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The relationship between vitamin D and muscle strength and function in UK South Asian older women

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The relationship between vitamin D and muscle strength and function in UK South Asian older women

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Abstract

Background: A positive association has been reported between circulating blood 25(OH)D concentration, muscle strength and physical function in older Caucasian women. However, little is known about this relationship in UK South Asian older women. This thesis aimed to explore this cross-sectional and causal relationship between circulating blood 25(OH)D concentration, muscle strength and function in this population.

Methods: Two investigations were conducted: i) a cross sectional study of 120 post-menopausal South Asian women and ii) a randomised control trial of vitamin D in older South Asian women with 25(OH)D concentration <50nmol/L. Inclusion criteria: able to communicate and able to give informed written or verbal consent. Blood 25(OH)D concentration was measured and the assessment of muscle strength and function included handgrip strength, single and repeated chair stand, balance, timed up and go and walking speed test. For the cross-sectional study these measurements were taken on one occasion. For the RCT, all assessments were performed at baseline and end of intervention.

Results: The cross-sectional study found that 47% of women had blood 25(OH)D concentration <50nmol/L and 41%of the women reported using vitamin D supplements. An inverse cross-sectional relationship was found between circulating blood 25(OH)D concentration and single chair stand (r=-0.27, p=0.004); repeated chair stand (r=-0.24, p=0.01) and timed up and go (r=-0.19,p=0.03). No association was found between vitamin D and handgrip strength or balance. In the randomised control trial, the 12-weeks vitamin D supplementation of 3000IU/day significantly increased blood 25(OH)D concentration however, did not improve muscle strength or function.

Conclusion: The prevalence of vitamin D inadequacy is high in older South Asian women not taking vitamin D supplementation. Significant weak negative cross-sectional association was observed between blood 25(OH)D concentration and some aspects of muscle strength and function. Twelve weeks supplementation with 3000IU vitamin D did not improve muscle strength and function in this population.

Dedication

I would like to dedicate this PhD to ALLAH who is most merciful and beneficent, Prophet MUHAMMAD (PBUH) and AHLE BAIT (as) for keeping grace and generosity on me. I am proud of being related to THEM. To my Mother, Nasim Shahbaz, for working hard her life to provide me a high standard education and allowing me and believing more in me than myself to fulfil my dreams. Mum, I can never pay you back of what you have been doing for me. To my father (Shahbaz Ali), uncle (Zafar Anjum), brother (Musharraf Raza) and sister (Noreen Zahra) for all their encouragement, support and trust in me. Equally, to my supervisors, Liz Williams and Bernard Corfe, for their kindness and support throughout my PhD. To all of the friends and colleagues, who made my everyday a pleasure and enjoyable.

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List of Abbreviations

25(OH)D	25 Hydroxyvitamin D
IU	international unit
μg	microgram
nmol/L	nanomoles per litre
ng	nanogram
BMI	Body Mass Index
SA	South Asians
ANOVA	Analysis of Variance
GP	General Practitioner
IOM	Institute of Medicine
SACN	Scientific Advisory Committee on Nutrition
COMA	Committee on Medical Aspects of Food and Nutrition Policy
HGS	Handgrip strength
SCS	Single chair stand
RCS	Repeated chair stand
TUG	Timed Up and Go
N	Sample size
PA	Physical Activity
WHO	World Health Organization
EFSA	European Food Safety Authority
NDNS	National Diet and Nutrition Survey
EAR	Estimated Average Reference
DRI	Dietary Reference Intake

DRV	Dietary Reference Value
RNI	Reference Nutrient Intake
UVB	Ultra-violet B radiation
G	gram
Sec	Second
Kg	Kilogram
VDR	Vitamin D Receptor
DBP	Vitamin D Binding Protein
DNA	Deoxyribonucleic acid
PTH	Parathyroid hormone
7-DHC	7-Dyhydrocholesterol
SD	standard deviation
RCT	Randomised Controlled Trial
KCal	Kilocalories
m/s	Metres per second
%	Percentage
ID	Identification
IPAQ	International Physical Activity Questionnaire

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1 Chapter 1. General Introduction

1.1 Background

Vitamin D was first described as a vitamin in the early 20th century and is now known to be a prohormone (2). Vitamin D, also known as calciferol, is fat soluble in nature and has two major forms: cholecalciferol (D_3) and ergocalciferol (D_2). Cholecalciferol is produced by exposure of 7-dyhydrocholesterol (7-DHC) in the epidermis of the skin to UVB rays of the sunlight at a wavelength of 290-320nm (2). It can also be obtained from the consumption of animal-based foods. In contrast, ergocalciferol is synthesised by plants and fungi. Both cholecalciferol and Ergo-calciferol undergo the same process of absorption and metabolism in the human body and have same biological activities. Both forms can be used in the fortification process and are available over the counter in a supplementary form. The units used for expressing vitamin D is IU (international unit) or μg (microgram). 1μg is equal to 40IU. The units used to measure circulating blood 25(OH)D concentration in the body is nmol/L (nano mol per litre) and ng (nano gram). 1ng is equal to 2.5nmol/L.

1.1.1 Sources of vitamin D

Vitamin D in the human body is derived from exogenous sources and endogenous synthesis. Exogenously vitamin D is derived from the diet and supplements, and endogenous synthesis is achieved through skin exposure to sunlight. This dual source of this vitamin makes it challenging in terms of measuring circulating blood 25(OH)D concentration and setting the Reference Nutrient Intake for this vitamin.

1.1.1.1 Cutaneous source

The natural precursor 7-dyhydrocholesterol is present in the epidermis layer of the skin and reacts with UVB radiation of sunlight during skin exposure and converts to pre-vitamin D_3 (3). Pre-vitamin D_3 is a temperature sensitive hormone in nature therefore undergoes a temperature dependent isomerisation reaction in the skin over the following 2-3 days and transforms to a thermostable form of vitamin D_3 which is also known as Cholecalciferol (3). The vitamin D_3 diffuses into dermal capillary and transport to the circulation.

Cutaneous vitamin D synthesis is estimated to have the potential to provide about 90% of the vitamin D requirement of the human body (4) however, this can be influenced by number of factors including season of the year (5), latitude (6), increasing age (7), skin tone (8) and the amount and duration of skin exposure to UVB rays of sunlight (9). Webb et al. (2010) in their

prospective cohort study measured circulating 25(OH)D level and personal sunlight exposure over a 12 month period in 125 white adults aged 20-60 years old in Greater Manchester. They reported the highest levels of circulating 25(OH)D to occur in the month of September (with zero deficiency), with declining vitamin D status across the winter and that levels in excess of 30ng/ml in September where required in order to maintain adequacy by February (5). The importance of sunlight exposure is further evidenced by the prevalance of vitamin D deficiency in people living in higher latitude regions (6).

The amount of vitamin D produced is inversly associated with increasing age and tone of the skin. A study by MacLaughlin and Holick (1985), identified an age dependent decrease in the amount of the epidermal 7-DHC precursor in the skin of older individuals that was associated with a limited capacity of those individuals to synthesize vitamin D (7). In terms of skin tone as a confounding factor to vitamin D synthesis, Clemens *et al.* (1982) conducted a study in which they exposed two light pigmented and three highly pigmented participants to same standard dose of UVB rays of sunlight and measured the vitamin D status after 24-48 hours of exposure (8). They concluded that a six fold greater sunlight exposure was required in participants with highly pigmented skins compared to the particpants with light skin pigmentation to achieve the same rise in vitamin D status. The underlying mechanism reported is that the high amount of melanin pigment allows less penetration of UVB rays into the skin for photochemical reaction to 7-dyhydrocholesterol which ultimately yields low production of vitamin D (8).

1.1.1.2 Dietary source

Diet, fortified foods and supplements provide the exogenous sources of both vitamin D_2 and D_3. The main dietary sources of vitamin D are oily fish, cod liver oil and eggs. The typical content of vitamin D in the principal food sources are shown in Table 1.1. Aside from natural foods rich in vitamin D, some foods such as cereals, margarines, cheeses and juices are fortified with vitamin D, details of which is given below in section 1.1.1.3. Vitamin D_2 can also be obtained from exposing yeast and mushrooms to UVB rays of sunlight (10). Vitamin D supplements are available in both vitamin D_2 and D_3 forms and both forms are available in the form of capsules, tablets or oral sprays over the counter.

Table 1.1 Principal food sources of vitamin D (µg/serving) Adapted from (11)

Food	Vitamin D (µg/100g)
Herring (grilled)	16.1
Salmon (farmed, grilled)	7.8
Salmon (farmed, steamed)	9.3
Salmon (pink, canned in brine, drained)	13.6
Sardines (grilled)	5.1
Mackerel (grilled)	8.5
Mackerel (smoked)	8.2
Tuna (baked)	3.1
Egg (whole, boiled)	3.2
Egg (yolk, boiled)	12.6
Liver (lamb, fried)	0.9

1.1.1.3 Vitamin D fortification

Mandatory or voluntary vitamin D fortification of dairy products is in process in a few countries including USA, Canada (10), Finland (10), Norway and Sweden (12) on the basis of beneficial effects of vitamin D on various health outcomes (10, 12). In England, margarine fortification by Statute has been in place since World War II (13) but came to end later in 2013 (14). In the United States, the voluntarily fortification of fluid milk with the amount of vitamin D of 385IU per one litre is in place (12).

Recent research has further demonstrated the potential of vitamin D food fortification. In the National Adult Nutrition Survey of the Irish population fortified foods were found to be a major contributor to the vitamin D intake of adults ≥50 years and it was predicted that the combined

consumption of fortified foods could enable 70% of the population to achieve their recommended intakes (15).

O'Neill and colleagues have proposed the use of step-wise approach to develop and validate an integrated model in regards to design and implement the vitamin D fortification plan for UK white and black and Asian minority ethnic (BAME) population (16). The model was based on the use of following components: available literature on ultraviolet B data, hours of sunlight, dose response relationship between 25(OH)D and UVB and dose response relationship between 25(OH)D and vitamin D in above ethnicity populations. This model helped to predict measure 25(OH)D concentration throughout the year in reflection to increase in vitamin D intake via food fortification. The model may help to fill a gap between circulating blood 25(OH)D concentration and vitamin D intake requirement in general population.

The efficacy of vitamin D food fortification was reported by Black et al (2012) in a meta-analysis of fifteen randomized controlled trials. This meta-analysis involved a total of 767 treated adults and 746 controls (both genders) who consumed a range of dairy products fortified with vitamin D dose of 3μg to 25μg per day for a duration of three weeks to one year. Their analysis demonstrated a 1.2nmol/L increase in circulating blood 25(OH)D concentration after the ingestion of each 1μg per day of fortified food (17).

1.1.2 Vitamin D absorption and metabolism

Absorption

Dietary vitamin D is fat soluble and hence is absorbed by fat-solubilising mechanisms in the upper gastrointestinal tract. Ingested vitamin D is localized into the mixed micelles or dissolved into lipid droplets in the duodenum (18). Therefore, efficient absorption of vitamin D is associated with the amount of fat present in the lumen which triggers the release of bile acid and pancreatic acid. Pancreatic acid causes hydrolysation of triglyceride present in the small intestine into mono-glycerides and free fatty acid molecules and bile acid facilitate this process of formation (19). People with bile acid or pancreatic lipase secretion problems are reported to have reduced absorption of vitamin D (19). Furthermore, people with taking cholesterol lowering medication may have impaired absorption of vitamin D (20).

Vitamin D along with these fat molecules are packed together into chylomicrons within the enterocytes (18) and transport to systematic circulation through lymphatic system (21).

The chylomicrons are hydrolysed in peripheral tissues such as adipose tissue and skeletal muscle therefore the vitamin D remaining content present in the chylomicrons can be picked up by these tissues which may cause reduction in the circulating vitamin D levels in plasma. Perhaps this is the reason vitamin D sequestration has reported to be associated with

decreased circulating levels (22). However, the rate of intestinal uptake or the absorption of dietary vitamin D is independent of obesity and ageing (20). Cutaneous vitamin D_3 binds to vitamin D binding protein (DBP) in the circulation and transport to the liver for metabolism (23). Dietary vitamin D_2 and D_3 are incorporated into chylomicrons and transport to circulation via lymph and blood plasma to the liver.

Metabolism

Vitamin D binds to vitamin D binding protein in the blood (23). Due to its lipophilic nature, this is all collectively transported to the liver for metabolism which is similar for vitamin D_2 and D_3 (23, 24), or taken up by adipose tissue and other tissues. The liver and kidneys are two known sites for three steps enzymatic hydroxylation of vitamin D. Primary hydroxylation takes place in the liver where vitamin D is hydroxylated by 25-hydoxylase into 25-hydroxyvitamin D (25(OH)D) which is the major indicator of circulating blood vitamin D level. The half-life of 25(OH)D metabolite is approximately 2-3 weeks whereas vitamin D, the parent compound, has half-life of 1 day only. Kidneys, parathyroid glands and the placenta are the main 25(OH)D uptake organs, through endocytosis by cubilin complex (24).

The kidneys are the site of secondary hydroxylation of 25(OH)D through 1~hydroxylase and yields $1,25(OH)_2D$, also known as calcitriol, which is biologically active metabolite of vitamin D. The half-life of calcitriol is reported as few hours (25). The secondary metabolism in kidneys is tightly regulated by parathyroid hormone due to calcium and phosphorous metabolism. The process of metabolism till here is same for vitamin D_2 and D_3 however, the next step of 24-hydroxylation to form 1,24,25(OH)3D is slightly different for both forms of vitamin D. As, with the formation of $1,24,25(OH)_3D_2$, the vitamin D_2 becomes deactivated and lose its ability to join VDR for its metabolic function (26). Whilst, vitamin D_3 remains in its binding capacity to combine with VDR to perform further action and stays activated even after the formation of $1,24,25(OH)_3D_3$ (27). The metabolites of vitamin D are excreted via bile into the faces and a fraction of it excreted through the urine (2). This difference may well explain on the basis of difference in the side chains of both forms of vitamin D as described in the section 1.1.4. Figure 1.1 shows the process of absorption and metabolism of vitamin D.

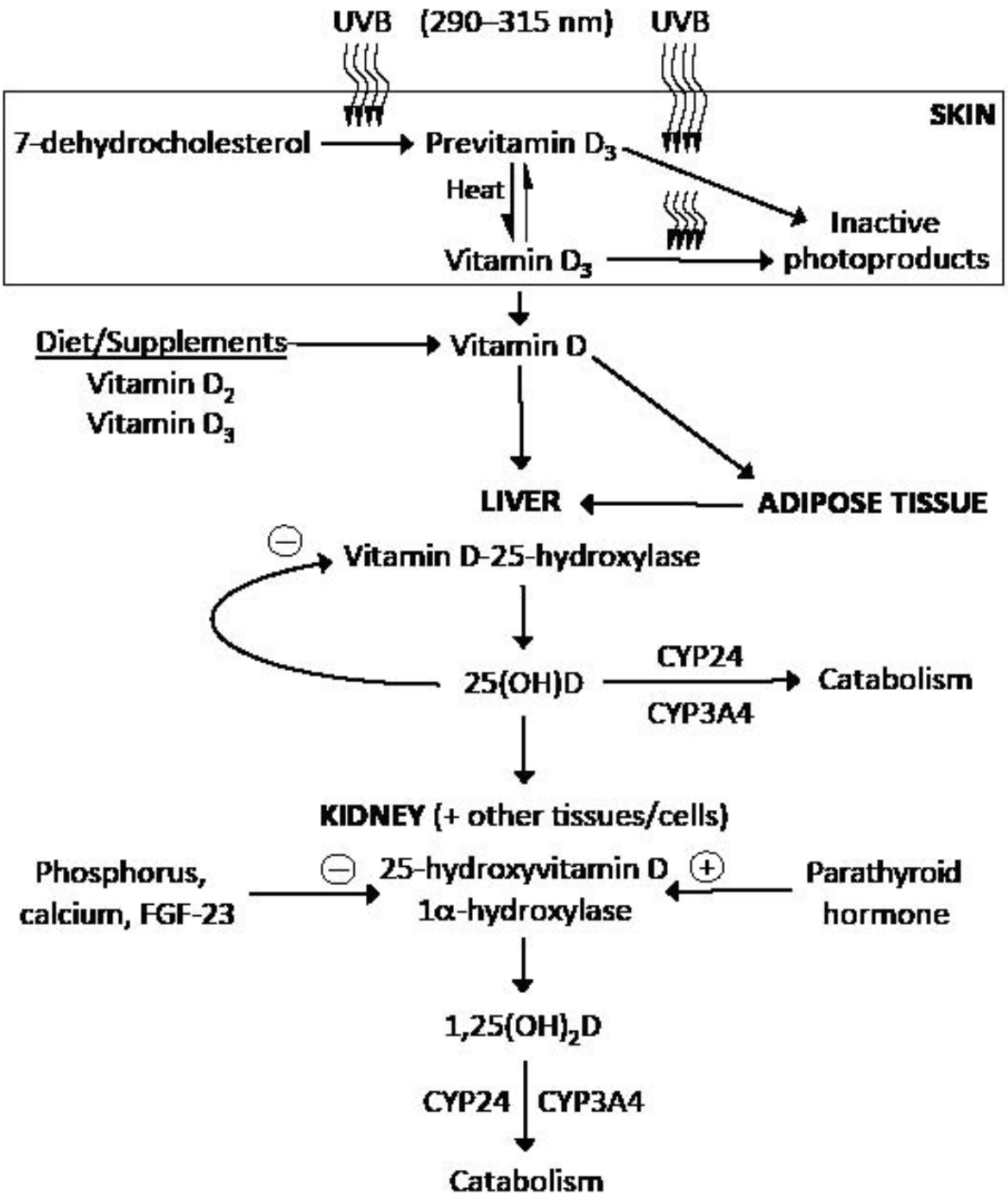


Figure 1.1 Metabolism of vitamin D (28).

This Figure is reused from the literature with the permission of author (28). Followed by the vitamin D absorption in the small intestine, the three steps enzyme hydroxylation take place in the liver and kidneys to form the biological active form of vitamin D.

1.1.3 Functions of vitamin D

1.1.3.1 Mechanism of action

After release from kidneys into the circulation, $1,25(OH)_2D$ has biological response through genomic (regulation of DNA transcription) or non-genomic pathways (through activation of signalling pathways). The action of $1,25(OH)_2D$ occurs through binding with a vitamin D receptor (VDR) which has similar binding affinity for $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$. The VDR is essential for uptake of $1,25(OH)_2D$ by muscle and its action within the muscle (29). There is evidence of the presence of VDR in the intestine, osteoblasts of bone and distal renal tubules of kidneys as calcitriol has calcium and phosphorus regulation activity in these cells. VDR also has reported to be present in number of other cells such as macrophages, lymphocytes, skin, pancreatic cells, ovarian tissue, mammary epithelium, lungs, gonads, placenta and adipose tissue (30). Vitamin D role in skeletal muscle is a focus of this thesis.

Genomic pathway

$1,25(OH)_2D$ binds with VDR which leads to conformational changes that allows VDR to form heterodimer complex with retinoid X receptor (RXR). The complex $1,25(OH)_2D$-VDR-RXR is translocated to the promoter region of gene within the muscle cells and binds to vitamin D response elements (VDRE) which promotes the interaction of VDR with DNA, target vitamin D specific gene and alter protein synthesis (31). Montenegro et al., 2019 has treated human skeletal muscle myoblast cells with 100nM vitamin D for 24h, 48h, 72h and five days, and reported that vitamin D enhanced myoblast cells differentiation. It has reported that vitamin D deficiency can have negative effects on skeletal muscle (32). Evidence for an effect of vitamin D on skeletal muscle includes:

- Studies have reported an increase in oxygen consumption rate of skeletal muscle when treated with $1,25(OH)_2D$-VDR (33). The effect of vitamin D on skeletal muscle appears to be specific to $1,25(OH)_2D$ with VDR and no effect has been seen with other forms of vitamin D (33).
- The presence of FokI polymorphism of VDR has reported to be associated with the reduction in fat free muscle mass and quadriceps strength and increased the risk of sarcopenia in humans (34, 35).
- The reduction in muscle atrophy and improvement in myopathy has been reported by eliminating vitamin D deficiency (36).
- Vitamin D deficiency has reported to be associated with decrease in the expression of VDR in muscle and increased oxidative stress in muscle. The down regulation or

overexpression of VDR can have negative consequences on skeletal muscle homeostasis (37).

Non-genomic response

This is characterised as rapid process of activation which allows the interaction of VDR with caveolae (membrane proteins) which are present in the plasma membrane of the cells (38). Followed by this interaction, VDR mediates a cellular response on calcium channels through activation of signalling pathway such as mitogen-activated protein kinase (39). This involvement of VDR with membrane invaginations and signalling pathways take place in intestine, vascular smooth muscle and monocytes (40).

1.1.3.2 Physiological function

Calcium and phosphorus homeostasis

The main function of vitamin D in its hormonal form (calcitriol) is in calcium and phosphorus homeostasis for bone mineralization (41). Calcium is a crucial part of bone health and the normal concentration of calcium in the human body is reported as 8.5-10.5 mg/dl that is strictly regulated by the function of vitamin D-related endocrine system and para-thyroid hormone (PTH) (2).

A decrease in plasma calcium concentration is detected by calcium sensing transmembrane proteins in parathyroid gland which triggers the secretion of PTH into the circulation. PTH cause osteoblasts and kidney to produce calcitriol which in turn elevates plasma calcium levels by three means:

- increasing intestinal calcium absorption (2): here calcitriol act with VDR in the intestine to increase the expression of calcium binding protein (known as calbindin 9k) which release calcium from lumen into the circulation.
- renal calcium reabsorption (42): calcitriol along with PTH acts on renal distal tubules of the kidneys for reabsorption and retention of calcium by the kidney.
- bone resorption (2): here calcitriol act with VDR in the osteoblast to increase the production of osteoclast which release calcium into the circulation.

In case of elevated extracellular calcium level, calcium sensing receptors suppress the action of the parathyroid gland to stop the secretion of PTH and calcitriol blocks the calcium resorption from bone (42). Moreover, the parafollicular cells (C cells) of the thyroid secretes the peptide calcitonin which blocks calcium reabsorption from bone. Thus, the calcitriol along with the VDR suppress the parathyroid gene expression and the parathyroid cell proliferation that ultimately controls the calcium levels (2).

Autoimmune function

In monocytes and macrophages of immune system, the presence of 1alpha-hydroxylase enzyme contributes to the production of $1,25(OH)_2D$ which is the biological active metabolite of vitamin D in body. However, the production of $1,25(OH)_2D$ upon stimulation of immune system is different than the renal synthesis of $1,25(OH)_2D$. Different regulation of macrophage 1alpha-hydroxylase has been reported previously which could cause increased production of $1,25(OH)_2D$ even in dysregulated calcium homeostasis. Therefore, in macrophages, the 1alpha-hydroxylase is highly regulated by the presence of immune signals such as IFNgamma and LPs (43). Figure 1.2 below shows the actions of vitamin D.

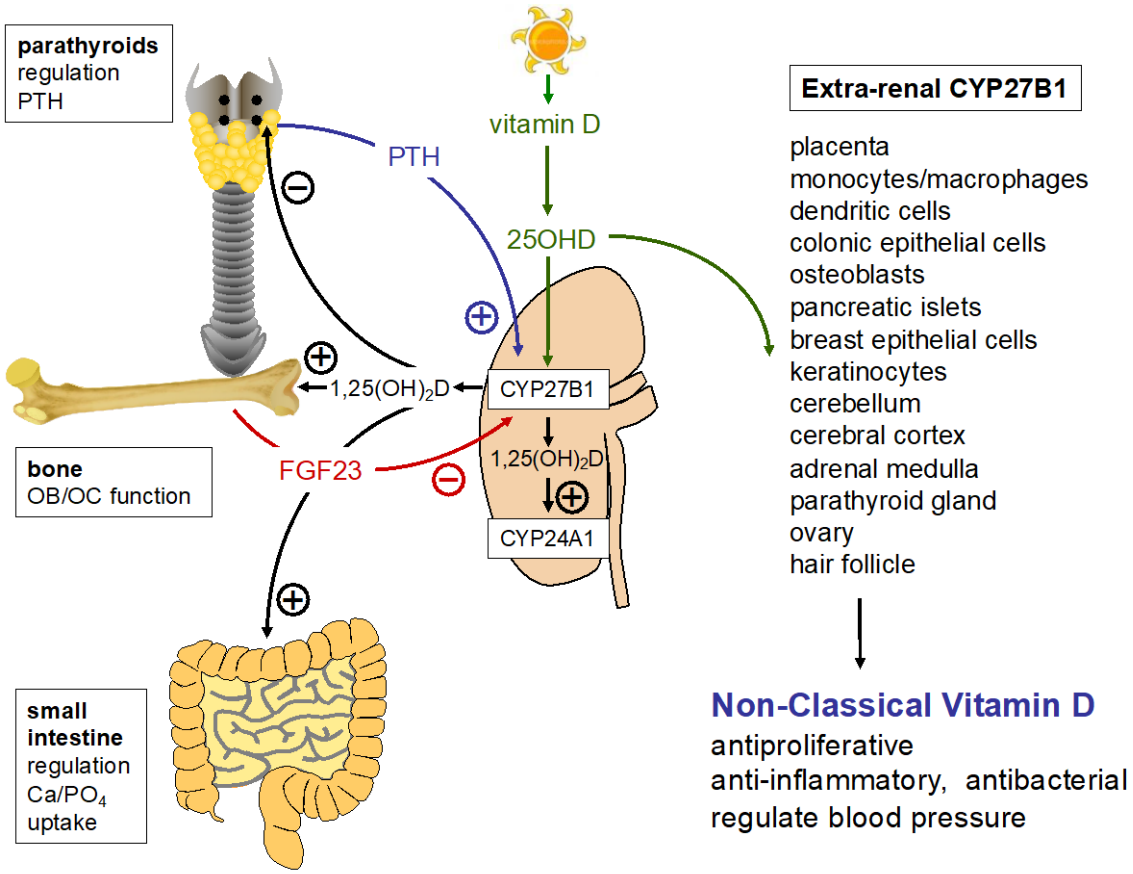


Figure 1.2 Action of vitamin D

This Figure is reused with the permission of the author (44).

1.1.4 Difference between vitamin D_2 and D_3

Structurally, vitamin D_2 differs from D_3 due to the addition of a methyl group to carbon twenty-four position of the vitamin D_2 ($C_{28}H_{44}O$) and double bond between carbon 22 and 23 compared to D_3 ($C_{27}H_{44}O$) (22). The molecular mass of the vitamin D_2 and vitamin D_3 has reported to be 396.65 g/mol and 384.64 g/mol respectively (43). A number of studies have explored difference in the function and efficacy of vitamin D_2 and D_3 intake in relation to change in the circulating blood 25(OH)D concentration. The results of these studies have been collated in a meta-analysis which involved ten trials with 1016 participants aged between18-97 years. This meta-analysis has compared the direct effect of vitamin D supplementation of both forms using various doses (as single large bolus, weekly, monthly or daily basis) and duration. In this meta-analysis, eight studies reported that the vitamin D_3 was more efficient to increase circulating blood 25(OH)D concentration from baseline regardless of the dose frequency and method of intake as compared to the vitamin D_2 (45). Two studies in this meta-analysis reported the equal efficiency of vitamin D_2 and D_3. This difference in the efficacy of two forms of vitamin D could be due to the difference reported in the structure (as discussed above) and metabolic state of two forms (discussed in section 1.1.1).

1.1.5 Reference nutrient intake (RNI) and circulating blood 25(OH)D concentration guidelines

1.1.5.1 RNI guideline

In 1991, the UK Committee on Medical Aspects of food policy (COMA) set Reference Nutrient Intakes for energy and nutrients. At that time a dietary RNI for vitamin D was only set for specific groups of population at risk of vitamin D deficiency (13). An RNI of 8.5µg/day was set for infants aged 0-up to 6 months; 7µg/day for children aged 6months to 3 years and 10µg/day for pregnant and breastfeeding women and adults aged ≥65 years. No recommendations were made for the population ages between 4-64 years because it was assumed that intakes of vitamin D from cutaneous sources during summer would be sufficient to fulfil the requirement of vitamin D in the following winter season. These recommendations were made on the basis of maintaining plasma levels of 25(OH)D above 20nmol/L during winter months.

In 2016, Scientific Advisory Committee on nutrition (SACN) updated the recommendation of COMA for vitamin D intake on the basis of evidence gathered from literature (46). The purpose of this review was as to whether the previous dietary reference value accurately covered the nutrient requirements of the UK population and still appropriate for reaching and maintaining

sufficient circulating blood 25(OH)D concentration. SACN's recommendation for vitamin D was designed to prevent rickets in children, osteomalcia in adults, and for maintenance of good muscle strength and function in older adults and for the prevention of falls and fractures. The updated recommendations were expanded to include the adult population. An RNI of 10µg/day for vitamin D has recommended for the UK population aged 4 years and above throughout the year to maintain serum 25(OH)D concentration =>25nmol/L when exposure to sunlight is limited. This was recommendation for general UK population includes pregnant and lactating women and risk deficient population. A safe intake of 8.5-10µg/day and 10µg/day has recommended for ages 0 up to 1 years and ages 1 up to 4 years respectively (46). The IOM has recommended different doses for daily vitamin D intake however this difference between two recommendations is due to the basis on which these recommendations were made. In contrast to SACN (as mentioned above), IOM has made recommendation for vitamin D intake on the basis of good bone health (2).

The dietary reference values commonly referenced in the literature include those from the US Institute of Medicine (2), the European Food Safety Authority (EFSA) (47) and the Endocrine Society (48) as presented in Table 1.2.

Table 1.2 Recommendations of vitamin D intake

Age group	SACN* µg/d	IOM** µg/d	EFSA µg/d	Endocrine society µg/d
Infants (0-12 months)	8.5-10	10	10 (for age 7-11months)	10
aged 1-18 years	10	15	15	15
Aged 19 years and above	10	15	15	15
71 years and above	10	20	15	15-20
Pregnant or lactating women	10	15	15	15

**SACN set a precautionary and safe intake for infants.*

***IOM set adequate intake rather than reference intake for infants.*

1.1.5.2 Circulating blood 25(OH)D concentration guideline

In terms of circulating blood 25(OH)D concentration, the above recommendations of vitamin D intake were made to achieve and maintain sufficient vitamin D status in blood. SACN's recommendation is based on the vitamin D requirements to achieve and maintain circulating blood 25(OH)D concentration at ≥25nmol/L during winter months. According to IOM guideline, a serum 25(OH)D concentration of 30nmol/L was considered as the threshold value below which is associated with an increased chance of poor bone health and risk of fracture. A serum 25(OH)D concentration of 40nmol/L was considered enough for half of the population (50%). The status at ≥50nmol/L was defined as sufficient vitamin D status for 97.5% of the population to maintain good bone health. The circulating blood vitamin D thresholds set by SACN and IOM are summarised in Table 1.3 (2, 46):

Table 1.3 Thresholds of circulating blood 25(OH)D concentration

Cut-off values	SACN, 2016	IOM, 2011
Deficiency	<25nmol/L	<30nmol/L
Inadequacy	n/a	30-49.9nmol/L
Sufficiency	≥25nmol/L	≥50nmol/L

1.1.6 Vitamin D storage/sequestration and toxicity

Vitamin D storage/sequestration

An accumulation of vitamin D in adipose tissue has been reported. An early study reported the administration of radiolabelled vitamin D in depleted mice and measured increase uptake of radiolabelled vitamin D by adipose tissue upon repletion (49). It has suggested that vitamin D stored in peripheral tissues that express lipoprotein lipase such as adipose tissue and skeletal muscle cannot be released into the circulation, even with the indication of deficiency in blood (2). Elderly participants with a 15kg excess body mass who were supplemented with vitamin D 700IU/day for one year were found to have serum 25(OH)D level 10nmol/L less than those with normal BMI (50). It was estimated that an additional 17% increase in vitamin D intake above 700IU/day was required to meet the serum 25(OH)D level as those in subjects

with normal BMI (50). A prospective study involving 2158 participants aged 55 years and older has confirmed the presence of higher body fat percentage in participants with vitamin D deficiency compared to those with vitamin D status ≥75nmol/L (51). Supporting this association some studies have reported increase in serum 25(OH)D concentration with weight loss (52, 53).

Toxicity

The European Food Safety Authority (EFSA) has considered the existing literature reporting the use of single large bolus or range of multiple doses of vitamin D for various time periods and its respective adverse health outcomes for setting the tolerable upper intake level (UL) of vitamin D for children and adults in order to prevent the side effects (54). They have set an UL of vitamin D intake in children aged 0-1 years at 25μg per day; aged 1-10 years at 50μg per day; and in children aged ≥11 years and adults at 100μg per day (54). The consumption of vitamin D on daily basis more than the upper limit has potential to cause the adverse effects of vitamin D.

Vitamin D toxicity as a result of excessive exposure of skin to UVB rays of sunlight is not possible due to variety of factors:

- Prolonged exposure triggers the conversion of pre-vitamin D into photo chemicals known as lumisterol and tachysterol (55).
- Once converted to vitamin D, the excessive exposure to UVB can triggers reversible isomerisation of vitamin D in the skin to the photochemicals suprasterol II and 5,6 transvitamin D_3 (5).

These photoproducts can be available in case of under production of pre-vitamin D_3 and otherwise prevent from vitamin D toxicity or over production of the cutaneous vitamin D_3 (55). The threshold concentration of circulating 25(OH)D related with the symptoms of vitamin D toxicity in humans reported to be at 750nmol/L level and the range of 710-1587 nmol/L could lead to detrimental effects such as hypercalcemia (56).

1.1.7 Biomarker for circulating vitamin D

Serum/plasma 25(OH)D concentration is known to be best biomarker to be used for the measurement of blood circulating vitamin D content. Vitamin D, the pro-hormone, is short lived as vitamin D intake through skin or lymph is taken by liver or adipose tissue within few hours so it is not appropriate to measure vitamin D content in the liver or adipose tissue and thus the biochemical assessment of vitamin D content in the blood is more appropriate to be measured (46). In the literature, the term ‘vitamin D’ used to define vitamin D level in the body is actually

the circulating blood 25(OH)D concentration. The amount of 25(OH)D concentration in blood represents the vitamin D deficiency or sufficiency in the body. The rationale for this metabolite as a choice of selection of indicator to measure the change/status in body vitamin D is as follows:

- Vitamin D, after entering into the body, undergoes rapid process of transportation to the liver and kidneys for metabolism and yield biological active metabolite $1,25(OH)_2D$ which amount is tightly regulated by kidneys.
- Calcitriol ($1,25(OH)_2D$), known as biological active metabolite of vitamin D, does not stay in the blood circulation for a long to be used as biomarker of vitamin D level of the body. The average half-life of 25(OH)D is approximately 2-3 weeks which makes this metabolite a suitable indicator to measure the blood circulating vitamin D level (2). The half-life of $1,25(OH)_2D$ is less than even 4 hours. The 25(OH)D metabolite of the nutrient does stay in the blood for longer and thus accessible for cells for utilisation either through direct action with enzyme or by conversion to $1,25(OH)_2D$ in order to perform physiological activities in the body.
- Vitamin D deficiency or sufficiency status in the body does not associate with the amount of $1,25(OH)_2D$ in body as even in the severe deficiency the level of $1,25(OH)_2D$ can found to be normal.
- The formation of blood 25(OH)D is directly regulated by vitamin D intake (through sunlight, diet and use of supplements) whilst the formation of $1,25(OH)_2D$ is regulated by the other factors such as parathyroid hormone.
- The plasma/serum 25(OH)D concentration has used previously in two main reports, SACN and IOM, as a measure of body vitamin D content which is required to be available for performing physiological activities in the body (2, 46). The other metabolites of vitamin D are not considered to represent vitamin D content of the body.
- However, the accurate concentration of circulating 25(OH)D as biochemical marker in response to vitamin D intake in body (through skin or diet) is limited by number of factors such as age, skin tone, season, latitude, skin exposure to sunlight and body mass index (2). The accuracy and validity of the laboratory method of measuring circulating 25(OH)D concentration is also important to consider.
- The concentration of 25(OH)D in circulation can also fluctuate depending on the amount and rate of vitamin D uptake by the liver, amount and rate of conversion by the liver and its half-life in the plasma (57).

1.1.8 Measurement of vitamin D profile in UK general population of older adults

The UK National Diet and Nutrition Survey (NDNS) rolling programme is a continuous survey of dietary intake and nutritional status of a representative sample of the UK population. According to the latest UK NDNS of combined years 7 and 8 (2014/15-2015/16) which is based on the completion of food diary from 1417 adults, the daily vitamin D intake from food sources was mean (SD) 3.9(2.4)μg and 3.2(2.0)μg in men and women aged 65-74 years old respectively (58). The daily vitamin D intake from food source only in men and women aged 75 years and older was mean (SD) 3.3(2.1)μg and 2.5(1.6)μg respectively. Even with the inclusion of supplemental vitamin D older adults on average still failed to achieve the recommended intake of 10 microgram of vitamin D per day. The daily total vitamin D intake from all sources (including supplements) was estimated as mean (SD) 5.5(4.4)μg and 6.5(7.3)μg in men and women aged 65-74 years respectively and 4.6(4.4)μg and 5.8(8.4)μg respectively in men and women aged 75 years and older (58). In terms of circulating 25(OH)D levels in older adults aged ≥65 years, men and women had 25(OH)D concentration mean (SD) 50.8(20.32) nmol/L and 51.6(21.06)nmol/L respectively (58). In this analysis, fifteen percent of included women (total *n*=89) aged ≥65 years had circulating 25(OH)D concentration <25nmol/L. These findings are based on a representative sample of 704 adults and 329 children from across four parts of the country on whom blood samples were available.

1.2 Aging

Aging brings a natural progressive decline in the physiological functions of different organs of the body often accompanied by pathological changes. The age-related decline in the heart rate output, which is supported by the evidence from the Framingham study, and increase in systolic and diastolic blood pressure has reported to be predominant cause of cardiovascular diseases among the elderly aged 65 years and over (59). The age-related decrease in the number of beta cells (which are responsible for producing insulin), increase in size of adipose tissue has been reported to increase the peripheral insulin resistance in elderly (60). This section describes briefly the impact of aging on the human body.

Skin

Starting from the skin, the atrophic changes in the skin epidermis in old age causes reduction in collagen and elastin which negatively affects the skin tone and elasticity. Of critical relevance to this thesis there is reduction in the concentration of 7-dyhydrocholesterol in the

epidermis with advance age has been reported which is required for the photochemical reaction in the synthesis of cutaneous vitamin D (7).

Gastrointestinal system

Advancing age is associated with the decline in the intestinal functions. Aging could cause decrease in peristaltic and increased non-peristaltic response of the oesophagus that could have negative impact on swallowing due to decreased relaxation of lower part of the oesophagus and may lead to dysphagia (60). Aging could also be etiological factor in diverticulosis by effecting intestinal motility negatively. The colon becomes hypotonic which cause the long-term storage of stool and the dehydration of the stool that could lead to chronic constipation condition in elderly (60). The effect of aging on the absorption of vitamin D has been studies in male rats aged 9-101 weeks previously (61). They have reported an increase absorption of vitamin D from age 9 to 41 weeks which remained consistent afterwards.

Respiratory system

Whilst the total volume of lung remains same however the residual volume increases with advance age (60). There is a decrease/mismatch in the expiration and ventilation capacity due to the decrease in the elastic recoil properties of the lung that could cause the airway collapse with advancing age. Elderly are also prone to pneumonia caused by bacterial or viral infection. The increased aspiration rate in elderly caused by the aspiration of oropharyngeal secretion make them at risk for infections (60).

Liver function

In the liver, aging causes the decrease in the size of the liver by substantial decline in the number of hepatocytes. The appearance of symptoms is unusual in most of the cases which may be due to the large reserve capacity of the liver. However, it may mirror by the decrease in the blood flow capacity which is related with the decrement in the clearance of various circulating drugs and alter the hepatic drug metabolism (60).

Kidneys

Studies has shown that there is age-related reduction in the size and volume of the kidneys (60). The degradation in the number of glomeruli has also been reported which hinders the capability of clearing waste proteins (urea and creatinine) into the urine and hence responsible for the accumulation of these proteins in blood. The change in the amount of creatinine is little

bit because there is decrease in the production of creatinine anyway due to the age-related decrease in the size of muscle mass which is responsible for its production (62). A decrease in the tubular function of the kidney has also associated with increasing age which enhances the glucose reabsorption by the tubules in elderly and therefore glycosuria could be mis leaded with diabetes mellitus in elderly patients. The capacity of bladder has observed to decrease in aging which could collectively decrease the sensation of needing to void in elderly (60).

1.2.1 Skeletal Muscle aging

As per the basic muscle physiology, motor units (MUs) and muscle fibres are the crucial part of skeletal muscles structure. The age-related loss of motor units and muscle fibres areas act in unison to reduce the cross-sectional skeletal muscle mass areas, strength and functional ability (63). The age-associated decrease in the number and size of MUs and cross-sectional muscle mass areas is shown equally distributed in both upper and lower limbs. Motor units are composed of motor neurons (MN) and motor nerves to which the muscle fibres are innervated and act as contractile units of the neuromuscular system. Muscle fibres are composed of a myosin heavy chain (MHC) type IIa, IIx and type I. MHC type IIa and IIx have been reported stronger in strength than type I and play an important role in muscle strength and contraction properties. The reduction in MHC type IIa and IIx muscle fibres area is displayed by the decrease in their expression of strengthening and contractile properties of muscle (64). Furthermore, the decrease in myosin content is coupled with the decrease in myofibrillar content, actomyosin interactions, structural changes in actin filaments and the decreasing power of cross-bridges between the fibres, all have negative influences on muscle strength and function in old age (64). The development of age-related changes in muscle and degenerative changes in bone can be evident through a decrease in muscle mass, strength and function due to muscle atrophy (60). The Health ABC study has reported increased in lipid content of skeletal muscle with increasing age was associated with reduction in the muscle attenuation (65). This was in accordance with increase body mass index and was prevalent in women than men.

1.2.1.1 Sarcopenia

Sarcopenia is comprised of two terms: sarc means muscle and penia means loss. Sarcopenia has reported to be associated with vitamin D deficiency (66). Sarcopenia is extensively defined and updated in the report of Asian Working Group on Sarcopenia (AWGS) that has targeted community-dwelling older adults aged 60 years and above (66). The decline in three parameters of healthy skeletal muscle has been used to diagnose the sarcopenia (Table 1.4).

The three parameters are: muscle mass, muscle strength and muscle function (66). The three stages of sarcopenia are described as below:

***Table 1.4 Stages of sarcopenia (*adapted from Asian Working Group on Sarcopenia (66)).**

Stages	Decrease muscle mass	Decrease muscle strength	Decrease function
Pre-sarcopenia	✓		
Sarcopenia	✓	✓ or	✓
Severe sarcopenia	✓	✓	✓

The decrease in muscle mass plus decline in either muscle strength or function was used by AWGS to confirm the diagnoses of sarcopenia. Sarcopenia has two categories: primary and secondary. Primary sarcopenia is exclusively age-related however secondary sarcopenia could be activity, disease or nutrition related (66). The cut-off points in muscle health parameters were set to diagnose sarcopenia such as: muscle mass 7 kg/m^2 for men and 5.7 kg/m^2 for women measuring by using bioelectrical impedance, muscle strength (handgrip strength) of <26 kg for men and <18 kg for women and for function, 6-m usual gait speed without deceleration <0.8 m/s. The adverse health outcomes related to sarcopenia in old age can be functional impairment, fractures following falls, hospital admission and poor quality of life which may lead to disability and eventually mortality.

1.2.1.2 Frailty

There are 2 commonly used definitions of frailty i) by Rockwood and Mitnitski. 2001 (67), and ii) Fried and colleagues 2001 (68).

1) The frailty index provided by Rockwood and Mitnitski. 2001, consists of 92 items which determines the onset and severity of frailty. This works by dividing number of frailty items present by the total number of items. This frailty index is the accumulation of

symptoms, signs, abnormal laboratory values, disease classifications and disabilities (67).

2) The other definition of frailty has provided by Fried et al (2001) which is based on a frailty phenotype (68). The diagnose was based on five physical criteria relating to decline in the muscle strength, functional performance, low physical activity, exhaustion and lean body mass (Table 1.5). The methodology used was as following: self-assessed health interview, physical function was assessed by administering questions about capability of performing instrumental activities of daily life (IADL) and activities of daily life (ADL). Fried and colleagues have reported that frailty was more prevalent in older age and was associated with low self-reported physical activity status (68).The table below presents the parameters of frailty as per Fried and colleagues (2001). The presence of three or more below components have reported to confirm the diagnoses of frailty in women with advance age.

Table 1.5 Characteristics of Frailty

**"Self-reported physical activity method was used to assess the activity level of the women. **The scoring of self-exhaustion question consisted of two statements: (1) Everything I get was an effort; (2) I could not get anything done. The question was how frequent did you feel any of this during last week?; 0=rare or none; 1=some time (1-2 days); a moderate amount of time (3-4 days) or 3=most of the time* (68)".

Parameters	Definition of parameters
Loss of muscle mass	≥10 pounds in last 12 months unintentionally
Decline of muscle strength	Handgrip strength ≤17.3 kg for normal BMI
Decline in muscle function	Cut-off time to walk 15 feet was 6-7 sec
Low activity*	spending <270 Kcal per week
Exhaustion**	Two statements consisted CES-D (Center for Epidemiological Studies)- depression scale (69).

1.2.2 Physical activity

Each week older adults of age 65 years and above should undertake at least 150 minutes of moderate intensity physical activity in a week or 75 minutes of vigorous physical activity or the combination of them. The updated recommendations for physical activity involves undertaking activities which aim to improve muscle strength, balance and flexibility at least twice a week (70). To undertake these recommended physical activity guidelines is reported to be beneficial for prevention of falls and chronic disease in older adults and (70). A decline in muscle functional capacity with increasing age has been reported previously. Therefore, recommendation to older adults to involve in different physical activities given as per suitable to their lifestyle has been made as long term sedentary behaviour could be associated with development of certain comorbidities such as diabetes, cardiovascular disease, obesity and all-cause mortality (70).

1.3 Vitamin D and muscle ageing

The relationship of vitamin D with muscle strength and function in community-dwelling post-menopausal women:

In the light of the above background, aging may have detrimental effects on skeletal muscle strength and functional performance given that high prevalence of vitamin D deficiency (hypovitaminosis D) in older adults can exaggerate these negative effects. Whilst, it is hypothesized that vitamin D can help prevent/maintain age-related poor muscle strength and function in older adults. There are numerous previous observational studies (Table 1.6) and randomised controlled trials (Table 1.7) which have examined the relationship of vitamin D with muscle strength and function in community-dwelling older adults. The evidence from observational studies which have used the following tests: handgrip strength, single chair stand test, repeated chair stand test, timed up and go test and balance test, for the estimation of capacity of skeletal muscle strength and function are considered here. For the consideration of evidence from randomised control trials, the studies with inclusion of walking speed test in addition to the above tests are considered.

1.3.1 Observational studies:

1.3.1.1 Relationship of circulating blood 25(OH)D concentration with muscle strength

Several cross-sectional studies have reported a null association between serum/plasma 25(OH)D concentration and handgrip strength, as indicator of upper limb muscle strength, in community-dwelling post-menopausal women of multiethnicity (71-76). Studies were found to

have high heterogeneity in terms of circulating blood 25(OH)D concentration of women of different ethnicity groups. In terms of handgrip strength, some have reported that women were found to have low handgrip strength against their age group than estimated as normative value defined by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) (71, 76, 77). The handgrip strength of women has reported to be same across circulating blood 25(OH)D concentrations (71, 73) and one study has reported an association between given age and handgrip strength capacity of the included women (74).

In terms of lower limb muscle strength, the observational studies have reported mixed findings with regards to relationship of circulating blood 25(OH)D concentration with single chair stand, repeated chair stand and balance test in community dwelling women of different ethnicity groups. Some studies have found a positive relationship of circulating blood 25(OH)D concentration with better performance of chair stands and balance test (76, 78) whilst others have not (71, 73, 74). Different parameters to define lower limb muscle strength have been used previously and hence different findings have reported based on the selected measure of variable.

Studies have identified different levels of circulating blood 25(OH)D concentration in regard to have positive relationship with lower limb muscle strength. A previous cross-sectional and longitudinal design study (78), the Longitudinal Aging Study Amsterdam (LASA), has reported significant cross-sectional association between risk of having poor performance of chair stands, tandem test and serum 25(OH)D concentration of <75nmol/L compared to the serum 25(OH)D concentration of >75nmol/L. From longitudinal perspective, they have reported the higher odds of 3-years decline in performance of chair stands and tandem test in participants with serum 25(OH)D concentration <75nmol/L than serum 25(OH)D concentration >75nmol/L (78). Whilst, the Rancho Bernardo Study (RBS), a longitudinal observational cohort study with duration of 2.5 years, has reported significant relationship between better performance of 5-times repeated chair stand test and circulating blood 25(OH)D concentration of >80nmol/L in 656 women of aged 74.7 (10.8) years. However, they have reported a 7.2% longitudinal decline in the performance of 5-times repeated chair stand and timed up and go test in those who had serum 25(OH)D concentration <80nmol/L at baseline than those with =>115nmol/L (76).

The HALST study has reported significant association between risk of vitamin D inadequacy (plasma 25(OH)D concentration of 30-<50nmol/L) and performance of 5-times chair stands and success in full tandem stands (balance test) in community-dwelling Taiwanese women (n=2585; aged >=55 years) as analysed by multiple linear regression model after adjusted for

covariates. For success in single leg stands, no relationship has observed at plasma 25(OH)D concentration <75nmol/L (71).

1.3.1.2 Relationship of circulating blood 25(OH)D concentration with muscle function

There are evidence reporting positive relationship between circulating blood 25(OH)D concentration and muscle function (timed up and go test) in community dwelling post-menopausal women (73).

A recent meta-analysis of 15 observational studies (12 cross-sectional and 3 longitudinal studies) has reported a positive relationship between vitamin D status at >75nmol/L and faster TUG performance (79). They have used vitamin D status at >75nmol/L as the 'normal' or reference population and compared the performance of walking speed and timed up and go test in the other quintiles against this. In the first model, they have reported the mean difference of -0.18m/s and -0.08m/s slower usual-pace walking speed among participants with severe vitamin D deficiency (SVDD) (≤25nmol/L) and vitamin D deficiency (VDD) (≤50nmol/L) respectively compared to the normal vitamin D level (NVD) (>75nmol/L). Second model has reported -0.04 m/s mean difference between people with VDD and VDI (≤75nmol/L) compared to NVD. Third model has showed mean difference of 0.48 s faster TUG performance in people with NVD compared to SVDD and fourth model has reported greater proportion of participants with SVDD had showed low walking speed compared to other groups (79).

Overall, the identification of circulating blood 25(OH)D concentration in relation to muscle function has found at concentration between 50-100nmol/L (76) with given the optimal dose for muscle function with target of at least 75nmol/L level (79) which has also been defined by US Endocrine Society (48, 80).

1.3.1.3 Summary

In summary, the findings of observational studies are mixed and inconsistent in women of different ethnicity groups with regards to reporting relationship of vitamin D with muscle strength and function. There is evidence of a relationship of vitamin D with lower limb muscle strength and function compared to the upper limb muscle strength (handgrip strength) however given the sample size of many of these studies caution should be taken while interpreting them. Studies possess high heterogeneity in their findings and study limitations impose the need for caution while interpreting the findings. The inconsistencies among studies may be due to the following reasons:

- The relationship between vitamin D and muscle strength was often not the primary research question and hence ample data has not been illustrated or discussed in order to rule out underpinning facts of null association (74).
- The use of strict/different selection criteria of the participants has found to be another limitation which may limit the comparison of the findings (73, 74).
- Studies also have used different method of measuring circulating blood 25(OH)D concentration which may produce a chance of variation in the accurate measurement of circulating blood 25(OH)D concentration (71, 73-76) and hence can impact on the potential relationship with muscle strength and function.
- Studies have used different methods of presenting serum/plasma 25(OH)D concentration such as quintiles or continuous manner and investigated the potential association by using different statistical approaches (71, 73-76).
- Finally, the use of different sample size among studies cause inconsistency while reporting the findings and effect the potential relationship.
- Use of different protocol (for example time duration) used for same assessment of muscle strength/function.

Table 1.6 Cross-sectional studies reporting association between vitamin D, muscle strength and function in populations of different ethnic groups

Author	Study design	Country	Age (years)	Sample size	Baseline characteristics	Aim	Results	Conclusion
Shardell et al., 2007 (72)	Cross-sectional	Italy	≥65	628	Community-dwelling, Serum25(OH)D groups: <25nmol/L, 25-52.9 nmol/L and ≥53 nmol/L	To investigate the association between serum25(OH)D concentration and grip strength, hip flexor strength, knee extensor strength, walking speed and time for chair stand	Vitamin D and hip flexor strength (*p*=0.90), knee extensor strength (*p*=0.5), grip strength (*p*=0.14), chair stands (*p*=0.5), walking speed (*p*=0.98)	No association found between vitamin D status and any variable
Boye et al., 2013 (73)	Cross-sectional	Netherlands	≥65	230 men, 370 women, Community-dwelling, visited emergency department because of fall	55 participants had serum 25(OH)D level <25nmol/L, 209 had 25-49.9nmol/L, 172 had 50-74.9nmol/L and 164 had ≥75nmol/L. 6% of men and 17% of women were taking vitamin D supplements	To investigate association between serum25(OH)D level and hand grip strength, timed up and go, five time sit-to-stand, tandem stand test	Association of vitamin D with hand grip strength (*p*=0.59;0.004), timed up and go (*p*=0.02; p= 0.07), five times sit to stand (*p*= 0.14; *p*=0.05) in women and men respectively	An association was found between timed up and go and vitamin D in both men and women. However, hand grip strength was significantly associated with vit D in men only.

Chuang et al., 2016 (71)	Cross-sectional	Taiwan, A nation wide study	≥55	2,498 men and 2,732 women	4 men and 34 women had serum 25(OH)D level <30nmol/L, 534 men and 939 women had 30-50nmol/L, 1405 men and 1440 women had 50-75nmol/L, 487 men and 311 women had 75-125nmol/L and 93 men and 22 women had ≥125nmol/L	To investigate the association between 25(OH)D status and hand grip strength, 5-times chair stands, standing balance, timed up and go, 6-min walk, single-leg stand	In group with serum 25(OH)D status 30-50nmol/L the association of vitamin D with HGS (p=0.002; p=0.2), TUG (p<0.001; p<0.001), 6-min walk (p=0.02; p=0.001), single leg stand (p=0.80; p=0.12), gait speed (p=0.03; p= 0.01) in men and women respectively.	Association of vitamin D with all variables reported only in group with serum 25(OH)D 30-50 nmol/L. No association between vitamin D and single leg stand in men and women. No association of vitamin D with hand grip strength reported in women.
Carrasco et al., 2014 (81)	Cross-sectional	Santiago de Chile	60-98	57 women and 47 men	26 men and 47 women had vitamin D deficiency (<50nmol/L), 17 men and 8 women had vitamin D in-sufficiency (50-74.75nmol/L) and 4 men and 2 women had sufficient vitamin D status (≥75nmol/L)	To investigate serum 25(OH)D status and its association with physical performance (gait speed and hand grip strength)	Significant correlation between vitamin D, grip strength and gait speed reported in females and males respectively (r=0.32 and 0.34; p <0.01)	High prevalence of vitamin D deficiency. Direct correlation reported between lower gait speed, hand grip strength and low vitamin D status.

Bentes et al., 2017 (74)	cross-sectional	Brazil	62.48 ± 7.67	40 women	mean (SD) vitamin D status was 68.67 (22.45) nmol/L	To investigate the association between vitamin D status and muscle function (timed up and go, 30-s chair stand, arm curl, sit to stand and grip strength	No association with vitamin D and hand grip strength (kg) (p=0.550), 30-s chair stand (p=0.076) and timed up and go (p=0.949) other than arm curl test (p=0.020).	No association with vitamin D and all variable other than arm curl test (p=0.020).
Meng et al., 2017 (75)	Cross-sectional	China	Over 60 years	316 men and 596 women	Community living, serum 25(OH)D (52.5(22.5) nmol/L,	To investigate the association of vitamin D with muscle strength and function	gait speed in men and women respectively (p 0.267; p 0.343), Grip strength in men (p 0.001) and women (p 0.493).	A strong association between vitamin D and grip strength found in men only.
Zamboni et al., 2002 (82)	Cross-sectional	Italy	68-75 years	94 men and 175 women	Community living, serum 25(OH)D status in men 56.5(37.5) nmol/L and in women 39.4 (24.1) nmol/L	To investigate the association of vitamin D with muscle strength and function	6-m walking speed (p=0.04), leg strength (p= -0.01) in men.	A significant association found between vitamin D, muscle strength and function in men.

(Bischoff et al., 2004) (83)	National health and Nutrition Examination Survey (NHANES III)	United State population	71.4 (7.9)	2003 males and 2097 females	Community dwelling serum 25(OH)D = 22.5-94nmol/L	To investigate the association of vitamin D with muscle strength and function	Sit to stands and walking test	A significant association of vitamin D with muscle strength (p=<0.0001) and physical function (p=<0.0001) found.
Mastaglia et al., 2011 (84)	Cross-sectional	Argentina	>65 years	54 women	2 groups: serum 25(OH)D level >50nmol/L and <50nmol/L.	To investigate the association between vitamin D nutritional status, muscle strength and function	Knee extensor, hip abductor, hip flexor, gait speed, grip strength, balance test	Vitamin D status >50nmol/L was significantly associated with muscle strength as compared to <50nmol/L level: knee extensor (p=<0.03), hip flexor

								(p=<0.05), hip abductor (p=<0.04).

1.3.2 Meta-analysis of RCTs

A recent systematic review and meta-analysis, with community dwelling post-menopausal women of different ethnic groups, investigated the casual relationship of vitamin D with muscle strength (handgrip strength, single chair stand, repeated chair stand and balance test) and function (timed up and go and walking speed test) and is described here. In addition, the findings of another meta-analysis with general older adults (men and women) have also considered.

1.3.2.1 Relationship of circulating 25(OH)D concentration with muscle strength and function

A recent systematic review and meta-analysis of 29 randomized controlled trials (RCTs) with community-dwelling post-menopausal women of different ethnic groups has summarized the effect of vitamin D supplementation (with/without calcium) of different doses and duration in improving muscle strength and function (1). Out of 29 included RCTs, only six studies have reported a beneficial role of vitamin D in improving muscle strength (handgrip strength, chair rising test (number of repetitions) and five times sit to stand test (85-89) and function (timed up and go test) (87, 90).

Overall, this is very low number supporting the benefits of vitamin D involvement to skeletal muscle compared to the total number of RCTs involved in this meta-analysis. Furthermore, the findings of these above six studies should be considered very carefully depending on the reliability/quality of these studies. Taking a closer look at the six positive studies, it is evident that sample size used in these studies was small which limits the credibility and transferability of their findings. The other limitations were lack of use of double blinded randomisation method/absence of declaration of type of randomisation which may question on the quality of the studies (86, 89) and inclusion of women with low bone mass (89) which may limit the generalisability of the study findings. In one of the above studies, no post-course change in serum 25(OH)D levels has reported (89). However, women had been undertaking exercise over the intervention period which may possibly bias the findings towards favourable role of exercise in improving handgrip strength and timed up and go performance.

The studies, in this meta-analysis, which have reported null causal relationship between vitamin D and muscle strength and function were inconsistent with regards to the characteristics of included women, sample size, baseline circulating blood 25(OH)D concentration, baseline muscle strength and function and dose/duration/type of vitamin D supplementation been used.

Some of the studies have recruited women with sarcopenia, low bone mass/osteopenia and/or obese/overweight (88, 89, 91-93). Women with such clinical history could have negative effect on handgrip strength and may require special treatment. It was also secondary endpoint to investigate the effect of vitamin D supplementation on muscle strength and function in some studies and is some instances the baseline and post-treatment 25(OH)D concentration were not reported (89, 93, 94). Therefore, it is not possible to report on change in circulating 25(OH)D concentration from baseline and its corresponding effect on muscle strength and functional capacity in women.

Overall, the use of high dose of vitamin D supplementation with weekly/monthly frequency of administration has not proven to be beneficial for improving muscle strength and function (86, 91, 95-97). Likewise, very low dose vitamin D supplementation with daily administration for longer interval has reported to have no advantages to skeletal muscle health (94, 98). However, it may favour an increase in circulating blood 25(OH)D concentration depending on the baseline profile (Table 1.7).

In summary, taken together the findings of positive studies in this meta-analysis, it may possibly suggest that baseline circulating blood 25(OH)D concentration between 20.5-65.95nmol/L and administration of medium dose of vitamin D supplementation on daily basis for 3-4 months may have beneficial effect in improving muscle strength and function in community dwelling post-menopausal women (1). The IOM report also has suggested that the use of at-least 800IU/day could benefit muscle function (2).

Another early systematic review and meta-analysis of 15 studies with older adults (men and women) aged 65 years and above has reported no effect of vitamin D supplementation (with/without calcium) on muscle strength and function (99). This study has also reported high heterogeneity between studies making it hard to compare the findings. Taking a closer look at the results, six studies included have reported a post-course improvement in muscle strength or function. Two of them were same positive studies as in above meta-analysis (89, 90). Two positive studies have used knee extension strength and quadriceps muscle strength, used 25(OH)D and alfacalcidol treatment and used very low sample size (100, 101). Overall, these six positive studies, in comparison to findings of the above meta-analysis (1), have included participants with baseline 25(OH)D concentration below 78nmol/L, use of different treatments with duration 3-12 months to report beneficial role of vitamin D to muscle strength and function.

Likewise, cross-sectional studies, the findings of the randomised control trails have also been inconsistence due to several factors which have discussed above. Nonetheless, a range of assessments methods have been used previously. Finally, there is recent prospective cohort

study, which is of relevant to this thesis, has showed a significant effect of calcifediol treatment (20μg, 4 oral drops per day for 6 months) to increase in baseline 25(OH)D concentration, muscle strength and physical performance in osteoporotic/vitamin D deficient (<75nmol/L) post-menopausal women (n=113) aged 50 years and above (102). A summary of randomized control trials with community dwelling post-menopausal women is shown in Table 1.7 below.

Table 1.7 Randomised control trials reporting the effect of vitamin D supplementation on muscle strength and function in post-menopausal women of different ethnicities (adapted from recent meta-analysis (1)).

Citation	Study design	Sample size (in all arms)	Country	Baseline mean 25(OH)D (Intervention, Control)	Intervention	Duration	Comparator	Outcome variables	Results
Verhaar et al., 2000 (103)	Open intervention	24	Italy	18.2, 80.6	Alphacalcidol 0.25μg/day for 1 month and 0.5μg/day alphacalcidol for 5 months	6 months	none	HGS, TUG, Walking test	no post-course improvement in outcome variables
Bischoff-Ferrari et al., 2003(104)	Double blinded randomised placebo controlled	62	Switzerland	13.95, 13.9ng/ml	800IUD_3/day plus 1200mg/day calcium	3 months	none	TUG, grip strength	Significant post-course improvement in outcome variables
Brunner et al., 2008 (94)	Double blinded randomised controlled	2347	America	not provided	400IUD_3/day plus 1000mg/day calcium	60 months	placebo	HGS, chair stand test, timed walk	no post-course improvement in outcome variables
Xia et al., 2009 (89)	Multicentre randomised open label controlled	140	China	not provided	125IUplus 0.25μg calcitriol/day	12 months	125IU/day calcitriol plus 600md/day calcium	HGS, five times sit to stand test	five times sit to stand test improve significantly in calcitriol plus calcium group
Janssen et al., 2010 (98)	Double blinded randomised	70	Netherland	32.6, 34.3nmol/L	400IU/day D_3 plus 500 mg/day calcium	6 months	placebo	HGS, TUG	No post-course improvement in HGS, TUG

	placebo controlled								
Zhu et al., 2010 (90)	Double blinded randomised placebo controlled	261	Australia	18.1, 17.7ng/ml	1000IU/day D_2	12 months	placebo	TUG	Significant improvement from baseline
Glendenning et al., 2012 (95)	Double blinded randomised placebo controlled	686	Australia	65.0, 66.5nmol/L	150,000IU/3 months D_3 plus 1300mg/day calcium	9 months	placebo	Grip strength, TUG	No improvement grip strength, TUG
Bischoff-Ferrari et al., 2012 (100)	Double blinded randomised	20	Switzerland	12.28, 14.18ng/ml	20µg/day (HyD_3) or 140µg/week (HyD_3)	4 months	800IU/day D_3 or 5600IU/week D_3	Repeated sit to stand test, TUG	No improvement in repeated sit to stand test, TUG
Wood et al., 2013 (92)	Double blinded randomised placebo controlled	293	Scotland	33.5nmol/L	Two arms: 400IU/day D_3 and 1000IU/day D_3	12 months	placebo	Grip strength	No improvement in grip strength
Gao et al., 2015 (105)	Prospective, open label	461	China	20.52ng/ml	Three arms: Calcium 600mg/day alone; Calcium 600mg/day plus	24 months	none	Chair rising test, grip strength	Significant increase in time required to perform chair rising test and

					800IU/day D_3; or Calcium 600mg/day and 0.25µg/day calcitriol				decrease in grip strength
Cavalcante et al., 2015 (86)	Randomised controlled	38	Brazil	22.4, 22.91ng/ml	6600IU/week D_3 in 2 cc olive oil	3 months	none	HGS	Significantly improved
Cangussu et al., 2015 (85)	Double blinded randomised placebo controlled	160	Brazil	15, 16.9ng/ml	1000IU/day D_3	9 months	placebo	HGS, chair rising test (number of repititions)	Significant increase chair rising test only
Hansen et al., 2015 (97)	Double blinded randomised placebo controlled	220	America	21ng/ml	Two intervention arms: 800IU/day; 50,000IU/day D_3 for 15 days and then twice monthly D_3 for 11.5months	12 months	placebo	Five sit to stand test, TUG	No improvement
Saito et al., 2015 (93)	Open label randomised controlled	35	Japan	Not provided	0.75µg/day eldecalcitol plus 35mg alendronate/week	6 months	35mg alendronate/week	HGS, TUG	TUG significantly improved in0.75µg/day eldecalcitol plus 35mg

									alendronate/week group
Uusi-Rasi et al., 2015 (106)	Double blinded randomised placebo controlled	370	Finland	26.8ng/ml	Two arms: 800IU/day D_3; 800IU/day D_3 plus exercise	24 months	Two arms: placebo; placebo plus exercise	Chair stand test, TUG, normal walking speed, short physical performance battery	No improvement
Grimnes et al., 2017 (91)	Double blinded randomised placebo controlled	297	Norway	64.7, 64.1nmol/L	800IU/day D_3 plus 20,000IU D_3 twice a week along with 1000mg/day calcium	12 months	Placebo plus 800IU/day D_3	HGS	No improvement in HGS
Cheng et al., 2018 (88)	Double blinded randomised placebo controlled	141	China	17.10, 15.8ng/ml	0.5μg/day calcitriol	3 months	placebo	HGS (right and left)	Left HGS significantly improved
Setiati et al., 2018 (87)	Double blinded randomised	88	Indonesia	49.75, 39.5ng/ml	0.5μg/day alfacalcidol plus	3 months	placebo	HGS, TUG	Significant improvement in HGS and TUG

	placebo controlled				500mg/day calcium				compared to placebo
Bislev et al., 2018 (107)	Double blinded randomised placebo controlled	81	Denmark	31, 35nmol/L	2800IU/day D_3	3 months	placebo	HGS, chair rising test, TUG	No improvement in intervention arm compared to placebo
Suebthawinkul et al., 2018 (96)	Double blinded randomised placebo controlled	60	Thailand	44, 16.2ng/ml	40,000IU/week D_2	3 months	placebo	HGS	There was within intervention group improvement however, no improvement compared to placebo

1.4 Circulating blood 25(OH)D concentration in the UK South Asian population

1.4.1 Younger adults

Previous studies have unanimously reported a lower circulating blood 25(OH)D concentration in community dwelling South Asians living in the UK compared with the Caucasian population. A cross-sectional study has reported the circulating 25(OH)D concentration in South Asians (Gujarati) and Caucasians of same age range of 20-40 years living in the Leicester (108). The study recruited a random sample of 51 Caucasian females, 37 Caucasian males, 71 Gujarati females and 42 Gujarati males through Leicestershire Health Authority. The mean circulating 25(OH)D concentration in each sub-groups was 32.5nmol/L in Caucasian females; 37nmol/L in Caucasian males; 23.5nmol/L Gujarati females and 25.75nmol/L in Gujarati males (108).

An observational prospective study has investigated the vitamin D status in South Asians and Caucasian population (male and female) of age range 20-60 years living in the Greater Manchester area with respect to season (5). They have reported that the median of serum 25(OH)D concentration during summer period was 22.5nmol/L and 65.5nmol/L in 95 South Asians and 109 Caucasians respectively. During the winter, the median of serum 25(OH)D concentration was shown as 14.5nmol/L in 87 South Asians and 47.25nmol/L in 109 Caucasians (5). Another prospective cohort study has recruited 46 South Asian women of mean age ± SD: 37.91 ± 9.04 and 135 Caucasians of mean age ± SD: 33.87 ± 5.62 to determine and compare the serum 25(OH)D status between two groups with respect to season. They have concluded that 81% of South Asians and 10.0% of Caucasians had serum 25(OH)D status <25nmol/L during the winter period. During the summer period, 0.8% of Caucasians and 53.5% South Asians had serum 25(OH)D status <25nmol/L (109).

1.4.2 Older adults

The low vitamin D status of the South Asian population is reflected in the UK Biobank: a survey of 500,000 people aged 40-69 years in 2006-2010 from across the country including 8024 South Asian individuals (110). The UK Biobank has reported that 29%, 60% and 93% of included South Asians have circulating blood 25(OH)D concentration <15nmol/L, <25nmol/L and <50nmol/L respectively.

A previous cross-sectional study involving community dwelling 66 postmenopausal South Asian (Pakistani) women and 42 Caucasian women of both aged 50-65 years living in the Blackburn area between May-September (111). They reported that the mean serum 25(OH)D concentration in South Asians and Caucasians was 16.6nmol/L and 45.2nmol/L respectively. A longitudinal study reported the seasonal difference in the circulating 25(OH)D concentration

of 36 Asians and 144 Caucasian post-menopausal women of same age 55-70 years living in the Surrey region (51°N) with compare to the circulating 25(OH)D concentration of 338 post-menopausal Caucasian women of same age living in the Aberdeen (57° N) (6). During the summer period, the median serum 25(OH)D concentration was found as 62.5nmol/L in Caucasians and 24.0nmol/L in Asians based in Surrey. During the winter season, the median serum 25(OH)D concentration in Caucasians was measured as 39.9nmol/L and 16.9nmol/L in South Asians living in Surrey. In Surrey, no Caucasian women was found to be below 20nmol/L at any season. In contrast, the serum 25(OH)D concentration in the population of Aberdeen was reported as 43.0nmol/L and 28.3nmol/L during summer and winter season respectively (6).

Despite South Asians have found to have vitamin D deficiency throughout the year, previous studies have reported difference in the circulating blood 25(OH)D concentration within South Asian sub-groups (Gujrati, Pakistani, Indian) as described above. This highlights the heterogeneity with South Asian sub-groups. Overall, the UK South Asians were found to possess an inadequate vitamin D status which is persistent throughout the year and could have detrimental effects on their skeletal muscle health. These different levels among sub-group population may possess different relationship with vitamin D and have included in Chapter 4 and 5 as part of this thesis.

1.5 Relationship of vitamin D with physical activity

To be involved in physical activity is very important for overall health and particularly skeletal muscle strength and functional performance. A recent cross-sectional analysis of PROPELS trial has reported South Asians to be less active versus Caucasians (p=0.001) (112). Another study has reported a generational difference in South Asians with regards to involving in physical activity with first generation found to be least active compared to the second generation (113). These first generation could be UK born but majority could be migrants too. The women included in cross-sectional and RCT as part of this PhD could be considered as first generation. A hypovitaminosis D has also reported in this community (111) and given literature reporting an association between vitamin D and muscle functional performance, there could be possibly an association between vitamin D and self-reported physical activity in UK South Asians but yet to be determined. A recent systematic review of ten randomised controlled trial with 200 elderly population with mean (SD) aged 75.1(4.4) years has reported that involvement in various kind of physical activity has improved muscle function including balance performance in this population (114).

A detailed information about physical activity, generational trends among South Asians for involving in physical activity and it's relationship with blood circulating 25(OH)D concentration in this target population is given in Chapter 5.

1.6 Heterogeneity within South Asian

Heterogeneity within South Asian subgroups has been reported previously. This includes variation in height, body size, waist circumference, culture, diet, lifestyle in relation to culture and presence of comorbidities (hypertension, diabetes) previously described in a case control study (115, 116). A difference in diet of Indian and Pakistani women has been reported previously as some Indians women particularly Indian Gujrati women are vegetarians, and some may be vegans. This limit their vitamin D intake through diet as the food that naturally contains vitamin D are meat, fish and dairy products which are not part of their diet. The socioeconomic difference has also been found previously which may have some effects on their knowledge of supplementation and hence limit their use.

1.7 Relationship of vitamin D with muscle strength and function in UK South Asian older women

The evidence above is from women of different ethnic groups and there is only one study which has investigated relationship between vitamin D and muscle strength and function (right grip strength and stand to walk time (3m)) in 19 UK South Asian women aged 58-71 years (117). This study had reported no relationship between vitamin D and either of the muscle strength test. However, the sample size in this study is so small to consider its findings and therefore a study with bigger sample size in this population is needed to investigate this potential relationship for robust evidence. There is another study which included 173 healthy Asian Indian females with mean (SD) age 21.7(4.4) years and baseline 25(OH)D concentration 23.25(8.4) nmol/L to investigate the effect of vitamin D and/or calcium on muscle strength (118). Females were randomised to receive 1) double placebo, 2) calcium/placebo, 3) cholecalciferol/placebo and 4) cholecalciferol/calcium for 6 months. The study used a cholecalciferol dose of 60,000IU/week for 8 weeks followed by 60,000IU/fortnight. The study has reported post-course increase in 25(OH)D concentration but no improvement in muscle strength and functional capability from baseline with the use of high dose (118). However, the dose in this study is high compared to the recommendation by authority bodies and could therefore not find improvement in muscle performance compared to baseline as discussed in section 1.3.2.1 of this thesis. A 10ug/day vitamin D intake is recommended for this population despite without knowing that whether it would be sufficient for the maintenance of good muscle

strength and function due to lack of high-quality scientific evidence. It is very important to investigate whether there is a relationship between vitamin D and muscle strength and function in UK South Asian older women given this population is at high risk of vitamin D deficiency throughout the year and low physical activity is reported in this population. This thesis is therefore aimed to address this research and investigate this relationship in cross-sectional and causal manner.

2 CHAPTER 2. Aims and objectives

2.1 Aim

The aims of this thesis were

1) to investigate the relationship between vitamin D and muscle strength and physical function in older South Asian women and
2) to test the hypothesis that vitamin D supplementation in South Asian post-menopausal women improves muscle strength and physical function.

2.2 Objectives:

2.2.1 Primary Objectives

1) To conduct a cross-sectional analysis of the relationship between vitamin D, muscle strength and physical function in South Asian women aged ≥60 years (Chapter 4).

2) To conduct a randomised, placebo control trial to determine the effect of vitamin D supplementation on muscle strength and physical function in vitamin D inadequate and deficient South Asian post-menopausal women living in the UK (Chapter 5).

2.2.2 Secondary Objectives

In meeting the primary objectives, the following secondary objectives were achieved:

1) To investigate self-reported physical activity in South Asian women aged 60 years and above living in the UK and to explore the relationship between circulating blood 25(OH)D concentration and self-reported physical activity in South Asian women (Chapter 6)
2) To report the strategies for successful recruitment of post-menopausal South Asian women living in the UK (Chapter 7)

3 Chapter 3. Methodologies common to both the cross-sectional study and the randomised control trial.

3.1 Study protocol

3.1.1 General interview

A general interview was used at the start of each study to collect information on: demographics, anthropometrics, presence of comorbidities and the use of supplementation. The participant reported data on comorbidities was GP diagnosed as confirmed by medication prescription of the participants. The proforma for this interview can be found in the appendix f.

3.1.2 Vitamin D measurement

Total vitamin D concentration (D2 and D3) was assessed in research participants using a finger prick blood spot sample test kit (Black Country Pathology Service, Sandwell & Birmingham).

Participants were asked for any known coagulation disorder, haemophilia or the use of blood thinning medication which may need additional precaution while performing finger prick. They were shown the test kit and verbally instructed how to perform the test.

Participants were required to prick their side of middle finger using a lancet and place 4 drops of blood on the test strip provided. All participants were asked to remain seated for about five minutes after performing the test for safety purposes. The vitamin D kits were then posted to the Department of Clinical Biochemistry, Sandwell and West Birmingham hospital for the measurement of 25 hydroxy vitamin D_2 and 25 hydroxy vitamin D_3 using Liquid Chromatography Mass Spectrometry (LC-MS) (119, 120). The average time of receiving the vitamin D reports was ≥7days.

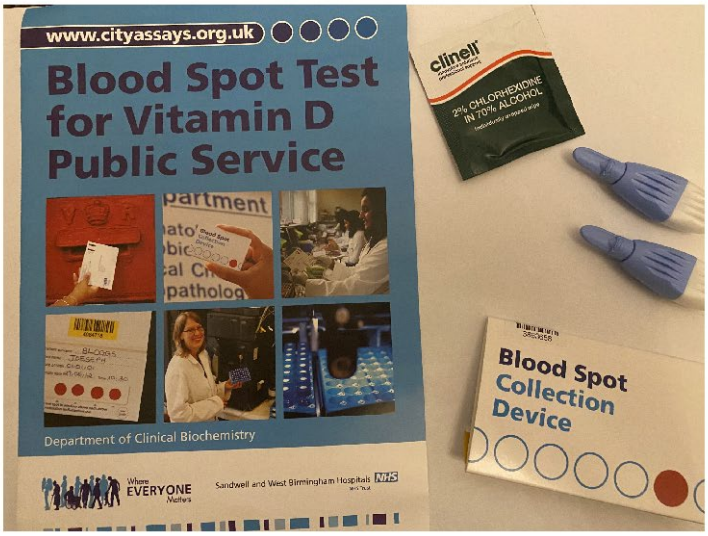


Figure 3.1 Vitamin D blood spot kit

Pre-preparation common to all functional assessments

Prior to the start of every test the researcher explained and demonstrated the technique to the participant. The purpose of the test was explained and the participant's understanding of the test procedure was checked. The researcher remained nearby to assist the participants if they needed support. Participants were reminded that they could stop the test at any point if they felt uncomfortable. Participants were asked if they felt safe prior to the start of the tests.

3.1.3 Muscle strength

3.1.3.1 Handgrip strength (kg)

Upper limb muscle strength was assessed by asking participants to perform a Hand grip strength test using a Jamar hand-held dynamometer (Patterson Medical, Warrenville, IL, USA).

Test performance

The participants stood with their right arm hanging free on the side of the body (with a little gap to avoid resistance) and squeezed the dynamometer with maximum power for few seconds until they were asked to release. Three consecutive readings (with a gap of about

one minute) were taken with the highest measured value for three attempts used for analysis purposes (71).

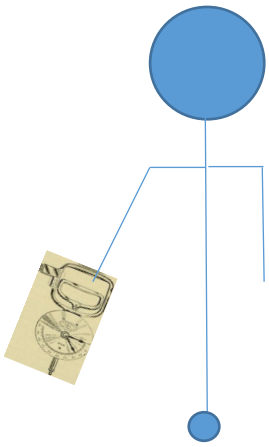


Figure 3.2 Performance of handgrip strength test (kg)

3.1.3.2 Single chair stand

A chair stand test was used to assess lower limb muscle strength (121).

Test performance

A straight back chair (used from the community centre/temple) without arms was placed against the wall. Participants were instructed to sit down on the chair with their feet properly placed on the floor and their back was touching with the chair's back. Participants were asked to fold their arms around the chest while sitting on a chair, rise up from the chair and sit back on the chair without using their arms. A stopwatch was used to record the time in seconds they took to perform the test procedure.

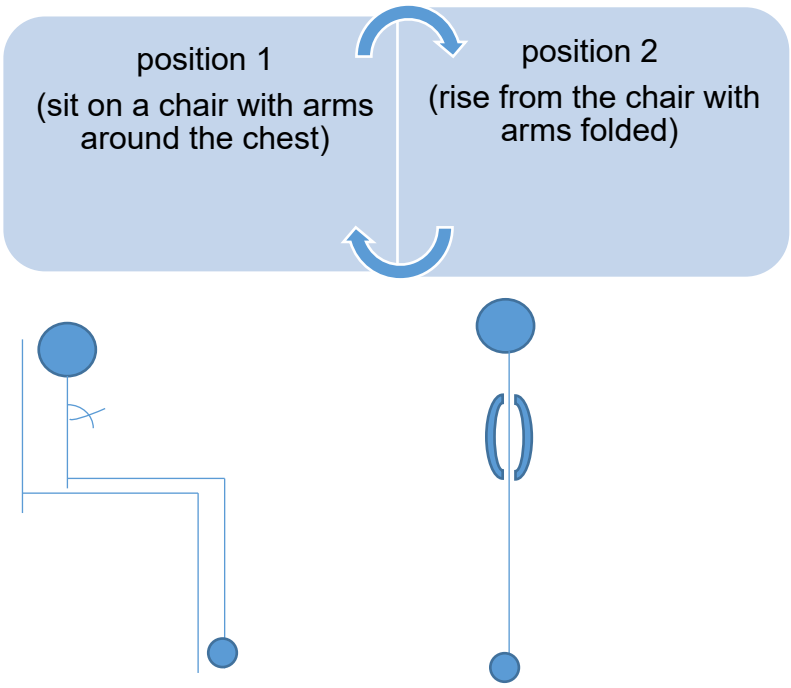


Figure 3.3 Performance of single chair stand test (s)

3.1.3.3 Repeated chair stand

If the participants had used their arm for support while rising up from the chair in a single chair stand test then the repeated chair stand test was not performed. Otherwise, if successful, they were asked to repeat the single chair stand test five consecutive times as quickly as possible (121). The pre-preparation of this test was the same as in single chair stand test. The same chair was used as in the single chair stand test. The test was conducted using the stopwatch and recorded the time in seconds for five stands. The scoring of repeated chair stand test was as below however, behind this scoring, the time (in seconds) taken to perform this test in continuous manner was used for analysis purpose (121).

Table 3.1. Scoring of repeated chair stand test performance *(121)*

Repeated chair stand test performance time (sec)	Scoring
Not performed/took >60	0
≥16.70	1
13.70-16.69	2
11.20-13.69	3
≤11.19	4

3.1.4 Muscle function

3.1.4.1 Timed up and test (s)

The timed up and go test was used to assess the functional mobility of the participants (122). It was defined by the time needed for the participant to stand up from a straight-backed chair, walk safely to a line at a 3 meters distance, turnaround at the line, walked back to the chair and sit down again (122).

Test performance

Participants were instructed to sit down on a chair (used from the community centre/temple) with their feet resting on the floor and their back against the chair's back. Upon researcher saying 'start' the participants stood up from the chair, walked 3 meters straight, turned around the line marked at 3 meters, walked back and sat down on a same chair. A walking time for 3 metres (9.8 feet) was measured in seconds using a stopwatch. They were informed before starting the stopwatch and stopped recording the time when their buttock touched the seat. Participants were allowed to use their walking aid if needed and wore regular footwear. The researcher and any assistive device remained near to the participant during the test.

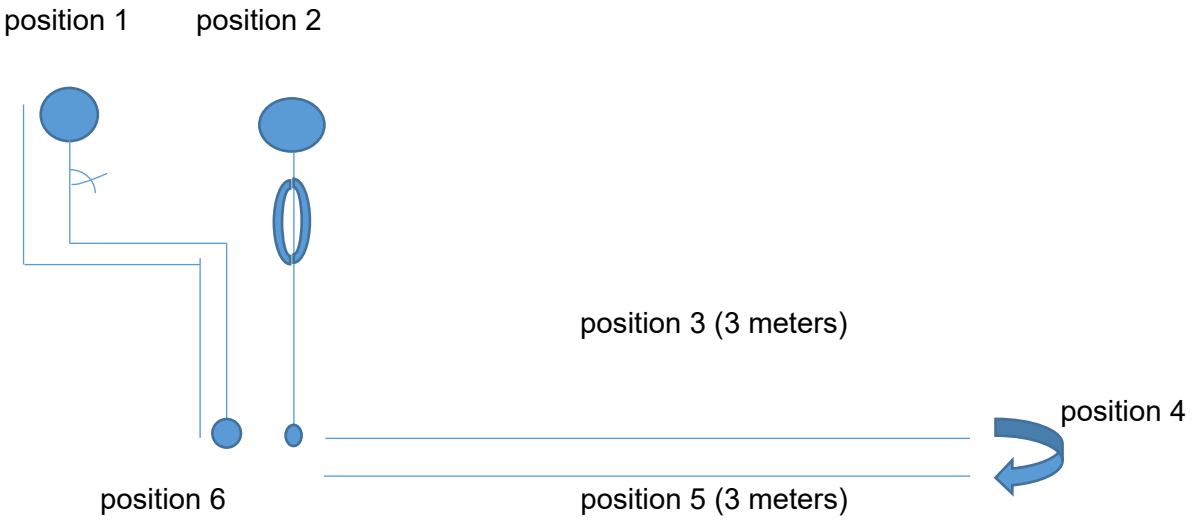


Figure 3.4 Steps taken in performing timed up and go test (s)

3.1.4.2 Balance test (s)

The balance test was used to assess the functional capability of the participants and counted as a muscle performance measure (121). It was consisted of three tasks: side by side, semi-tandem and full-tandem. The participants were asked to perform all three tasks of the test one by one at single occasion. Participants were asked and informed before start counting the time on a stopwatch. The score of balance test was the sum points from all three tasks as described below.

Side-by-side test

Test performance

The participant held their feet together side by side and they were asked "*are you ready*?" and upon their response the researcher said "test begin" and started recording the time. After 10 seconds, the researcher said "stop" and stopped the stopwatch so the participant stepped out the position. They could move their body without moving their feet. A stopwatch was used to record the time the participant needed to maintain this position. The scoring of this task was as such:

Table 3.2. Scoring and the reasons for not attempting side by side balance task *(121)*

Time needed to hold position	Score	Reason for not attempting	Correspond number
Held for 10 sec	1	tried but unable	1
Not held for 10 sec	0	could not hold the position unassisted	2
Not attempted	0	not attempted, the researcher felt unsafe	3
		not attempted, the participant felt unsafe	4
		unable to understand instructions	5
		Other	6
		Participant refused	7

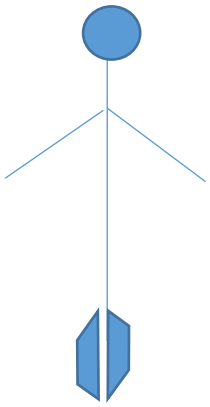


Figure 3.5 Position in performing side by side balance task (s)

Semi-tandem

This test was performed by standing in a position with the side of heel of one foot touching the side of big toe of the other foot with either foot in front for about 10 seconds (121). The instruction given to the participant and the pre-preparation of the test was same as above. A

stopwatch was used to record the time needed as above. The scoring protocol and the reasons for not to attempt the test were same as above for side-by-side task.

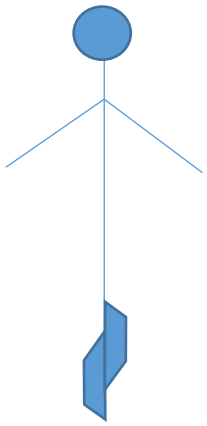


Figure 3.6 Position in maintaining semi-tandem balance task (s)

Tandem stand

In this third task of the balance test, each participant was asked to stand with the heel of one foot in front of and touching the big toe of the other foot with either foot in front for about 10 seconds (121). A stopwatch was used to record the scored in seconds and participants were informed before starting the stopwatch. The list of the reasons to demonstrate the failure for attempting the test were same as above.

Table 3.3. Scoring of maintaining tandem balance position *(121)*

Time needed to hold position (seconds)	**Score (points)**
Held for 10	2
Held for 3 to 9.99	1
Held for less than 3	0

Not attempted	0
Number of seconds held the position	…

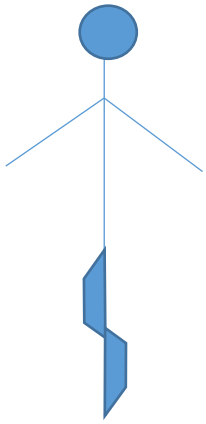


Figure 3.7 Position in maintaining tandem balance task (s)

3.1.5 Approach taken for methodologies

Both theorectical and scientific framework approaches were taken to perform methodologies for this thesis. A qualitative approcah was taken for chapters 7 methodology (Loraine Busetto et al., 2020) and scientice approach was apapted for chapter 4 to 6 methodologies.

4 Chapter 4: Association between circulating blood 25(OH)D concentration, muscle strength and function in UK South Asian older women: A cross-sectional study

4.1 Background

There is evidence from the UK Biobank of low vitamin D intake (through food and supplements) in South Asians (Bangladeshi, Indian and Pakistani) living in the UK (110). The vitamin D intake in South Asians through diet was found to be 1.0-3.0µg/day and only 22% of Bangladeshi, 32% Indians and 25% of Pakistanis were taking a vitamin D containing supplement (110). This survey also has reported corresponding high vitamin D deficiency in this population (section 1.4). Sun avoidance behaviour and limited outdoor activities (123) has also previously reported in UK South Asians which all collectively contributes towards high prevalence of low circulating 25(OH)D concentration in UK based South Asian older women (section 1.4).

The involvement of sufficient circulating 25(OH)D concentration in maintenance of muscle strength and function with advance age has been investigated and reported previously in the SACN report of 2016 and in other cross-sectional studies (section 1.3.1). Although, there is considerable heterogeneity in study design and outcomes taken together the findings of mainly cross-sectional studies have suggested a relationship of vitamin D with lower limb muscle strength and function (chair stand test, balance test, timed up and go and walking speed test) in community-dwelling post-menopausal women of different ethnic groups.

SACN (2016) recommended a vitamin D intake of 10µg/day to achieve circulating 25(OH)D concentration of ≥25nmol/L for musculoskeletal health in the general population of older adults in the UK (46). Despite a high prevalence of vitamin D deficiency in South Asian post-menopausal women there is little data available on requirements for good muscle strength and function in this target population. All the evidence of a relationship between vitamin D and muscle are from Caucasian post-menopausal women and there is no cross-sectional data from South Asian older women in the UK. This is very important and would be interesting to study whether such low vitamin D levels has links with skeletal muscle strength and function in South Asian older women.

4.2 Hypothesis

It is hypothesised that there is a positive linear relationship between circulating blood 25(OH)D concentration and muscle strength and physical function in older South Asian women (Indian and Pakistani) living in the UK.

4.2.1 Aim

To test the hypothesis that a relationship exists between circulating blood 25(OH)D concentration (nmol/L), muscle strength and function in UK South Asian older women.

4.2.2 Objectives

- To obtain ethical approval for the study
- To recruit one hundred and twenty community-dwelling South Asian women aged 60 years and above.
- To measure circulating blood 25(OH)D concentration
- To assess handgrip strength as indicator of upper limb muscle strength
- To assess single chair stand as indicator of lower limb muscle strength
- To assess repeated chair stand as indicator of lower limb muscle strength
- To assess balance test and timed up and go performance as parameter of muscle function.

While meeting these objectives, the dietary intake was assessed by using single 24-hour diet recall by using multiple pass method. The self-reported physical activity level was also assessed, details of which are provided in Chapter 6.

4.3 Methods

4.3.1 Study design

This cross-sectional study was designed to investigate the relationship between 25(OH)D concentration (nmol/L), muscle strength and function in UK South Asian older women. No formal power calculation was conducted and the sample size was based on similar studies that reported a relationship between vitamin D and musculoskeletal health. It was also a pragmatic decision based on what could practically be achieved. Participants who expressed an interest in taking part in the study were invited to meet with the researcher (SZ) on a single appointment visit at their preferred location (home, community centre, temple) when consent was obtained and the study assessments were performed. Participant eligibility was re-checked before the study commenced and informed, written consent was obtained from each participant. For participants unable to read the consent form was read to the participant by the researcher. Each participant was asked to sign (or mark) two copies of the written consent forms (one for the participant record and other for the researcher to keep in the site file). After that, the study protocol was performed i.e., on a single occasion. The study protocol took around 60-90 minutes in total to perform.

4.3.2 Ethical approval

Ethical approval for the cross-sectional study was gained from the University of Sheffield's Medical School Research Ethics Committee. The study was granted approval on 11/10/2017 (reference number 015586, see appendix (c).

4.3.3 Participants

One hundred and twenty Indian (Sikh, Gujarati, Hindu) and Pakistani (Punjabi and Mirpuri) women were recruited from Rochdale and Sheffield between January-May 2018. The study recruited healthy and community-dwelling women.

The inclusion criteria were:

- Aged 60 years and above
- Community-dwelling
- Vitamin D supplement users and non-users
- Able to give written and verbal consent.

The exclusion criteria were:

- Unable to communicate
- Hearing impairment or vision loss and walking disability or
- being institutionalized.

4.3.4 Advertisement and recruitment

4.3.4.1 Advertisement

Convenience sampling was used to recruit potential participants. Figure 4.1 shows the CONSORT flow diagram of participant recruitment. The study was advertised using a variety of methods. A leaflet was prepared in English and Urdu/Hindi language (two-sided print) to advertise the study and provide study information and researcher contact details to potential participants (Appendix b). South Asian community centres, Indian temples (Gurdwara) and mosques were approached in order to invite target participants. In addition to the contacts made via community centres and places of worship, the project was advertised by word of mouth. The participant information sheet was prepared with the inclusion of the study protocol to aid participants understanding of the study requirements.

4.3.4.2 Recruitment procedure

With the permission of authorities at South Asian community centres, face to face sessions were conducted with potential participants in which a talk was given in the common language

of the centre attendees. The purpose of this was to provide information about the study and to answer any questions. Participant information sheets were distributed to interested participants at the end of the session and contact details were shared. Participants were provided with sufficient time to consider their involvement with the study and were encouraged to discuss the study details with family members before making a decision as whether or not to take part.

Participants who expressed an interest in taking part were invited to contact the researcher (usually by telephone) and a one-to-one appointment was arranged. The appointment was typically arranged for the same day/place that the women visited the community centre/venue. The authorities of community centres were informed in advance and arrangements made for access to a dedicated room in order to perform the study procedure as per timetable.

The researcher arrived at the destination at least 15 minutes before the volunteer in order to set up the study equipment. It involved setting up a folder for each woman which consisted of all documents for assessment which has to perform that day, setting up the stadiometer, stopwatch, straight backed chair for performing muscle strength test and marking the floor for performing timed up and go test. Individual time slots were given to each woman. Fifteen minutes gap was maintained between each appointment. A prize draw containing five prizes of £100, £75, £50, £25, £25 high street vouchers was held at the end of the study. As part of one-to-one appointment, the following study measurements were performed on a single occasion.

4.3.5 Study protocol

4.3.5.1 Anthropometry

Anthropometric data on height, weight, waist and mid-upper arm circumference was collected. Body height was measured in centimetres using a portable stadiometer (Seca 213 Leicester portable height measure, UK) and body weight was measured in kilograms using a portable Tanita weighing scale (BC-601). Participants were required to remove their shoes and socks off before height and weight measurements were taken. Waist and mid-upper arm circumference were measured in centimetres with a plastic measuring tape.

4.3.5.2 Vitamin D measurement

The finger prick blood spots were collected and posted on the same day for the measurement of circulating 25(OH)D concentration of the participants (120) (Figure 3.1). Samples were sent directly on collection for analysis to a service provider (Department of Clinical Biochemistry, Sandwell and West Birmingham Hospital). Further detail is provided in Chapter 3 of the thesis (section 3.1.2).

4.3.5.3 Assessment of muscle strength and function

Muscle strength were assessed by measuring handgrip strength, single chair stand and repeated chair stand tests. Muscle function was collected by assessing balance and timed up and go test. Handgrip strength (kg) was measured using a Jamar hand-held dynamometer (Patterson Medical, Warrenville, IL, USA). Three consecutive readings were taken in a standing position using the right hand hanging free on the side of the body with a one-minute interval between each attempt (Figure 3.2). The highest measured value for three attempts was used for analysis (71) (section 3.1.3.1).

The single chair stand test (s) was used to examine the lower limb muscle strength. This measurement records the time taken for a participant to stand up from a straight back chair with arms folded around the chest and sit down back (section 3.1.3.2) (Figure 3.3). The repeated chair stand test (s) was the time taken to performing a single chair stand test five times consecutively (121) (section 3.1.3.3).

The timed up and go test (TUG) was used to examine muscle function and walking ability. This was the time taken by a participant to stand up from a chair, walk 3 meters straight, turn around a marker, walk back to the chair and sit down (122) (section 3.1.4.1) (Figure 3.4). The same chair which was used for chair stand test was used for this test. The balance test was used to examine functional capacity. It consisted of side by side (Figure 3.5), semi-tandem

(Figure 3.6) and tandem tests (121) (section 3.1.4.2) (Figure 3.7). Further detail of each test is provided in chapter 3 of the thesis (section 3.1.3). The balance test was also used to assess muscle functional capacity of this population. It was consisted of maintaining side by side, semi-tandem and tandem balance test (section 3.1.4.2 of Chapter 3). The walking speed test (s) was not possible conduct given that the time required for rest of the protocol and recruitment challenges.

4.3.5.4 Dietary assessment

A 24-hour single diet recall using the multiple pass method was used to collect the dietary intake of the participants (124). Each participant was asked to recall their diet of the previous day, including all food, drinks and snacks they consumed. Participants were given about five minutes to think and recall their diet prior to start the interview which was conducted in their preferred language.

In brief, participants were asked to recall everything they consumed on a previous day at home or from outside, then time of the day when they had eaten/drunk each product, recipe of the product that how it had been made, which ingredients were used in making it, of which brand they were and how much they had consumed. In the end, participants were asked again to recall if they had forgotten to tell anything of what they had consumed on the previous day. They were then asked about other food commonly consumed in combination with the foods they had reported or other options i.e., any side dish along with main course of meal, dessert, any snack they were offered and shared with grandchildren. Portion size and food type was identified and noted in a code form written on a food book. Data were analysed using DietPlan7 (Forestfield Software).

4.3.5.5 Self-reported physical activity

The International Physical Activity Questionnaire (IPAQ)-short form was self-administered to assess the physical activity of the participants (125). This questionnaire assessed the time spent in minutes on vigorous, moderate and walking activity during the last 7 days. Vigorous activity included: heavy lifting, digging, aerobics or fast bicycling and moderate activity included: carrying light loads, bicycling at a regular pace or double tennis. The time spent in minutes on each activity was multiplied by the Metabolic Equivalent Task (MET) and number of days that activity was undertaken during last 7 days period. One MET was defined as equal to the energy expenditure in a resting position. Vigorous activity was equal to 8 MET, moderate was equal to 4 METS and walking was equal to 3 METS multiplied by the time in minutes spent on carrying each activity and the number of days that activity was undertaken during last 7 days period (125). A copy of the questionnaire is included in the appendix.

4.3.6 Data management

All the collected information during the course of the research was kept strictly confidential and was only accessible to members of the research team. The handling of personal data was controlled by the General Data protection Regulation (GDPR) and associated legislation.

Ethical approval and all study related documents were kept in a site file. Consent forms were kept as hard copies in a site file and store in a locked filing cabinet at the Department of Oncology & Metabolism, Medical School premises at the University of Sheffield. All collected data was pseud-anonymised. After taking consent, participants were given a unique identification number that was used on all other records. Participants names were written on two hard copies of consent form only. One copy was provided to participant and we kept the other copy for our records. The collected data was saved as hard copies and in electronic format on a password encrypted protection for analysis purposes. The electronic format of collected data was transferred into SPSS file and stored in google drive at the University of Sheffield.

4.3.7 Statistical analysis

Data was analysed using IBM SPSS statistics software (Version 26). The one-sample Kolmogorov Simonov test was used to test the distribution of the data. Continuous variables were presented as mean, median, range and standard deviation and categorical variables were reported as numbers and percentages. Mann-Whitney U test and chi-square test were used to analyse continuous and categorical scale variables respectively. The relationship between blood 25(OH)D concentration, muscle strength and physical function was assessed by Spearman's correlation analysis. A *p*-value of ≤0.05 used to define significance.

4.4 Results

4.4.1 Recruited Participants

One hundred and twenty community living South Asian (Indian and Pakistani) women aged 60 years and above were recruited to the study and completed all the measurements.

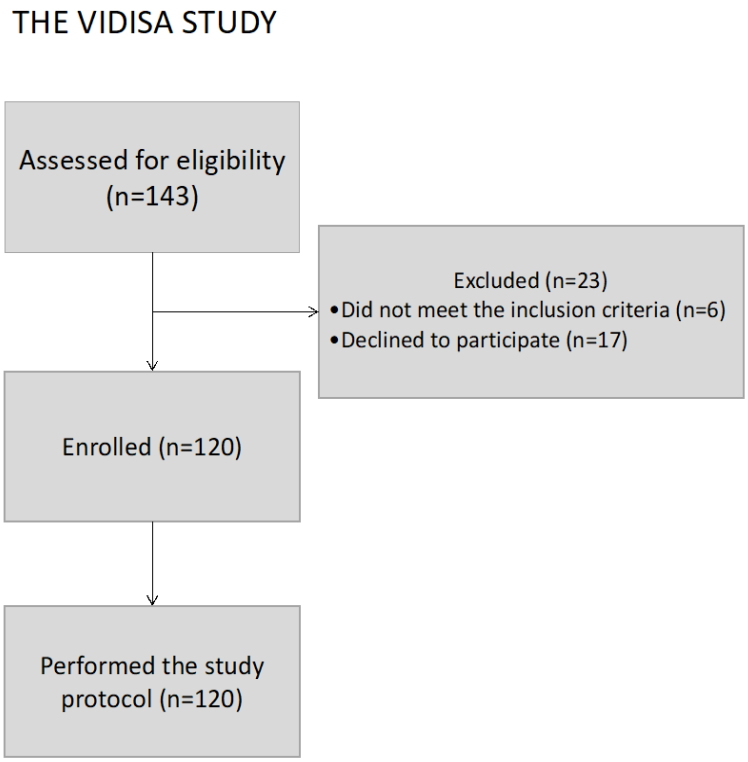


Figure 4.1 CONSORT flow-diagram showing participant recruitment

4.4.2 Participant's characteristics

One hundred and twenty women were successfully recruited to the study. Table 4.1 shows the characteristics of all the recruited participants, which is then presented according to blood 25-dihydroxy vitamin D concentration cut offs as defined by Institute of Medicine (2011). The vitamin D concentration of the women was mean (SD) 58.3 (37.8) nmol/L. Geographically, the 25-hydroxyvitamin D concentration in participants from the Sheffield and Rochdale region was measured as mean (SD) 75.3 (32.4) and 38.1 (25.6) nmol/L respectively. Fifty-six women (47%) were found to have an inadequate/deficient vitamin D status (<50nmol/L) and 64 women (53%) were found to have a sufficient status (≥50nmol/L). Majority of the women (67%) were recruited from the Pakistani community. There was no difference in the blood 25(OH)D concentration of Indian and Pakistani women.

The mean age of the women at the time of recruitment was 69 years and there was no significant difference in the age of women with inadequate/deficient status compared with those with sufficient vitamin D status (Table 4.1). Majority of the women were recruited from Rochdale. Overall, the BMI of the women was in the obese category as none of the women

were underweight and the majority were obese. No difference in BMI of the women according to status was found. More than half of the included women had no education history though there was significant difference in literacy level of women with inadequate/deficient and sufficient status with women with sufficient status were found to be more educated than others.

Eighty-six percent of current vitamin D supplement users had sufficient 25(OH)D concentration and only 14% of participants who reported to be current user of vitamin D supplement were deemed to have inadequate/deficient 25(OH)D concentration (Table 4.1). Sixty-eight percent of vitamin D supplement non-users had inadequate/deficient 25(OH)D concentration and only 32% had sufficient levels. The presence of ≥3 comorbidities was [n=23(19%)] and it has found to have no relationship with inadequate or sufficient levels (p=0.67) (Table 4.1). The high prevalence of diabetes, hypertension and arthritis was found in this target population.

Table 4.1. Participant characteristics according to blood 25(OH)D concentration

Characteristics	All Mean (SD), median, IQR (*n*=120)	Inadequate/deficient (<50nmol/L) Mean (SD), median, IQR (*n*=56)	Sufficient (≥50nmol/L) Mean (SD), median, IQR (*n*=64)	*p*-value†
Age (years)	68.9 (7.2), 66.3, 9.67	68.1 (6.5), 66.0, 7.5	69.6 (7.6), 66.9, 12.2	0.335
Ethnicity [n (%)] **Pakistani** **Indians**	 80 (67) 40 (33)	 39 (69.6) 17 (30.3)	 41 (64.0) 23 (35.9)	0.518‡
Regionally [n (%)] **Sheffield** **Rochdale**	 33 (28) 87 (72)	 7 (12.5) 49 (87.5)	 26 (40.6) 38 (59.3)	0.03*
Waist (cm)	107.3 (11.4), 107.5, 14	108.6 (12.7), 108.0, 20	106.2 (10.0), 106.0, 13	0.225
Mid upper arm circumference (cm)	32.4 (4.9), 32.0, 7	33.1 (4.4), 32.5, 7	31.8 (5.3), 31.5, 6	0.133
BMI (kg/m^2)	32.1 (5.7), 32.1, 8.0	32.8 (6.0), 32.9, 9.2	31.6 (5.4), 31.5, 7.1	
Underweight (<18.5) [n (%)]	0 (0%)	0 (0%)	0 (0%)	0.210
Normal weight (18.5-24.9) [n (%)]	12 (10)	7 (13)	5 (8)	
Overweight (25-29.9) [n (%)]	34 (28)	13 (23)	21 (33)	
Obese (≥30) [n (%)]	74 (62)	36 (64)	38 (59)	
Education [n (%)] **No education** **School level** **College level** **University level**	 63 (52.5) 38 (31.6) 14 (11.6) 5 (4.2)	 35 (62.5) 15 (26.7) 5 (8.9) 1 (1.7)	 28 (43.7) 23 (35.9) 9 (14.0) 4 (6.2)	0.032*
Supplement use (n (%))***	49 (41)	8 (14)	41 (86)	0.000***
Rochdale	21 (42.8)	5 (62.5)	16 (39)	

Sheffield	28 (57.1)	3 (37.5)	25 (61)	
Blood 25(OH)D concentration (nmol/L)	58.3 (37.8), 53.0, 59.6	25.5 (10.7), 24.6, 14.9	86.9 (28.7), 84.3, 37.9	0.000*
Total number of Comorbidities	1.9(0.9), 2.0, 1.0	2.0 (1.0), 2.0, 2.0	1.8 (0.9), 2.0, 1.0	
Hypertension [n (%)]	71 (59.1)	32 (57.1)	39 (60.9)	
Diabetes [n (%)]	61 (50.8)	30 (53.5)	31 (48.4)	
Asthma [n (%)]	27 (22.5)	11 (19.6)	16 (25)	
Liver disease [n (%)]	0	0	0	0.724
Kidney disease [n (%)]	5 (4.1)	2 (3.5)	3 (4.6)	
Heart disease	18 (15)	10 (17.8)	8 (12.5)	
Previous history of Cancer [n (%)]	12 (10)	7 (12.5)	5 (7.8)	
Arthritis n (%)* **	84 (70)	40 (71.4)	44 (68.7)	0.75

"n" represents the number, kg/m2 is kilogram/meter square, BMI=body mass index.

The age of participants is the age in years at the time of recruitment.

*Mean values within a column like superscripts letters were significantly different $p<0.05$.

†Mann-Whitney U test was used (unless indicated).

‡Chi-square test.

4.4.3 Muscle strength and function according to 25(OH)D concentration

Table 4.2 shows the means (SD), median and range values of muscle strength and function parameters in all participants and then further shows the muscle strength and function according to inadequate and sufficient 25(OH)D levels. Participants who had sufficient 25(OH)D concentration performed single chair stand, repeated chair stand, balance test and timed up and go test better that those who had inadequate 25(OH)D concentration (Table 4.2). In all of these tests women who were vitamin D inadequate were slower at completing these tasks. There was no difference in the capacity of handgrip strength (kg) (p=0.35) between participants with inadequate/deficient and sufficient levels. For single chair stand and repeated chair stand test, the data of 104 women has showed with 56 and 48 women with sufficient and inadequate/deficient status respectively.

Table 4.2. Muscle strength and function according to 25(OH)D concentration

Outcome variables	All Mean (SD), median, IQR (*n*=120)	Inadequate/deficient (<50nmol/L) mean (SD), median, IQR (*n*=56)	Sufficient (≥50nmol/L) Mean (SD), median, IQR (*n*=64)	*p*-value†
Handgrip strength (kg)	18.2 (4.6), 18.0, 5.9	17.8 (4.6), 18.0, 5.3	18.5 (4.7), 18.7, 5.8	0.357
Single chair stand (s) (*n*=104)	4.3 (1.6), 3.8, 1.9	4.7 (1.7), 4.4, 1.9	3.9 (1.5), 3.6, 1.1	0.006*
Repeated chair stand test (s) (*n*=104)	23.0 (8.2), 21.7, 7.9	25.1 (8.2), 23.1, 7.9	21.2 (7.7), 19.8, 8.9	0.002*
Timed up and go test (s)	17.3 (10.3), 14.2, 7.8	18.3 (9.3), 15.1, 7.9	16.4 (11.1), 13.0, 6.3	0.028*
Balance test (s) (*n*=118)	3.1(1.0), 3.0, 2	2.8 (1.0), 3.0, 2	3.2(0.9), 4.0, 2	0.04*

"kg" and s stand for kilogram and second respectively.

*Mean values within a row were significantly different ($p<0.05$).

†Mann-Whitney U test.

4.4.3.1 Baseline characteristics of all participants by ethnic sub-groups

Due to heterogeneity reported between Indian and Pakistani women vitamin D level as described in section 1.6 it was thought to perform analysis of muscle strength and function as per vitamin D status cut off in ethnic groups to explore further as to whether muscle performance has same appearance in Indian and Pakistani women. Table 4.2 shows the muscle strength and function of one hundred and twenty Indian and Pakistani women. The data of sixteen women for single and repeated chair stand test and two for balance test was missing. The performance of handgrip strength, single chair stand test, repeated chair stand test and timed up and go test was found to be same across Indian and Pakistani women except balance test performance which was different between two sub-groups. This difference could be cultural/sub-ethnic population specific and required further investigation.

Table 4.3 Baseline characteristics/skeletal muscle performance by ethnic sub-groups

Outcome variables	Indian (*n*=36) Mean (SD), median, IQR	Pakistani (*n*=66) Mean (SD), median, IQR	*p* value†
Age (years)	71.1(8.4), 68.5, 14.2	67.3(5.2), 66, 6.9	0.004
25(OH)D concentration (nmol/L)	61 (36.2), 61, 60	57.5 (38.2), 51, 61	0.260
Right handgrip strength (kg)	18.1 (3.6), 17.8, 5.5	19.2 (4.9), 19.1, 6.1	0.369
Single chair stand (s)	4.2 (1.9), 3.8, 2.3	4.3 (1.5), 3.8, 1.6	0.239
Repeated chair stand (s)	21.9 (6.8), 21.7, 9.9	23.5 (8.9), 21.7, 7.2	0.494
Timed up and go (s)	15.0 (7.1), 13.6, 5.5	14.5 (5.7), 13.1, 4.8	0.982
Balance test (s)	2.9 (0.9), 3, 2	3.3 (0.9), 4, 1	0.037

†Mann-Whitney U test.

**p*-value was significant at <0.05

4.4.4 Muscle strength and function across vitamin D status cut-offs by ethnic groups

Further to findings in Table 4.3, it was thought to understand muscle strength and functional performance across vitamin D status thresholds as set by SACN and IOM as shown in Table 4.4. The performance of single and repeated chair stand test in Indian women and single chair stand, repeated chair stand and timed up and go test in Pakistani women was found to be different across vitamin D threshold as the women with sufficient vitamin D status had given better performance on these tests compared to with having inadequate/deficient status. The performance on other variables were found to be same/independent of vitamin D thresholds.

Table 4.4 Muscle strength and function across vitamin D cut-offs by ethnic sub-groups

Outcome variables	**Indian**		***p* value†**	**Pakistani**		***p* value†**
	n= 16 Vitamin D status <50nmol/L Mean (SD), median, IQR	*n*= 20 Vitamin D status ≧50nmol/L Mean (SD), median, IQR		*n*=31 Vitamin D status <50nmol/L Mean (SD), median, IQR	*n*=35 Vitamin D status ≧50nmol/L Mean (SD), median, IQR	
Age (years)	66.5(6.0), 66.2, 6.2	74.7(8.3), 75, 14.2	0.001	67.9(5.8), 66, 7	66.7(4.6), 66, 7	0.193
Right handgrip strength (kg)	18.5(4.1), 18.3, 5	17.8(3.2), 17.5, 6	0.588	18.4(4.8), 18.5, 6.9	19.9(4.9), 19.6, 6	0.129
Single chair stand (s)	4.8(1.9), 4.9, 1.9	3.7(18), 3.5, 1.9	0.068	4.6(1.7), 4.2, 1.9	4.0(1.2), 3.6, 1.6	0.043
Repeated chair stand (s)	24.3(5.9), 23, 8.8	20.0(7.0), 18.4, 8.9	0.02	25.4(9.4), 23.1, 7.2	21.9(8.2), 20.1, 7.5	0.028
Timed up and go (s)	15.8(5.4), 14.6, 6.6	14.3(8.2), 12.9, 6.1	0.37	15.7(6.7), 14.3, 4.4	13.5(4.4), 12.8, 5.5	0.039

Balance test (s)	2.7(0.7), 3.0, 1	3.1(0.9), 3.5, 2	0.37	3.1(1.0), 4, 2	3.5(0.8), 4, 1	0.064

†Mann-Whitney U test.

**p* value was significant at <0.05

4.4.5 Relationship between 25(OH)D concentration, muscle strength and function in overall participants

Figure 4.2 shows the Spearman's correlation between blood 25(OH)D concentration and muscle strength and function in all participants. A significant weak negative association was observed between 25(OH)D concentration and the performance of single chair stand test (r=-0.24; *p*=0.01), repeated chair stand test (r=-0.27; *p*=0.004) and timed up and go test (r=-0.19; *p*=0.03). However, no association was found between 25(OH)D concentration and handgrip strength (r=0.09; *p*=0.3).

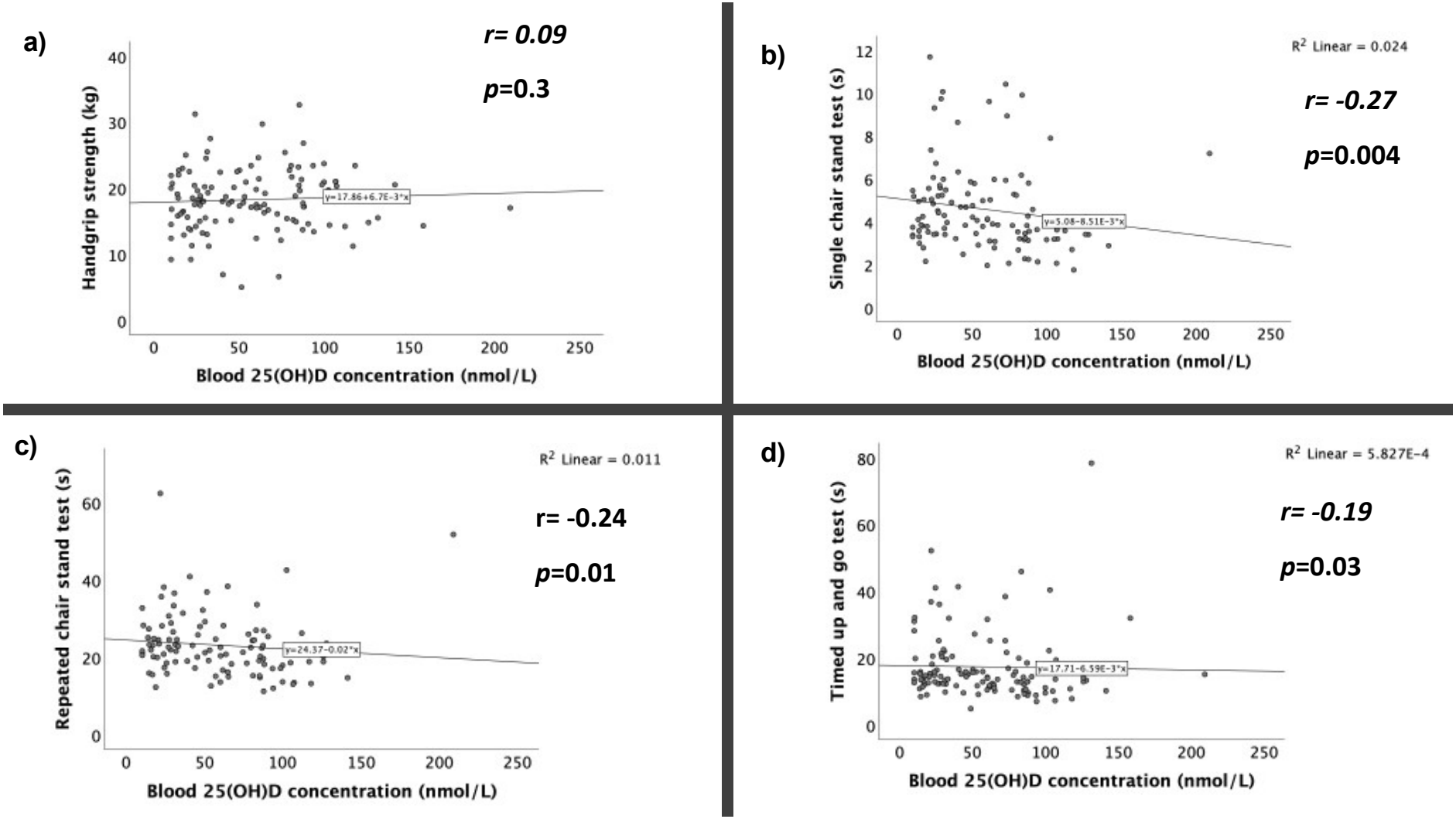

a)
r= 0.09
p=0.3
Handgrip strength (kg)
Blood 25(OH)D concentration (nmol/L)
b)
R² Linear = 0.024
r= -0.27
p=0.004
Single chair stand test (s)
Blood 25(OH)D concentration (nmol/L)
c)
R² Linear = 0.011
r= -0.24
p=0.01
Repeated chair stand test (s)
Blood 25(OH)D concentration (nmol/L)
d)
R² Linear = 5.827E-4
r= -0.19
p=0.03
Timed up and go test (s)
Blood 25(OH)D concentration (nmol/L)

Figure 4.2 Circulating blood 25(OH)D concentration associates with lower limb muscle strength and function

Weak relationship was found between 25(OH)D concentration and b) single chair stand, c) repeated chair stand and d) timed up and go test. No apparent relationship between 25(OH)D and handgrip strength found (a).

p and r value were obtained from Spearman's correlation analysis.

Correlation is significant at 0.05 and 0.01 level.

- **Further analysis was performed to explore difference in muscle strength and function according to vitamin D quintiles, across SACN threshold and age of the participants. Relationship between 25(OH)D concentration, muscle strength and function was performed according to vitamin D threshold set by SACN and IOM too but none of them has found any additional information regarding relationship than above (Appendix g).**

4.4.6 Dietary intake analysis

Dietary intake data is not shown in this thesis due to apparent underreporting of reported dietary intake. Dietary intake analysis of a random sample of 37 women was performed using Dietplan7. The reported energy intake was compared against estimated basal metabolic rate as determined by Harris Benedict formula, and cut-offs applied as described by Black (2000) (126). Out of 37, twenty-four women were found to be under-reporting, nine women were in acceptable range and four women were found to be over-reporting. On this basis, a decision was taken not to analyse the dietary records any further.

4.4.7 Self-reported physical activity

A detailed information on analysis of level and frequency of physical activity is provided in chapter 5 of this thesis as this could be a separate interest of study in this population and whether it has any relationship with circulating blood 25(OH)D concentration. In Brief, majority included women were found to have low physical activity level calculated by automated tool for categorising self-reported physical activity data (125). We have not found to have relationship between vitamin D and self-reported physical activity in these group women.

4.5 Discussion

4.5.1 Main findings

The purpose of this cross-sectional study was to explore the relationship between circulating 25(OH)D concentration, muscle strength and function in community-dwelling South Asian women aged 60 years and above living in the UK. The study found forty-seven percent of participants with inadequate 25(OH)D concentration (<50nmol/L) and the majority of this vitamin D inadequate population did not use vitamin D supplements.

The main finding of the current study was that participants with sufficient 25(OH)D concentration had better lower limb muscle strength and functional performance than those with inadequate/deficient 25(OH)D concentration (Table 4.2). However, there was no significant difference in the handgrip strength of those who were sufficient compared with those who were deficient. There was no apparent association between circulating blood 25(OH)D concentration and hand-grip strength of the target population. A significant weak negative relationship was observed between circulating blood 25(OH)D concentration and single chair stand, repeated chair stand and timed up and go performance of the women (Figure 4.2). Furthermore, a high percentage of prevalence of arthritis was found in this target population which could possibly affect the capacity of performing handgrip strength test and thus the relationship with circulating 25(OH)D concentration. Muscle strength and functional performance was also analysed in Indian and Pakistani women separately as per vitamin D thresholds defined by IOM. Except repeated chair stand test none of the other muscle performance outcome variable were found to be differed across vitamin D thresholds (Table 4.3). In comparison among Pakistani women, the participants with sufficient vitamin D status had given better performance at single chair stand, repeated chair stand and time up and go test.

4.5.2 Critical analysis with literature

4.5.2.1 Relationship between circulating blood 25(OH)D concentration and handgrip strength

It is important to consider the current study within the context of the revised version of European Working Group on Sarcopenia in Older People 2 (EWGSOP2) which has reported the mean (SD) normative value of handgrip strength of 26.5(6.2) kg in women aged 60 years old however with the estimation of at least 2.5SDs below the peak mean increased rapidly with increasing age (77). EWGSOP2 has also recently updated the criteria to define the

probability of diagnosis of sarcopenia in women with increasing age is based on decline in the handgrip strength. Given the average age of our study population the average handgrip strength was lower than expected, which may be explained by loss of muscle or by co-morbidity such as arthritis.

In agreement with our results a number of other studies have reported no relationship between 25(OH)D concentration and handgrip strength in community-dwelling women aged 60 years and above (71, 73-76). Furthermore, studies have reported a direct association between increasing age and decline in capacity of hand grip strength rather with 25(OH)D concentration (74) and this age-related decline in handgrip strength was reported to be independent of 25(OH)D concentration (76). In contrast the current study has not found any difference in handgrip strength according to age (Appendix g).

The current study population had a mean handgrip strength of 18.7kg and a mean age of 68.7 years. This is comparable with a previous study (71), in which Taiwanese women with a mean age of 62 years had a mean handgrip strength of 21.7 kg. Similarly, Dam and colleagues reported a handgrip strength of mean (SD) 17.6 (5.0) kg in older women with a mean age of 75 years. Interestingly, this study also reported no association between handgrip strength and 25(OH)D concentration, however this was a Caucasian population living in Southern California which may explain a high mean and relatively homogeneous 25(OH)D concentration mean(SD) (100.8 (33.1)nmol/L (76).

4.5.2.2 Relationship between 25(OH)D concentration and single chair stand and repeated chair stand

Regarding lower limb muscle strength, the current study has found significant weak negative relationship between blood 25(OH)D concentration and performance of single chair stand and repeated chair stand test (Figure 4.2). The current study has used circulating blood 25(OH)D concentration cut-offs defined by IOM classification (2) to compare participant's muscle strength and function accordingly (Table 4.2). Participants with 25(OH)D concentration =>50nmol/L were found to have better lower limb muscle strength and functional performance compared to those with <50nmol/L (Table 4.2). These findings are in line with IOM recommendation of vitamin D repletion level for good musculoskeletal health. These findings also are corroborated with previous cross-sectional study, with healthy Argentinian women (n=54; aged >65 years), which has reported the association between 25(OH)D concentration of >50nmol/L and higher lower limbs muscle strength as compared to those with 25(OH)D concentration <50nmol/L (knee extensor: p=<0.03; hip flexor: p=<0.05 and hip abductor: p=<0.04) (84).

Our findings are in contrast to two previous studies (73, 74). However, these studies have used strict selection criteria for participants. Such as, the rate of completion of five times sit to stand test was reported to be independent of serum 25(OH)D concentration whilst the women recruited in this study had previous history of falls and were consuming fall prevention medication (73). The later study, which has reported no association between vitamin D and 30s chair stand in Brazilian women, recruited women with type-2 diabetes. The study also had a modest sample size of 40 (74).

In terms of circulating blood 25(OH)D concentration threshold for better lower limb muscle strength, we have used IOM classification of vitamin D threshold. Several previous studies have reported better lower limb muscle strength at 25(OH)D concentration >=75nmol/L in community dwelling women aged 60 years and above (71, 76, 78, 127). Whilst, some studies have used vitamin D level quintiles or quartiles to report the relationship with lower limb muscle strength accordingly (71, 76, 78, 79, 127). Studies also have used different statistical approaches, some have used correlation analysis to explore relationship between circulating 25(OH)D concentration and lower limb muscle strength in a continuous manner (74, 76, 78, 127) while others have reported odds of good muscle strength according to 25(OH)D concentration (71, 73).

4.5.2.3 Relationship between circulating 25(OH)D concentration, balance and timed up and go

In terms of muscle function, the current study did not find a relationship between blood 25(OH)D concentration and balance test performance. In line with our findings, a considerable volume of the literature has reported an association between blood 25(OH)D concentration and timed up and go performance in community-dwelling women aged 60 years and above (71, 73, 79, 128). In contrast, Bentes and colleagues did not find a relationship between 25(OH)D concentration of mean (SD) 68.7(22.45) and muscle function (74). The study has not shown the data for timed up and go test which makes it hard to further critique the research.

4.5.2.4 Vitamin D deficiency in South Asians and use of supplementation

The current study confirms previous reports of hypovitaminosis D in UK South Asian post-menopausal women (6, 111) particularly in those who were not consuming vitamin D supplementation at the time of recruitment (Table 4.1). The findings from the UK Biobank also has reported low use of vitamin D supplementation and corresponding high prevalence of vitamin D deficiency in the South Asian population (110).

The current study has found sufficient 25(OH)D concentration in the majority of women who were vitamin D supplement users at the time of recruitment. Eighty six percent of users of

vitamin D supplement had sufficient 25(OH)D concentration (=>50nmol/L). Our findings are in line with a previous study which has reported a higher level of 25(OH)D concentration in vitamin D supplement users than non-users (76).

A significant difference in vitamin D supplement use between the 2 geographical regions (Rochdale and Sheffield) studied was identified (Table 4.1). A smaller proportion of women from Rochdale were using vitamin D supplements than from Sheffield (Table 4.1). These findings are supported by UK Biobank data which has reported geographical differences in the vitamin D intake of UK South Asians (110). Another recent study has highlighted the variation in GP prescribing and thus consumption of vitamin D supplementation in different postcode regions (129). It has further reported the difference in dose of prescribed vitamin D supplementation among different regions which is reflected the fluctuation in a healthcare setting. The current study suggests that post-menopausal South Asian women living in the North of England who are not prescribed vitamin D supplementation are at risk of hypovitaminosis D.

In terms of education level in this target population, over half of the women had no formal education. A higher level of education was evident in the women with a sufficient vitamin D status than those with having inadequate status (Table 4.1). Among those who had achieved the university level education, eighty percent had a sufficient vitamin D status. These findings are in line with previous cross-sectional studies in UK Caucasian and Taiwanese women respectively (71, 130). The current study has found under-reporting of dietary intake in included women. With regards to dietary intake and analysis in this population, a previous systematic review has suggested the importance of portion size specific to age and gender, culture-specific utensil usage and the education level ethnic groups for the development of portion size estimation tool for dietary assessment in this community (131).

4.5.2.5 Dietary intake

A Food Frequency Questionnaire (FFQ) has been developed for UK South Asians and validated twice previously with South Asian women (132, 133). This tool includes 207 questions of list of commonly used foods and dishes recorded according to the geographical and religious region of the respondents. This needs to interviewing individuals and ask them to recall information about their dietary intake of previous week/month/year. Therefore, this may not be appropriate and suitable method to assess dietary intake of older South Asian women. In addition, it collects information on recipes which may not be relevant to older South Asian women as it is unlikely older South Asian women to be involved in cooking at home due to culture and tradition and therefore, they may not be aware of current ingredients status. With that in mind, it was thought to use 24-hour diet recall could be a right choice for this

population to obtain possible accurate estimate of dietary intake data. Though the under-reporting has been found in data and this could be by the participants rather the tool itself.

4.5.2.6 Self-reported physical activity

We have found majority included women were involved in low physical activity which perhaps shows sedentary behaviour in this population. This is in line with literature as previous studies also reported South Asians to be less active compared to the White British (112). However, the Department of Health and Social care in the UK has recently updated the guidelines and recent recommendations are suggesting breaking up prolonged periods of sedentary time into small and light regular activities. This could have positive impact on quality of life as small changes make a difference to individuals in the longer term. The updated recommendations involved undertaking activities which aim to improve muscle strength, balance and flexibility at least twice a week (70). A previous RCT has also reported beneficial effects of involving in physical activity and exercise in improving muscle strength and function (89). The detailed information and discussion is provided in Chapter 5 of this thesis.

4.5.3 Clinical relevance

The findings of this study have the potential to have beneficial impact on public health and well-being as these findings are exploring the relationship between blood 25(OH)D concentration and aspects of muscle strength and function in South Asian women. This study has highlighted the difference in use of vitamin D supplementation between two regions which can be the basis for evaluating and understanding involved care services provided. The current study has highlighted the importance of using vitamin D supplementation for maintaining sufficient circulating blood 25(OH)D concentration which is important for good muscle strength and function in older adults.

4.5.4 Strengths and limitations

The following limitations should be considered while interpreting the results of this study. The main limitation in the design of the study is that causality cannot be inferred from cross-sectional studies. There are three routes of vitamin D intake: through exposure to sunlight, diet and supplementation. The current study did not quantify sunlight exposure because of the nature of the study. Dietary intake data was collected for the purpose of determining vitamin D intake, however extensive underreporting was evidence and thus data analysis was not performed. The handgrip strength of right hand was measured only which could not be dominant hand in all cases. The major strength of this study is that this is the largest study of its kind in South Asian women living in the UK. It provides a substantial population reporting

inadequate/sufficient vitamin D status which makes it possible to evaluate the vitamin D relationship with muscle strength and function.

4.6 Conclusion

- Vitamin D inadequacy is prevalent in the UK South Asian older women population, particularly in those who were not consuming vitamin D supplementation.
- The use of supplementation and vitamin D inadequacy was observed to differ in two geographical recruitment areas.
- The reason for this is unknown but may reflect concerted efforts within primary care to tackle vitamin D deficiency in the South Asian community in the Rochdale area.
- There is a relationship found between 25(OH)D concentration and some aspects of muscle strength and function in this target population.
- Further studies in this population are merited on a bigger sample size.

4.7 Summary

This cross-sectional study has investigated the relationship between circulating blood 25(OH)D concentration, muscle strength and function in UK South Asian women aged 60 years and above.

The data analysis of this study has demonstrated the following interesting findings:

- There is no apparent relationship between circulating blood 25(OH)D concentration and handgrip strength of this target population. Women were found to possess poorer handgrip strength than required in this age group, based on the suggestion of European Working Group on Sarcopenia in Older People 2 (EWGSOP2), regardless of measured circulating 25(OH)D concentration. These findings are in line with existing literature.
- Participants with sufficient circulating blood 25(OH)D concentration performed single chair stand test and repeated chair stand test better and faster than those who had inadequate/deficient levels.
- Participants with sufficient circulating blood 25(OH)D concentration had better performance on balance and timed up and go test compared to those with 25(OH)D concentration <50nmol/L.
- A higher prevalence of hypovitaminosis D was found in those participants who were not consuming vitamin D supplements at the time of recruitment.

- A significant difference in the use of vitamin D supplementation was found between Sheffield and Rochdale areas and this was reflected in their circulating blood 25(OH)D concentration.

5 Chapter 5. Relationship between circulating blood 25(OH)D and self-reported physical activity in South Asian women aged 60 years and above

5.1 Introduction

5.1.1 Physical activity

The Department of Health and Social care in the UK has recently updated the recommendation of physical activity guidelines for older adults aged 65 years and above (70). The reviewed guidelines have suggested breaking up prolonged periods of sedentary time into small and light regular activities. The updated recommendations involved undertaking activities which aim to improve muscle strength, balance and flexibility at least twice a week (70). Each week older adults of this age group should undertake at least 150 minutes of moderate intensity physical activity in a week or 75 minutes of vigorous physical activity or the combination of them. To undertake these recommended physical activity guidelines is reported to be beneficial for falls prevention in older adults and for the prevention of chronic disease (70).

Overall, there are five levels of physical activity: sedentary, light, moderate, vigorous and very vigorous. The sedentary level is defined by sitting and/ or lack of any activity; light activity includes cleaning, carrying light weights and yoga; moderate activity could be walking, cycling and going for shopping; vigorous activity is playing games, swimming and undertaking dancing performance; and finally, the very vigorous activity includes weight-lifting activities and push ups (134). Likewise, three categories of older adults (active older adults, transition phase, and frail older adults) have been identified and physical activity have recommended according to the category (70).

5.1.1.1 Active older adults

Active older adults are defined as active individuals who are already involved in regular recreational activity. This could be the performance of resistance exercise (weight-lifting or resistance bands), balance maintenance activities (one leg stand, backward walking or 3-dimensional movements etc) and impact exercises (swimming, jumping, skipping etc) twice a week. This level of activity is beneficial for overall health and muscle and bone capacity with advancing age (70).

5.1.1.2 Transition age

The transition phase of older adults is the period in which there is a decline in muscle functional capacity of older adults. This could be due to age-related decline in muscle strength/muscle

mass or being overweight otherwise remained healthy (70). This is accompanied by the lack of being physically active and adapting sedentary lifestyle due to not being able to perform any level and type of activity. This group is recommended to undertake simple basic light activities 'walk and rest for 5 minute' to start with and gradually build up to moderate level of performance as per guideline over the course of time (70).

5.1.1.3 Frail older adults

Frailty is a composition of five indicators which collectively define the inability of an individual to undertake any such activities. These factors are as follows: unintentional weight loss in last 12 months, loss of muscle mass, muscle strength below cut-offs (according to gender and age), muscle function below cut-offs and energy expenditure <270 kcal/week (68). The frail older adults have defined to have low capacity of performing any level of physical activity therefore any light activity such as walking or stair climbing is to be encouraging (70).

5.1.2 Importance of physical activity in older adults

The long-term sedentary behaviour in older adults may increase the risk of developing obesity, cardiovascular disease, type 2 diabetes and all-cause mortality (70). Undertaking physical activity recommendations could decrease the risk of poor physical function, frailty and disability in older adults compared to those who do not (135). Meeting these recommendations has also been reported to have positive role in the improvement of cognitive impairment (136).

Physical activities targeting muscle and bone health are especially important and emphasized in older adults for the prevention of age-related muscle loss and functional capacity. Muscle loss is reported to start from forty years of age with 50% loss by the 8th decade of life (137). However, having a sedentary lifestyle can increase this percentage along with risk of sarcopenia and frailty with increasing age (137). A previous RCT has reported beneficial effects of physical activity/exercise in improving muscle strength and function in community dwelling post-menopausal women (89). In addition, a recent systematic review of ten randomised controlled trial with 200 elderly population with mean (SD) aged 75.1(4.4) years has reported that involvement in various kind of physical activity has improved muscle function including balance performance in this population (114).

The Global Action Plan on Physical Activity (GAPPA) in 2018 by WHO has also supported and confirmed the importance of regular walking and cycling to contribute to health benefits. However, there is lack of evidence reporting such activities to be undertaken in low-and-middle income countries population which perhaps reflect the prevalence of higher risk of developing chronic diseases in these populations (138).

5.1.3 Measurement of physical activity

Several different ways to assess physical activity in older adults are available and have been used in previous studies depending on the design of the study, cost effectiveness and target population as per lifestyle or ethnic background. The suitability of using questionnaires to assess duration, type and intensity of physical activity undertaken has been investigated in various studies (70, 139, 140). Questionnaires are typically delivered via interview, self-administered or over the telephone call.

Other measures include pedometer and accelerometer (141, 142). Pedometers work by counting the vertical steps taken by the body and horizontal movements of the hip. These are light weight, portable and cost effective. Alternatively, accelerometers are motion sensors that are sensitive to movement or acceleration of the body and inform about duration, type, and intensity of the movement (141, 142). Both the accelerometer and pedometer can be attachable to hip, waist, or clothing with a belt.

A systematic review of 16 articles has analysed the protocol commonly used for questionnaire, pedometer and accelerometer for measuring physical activity in older adults (143). This review suggested that the use of questionnaire (interview format) to quantifying physical activity has been undertaken during last seven days along with the use of accelerometer can be accurate and reliable way of assessing physical activity in older adults (143).

5.1.4 Vitamin D, body mass index and physical activity

A systematic review of 32 studies with 50,000 participants, aged over 65 years, has reported positive association between sufficient vitamin D level and vitamin D intake along with physical exercise performance (144). An indirect association has also been reported between circulating 25(OH)D level and body mass index (BMI) in the report of Institute of Medicine (2). Elderly participants aged ≥65 years with 15kg above in weight than normal supplemented with vitamin D 700IU/day for one year were found to have circulating 25(OH)D level 10nmol/L less than those of with normal BMI (50). It was estimated that a 17 percent increase in vitamin D intake above 700IU/day was required to achieve circulating 25(OH)D levels the same as those in subjects with normal BMI (50). It was interpreted that vitamin D, following ingestion stored in peripheral tissues, particularly adipose tissue, and does not release into the circulation even with indication of deficiency in the blood (2). Some studies have also reported an increase in serum 25(OH)D concentration with weight loss (52, 53).

5.1.5 Evidence of low physical activity and generational difference in the physical activity in South Asian population

A systematic review has investigated the difference in physical activity in the following groups: UK South Asian compared to White British, within South Asian subgroup (Bangladeshi, Indian and Pakistani) and in different age groups/generations among South Asians (113). They have reported South Asians to be less active compared to the White British. Within South Asian subgroups, the Bangladeshi population was observed to be highly active, and Indians were reported to be least active. They further found generational difference in South Asians as indicator of physical activity given that the second generation (born in the UK) was observed to be more active than first generation or migrants to the UK (113).

Furthermore, a recent cross-sectional analysis of PROPELS trial has quantified the involvement in moderate to vigorous physical activity and prevalence of sedentary activity (sitting and standing) in South Asians compared to the Caucasian population (112). They have also reported South Asians to be less active versus Caucasians (p=0.001). They further reported gender differences among South Asians with regards to involving in moderate-to-vigorous activities given that women were less active than men although women were found to stand more and spend less time sitting (112).

5.1.6 Vitamin D and physical activity in South Asians

A recent narrative literature review has suggested an increase in plasma vitamin D concentration with increased engagement in indoor and outdoor physical activities (145). The UK based South Asian older women is reported to be high risk of vitamin D risk deficiency (111) and less physically active (123). The findings of the Chapter 4 has also reported hypovitaminosis D in South Asian women aged ≥60 years particularly in those who were non-users of vitamin D supplementation. Therefore, given the literature reporting South Asians being less active (section 6.1.5) and highly vitamin D deficient population, we wished to investigate whether there is an association between circulating blood 25(OH)D concentration and physical activity in this population. To meet this aim, the current chapter explored the potential relationship between circulating blood 25(OH)D level and self-reported physical activity in South Asian women.

5.2 Aims and objectives

To investigate the potential relationship between circulating blood 25(OH)D concentration and self-reported physical activity in South Asian women aged ≥60 years.

5.3 Methods

5.3.1 Study design

The data in this chapter was collected as part of the cross-sectional (VIDISA study). The physical activity of the women was assessed by interview format using International Physical Activity Questionnaire (IPAQ)-short form (125). The detail of the study was given in Chapter 4 and methodology used in detail is provided in Chapter 4.

5.3.2 Participants

In the VIDISA study, one hundred and twenty community-dwelling Indian and Pakistani women aged ≥60 years were enrolled. The inclusion criteria was vitamin D supplement user and non-user, able to give written and verbal consent. Participants were excluded if they had cognitive impairment.

5.3.3 Study protocol

The international physical activity questionnaire (IPAQ)-short form contains information on intensity level (mild, moderate or vigorous) and duration (how many minutes per day) of physical activity undertaken during last 7 days in MET minutes (125). One MET minute is equivalent to energy expenditure in the resting stage. Detail method of analysis is provided in Chapter 4. The circulating blood 25(OH)D concentration was measured in each participant by taking finger prick blood spot sample using vitamin D test kits and analysed by Liquid-mass chromatography (120). The detail of the test has provided in Chapter 3. Age and BMI was also collected as part of the study.

5.3.4 Statistical analysis

Data was analysed using the IBM SPSS statistics software (Version 26). The one-sample Kolmogorov Simonov test was used to determine the distribution of the data. Age, body mass index, circulating blood 25(OH)D concentration and self-reported physical activity were all normally distributed so therefore Pearson correlation analysis was performed to explore the potential cross-sectional relationship between outcome variables. Paired-Samples T test was used to compare means of age, body mass index and blood 25(OH)D concentration in women with low and moderate-high physical activity. Multiple linear regression analysis was performed to explore the relationship of self-reported physical activity with other variables. Continuous variables were expressed as mean (±SD). *p*-value was set at ≤0.05 to define significant.

5.4 Results

5.4.1 Participant's demographics

Table 5.1 shows the overall characteristics of the South Asian women and then according to physical activity level. Overall, sixty two percent of South Asian women had BMI ≥30 kg/m^2 and none was found to be underweight. Forty seven percent of South Asian women were found to have circulating blood 25(OH)D concentration <50nmol/L. Forty one percent of South Asian were users of vitamin D supplement at the time of recruitment. A significant difference in blood 25(OH)D concentration and self-reported physical activity was found between vitamin D supplement users and non-users. The detail characteristics of the study population are described in Chapter 4 of this thesis.

Table 5.1. Participant's characteristics

Outcome variables	**All (*n*=120) Mean (SD), median, IQR**	**Low active (*n*=96) Mean (SD), median, IQR**	**Moderate-High active (*n*=24) Mean (SD), median, IQR**	***p* value†**
Age (years)	68.9(7.2), 66.3, 9.6	69.6(7.6), 67.0, 11.7	66.1(4.3), 66.0, 5.3	0.14
Body mass index (kg/m^2)	32.1(5.7), 32.1, 8.0	32.3(5.6), 32.3, 8.1	31.4(5.9), 29.8, 7.2	0.35
Blood 25(OH)D concentration (nmol/L)	58.3(37.8), 53.0, 59.6	56.2(39.3), 49.7, 58.6	66.7(30.7), 76.1, 49.6	0.96
Vitamin D supplement use [n(%)]††	49(41)	30(31)	19(79)	0.00*

†Paired-Samples T test.

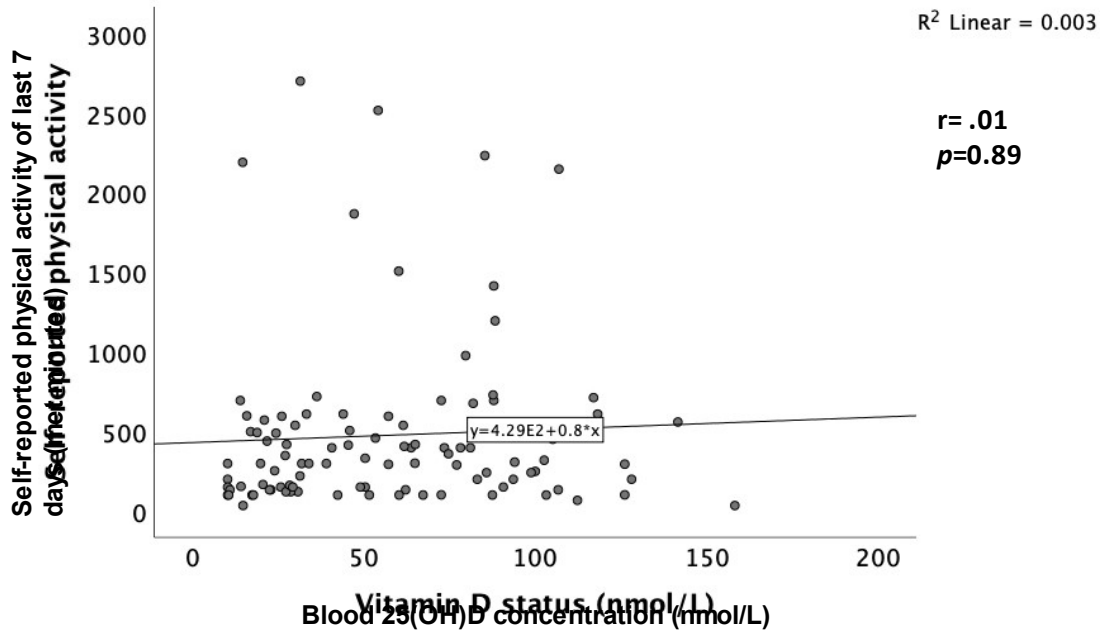
††Chi-square test

**p* value is significance at <0.05

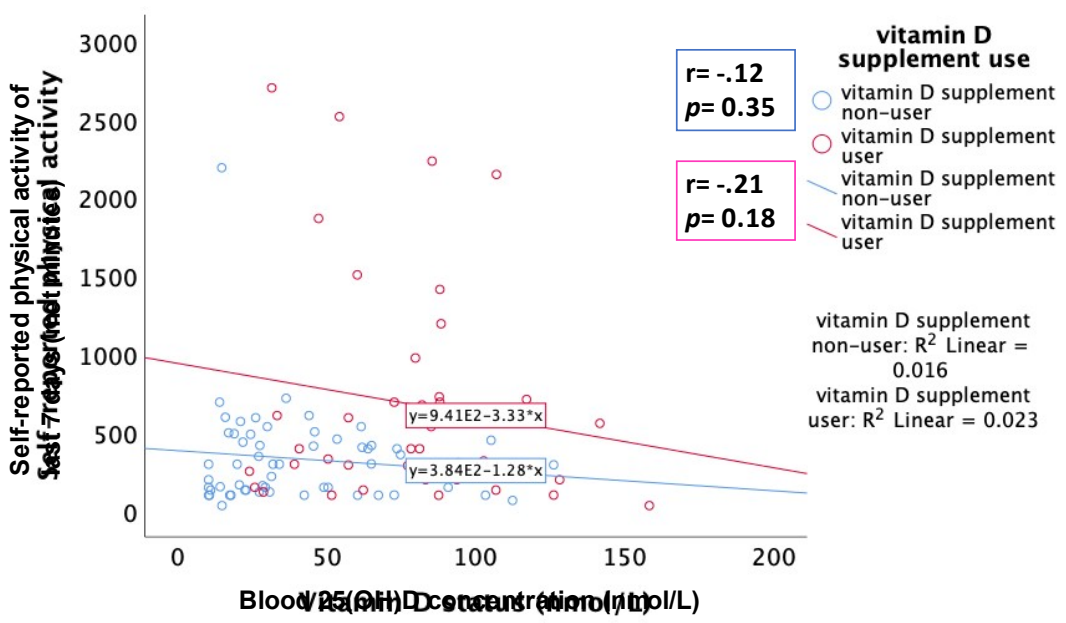
5.4.2 No apparent association between circulating blood 25(OH)D concentration, self-reported physical activity and body mass index in South Asian older women

Figure 5.1 (a) shows null association between circulating blood 25(OH)D concentration and self-reported physical activity in South Asian women. Most of the women were found to have low levels of active regardless of circulating blood 25(OH)D concentration. Following on from the findings of Table 5.1, a Pearson correlation analysis was performed to explore the relationship of blood 25(OH)D concentration with self-reported physical activity level in vitamin D supplement users and non-users. However, despite the significant difference in the proportion of women who were vitamin D supplement users that had moderate-high physical activity, Figure 5.1 (b) shows no relationship between vitamin D and self-reported physical activity level against the use of vitamin D supplementation. Figure 5.1 (c) shows null association between blood 25(OH)D concentration and body mass index South Asian women.

a)



b)


vitamin D supplement use
vitamin D supplement non-user
vitamin D supplement user
vitamin D supplement non-user
vitamin D supplement user
r= -.12
p= 0.35
r= -.21
p= 0.18
vitamin D supplement non-user: R2 Linear = 0.016
vitamin D supplement user: R2 Linear = 0.023
y=9.41E2-3.33*x
y=3.84E2-1.28*x
3000
2500
2000
1500
1000
500
0
0
50
100
150
200

c)

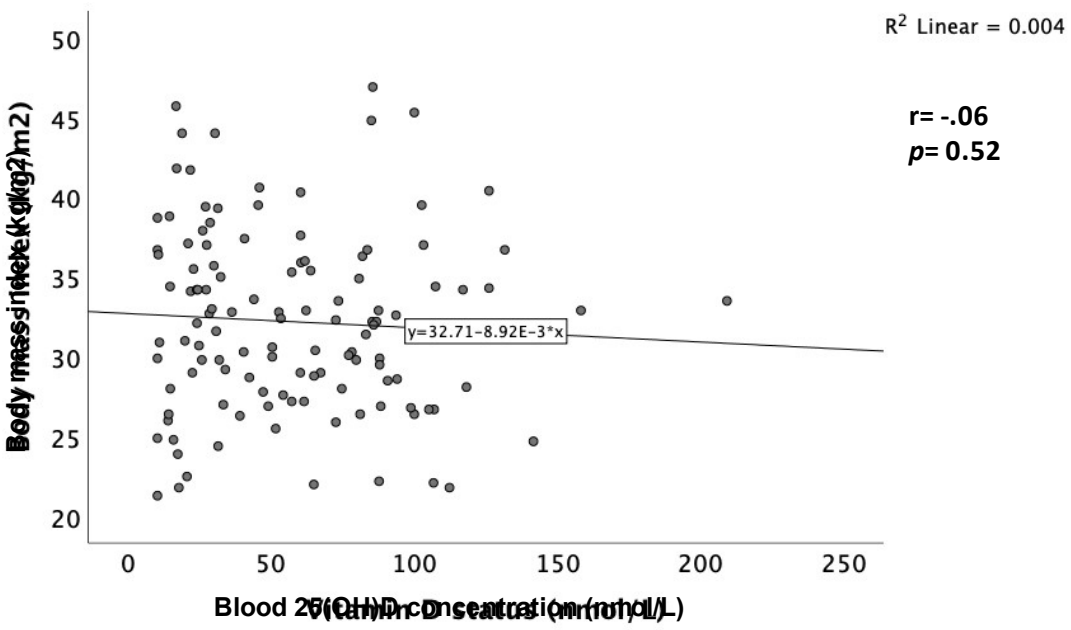

R2 Linear = 0.004
r= -.06
p= 0.52
y=32.71-8.92E-3*x
50
45
40
35
30
25
20
0
50
100
150
200
250

Figure 5.1 Relationship of circulating 25(OH)D concentration with self-reported physical activity (met minutes) (a), stratified by vitamin D supplement users and non-users (b) and body mass index (c) in South Asian women.

5.4.3 Self-reported physical activity performance

Table 5.2 shows a significant negative relationship of self-reported physical activity with age. The women were found to have decline in the physical activity with increasing age.

Table 5.2 Relationship of self-reported physical with other variables

Variables	**beta**	**t**	**sig**	**95% confidence interval**	
				lower	upper
Circulating 25(OH)D concentration (nmol/L)	0.126	1.25	0.21	-1.4	6.2
Body mass index (kg/m^2)	0.07	0.75	0.45	-14.6	32.3
Age (years)	-.21	-2.05	0.04	-39.2	-.64

Multiple linear regression analysis was used.

5.4.4 Physical activity in South Asian older women against guidelines

Table 5.3 shows high prevalence of low activity (or sedentary behaviour) in South Asian older women. The physical activity status among them was found to be independent of body mass index and blood vitamin D level. However, a higher proportion of the active women were consuming vitamin D supplements which may reflect a greater awareness of their health.

Table 5.3 Prevalence of physical activity among South Asian women against guideline

***PA category score [n(%)]**	**Percent of participants (*n*=120)**
Low active**	96 (80)

Moderate active***	22 (18)
highly active****	2 (2)

*PA stands for physical activity.

“Categories of physical activity is defined by Department of Health and Social care guideline as follows: (70, 125)

**Low active: if not moderate or vigorous.

***Moderate: (a) 3 or more days of vigorous intensity activity of at least 20 minutes per day or

(b) 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day or

(c) 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum T=total physical activity of at least 600 MET-minutes/week.

****High: (a) vigorous-intensity activity on at least 3 days (20min minimum, achieving a minimum Total physical activity of at least 1500 MET-minutes/week or

(b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum Total physical activity of at least 3000 MET-minutes/week”.

5.5 Discussion

5.5.1 Main findings

The International Physical Activity Questionnaire (IPAQ)-short form was used to access self-reported physical activity (SRPA) in South Asian women. A previous systematic review has reported IPAQ to be suitable and accurate marker of physical activity (143). The current chapter has found no relationship between blood 25(OH)D concentration and self-reported physical activity (SRPA) of South Asian women aged 60 years and above (Figure 5.1). Likewise, body mass index in this population was found to have no association with blood 25(OH)D concentration (Figure 5.1). A significant difference in vitamin D supplement use was found between women with low and moderate-high physical active (Table 5.1) which may suggest overall greater awareness of their health. However, SRPA has no direct relationship with blood 25(OH)D concentration in either supplement users or non-users (Figure 5.1 (b)). An independent age-related decline in SRPA has found in this target population (Table 5.2).

5.5.2 Critical analysis of relationship between blood 25(OH)D concentration, BMI and self-reported physical activity in South Asians

The null association between blood 25(OH)D concentration and self-reported physical activity level in South Asian women can be understood in the light of evidence of limited physical activities in this target population as reported previously. In line with literature (113), the low physical activity was found to be prevalent in South Asian women (Table 5.3) which was independent of blood 25(OH)D level or body mass index. In contrast to the literature (2), no relationship was found between body mass index and blood 25(OH)D concentration in this target population (Figure 5.1 (c)). However, most women included in this study were found to have higher body mass index which may possibly be lifestyle related or cultural-specific. However, no association was found between self-reported physical activity and body mass index in South Asian women. The prevalence of increase body mass index and low physical activity level has been suggested previously in cross-sectional survey of 137 Sikh Indians (146).

A significant negative weak relationship was found between age and self-reported physical activity level in South Asian women which is indicative of a trend of declining physical activities with increasing age (Table 5.2). These findings are in line with the previous systematic review which reported generational differences in the physical activity of South Asians given that first generation or migrants South Asians were reported to be less active compared to second generation (born in the UK) (113). This can further be evidenced by our finding of a higher prevalence of low physical activity in South Asian women as the majority were first generation or migrants (Table 5.3). As described in section 5.1.2, engagement in physical activity can play an important role in the prevention of muscle loss and function. A recent systematic review of ten randomised controlled trial with 200 elderly population with mean (SD) aged 75.1(4.4) years has reported that involvement in various kind of physical activity has improved muscle function including balance performance in this population (114).

5.5.3 Strength and limitation

The limitation of this study is that a subjective self-reported assessment of physical activity was used rather than an objective method such as accelerometery. This is a cross-sectional analysis investigating the association between vitamin D level and physical activity and thus the casual relationship could not be observed. The strength of the current study is that it has provided a large sample size of target population to compare the potential relationship between outcome variables. It has provided information on the use of IPAQ (short form) with South Asian women. This study has also explored the trend of involvement in physical activity in this target population.

5.6 Conclusion and possibilities of future research

Vitamin D has no relationship with self-reported physical activity in South Asian women aged ≥60 years. South Asian older women were found to have decreasing activity with advancing age and do not meet the physical activity guidelines.

An objective measure of physical activity should be conducted to determine the accurate physical activities, level and duration in this population by using different measures. A vitamin D administration intervention along with muscular exercise programme could also be another way to evaluate the casual relationship over the time course. Alternatively, a longitudinal study to investigate the trend and confounding factors involved in commencing physical activity in South Asian older women could be a better way to study this population attributes.

6 Chapter 6. The effect of vitamin D supplementation on muscle strength and function in vitamin D inadequate/deficient UK South Asian older women (VITD trial)-A randomised controlled trial

6.1 Introduction

The previous chapter demonstrated an association between vitamin D and muscle strength and function in the South Asian older women, an observation that has been replicated in many other populations around the globe. However, such observational associations do not demonstrate causality and a higher quality of scientific evidence is generally provided by randomised control trials. Numerous RCTs have been conducted over recent years to investigate the effect of vitamin D at various doses and for various durations on community-dwelling post-menopausal women of different ethnic groups (reviewed in detail in Chapter 1). However, the relationship between vitamin D and muscle strength and function remains controversial. Studies are varied in baseline 25(OH)D concentration of the participants and some participants have sufficient vitamin D status at baseline which may limit intervention response. Chapter 1 has provided the insights of a recent meta-analysis which highlighted some aspects of the studies may impact the investigation of vitamin D on muscle strength and function in community dwelling post-menopausal women (1) . The data of this meta-analysis has demonstrated high degree of variation among studies whilst, accumulating overall findings, it has suggested baseline circulating 25(OH)D concentration between 20.5-65.95nmol/L and benefits of medium dose of vitamin D supplementation on daily basis for 3-4 months in improving muscle strength and function (handgrip strength, repeated chair stand and timed up and go test) in community living post-menopausal women of different ethnic groups (section 1.3.3).

The findings of previous studies and of our own, from cross-sectional study, has suggested high prevalence of vitamin D deficiency in those women who were non-user of vitamin D supplementation and found poor muscle strength (handgrip strength) in this population (section 4.5.2.4). These findings were supportive of our objective to conduct a randomised controlled trial. Despite earlier evidence that South Asian older women have a high risk of vitamin D deficiency, there has been no randomised controlled trial, yet, with the UK based South Asian older deficient women to investigate whether vitamin D administration would improve muscle strength and function in this highly deficient population.

6.2 Hypothesis

It is hypothesized that muscle strength and function will improve in vitamin D inadequate/deficient UK South Asian older women upon repletion with vitamin D.

6.2.1 Aim

To investigate the effect of vitamin D_3 supplementation (3000IU/day) for 12 weeks on muscle strength and function in UK based, vitamin D inadequate/deficient South Asian older women.

6.2.2 Objectives

- To obtain ethical approval for study.
- To conduct a double-blinded randomised, placebo-controlled trial.
- To identify, recruit and screen a population of South Asian older women for vitamin D inadequacy/deficiency.
- To randomise women with a 25(OH)D concentration <50nmol/L, to receive either 3000IU/day vitamin D supplementation or placebo for 12-weeks.
- To measure circulating blood 25(OH)D concentration, muscle strength (handgrip strength, single chair stand test, repeated chair stand test) and muscle function (timed up and go test, balance test and walking speed test) at week 0 (baseline) and at 12-weeks (end of intervention).

6.2.2.1 Primary endpoints

- To assess handgrip strength at baseline and end of intervention.
- To assess the performance of single chair stand test at baseline and end of intervention.

6.2.2.2 Secondary endpoints

- To collect information on demographic characteristics, anthropometry, medical history and use of supplementation.
- The following secondary outcome measurements were made at baseline and end of intervention:
- 25(OH)D concentration
- repeated chair stand
- balance test
- timed up and go test

- To assess the performance of walking speed test at baseline and end of intervention.

6.3 Methods

6.3.1 Ethical approval/ MHRA/Trial database registration

Ethical approval for the study was obtained in advance from the University of Sheffield Medical School Research Ethics Committee (reference number 022981) (appendix c). The Medicine and Healthcare products Regulatory Agency (MHRA) was contacted at the start of the study as to whether the trial fell under their regulations. The MHRA concluded that the trial protocol was not a Clinical Trial of an Investigative Medicinal Product (CTIMP) i.e., vitamin D deficient women recruited to vitamin D intervention study. The study was registered in the clinical trial registry (ISRCTN) with the reference number 41610200.

6.3.2 Participants

The UK South Asian (Indian and Pakistani) community-dwelling post-menopausal women were invited to take part in this study. The study aimed to recruit vitamin D deficient (< 50nmol/L) women to the intervention study.

6.3.2.1 The inclusion criteria:

- Post-menopausal
- Circulating 25(OH)D concentration <50nmol/L.
- Able to give written and verbal consent.

6.3.2.2 Exclusion criteria:

- Unable to communicate
- Hearing impairment
- Blindness
- Walking disability
- Being institutionalized.
- Vitamin D/multivitamin supplement users
- Women who plan to/just came from holidays in sunny countries.

6.3.3 Advertise and recruitment

The same approach, as of our cross-sectional study (Chapter 4), was used and a variety of methods were used to advertise the study to potential volunteers. Leaflets prepared in Urdu and English language were distributed to relevant organizations to give to the potential women.

In cross-sectional study, the recruitment waws conducted in Sheffield and Greater Manchester area and majority women from Sheffield were found to be vitamin D supplement users, compared to women in Greater Manchester, which has positive impact on their circulating 25(OH)D concentration (section 4.4.2). Therefore, for RCT, the recruited was only planned to be conducted around Greater Manchester area only with a potential to find high proportion of vitamin D supplement non-users which ultimately could have reflection on their circulating 25(OH)D concentration. The Indian Mandirs, as an additional new potential place, were approached to advertise this study and to invite new Indian women to the study that has not taken part in the cross-sectional study. Otherwise, same South Asian community centers/temple(s), which were approached for cross-sectional study, were also approached for RCT and some same (as in cross-sectional study) and other new eligible women were recruited in this study. However, compared to previous study (Chapter 4), Mosques were not approached to advertise this study given that no participant could be recruited through this route. The home visits were also performed for the women who were recruited by word of mouth.

A same method, as of our cross-sectional study (section 4.3.4), was used to introduce the study to potential women and to invite the women to the study. Compared to the previous study, the participant information sheets in this study were prepared with the inclusion of diagrams to aid participants' understanding of the study requirements i.e., methods of muscle strength and function assessment (see Appendix b). An informed, written consent was obtained from all participants prior to start the study protocol. The recruitment was conducted in Rochdale, Ashton, Oldham and the Manchester area. Each participant (who completed the intervention) was reimbursed with a £30 high street voucher as thanks for their time and participation. Women who were screened and found not to be eligible for intervention were reimbursed with a £10 high street voucher.

6.3.4 Study design

This study used a double-blinded randomized placebo controlled two arm parallel design to investigate the effect of vitamin D_3 supplementation on muscle strength and function in vitamin D inadequate/deficient UK South Asian older women. The study was conducted between March 2019 (first participant recruited) to November 2019 (last participant visit). The primary outcome of the study was to investigate the effect of vitamin D_3 supplementation on handgrip strength and the performance of single chair stand test (s) in a population of UK South Asian older women with low vitamin D status. The dose of 3000 IU/day was selected on two basis: 1) feasible availability of this dose by BetterYou company Ltd, 2) in line with findings of a previous RCT with community dwelling post-menopausal women which has reported a significant post course increase in circulating 25(OH)D concentration at 12 weeks with using 2800IU/day (107). All study procedures were conducted at a venue that was convenient for the participants. The study was conducted in four stages and required eligible women to meet with the researcher on 4 occasions.

6.3.4.1 First stage:

This was first face to face study introduction session with all women. The study information and protocol was delivered to women in their preferred language. The participant information sheet was provided, interested women were identified and invited to participate in the study.

6.3.4.2 Second stage:

At the 2nd visit (screening visit) women's eligibility to take part in the study was checked during one-to-one appointment and they were asked to sign a written, informed consent form (see Appendix d). A total of one hundred and thirteen women were screened at baseline. A finger-prick blood spot for circulating blood 25(OH)D concentration was taken and assessments for muscle strength and function were performed. Blood spots were sent off to the Department of Clinical Biochemistry, Sandwell and West Birmingham hospital and the reports for circulating 25(OH)D concentration were returned to researchers an average of 7 days later. The following baseline muscle strength and function assessment were performed on this occasion: right and left handgrip strength, single chair stand, repeated chair stands, timed up and go test, balance test (Chapter 3) and walking speed test. Participants were interviewed to collect information about demographics and medical history.

Only those participants who had 25(OH)D concentration <50nmol/L were entered into the intervention study. Participants found to have a sufficient 25(OH)D concentration =>50nmol/L

were excluded and not entered to the intervention study. The excluded participants were provided with their vitamin D report and given a £10 high street voucher to thank them for their participation.

6.3.4.3 Third stage:

In the third visit of the study i.e., within 2 weeks of first visit, all eligible participants, with blood 25(OH)D concentration <50nmol/L, were entered into the intervention after randomly allocated to intervention arm. (The detail of randomisation is provided below in section 5.3.5). The researcher visited the community centre/temple/participants home to inform/provide participants with vitamin D test report, to provide placebo/vitamin D_3 supplements for intervention and provide instruction for use of the supplement.

6.3.4.4 Fourth stage:

In the fourth visit i.e., after 12 weeks of being on the intervention study, the researcher revisited the designated place or performed home visits for a final meeting with the participant. Participants were asked for a finger-prick blood spot sample so circulating blood 25(OH)D concentration could be checked again and same assessments of muscle strength and function which were performed at screening visit were repeated. At the 12-week of intervention, weight, height and body composition of the participants was also performed. The interventional product was taken back and £30 high street vouchers and vitamin D supplement was supplied to all participants who have completed the intervention period. Figure 6.1 illustrates the study procedure and timeline.

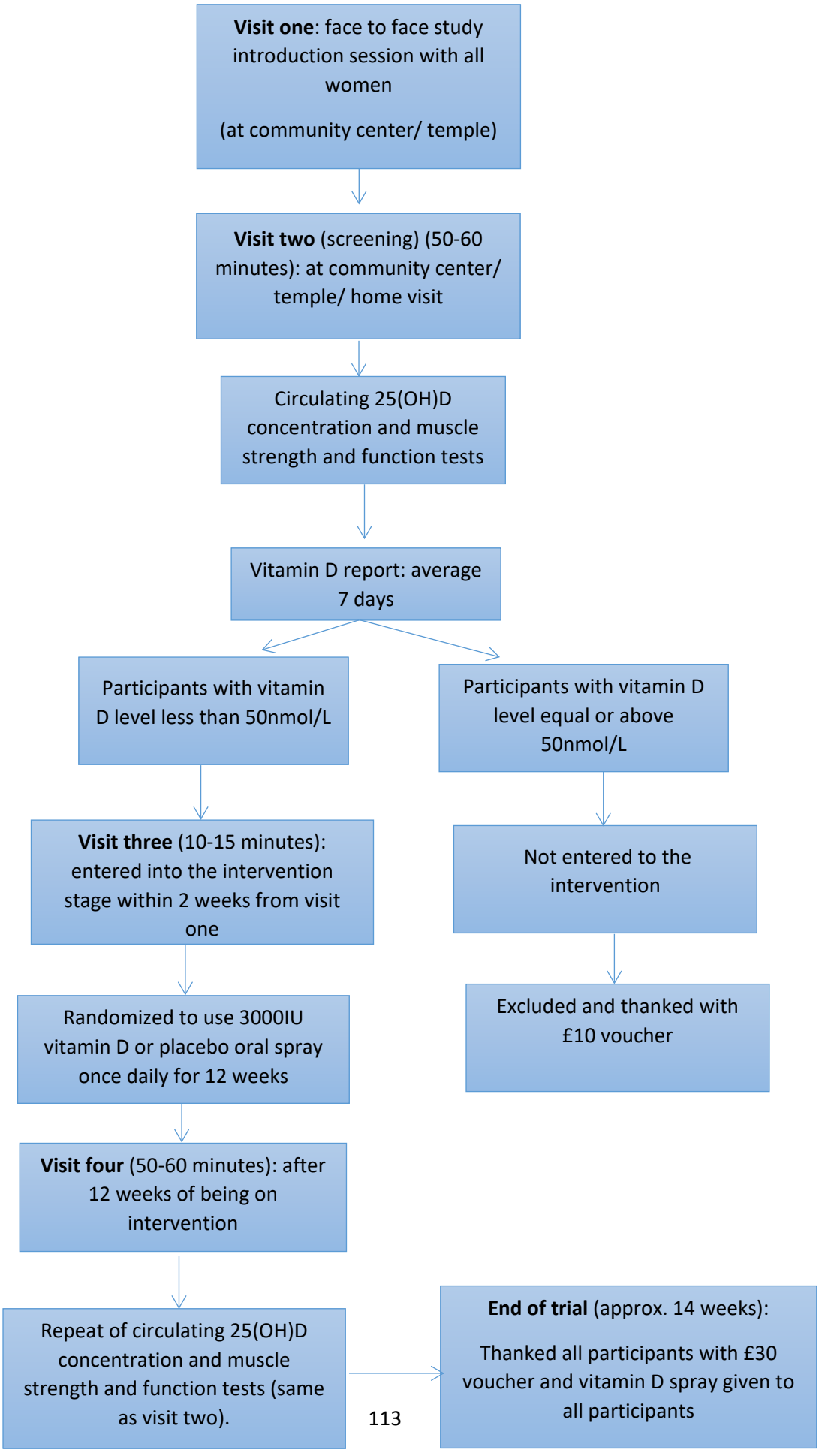

Visit one: face to face study introduction session with all women
(at community center/ temple)
Visit two (screening) (50-60 minutes): at community center/ temple/ home visit
Circulating 25(OH)D concentration and muscle strength and function tests
Vitamin D report: average 7 days
Participants with vitamin D level less than 50nmol/L
Participants with vitamin D level equal or above 50nmol/L
Visit three (10-15 minutes): entered into the intervention stage within 2 weeks from visit one
Not entered to the intervention
Randomized to use 3000IU vitamin D or placebo oral spray once daily for 12 weeks
Excluded and thanked with £10 voucher
Visit four (50-60 minutes): after 12 weeks of being on intervention
Repeat of circulating 25(OH)D concentration and muscle strength and function tests (same as visit two).
End of trial (approx. 14 weeks):
Thanked all participants with £30 voucher and vitamin D spray given to all participants

Figure 6.1 Study procedure and timeline

6.3.5 Randomisation and Allocation

Figure 5.3 shows the CONSORT diagram of randomization and allocation of the participants. Seventy participants were found to have circulating blood 25(OH)D concentration <50nmol/L and hence were eligible for the intervention. The block randomisation in blocks of 4 was created by using computer-generated method by an independent source. Participants were allocated to each code in a sequence in which they were recruited to the study to allow fair randomisation. The randomization sequence remained locked and confidential till the completion of data analysis of the study.

6.3.5.1 Intervention protocol

Participants were randomly allocated to two parallel arms i.e., 36 women in active arm and 34 women in placebo arm to receive intervention as below:

1. 3000IU vitamin D_3 oral spray, per day for 12 weeks.
2. Placebo oral spray per day for 12 weeks.

Participants were required to remain in the study for 12-weeks. Detail about strategies used to retain participants in a study are provided in Chapter 7 of the thesis. Participants were requested not to self-supplement with vitamin D/multivitamin during intervention period. According to European Food and Safety Agency, the upper tolerable intake of vitamin D is 4000IU/day and the dose used in the current study was 3000IU/day which was within safe limits (19). The vitamin D_3 supplement/placebo used in this study were provided by BetterYou Ltd (Barnsley, South Yorkshire, UK). Each spray bottle provided 15ml preparation and each spray was provided 3000IU vitamin D_3 per dose, participants were instructed to use a single pump of spray per day for 12 weeks.

6.3.5.2 Further to end of intervention

The post-intervention vitamin D reports (after 12-weeks) of the participants were directly received by independent senior staff member of the research team and the researcher was not aware of results. The vitamin D reports were held confidentially until the completion of all exits and data entry was performed. After all data entry, the vitamin D reports were disclosed for the purpose of analysis. At this stage, the end of study vitamin D reports were posted to participants.

6.3.6 Study measures used

Right and left handgrip strength was used as a parameter of upper limb muscle strength and was measured using Jamar hand-held dynamometer. Single chair stand and repeated chair stand, a parameters of lower limb muscle strength, was assessed using a stopwatch. Balance test and timed up and go test, an indicator of muscle function, was assessed using a stopwatch. Details of each method of assessment is provided in Chapter 3. A walking speed test, as indicator of muscle function, in addition to the methodology used in the cross-sectional study was performed.

6.3.6.1 Walking speed

Walking speed was assessed as an indicator of muscle function. Participants were asked to walk 6 metres in a straight line as quickly as possible with the addition of 2 metres acceleration and 2 meters deceleration on each side (total 10 meters) (147). Figure 6.2 shows the protocol of walking speed test.

Pre-preparation

The researcher explained the purpose and procedure of the test to participants showing them the four marks (a,b,c,d) on the ground. They were asked to walk as quickly as possible (but safely) from the mark ‘a’ to the mark ‘d’ (10 meters total). The researcher started to record the time when the participant feet crossed the mark ‘b’ and stopped to recording when the participant feet crossed the mark ‘c’. Researcher informed the participants that ‘*I will now perform the test in front of you to observe me that how I do in the test before I asked you to do so’*. The participants observed the researcher performing the test before they did it. Prior to the task, they were asked that if they understood the procedure and ready to do so. They were asked to start the test when the researcher said the word ‘start’.

Test performance

They were asked to stand at the mark ‘a’ then researcher asked ‘are you ready’? and then said ‘Start’ and the participants started the test. The researcher was there for any assistance and the time was recorded in seconds by using the stopwatch.

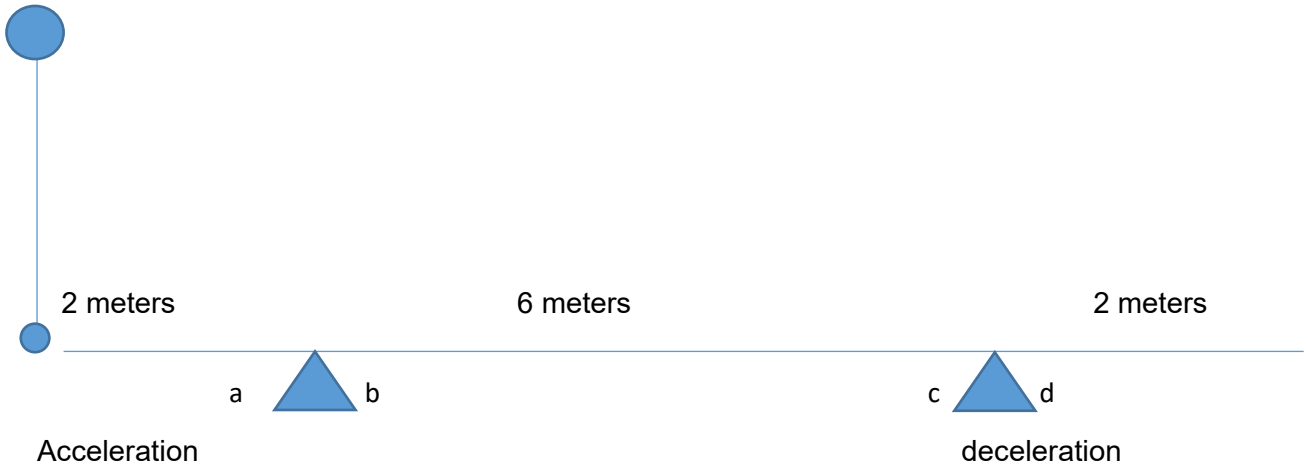


Figure 6.2 Protocol of walking speed test (s)

6.3.7 Sample size and power calculation

A sample size of 35 per group was calculated based on an improvement in handgrip strength in response to vitamin D supplementation with an effect size of 3kg (SD 4.21) at 5% significance level and a power of 80%. Sample size was based on the effect of vitamin D supplementation on handgrip strength in post-menopausal women with a 25(OH)D concentration of ≤75nmol/L (102). Iolascon and colleagues saw an effect on grip strength following 6-months supplementation with vitamin D in post-menopausal women. Vitamin D status was actually good at baseline, nonetheless they saw a post-course improvement in circulating 25(OH)D concentration and grip strength in the population (102). When they looked just at people with a baseline 25(OH)D concentration ≤75nmol/L (30ng/ml) they saw an improvement in grip strength of >3kg with a SD of around 4.21 therefore giving a sample size of around 32 per group (increased to 35 per group to allow for drop out).

6.3.8 Data management

Ethical approval and all study related documents were kept in a site file stored in locked cabinet at the department of Oncology & Metabolism. The collected data was saved as hard copies and in electronic format for analysis purposes. Participants' identification was pseudo-anonymised and participants name was written on consent forms only. Participants were given a unique identification ID numbers. The electronic format of collected data was transferred into SPSS file and stored in google drive at the University of Sheffield. The computer used for analysis was password encrypted.

6.3.9 Statistical analysis

Data was analysed using the IBM SPSS statistics software (Version 26). The one-sample Kolmogorov Simonov test was used to determine the distribution of the data of all outcome variables. The only variable found to be normally distributed was right and left handgrip strength. A log transformation was performed on the remaining outcome variables but failed to normalise the data and so non-parametric analysis was performed throughout the analysis. *Wilcoxon Signed ranks test* was used to analyse the within-group (active and placebo) effect on all outcome variables at 12 weeks compared to baseline. Data computation was performed to compute the variables at baseline, and Mann-Whitney U test was used to determine the effect size in outcome variables at 12-weeks compared to baseline in active versus placebo group. Mann-Whitney U test was used to analyse the age, number of comorbidities, muscle strength and function of sufficient and inadequate/deficient participants. Continuous variables were expressed as mean and standard deviation in brackets and categorical variables presented as numbers and percentages. A *p*-value was set at <0.05 to define significance.

6.4 Results

6.4.1 Recruitment

In total 113 participants were assessed for eligibility, 43 did not meet the inclusion criteria, Seventy-women were randomised, 36 in treatment arm and 34 in the placebo arm. There were participants who withdrew from the trial and fifty-seven participants completed the 12-weeks intervention trial period with 29 participants remaining in the active arm and 28 in the placebo arm. The reasons for dropouts were provided in below CONSORT flow chart diagram Figure 6.3.

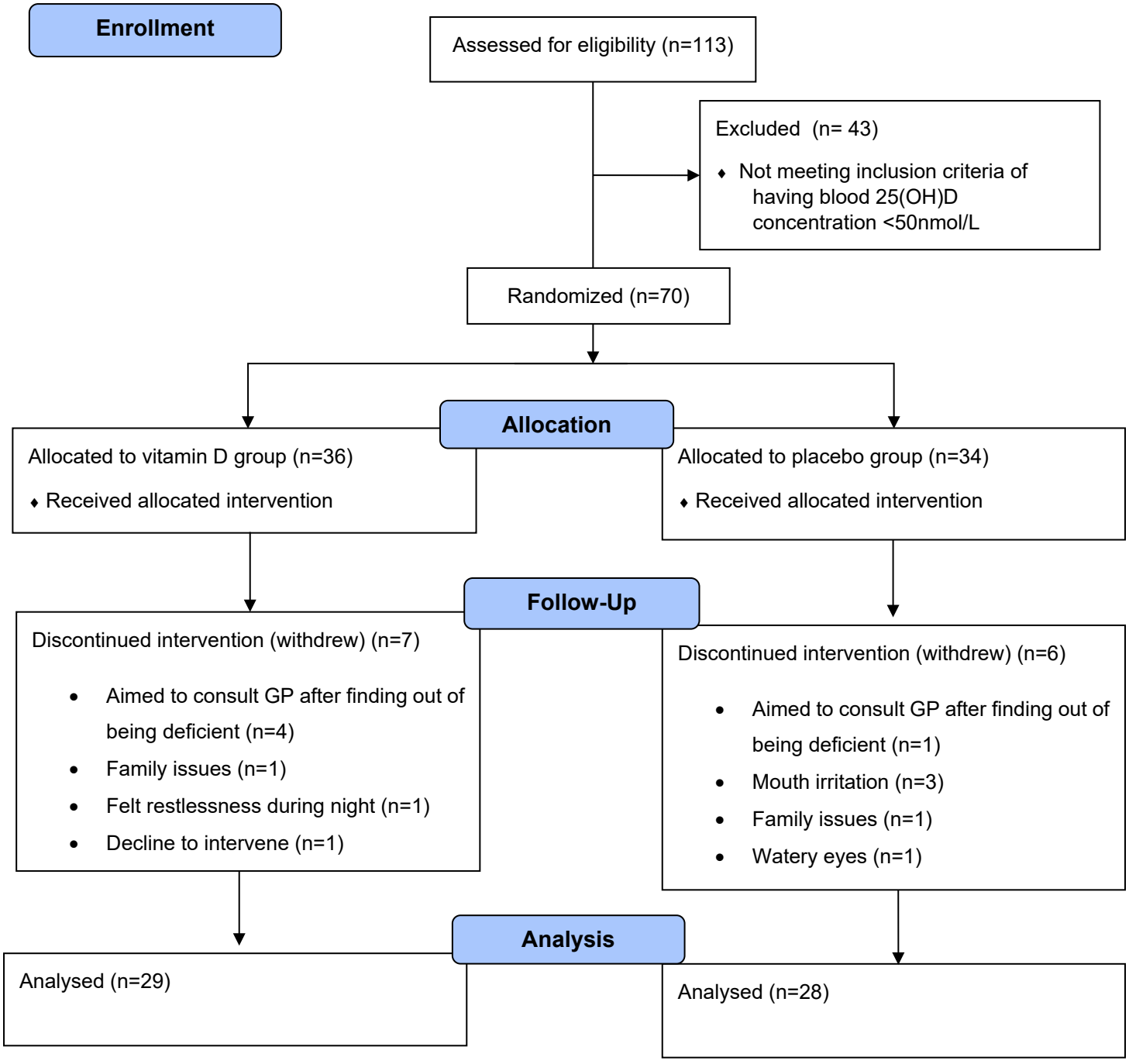


Figure 6.3 CONSORT flow-diagram of participant recruitment and allocation

6.4.2 Baseline analysis

6.4.2.1 Baseline characteristics of all recruited participants

A total of 113 women were screened at baseline to meet the eligibility criteria to take part in the study and the circulating blood 25(OH)D concentration was measurement and all assessments of muscle strength and function were performed with this screening population. 62% women were found to have 25(OH)D concentration <50nmol/L (Table 6.1). Below table shows baseline characteristics of 113 participants.

Table 6.1 Baseline characteristics of all participants

Variables	All (*n*=113) Mean (SD), median, IQR	Vitamin D status <50nmol/L Mean (SD), median, IQR (*n*=70)	Vitamin D status≧50nmol/L Mean (SD), median IQR (*n*=43)	*p* value†
Age (years)	69.4(6.6), 69, 10	69.1(6.4), 69.5, 11.0	69.9(6.9), 68.0, 7.0	0.73
Ethnic sub-groups **Indian [n (%)]** **Pakistani [n (%)]**	 58 (51.3) 55 (48.6)	 36 (51.4) 34 (48.5)	 22 (51.2) 21 (48.8)	0.978‡
Waist (cm)	94.9 (12.9), 96, 18.0	93.0(12.8), 92.0, 19.5	97.8(12.7), 99.0, 14.0	0.061
Mid upper arm circumference (cm)	30.03 (4.0), 30.0, 6	29.5(4.0), 29.0, 7	30.8(3.9), 31.0, 4	0.046
Number of Comorbidities	2.6(1.1), 2.0, 1	2.6(1.2), 2.0, 1	2.7(0.9), 3.0, 1	
Hypertension [n (%)]	74 (65.4)	45 (64.2)	29 (67.4)	
Diabetes [n (%)]	49 (43.3)	28 (40)	21 (48.8)	0.439
Asthma [n (%)]	30 (26.5)	20 (28.5)	10 (23.2)	
Kidney disease [n (%)]	4 (3.5)	2 (2.8)	3 (6.9)	
Heart disease [n (%)]	15 (13.2)	9 (12.8)	6 (13.9)	

Previous history of Cancer [n (%)] **Arthritis [n (%)]**	3 (2.6) 76 (67.2)	3 (4.3) 43 (61.4)	0 33 (76.7)	
25(OH)D concentration (nmol/L)	45.2(29.0), 41.4, 39.4	27.2(11.8), 24.0, 18.2	74.7(24.4), 66.2, 21.4	0.001***
Right handgrip strength (kg)	16.4(4.6), 16.0, 6.7	16.6(5.0), 16.4, 6.8	16.2(4.1), 15.8, 6.6	0.59
Left handgrip strength (kg)	14.9(4.7), 15.5, 6.4	14.8(4.8), 15.7, 6.3	15.2(4.5), 15.4, 6.5	0.98
Single chair stand (s) (*n*=112)	4.9(3.3), 4.1, 2.3	5.1(4.0), 3.9, 2.7	4.4(1.4), 4.1, 1.8	0.55
Repeated chair stand (s) (*n*=108)	27.0(16.1), 22.6, 14.8	27.6(16.9), 22.0, 15.0	25.9(14.4), 23.2, 13.5	0.99
Timed up and go (s)	14.5(6.4), 12.8, 5.4	13.8(5.3), 12.5, 4.8	15.6 (7.6), 13.3, 7.5	0.13
Walking speed (s)	6.4(2.8), 5.7, 3.2	6.3(2.6), 5.4, 2.9	6.7(3.1), 5.9, 3.2	0.46
Balance test (s)	3.2(0.9), 4.0, 2.0	3.2(0.9), 4.0, 2.0	3.1(1.0), 3.0, 2.0	0.58

†Mann-Whitney U test

‡Chi-square test

****p*-value is significant at <0.05.

6.4.2.2 Baseline characteristics/skeletal muscle performance of all participants by ethnic sub-groups

In line with findings of Table 4.3, muscle strength and function was assessed between Indian and Pakistani women at baseline as shown in Table 6.2. The data of five women was missing. Compared to Table 4.3, the performance of repeated chair stand test only was significantly different between Indian and Pakistani women given the Indian women had a better performance than Pakistani women. Given that, the data of below Table 6.2 has suggested the same distribution of muscle strength and function across vitamin D thresholds in Table 6.1 could be cultural/sub-ethnic population specific and required further sub-investigation.

Table 6.2 Baseline characteristics/skeletal muscle performance by ethnic sub-groups

Outcome variables	Indian (*n*=54) Mean (SD), median, IQR	Pakistani (*n*=54) Mean (SD), median, IQR	*p* value†
Age (years)	69.9 (5.8), 71.0, 9.0	68.5 (6.9), 67.0, 10.3	0.079
25(OH)D concentration (nmol/L)	43.5 (32.8), 32.3, 36.7	44.6 (25.1), 42.2, 39.2	0.636
Right handgrip strength (kg)	17.1 (4.5), 16.1, 7.1	16.3 (4.5), 16.2, 6.3	0.863
Left handgrip strength (kg)	15.2 (4.2), 15.6, 5.8	15.1 (5.1), 15.5, 6.6	0.603
Single chair stand (s)	4.2 (1.9), 3.8, 2.0	5.5 (4.2), 4.2, 2.7	0.059
Repeated chair stand (s)	22.3 (8.1), 20.2, 14.9	31.7 (20.2), 24.3, 13.1	0.009*
Timed up and go (s)	12.9 (4.8), 11.8, 5.5	14.9 (4.6), 13.5, 5.2	0.069
Walking speed (s)	5.7 (2.3), 5.2, 2.7	6.6 (2.4), 6.1, 3.6	0.215
Balance test (s)	3.3 (0.9), 4.0, 1.0	3.2 (0.9), 3.0, 1.3	0.786

†Mann-Whitney U test.

**p*-value was significant at <0.05

6.4.2.3 Baseline skeletal muscle performance across vitamin D threshold in ethnic sub-groups

Further analysis was performed to understand and confirm the cultural-specific difference of skeletal muscle health across vitamin D thresholds (define by IOM) in Indian and Pakistani women separately. In line with the findings of cross-sectional study (Table 4.4), a difference in muscle strength and function across vitamin D thresholds in Indian and Pakistani women were found in this study. Table 6.3 shows a significant difference in all parameters of skeletal muscle strength and function across vitamin D thresholds in Indian women except balance test performance. Contrary to expectation, the Indian women with vitamin D inadequacy/deficiency had better muscle strength and function than those with sufficient status. However, this could also be due to the different inclusion criteria of both studies as compared to cross-sectional study, the women using vitamin D supplement at the time of recruitment were approached only with a potential to recruit with deficient/inadequate vitamin D status. Whereas, in Pakistani women, a significant difference was found in the performance of right and left handgrip strength and repeated chair stand test and those women with sufficient vitamin D status had better performance at these above tests than those with inadequate/deficient status.

Table 6.3 Baseline muscle strength and function across vitamin D threshold in ethnic sub-groups

Outcome variables	**Indian**		***p* value†**	**Pakistani**		***p* value†**
	n=36 Vitamin D status <50nmol/L Mean (SD), median, IQR	*n*=18 Vitamin D status ≧50nmol/L Mean (SD), median, IQR		*n*=34 Vitamin D status <50nmol/L Mean (SD), median, IQR	*n*=20 Vitamin D status ≧50nmol/L Mean (SD), median, IQR	
Age (years)	69.2 (5.5), 71.0, 10.8	71.2 (6.4), 70.5, 7.5	0.247	68.9 (7.3), 68, 11.5	67.7 (6.4), 67, 7.8	0.538

Right handgrip strength (kg)	17.8 (4.8), 18.2, 8.0	15.6 (3.6), 15.3, 4.9	0.011*	15.2 (4.9), 15.5, 5.4	18.1 (3.4), 18.9, 5.4	0.048*
Left handgrip strength (kg)	15.7 (4.4), 16.6, 5.6	14.1 (3.6), 14.9, 5.3	0.023*	13.8 (5.1), 14.4, 6.9	17.3 (4.3), 17.4, 5.3	0.027*
Single chair stand (s)	4.1 (2.2), 3.4, 1.3	4.4 (1.1), 4.3, 1.6	0.009*	6.1 (5.1), 4.7, 3.1	4.4 (1.7), 3.8, 1.8	0.070
Repeated chair stand (s)	20.8 (8.2), 18.3, 12.5	25.3 (7.2), 25.7, 10.1	0.035*	34.8 (20.6), 26.8, 20.8	26.5 (18.9), 21.2, 10.3	0.014*
Timed up and go (s)	12.1 (4.8), 11.1, 4.7	14.6 (4.4), 14.1, 8.4	0.002*	15.7 (5.2), 14.4, 7.3	13.4 (2.9), 12.3, 4.1	0.111
Walking speed (s)	5.5 (2.5), 4.9, 2.3	6.1 (1.9), 5.9, 3.4	0.0278	7.0 (2.6), 6.2, 4.6	5.9 (1.9), 5.7, 1.7	0.155
Balance test (s)	3.3 (0.9), 4.0, 1.8	3.2 (0.9), 3.5, 1.0	0.157	3.1 (0.9), 3.0, 2.0	3.3 (0.8), 4.0, 1.0	0.473

†Mann-Whitney U test.

**p* value was significant at <0.05

6.4.2.4 Baseline characteristics of participants who were randomised (n=70)

Table 6.4 shows the baseline characteristics of the seventy participants, who were found to have circulating blood 25(OH)D concentration <50nmol/L, in active and placebo group prior to randomisation. There was no significant difference in any of the musculoskeletal outcome measures of the placebo and treatment arm at baseline.

Table 6.4 Baseline characteristics of randomised participants only

Variables	**Active (*n*=36)* Mean (SD), median, IQR**	**Placebo (*n*=34)* Mean (SD), median, IQR**	***p*-value†**
Body mass index (kg/m^2)	29.0(6.3), 28.5, 7.8	29.2(7.1), 26.9, 10.2	0.82

Total body fat (%) active (*n*=29), placebo (*n*=28)	41.5 (6.4), 41.8, 9.1	40.8 (7.6), 41.5, 11.5	0.86
Whole body muscle mass (kg) active (*n*=29), placebo (*n*=28)	36.5 (5.6), 35.8, 9.0	36.9 (5.7), 36.5, 6.8	0.79
25(OH)D concentration (nmol/L)	26.5(10.2), 24.2, 13.6	27.9(13.3), 22.6, 25.8	0.85
Right handgrip strength (kg)	15.8(4.8), 16.2, 5.5	17.4(5.1), 17.3, 8.5	0.23
Left handgrip strength (kg)	13.7(5.2), 15.1, 7.5	15.9(4.2), 16.2, 6.0	0.11
Single chair stand (s)	4.8(2.4), 3.8, 3.2	5.4(5.2), 4.0, 1.4	0.80
Repeated chair stand (s)	29.3(17.8), 25.0, 18.7	25.8(16.0), 21.6, 13.6	0.52
Timed up and go (s)	14.6(5.2), 12.9, 7.9	13.1(5.3), 12.3, 5.4	0.18
Balance test (s)	3.1(0.9), 3.0, 2.0	3.4(0.9), 4.0, 1.0	0.07
Walking speed (s)	6.5(2.9), 5.7, 4.6	5.9(2.3), 5.5, 2.5	0.58

†Mann-Whitney U test.

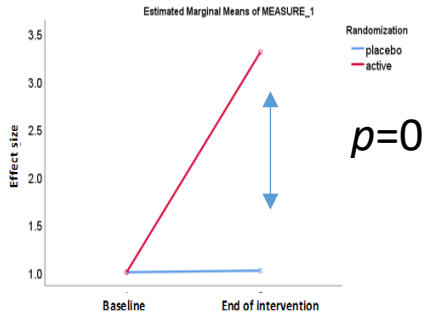
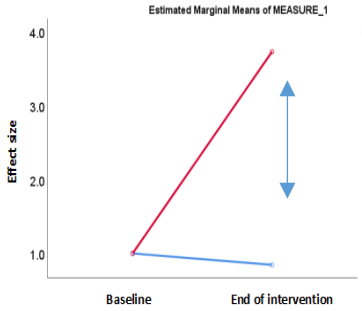
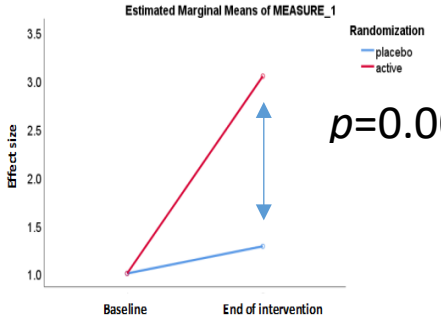
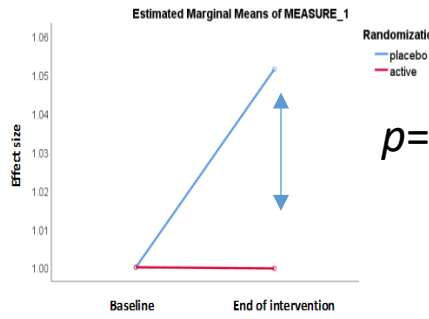
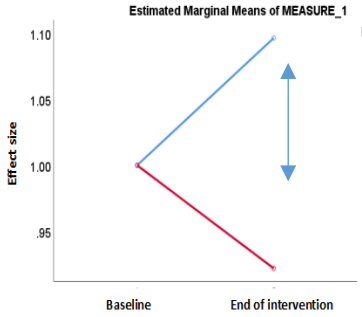
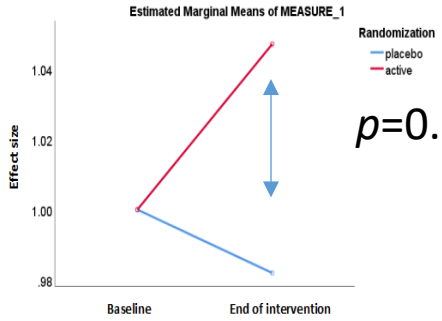
*Mean (SD)

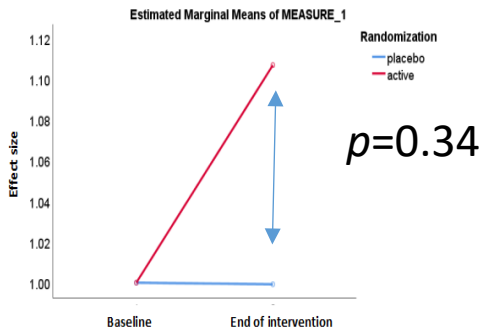
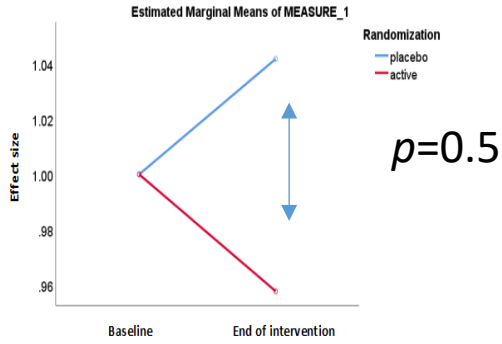
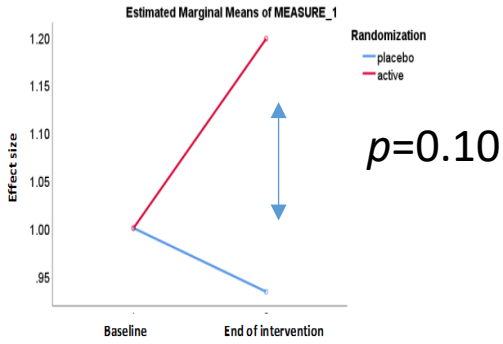
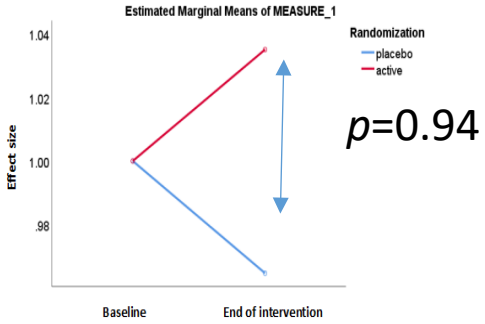
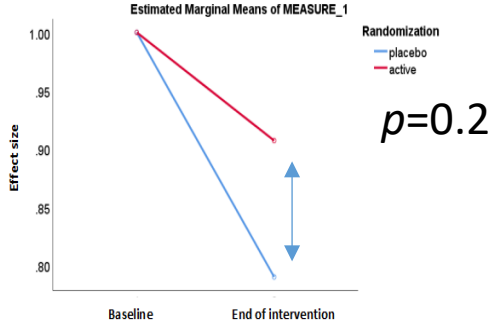
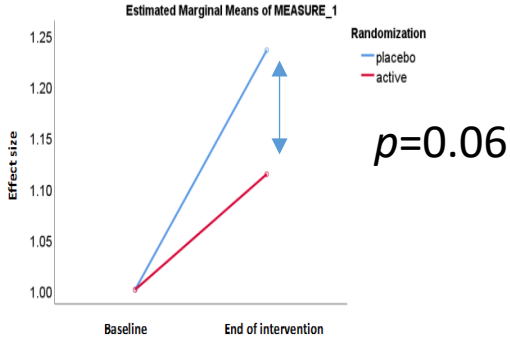
6.4.3 End of intervention findings

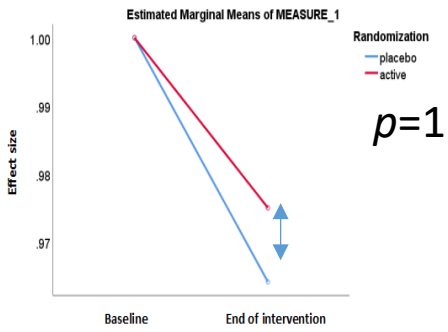
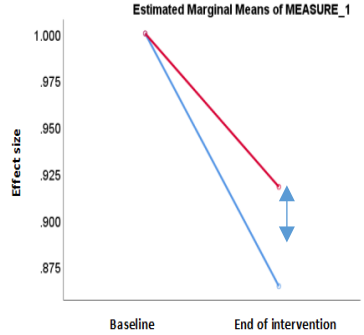
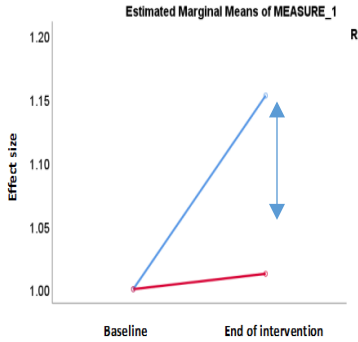
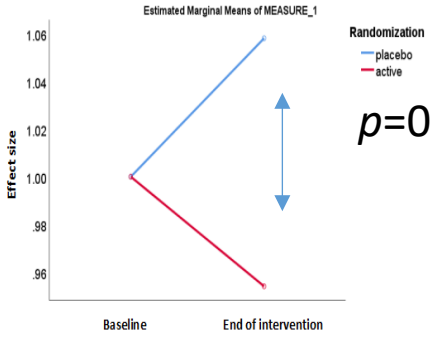
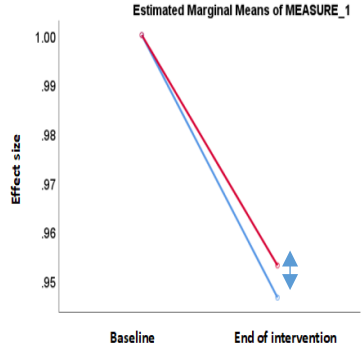
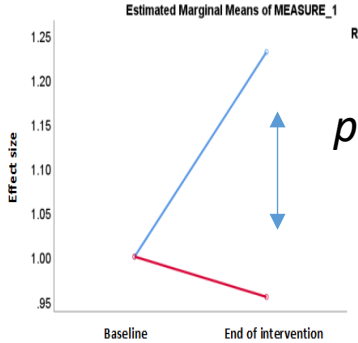
6.4.3.1 No between-group (active versus placebo) post-course effect found on muscle strength and function

Fifty-seven participants completed the intervention and were included in the final analysis at 12-weeks. Figure 6.4 shows a significant increase in circulating blood 25(OH)D concentration in the active arm compared to placebo at 12 weeks (p<0.001). No between-group post-course (active versus placebo) effect on right handgrip strength, left handgrip strength, single chair stand, repeated chair stand, timed up and go and the performance of walking speed test was found (Figure 6.4). The between-groups post-trial effect as per ethnicity groups was also analysed (Figure 6.4) which showed no post-course improvement in muscle strength and function in active versus placebo in any sub-ethnicity group. However, a significant decrease

in right handgrip strength in Indian women treated with vitamin D was observed at 12-weeks (Figure 6.4).

Outcome variables	All (*n*=57)	Indians (*n*=29)	Pakistani (*n*=28)
25(OH)D concentration (nmol/L)	 *p*=0.000	 *p*=0.000	 *p*=0.003
Right handgrip strength (kg)	 *p*=0.51	 *p*=0.03	 *p*=0.34

Left handgrip strength (kg)	 p=0.34	 p=0.5	 p=0.10
Single chair stand (s)	 p=0.94	 p=0.21	 p=0.06

Repeated chair stand (s)	 *p*=1.0	 *p*=0.48	 *p*=0.22
Timed up and go (s)	 *p*=0.09	 *p*=0.89	 *p*=0.002

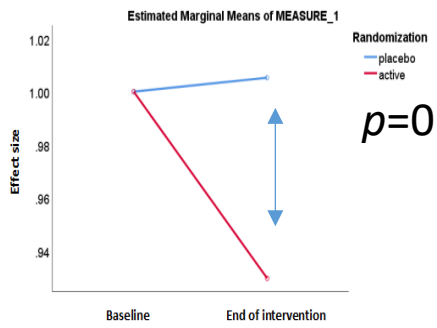
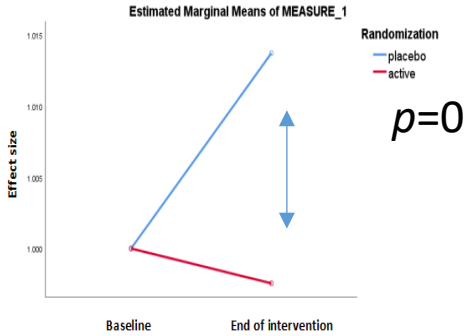
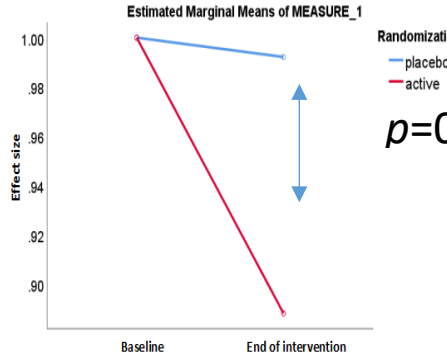
Walking speed (s)	 *p*=0.35	 *p*=0.9	 *p*=0.49

Figure 6.4 Between-groups (active vs placebo) post-course effect on muscle strength and function

6.4.3.2 No within-group change in muscle strength and function at 12 weeks from baseline

Table 6.5 shows a significant within-group improvement in circulating blood 25(OH)D concentration within the active arm at 12-weeks from baseline. No within-group change in the capacity of performing right and left handgrip strength, single chair stand, repeated chair stand, timed up and go, balance and walking speed was found at 12 weeks from baseline.

Table 6.5 Within-group change in muscle strength and function at 12-weeks compared to baseline (n=57)

Variables	Baseline	End of intervention	*p* value†
25(OH)D concentration (nmol/L)			
Active	25.7(10.6)	71.4(24.2)	0.000**
placebo	28.6(13.4)	30.0(23.7)	0.28
Right handgrip strength (kg)			
Active	14.9(4.6)	14.3(4.4)	0.109
placebo	17.2(5.1)	17.1(4.2)	0.764
Left handgrip strength (kg)			
Active	13.2(4.8)	13.5(4.6)	0.820
placebo	15.4(4.1)	14.8(4.2)	0.428
Single chair stand test (s)			
Active	5.0(2.5)	5.1(3.8)	0.230
placebo	5.8(5.6)	5.4(5.1)	0.350
Repeated chair stand test (s)			
Active	31(19.1)	26.7(13.6)	0.246
placebo	27.5(17.1)	23.2(13.9)	0.280
Timed up and go test (s)			
Active	15.1(5.3)	14.3(5.6)	0.054
placebo	13.6(5.6)	14.6(8.2)	0.699

Walking speed test (s)			
Active	6.7(2.5)	6.0(2.3)	0.067
placebo	6.2(2.4)	6.2(3.1)	0.459
Balance test (s)			
Active	3.0(0.9)	3.3(1.0)	0.080
placebo	3.2(0.9)	3.3(0.9)	0.830

†Wilcoxon signed ranks test was used.

***p* value was significant at <0.05.

6.4.3.3 Compliance to the measurements and intervention period

Table 6.6 shows compliance of the participants to the study assessments at baseline and end of intervention. The study participants had 100% compliance to all study assessments at baseline and end of intervention except single and repeated chair stand tests. With regards to the compliance to 12-weeks intervention period, 52 participants (out of 70) have completed 12-weeks intervention period, one participant had exit one day before completion of 12-weeks, three participants were exit two days before completion of 12-weeks and one participant had exit 19 days prior to completion of the 12-weeks intervention period.

Table 6.6 Compliance to measurements

Variables	Baseline (*n*=113)	End of intervention (*n*=57)
Circulating 25(OH)D concentration (nmol/L)	100%	100%
Right handgrip strength (kg)	100%	100%
Left handgrip strength (kg)	100%	100%
Single chair stand test (s)	99%	100%
Repeated chair stand test (s)	95%	93%
Timed up and go test (s)	100%	100%
Balance test (s)	100%	100%
Walking speed test (s)	100%	100%
Compliance to complete 12-weeks Intervention period	100% (*n*=70)	74.2 (*n*=52)

6.4.3.4 Vitamin D status at 12 weeks compared to baseline by ethnic group (n=57)

Figure 6.5 shows vitamin D status of the fifty-seven participants only by sub-ethnic, who have completed 12 weeks intervention period and hence were included in the final analysis. Among Pakistani participants (*n*=29), only 21% (*n*=6) participants had vitamin D status <25nmol/L, five of them were in placebo group. Whilst, among Indian participant (*n*=28), 39% (*n*=11) were found to vitamin D status <25nmol/L and they were all allocated to placebo group. The 100% Indian participants in active arm had achieved sufficient vitamin D status of >50nmol/L at 12 weeks. Among Pakistani participants (*n*=29), the 25(OH)D concentration measured at 12-weeks ranged from 10.3 to 114.10nmol/L (Figure 6.5) and in Indian participants ranged from 10.30nmol/L to 121.30nmol/L.

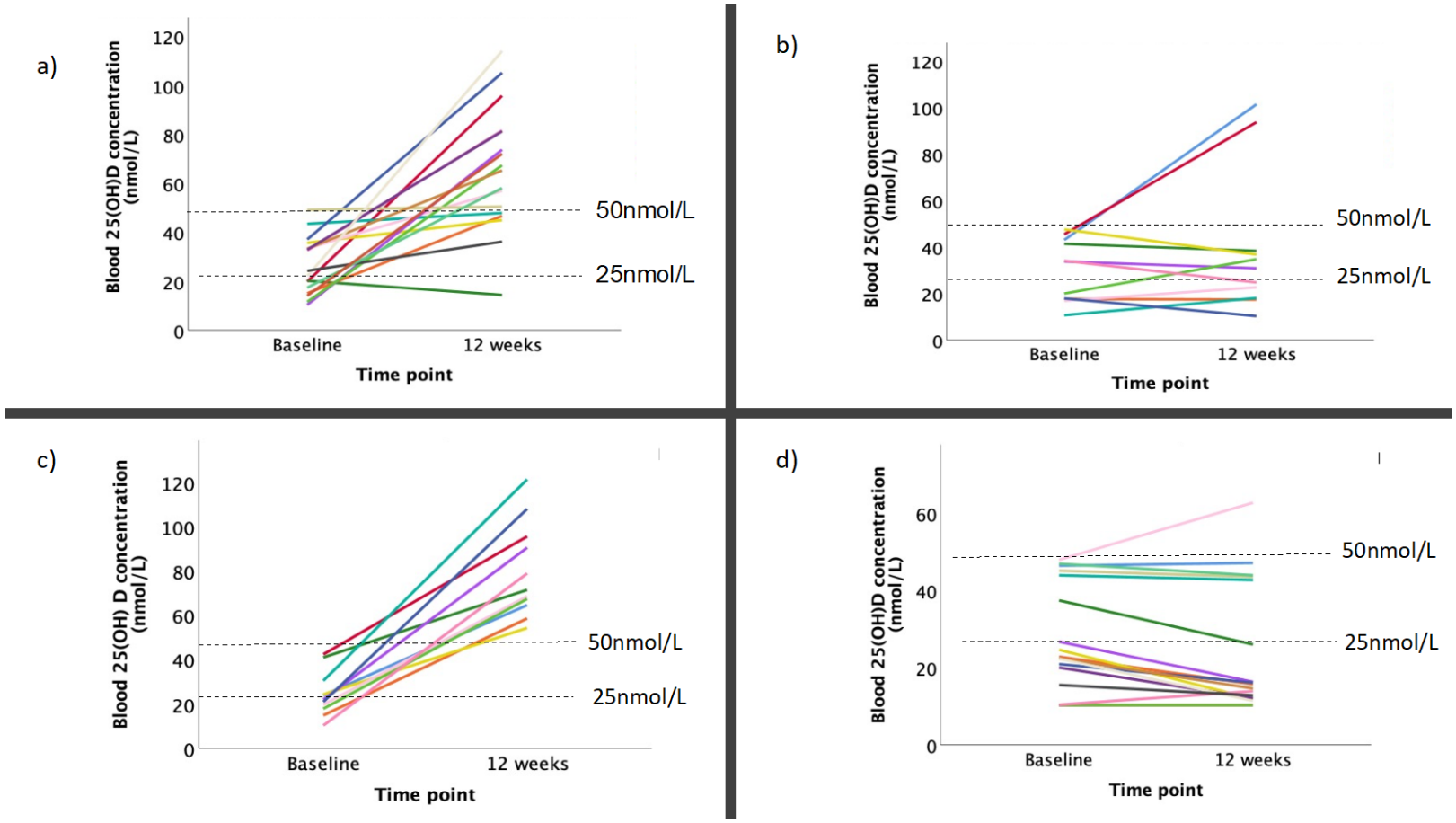


Figure 6.5 Change in blood 25(OH)D concentration at 12 weeks from baseline of randomised women in active arm versus placebo according to the ethnic sub-groups (n=57)

a) Pakistani women (active group), b) Pakistani women (placebo group), c) Indian women (active group), d) Indian women (placebo group)

6.5 Discussion

6.5.1 Purpose of study and main findings

The purpose of this randomised placebo-controlled trial was to investigate the effect of vitamin D supplementation for 12 weeks on muscle strength and functional performance in community-dwelling vitamin D inadequate/deficient UK South Asian post-menopausal women. The study found a significant increase in circulating blood 25(OH)D concentration at 12-weeks compared to baseline in the active arm compared to placebo. However, 12 weeks vitamin D supplementation in this deficient population did not significantly alter muscle strength and function parameters (Figure 6.4).

6.5.2 Critical analysis with literature

6.5.2.1 Effect of vitamin D supplementation in improving 25(OH)D concentration

Twelve-weeks supplementation with 3000IU of vitamin D_3 significantly increased circulating blood 25(OH)D concentration from baseline (Table 6.5) (Figure 6.4). This is in close agreement with previous research which has used 2800IU/day of vitamin D supplementation for same duration with post-menopausal women with baseline vitamin D <50nmol/L and reported a significant post-course increase in 25(OH)D concentration (p <0.001) (148). The efficacy of vitamin D supplementation is supported by several other trials with community-dwelling post-menopausal women belonging to different ethnic groups, which have used different doses of vitamin D with various duration of intervention and reported a significant post-course improvement in 25(OH)D concentration from baseline (85, 88, 92, 93, 95-97, 100, 106, 148, 149). Some trials also have reported significant combined beneficial effect of vitamin D and calcium supplementation in increasing 25(OH)D concentration from baseline (90, 105).

Studies which used low dose of vitamin D with women having better 25(OH)D concentration at baseline has not reported any post-course increase in 25(OH)D concentration over the time course (86, 87). Previous trials have suggested the use of high dose vitamin D supplementation and long intervals, such as 20,000IU twice a week or 50,000IU twice monthly, with sufficient 25(OH)D concentration at baseline in order to observe a significant post-course increase in 25(OH)D concentration compared to baseline (91, 97). Overall, the higher levels can be achievable through using high dose of supplementation however there is no evidence of an improvement to good muscle strength and function (95-97).

6.5.2.2 Muscle strength and function at 12 weeks

6.5.2.3 No post-course effect of vitamin D supplementation on muscle strength and function

In agreement to our findings, previous trials have reported no post-course improvement in muscle strength and function of community-dwelling post-menopausal women (85, 89, 91-98, 103, 105, 148).

Our findings are in line with a recent randomised controlled trial (148), which intervened in vitamin D deficient (<50nmol/L) post-menopausal women (*n*=81) with 2800IU/day for 3-months and reported no post-course beneficial effect on muscle strength and function. Similarly, the trials which have used very high dose of vitamin D supplementation for longer duration of intervention have reported no post-course improvement in muscle strength and function of community-dwelling post-menopausal women (91, 95-97, 149). Furthermore, the selection of long intervention period with standard or low dose of vitamin D supplementation also has reported to have no beneficial effect in improving muscle strength in community-dwelling post-menopausal women (94, 105).

6.5.2.4 Studies reporting significant post-course beneficial effect of vitamin D supplementation on muscle strength and function

In contrast to our findings, some previous studies have reported a post-course beneficial effect of vitamin D supplementation on muscle strength and function (handgrip strength, repeated chair stands and timed up and go test) in community-dwelling post-menopausal women using low to medium dose vitamin D supplementation (125IU-1000IU/day) for 3-4 months (86, 88-90, 100).

With regards to the relationship between optimal replete levels and better muscle strength and function, as it was identified in previous cross-sectional studies (section 4.5.2.2), it is hard to identify the optimal level for good muscle strength and function in community dwelling post-menopausal women in the light of the mixed evidence from RCTs. As some studies have reported a post-course improvement in muscle strength and function without finding a post-course increase in 25(OH)D concentration. It may be possibly due to adequate vitamin D status at baseline (86-88). It can also be interpreted in the context of IOM report which has reported vitamin D saturation after repletion (2). Hence, vitamin D administration, after reaching saturation levels in the body, may be clinically important with regards to maintaining/improving muscle strength and function rather than elevating circulating blood 25(OH)D concentration. Furthermore, Setiati et al (2018) have reported a significant post-

course improvement in muscle strength and function with alfacalcidol 0.5µg/day for 90 days without an increase in 25(OH)D concentration. They have interpreted that alfacalcidol goes into the body and directly reacts with the VDR rather than causing an increase in circulating blood 25(OH)D levels (87).

The 25(OH)D levels of women in previous RCTs, which have reported a post-course improvement in muscle strength and function with the use of medium dose for 3-4 months, was around the threshold for sufficiency set by IOM (86-90). Collectively these findings support the threshold vitamin D level of >=50nmol/L set by IOM for keeping good muscle strength and function in community dwelling post-menopausal women. The findings of our cross-sectional study also are in support of keeping >=50nmol/L levels for better muscle strength and function (Chapter 4), however the reason why we have not found this substantiated in the current RCT is unknown but could be due to ethnicity sub-group and cultural-specific, dose or duration of intervention (Table 6.3).

6.5.3 Analysis by ethnic sub-groups

It is also important to recognise that the intervention was not sufficiently powered to explore differential responses according to ethnic sub-groups, but this analysis has been done in an attempt to understand why the cross-sectional data collected at baseline in this study differed from that in Chapter 4. The women included in this study differed in their muscle strength and functional capability at baseline with Pakistani women with baseline circulating blood 25(OH)D concentration >=50nmol/L maintaining better musculoskeletal health (in agreement with the trends observed in Chapter 4) than Indian women (Table 6.2 and 6.3)

After using vitamin D supplementation for 12 -weeks, the circulating blood 25(OH)D concentration was significantly increased in both sub-ethnic groups. Regarding post-course effect on muscle strength and function, no beneficial effect of vitamin D supplementation was found in Indian or Pakistani women. However, the right handgrip strength in Indian women in active arm was decreased significantly at 12-weeks compared to placebo (Figure 6.4). The timed up and go performance was significantly improved in active group of Pakistani women at 12 weeks compared to baseline (Figure 6.4).

6.5.4 Strength of this study

The strength of this study is the nature of its originality. This study is, to my knowledge, the first to investigate the effect of vitamin D role on muscle strength and function in UK South Asian post-menopausal women. The sample size at baseline provides a snapshot of circulating blood 25(OH)D concentration, muscle strength and function measurements in this target population. Due to the initial screening step to identify only those women with low

vitamin D status the intervention population was fairly homogeneous in their baseline status. Other strengths include the randomised, double blind nature of the intervention, the measurement of circulating 25(OH)D concentration at baseline and end of intervention, the high compliance of the participants and the measurement of vitamin D status the gold standard method of Liquid Chromatography Mass Spectrometry (LC-MS) for accurate and precise results (119, 120).

6.5.5 Limitations

The main limitation of the study is that the inclusion of different South Asian populations has resulted in a possible dilution of the results. It has however identified a possible differential effect of vitamin D in these sub-populations that warrants further investigation and highlights the importance of careful interpretation with regards the generalisability of research. Other limitations include the outcome variables were measured at two points only and so therefore the rate of change in 25(OH)D concentration, muscle strength and function, with vitamin D supplementation, over the time course could not be quantified. The study has only used one dose of vitamin D supplementation for intervention and therefore it is not possible to explore any dose response relationship. The body composition was performed at the end of intervention only and dietary intake of vitamin D was not measured. The dropout rate was 18% which confirmed the challenges in researching this target population. The compliance to intervention could not be measured however the vitamin D status measurement may indicate that >20% of the Pakistani women failed to comply (Figure 6.5). The primary outcome was to measure single chair stand test however the study was not adequately powered for that and sample size was based on what was practical.

6.5.6 Future recommendations

The findings of this study will be useful to plan prospective study to investigate the dose response relationship between circulating 25(OH)D concentration, muscle strength and function over the time course in ethnic minorities for the better understanding of underlying mechanism in a bigger sample size. In particular, the dose response with 10µg/day and 20µg/day vitamin D supplementation, as recommended by SACN and IOM, on muscle strength and function in South Asian women would be of interest to investigate. Future studies are required to define optimal dose and duration for vitamin D administration in order to improve/maintain muscle strength and function in this population. Furthermore, as this study was performed with South Asian post-menopausal women so future studies in ethnic minority populations could consider different age groups and males. The differential response of different South Asian sub-populations also warrants further consideration as discussed above.

6.6 Conclusion

The vitamin D supplementation course of 3000IU/day for 12-weeks did not improve muscle strength and function in UK South Asian post-menopausal women. The post-course blood 25(OH)D concentration has significantly increased in the active arm of both ethnic sub-groups (Indians and Pakistani) with the achievement of 100% repletion rate in Indian women.

6.7 Summary

This randomised placebo-controlled trial has investigated the effect of vitamin D supplementation on muscle strength and function in vitamin D deficient UK South Asian post-menopausal women.

The data analysis of this study has demonstrated the following:

- The post-course circulating 25(OH)D concentration was significantly increased in the active group versus placebo at 12-weeks compared to baseline.
- The vitamin D repletion, upon receiving vitamin D supplementation at 12-weeks, did not improve right and left handgrip, single chair stand, repeated chair stand, timed up and go, balance test and walking speed performance in active versus placebo compared to baseline.

7 Chapter 7: A narrative reflection on the experience of recruiting older South Asian women to research: a narrative report

7.1 Introduction

7.1.1 Recruitment and impact on delivery of research

Recruitment of research participants to research and in particular to Randomised Control Trials is recognised as critical for the success or failure of research (150). Recruitment can often be slower than anticipated and can jeopardise the success of a study. It is important to understand the factors that influence recruitment, particularly for hard-to-reach groups. The approach to recruitment is crucial and has an ultimate impact on the fate of recruitment. The achievement of target sample size is important to reach statistical significance of outcome findings. The quantification of time required to achieve target of recruitment may vary widely and will depend on a variety of factors including the approach to recruitment used. The study protocol and study specific procedure should collate with recruitment target set and selection of the target population. The design of methodologies used in recruitment can be considered as a separate research area given that it may have impact on the quality of research excellence and strengthen the outcomes of the study. The All-Ireland Hub has established SWAT in 2012, which is a Study Within a Trial, to introduce and develop the investigation of methodologies involved within a study and their effectiveness for successful delivery of study outcomes and for a robust approach to recruitment (151).

7.1.2 Recruitment of older adults

Recruiting older people to health research can present particular challenges and adaptation of recruitment strategies may be required (152). There are a number of reviews of recruitment procedures, highlighting underpinning barriers and methods used for successful recruitment rate and retention target. A systematic review of twelve papers has investigated the effectiveness of different recruitment strategies to recruit a general population of older adults aged 50 years and above in health research and for quantification of response rate or enrolment (153). They concluded that the strategy of face-to-face contacts with potential participants can be a more effective recruitment method than indirect methods (such as mail recruitment pack with a follow-up phone call).

In contrast to a community-dwelling population, another review of 41 RCTs, with older adult participants (aged 65 years and above) in institutional settings, has investigated recruitment, attrition and adherence to follow-up at 12 months in falls prevention trials (154). They have reported that older people in residential settings were more likely to be excluded from a study

rather than refuse to participate in the first place (154). They have estimated 50% success in recruitment rate and 16% attrition rate (including mortality) to 12-months follow-up period with a single intervention component compared to multifactorial interventions in residential settings. The recruitment and retention rate was observed to be decrease in studies of longer period. They have also highlighted the importance of motivating staff members for successful recruitment (154).

7.1.3 Recruitment of ethnic minority population in the UK

The UK based South Asian community is under-represented in research (155) and this is likely to be due to a combination of factors including inadequacy of research funding for the BAME community, exclusion of the BAME community from research, mistrust of the community in research (156), but may also be due to failure to recruit (155). A recent national longitudinal study, the community ageing research 75+ study, has collected data on frailty, independence and quality of life and reported potential barriers and facilitators to successful recruitment of UK South Asian older people (156). They have reported a 33% recruitment rate out of 233 South Asian older people approached in a population residing in Bradford, West Yorkshire, England which has a large South Asian community (156).

They reported potential barriers to recruitment with older South Asians were: different spoken languages and mode of communication of this target population, involvement of family members while consenting to take part in a study and ensuring confidentiality (156). The potential facilitators to recruitment have been reported as: knowledge of cultural norms, a researcher with bilingual skills and to build trust with research participants (156). The South Asian older women in particular may have responsibilities of taking care of their grandchildren which may limit their outdoor activities and occupy their daily schedule significantly (156).

A knowledge of the strategies adopted by previous researchers to aid recruitment in older adults and in BAME community is of value to other researchers for the planning purposes and information on drop-out rates may aid estimation of sample size for this community in future studies. This chapter describes my experience of recruiting post-menopausal South Asian (Indian and Pakistani) women to a cross-sectional study and to a randomised controlled trial. This chapter highlights some of the practical barriers encountered when approaching South Asian women, recruitment process, strategies adopted to aid recruitment and retention of this target population to the studies across this PhD.

7.2 Methods

7.2.1 Study design

Two research designs were used in this PhD and provide the basis for this recruitment process narrative i) a cross-sectional study (as described in Chapter 4) and ii) a double-blind randomised control nutritional intervention trial of 12-weeks duration (as described in Chapter 5). The former required participants to meet with the researcher on a single occasion to perform the study protocol while the later required participants to attend meetings on 3 different occasions and perform assessments with the researcher on 2 different occasions. Below is the brief overview of the two studies to understand the level of involvement of potential participants in each study.

7.2.2 Overview of the studies

7.2.2.1 Cross sectional study

This was an observational study design and required the delivery of study protocol with each woman in one setting. The target population in this study was community-dwelling South Asian (Indian and Pakistani) women aged 60 years and above. Therefore, the methodology involved was approaching South Asian community centres, Indian temples (Gurdwaras), mosques around Sheffield and Rochdale areas. This study involved two face-to-face meetings with participants. The first meeting was with groups of women in designated community venues with the purpose sharing information about the study and inviting people to take part. The second meeting was a one-to-one appointment with interested and eligible women who took part in the study and completed the study protocol. The below Figure 7.1 shows participants involvement in cross-sectional study.

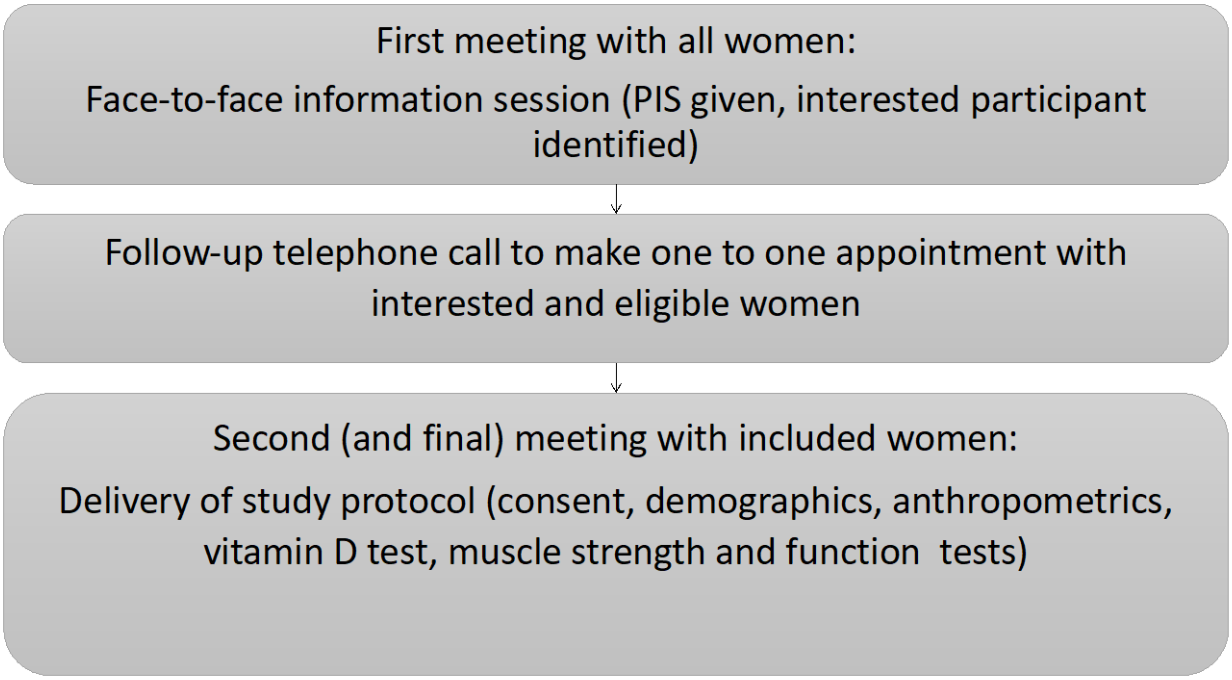


Figure 7.1 Participant involvement in cross-sectional study

7.2.2.2 Overview of randomised control trial

The second study of this thesis was a 12-weeks RCT with community-dwelling South Asian (Indian and Pakistani) post-menopausal women. The study required participants to meet with the researcher on 4 occasions: the first meeting was with groups of women in community settings to provide a face-to-face information session, PIS were distributed, interested and eligible women were invited to take part in the study. The second meeting was a one to one, face-to-face meeting with interested and potentially eligible women for screening purposes and collection of baseline information (consent, demographics, finger prick blood spot collection, muscle strength and function assessments). The third meeting was required to allocate eligible women to the intervention and to supply them with intervention regimen. The fourth meeting was (after 12 weeks intervention) required post-intervention repetition of all study assessment made at second meeting with the intervention participants. Figure 7.2 shows participant involvement at each stage of the randomised control trial.

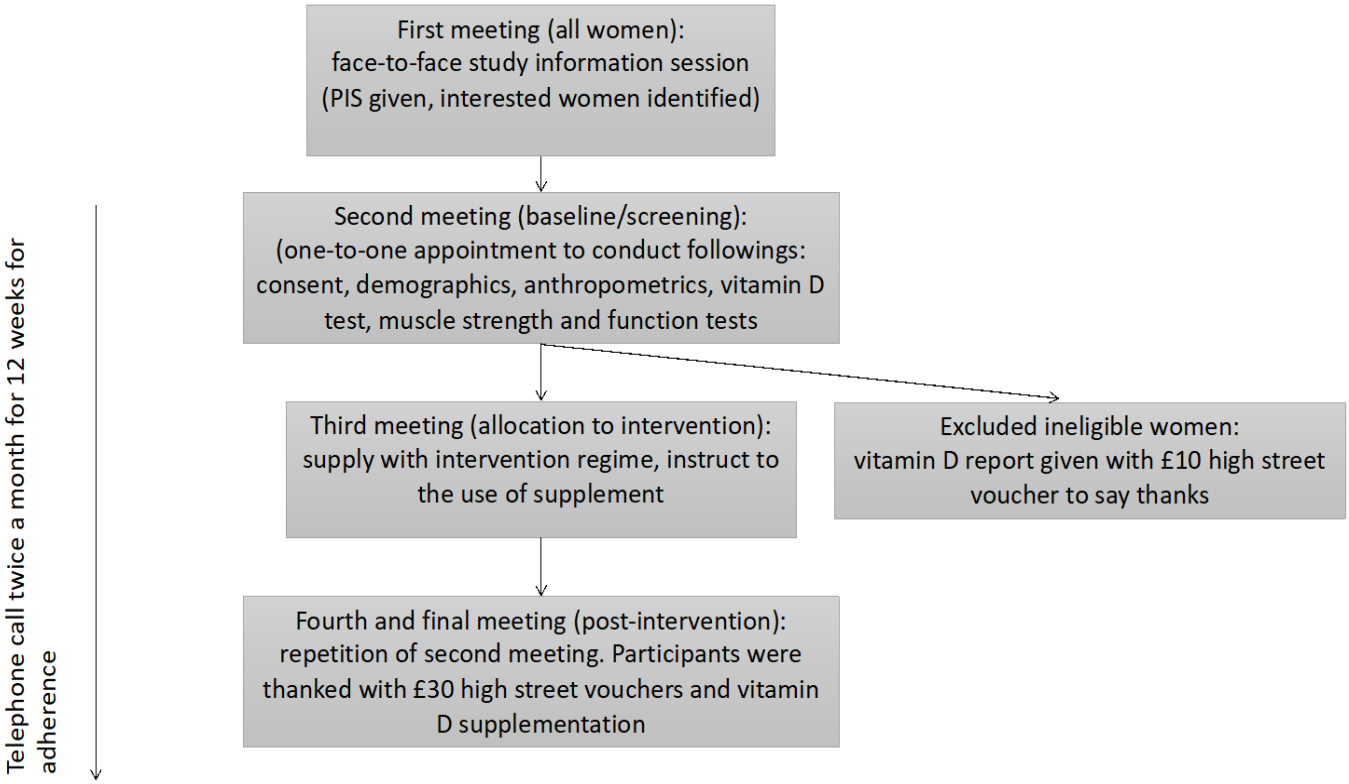


Figure 7.2 Participant involvement in randomised control trial

7.2.3 Research aid documents to both studies

Ethical approval of cross-sectional study and randomised control trial was sought in advance (Chapter 4 and 5 respectively). Leaflets, to advertise the study, were prepared in Urdu and English languages. The participant information sheets were prepared in simple English language and were provided to interested and eligible participants. Participants were asked to share the PIS with family members which explained the purpose of the studies.

7.2.4 Study protocol

The details of the study protocols for both studies have been provided in Chapters 4 and 5. Common methodologies are given in Chapter 3. The study protocol was completed on a single occasion with each woman in the cross-sectional study. The study measurements in the RCT were performed at baseline and end of intervention. The RCT has an additional muscle function test (walking speed test) however compared to cross-sectional study, the randomised control trial did not include the assessment of dietary intake of women.

7.3 Results

The section is written with an exclusive reporting of how the cross-sectional study was delivered and how this guided the design and delivery of the RCT. The changes made and strategies adapted for the RCT with lessons learnt from a cross-sectional experience is narrated under this section.

7.3.1 Delivery of cross-sectional study

7.3.1.1 Advertisement

Both studies used convenience sampling. Figure 7.3 shows the approaches made during the recruitment to the cross-sectional study. There were 4 main methods of advertisement and recruitment: leaflets, visiting community centres (Indian and Pakistani), mosques, visiting Indian temple (Gurdwara) and by word of mouth. The leaflets were distributed at the local Asian supermarket and lady's boutique shop in Sheffield. The South Asian community centres (Indian and Pakistani) were approached in Sheffield and Greater Manchester region (Rochdale, Oldham and Manchester). Five Indian temples (Gurdwaras) were identified and approached around Manchester area. Several mosques were approached and visited in Sheffield however, it became apparent that the majority of women there were same as those attending the community centres. Women in mosques were found to have lack of interest to take part in a research study.

For all the venues, the study was introduced first to the authorities (prior to access to the participants), who are known to be gatekeepers to this community, and research documents (ethical approval and participant information sheet) were provided upon request. The permission to conduct face-to-face study information session with women was sought to the relevant authorities of these places. Two temples granted permission to visit, but only one temple then granted permission to run information sessions with women and conduct face-to-face session. The Indian temple in Sheffield was approached however, no permission was granted to visit due to lack of interest and busy schedule.

Upon permission, the face-to-face sessions were arranged with potential women at designated places. During the session, I delivered a talk about the study to the women in the preferred language in which knowledge of vitamin D, study purpose, protocol was provided. The sessions covered the information in the PIS. By end of the sessions, women were provided an opportunity to ask questions. The leaflets and PIS were distributed among interested and eligible participants and women were asked to share it with family members. At the end, an initial list of women was made who expressed their interest in the study and contact details were obtained.

7.3.1.2 Documentation

During the face-to-face session of the cross-sectional study participants were not found to be familiar with all of these assessments and hence in the subsequent RCT the PIS included diagrams to illustrate the procedures of muscle strength and function assessments. Informed, written consent for both studies was read to each participant in a preferred language and asked if they understand it. Participants who were accompanied by a family member at the time of consent were asked to bring along the same family member to aid future appointments. Upon agreement, written consent was obtained and one copy was provided to participants.

7.3.1.3 Recruitment

After face-to-face session, women were provided with one to two weeks to make a decision of taking part in the study. A follow-up phone call was made to women, who had expressed an initial interest as a result of face-to-face session, to ask about their decision. A one-to-one appointment was made with women who wanted to take part. Appointments were made on the same day that women usually visited the community centre/temple/mosque. The authorities were informed and arrangements were made for room availability to conduct study protocol. Appointments at the temple were made either over the weekends or in evenings during the week as per women suitability. Home visits were performed for women who were recruited by word of mouth. The home visits were not found to be ideal place to recruit and conduct study protocol.

It was found that South Asian community centres were proven to be more effective places to access this community than religious places. The major proportion of recruitment in this study was achieved through community centres followed by Gurdwara. The least effective recruitment methods were word of mouth, mosques and no response was received through the distributed leaflets. Table 7.1 shows researcher's perceived challenges encountered during recruitment.

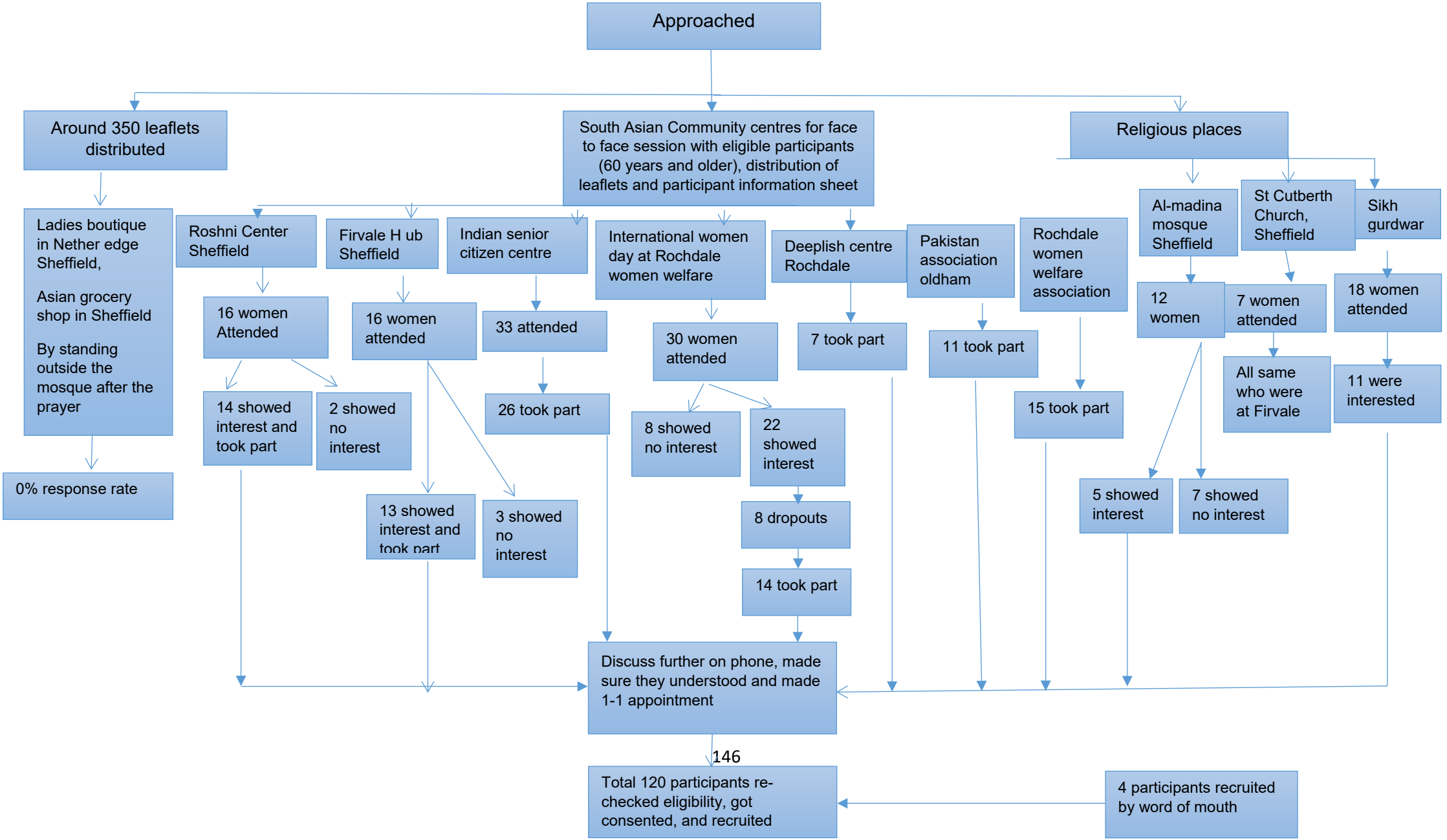

Approached
Around 350 leaflets distributed
Ladies boutique in Nether edge Sheffield,
Asian grocery shop in Sheffield
By standing outside the mosque after the prayer
0% response rate
South Asian Community centres for face to face session with eligible participants (60 years and older), distribution of leaflets and participant information sheet
Roshni Center Sheffield
16 women Attended
14 showed interest and took part
2 showed no interest
Firvale H ub Sheffield
16 women attended
13 showed interest and took part
Indian senior citizen centre
33 attended
26 took part
3 showed no interest
International women day at Rochdale women welfare
30 women attended
8 showed no interest
22 showed interest
8 dropouts
14 took part
Deeplish centre Rochdale
7 took part
Pakistan association oldham
11 took part
Rochdale women welfare association
15 took part
Religious places
Al-madina mosque Sheffield
12 women
5 showed interest
7 showed no interest
St Cutberth Church, Sheffield
7 women attended
All same who were at Firvale
Sikh gurdwar
18 women attended
11 were interested
Discuss further on phone, made sure they understood and made 1-1 appointment
146
Total 120 participants re-checked eligibility, got consented, and recruited
4 participants recruited by word of mouth

Figure 7.3 Flow chart showing advertisement and recruitment of cross-sectional study

7.3.2 The delivery of the randomised control trial (lessons learnt from cross-sectional study)

7.3.2.1 Advertisement

Many lessons were learnt while conducting the cross-sectional study hence new recruitment strategies were used for the RCT. Firstly the randomised control trial was conducted only in greater Manchester area as during the cross-sectional study, low levels of supplementation was identified in this region and for the RCT non-users of vitamin D supplement were needed.

The recruitment to the RCT was more challenging as it required participants to stay for a longer period and hence involved additional initial meetings with authorities (details given below) for ensuring participants safety and confidentiality. The same South Asian community centres and one Indian temple (Gurdwara), who granted a permission in previous study, was contacted to advertise the study. In addition, some new community centres (around Ashton), Shelter House (Rochdale) and Indian mandir (Ashton) were identified and approached. The study was advertised on a WhatsApp group too of the lay member of one Pakistani community centre in Rochdale however, this did not contribute to recruitment in anyway.

Community centres and Shelter House were found to be the most effective places to recruit Pakistani women and overall, the Indian community were found to be more interested than Pakistani women. Similar to the cross-sectional study, a direct method of contact via face-to-face sessions were found to be effective in demonstrating the importance of the study to women and building trust to help making a decision of taking part in a study. Mosques were not approached in this study and no particular attempt was made to distribute leaflets. The same approach, as used in the cross-sectional study, was adopted to recruit potential participants.

7.3.2.2 Initial meeting with authorities of community centres/religious leaders at Indian temple

A formal meeting was arranged with gatekeepers to this community in which study protocol was explained and research documents were presented. Due to the nature of the intervention study additional anxieties were raised about the researcher qualification therefore an initial discussion with authorities was effective with regards to build trust. The study's ethical approval and participant information sheet were also provided during the meeting and via

email. A participant insurance letter was provided to one community centre (in Rochdale) by the University of Sheffield upon request. The meetings were conducted in a preferred language.

7.3.2.3 Meeting with participants

Upon permission from gatekeepers, a face-to-face information session was conducted with potential women. The theme of this session and provision of leaflets and PIS was same as it was in previous study. Three Indian Mandir (in Ashton and Oldham), one Indian temple (Gurdwara) (in Manchester) and five South Asian community centres (one shelter house) (in Rochdale, Oldham, Ashton and Manchester) were visited and recruited participants from.

Building rapport and trust to this community was found to be important. I have attended activities and prayer with Indian participants at Mandir and temple prior to start face to face session. This helped to build a good rapport of the researcher with the women. The session was conducted in the same way as in cross-sectional study. However, simple language was used to translate and convey some scientific terms accurately. This was a bit longer session comparatively in order to convey all study details and let participants to understand it properly. Women of all ages and family members who were present attended the session.

7.3.2.4 Recruitment

The similar, to cross-sectional study, approach was used to deliver the RCT. The numerous visits to recruitment sites were performed to deliver the study protocol at baseline and end of intervention. However, like wise in cross-sectional study, the study protocol, at each stage, with each woman was completed in one sitting. Few home visits were performed too for the women who were recruited by word of mouth. All study procedure was conducted in a preferred language. Figure 7.2 above shows study protocol and correspond participants involvement to study. Below Table 7.3 shows challenges encountered during recruitment to RCT.

7.3.3 Barriers to recruitment

7.3.3.1 Challenges encountered to recruit South Asian post-menopausal women to cross-sectional study

The challenge encountered is split into two sections: researcher's perspective and participant's perspective. The former includes the challenges based on the researcher thoughts and experience which may have influence this community with the decision of taking part in the study. Whilst, the later challenges are built on the basis of participants questions to

the researcher and illustrate participants' initial responses/thoughts of taking part in a study as received/observed by researcher. Some of them could be generally applicable to all research studies and hence to population accordingly whereas some are specially related to researching South Asians and mentioned accordingly.

Table 7.1. Challenges encountered in cross-sectional from researcher's perspective

Limited knowledge (specific)	• Women were found to have limited knowledge about research, vitamin D, muscle strength and function assessments. • This could possibly reflect education/knowledge level as only 4.2% of women were found to have university level education in the cross-sectional study (Chapter 4).
Lack of enthusiasm (specific)	• The lack of enthusiasm and no interest at all to take part in research study was observed in target women.
Influence of family members (specific)	• Women were found to be strongly influenced by their family members when deciding whether or not to consent and family members often accompanied them on the initial visit (for transparency this was encouraged for future meetings). It was also more work for researcher to explain study protocol to participant's family members.
Long study protocol (general)	• It was hard to keep hold of participants for the duration of the study procedure and some participants appeared bored.
Labour intensive (specific)	• The delivery of the research methods with post-menopausal South Asian women was found to be labour intensive as each test needed demonstration and explanation (sometimes more than once).
Time intensive (specific)	• As per above method of recruitment, it involved huge travelling to designated places across Rochdale and Sheffield regions. • Researcher waited for the completion of women's routine activities at community centres/Mandir/temple prior to start initial face to face informative session. • Indian temple (Gurdwara) required visiting over the weekends and evenings to recruit Indian women to the study. As Sikh Indian community visit temple in evening and over the weekends to perform worship/religious activities. • The conduct/completion of study protocol with post-menopausal South Asian women was found to be time consuming. • The delivery of face-to-face session was also found to be time consuming as it took longer to explain the study details in simple and translated language.

Home visit (specific)	• Home visits were performed for women recruited by word of mouth. It was challenging to deliver study protocol during home visit due to the presence of grand children and family members around. The study setup during home visit was more time consuming than at community centres/temples.

Table 7.2. Challenges encountered in cross-sectional from participant perspectives

Busy routine (could be general in all research studies however a little more to South Asians at some extent)	• Post-menopausal women in this target population were found to be very busy with family and grandchildren which made it challenging for them to take part in research activities. Women did visit to community centres/Mandir/temple once a week for activities and were not keen to interrupt their tight schedule.
Safety concern (could be general to all research studies however a little more to South Asians at some extent)	• Women were found to be concerned about the safety of the muscle strength and function tests so they were informed that this study did involve common methods of assessing muscle performance which were safe to do in a community setting. • Women were ensured of researcher's presence and instructed to stop the test if they felt unsafe.
Ensuring confidentiality (could be general in all research studies)	• Women were found to have perception that this project involved NHS and thus could interfere with their medication/prescription so women were told that this study did not involve NHS and solely conducted by the University of Sheffield. • Women were informed that taking part in this study did not interfere with their medication use/supply.
Burden of study (could be general in all research studies)	• Women were found to be concerned about the burden of the study and level of their involvement required in study protocol. So, women were told that they did not have to go anywhere to take part in the study and that visits would take place at a venue of their convenience.

7.3.3.2 Additional challenges to recruit and retain South Asian women to randomised controlled trial

The main challenge of recruitment to randomised controlled trial was due to the duration of the intervention (12-weeks) and use of intervention regime which affects recruitment at the first place. Table 7.3 shows extra challenges in addition to common challenges encountered during cross-sectional study.

Table 7.3. Additional challenges encountered in RCT from researcher's perspective

Lack of knowledge	The lack of knowledge about placebo and intervention was found in this target population.
Labour intensive	• More labour intensive for researcher compared to cross-sectional study. • It was required number of visits/travelling to community centres/temple/Mandir during conduction of randomised controlled trial as compared to cross-sectional study in order to recruit target number of women. • The demonstration and explanation of each test was required at multiple occasions and multiple times. • Telephone calls were made to included women 6 times (during 12-weeks) to maintain adherence rate. • The lack of self-response was found in recruited women as women who dropped out during intervention period have not informed researcher prior/after stop using intervention regimen. It was to be found on next follow-up call.

Table 7.4. Additional challenges encountered in RCT from participants perspective

Health issue	• Women were found to have the perception that some kind of drug treatment was going to be tested on them and so they were reassured that this trial did not involve the investigation of any medicinal product. • Women were informed about the safety of the use of vitamin D supplementation dose used in this study.

	• Women were observed to be worried about the use of placebo as the randomisation was double blinded. Women were informed about the importance of inclusion of placebo in a study. • As this study recruited vitamin D deficient women were concerned about living with vitamin D deficiency for 12-weeks while being on intervention blindly, but were reassured that the study had been ethically approved and that the deficiency would be corrected on completion of the study with the supply of free vitamin D.

7.3.4 Common strategies to both studies adopted to recruit women

The following common strategies were adopted to recruit women to both above studies and are recommended.

Table 7.5. Strategies adopted to recruit South Asian post-menopausal women to research study.

Generally applicable to research studies	Specific to research South Asian population
Conduct research study at place convenient to them	Conduct initial face to face information session in a preferred language as it did illustrate the importance of the study and build rapport of the researcher with the women.
Booking appointment and conducting research activities at time suitable to participant's schedule.	Deliver all aspects of the research study in a preferred language (as far as possible).
Give respect and dignity to research participants.	Read the informed written consent form to the participants in the preferred language.
Provide an assistance as and when required such as helping them in standing on and off the weighing scales given the age of the women.	Used the title "aunti" to call women and "uncle" to call man as this is cultural.
Positive attitude of the researcher.	Allow sufficient time to work with this community.
Inclusion of incentives for all participants.	Adopt flexible working (over the weekends and evenings).
Use a friendly and warm approach with participants to build trust	Attend religious activities with Indian participants at temple.
	Explain study details in a simple manner to participants and family members.

7.3.4.1 Additional strategies adopted to recruit and retain women to randomised control trial

Some additional approach was adopted to recruit and retain South Asian post-menopausal women to randomised control trial.

Table 7.6. Additional strategies to recruit and retain South Asian post-menopausal women to randomised control trial

• The diagrams illustrating study procedures were included in participant information sheet to provide participants with initial understanding of assessments involved in a study protocol.
• Become a part of religious activities/worship at Mandir with Indian participants as this has built trust and respect to them.
• Phone calls to participants to maintain adherence to the study.
• Patience to complete study measures with target population.
• Given clarification and explanation of study details to family members which has high influence on decision making of older women in this community.
• Provided vitamin D supplement to women who were intervened at the end of intervention.

7.3.5 Factors proven to the successful recruitment of South Asian women

The number of useful strategies were used and identified for successful recruitment of this target population to research studies. These factors were found to be cultural-specific and important while researching this community.

Table 7.7. Factors contributed to successful recruitment of South Asian post-menopausal women

• A face-to-face session with potential participants as it allows the researcher to deal with any concerns and elaborate the importance of study.
• Same/match gender as post-menopausal South Asian women were observed to be more comfortable with female researcher
• Researcher to have same language skills as per participants as the inclusion of translator could be challenging to convey accurate information.
• Knowledge of cultural sensitivity of the participants.
• Respect and dignity to participants, to build rapport and trust with participants and good way is to engage in their religious activities.
• A warm approach and smile on face of researcher to the participants.
• Inclusion of incentives.
• Allow extra time to become a part of general discussion with South Asian women participant.

7.4 Discussion

7.4.1 Main findings

The studies have identified potential challenges encountered (Table 7.1 and 7.3) and strategies adapted (Table 7.5) to successful recruitment of South Asian post-menopausal women to cross-sectional study and randomised control trial which were conducted as part of this PhD thesis. The challenges involved with the RCT were greater than those experienced in the cross-sectional study. The authorities of South Asian institutions acted as Gatekeepers to the participants and therefore their satisfaction or involvement at trial design phase, if possible, can grant access to this community. The post-menopausal South Asian women were influenced by their family members in decision making of taking part to the study and hence required additional explanation of study to family members. South Asian community centres were found to be more successful place to approach Pakistani women and hence yielded high proportion of recruitment of this community compared to the religious places. Overall, Indian

women were found to be more enthusiastic to take part in a research study (both from community centre and Gurdwara/mandir) than Pakistani women.

Likewise, women in Greater Manchester area were found to be more interested/responsive to take part in research study than women in Sheffield area. Inequality in terms of access to prescribed vitamin D was evident between women from Sheffield and Greater Manchester area. However, these findings are specifically related to these two studies and may vary depending on the nature of the study. The numerous culture-specific recruitment strategies have been identified and applied, as in line with literature, to meet challenges and achieve the recruitment target successfully. Overall, initial face to face session with this community were found effective method to recruit the potential women. A follow-up phone call held very importance to achieve/maintain recruitment rate.

7.4.2 Simplified information sheets and consenting

Our approach of preparing PIS and consent forms in simplified and easy manner to improve understanding of older population is in agreement with previous systematic review of 99 articles (157), which suggested a link between understanding of informed consent and old age and education level of the participants. They have further supported our approach of including visual aids/diagrams to illustrate the study procedures as it helps better understanding and can increase the chances of recruitment rate. Another previous study has suggested the design of the study to be relevant and feasible in order to increase interest and participation rate of the older people to the study (158).

We have observed the presence of family members while consenting to take part in a study. A recent longitudinal study has reported taking proxy consent from family member, in instances where older South Asian participant were not able to write their name (156).

7.4.3 Approach to recruit South Asians postmenopausal women

In support of our findings, a recent national longitudinal cohort study has reported challenging to recruit older South Asian population to research study (156) and thus a pragmatic approach was adopted to access this population in current studies.

7.4.3.1 Face to face sessions

We found an initial face-to-face information session with this community to be useful in number of terms such as: initial effective introduction to researcher, a meeting with warm approach to potential participants and family members, demonstrate respect and dignity to women in turn to build rapport, to emphasize the importance of the study, delivering knowledge of the study,

providing opportunity to ask questions and achieving an initial interest of the women. The use of preferred language to participants have provided participants with clear knowledge of the study and better understanding. This also helped in decision making of taking part in a study. Women asked questions during session which could possibly be a good way of ensuring their involvement and understanding of the study.

Our approach of conducting face-to-face session was in line with previous recent study which has reported the effectiveness of face-to-face contacts with South Asian potential participants in building trust and rapport (156). Previous studies have also highlighted the importance of face-to-face contact with African American and Caucasian older adult research participants help in achieving of higher rate of enrolment over indirect methods (153, 159).

7.4.3.2 Follow-up phone call

Our approach of arranging a follow-up phone call to the participants who expressed their interest as a result of face-to-face information session is supported by previous longitudinal study with older South Asians (156). We have found that the follow-up phone call approach to be useful to demonstrate the reliability of the study to the participants and encourage them to take part in. It was also found to be an appropriate way of estimating the number of participants who expressed their interest and to confirm initial eligibility check against study. Our approach is supported by previous study which has suggested the importance of follow up phone call or contact to the research participants after initial post-card or mailing approach to increase the response rate to enrolment to the study (160). The timing of the follow-up phone calls, was mindful of prayer times when possible. This approach is in agreement with previous study with older South Asian people (70, 156).

7.4.3.3 Word of mouth approach

Word of mouth was found to be least effective way of recruiting this population. The home visits were performed to recruit women for this method and it was found to be time extensive and burdensome for the researcher. The researcher has to effort in setting-up study at home environment. The presence of grandchildren and family members were found to be distracting. These findings are in agreement with previous study with older South Asian people which also has reported the involvement of family members in research study (156).

7.4.4 Recruiting South Asian women to study

All recruitment procedures were performed at the place convenient for participants to build comfort and hence to increase recruitment rate. A systematic review of 15 studies has supported our strategy of building trustful approach with research participants and identifying

a place and time convenient to participants as to be useful practice of improving enrolment and adherence rate to the study (161). They further reported distrust to research team as barrier to recruit older adults in research trial (161).

In line with our findings, a previous study has reported the influence of family members to consenting to research study. They have reported it to be a labour intensive for researcher to deal with different questions of family members of South Asian older participants compared to the family members of Caucasian participants who usually not present at the time of recruitment (162).

In line with previous narrative review (155), we also observed that women were concerned that some experimental product would have been testing on them therefore women were reassured that vitamin D is found in foods, is a dietary supplement and can naturally be achieved through skin exposure to sunlight.

7.4.5 Retaining South Asian women to study

Different strategies were adapted to retain participants to RCT (Table 7.6). Our strategy of involving the same sex of researcher as to the participants is in agreement with recent national cohort study which has reported this method to be useful to successfully delivery of study with South Asian community. South Asian older women were reported to be more comfortable with a female researcher performing study protocol and providing study information (156), and further suggested that the researcher's willingness to have personal discussion with participants was helpful in terms of building trust and ensuring confidentiality (156).

7.4.6 Cultural competence

Knowledge of South Asian cultural norms and tradition have found to be very important and useful while dealing with this population of age group. The researcher from having same background have an advantage of translating all research information to potential participants in a preferred language during face-to-face session and while undertaking recruitment activities. In addition to presence of family members with some participants, the researcher had read out the consent in a preferred language to all participants to ensure their understanding prior to taking written informed consent. Being a South Asian it helped researcher to access this population and facilitate two ways delivery of research. In comparison to the researcher from a different ethnic background, I was more familiar and had better understanding of level of respect and warm approach required to research this target women.

7.5 Conclusion

It is hard to conclude the challenges and strategies needed to adopt to cope those challenges for achieving target recruitment and retention rate in community-dwelling South Asian older adults. However, based on our experience, we found that the researcher belonging to the same cultural background and able to converse freely with participants without the aid of a translator was likely to be the primary factor that enabled recruitment to these studies. We have also found that a very warm approach and motivation can play moderating role in it. These findings are with respect to South Asian ethnic population however, some of the recruitment issues experienced are similar among older adults regardless of ethnicity.

7.6 Future recommendations

Future work should include qualitative semi-structured interviews with participants to understand their responses for non-participation, withdrawal from the study and factors contributing to this. There is also a need to include feasibility studies prior to the development of full trials to quantify recruitment, retention and adherence to such trials. The identification of the successful ways of approaching, recruiting and retaining South Asians in research study is also important because the participants declined from the study may possess a different features and characteristics which left the remaining cohort non-representatives (163).

7.6.1 Site location for conduction of study protocol

The study with this population could be advertised via community centres or religious places, however the delivery of study protocol/assessments should ideally be conducted at a study specific site to reduce time and labour related issues as discussed above. To make arrangement of providing taxis to potential older participants may help in implementation of this approach.

7.6.2 Research facilitator

In the current studies, the researcher was from South Asian background and had ability to speak the same language as the target population. This approach is in line with previous study which has reported the importance of having researcher from same ethnic background, with sensibility of culture and bilingual for the successful way of recruiting and retaining older South Asians (156).

The researcher in these included studies could speak English, Hindi, Punjabi and Urdu. The researcher could also understand Gujarati and Potwari language. The majority of participants recruited to in this study were from “Mirpur” ethnic background which created minor differences

in language, cultural tradition and life-style from Punjabi origin Pakistani women. The main language of speaking in Mirpur is “Potwari” however, the recruited population have capacity to understand and speak Urdu/Punjabi which is main speaking language in Punjab Pakistan. In contrast, the majority Indian participants were Gujarati and Sikh women. The main language of speaking in Gujarati and Sikh women was Marathi and Punjabi however, they had capacity to understand Hindi language well.

It has been observed that having a researcher of same ethnic background can also provide insight of cultural sensitivities of this target population. This population needs motivating with a warm approach and deliverance of knowledge about study project help them to understand and make a decision of taking part in a study. I have also found very useful the researcher to be highly enthusiastic about study to the participants. However, all this together can increase burden on the researcher and hence a research team of more than one member can share work and ensure the delivery of quality of research.

8 Chapter 8: General Discussion

8.1 Main findings in relation to the literature

Vitamin D, a pro-hormone, is primarily obtained from skin exposure to ultraviolet rays of sunlight that catalyses the reaction of 7-dihydrocholesterol in skin to yield pre-vitamin D_3. Pre-vitamin D_3 is subject to three-step hydroxylation to produce biological active form of vitamin D which is known as calcitriol or 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). The first step of hydroxylation in liver produces 25-hydroxyvitamin D (25(OH)D) which is considered a biomarker for circulating blood 25(OH)D concentration. The vitamin D obtained from sunlight is called cholecalciferol however it does naturally present in few food products which is called as ergocalciferol. Both forms are available in supplement form over the counter.

Vitamin D may play a role in many tissues of the body, suggested by the presence of the vitamin D receptor (VDR) in these tissues. The role of vitamin D on muscle function has been my focus. There is a decrease in MHC type IIa and IIx in skeletal muscle fibre area with increasing age which is expressed by decrease in the strengthening and contractile properties of muscle (164). Furthermore, the decrease in myosin content is coupled with decrease in the myofibrillar content, actomyosin interactions, structural changes in actin filaments and the decreasing the power of cross-bridges between the fibres, all have negative influences on muscle strength and function in old age (64) . Vitamin D has been reported to be involved in maintaining skeletal muscle health due to the reaction of $1,25(OH)_2D$ with the VDR present in skeletal muscle tissue through genomic and non-genomic pathways (31) . The complex $1,25(OH)_2D$-VDR is reported to increase oxygen consumption rate of skeletal muscle (33). The reduction in muscle atrophy and improvement in myopathy has also been reported by resolving the vitamin D deficiency (36).

A substantial volume of cross-sectional and intervention trials have been performed to investigate the relationship between vitamin D, skeletal muscle strength and function in women of multi-ethnicity with advance age. The following parameters for muscle strength and function were included and are discussed here: handgrip strength test (kg), single chair stand test (s), repeated chair stand test (s), timed up and go test (s), balance test (s) and walking speed test (s). The investigation of an association between vitamin D, muscle strength and function in South Asian women of increasing age living in the UK was identified as a gap in the literature.

Previous studies with Caucasian women have reported mixed findings depending on the characteristics of the selected population, baseline profile of vitamin D, muscle strength and function, dose and duration of intervention used. This PhD project aimed to explore cross-

sectional relationship between vitamin D, muscle strength and function in UK South Asian women aged =>60 years and casual relationship, through conducting randomised control trial, to investigate the effect of vitamin D supplementation on muscle strength and function in UK South Asian post-menopausal women. Studies are summarised in Table 8.1.

Table 8.1 Summary of studies conducted in this PhD project

Study design and date conducted	**Objective**
Literature review (2017)	To identify evidence reporting an association between vitamin D, muscle strength and function in women of multi-ethnicity ages 60 years and above and to find a gap in a literature in the investigation of this association in UK based community-dwelling South Asian post-menopausal women.
Cross-sectional study (2018)	To explore the relationship between vitamin D, muscle strength and function in UK South Asian women aged 60 years and above.
Randomised control trial (2019)	To investigate the effect of vitamin D supplementation on muscle strength and function in vitamin D inadequate/deficient UK South Asian post-menopausal women.

The two studies and a literature review were conducted between June 2017-November 2019. During this time, a literature review was conducted, ethical approval was obtained for both the cross-sectional study and randomised control trial, the studies were advertised, participants were recruited and screened against the study eligibility criteria and study protocol was delivered.

The main findings from these studies were:

1. The literature review identified a high prevalence of hypovitaminosis D in community-dwelling UK South Asian women in advance age (6, 111) which could have an impact on their muscle strength and functional capacity. This association was explored in the cross-sectional study in which forty seven percent of participants were found to have inadequate/deficient vitamin D status (<50nmol/L). When this data was compared with previous studies in South Asians (6, 111), this suggests that there is higher prevalence

of hypovitaminosis D in South Asian women of this age group living in different regions of the UK. The review also identified that South Asian women aged =>60 years living in the UK were a largely under-researched population.

2. This cross-sectional study confirmed a weak negative association between circulating blood 25(OH)D concentration and lower limb muscle strength (single chair stand and repeated chair stand test) in community-dwelling UK South Asian women aged =>60 years. In summary, the women with sufficient vitamin D status took less time to perform single chair stand and repeated chair stand tests compared with those with inadequate/deficient status. When I compared these findings with previous cross-sectional studies in community-dwelling Caucasian women of this age group, studies reported mixed and inconsistent findings in women of different ethnicity groups with regards to reporting relationship of vitamin D with muscle strength and function. This could be due to high heterogeneity among studies with regards to blood 25(OH)D concentration and the use of different methods of assessments for muscle strength. Only a few studies reported positive associations between vitamin D and repeated chair stand, 30-s chair stand and balance (tandem test) as indicator of lower muscle strength in Dutch and Brazilian women (76, 78)compared to the upper limb muscle strength (handgrip strength) however given the sample size of many of these studies caution should be taken while interpreting them. Studies possess high heterogeneity in their findings and study limitations impose the need for caution while interpreting the findings. The reasons for inconsistencies among studies are listed in Chapter 1.
3. I have used circulating blood 25(OH)D concentration at =>50nmol/L, in line with guidelines of the IOM (2011), for better muscle strength and function in South Asian women. When I compared these findings with the literature, studies have identified different thresholds for better lower limb muscle strength. A nationwide cross-sectional study in Taiwan, has reported a significant association between plasma 25(OH)D concentration of 30-<50nmol/L and performance of 5-times chair stands and success in full tandem stands (balance test) (71). Another cross-sectional and longitudinal design, the Longitudinal Aging Study Amsterdam (LASA), has reported better performance of chair stands and tandem test at serum 25(OH)D concentration >75nmol/L in 1234 Dutch older population (78). Whilst, another longitudinal observational cohort study of duration 2.5 years, has reported a better performance of 5-times repeated chair stand test at 25(OH)D concentration at >80nmol/L in 656 women with aged 74.7 (10.8) years (76). Overall, the identification of circulating blood 25(OH)D concentration in relation to muscle function has found at concentration between 50-100nmol/L (76) with given the optimal dose for muscle

function with target of at least 75nmol/L level (79) which has also been defined by US Endocrine Society (48, 80).

4. In my cross-sectional study, a weak negative association between circulating blood 25(OH)D concentration and muscle function (timed up and go test) was found in UK South Asian women. In summary, women with sufficient vitamin D status performed timed up and go test better than those with inadequate/deficient status. When I compared these findings with literature, I found a considerable volume of the literature has reported an association between blood 25(OH)D concentration and timed up and go performance in community-dwelling women aged 60 years and above (71, 73, 79, 128). I have identified better performance of timed up and go test at blood 25(OH)D concentration at =>50nmol/L. A recent meta-analysis of 15 observational studies (12 cross-sectional and 3 longitudinal studies) has reported faster timed up and go performance in participants with vitamin D status >75nmol/L than others (79).
5. The findings of the cross-sectional study suggested null association between circulating blood 25(OH)D concentration and handgrip strength and balance test in community-dwelling UK South Asian women aged =>60 years. When I compared these findings with previous studies in Caucasian women of multi-ethnicity, the majority of cross-sectional studies have reported no association between vitamin D and handgrip strength (71, 73-76). However, some women in this cross-sectional study were found to have lower handgrip strength than it should be in this age group as estimated by recently revised findings of the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) (71, 77, 127). The presence of low handgrip strength in this cross-sectional study may be explained by loss of muscle or by co-morbidities such as arthritis.
6. In this cross-sectional study, forty one percent of South Asian women were using vitamin D supplementation at the time of recruitment. The use of vitamin D supplementation was found to be directly associated with having sufficient vitamin D status (=>50nmol/L) in this study as eighty six percent women who were users of vitamin D supplementation at time of recruitment had possessed sufficient vitamin D status compared to the non-users. These findings suggest the benefit of using vitamin D supplementation to maintain sufficient vitamin D status in highly risk deficient known population and having better lower limb muscle strength and functional performance. There is no previous cross-sectional data reporting the use of supplementation in this population to compare our findings with. However, the UK Biobank data has reported that only 22% Bangladeshi, 32% Indian and 25% included Pakistani participants were

found to be using vitamin D supplementation that had reflection on their low vitamin D level in included population (110) .

7. In this cross-sectional study, there was a significant difference in the vitamin D supplement use between women of Sheffield and Greater Manchester at the time of recruitment. In summary, the majority included women from Sheffield were found to be using vitamin D supplementation at time of recruitment (57%) compared to women enrolled from the Greater Manchester region (43%). A previous cross-sectional study has reported variation in the prescribing and the consumption of vitamin D supplementation in different postcode regions in the UK (129). This study has further reported the difference in prescribing the dose of vitamin D supplementation according to the region. Based on the link between sufficient vitamin D status and the use of vitamin D supplementation, this may be possible that the areas where the practice of prescribing vitamin D supplementation to women in advance age is not implemented, these areas could be at the risk of hypovitaminosis D.
8. In this cross-sectional study, no association between circulating blood 25(OH)D concentration and self-reported physical activity (using IPAQ-short form) of UK South Asian women aged $\geqslant 60$ years was found. This study has further found a high prevalence of low level self-reported physical activity status in this population as 80% women were found to be less active (energy expenditure of <270kcal per week) against DoH physical activity guidelines (139). Furthermore, we found a significant weak negative relationship between self-reported physical activity and increasing age which could interpret that the women became less active with increasing age. When I compared these findings with literature, we have found similar results as a recent cross-sectional analysis of PROPELS trial has also reported South Asians to be less active versus Caucasians (p=0.001). They further reported gender differences among South Asians with regards to involving in moderate-to-vigorous activities given that women were less active than men although women were found to stand more and spend less time sitting (112).
9. The recruitment of UK South Asian community-dwelling women aged 60 years and above in cross-sectional study was best achieved through initial face to face information session with women at community centres, gurdwara and mandir in which the study details were delivered in a preferred language (English, Hindi, Urdu and Punjabi) and an invitation to take part in a study was given to women. This initial contact with women proved to be useful to build rapport and trust with me. A researcher from same ethnic background able to speak these languages and have cultural knowledge also aided with recruitment and working with this community. A previous

national longitudinal study with South Asian older people also has reported the knowledge of cultural norms and researcher with bilingual skills as potential facilitators to recruitment (156). However, this population was interested in listening and taking part in a research study if knowledge/importance of study was delivered and a warm approach in a respectful manner was used. Overall, I have found this population required motivation to take part in a study. I also have found researcher being a good listener, patience, friendly nature and to communicate well with this population for achieving successful rate of recruitment. From researcher perspective, I have found researching South Asian women of this age group was labour and time intensive. South Asian community centres were found to be appropriate place for approaching and recruiting participants compared to the religious places (particularly mosque) as women have very tight schedule there with a complete focus on the religious activities session.

10. Following on from the findings of our cross-sectional study, the randomised placebo control trial was designed for community-dwelling UK South Asian older women with vitamin D inadequate/deficient status to investigate the effect of 12-weeks vitamin D supplementation course of 3000IU/day on muscle strength and function. As the cross-sectional study showed that the majority vitamin D supplement user women possessed sufficient vitamin D status we decided to approach women who were non-user of vitamin D supplement to be screened with the intention of recruiting women with inadequate/deficient vitamin D status to meet the eligibility criteria of the intervention study. The sample size of seventy women was estimated for intervention and by adopting this approach we have screened one hundred and thirteen women to achieve sample size of 70 women with inadequate/deficient status to be entered onto intervention study.
11. The 12-week randomised control trial with vitamin D supplementation of dose 3000IU/day increased circulating blood 25(OH)D concentration significantly at the end of intervention. This trial found no beneficial effect of vitamin D supplementation, upon vitamin D repletion, on handgrip strength, single chair stand, repeated chair stand, balance test, timed up and go and walking speed performance at 12-weeks compared from baseline in UK based community-dwelling South Asian post-menopausal women. These findings are in agreement with a recent meta-analysis summarising 29 randomised control trials that reported no post-course improvement in muscle strength and function with administration of vitamin D supplementation with various doses and duration in community-dwelling Caucasian post-menopausal women too (1). The detailed analysis of this meta -analysis is given in the section 1.3.2 of this thesis. Only

six studies in that meta-analysis had found a beneficial effect of administrating vitamin D supplementation in improving muscle strength and function from baseline in community dwelling post-menopausal women. We further scrutinised the study designs of those six studies and they have used medium dose of vitamin D with duration of 3-4 months to see remedial effects of vitamin D.

12. The findings of our RCT suggests that vitamin D did not favour muscle strength and function in post-menopausal South Asian women. However, as per the evidence of presence of VDR in number of human tissues, vitamin D may have overall beneficial effects to human health and awareness, upon sufficient circulating blood 25(OH)D concentration, rather than just improving muscle strength and function parameters included in this PhD project.
13. Finally, the experience I gained from the cross-sectional study was applied to recruit South Asian post-menopausal women to the RCT. For RCT, we performed recruitment in Greater Manchester area as majority population living there were found to be non-users of supplementation/deficient in cross-sectional study. Same approach was adapted, as in cross-sectional study, to contact recruitment sites. The study did not rely advertisement through leaflets as lesson learnt from previous study. The maximum recruitment was achieved through arranging direct face to face session with potential women which I found very effective method for successful rate of recruitment in both studies. The follow up telephone calls to women were found to be effective for achieving sustainability of the women to the study. The compliance to study period was 81% (i.e. 57/70 number of participants completed intervention study) which is an achievement when considering challenges to recruit and retain this population to RCT.

8.2 Integrated critical discussion of this PhD

This PhD has identified a high prevalence of vitamin D deficiency in UK South Asian older women. This observation agrees with literature (6, 111). The hypovitaminosis D in this population could be due to various possible confounding factors such as, but not limited to increasing age (7), darker skin pigmentation (8), covering most of the body area and limited outdoor activities which could possibly translate to less exposure to UVB rays of sunlight to synthesize vitamin D. The hypovitaminosis D could also be better understood in the light of our findings around high prevalence of low physical activity in included women (Chapter 5). As South Asian older women were found to have limited involvement in physical activity this could have possible contribution to their low circulating 25(OH)D concentration. This is also suggested in SACN report of 2016 that limited outdoor activities could be an indication of vitamin D deficiency (46). Our findings of low physical activity are

in line with previous studies with South Asians (112). We have also observed an association between increasing age and decreasing physical activity level, this is also supported by previous research (113) which reported generational difference for physical activity engagement in this population as first-generation migrants were reported to be least involved. The reason for low physical activity in this population could be due to cultural reasons, lifestyle or could be presence of high body mass index (Chapter 4). However, the distribution of body mass index was same across all women independent of physical activity and the majority had a high body mass index. Finally, an explanation for the low self-reported physical activity could result from the use of the International Physical Activity Questionnaire (IPAQ) which may not be a suitable choice for this population and may correct by its use with an objective measure such as pedometer or accelerometer to obtain more robust and accurate results.

Considering the results of this research the most reliable way to achieve sufficient level of vitamin D in this population is likely to be through food fortification or supplementation. Dietary intake of the women was measured as part of cross-sectional study (Chapter 4) through using multiple-pass 24-hour diet recall method. However, intakes were found to be underreported. This could cause by the participants as this need women to recall information of a previous day however this tool is still better suitable for this population versus others such as Food Frequency Questionnaire. We also know from literature the low vitamin D intake through food in this population as UK Biobank findings reported mean vitamin D intake through food was 1-3ug/d in UK South Asians aged 18-64 years (110). As there are only limited foods naturally rich in vitamin D such as oily fish, cod liver oil, eggs, and meat. The low vitamin D dietary intake in South Asians may reflect less consumption of fish, meat or even dairy products with increasing age based on the assumptions that they may have contradiction with hypertension which is prevalent comorbidity in this community (Table 4.1). Moreover, some Indian women (Gujarati) are vegans which restrict their dairy products intake and hence can cause decrease vitamin D intake through food. Dietary vitamin D intake seems to be least beneficial route for this population to increase circulating 25(OH)D levels.

The third and only apparently effective route for increasing circulating levels in this population is through vitamin D supplementation. We have tested this in our studies and found a relationship between having sufficient vitamin D status and the use of vitamin D supplementation as the women who had vitamin D intake were found to have sufficient vitamin D status compared to non-users (Chapter 4 and Chapter 5). However, we have found the consumption of vitamin D supplementation was not the same between two

regions (Sheffield and Greater Manchester) as large community recruited from Sheffield in cross-sectional study were using supplementation at the time of recruitment versus women from Greater Manchester area. These findings are in line with previous research which has reported variation in the prescribing and dose for vitamin D supplementation (129). This difference may reflect local practice and procedures as some GPs may not recommend vitamin D supplements on prescription given vitamin D as being dietary nutrient rather medicine and hence the geographical difference is. As through this PhD we now know positive impact of using vitamin D supplementation in increasing circulating 25(OH)D concentration in South Asian older women. In line with our and UK Biobank findings evidencing low trends of using supplementation in UK South Asians a future work is warranted to address this as deficiency may have long term detrimental effect on overall health of this community.

Regarding the relationship between blood 25(OH)D concentration and muscle strength and function in UK South Asian older women, we have not found consistency from this perspective as we did find weak relationship in cross-sectional study, however no causal relationship was found in the RCT. The exception is handgrip strength, as no apparent association was found between blood 25(OH)D concentration and handgrip strength of women (Figure 4.2) in cross-sectional study (Chapter 4). These findings however are in line with literature (71, 73). The reason for this is unknown however based on our findings we can anticipate that presence of arthritis (70% women) might pose negative effects on performing handgrip strength test and might have not allowed to capture accurate capacity. Therefore, the women in this study were found to have low handgrip strength than it should be as suggested by the European Group on Sarcopenia in Older People 2 (EWGSOP2) in this age group (76, 77). We're unsure, unless if tested, the use of an alternative muscle strength assessment methods such as isometric and isokinetic muscle testing may give different (actual) strength of upper limb and may alter its relationship with circulating level accordingly. Previous studies did report different results as per assessment tool so this could be an option here.

A cross-sectional association was found between having sufficient vitamin D status and better lower limb muscle strength and function (single chair stand, repeated chair stand and timed up and go test performance) (Figure 4.2). These findings are supported by previous studies as some reported positive relationship between vitamin D and lower limb muscle strength (76, 78) while others not (71, 73, 74). The difference in findings among previous studies could be due to the selection of various tests to define muscle strength.

In contrast to cross-sectional findings, we didn't find beneficial effect of 3000IU/day vitamin D oral spray for 12 weeks in improving muscle strength and function in included women. These findings are in line with previous research with community dwelling women which has used 2800IU/day for the same duration but reported no improvement in muscle strength and function in response to repletion (148). This null causal association could be due to selection of this dose or duration. As the recent meta-analysis (1) of 29 RCTs including community dwelling post-menopausal women has summarised and suggested administration of medium dose of vitamin D administration on daily basis for 3-4 months could have beneficial role in improving skeletal muscle health. However only six studies in this meta-analysis have reported beneficial role of vitamin D supplementation in increasing handgrip strength, chair rising test (number of repetitions), five times sit to stand test and timed and go test). This suggestion is also in line with IOM recommendation of using 800IU/day for better muscle strength (2).

As outlined above dietary difference within Indian and Pakistani women, a difference in their muscle strength and function performance was also found at baseline and at 12 weeks in RCT. The subgroup difference within South Asians has previously been highlighted in the literature as this possibly a reflection of cultural, diet and lifestyle difference between two groups. There are further subgroups within Indian population such as Gujarati and Sikh, both possess some difference in their diet and lifestyle. Therefore, researching each group separately should be considered in future studies as it may help in better understanding of this relationship specifically to that population. This may also help in understanding where the difference occurs in relation to their lifestyle and dietary patterns and will also help in sustaining the integrity of sample size.

In summary, effort has been made to understand the role of vitamin D in skeletal muscle strength and function in UK based older South Asian women however uncertainty remains due to lack of robust evidence and a need of further investigation. There was only one previous RCT that assessed the role of vitamin D on muscle strength and function in South Asians however the sample size was too small to interpret their findings (117). Therefore, the studies designs were based on pragmatic approach to what could possibly be achieved through researching this population as part of this PhD. The findings from this PhD can be utilised to better design future studies with this community to understand chemistry of vitamin D with skeletal muscle health.

8.3 Recommendations and future studies

8.3.1 Recommendations from this PhD

The results of this study can be used to design future studies with South Asian population living in the UK.

1. Based on these findings, it may be valuable to establish a prospective cohort study with South Asian older women to study their characteristics, lifestyle, diet, indoor and outdoor activities, socio-economic status, work and education, health and medical history, family size, research interest, factors acting as barriers and facilitators to them of taking part in research studies and biochemical profile of micronutrients. This would help future researchers gain knowledge of this population and help them in adopting right approach to researching them. This also would help to understand and evaluate confounding factors effecting nutritional status in this population and help identify research priorities. The subgroup analysis would be helpful to identify and explore any region-wise difference in outcome variables and address them accordingly in future research studies. By conducting two studies with this community, I have observed that having a cultural knowledge of this population is very important and useful to research them. This design would require recruiting this population and could estimate sample size based on power calculations from existing studies of reporting barriers and facilitators to recruit South Asians and vitamin D intake (110, 156) and by incorporating recommendations from this PhD project.
2. We have conducted single 24-hour diet recall interview by using multiple pass method to collect data on the dietary intake of this population. We have found that dietary intake was under-reported. Therefore, the collection of dietary intake in this population would be useful to understand dietary patterns as health determinant and its contribution to biochemical status and reference nutrient intakes in future studies.
3. As we have found a higher prevalence of low physical activity in the participants which could contribute to their hypovitaminosis status, high BMI and overall health and well-being. Therefore, future studies should focus on increasing physical activity in the population and may highlight de-motivating and motivating determinants to be targeted. The current physical activity guidelines strategies can be adapted in this low active population to evaluate whether these guidelines can be effectively applied to ethnic minorities. Women recall and estimated the time of involving in physical activities during last 7 days. This method was highly dependent on their memory and had high chance of missing reporting any physical activity in which participants been involved.

There should be use of accelerometer to have accurate assessment of physical activity status of this population.

4. It is recommended to design prospective longitudinal study to investigate the dose response relationship of 10µg/day and 20µg/day vitamin D supplementation (as per SACN and IOM recommendations) in improving muscle strength and function in this target population. It will also help to determine optimal levels in relation to vitamin D intake throughout year and corresponding muscle strength and function and overall general health benefits. It will also help determine whether 10µg/day vitamin D intake is sufficient to meet the requirements in this population.

8.4 Studies strengths and limitations

8.4.1 Strengths

The literature has revealed that the relationship between vitamin D, muscle strength and function was not known in UK based South Asian (Indian and Pakistani) community dwelling women aged ≥60 years though the presence of vitamin D deficiency has been reported in this population (6, 111). The cross-sectional design of the study was important to investigate whether a relationship existed between vitamin D, muscle strength and function in UK South Asian women aged ≥60 years. The design of this study has allowed me to produce quantitative data to analyse this relationship between study outcomes and vitamin D status of this population. The inclusion criteria of study participants in this cross-sectional study provided a broader picture of study population. The inclusion of user and non-user of vitamin D supplementation has allowed me to analyse the relationship between vitamin D intake and circulating blood 25(OH)D concentration in this study population. It also has allowed me to observe the benefits of using vitamin D supplementation as 86% of user of vitamin D supplementation had sufficient vitamin D status compared to those who were non-users. The inclusion of users and non-users of vitamin D supplementation has provided information of having better lower limb muscle strength and function with sufficient vitamin D status compared to with of inadequate/deficient. The use of vitamin D supplementation and hence prevalence of vitamin D inadequacy/deficiency in this cross-sectional study has found to be varied between women from Sheffield and Greater Manchester, therefore these findings will be useful to select the region for potential participants in future intervention studies with this study population. A previous cross-sectional study also has reported the variation in postcodes for prescribing vitamin D supplementation (129).

Another strength of the cross-sectional study was the inclusion of assessment of physical activity of study population by using self-reported method through International Physical

Activity Questionnaire (IPAQ)-short form. The use of IPAQ has validated previously in older Australian population aged 60 years and above (165). Another strength is the inclusion of assessment of dietary intake of study population by using single 24-hour diet recall multiple pass method. The use of single 24-hour diet recall for dietary intake assessment was appropriate against other methods of assessments in considering the study population particulars and target age group.

A major strength of this cross-sectional study was the recruitment of South Asian (Indian and Pakistani) women aged ≥60 years. I recruited participants from South Asian community centres, gurdwara and mandir rather than approaching a GP registry in order to meet the inclusion criteria of including community-dwelling women only in this study. This approach enabled to prevent contamination of sample size with those with particular underlying condition or disease or receiving any particular treatment. While this may make recruitment strategy more labour and time intensive and unpredictable in terms from response from participants. However, in the long run I expect this approach of participants recruitment is unbiased and transparent. The inclusion of community-dwelling population in this cross-sectional study has increased the likelihood of generalisability of cross-sectional findings to general public.

The RCT with South Asian post-menopausal women is novel in that it was the first which has investigated the effect of vitamin D supplementation course in improving muscle strength and function in vitamin D deficient/inadequate community dwelling South Asian post-menopausal women. I recruited women with baseline 25(OH)D concentration of <50nmol/L as deficient/inadequate blood 25(OH)D concentration at baseline is more likely to be increased with vitamin D supplementation administration than sufficient levels at baseline (148). In our results of RCT, the baseline deficient/inadequate vitamin D status has increased to sufficient vitamin D status with 12 weeks vitamin D supplementation course of 3000IU/day. In this RCT, we used 12 weeks duration of the vitamin D supplementation course with women having baseline status <50nmol/L. The findings of IOM (2011) has concluded the dose response relationship at baseline serum 25(OH)D levels =<40nmol/L and duration of the supplementation course =<3months (2). The sample size given, in our RCT, at baseline is the largest to provide the snapshot of 25(OH)D concentration, muscle strength and function measurements in this target population. The study design in double-blinded manner is also another strength of this study as neither the researcher nor the participants were aware of the sequence of allocation group for receiving vitamin D or placebo oral spray. Finally, the blood 25(OH)D concentration was measured by gold standard method of Liquid Chromatography Mass Spectrometry (LC-MS) for accurate and precise results ((119, 120).

8.4.2 Limitations

The first limitation is study design of this cross-sectional study as causal relationship could not be inferred. In this cross-sectional study, the right handgrip strength of women was measured only therefore it may possible that some women might have left hand as a dominant hand and could have more strength than right hand therefore, the collected data may not reflect accurate maximal handgrip strength capacity in some women. The dietary intake of this study population has assessed using single 24-hour diet recall however complete dietary analysis for 120 women was abundant to perform due to under-reporting found in the dietary analysis of 37 women which were analysed.

The main limitation in this randomised control trial is that sample size of seventy women was calculated for 80% power to be significant at 0.05 however, due to unexpected high withdrawal rate (18%) in this study population, fifty-seven women completed the intervention study and therefore may influence the power of effect size of post-course findings at 12 weeks. Since there should be some extra number of recruits to allow dropouts and secure significance of effect size however due to the challenges encountered in recruiting this study population this was not possible. The final sample size who has completed the intervention study and included in analysis is small therefore while interpreting to generalise the findings of this study to general public should be undertaken carefully and may require investigation on bigger sample size.

Another limitation in this RCT is that vitamin D intake through UVB source of sunlight was not measured which might have contribution to total change in circulating blood 25(OH)D concentration at 12 weeks. However given this study population, previous study has reported higher skin pigmentation influencing negatively the cutaneous synthesis of vitamin D through skin exposure to UVB rays of sunlight (3). However, the skin tone was not measured in this study population. Another study has measured seasonal change in serum 25(OH)D concentration in UK South Asians and found this population to be vitamin D deficient throughout the year including summer season (6). Another study has reported limited outdoor activities in UK South Asians which showed sun avoidance behaviour in this population (123). The vitamin D intake through diet in this study population was not measured. However, as per our cross-sectional findings the analysis was abandoned due to under-reporting. The findings of UK biobank data has also reported vitamin D intake through food in this population was 1.0-3.0µg per day (110). Lastly, this randomised control trial has used single dose of vitamin D supplementation only for intervention therefore dose response relationship could not be obtained. The outcome variables were measured at baseline and exit only therefore rate of change in circulating blood 25(OH)D concentration and its corresponding effect on muscle

strength and function over the time course and underlying chemistry of mechanism involved could not be studied.

This was first study of its kind with this population; future studies with UK South Asians will benefit from our findings to improve study design by minimising confounding factors with the assistance of determinants of influencing circulating blood 25(OH)D concentration, muscle strength and function.

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Appendix a) Leaflets of both studies

The University Of Sheffield.

South Asian women

(Pakistani, Bengali and Indians)

Aged 60 years and above

The Human Nutrition Unit at the University of Sheffield is looking to recruit 120 South Asian women to take part in a research project investigating vitamin D, muscle strength and physical function.

The study involves meeting with the researcher Sabeen Zahra, On 2 occasions in your home or at a location convenient to you.

On completion of the study volunteers will have chance to win a £100, £75, £50 or £25 high street voucher.

vidisa@sheffield.ac.uk
Tel: 07925034704

vidisa@sheffield.ac.uk Tel: 07925034704 (repeated)

The University Of Sheffield.

South Asian women

(Pakistani and Indian)

Aged 60-75 years

Researchers at The University of Sheffield are looking to recruit South Asian women to a study investigating the effect of vitamin D supplementation on muscle strength and function.

The study involves meeting with the researcher, Sabeen Zahra, on 3 occasions at a community centre or location convenient to you.

Volunteers will receive a £30 high street voucher and a free supply of vitamin D supplements on completion.

For more information please contact Sabeen Zahra on email: vidisa@sheffield.ac.uk; Telephone: 07925034704

vidisa@sheffield.ac.uk
Tel: 07925034704

vidisa@sheffield.ac.uk Tel: 07925034704 (repeated)

جنوبی ایشیائی عورتیں

(پاکستانی اور انڈینز)

کی ریسرچ پراجیکٹ میں ضرورت ہے

عمر 60 - 75 سال

The University Of Sheffield.

یونیورسٹی آف شیفیلڈ کے ریسرچرز جنوبی ایشیائی عورتوں کو اس ریسرچ پراجیکٹ میں حصہ لینے کی دعوت دیتے ہیں جس میں وٹامن ڈی کا پٹھوں کی طاقت اور کارکردگی پر اثر چیک کیا جائے گا۔

اس پراجیکٹ میں ریسرچر سبین زہرا سے کمیونٹی سنٹر یا آپکی سہولت کے لیے کسی اور جگہ پر 3 دفعہ ملاقات شامل ہے۔

پراجیکٹ کے آخر میں حصہ لینے والوں کو 30 پاؤنڈ کے واؤچر اور ایک فری سپلائی فراہم کی جائے گی۔

مذید معلومات کے لیے سبین زہرا سے رابطہ کریں:

ٹیلیفون: 07925034704; ای میل: vidisa@sheffield.ac.uk

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Appendix b) Participant information sheets for cross-sectional study and RCT

Participant Information Sheet

The University Of Sheffield.

Study name: vidisa

Vitamin D, muscle strength and physical function in UK South Asian older women

You are being invited to take part in a research project. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please free to ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the project's purpose?

Vitamin D plays an important role in musculoskeletal health. Little research has been done in the South Asian community but there is some evidence of low vitamin D in UK South Asian older women. Public Health England (PHE) has categorised the UK South Asian population to be a at risk group for low vitamin D status. PHE recommends consumption (through diet or supplements) of 10µg of vitamin D each day. We plan to recruit 120 South Asian women aged 60 years and above living in the UK to look at the link between vitamin D levels in the blood and examine the relationship with muscle strength and physical function.

Why have I been chosen?

All South Asian women aged 60 years and above are eligible to take part in the research. Participants will be community-dwelling. People who live in care homes or already using vitamin D supplement will not eligible to take part.

Do I have to take part?

No. It is entirely up to you whether you take part or not. If you do decide to take part you can withdraw anytime during the research without giving any reason. If you decide to withdraw the data already collected from you may be used for analysis purposes.

What will happen to me if I take part?

If you decide to take part in the research you will be given a consent form to sign. A copy of participant information sheet and signed consent form will be given to you.

The study will involve two meetings with the researcher. At the first meeting we will ask you some general questions about your health and medical history. We will then measure your weight, height, waist and upper arm circumference. You will be asked to give a small finger prick blood sample using blood spot test kit that will be used to check your vitamin D level. Then, we will check your muscle strength [upper body strength (handgrip strength with dynamometer) and lower body strength (chair stand test)] and physical function level (balance test and timed up and go test). We will ask you questions in order to access your diet. This involves asking you to report everything you have eaten in the previous 24 hours. Finally, we will ask you questions of your physical activity. The second meeting will consist of repeat diet interview only.

What do I have to do?

You are free to live daily normal life as this is observational research and will not involve any intervention.

What are the possible disadvantages and risks of taking part?

Taking a finger prick blood sample may cause you some anxiety but the researcher will guide through the process. A finger prick causes a brief needle pain but this is short-lived and has no long term harm. Short physical performance battery tests; timed up and go (to measure the time you rise from the chair, walk three meters, turn around, walk back and sit down on chair), balance test (you will ask to stand on both feet with different positions without support for ten seconds) and chair stand test (you will ask to tie your both arms across your chest and lift up from chair) involve mild physical activity and may cause some tiredness. Physical performance tests are safe and commonly used in previous research studies for accessing muscle strength and physical function. The researcher will be there to give instructions before starting the test and support you during the procedure. The test will be stopped immediately if you feel discomfort. We will perform these tests at your home for your convenience and the time duration will be 60-90 minutes.

What are the possible benefits of taking part?

One benefit of taking part in the study is that you will be informed of your vitamin D level. You can expect these results 2-3 weeks after the 1st meeting. Physical performance tests will make you aware of your muscle strength and functional ability. In addition, participants will be eligible for inclusion in a prize draw of £100, £75, £50 and £25 that will take place at the

Elizabeth Williams (e.a.williams@sheffield.ac.uk).

Bernard Corfe (b.m.corfe@sheffield.ac.uk).

Telephone: 07925034704

Participants will be given a copy of this information sheet and a copy of their signed consent form.

Thank you for reading this information sheet.

The University Of Sheffield.

Research Project Title

The effect of Vitamin D on muscle strength and physical function in UK South Asian women.

'You are being invited to take part in a research project. Before you decide whether or not to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.'

1. What is the project's purpose?

Vitamin D is an important nutrient associated with bone health and thought to be important for muscle function. Vitamin D comes from certain foods and can be made by the body through sunlight exposure on the skin. It is also available in some nutritional supplements. Vitamin D status in the UK population is poor and the South Asian population is at high risk of deficiency due to limited sunlight exposure. Because of this Public Health England now recommends everyone over the age of 4 years consumes 10µg vitamin D (from food and supplements) per day.

A number of studies have shown a beneficial effect of vitamin D supplementation on strength and physical function in Caucasian post-menopausal women. However, whether vitamin D also benefits post-menopausal South Asian women is unknown. This study aims to investigate the effect of vitamin D supplementation for 12 weeks on muscle strength and function in South Asian women aged 60-75 living in the UK.

2. Why have I been chosen?

Community living UK South Asian women aged 60-75 years are eligible to take part in this study. If you are a recent/current user of vitamin D supplement, cod liver oil, omega-3 or multivitamins then you are not eligible for this study. We are looking to recruit a total of 70 women from the South Asian community to take part in this study.

3. Do I have to take part?

No. Taking part in this project is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep (and be asked to sign a written consent form). You can still withdraw at any time without any negative consequences and you do not have to give a reason.

If you wish to withdraw from the research, please contact lead researcher Sabeen Zahra at email address: vidisa@sheffield.ac.uk . If you do withdraw from the study any data that we have collected from you may still be used in an anonymised manner.

What will happen to me if I take part? What do I have to do?

The purpose of this project is to investigate the effects of 12 weeks vitamin D supplementation on muscle strength and physical function in UK South Asian post-menopausal women.

There will be three meetings with the researcher in total. In first visit, your eligibility for this study will be checked and you will be asked to sign a written consent form. Your finger-prick blood spot for vitamin D measurement and assessments for muscle strength and function will be performed. Blood vitamin D level will be measured using the finger-prick blood spot kits and the report will come after 7-8 days.

Only those participants who will have low vitamin D level (below 50nmol/L) will be entered into intervention study (second stage). Any participant found to have an adequate vitamin D level (equal to or above 50nmol/L) will be excluded and will not be entered onto the intervention study. This is because this vitamin D level is thought to be adequate for health.

In the second stage i.e., within 2 weeks of first visit, all eligible participants will be randomized into two groups to receive either 75µg vitamin D oral spray or placebo oral spray once on daily basis for 12 weeks. Neither the participant nor researcher will know which participant is taking the oral vitamin D or placebo oral spray. At this visit we will also ask you some simple questions about your diet and about nutrition.

The vitamin D oral spray is suitable to use for vegetarians however it might not be suitable for strict vegans.

In the third stage i.e., after 12 weeks of being on the supplement you will be invited back to the community centre or temple for your 3rd and final meeting with the researcher. You will be asked for a finger-prick blood sample so your vitamin D status can be checked again and we will repeat the measurements we made at the 1st visit. Below is the flow diagram to understand the study procedure.

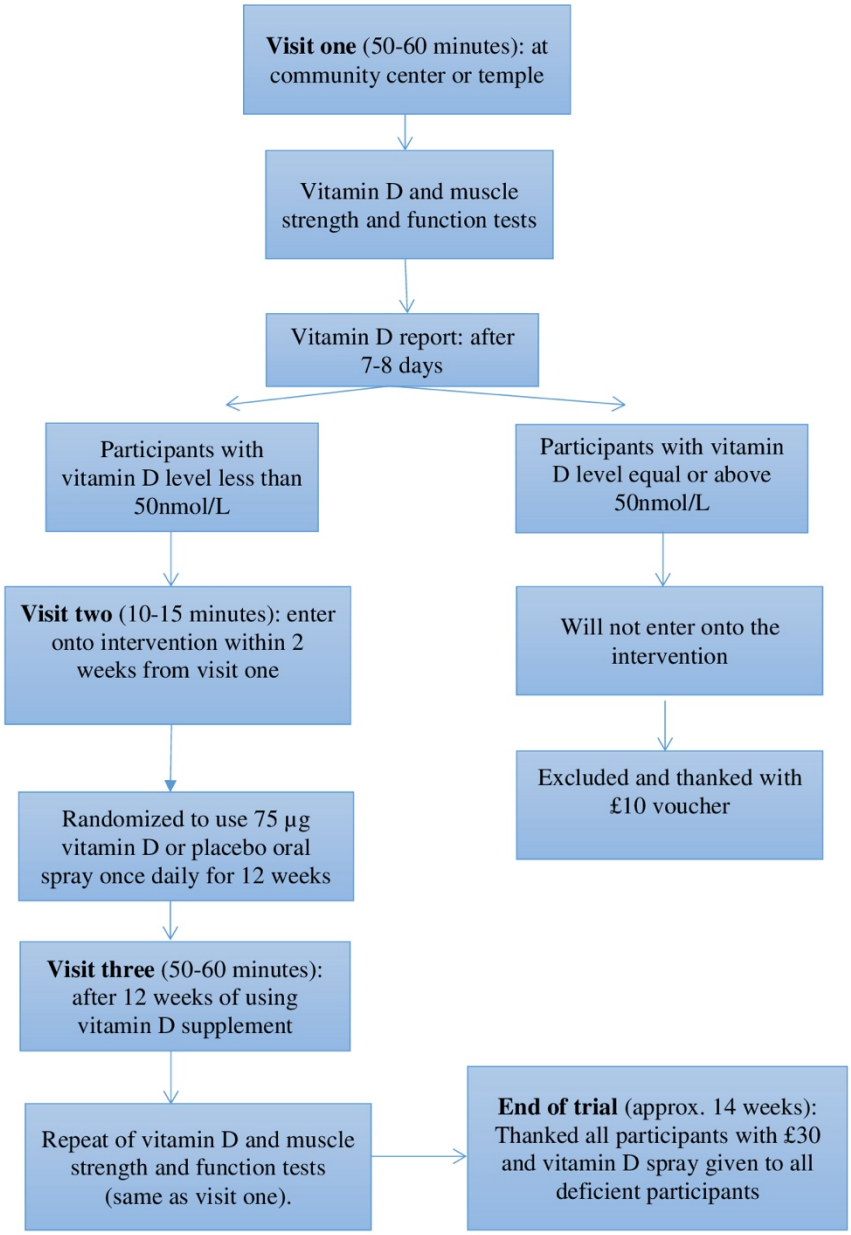

Visit one (50-60 minutes): at community center or temple
Vitamin D and muscle strength and function tests
Vitamin D report: after 7-8 days
Participants with vitamin D level less than 50nmol/L
Participants with vitamin D level equal or above 50nmol/L
Visit two (10-15 minutes): enter onto intervention within 2 weeks from visit one
Will not enter onto the intervention
Excluded and thanked with £10 voucher
Randomized to use 75 µg vitamin D or placebo oral spray once daily for 12 weeks
Visit three (50-60 minutes): after 12 weeks of using vitamin D supplement
Repeat of vitamin D and muscle strength and function tests (same as visit one).
End of trial (approx. 14 weeks): Thanked all participants with £30 and vitamin D spray given to all deficient participants

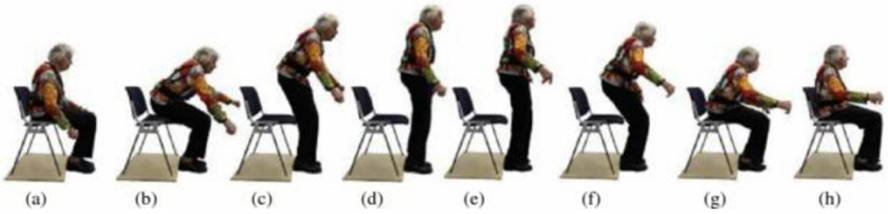
Figure1. Study procedure and timeline.

The following assessments will be conducted at the start and end of the study to assess muscle strength and function. These include: general interview (demographic, and health history), single chair stand test (s), repeated chair stand test (s), hand grip strength test (kg), timed up and go test (s), walking speed (s), balance test (s), some simple questions about physical activity level and finger-prick blood sample for checking vitamin D level in the body. The researcher will demonstrate each of the assessments in advance and you can refuse to do any that you don't feel confident doing.

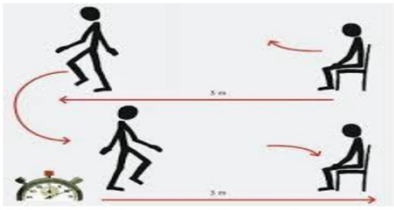
1) Vitamin D blood test kits will be used to take finger prick blood sample for checking vitamin D level in the blood. You may do it yourself or researcher will do for you. It is a quicker method (just like checking blood sugar at home) and causing little discomfort.

2) Handgrip strength tests is an easy task where you will be asked to hold an instrument tightly in your hand (as shown in picture) for few seconds and the researcher will note down your strength in both hands.

3) Chair stand test (s) is a simple exercise to assess strength in the legs. You will be asked to sit down on a comfortable normal chair with arms folded around chest, stand up without assistance and then sit down properly on a same chair. The researcher will note a time (using stopwatch) you will take to perform it. The repeated chair stand test (s) is about performing this test five times continuously.

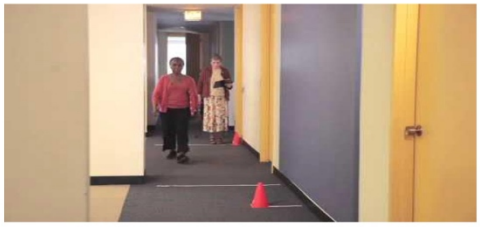


4) In the timed up and go test (s), you will be asked to stand up from a chair, walk straight in a room for a 3 metres distance at a normal speed, turn around, walk back in a same way and sit down on a chair. Researcher will note a time you will take using a stopwatch.



5) In the balance test, you will be asked to maintain your balance in three different positions (as shown below) for 10 seconds. Checking balance is important to prevent fall and vitamin D plays important role to maintain it.

6) In walking speed, you will be asked to walk straight in a room with normal speed for 6 metres. The researcher will be there for any assistance and will note a time you will take using stopwatch.



7) Height, weight and body fat will be measured using scales, a height measure and a body fat monitor.

The researcher will be with you throughout the time for any assistance and will perform each test herself in front of you before ask you to perform so. In self-reported physical activity interview, we will ask you few simple questions about your last 7 days physical activity level (for example heavy work, walk and sitting time etc).

Researcher will give you all the instructions in your preferred language (English, Hindi, Punjabi or Urdu). You may contact the researcher at any time via email or the telephone number given below. The researcher will ask your contact number in order to call you during the study period to make sure that you are taking supplement as advised.

At the end of the trial participants who have completed the intervention and have attended all 3 appointments will be reimbursed £30 to thank you for your time and effort. If you are found not to be eligible after visit 1 then you will receive a £10 voucher to thank you for your involvement. All volunteers found to have low vitamin D at the start of the study will be given a supply of vitamin D supplements at the end of study.

4. What are the possible disadvantages and risks of taking part?

You have to use oral vitamin D or placebo spray once a day on daily basis for 12 weeks. The vitamin D spray is commercially available and can be purchased from health food stores and is safe to use. It is very unlikely that you will have any side effects from the vitamin, but if you do have any unpleasant side effects that you think may be related to the supplement then you should stop taking it immediately and contact the researcher.

You will be asked not to use any other vitamin D supplement during this period, any multivitamin that contains vitamin D, liver cod oil or omega-3 capsules. The finger-prick method of taking blood for checking vitamin D may cause little pain but it would be temporary and you may perform it yourself. Physical function tests described above may cause some exertion however they are safe and easy to perform and the researcher will be with you.

5. Is this a medicine and does it involve the NHS?

No. Vitamin D is a dietary nutrient and found in some foods. This project is not about testing a medicine. Taking part in the study does not stop you taking your usual medicine. This study is being entirely run by the University of Sheffield and does not

involve the NHS in anyway. Taking part in this project will not affect your access to NHS or any future medical treatment.

6. What are the possible benefits of taking part?

At the end of the study you will be told your blood vitamin D level and muscle strength (strength in hands and legs) and function level that might not come into routine assessment. If you are entered onto the intervention then you will also be supplied with vitamin D at the end of the study.

7. Will my taking part in this project be kept confidential?

All the information that we collect about you during the course of the research will be kept strictly confidential and will only be accessible to members of the research team (Sabeen Zahra, Dr Elizabeth Williams and Dr Bernard Corfe). The handling of personal data is controlled by the General Data Protection Regulation (GDPR) and associated legislation.

After you sign the written consent form you will be given a unique identification number and all your collected data will belong to that ID number. Your name will be written on two hard copies of consent form. One copy will go with you and we will keep the other copy for our records. We will keep that copy in a locked filing cabinet locker based in Medical School premises at the University of Sheffield.

All collected data will be anonymised. You will not be identified in any reports or publications.

The desktop computers of the University of Sheffield will be used to process and analyse the data and they will be password encrypted. The information collected about you will remain strictly confidential.

8. What is the legal basis for processing my personal data?

In most cases, under the General Data Protection Regulation (applicable in the UK and EU from 25 May 2018) the appropriate legal basis for research purposes will be 'a task in the public interest'.

According to data protection legislation, we are required to inform you that the legal basis we are applying in order to process your personal data is that 'processing is necessary for the performance of a task carried out in the public interest (Article 6(1) e).

As we will be collecting some data that is defined in the legislation as more sensitive (information about contact details, health and medical history), we also need to let

you know that we are applying the following condition in law: that the use of your data is 'necessary for scientific or historical research purposes.

Further information, including details about how and why the University processes your personal information, how we keep your information secure and confidential, and your legal rights (including how to complain if you feel that your personal information has not been handled correctly), can be found in the University's Privacy Notice https://www.sheffield.ac.uk/govern/data-protection/privacy/general.

9. What will happen to the data collected, and the results of the research project?

The data will be used by research team members Sabeen Zahra, Dr Elizabeth Williams and Dr Bernard Corfe for research and analysis purposes only. The results of this research project will be presented in conferences, used in the thesis of Sabeen Zahra and for publication in journal. Your participation in this research will remain strictly confidential and it will not be possible to identify you from any of these reports.

Who is organising and funding the research?

This is a PhD project of Sabeen Zahra, supervised by Dr Elizabeth Williams and Dr Bernard Corfe, senior academic staff members at the Department of Oncology and Metabolism, The University of Sheffield. This is a self-funded project. The vitamin D and placebo oral sprays for this project will be provided by a company named BetterYou Ltd.

10. Who is the Data Controller?

The University of Sheffield will act as the Data Controller for this study. This means that the University is responsible for looking after your information and using it properly.

11. Who has ethically reviewed the project?

This project has been ethically approved by Medical School Ethics Review Committee on the date 05/02/2019. The reference number is 022981.

12. What if something goes wrong and I wish to complain about the research?

If you wish to complain about any aspect of this research then please contact the primary supervisor: Dr Elizabeth Williams at email: e.a.williams@sheffield.ac.uk : Tel: 0114 2159065 or secondary supervisor: Dr Bernard Corfe at email: b.m.corfe@sheffield.ac.uk. However, if you feel that your complaint has not been

handled to satisfaction then you can contact the Head of Oncology and Metabolism department, Dr Allan Pacey at email: A.Pacey@sheffield.ac.uk, who will then escalate the complaint through the appropriate channels.

If the complaint relates to how the participants' personal data has been handled, information about how to raise a complaint can be found in the University's Privacy Notice: https://www.sheffield.ac.uk/govern/data-protection/privacy/general.

13. Contact for further information

If you wish to obtain further information about the project please contact lead researcher:

Sabeen Zahra at email: vidisa@sheffield.ac.uk or telephone 07925034704.

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for reading this information sheet.

Appendix c) Ethics approvals for cross-sectional study and RCT

The University Of Sheffield.

Downloaded: 19/10/2017
Approved: 11/10/2017

Sabeen Zahra
Registration number: 160262724
Oncology
Programme: PhD Human Nutrition

Dear Sabeen

PROJECT TITLE: Vitamin D, muscle strength and physical function in UK South Asian older women
APPLICATION: Reference Number 015586

On behalf of the University ethics reviewers who reviewed your project, I am pleased to inform you that on 11/10/2017 the above-named project was **approved** on ethics grounds, on the basis that you will adhere to the following documentation that you submitted for ethics review:

- University research ethics application form 015586 (dated 26/09/2017).
- Participant information sheet 1035567 version 1 (20/09/2017).
- Participant consent form 1035568 version 2 (26/09/2017).

The following optional amendments were suggested:

1. Data should be anonymised before computer data entry (it sounds like it will be but this wasn't categorically stated). 2. It may be beneficial to make the study documentation available in different languages. This seems pertinent for this study population. The lead reviewer feels that the following points do not need to be addressed for ethical approval but have been conveyed to the researcher as they are things they might consider when writing their literature review and mini-thesis: Will patient recruitment occur only during summer or winter months or both? Has the scientific validity of the study been assessed? e.g. justification of sample size, aims in the context of past research, lack of a control group etc

If during the course of the project you need to deviate significantly from the above-approved documentation please inform me since written approval will be required.

Yours sincerely

Laura Williams
Ethics Administrator
Medical School

The University Of Sheffield.

Downloaded: 01/03/2019
Approved: 05/02/2019

Sabeen Zahra
Registration number: 160262724
Oncology
Programme: PhD human nutrition

Dear Sabeen

PROJECT TITLE: A randomized controlled trial to investigate the effect of vitamin D supplementation on muscle strength and function in UK South Asian post-menopausal women
APPLICATION: Reference Number 022981

On behalf of the University ethics reviewers who reviewed your project, I am pleased to inform you that on 05/02/2019 the above-named project was **approved** on ethics grounds, on the basis that you will adhere to the following documentation that you submitted for ethics review:

- University research ethics application form 022981 (dated 11/01/2019).
- Participant information sheet 1055140 version 1 (11/01/2019).
- Participant consent form 1055141 version 1 (11/01/2019).

If during the course of the project you need to deviate significantly from the above-approved documentation please inform me since written approval will be required.

Yours sincerely

Daniele Swain
Ethics Administrator
Medical School

The University Of Sheffield.

Sabeen Zahra <szahra1@sheffield.ac.uk>

Scope protocol review - Vitamin D and muscle function

Clinical Trial Helpline <ctdhelpline@mhra.gov.uk> 3 January 2019 at 11:35
To: Liz Williams <e.a.williams@sheffield.ac.uk>
Cc: Sabeen Zahra <szahra1@sheffield.ac.uk>, Bernard M Corfe <b.m.corfe@sheffield.ac.uk>

Notification that a Clinical Trial Authorisation (CTA) is not required

Dear Dr Williams

Thank you for your email dated 19 December 2018.

I can confirm that your proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC and no submission to the Clinical Trials Unit at the MHRA is required.

Kind regards

Clinical Trial Helpline

MHRA

Medicines & Healthcare products Regulatory Agency

Your views matter. Please tell us what you think of the service you have received from us by following the link below:
https://www.surveymonkey.com/s/ClinicalTrialHelplineFeedback

From: Liz Williams <e.a.williams@sheffield.ac.uk>
Sent: 19 December 2018 13:46
To: Clinical Trial Helpline <ctdhelpline@mhra.gov.uk>
Cc: 'Sabeen Zahra' <szahra1@sheffield.ac.uk>; 'Bernard M Corfe' <b.m.corfe@sheffield.ac.uk>

[Quoted text hidden]

[Quoted text hidden]

Appendix d) Consent forms for cross-sectional study and RCT

Participant Consent Form

Title of Research Project: Vitamin D, muscle strength and physical function in UK South Asian older women

Name of Researcher: Sabeen Zahra Participant identification number: ____

Participant Identification Number for this project: **Please initial box**

1. I confirm that I have read and understand the information sheet/letter dated 26/09/2017, explaining the above research project and I have had the opportunity to ask questions about the project. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without there being any negative consequences. In addition, should I not wish to answer any particular question or questions, I am free to decline. ☐

3. I understand that my responses will be kept strictly confidential. I give permission for members of the research team to have access to my anonymised responses. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research. ☐

4. I understand that my blood sample will be taken by using blood spot test kit to check the vitamin D status only. Blood test result will be used by research team. ☐

5. I agree to take part in the above research project. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Lead Researcher	Date	Signature

Copies: 2 copies are required: one copy for volunteer and one copy to keep in a file along with corresponding research material in a secure locker.

26/09/17 Version 2

Appendix e) Checklists

The University Of Sheffield.

The effect of Vitamin D on muscle strength and physical function in UK South Asian women.

Consent Form

Participant identification number:

	Please initial box
Taking Part in the Project	
1. I have read and understood the project information sheet dated 10/01/2019 for the above study and have had the opportunity to ask questions.	
2. I agree to take part in the project. I understand that taking part in the project will include being interviewed, measurement of blood vitamin D level, and some exercise tests. The use of 3000IU vitamin D/placebo supplementation for 12 weeks will depend on whether I am found to have a low vitamin D status (below 50nmol/L).	
3. If eligible I agree to consume the supplement/placebo as described in the participant information sheet and understand that neither the researcher not myself will be aware of the treatment allocation.	
4. I understand that my taking part is voluntary and that I can withdraw from the study at any time. I do not have to give any reasons for why I no longer want to take part and there will be no adverse consequences if I choose to withdraw.	
How my information will be used during and after the project	
5. I understand my personal details such as name, phone number, address and email address etc. will not be revealed to people outside the project.	
6. I understand and agree that authorised researchers (Sabeen Zahra, Dr Elizabeth Williams and Bernard Corfe) may use my data in publications, reports, web pages, and other research outputs, only if they agree to preserve the confidentiality of the information as requested in this form.	
7. I give permission for the anonymised data collected from me to be used for future research.	
8. I agree to take part in the above study	

Name of participant	Signature	Date
Name of Researcher	Signature	Date

Project contact details for further information:
Lead researcher: Sabeen Zahra (vidisa@sheffield.ac.uk)

Primary Supervisor: Dr Elizabeth Williams (e.a.williams@sheffield.ac.uk)

Date: 10/01/2019 Version 5

Appendix f) Questionnaires

RCT

Checklist

Participant Eligibility	☐
Signed consent form	☐
General interview	☐
Height	☐
Waist	☐
Weight	☐
Body fat and muscle mass	☐
Hand grip strength	☐
Chair stand test	☐
Balance test	☐
Timed up and go test	☐
Walking speed test	☐
Vitamin D test	☐

General and health related interview

The University Of Sheffield.

Date: _____ Participant number: _____

Contact details: _____ Mobile: _____

Ethnic group

Pakistani ☐

Bangladeshi ☐

Indian ☐

Anthropometric data

Age: _____ years

Height: _____ cms

Weight: _____ kgs

Waist circumference: _____ inches

Muscle mass (mid upper-arm circumference (MUAC): _____ cms

Triceps skin fold (TSF) measurement: _____ mm

Age at which left education _____

Health related interview	**Yes**	**No**
Weight loss in the last 12 months	☐	☐
If yes, how much	☐ kgs	
Do you smoke	☐	☐
No. of cigarettes per day	☐	
Alcohol No. of units per week	☐	☐
Spirit	☐	
Beer	☐	
Wine	☐	

Self-reported exhaustion

Evaluation of two statements of the CES-D scale (criterion positive if at least one condition is present for 3 days or more during the last week):

a) I felt that everything I did was an effort ☐

b) I could not get going ☐

Note: (This self-reported exhaustion criteria is adapted from Fried et al, 2001; CES-D = Centre for Epidemiological Studies Depression)

Medical History	**Yes**	**No**
1) Hypertension	☐	☐
2) Diabetes	☐	☐
Type 1	☐	
Type 2	☐	
Unspecified	☐	
3) Respiratory disease:	☐	☐
Bronchiectasis	☐	
Pulmonary fibrosis	☐	
Asthma	☐	
Other _____		
4) Cardiac disease:	☐	☐
Heart failure	☐	
Ischemic heart disease	☐	
Angina	☐	
Myocardial infarction	☐	
Coronary angioplasty	☐	

Other _____

5) Liver problem ☐ ☐

Hepatitis B ☐

Hepatitis C ☐

Other _____

6) Renal impairment ☐ ☐

Chronic kidney disease ☐

Acute renal failure ☐

Other _____

7) Gastrointestinal problems ☐ ☐

Inflammatory bowel disease ☐

Diverticulitis ☐

Irritable bowel syndrome ☐

Other _____

8) Arthritis ☐ ☐

Generalised osteoarthritis ☐

Rheumatoid osteoarthritis ☐

Unknown ☐

9) Muscular pain (lower back, legs and arms) ☐ ☐

10) Fall ☐ ☐

11) Fracture of upper limbs ☐ ☐

12) Fracture of neck of femur ☐ ☐

13) Cancer (diagnosed in the past 5 years) ☐ ☐

If Yes, year/month when you were diagnosed _____

Please tick which one applies

I have received a diagnosis and am due to start a treatment ☐

I am under active watch and wait but have not started receiving treatment ☐

I am currently receiving treatment ☐

I have finished treatment and am currently being monitored ☐

I am receiving treatment for my symptoms but I am no longer receiving active treatment for my cancer ☐

Type of cancer you were diagnosed with:

Anus	☐	Nasopharynx	☐
Oesophagus-upper	☐	Oesophagus-lower	☐
Bladder	☐	Ovary	☐
Blood, bone marrow and lymph	☐	Pancreas	☐
Bone	☐	Pharynx	☐
Brain	☐	Rectum	☐
Breast	☐	Salivary gland	☐
Endometrium	☐	Skin-melanoma	☐
Gall bladder/bile duct	☐	Skin-non melanoma	☐
Kidney	☐	Stomach	☐
Larynx	☐	Colon	☐
Liver	☐	Lung	☐
Thyroid	☐	Mouth	☐
Tonsil	☐	Muscle	☐

Vitamin D status related variables:

Taking vitamin D supplement ☐ ☐

If Yes, Dose _____ Duration _____

Name _____ Start date _____

Taking multivitamins ☐ ☐

If Yes, Dose _____ Duration _____

Name _____ Start date _____

Taking liver cod oil ☐ ☐

The University Of Sheffield.

General and health related interview

Date: _____ Participant number: _____

Contact details: _____ Telephone: _____

Ethnic group

Pakistani ☐

Indian ☐

Anthropometric data

Age: _____ years; Date of birth: _____

Height: _____ cms

Weight: _____ kgs

Waist circumference: _____ inches

Mid upper-arm circumference (MUAC): Length: ____ cm; Circumference: _____cm

Calf circumference: Length _____ cm; circumference _____ cm

Age at which left education _____

Health related interview	**Yes**	**No**
Weight loss in the last 12 months	☐	☐
If yes, how much	☐ kgs	
Do you smoke	☐	☐
No. of cigarettes per day	☐	
Alcohol No. of units per week	☐	☐
Spirit	☐	
Beer	☐	
Wine	☐	

Self-reported exhaustion

Evaluation of two statements of the CES-D scale (criterion positive if at least one condition is present for 3 days or more during the last week):

a) I felt that everything I did was an effort ☐
b) I could not get going ☐

Note: (This self-reported exhaustion criteria is adapted from Fried et al, 2001; CES-D = Centre for Epidemiological Studies Depression)

Medical History	**Yes**	**No**
1) Hypertension	☐	☐
2) Diabetes	☐	☐
Type 1	☐	
Type 2	☐	
Unspecified	☐	
3) Respiratory disease:	☐	☐
Bronchiectasis	☐	
Pulmonary fibrosis	☐	
Asthma	☐	
Other _____		
4) Cardiac disease:	☐	☐
Heart failure	☐	
Ischemic heart disease	☐	
Angina	☐	
Myocardial infarction	☐	
Coronary angioplasty	☐	
Other _____		

5) Liver problem ☐ ☐

Hepatitis B ☐

Hepatitis C ☐

Other _____

6) Renal impairment ☐ ☐

Chronic kidney disease ☐

Acute renal failure ☐

Other _____

7) Gastrointestinal problems ☐ ☐

Inflammatory bowel disease ☐

Diverticulitis ☐

Irritable bowel syndrome ☐

Other _____

8) Arthritis ☐ ☐

Generalised osteoarthritis ☐

Rheumatoid osteoarthritis ☐

Unknown ☐

9) Muscular pain (lower back, legs and arms) ☐ ☐

10) Fall ☐ ☐

11) Fracture of upper limbs ☐ ☐

12) Fracture of neck of femur ☐ ☐

13) Cancer (diagnosed in the past 5 years) ☐ ☐

If Yes, year/month when you were diagnosed _____

Please tick which one applies

I have received a diagnosis and am due to start a treatment ☐

I am under active watch and wait but have not started receiving treatment ☐

I am currently receiving treatment ☐

I have finished treatment and am currently being monitored ☐

I am receiving treatment for my symptoms but I am no longer receiving active treatment for my cancer ☐

Type of cancer you were diagnosed with:

Anus	☐	Nasopharynx	☐
Oesophagus-upper	☐	Oesophagus-lower	☐
Bladder	☐	Ovary	☐
Blood, bone marrow and lymph	☐	Pancreas	☐
Bone	☐	Pharynx	☐
Brain	☐	Rectum	☐
Breast	☐	Salivary gland	☐
Endometrium	☐	Skin-melanoma	☐
Gall bladder/bile duct	☐	Skin-non melanoma	☐
Kidney	☐	Stomach	☐
Larynx	☐	Colon	☐
Liver	☐	Lung	☐
Thyroid	☐	Mouth	☐
Tonsil	☐	Muscle	☐

Vitamin D status related variables:

Taking vitamin D supplement ☐ ☐

If Yes, Dose _____ Duration _____

Name _____ Start date _____

Taking multivitamins ☐ ☐

If Yes, Dose _____ Duration _____

Name _____ Start date _____

Taking liver cod oil ☐ ☐

Taking omega-3 capsules ☐ ☐

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

Participant identification number: _____

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Single 24-hour diet recall vidisa study Participant number _____

Time	Quick list of food	Description	Portion size	Portion code

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Dear Participant

Thank you for taking part in a research study looking at vitamin D and muscle function. You may remember that as part of the study a finger prick blood sample was taken for the analysis of vitamin D status. Your blood sample has now been analysed and a report is enclosed.
The normal level of vitamin D is considered as 50nmol/L or above. You may take this report to your GP for further guidance.

There is a £10 high street voucher enclosed for a thanks towards your valuable time and effort.

Thanks once again for your participation in this study. We hope that this study gave you the better understanding of the importance of vitamin D.
Yours sincerely,
Sabeen Zahra

VIDISA study team

The Queen's Anniversary Prizes
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Appendix g) Further analysis from cross-sectional study

Table 1. Muscle strength and function across SACN vitamin D status cut-off points

	SACN vitamin D status guideline		
Variables [mean (SD)]	<25nmol/L	≥25nmol/L	*p* value
Vitamin D status (nmol/L)	17.3 (4.7)*	70.78 (33.5)	0.001
Handgrip strength (kg)	18.5 (4.6)	18.8 (4.5)	0.296
Single chair stand (s)	4.5 (1.9)	4.2 (1.6)	0.730
Repeated chair stand (s)	25.1 (9.9)	22.4 (7.5)	0.165
Timed up and go (s)	15.6 (6.3)	14.5 (6.1)	0.216

Mann-Whitney U test.

**Means are outside the brackets and standard deviation are in brackets.*

***The significance level is <0.05.*

Table 2. Muscle strength and function according to vitamin D (nmol/L) quintiles

Vitamin D status	*0-24.9 nmol/L*	*25-49.9 nmol/L*	*50-74.9 nmol/L*	*75-99.9 nmol/L*	*≥100 nmol/L*	*p value*
Handgrip strength (kg)	*18.5 (4.6)*	*18.0 (4.6)*	*18.2 (4.8)*	*19.9 (4.8)*	*19.0 (3.4)*	*0.480*
*Single chair stand (s)**	*4.5 (1.9)*	*4.9 (1.5)*	*3.8 (1.1)*	*4.0 (1.7)*	*3.7 (1.6)*	*0.009*
Repeated chair stand (s)	*25.1 (9.9)*	*25.2 (6.3)*	*21.5 (7.1)*	*20.5 (5.4)*	*21.8 (11.1)*	*0.034*
Timed up and go (s)	*15.6 (6.3)*	*16.1 (6.3)*	*14.5 (4.6)*	*13.8 (7.8)*	*12.9 (4.1)*	*0.10*

Kruskal-Wallis test.

Outside the brackets are means and inside the brackets are standard deviation.

**'s' means seconds.*

Table 3. Relationship between vitamin D status, muscle strength and function according to SACN and IOM guidelines

	SACN guideline (r; *p* value)	IOM guideline (r; *p* value)*

Variables	<25nmol/L	≥25nmol/L	<50nmol/L	≥50nmol/L
Handgrip strength (kg)	0.001; 0.995	0.031; 0.773	0.079; 0.562	-0.026; 0.836**
Single chair stand (s)	0.372; 0.061	-0.389; 0.000	0.275; 0.048	-0.235; 0.071
Repeated chair stand (s)	0.098; 0.649	-0.307; 0.006	0.137; 0.352	-0.148; 0.277
Timed up and go test (s)	0.141; 0.465	-0.212; 0.043	0.003; 0.985	-0.099; 0.439
Balance test (s)***	OR=0.51; *p*=0.26		OR=3.2; *p*=0.09	

*Spearman's correlation.

**Correlation is significant at <0.05 level.

***Binary logistic regression analysis was used to explore the relationship between balance test and vitamin D status.

Table 4. Relationship between 25(OH)D concentration (nmol/L), muscle strength and function across different age groups

Outcome variables	60-69.9 years	70-79.9 years	≥80 years	60-74.9 years	≥75 years
Handgrip strength (kg)	0.223 (0.051)*	-0.047 (0.809)	-0.372 (0.190)	0.117 (0.261)	-0.006 (0.978)
Single chair stand test (s)	-0.173 (0.147)	-0.444 (0.016)	-0.464 (0.151)	-0.157 (0.146)	-0.482 (0.015)
Repeated chair stand test (s)	-0.202 (0.101)	-0.466 (0.011)	-0.619 (0.102)	-0.262 (0.017)	-0.347 (0.113)
Timed up and go test (s)	-0.234 (0.041)	-0.389 (0.037)	0.116 (0.692)	-0.116 (0.264)	-0.394 (0.046)

**Spearman's correlation.*

The values outside the bracket represent 'r' and the values inside the closing brackets represent significance 'p' values.

Table 5. The advance age and skeletal muscle performance in South Asian women

Outcome variables	**60-69.9 years**	**70-79.9 years**	**≥80 years**	**P value**
Vitamin D status (nmol/L)	56.4 (38.6)*	59.3 (34.4)	72.1 (35.3)	0.335

Handgrip strength (kg)	19.2 (4.8)	18.2 (3.7)	16.7 (3.8)	0.163
Single chair stand (s)	4.4 (1.7)	3.7 (1.2)	5.3 (2.0)	0.001**
Repeated chair stand (s)	22.9 (8.9)	22.2 (6.1)	26.4 (8.4)	0.491
Timed up and go (s)	14.2 (5.3)	14.7 (5.9)	19.5 (11.1)	0.005**

Kruskal-Wallis test.

**Means are outside the brackets and standard deviation are in brackets.*

***The significance level is <0.05.*