hospital, Newcastle) for analysis of high-sensitivity C-reactive protein (hsCRP), folate, vitamin B12, vitamin D (total 25(OH)D) and parathyroid hormone (PTH). The remaining samples were frozen immediately and stored at -80°C for subsequent analyses.

Human colorectal tissue samples. Ten biopsies were taken from the mid-rectum (10 cm from the ano-rectal verge) from the apparently-normal, healthy mucosa using Sarratt biopsy forceps (Stericom, 2.3 mm diameter, smooth without spike REF STE1500). No bowel preparation was used. Following the rigid sigmoidoscopy procedure, participants were given an aftercare advice information sheet. The ten circumferential biopsies taken from each participant were processed as detailed in Table 1. Snap-frozen biopsies and those in RNAlater® were immediately placed in liquid nitrogen and then stored at −80°C. Two biopsies were formalin fixed and paraffin-embedded and blocked at Northumbria Healthcare NHS Foundation Trust and stored at room temperature. Biopsies preserved in Carnoy's solution (70% ethanol, 30% acetic acid) were kept at 4°C for a minimum of 2 hours and maximum of 12 hours and then transferred to 70% ethanol for long-term storage at 4°C.

Table 1. Biopsy sample collection and storage.

Sample processing/storage	Number of biopsies
Snap-frozen	4
RNAlater® (Ambion, USA)	3
Formalin-fixed paraffin-embedded	2
Carnoy's solution	1

CRC risk outcome measures and statistical analyses

In the BFU Study, we will measure similar outcomes to those measured at baseline in the BORICC Study. This will include miRNA expression and patterns of DNA methylation in rectal mucosal biopsies and in potential surrogate tissues, for example, blood and buccal cells. We will quantify gut-specific and systemic inflammatory markers including faecal calprotectin and hsCRP, respectively. We will assess colonic crypt cell proliferative state, including proliferation rates and the distribution of proliferating cells along the crypt, as a marker of CRC risk. We will investigate effects of ageing and of lifestyle factors such as obesity on mitochondrial function, as well as the involvement of the mitochondria in CRC risk. We will characterise the faecal microbiome and quantify short-chain fatty acids.

Relationships between lifestyle factors measured at baseline with markers of CRC risk at follow-up (12+ years later) will be investigated longitudinally, as well as cross-sectionally at follow-up. Relationships between potential predictors measured at baseline, for example, BMI and outcome variables e.g. faecal calprotectin will be examined by regression controlling for potential confounders such as sex, age, smoking, dietary factors and physical activity. We will also investigate relationships between change in lifestyle factors over 12+ years, such as a change in markers of adiposity, and our outcome measures. For example, to test whether change in adiposity over 12+ years is associated with faecal calprotectin concentration, we will divide our participants into those who gained body weight, those who lost body weight and those whose weight was unchanged, with faecal calprotectin as our outcome measure, using the analysis of variance general linear model.

Table 2. Characteristics of Biomarkers Of RIsk of Colorectal Cancer Follow-Up Study participants.

	All	Males	Females
n	47	23	24
Age (years)	66.7 (49–79)	66.3 (49–78)	67.2 (52–79)
Menopause status	–	N/A	Post-menopausal
Body mass index (kg/m^2)	28.3 (18.9–39.3)	28.8 (18.9–39.3)	27.8 (21–39)
Weight (kg)	81.5 (56.8–124.5)	91.4 (60–124.5)	72 (56.8–107.5)
Body fat (%)	34.2 (10.9–46.2)	29.7 (10.9 – 41.2)	38.6 (28.9 – 46.2)
Waist circumference (cm)	96.5 (72.7–137.7)	102.4 (74.8–137.7)	80.3 (72.7–115.5)
Hip circumference (cm)	105 (90.3–136.8)	106.3 (90.3–136.8)	103.7 (92.9–128.5)
Smoking status			
Current smokers	1 (2)	1 (4)	0 (0)
Ex-smokers	22 (47)	11 (48)	11 (46)
Non-smokers	24 (51)	11 (48)	13 (54)

Note: data are presented as means and ranges are in parentheses. For smoking status, data are presented as number of participants and proportion in parentheses (%).
N/A: Not applicable.

Table 3. Proportions (%) of participants in each body mass index (BMI) category in the Biomarkers Of Risk In Colorectal Cancer Follow-Up (BFU) Study, in England and in the North East of England using data from the Health Survey for England 2017 (Health Survey for England, 2018).

BMI category	BFU Study	England (persons aged 16 and over)	England (persons aged 65–74)	North East England (persons aged 16 and over)
Underweight	0.0	2.0	1.0	2.0
Normal weight	29.8	34.0	24.0	32.0
Overweight	38.3	36.0	43.0	37.0
Obese	31.9	29.0	33.0	29.0

Results

Baseline participant characteristics

A total of 47 participants were recruited to the BFU Study, comprising 37 initially-healthy participants (originally recruited to the BORICC 1 arm) and 10 polyp participants (originally recruited to BORICC 2) (Table 2). All participants were Caucasian. 51% of participants were females, all of whom were post-menopausal. The mean age of participants was 67 years (range 49–79 years). The mean BMI was 28.3kg/m^2 and the means of other markers of adiposity were above recommended cut-offs for healthy body composition (Table 2). Only one participant was a current smoker.

The proportion of BFU participants in each BMI category and comparisons with data from England acquired from the Health Survey for England (HSE) 2017 are summarised in Table 3. Approximately 38% and 32% of BFU study participants were overweight and obese, respectively, which is similar to data for England for persons aged 65–74 years. In the 2017 HSE, overweight and obesity prevalence increased with age, peaking in the 55–64 age range (Health Survey for England, 2018). Within England, overweight and obesity rates vary by region and are greatest in the North of England. In the North East of England, 37% and 29% of persons aged 16 and over are overweight or obese, respectively (Health Survey for England, 2018) which is similar to data for the BFU Study participants (Table 3).

Blood-based phenotypic markers for BFU Study participants are summarised in Table 4. Values were comparable for male and female participants. Almost half (19 participants) had hsCRP concentrations ≥2mg/L. For the majority of participants, values were within the normal range for the other analytes. All participants had 25(OH)D concentrations classified as vitamin D sufficient (>25 nmol/L) using the UK criteria (National Institute for Health and Care Excellence, 2016). For vitamin B12, two participants (one male and one female) had concentrations below the recommended 145 pmol/L and one female participant had a vitamin B12 concentration above the normal range. One female participant had a serum folate concentration suggestive of folate deficiency (<3ug/L). PTH concentrations were above the recommended value of 6.4 pmol/L for six participants but none was below 1.1 pmol/L. HbA1c concentration was above the diabetes cut-off (48 mmol/mol) for five participants all of whom were already diagnosed with type 2 diabetes. A total of 23 participants had total cholesterol concentrations >5 mmol/L and 17 had a total cholesterol to high-density lipoprotein (HDL) cholesterol ratio greater than 4. Two female

Table 4. Blood phenotype markers: inflammatory, glucose control, lipid profile and nutrient status markers.

	Optimal values	Proportion of participants within optimal range (%)	All	Males	Females
High-sensitivity C-reactive protein (mg/L)[a]	<2.00	60	3.41 (0.838)	2.43 (0.524)	4.35 (1.55)
Haemoglobin A1c (mmol/mol)[a]	<48.0	89	40.5 (1.06)	40.2 (1.40)	40.8 (1.61)
Cholesterol (mmol/L)[a]	<5.0	51	5.25 (0.19)	5.03 (0.179)	5.46 (0.328)
High-density lipoprotein (HDL) (mmol/L)[b]	1.2–1.8 (female) 1.0–1.5 (male)	64	1.50 (0.058)	1.41 (0.097)	1.60 (0.0583)
Total cholesterol to HDL-cholesterol ratio[b]	<4.0	64	3.68 (0.155)	3.85 (0.219)	3.51 (0.217)
Non-HDL cholesterol (mmol/L)[b]	<4.9	81	3.77 (0.183)	3.62 (0.179)	3.93 (0.321)
Serum folate (ug/L)[a]	≥3	98	7.26 (0.422)	8.18 (0.687)	6.33 (0.422)
Vitamin B12 (pmol/L)[a]	145–569	94	284 (15.9)	289 (14.7)	281 (27.6)
25(OH) vitamin D (nmol/L)[a]	>25	100	66.3 (4.20)	61.3 (6.20)	71.2 (5.70)
Parathyroid hormone (pmol/L)[a]	1.10–6.40	87	4.50 (0.200)	4.20 (0.300)	4.90 (0.300)

Note: data are presented as means (standard error of the mean): [a]$n = 47$; and [b]$n = 46$.

participants were below, and four were above, the recommended guidelines (1.2–1.8 mmol/L) for HDL cholesterol. Three and eight male participants were below and above, respectively, the reference values for males (1.0–1.5 mmol/L). Nine participants had high non-HDL concentrations (≥4.9 mmol/L).

Discussion

The BFU Study is a 12+ years follow-up of participants in the BORICC Study that has been designed to investigate relationships between ageing and lifestyle factors, such as diet, adiposity and physical activity, and markers of healthy ageing and of CRC risk. We re-recruited 47 of the original 363 BORICC Study participants; approximately half of the BFU Study participants were female and the mean age of all participants was 67 years. Within the BFU Study, 70% of participants were overweight or obese. This is comparable to the proportion of overweight and obese persons aged 16 and over in England (64%) and in the North East (63%) and slightly lower than the national prevalence reported for those aged 65–74 (75%) (Health Survey for England, 2018).

Our *a priori* power calculation estimated that we would require 53 participants to detect a statistically significant effect of age on FCP, a local marker of intestinal inflammation. Although we re-recruited just under this number, this power calculation was based on an age difference of 10 years whereas the longitudinal aspect of this study was 12+ years. In addition, the original power calculation was based on cross-sectional data and, since this is a longitudinal study in which we will make within-participant comparisons over time, it is likely that we will have greater power to detect effects.

Study implications

Samples and data from the BFU Study will be used to investigate the mechanisms through which ageing and lifestyle factors, particularly diet, physical activity and adiposity, influence markers of large bowel health and CRC risk and of healthy ageing (primarily musculoskeletal function). We will focus on mitochondrial function, epigenetic mechanisms, WNT signalling and colonic crypt cell proliferative state in colonocytes and on the intestinal microbiome and their short-chain fatty acid metabolites. We will investigate the effects of lifestyle measured at baseline, particularly diet, adiposity and physical activity, on biomarkers of CRC risk 12+ years later and also examine these cross-sectionally in the BFU Study. Understanding the underlying mechanisms through which protective or detrimental effects of lifestyle factors are achieved could lead to more effective early prevention strategies and lifestyle interventions.

We will also investigate whether these ageing and lifestyle-related markers of CRC risk can be detected in surrogate tissues, such as in blood or buccal cells, and so reduce the need for invasively-collected biopsies from the large bowel in the future. The ability to make these biomarker measurements in surrogate tissues will: (i) make it easier for participants in studies; (ii) lower the costs for research by avoiding the need for expensive investigations of the large bowel by specialist doctors; and (iii) speed up the process of biomarker measurement.

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Authors' contributions

JCM, DMB, LCG and FCM designed the study. FCM, SPB, KEG, AJ and RMTKR performed the clinical study, data collection and sample collection and processing. FCM performed data processing and statistical analyses. FCM and JCM wrote the manuscript. SPB, RMTK and

TRH edited the manuscript. All authors read and approved the final manuscript.

Ethical approval

Ethical approval for the Biomarkers Of RIsk of Colorectal Cancer (BORICC) Follow-Up (BFU) Study was granted by the West Midlands – Coventry and Warwickshire Research Ethics Committee on 29th November 2016 (REC No. 16/WM/0424).

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental material

Supplemental material for this article is available online.

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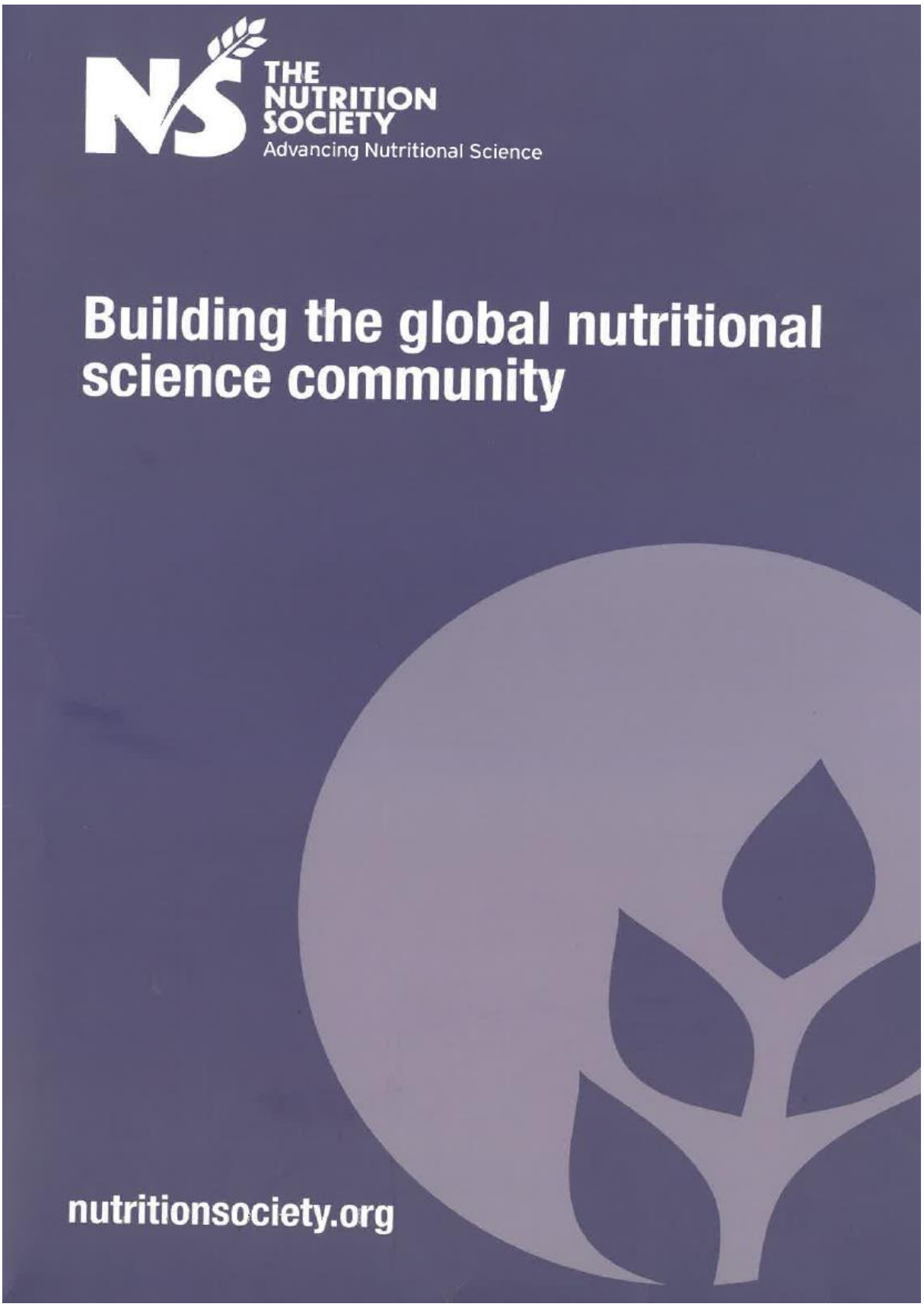
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Relationship between sun exposure behaviors and muscle function and bone strength of older adults in North East England. By RMTK Ranathunga, FC Malcomson, TR Hill and JC Mathers *Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, NE2 4HH, UK*

Use of sun exposure behavior as a proxy indicator of vitamin D status and function is still debated [(1)]. The aim of this study was to investigate relationships between sun exposure behaviors and muscle function and bone strength in older adults. A total of 47 older adults were re-recruited from those who participated in a study of biomarkers of risk of colon cancer 12 years ago in North East England (N°55) [(2)]. Current sun exposure behaviors including frequency and duration of sun exposure during weekends, weekdays and holiday visits, sunblock usage and sun avoidance behavior were collected using a sun exposure questionnaire. Muscle function was measured by Grip Strength (GS (kg) using a hand grip dynamometer) and Timed-Up and Go test (TUG) as the time (s) to walk 3 m distance. Bone strength was measured as Stiffness Index (SI) using a bone heel ultrasound scanner. One-way ANOVA was used to identify associations between sun exposure variables, muscle function and bone strength using SPSS package. Mean age of the adults was 66.8 (SD 8.3) years and the mean BMI was 28.3 (SD 4.7) kgm^{-2}. Approximately 28% of the participants reported sun exposure 2 times per week, while 72% reported sun exposure every day in the sunny months. About 15% of participants avoided sun exposure and 30% enjoyed the sun exposure during the sunny months. Approximately half of the adults (49%) had had a holiday during the last three months. Individuals who always enjoyed the sun exposure had greater GS, SI and lower TUG test value compared with those i who avoided sun exposure or sometimes enjoyed the sun exposure. This difference was significant for GS (p=0.005) (Table 1). There were no significant associations between measures of musculoskeletal health and other sun exposure behaviors including holiday visits and the frequency of sun exposure.

Sun exposure behaviors	GS (kg) Mean (SD)	TUG (s) Mean (SD)	SI Mean (SD)
Sun avoidance			
Avoid (n=7)	18.72 (14.5)	11.69 (6.7)	79.21 (7.6)
Sometimes avoid (n=24)	27.34 (18.1)	8.70 (4.0)	90.40 (8.4)
Always enjoy (n=14)	32.11 (20.2)*	8.49 (1.4)	97.83 (8.3)
p value	*0.005*	*0.184*	*0.100*
Holiday visits			
Yes (n=21)	29.48 (10.3)	9.56 (4.1)	89.95 (18.5)
No (n=23)	26.04 (7.9)	8.57 (4.1)	92.37 (20.0)
p value	*0.219*	*0.424*	*0.68*
Frequency of sun exposure			
More than 2 times/week (n=13)	29.16 (8.30)	9.57 (5.26)	99.11 (15.39)
Everyday (n=33)	26.87 (9.52)	8.92 (3.61)	88.34 (19.40)
p value	*0.645*	*0.638*	*0.102*

GS – Grip Strength *TUG – Timed-Up and Go test* *SI – Stiffness Index*

** Significantly differ between sun avoidance and always enjoy groups.*

Table 1 : Relationship between sun exposure behaviors, muscle function and bone strength

In conclusion, older adults who avoided sun exposure had lower muscle strength than those who always enjoyed sun exposure.

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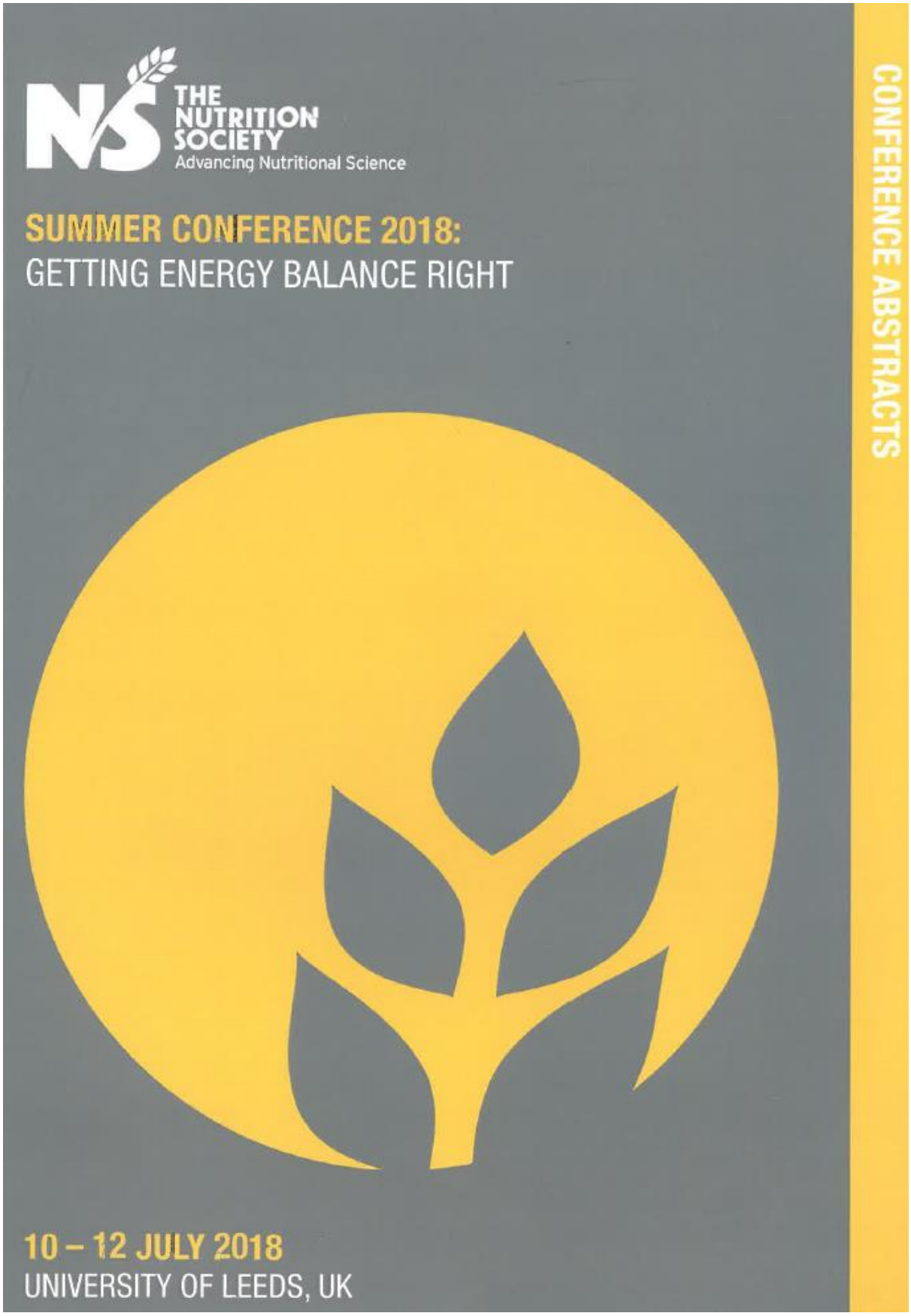
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CONFERENCE ABSTRACTS
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The association between circulating 25-hydroxyvitamin D concentration and Grip Strength and Timed-Up and Go test in 70+ year old adults : Baseline findings from the Vitamin D Supplementation in Older People (VDOP) study. RMTK Ranathunga[1], TR Hill[1], JC Mathers[1], I Schoenmakers[2] and TA Aspray[3] [1]Institute of Cellular Medicine, Human Nutrition Research Center, Newcastle University, Newcastle-upon-Tyne, NE2 4HH, UK [2]MRC Human Nutrition Research, Cambridge, Medical School, University of East Anglia, Norwich, NR4 7TJ, UK. [3]Institute of Cellular Medicine, Freeman Hospital, Newcastle-upon-Tyne, NE7 7DN, UK.

Vitamin D has important regulatory effects in muscle tissue(1). Cross sectional studies demonstrate that circulating 25-hydroxyvitamin D (25OHD) [the indicator of vitamin D status] is associated with better muscle function but the optimal 25OHD concentration for muscle function is not universally agreed. The objective of this study was to identify the association between serum 25OHD concentration and Grip Strength (GS) and Timed-Up and Go test (TUG) using 25OHD thresholds used in North America (≥50 nmol/l)(2) and the UK (≥25 nmol/L)(3) used to define nutritional adequacy for vitamin D.

A total of 377 male and female adults aged >70 years living in the North-East of England were recruited. GS (in kg) was measured by a hand grip dynamometer and TUG was measured as the time (in seconds (s)) to walk 2m distance. Serum 25OHD concentration was measured by LC-MS. Weight, height and body composition were also measured. In separate analyses, individuals were grouped according to the circulating 25OHD concentration < or ≥ 25 nmol/L and < or ≥ 50 nmol/L. Male and female older adults were grouped into two separately, based on the gender specific median values of GS and TUG test values. The odds ratios to have high GS and low TUG test values were identified using logistic regression analysis after adjusting for age, weight, height and body composition.

			Males			Females		
Classification		**n**	**OR***	**CI**	**p**	**OR***	**CI**	**p**
	GS							
SACN	25OHD < 25 nmol/L	102	0.371	0.163- 0.841	0.018	0.652	0.334- 1.272	0.209
	25OHD ≥25 nmol/L	275	**Reference**			**Reference**		
IOM	25OHD < 50 nmol/L	271	1.202	0.61 – 2.341	0.588	0.739	0.355 – 1.538	0.419
	25OHD ≥ 50 nmol/L	106	**Reference**			**Reference**		
	TUG test							
SACN	25OHD < 25 nmol/L	102	1.274	0.59 – 2.741	0.536	0.695	0.358 – 1.350	0.283
	25OHD ≥25 nmol/L	275	**Reference**			**Reference**		
IOM	25OHD < 50 nmol/L	271	0.910	0.474-1.74	0.777	0.537	0.257-1.120	0.098
	25OHD ≥ 50 nmol/L	106	**Reference**			**Reference**		

Mean serum 25OHD concentration of older adults was 39.8 (±19.9) nmol/L. About 21.0 % of males and 33.7% of females had serum 25OHD concentration less than 25 nmol/L. Similarly, 67.9 % of males and 76.2% of females had serum 25OHD concentration less than 50 nmol/L. Only for male, those with serum 25OHD concentration lower than 25nmol/L were significantly more likely to have lower GS compared to the individuals who had serum 25OHD concentration higher than 25 nmol/L. However, there was no significant relationship between 25OHD status and TUG for either gender. In conclusion, in male older adults, serum 25OHD concentration lower than 25 nmol/L is associated with lower GS but there was no evidence of musculoskeletal benefit from having serum 25OHD concentrations higher than 50 nmol/L for either gender. Therefore, based on this cross sectional analysis, the optimum serum 25OHD concentration to maximize the muscle strength for older males is likely to be between 25-50 nmol/L.

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PROGRAM BOOK
2018
VITAMIN D WORKSHOP

NO EFFECT OF MONTHLY SUPPLEMENTATION WITH 12000IU, 24000IU AND 48000IU VITAMIN D_3 FOR 1 YEAR ON MUSCLE FUNCTION: THE VITAMIN D SUPPLEMENTATION IN OLDER PEOPLE (VDOP) STUDY. RMTK Ranathunga[1], TR Hill[1], JC Mathers[1],I Schoenmakers [2] and TA Aspray[3] [1]Institute of Cellular Medicine, Human Nutrition Research Center, Newcastle University, Newcastle-Upon-Tyne, NE2 4HH,UK [2]MRC Human Nutrition Research, Cambridge, Medical School, University of East Anglia, Norwich, NR4 7TJ, UK [3]Institute of Cellular Medicine, Freeman Hospital, Newcastle-upon-Tyne, NE7 7DN, UK.

The optimal dose of vitamin D supplementation for muscle function is not universally agreed. The objective of this study was to determine the effect of monthly supplemental vitamin D_3 (12000IU, 24000IU and 48000IU) for 12 months on Grip Strength (GS) and Time-Up and Go test (TUG) in 75+ years old adults living in the North-East of England. GS was measured by a hand grip dynamometer in kilograms (kg) and the TUG test measured by the time taken to walk 2m distance in seconds (s) in participants before and after vitamin D_3 treatment. Fasting serum total 25(OH)D concentration of pre and post intervention was measured by LC-MS. Pre-intervention serum 25(OH)D, GS and TUG were 39.9($\pm$19.9) nmol/L, 28.5($\pm$12.8) kg and 11.4($\pm$3.1) s, respectively. Using, Analysis of Variance (ANOVA), there was a significant dose-related response in serum 25(OH)D concentration after 12 months of vitamin D supplementation ($p<0.000$). The change (Δ) in GS and TUG from pre to post-intervention was unaffected by the three doses of vitamin D_3. Results remained unchanged after adjustment (using ANCOVA) for age, gender and baseline serum 25(OH)D, GS and TUG. Stratifying participants into having baseline serum 25(OH)D concentrations above and below the median in each vitamin D treatment arm had no effect on the change in GS and the TUG test. In conclusion, vitamin D supplementation at 12000IU, 24000IU and 48000IU monthly for 12 months had no effect on GS and TUG test in older adults aged >75+ years. This study was supported by a number of funding sources including Arthritis Research UK, MRC Human Nutrition Research (funded by the UK MRC) and Newcastle University.

	12000IU(n=112)		24000IU(n=113)		48000IU(n=114)			
	Mean	SD	Mean	SD	Mean	SD	P	p*
Δs 25(OH)D (nmol/L)	14.27	16.63	25.32	18.02	40.86	19.75	<0.000	<0.000
Δs GS (kg)	-2.78	11.64	-3.62	8.67	-2.57	7.84	0.670	0.251
Δs TUG test (s)	0.56	2.32	0.46	2.77	0.15	2.5	0.449	0.439

*Adjusted for age, gender, baseline values of 25(OH)D, TUG and GS

Nutrition Society summer conference 2017, Abstract

Sunshine exposure and serum 25OHD concentrations during a 12 month randomized control trial with high dose vitamin D supplementation: results from the Vitamin D Supplementation in Older People (VDOP) study[(1)]. By RMTK Ranathunga[1,2], TR Hill[1,2], JC Mathers[2,3], I Schoenmakers[4] and TA Aspray[3,5] on behalf of the VDOP study team.*[1]Agriculture Food and Rural Development, Newcastle University, NE1 7RU, [2]Human Nutrition Research Center, Newcastle University, NE1 7RU, [3]Institute of Cellular Medicine, Newcastle University, NE4 4PL, [4]MRC Human Nutrition Research, Cambridge, Medical School, University of East Anglia, Norwich, NR4 7TJ, [5]Freeman Hospital, Newcastle upon Tyne, NE7 7DN.*

Assessing the relative contribution of sunshine exposure to vitamin D status in randomized control trials with vitamin D supplementation is of paramount importance. The objective of this study was to determine the effect of yearly sun exposure on serum 25OHD concentrations in older adults supplemented with 12 monthly doses of 12000IU, 24000IU and 48000IU vitamin D_3. Serum 25OHD concentration was measured by Tandem Mass Spectrometry at baseline and after 12 month supplementation in 327 adults aged >70 years[(1).] Sunshine exposure of the participants was assessed every three months (baseline, after 3 months, 6 months, 9 months and after 12 months) by a questionnaire which assessed the frequency of outdoor activities, skin exposure area exposed to sun, sunblock usage, holiday visits and Fitzpatrick skin types. Of the 327 participants, 62% of participants began the 12 months supplementation during the time of year where there is no UVB radiation at the study location (55^0N), defined as October to March[(2)]. Composite scores for each sun exposure variable were calculated from the mean of the five questionnaires (including baseline and 12 months follow-up visits). For each treatment arm of the trial, sunshine exposure variables as well as other socio-demographic, lifestyle and dietary factors were included as potential predictors of endpoint serum 25OHD using backward linear regression.

In those participants receiving 12000IU monthly, summer sun exposure, skin exposure area exposed to sun and female gender were positively associated with endpoint vitamin D status while skin type IV, calcium intake and BMI were negatively associated with endpoint vitamin D status. For participants randomized to 24000 IU and 48000 IU monthly, none of the sunshine exposure variables were significantly related to vitamin D status. The proportion of the variance in endpoint serum 25OHD concentration explained by the variables in the model were 36%, 21% and 16% for the 12000IU, 24000IU and 48000IU treatment arms, respectively.

Sun exposure variables	ß	SE	95%CI	P-value
12000IU group (n=100, R^2=0.36)				
Summer sun exposure (vs winter sun exposure)	0.321	2.702	4.311, 15.059	0.001
Skin exposure area	0.396	10.017	24.234, 64.080	<0.001
Fitzpatrick skin type IV (vs type I)	-0.207	4.134	-17.74,-1.292	0.024
Calcium intake	-0.185	0.005	-0.021, 0.000	0.041
Females (vs males)	0.212	2.808	0.817,11.985	0.025
BMI	-0.237	0.373	-1.703,-0.219	0.012
24000IU group (n=112, R^2=0.21)				
Sunblock use	0.191	2.963	-0.152, 11.633	0.056
BMI	-0.392	0.379	-2.262, - 0.754	<0.001
48000IU group (n=115, R^2=0.16)				
Age	-0.216	0.349	-1.436,-0.050	0.036
BMI	-0.359	0.379	-2.048, -0.559	0.001

Endpoint serum 25OHD as dependent variable and frequency of outdoor activities, skin exposure area exposed to sun, Fitzpatrick skin types, sunblock usage, sun exposure season, length of holiday visits, holiday season, calcium and vitamin D intake, age, gender and BMI as dependent variables.

In conclusion, a number of sunshine exposure variables predicted endpoint vitamin D status in participants receiving 12000 IU monthly but vitamin D supplementation appears to overwhelm the effect of sun exposure on 25OHD concentration at higher doses in older adults.

The VDOP study is supported by a grant (MP/ID19544) from Arthritis Research UK and partly supported by Newcastle University and funding from the core programme of the MRC Nutrition and Bone Health Group at MRC Human Nutrition Research funded by the UK MRC, grant code U10596037.

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