# Effect of Vitamin D and Whole Body Vibration on High Resolution peripheral Quantitative Computed Tomography (HR-pQCT) Parameters of the Distal Tibia

By

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Thesis

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# Contents

**Thesis overview**

This thesis is divided into four sections. Section 1 (Chapter 1) is an introductory chapter providing a literature review of the topic followed by rationale, aims and objectives of the thesis.

Section 2 (Chapter 2) is a cross-sectional prospective observational study. This chapter was conducted in collaboration with Hana Alloub (BMedSci Student) and Professor Derek Burke (Consultant in Paediatric Emergency Medicine at Sheffield Children's Hospital). Dr Amaka C Offiah provided the academic supervision. In this chapter, we determined vitamin D intake, calcium intake, physical activity levels and their association with fracture risk among children (aged 6 to 15 years) with wrist and ankle injuries. This chapter is presented in the format of a paper currently undergoing major revision for peer-reviewed publication in Nutrition and Health journal.

Section 3 contains the major work undertaken during my PhD. This section consists of four chapters (Chapters 3, 4, 5 and 6). The work in this section was conducted by myself under the supervision of Dr Amaka C Offiah (my primary supervisor), Professor Nick Bishop and Dr Margaret Paggiosi (my co-supervisors). Statistical support was received from Professor Alan Rigby (University of Hull). This section is a double blind pilot randomised control trial of the effect of vitamin D and whole body vibration on high resolution peripheral quantitative computed tomography (HR-pQCT) parameters of the distal tibia. In Section 3, Chapter 3, I provide details of the methods used to undertake this study.  In chapter 4, I present the results. In Chapter 5, I provide a discussion of the results. In Chapter 6, I provide the conclusion, strengths and limitations and potential future directions for the project. In Section 4 (Chapter 7), I provide the reference list and appendices.

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**Abstract**

Vitamin D, calcium and physical activity are important factors for bone health. A cross sectional study was conducted in Chapter 2 to assess vitamin D intake, calcium intake and physical activity among children with wrist or ankle injuries and their association with fracture risk. The majority of children had low vitamin D and calcium intake. The logistic regression indicated that there was a small but significant relationship between calcium intake and fracture risk (OR per SD increase = 0.998; 95% CI, 0.997 – 0.999) but no significant relationship was found between vitamin D intake and fracture risk.

In the following section (Chapters 3, 4, 5 and 6), a randomised controlled trial was conducted to determine the effect of whole body vibration and a large single dose of vitamin D (150,000 IU) on bone density of the distal tibia as measured by HR-pQCT. The study consisted of four parallel groups of equal numbers (40 in total). WBV and placebo group, placebo group, vitamin D group and vitamin D+ WBV group. Measurements (HR-pQCT, serum 25 (OH)D, PTH, bone profile) were collected at baseline and after 12 weeks (during week 13).

The median baseline serum 25(OH)D for all participants was 23 nmol/L. The high dose of vitamin D was well tolerated. There was a significant increase in serum 25 (OH)D in vitamin D and vitamin D+WBV groups relative to the placebo group (all p<0.01). There was a significant decrease in PTH in vitamin D group relative to the placebo group (p=0.013). After 12 weeks, vitamin D group showed the greatest increase in total bone density (increased by 2.7 mg/cm$^3$ relative to the placebo group, p= 0.05). The main findings of this study indicated that the large single dose of vitamin D and 12 weeks of WBV did not improve bone density, bone microarchitecture or bone strength.

# Acknowledgements and dedication

(In the Name of Allah, the Most Beneficent, the Most Merciful)

I would like to acknowledge the many important individuals who have helped me during my PhD journey. First and foremost, I would like to express my sincere gratitude and appreciation to my supervisors; my primary supervisor Dr Amaka C Offiah, and my co-supervisors Professor Nick Bishop and Dr Margaret Paggiosi for their support, guidance and encouragement throughout my PhD. Without whom, this work would not be possible. It has been a wonderful opportunity to work under their supervisions. In addition, I would like to thank Professor Derek Burke and Ms Hana Alloub for co-authoring Chapter 2. My thanks go also to Professor Alan Rigby for providing support with the statistical analysis in Section 3 of this thesis.

 I would like to thank all the staff of Sheffield Children's Hospital (SCH), especially those within the clinical research facility (CRF), pharmacy and the clinical laboratory for their support. Special thanks to Samya Armoush, Rachel Harrison, Susan George, Jayne Clements, Emilia Bettell and Carolyn Clark, for their administrative support at various points during my PhD. In addition, I wish to thank all volunteers for their participation in this research.

My sincere thanks go to my brothers and sisters for their care. My thanks go also to my colleagues and friends; Mohammad, Khalaf, Fawaz and Hani who were the best companions in Sheffield.

**Attribution Statement**

For the cross-sectional study (Section 2, Chapter 2), Dr Offiah proposed and contributed substantially to the study design, provided general supervision, applied for and obtained ethical approval, and revised and approved the final version of the manuscript. Professor Burke contributed to the study design, and revised and approved the final version of the manuscript. Ms Alloub contributed to the study design and ethical approval, recruited and interviewed patients, contributed to data analysis and revised and approved the final version of the manuscript. I contributed to the study design, recruited and interviewed patients, analysed and interpreted the entire data set. I wrote the manuscript, and submitted it to the Nutrition and Health journal and was responsible for all communication with the journal. I also responded to the reviewers' comments and amended the paper accordingly.

For the randomised control trial (VibeD study) (Section 3, Chapters 3, 4, 5 and 6), Dr Offiah and Professor Bishop proposed the study. I wrote the original protocol for the VibeD study and the subsequent amendments and I responded to the reviewers' comments regarding the protocol. I also coordinated and liaised with various departments including the Research and Development (R&D) and the Clinical Research Facility (CRF) at Sheffield Children's Hospital (SCH) and Northern General Hospital (NGH), pharmacy and clinical laboratory at SCH in order to set-up and organised the VibeD study.

I wrote the Participants' Information Sheets (PIS), consent forms, eligibility checklist, the advertising materials and all other supporting documentation and I was responsible for adverting the study and participants' recruitment.

I applied for and acquired the Research Ethics Committee (REC) approval and the Health Research Authority (HRA) approvals and I responded to all the queries and did all the amendments requested by REC and HRA. I also acquired the Research and Development (R&D) approval for the study. I completed the Integrated Research Application System (IRAS) and obtained medical imaging and medical physics approval for the study. I was responsible for site file maintenance and general study management. I sent letters to participants with their vitamin D results and wrote to participants' GPs in case of abnormal results. Dr Offiah approved the letters before sending them out.

I assessed the participants' eligibility, and I collected the consent forms from them. I booked all study visits for all participants and I organised the travel for the participants between SCH and NGH. I performed the anthropometry measurements. I was also responsible for obtaining and distributing participants' thanks vouchers. I booked the venue for the vibration training in the CRF throughout the duration of the study, set-up the vibration machines, supervised all the vibration session, and recorded all study activities including the vibration visits and the clinical visits.

I entered the data into the spreadsheet and performed the preliminary data analysis. Statistical support was received form professor Alan Rigby (University of Hull). Dr Margaret Paggiosi performed all HR-pQCT scanning and analysis. Bone profile, parathyroid hormone and urine samples were analysed by the Clinical Chemistry Laboratory Department at SCH. Vitamin D analysis was performed by the Biochemistry Department at Bristol Royal Infirmary.

Dr Amaka Offiah, Professor Nick Bishop and Dr Margaret Paggiosi provided general supervision and guidance to the study.

## Conferences and publications

**HA Alshamrani**, H Alloub, D Burke, AC Offiah**.** Vitamin D intake, calcium intake and physical activity among children with wrist and ankle injuries and the association with fracture risk**.** *Nutrition and Health journal*, *2019* (*in press*), *available online at*: *https://doi.org/10.1177/0260106019826422*

**HA Alshamrani**, MA Paggiosi, NJ Bishop, AC Offiah. Effect of vitamin D and whole body vibration on high resolution peripheral quantitative computed tomography (HR-pQCT) parameters of the distal tibia (VibeD study). Bone Research Society (BRS) Annual Conference, June 2018, Winchester, UK. Poster presentation. *JBMR Plus (2018)* *2, https://doi.org/10.1002/jbm4.10073*

**HA Alshamrani**, MA Paggiosi, NJ Bishop, AC Offiah. Correlation between serum 25 hydroxyvitamin D, parathyroid hormone (PTH) and high resolution peripheral quantitative computed tomography (HR-pQCT) parameters of the distal tibia. Bone Research Society (BRS) Annual Conference, June 2018, Winchester, UK. Poster presentation. *JBMR Plus (2018)* *2, https://doi.org/10.1002/jbm4.10073*

**HA Alshamrani**, MA Paggiosi, NJ Bishop, AC Offiah. Effect of vitamin D and whole body vibration on high resolution peripheral quantitative computed tomography (HR-pQCT) parameters of the distal tibia (VibeD study). Mellanby Centre Internal Seminar, May 2018, Sheffield, UK. Oral presentation.

**HA Alshamrani**, H Alloub1, D Burke, AC Offiah. Impact of age, sex, location of injury, physical activity, vitamin D and calcium intake on the injury outcome of wrist and ankle in children. The 8th International Conference on Children's Bone Health (ICCBH), June 2017, Würzburg, Germany, Poster presentation. *Bone Abstracts (2017)* *6 P082, doi: 10.1530/boneabs.6.P082*

**HA Alshamrani,** H Alloub, D Burke, AC Offiah. Fracture risk in children and its association with physical activity and Vitamin D and calcium intake. United Kingdom Radiological Congress (UKRC), June 2016, Liverpool, UK, Oral Presentation.

**HA Alshamrani,** H Alloub, D Burke, AC Offiah. Inadequate Intake of Vitamin D and Calcium among Children with Wrist and Ankle Injury. The 9th Saudi Students Conference in the UK, February 2016, Birmingham - UK, Poster Presentation.

**HA Alshamrani,** H Alloub, D Burke, AC Offiah. Fracture risk in children and its association with physical activity and vitamin D and calcium intake: A cross sectional study. The 6th Annual Mellanby Centre Research Day, December 2015, Sheffield - United Kingdom, Poster Presentation.

## List of figures

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## Abbreviations

| | |
|---|---|
| 25 (OH) D | 25-hydroxyvitamin D |
| A | Amplitude |
| aBMD | Areal bone mineral density |
| ACO | Dr Amaka C Offiah |
| $a_{peak}$ | peak acceleration |
| BA | bone area |
| BHI | Bone health index |
| BMC | Bone mineral content |
| BMD | Bone mineral density |
| BMI | Body mass index |
| CI | Confidence interval |
| Conn.D. | Connectivity density |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRF | Clinical research facility |
| Ct.Ar | Cortical area |
| Ct.Pm | Cortical perimeter |
| Ct.Po | Cortical porosity |
| Ct.Po.Dm | Cortical pore diameter |
| Ct.Po.Dm.SD | SD mean cortical pore diameter |
| Ct.Th | Cortical thickness |
| Ct.TMD | Cortical tissue mineral density |
| Ct.vBMD | Cortical density |
| CV | Coefficient of variation |
| D | Peak-to-peak acceleration |
| Dinn | Inner trabecular density |
| Dmeta | Meta trabecular density |
| DXA | Dual energy x-ray absorptiometry |
| DXR | Digital x- ray radiogrammetry |
| ED | Emergency Department |
| EU | European Union |
| f | Frequency |
| F.ult | Estimated ultimate failure load |

| | |
|---|---|
| FE/ FEA | Finite element / finite element analysis |
| G | Gravity force |
| GP | General practitioner |
| HA | Hydroxyapatite |
| HRA | Health Research Authority |
| HR-pQCT | High resolution peripheral Quantitative Computed Tomography |
| HU | Hounsfield number |
| Hz | Hertz |
| ICH GCP | International Conference for Harmonisation of Good Clinical Practice |
| IGF-1 | Insulin-like growth factor-1 |
| IMD | Index of Multiple Deprivation |
| IOF | International Osteoporosis Foundation |
| IOM | Institute of Medicine |
| IU | International Units |
| JoSLE | Juvenile-onset Systemic Lupus Erythematosus |
| LFFQ | long food frequency questionnaire |
| MAP | Dr Margaret A Paggiosi |
| MENA | Middle East and North Africa |
| MET | Metabolic equivalent of task |
| NGH | Northern General Hospital |
| NHS | National Health Service |
| NJB | Professor Nick J Bishop |
| OR | Odds ratio |
| OPG | Osteoprotegerin |
| PBM | Peak bone mass |
| PI | Public Involvement |
| pQCT | peripheral Quantitative Computed Tomography |
| PTH | Parathyroid Hormone |
| QC | Quality Control |
| QCT | Quantitative Computed Tomography |
| QMRI | Quantitative Magnetic Resonance Imaging |

| | |
|---|---|
| QUS | Quantitative Ultrasonography |
| R&D | Research and Development |
| RANK | Receptor activator of nuclear factor $\kappa\beta$ |
| RANKL | Receptor activator of nuclear factor $\kappa\beta$ ligand |
| RCPCH | Royal College of Paediatrics and Child Health |
| RCT | Randomised controlled trial |
| REC | Research Ethics Committee |
| RMSCV | Root mean square coefficient of variation |
| RNI | Reference nutrient intake |
| SACN | Scientific Advisory Committee on Nutrition |
| SAE | Serious adverse event |
| SCH | Sheffield Children's Hospital |
| SD | Standard deviation |
| SDS | Standard deviation scores |
| SFFQ | Short Food Frequency Questionnaire |
| SOP | Standard operating procedure |
| Tb.Ar | Trabecular area |
| Tb.N | Trabecular number |
| Tb.N.SD | Trabecular inhomogeneity |
| Tb.Sp | Trabecular separation |
| Tb.Th | Trabecular thickness |
| Tb.vBMD | Trabecular density |

**List of appendices**

# SECTION 1

# CHAPTER 1

# INTRODUCTION

**1.1 Bone**

1.1.1 Bone structure, composition and physiology

Bone is a dynamic and adaptive tissue that has structural and reservoir functions. More specifically, the bony skeleton provides the mechanical support for the body, protects internal organs, provides the muscles with sites of attachment and facilitates the movement of the body (Boskey and Robey, 2013). Bone also represents a reservoir for two main minerals; calcium and phosphate ions, both of which are essential minerals for all other organs in the body. The skeleton is one of the hardest materials in the body and is commonly classified into two types: the axial skeleton, which includes the skull, the whole spine and the ribs; and the appendicular skeleton, which includes the extremities and the pelvis (Grabowski, 2015). Aside from its functions, bone has some outstanding features, such as the ability to adapt to mechanical loading and its excellent self-repairing ability.

In terms of its composition, bone is comprised of minerals, water, lipids, collagen and collagenous proteins (Boskey and Robey, 2013). Collagen fibres (type 1 collagen) are the basic and main components of the bone matrix, accounting for 85 to 90% of the mass with the remainder comprising non-collagenous proteins. The collagen matrix itself contains minerals that are deposited on it. In the bone, minerals are transformed to hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, a naturally occurring mineral (Boskey and Robey, 2013) . In terms of structure, bone is mainly hollow with two compartments: cortical (also known as compact bone) and trabecular (also known as spongy or cancellous bone) (Figure 1.1, next page). Cortical bone is a thick and dense layer of the bone that surrounds the trabecular layer of the bone. This type of bone is found in abundance in the shaft of the long bones, provides strength and protection, and represents approximately 75% of the

total human bone mass. Trabecular bone is more porous and is found at the end of the long bones, in the vertebrae and in the pelvic bones. This porosity and trabecular bone's microstructure impart excellent absorptive ability, which results in a natural tendency to distribute external force and to allow the bone to adapt to various external loading conditions.
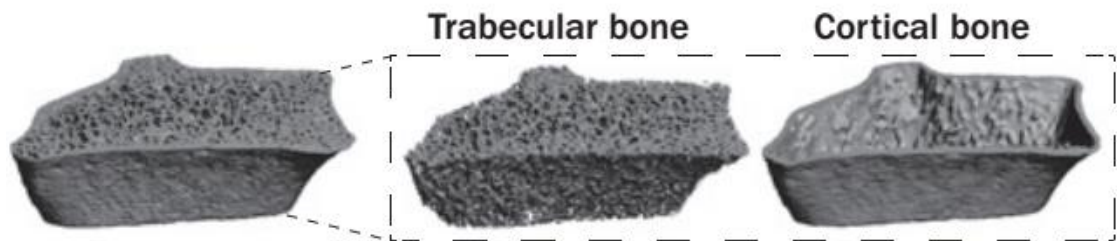


*Figure 1.1: Cortical and trabecular bone. From Geusens et al., (2014) with permission.*

Bone is one of the most metabolically-active systems in the body, with trabecular bone being considerably more active than cortical bone. According to Grabowski (2015), bone physiology in adulthood is related to maintaining the metabolic activities of the bone through the process of bone turnover in response to the body's needs. During childhood, bone physiology is related to skeletal increase in size, mass and bone mineral density (BMD) until the skeleton reaches its final shape. Bone contains several different types of cells that regulate its physiological functions. These include osteocytes, osteoblasts, and osteoclasts. Osteocytes are the most abundant cell types in the adult skeleton by far, comprising approximately 90-95 % of all bone cells. Osteoblasts represent 4-6 % and osteoclasts represent a mere

1-2% (Bonewald, 2011). These cells are connected to each other and to other cells on the surface of the bone via dendritic processes that travel through small canals known as canaliculi. The gap junction between them maintains their effective communication (Clarke, 2008). It has been reported that osteocytes play a significant role in bone resorption and formation as they respond to the mechanical loading. Bone turnover is an ongoing process that resorbs the old bone and forms new bones in a process known as remodelling.

## 1.1.2 Bone modelling and remodelling

Bone undergoes a continuous modelling and remodelling process at all times during a human life. This process is essential for maintaining the integrity of the skeleton and its proper metabolic functioning. Bone modelling is the process by which bone size and shape is changed as a result of the influence of physiological or mechanical stimulation (Clarke, 2008). In the bone remodelling process, bone is renewed at the same site in order to maintain skeletal integrity and strength as well as to maintaining calcium homeostasis.

Bone remodelling is a complex and highly organised process involving four stages which are regulated by the interaction of two main bone cell types: osteoclasts and osteoblasts (Raisz, 1999). These stages follow in sequential order: the activation phase is followed by the resorption phase, which is followed by the inversion phase, which is followed by the final formation phase (Clarke, 2008). The specific processes each stage are as follows. In the activation phase, the osteoclast interacts with osteoblast precursor cells. The large osteoclasts are differentiated, migrated, and fused as a result of this interaction which leads to the resorption phase (Raisz, 1999).

Osteoblast-lineage cells produce receptor activator of nuclear factor kappa-B ligand (RANKL), which bind to a recptor on the osreoclats precursors (RANK) and stimluaate their devoloment into diffrencteed osteclasts (Dempster et al., 2012). Oestoblast celles can also produce another protein called osteoprotegerin (OPG), which prevents the binding of RANK to RANKL, thus preventing the recruitment, proliferation, and activation of osteoclasts (Schoppet et al., 2002). The RANKL/RANK/OPG system plays a vital role in coordinating the bone remodelling process, which maintains the balance btween the bone resorption and bone formation process.

During the resorption phase, hydrogen ions and lysosomal enzymes are secreted which initiate resorption. This process leads to the breakdown of the bone matrix leaving resorptive cavities known as Howship's lacunae on the trabecular bone and Haversian canals in the cortical bone (Clarke, 2008, Raisz, 1999). The inversion or reversal phase of the remodelling process represents a transition phase from bone resorption to bone formation. During this phase, different cell types such as monocytes and osteocytes are seen in the resorption cavities (Clarke, 2008). In addition, the rough surfaces of the resorption cavity are made smooth. According to Clarke (2008), the exact mechanism of coupling the resorption phase to the formation phase is not fully understood. In the final phase, osteoblasts fill the cavities created in the resorption phase and begin producing and mineralising newly-formed bone matrix. Figure 1.2 in the next page illustrates the bone remodelling cycle.

Bone remodelling occurs in a small units or packets, with each packet usually at a different stage of the remodelling process in order that the whole bone is not in the resorption phase (Clarke, 2008). Bone remodelling can occur randomly (non-

targeted) but can also be targeted. Targeted remodelling occurs as a response to microdamage of bone while the non-targeted remodelling can occur at any site of the skeleton, seemingly in a random manner (Shabestari and Eriksen, 2013).
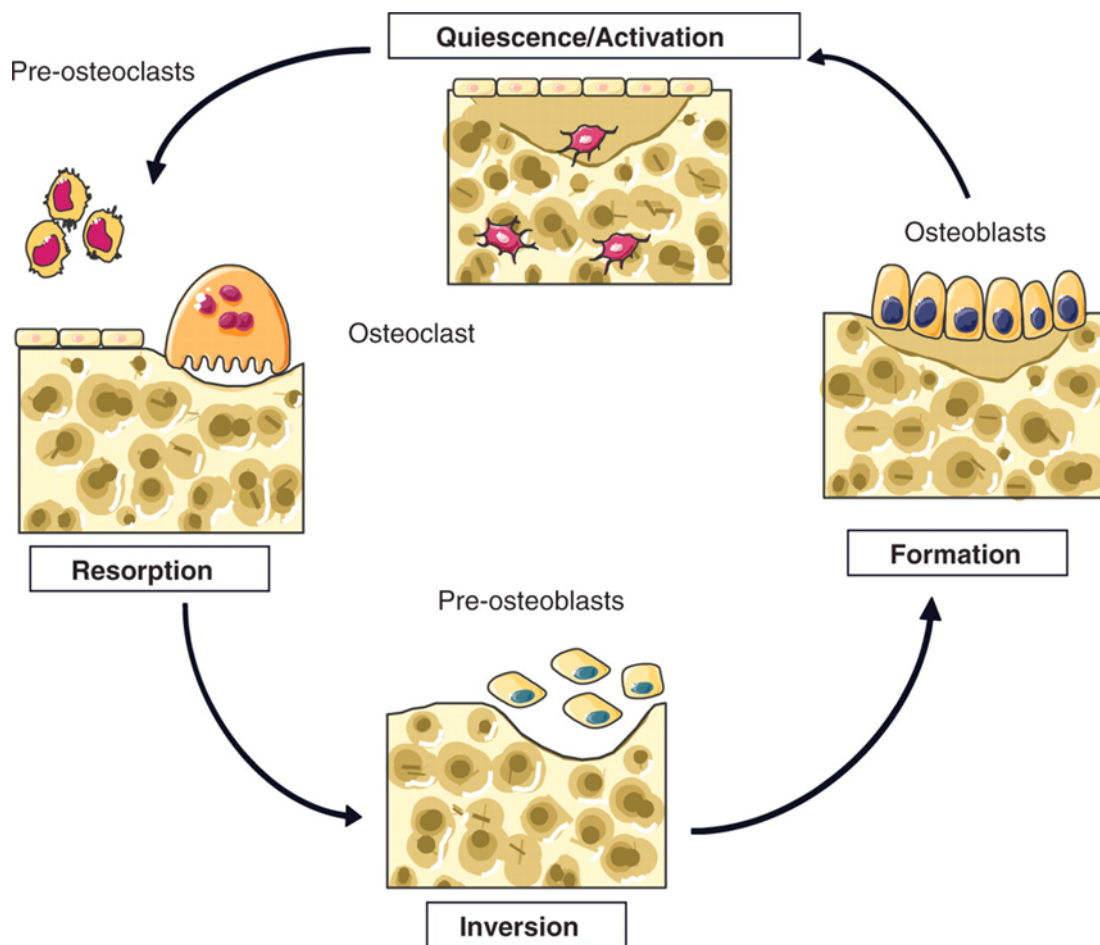


*Figure 1.2: Diagram of the bone remodelling process which consists of 4 stages in a complete cycle: 1- Activation phase; 2-Resorption phase; 3- inversion phase; and 4- Formation phase. From Brandi, (2009) with permission.*

Bone resorption and formation cycle is a highly organised and balanced process which is regulated by many factors in response to skeletal needs. Any disassociation between the two processes can cause abnormalities such as in the case of

osteoporosis, where the ratio of bone loss is significantly higher than bone gain, or in osteopetrosis, where bone gain is significantly higher than bone loss (Shabestari and Eriksen, 2013).

It should be noted that bone loss naturally occurs with aging due to a variety of factors. These include changes in hormone levels, especially in women; increased bone resorption due to suboptimal calcium and vitamin D levels; and defective osteoblast functioning which occurs due to aging (Shabestari and Eriksen, 2013). Bone health is regulated by several hormones, the most important of which are parathyroid hormone (PTH), calcitriol, calcitonin, oestrogen and testosterone, human growth hormone, insulin-like growth factor-1 (IGF-1), thyroid hormone and cortisol  (U.S. Department of Health and Human Services, 2004).

### 1.1.3 Peak bone mass

The amount of bone tissue accrued once the skeleton becomes mature is known as the peak bone mass (PBM), where bones reach their maximum strength. There is no absolute age at which PBM is reached by humans (Heaney et al., 2000), but according to Welten et al. (1994) it is usually assumed that PBM is achieved towards the end of the twenties. This was confirmed in a longitudinal study, which showed that although the majority of lumber spine PBM was achieved by the age of 18, increases in lumber spine mass and size continue to occur until the third decade of life (Walsh et al., 2009). It has been suggested that achieving the maximum potential PBM is important as it can protect from fracture and osteoporosis later in life (Weaver et al., 2016, Rizzoli et al., 2010).

Several factors have been noted as influencing the level of PBM including genetics, nutrition, lifestyle factors, hormones and exercise. Although some of these factors

cannot be modified, such as genetics, other factors such as nutrition and exercise can, which eventually can help to achieve maximum PBM (Schonau, 2004). Lifestyle factors are the most important modifiable factor for bone mass as it has been reported that such factors account for 20-40% of adult PBM (Weaver et al., 2016). Suboptimal optimisation of lifestyle factors may lead to more fragile bone later in life (Figure 1.3, next page). In review of the lifestyle factors that influence achieving the full genetic PBM; calcium intake, physical activity and exercise, vitamin D and diary intake have all been strongly and positively associated with PBM (Weaver et al., 2016).

PBM can also be influenced by ethnicity. Most studies in this regard have focused on the difference between Africans and Caucasians. There is however a lack of studies on the comparison of other ethnic groups. It has been reported that African-Americans have a greater bone mass compared with Caucasians. This has been linked to the lower fracture risk among African-Americans (Norris and Nelson, 2013). Using peripheral quantitative computed tomography (pQCT), it has also been shown that volumetric density and bone geometry is greater in African-American children than in white children (Wetzsteon et al., 2009, Micklesfield et al., 2011). The same conclusion was drawn by another study which demonstrated that young African-American adults have more favourable bone microarchitecture compared to young white adults. This was concluded using high-resolution peripheral quantitative computed tomography (HR-pQCT) to compare the two populations (Popp et al., 2017).
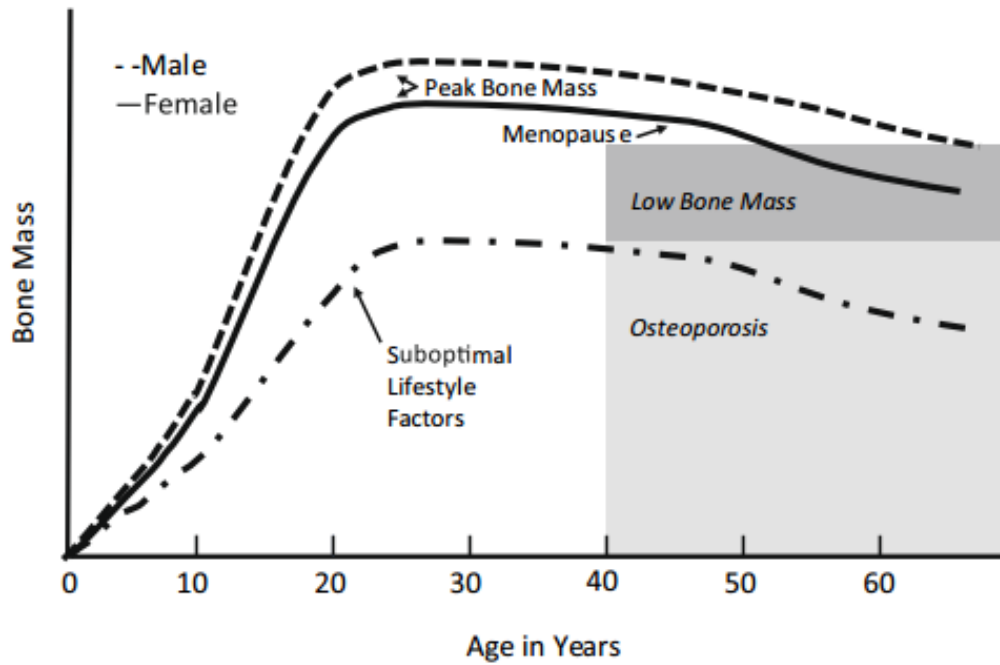
*Figure 1.3: Bone mass throughout a human lifespan and the influence of suboptimal lifestyle factors. A substantial proportion of bone mass is built between the age of 10 and 19 years. Bone mass continues to increase until the third decade of life. Optimal choice of life style factors can help in achieving the maximum potential PBM and maintaining good bone health throughout life. From Weaver et al., (2016) with permission.*

## 1.2 Osteoporosis

### 1.2.1 What is osteoporosis?

Osteoporosis is one of the most common diseases to affect the skeleton, especially in the elderly population. Osteoporosis causes a progressive decline in bone mass and microstructure which leaves the bone weak and increases its susceptibility to fracture (Kanis et al., 1994). The microstructure of a healthy trabecular bone consists of plates that are well-connected to each other, making a strong network of bone tissue which provides protection against fracture (U.S. Department of Health and Human Services, 2004). When the connections between these plates weaken and become disrupted due to the osteoporotic condition, bone frailty increases, reducing its resistance to fracture (Figure 1.4, next page).

There are number of factors that can contribute to loss of bone mass, which in turn may eventually lead to osteoporosis. These are a reduced PBM, excessive resorption of bone mass due to genetic and environmental factors, and defective functioning of bone formation (Raisz, 1999). In the adult population, osteoporosis is diagnosed when the bone mineral density measured by dual energy x-ray absorptiometry (DXA) is 2.5 standard deviations below the average adult bone mass (Lippuner, 2012).
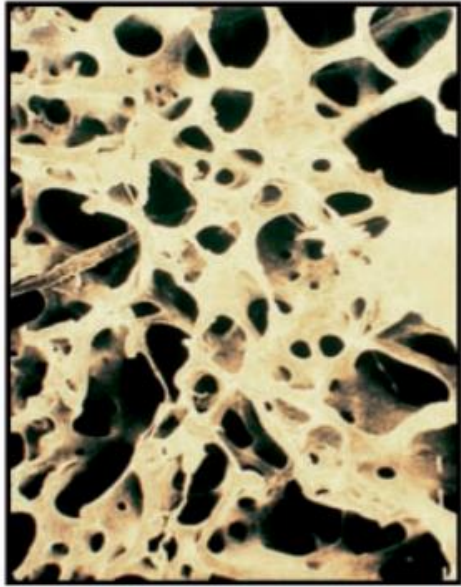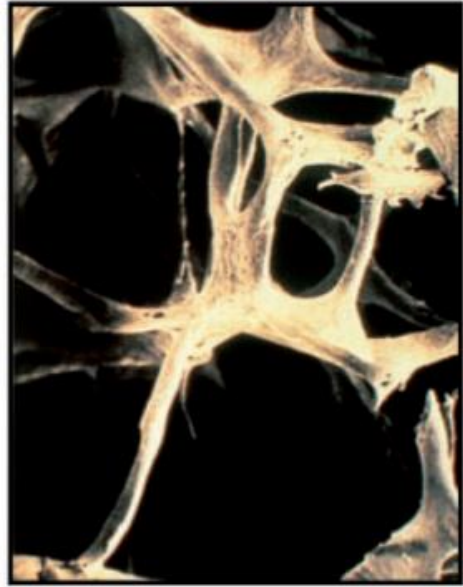
*Figure 1.4: Visual comparisons of the trabecular microstructure of normal and osteoporotic bones showing healthy (left) and osteoporotic (right) bones. From Brandi, (2009) with permission.*

Osteoporosis is associated with adverse health and socio-economic consequences including mortality, morbidity and increases demand on the healthcare system, especially in economic terms. For instance, the European Union (EU) countries spent €37 billion on osteoporosis treatment in 2010, and in the UK alone around 3.5% of its healthcare budget was allocated to treating osteoporosis and its complications (Hernlund et al., 2013). Of these complications, fragility fractures can have fatal consequences, especially when occurring in the hip and vertebrae. These types of fractures are associated with a 20% reduction in survival rates for sufferers (Harvey et al., 2010).

The osteoporosis treatment strategy aims to reduce bone resorption and/or boost bone formation. Pharmacological treatment using bisphosphonates is currently the main treatment for osteoporosis (Lippuner, 2012). However, bisphosphonates are like any other drugs; they are associated with some unwanted side effects such as osteonecrosis especially when used for prolonged periods of time (Rizzoli et al., 2010). Preventative strategies should therefore be implemented, especially in terms of optimising lifestyle factors. As such, the overarching goal of this project was to investigate the effectiveness of physical stimulation and optimising vitamin D status as an alternative non-pharmacological intervention in optimising bone health.

### 1.2.2 Prevalence of osteoporosis

In western countries, osteoporosis prevalence ranges from 9% to 16% for females and from 1% to 8% for males (Wade et al., 2014). In terms of geographical variance in prevalence, the literature indicates that it is highest in the Middle East and North Africa (MENA) region. In Saudi Arabia for example, the prevalence of osteoporosis has been reported to be 34% for women (Sadat-Ali et al., 2012). In another example from the MENA region, the prevalence of osteoporosis in Jordan and Egypt was reported to be 29.6% among Jordanian women and 28.4 among Egyptian women (Shilbayeh, 2003, Taha, 2011). Shockingly, it has been claimed that the prevalence of osteoporosis is underestimated in males in the MENA region (Al-Saleh et al., 2015). Several studies have showed significantly higher prevalence of both osteoporosis and osteopenia among Saudi males compared to males in industrialised nations, ranging from 21% to 38% and 34% to 54% for osteoporosis and osteopenia, respectively (Ardawi et al., 2005, Sadat-Ali and AlElq, 2006, El-Desouki and Sulimani, 2007). The burden of the disease is only expected to rise in

the future in the MENA region due to the increase in the proportion of the aging population (Maalouf et al., 2007).

Cultural factors may explain this phenomenon in the MENA region: the code of dress in these countries encourages the covering of most of the body. This restricts the amount of sun exposure that is necessary for producing vitamin D which can lead to poor musculoskeletal health (Edwards et al., 2013). Clearly then, intervention is required to optimise bone health and reduce the prevalence of osteoporosis and osteopenia in the MENA region. The Saudi Osteoporosis Society for instance emphasises the importance of focusing on the encouragement of factors that positively influence bone health such as vitamin D and physical activity regardless of age and gender (Al-Saleh et al., 2015). There is a lack of studies of the minor populations who emigrated from the MENA region to Europe. It is not clear if this ethnic group who live in Europe have a similar patterns of osteoporosis prevalence.

## 1.3 Bone and physical activity

### 1.3.1 Bone adaptation to loading

Bone is attached to the muscle anatomically and physiologically, and therefore any improvement in muscle strength will be matched by an improvement in bone strength (Andreoli et al., 2001). Muscle strength plays a dominant role in bone mineral density, geometry and microarchitecture (MacDonald et al., 2006). Muscle activities generate a load on bone, which creates adaptive response by bone cells. It has been reported that the changes in bone mass are preceded by changes in muscle strength (Robling et al., 2009). A suggested mechanism for muscle action on bone is that the bone mechanoreceptors in the bone periosteum is activated in response to muscle contraction (Cowin and Interactions, 2002). It has also been suggested that the Insulin-like growth factor 1 (IGF-1) and insulin receptors in muscle are activated in response to the loading placed on the muscle, which leads to the activation of the mechanoreceptors in the bone (Bolster et al., 2004).

As has been stated earlier, bone has an excellent ability to adapt to a variety of loading conditions. Wolff's law explains the relation between bone mass and loading in the expression "use it or lose it". In more scientific terms, bone responds to loading by increasing bone formation while stimulating bone resorption in the absence of loading on bone (Frost, 1987). When the bone is exposed to external loading, the remodelling process adapts the bone's properties so that it can resist the external loading and vice-versa. The Mechanostat Theory refinement of Wolff's law explains that there is a load threshold that the strain has to exceed in order to induce bone adaption (Frost, 1997). A small strain below the threshold leads to bone loss (disuse mode) while a too high level of strain can cause fracture (Frost, 1997,

Frost, 2003). There is therefore a desirable range (2000-3000 µ-strains) between the disuse mode and fracture in which the strain should remain to induce bone formation (Frost, 1997, Frost, 2003). It has been reported that there are three rules for bone adaption to mechanical stimuli. According to Turner (1998), these three rules are: 1) dynamic loading drives bone adaption rather than static loading; 2) mechanical loading is only necessary for a short period to initiate an adaptive response and 3) bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals (Turner, 1998).

Physical activity causes repeated strains, which in turn cause microdamage that needs to be repaired through bone remodelling. Over time, this leads to more bone formation. Mechanotransduction is the process by which the mechanical loading of physical activity is converted into metabolic signals (Kohrt et al., 2004). It has been suggested that the metabolic signals are transferred through fluid-flow movement within the canaliculae (Duncan and Turner, 1995, Turner et al., 1995, Han et al., 2004). This fluid-flow movement is likely responded to by osteocytes, which (as previously noted) are the most abundant cell type in the bone (Huiskes et al., 2000, Xiong et al., 2011). Osteocytes are mechanosensory cells that have long processes that span in different directions and penetrate the matrix in what is known as canaliculi (Bonewald, 2011). It is widely believed that these cells play a major role in the process translating the mechanical signal into biological action due to their location in bone and their complex dendritic network (Huiskes et al., 2000, Xiong et al., 2011, Bonewald, 2011).

Mechanical loading has a direct effect on osteocyte secretion of sclerostin. Sclerostin decreases in response to mechanical loading and increases in response to unloading (Gaudio et al., 2010). Sclerostin is an antagonist of the Wnt-signalling pathway and thus can prevent bone formation by inhibiting osteoblast differentiation (Robling et al., 2008). However, mechanical loading can down regulate sclerostin allowing for increased osteoblast activity through Wnt/ β-Catenin signalling, which in turn can lead to increased bone formation (Lin et al., 2009). However, the exact mechanisms of mechanical stimulation of osteocytes is not fully understood and there may be factors that influence this process which remain to be discovered (Kohrt et al., 2004, Klein-Nulend et al., 2013).

Since there is a threshold that external loading has to exceed in order to induce bone formation, moderate to intensive exercise has been recommended to enhance muscle strength, cause a reduction in the risk of falls, and induce bone formation, although the resulting increase in bone mass might be modest and site specific (Forwood and Burr, 1993, Wallace and Cumming, 2000). However, because most people are not exposed to high magnitude loads during normal daily activity it possible that a small magnitude may have a role in bone adaption through muscle contractions (Fritton et al., 2000). The role of low magnitude at high frequency loading on bone adaption was demonstrated in several experimental studies (Rubin et al., 2001, Judex et al., 2003, Qin et al., 1998), and technological advances in recent decades have led to the utilisation of vibration platforms that can be used to provide mechanical loading in the clinical setting. This form of physical stimulation is known as whole body vibration (WBV). This type of physical stimulation forms the focus of Section 1.4.

## 1.3.2 Physical activity and bone density

Physical activity and inactivity are well-known factors that influence bone health. For instance, observational studies indicate that professional tennis players have around 35% more bone mass in their dominant arms compared to their non-dominant arms (Jones et al., 1977). On the other end of the activity scale, long-term immobilisation has been found to induce bone loss (Uhthoff and Jaworski, 1978, Frost, 2003). In addition, astronauts lose bone during long airspace flights due to the absence of gravitational force (Lang et al., 2004).

Several systematic reviews have demonstrated the positive impact of physical activity on bone density across all life stages (Hind and Burrows, 2007, MacKelvie et al., 2002, Macdonald et al., 2009, Nikander et al., 2010, Behringer et al., 2014, Tan et al., 2014, Wallace and Cumming, 2000, Bolam et al., 2013). Weight bearing activities in particular, such as jogging and dancing, are the most effective at improving bone mass (Kohrt et al., 2004).

Most of the literature that determines the relation between physical activities and bone mass relies on measurements of areal BMD as measured by DXA. The recent advance in high-resolution bone imaging allows for deeper evaluation of the effect of physical activity on bone structure and strength. In a review of the effect of exercise on peripheral quantitative computed tomography (pQCT) parameters in postmenopausal women, it has been concluded that bone loss might be preventable through exercise with its effects being more obvious in early menopause (Polidoulis et al., 2012). To assess the young adult population, two studies used pQCT to determine bone response to unaccustomed physical activity. These two studies showed a significant increase (from 0.8 to 1.3 mg/cm$^3$) in volumetric trabecular

bone mineral density after 13 weeks of training (Evans et al., 2012, Lester et al., 2009). However, in a recent study that used high-resolution peripheral quantitative computed tomography (HR-pQCT) to determine the tibial bone responses of female recruits to an army training regimen, volumetric trabecular bone mineral density significantly increased by 2% after only 8 weeks of training. It should be noted however, that the results of this study are limited in reliability due to the absence of a control group (Hughes et al., 2018). Nonetheless, the results of all these studies may indicate that anabolic changes in bone occur shortly after starting intensive physical activity. Future randomised control trials should consider the value of high-resolution imaging in determining the effect of physical activity on bone health.

A few trials have investigated the effect of exercise on bone when combined with calcium and vitamin D supplementation and their findings are equivocal. For example, there was no advantage of calcium–vitamin-D3-fortified milk plus exercise over exercise alone to bone structure and strength at 12 months (Kukuljan et al., 2009) and at 18 months (Kukuljan et al., 2011) in a study of older men's (50 -79 years) response to exercise and calcium-vitamin D fortified food. In contrast, calcium and vitamin D supplementations in addition to 9 weeks of exercise were found to significantly improve volumetric bone mineral density at the tibia compared to exercise alone in a subset of 46 military recruits who underwent pQCT scan among 247 participants (Gaffney-Stomberg et al., 2014). These differences between the trials' results could have arisen from the differences in the age and sex of the populations in the two trials or perhaps due to the difference in the period of intervention.

Although physical activity has been shown to have beneficial effects on bone, many potential confounding factors have to be borne in mind. These factors include the intensity and the duration of the training, the hormonal status, the skeletal site where the effect of the physical activity is being measured, the exercise history, the initial bone mass, and the sex and age of the person undertaking the physical activity (Forwood and Burr, 1993, Hind and Burrows, 2007, Boreham and McKay, 2011). Given the seeming importance of diet, especially in terms of vitamin D and calcium intake, on the effects of physical exercise on bone, Bolam et al. (2013) indicted that such data should be included in any future trials on the effects of exercise on bone.

Another advantage of weight-bearing exercise, especially over pharmacological intervention which can only address bone mass, is that fall risk decreases. However, there are issues associated with conventional methods of exercising. These issues include low compliance, and the risk of stress fracture and tissue injuries which are commonly seen in military recruits and marathon runners (Rittweger, 2010). It has been shown that low magnitude mechanical stimulus has an anabolic effect on bone (Rubin et al., 2001) which might be an alternative and safer approach to conventional exercise.

## 1.4 Whole Body Vibration (WBV)

WBV is a relatively new training approach that has garnered interest from the fitness industry and the medical community in recent years. The research on WBV includes its application to a range of clinical conditions in terms of its efficacy as a potential alternative to conventional exercises to stimulate the growth and development of muscle and bone (Prisby et al., 2008, Fratini et al., 2016). This form of training has been found to be able to overcome some of the issues which are associated with conventional methods of exercising such as falls, small injuries and stress fractures, especially in elderly and obese populations (Rittweger, 2010). The first application of WBV as a scientifically-studied training method was conducted in the mid-nineteen eighties by Nazarov and Spivak (1985). However, WBV as a training modality only gained the interest of the scientific community more than a decade after Nazarov and Spivak's intial paper, with several publications and commercial devices emerging in the 1990s and early 2000s (Dakota, 1998, Issurin and Tenenbaum, 1999, Rittweger et al., 2000, Kerschan‐Schindl et al., 2001).

Since then, the literature on WBV has been diverse in terms of its specific focus, with different studies reporting various outcomes of WBV, such as neuromuscular performance (Von Stengel et al., 2012), hormonal response (Bosco et al., 2000), cardiovascular response (Hazell et al., 2008), body composition (Marín-Cascales et al., 2017), balance and mobility (Bautmans et al., 2005), and bone density (Verschueren et al., 2004). The focus of the current thesis is to contribute to this field by studying the effect of WBV on bone density, microarchitecture and estimated bone strength.

### 1.4.1 Parameters of WBV

In WBV, a vibration platform is used to generate mechanical stimuli which are transmitted through the skeleton and apply mechanical loading to the muscle and bone (Verschueren et al., 2004). The parameters associated with WBV are summarised in Table 1.1 (next page). In short, the intensity of WBV is controlled by its frequency and peak-to-peak displacement (Slatkovska et al., 2011). The frequency of the vibration as well as the peak-to-peak acceleration is used to determine the peak acceleration of the WBV, which is commonly used to describe the intensity of the WBV (Rauch et al., 2010). Peak acceleration is preferably expressed as a g force, which can be calculated by dividing the peak acceleration by Earth's gravity ($a_{peak}/9.81g$) (Beck, 2015). The following formula can be used to calculate the peak acceleration of the WBV (Rauch et al., 2010):

$$a_{Peak} = 2 \text{ x } \pi^2 \text{ x } f^2 \text{ x } D$$

$$a_{Peak} \approx 20 \text{ x } f^2 \text{ x } D$$

$a_{Peak}$: peak acceleration

f: frequency

D: peak-to-peak acceleration

It is not certain what is the strain induced by WBV. It has been reported that strain depends on other factors such as the type of the vibration (horsetail or vertical), the anatomical site where the strain is being measured and the positioning/posture of the body on the platform and musculoskeletal stiffness (De Zepetnek et al., 2009). However, some instruments can be used to determine the strain that is induced by WBV such as the strain gauge. Nonetheless, this is outside the scope of this project due to the limited timescale and the financial resources available for the project.

The WBV training protocols vary widely among studies. In addition, WBV has been applied to different age groups including children, adults and elderly and to samples that comprise apparently healthy people and people with different bone diseases such as osteopenia, osteoporosis and osteogenesis imperfecta. However, despite using various WBV training protocols and targeting various populations, no ideal WBV training protocol has been identified nor any specific population which benefits most greatly from WBV (Wysocki et al., 2011). Thus, research is ongoing to find an ideal training protocol and determine a target group that can benefit most significantly from such intervention.

Table 1.1: WBV parameters

| Parameter | Definition | Unit | Symbol |
|-----------|------------|------|--------|
| Frequency | The number of oscillations per second | Hertz (H) | f |
| Peak to peak displacement | Distance between the bottom and the peak points in vibration excursion | Millimeter (mm) | D |
| Amplitude | The range of perpendicular displacement from the equilibrium condition | Millimeter (mm) | A |
| The peak acceleration | The force gendered by the platform | $ms^{-1}$ | $a_{Peak}$ |

WBV platforms can be broadly classified either based on their vibrating technique or on their intensities. When based on the former, WBV platforms can be divided into two categories (Figure 1.5, next page). The first category includes platforms that produce sinusoidal vibration (side-alternating) which have a greater effect on the lateral axis, and platforms that produce vertical vibration (synchronous) and have greater effect on the vertical axis (Beck, 2015). In the side-alternating platforms, the vibrating plate oscillates around the central axis, and therefore, peak acceleration depends on the peak-to-peak displacement and differs according to the distance of the feet from the centre of the platforms (Rauch et al., 2010). In contrast, the peak acceleration and peak-to-peak displacement in synchronous platforms are the same and therefore the intensity of WBV is not affected by the position of the feet on the platforms (Rauch et al., 2010). There have been some attempts to introduce a third type which involves random movement, but this type is not yet been fully commercialised (Rittweger, 2010). In a recent systemic review of the effect of WBV on bone density in postmenopausal women, side-alternating platforms were associated with heterogonous outcomes, specifically at lumber spine and trochanter, while the results of synchronous platforms were more homogenous (Oliveira et al., 2016). Despite this heterogonous outcome of side alternating platforms, a recent meta-analysis found side-alternating platforms to be more effective in enhancing bone density (Fratini et al., 2016)

The alternative classification system of WBV platforms is based on the magnitude of the WBV. Depending on the magnitudes of WBV protocol, it can be grouped into two categories: low intensity (g force < 1 g) and high intensity platforms (g force >1g) (Beck, 2015). The intensity of WBV is determined by the peak

acceleration (g) regardless of its frequency (Wysocki et al., 2011). The "Galileo" is an example of the high intensity platform while "Juvent 1000" is an example of a low intensity platform (Elmantaser et al., 2012). It has been reported that the high magnitude platforms are associated with greater effects of the WBV on bone and this effect is more pronounced when the intensity of the WBV was more than 3 g (Fratini et al., 2016). A possible explanation for this could be that the high intensity WBV protocol generates more intense signals which have more of an ability to penetrate soft tissue and reach target locations effectively (Fratini et al., 2016).



Side alternating vibration          Synchronous vibration
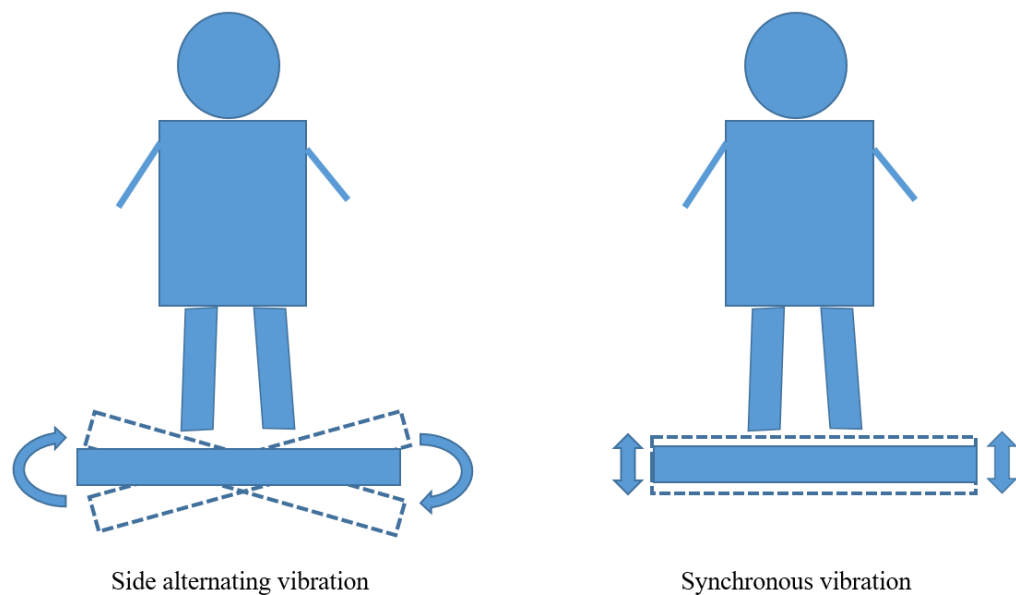
*Figure 1.5: Two main types of vibration platform according to their movement direction. Adapted from Rauch et al., (2010).*

### 1.4.3 Transmission of WBV

In spite of the numerous publications that have studied WBV in the past decade, the mode of action of WBV is not clearly understood. However, it has been suggested that WBV stimulates the bone directly by transmitting the mechanical signals into bone cells, which in turn change the flow of the fluid in the bone and produce an osteogenic effect (Lam et al., 2013). This osteogenic effect may occur through osteocytes or by activation of skeletal muscles, perhaps due to the stretch reflex (Ozcivici et al., 2010). It has been also hypothesised that the low intensity vibration signals are amplified inside bone tissue and act as mechanosensors for bone cells (Rubin et al., 2006). It has been also suggested that WBV may indirectly alter bone remodelling by affecting the hormones that can influence bone mass such as testosterone and growth hormone (De Zepetnek et al., 2009). Regardless of the specific mechanisms, it has been convincingly argued that the general mode of action of WBV is driven through a series of pathways and involves a complex set of several parallel mechanisms, as is the case with other biological systems (Beck, 2015).

It should be noted that there are also many factors that can affect the transmission of WBV signals from the vibration platform to the body. These factors include the vibration intensity and frequency, the subject's posture on the platform, and the tissues' attenuation to the vibration signals. For instance, Rubin et al. (2003) investigated the transmission of the vibration signals at certain frequencies (15 to 35 Hz) to the hip and lumbar spine. The authors reported that at frequencies of 15 Hz and 35 Hz when the subject was standing in a relaxed stance, the transmission was 130% and 60%, respectively to the hip and was 80% and 60% at 15 Hz and 25

Hz to lumbar spine, respectively (Rubin et al., 2003). However, when the position of the subject was changed to a bent knee posture, the transmission to the hip decreased to below 50% at 15 Hz and below 30% at 35 Hz (Rubin et al., 2003). Kiiski et al. (2008) further expanded these findings by investigating a wider range of frequencies (10 to 90 Hz) at multiple anatomical sites (ankle, knee, hip, and lumbar spine). The authors found that peak acceleration is site-specific and it is greatest at the feet at frequencies between 10 and 40 Hz. However, at higher frequencies ($> 40$ Hz), the transmission declines with the greatest decline observed in the lumbar spine, suggesting that high frequency vibration signals are attenuated by soft tissue during the journey inside the body.

### 1.4.4 Experimental studies of WBV

The first studies of the effect of WBV on bone density were carried out on animal models. For instance, Flieger et al. (1998) vibrated ovariectomised rats for five weeks and reported a significant increase in bone density in rats that underwent vibration compared to the control group. The strongest evidence regarding the anabolic effect of WBV on bone density has been provided by two studies that were carried out on sheep for 12 months (Rubin et al., 2001, Rubin et al., 2002). The vibration protocol consisted of low-magnitude high frequency WBV for 20 minutes daily for 12 months. The two studies reported significant increases in BMD in the tibia of sheep that underwent vibration compared to the sheep in the control group. In addition, a 34.2 % increase in tibial volumetric BMD was found in the active group compared to the control group (Rubin et al., 2001). However, these improvements were observed in the hind limbs, not in the front limbs, which may suggest that the osteogenic effects of WBV are more pronounced at weight-bearing

sites when translating this into the clinical context (Rubin et al., 2002). Since then, several animal studies have reported anabolic effects of WBV on bone in rats and mice (Xie et al., 2006, Garman et al., 2007, Oxlund et al., 2003).

Various additional bone outcomes have been reported in animal studies that focus on the anabolic effect of WBV on bone. For instance, a significant increase in the trabecular bone volume of the proximal tibia in mice that were vibrated for five weeks was observed. No other significant changes were detected at distal sites away from the vibration surface (Christiansen and Silva, 2006). In another study, Judex et al. (2007) reported a significant increase in the trabecular bone volume (22% to 25% and trabecular thickness (11% to 12%) in rats that were subjected to high frequency (95 Hz) WBV for 28 days. Several studies have also reported improvement in bone formation rates (Oxlund et al., 2003, Garman et al., 2007, Judex et al., 2007).

It should be noted that most recent animal studies have used a low intensity, high frequency WBV protocol due to two studies which compared different magnitude and frequencies on bone indicating that the low magnitude high frequency WBV protocols were more effective in enhancing trabecular bone (Judex et al., 2007, Garman et al., 2007). This finding suggests that the frequency of WBV plays a more important role than WBV magnitude in animal studies.

Furthermore, factors such as vitamin D status may also alter the bone's response to mechanical loading. In a recent study, vitamin D deficient or replete pregnant mice were used to determine the effect of early vitamin D status on skeletal health later in life (Borg et al., 2018). The offspring of the mice in the two groups received a vitamin D-sufficient diet at weaning and were loaded at 10 weeks and 18 weeks of

age. The offspring of the replete mice showed an increase in both trabecular and cortical bone mass in the younger animals, but only in the cortical bone of the older animals suggesting a positive role of early vitamin D on the response to loading (Borg et al., 2018).

## 1.4.5 Clinical studies of the effect of WBV on bone

Due to the promising results of the effect of WBV on bone in animal studies, a significant number of clinical studies have been conducted in the past 15 years. However, the results of the clinical studies have not matched the encouraging results of experimental models, and so the role of WBV in enhancing bone health in humans is currently debated in the literature. It is not surprising that most of the clinical trials that investigated the effect of WBV on bone density were conducted on postmenopausal women (Russo et al., 2003, Rubin et al., 2004, Verschueren et al., 2004, Iwamoto et al., 2005, Gusi et al., 2006, Ruan et al., 2008, Beck and Norling, 2010, Bemben et al., 2010, Von Stengel et al., 2011, Verschueren et al., 2011, Slatkovska et al., 2011, Karakiriou et al., 2012, Lai et al., 2013, Slatkovska et al., 2014, Leung et al., 2014, Kiel et al., 2015, Liphardt et al., 2015, Marín-Cascales et al., 2017, Zha et al., 2012b). This is because postmenopausal women have a greater risk of developing osteoporosis and thus WBV might be a non-pharmacological strategy to preserve their bone mass and reduce their risk of osteoporosis. Nevertheless, WBV trials have also included different populations such as men (Elmantaser et al., 2012), children with osteogenesis imperfecta (Ward et al., 2004, Högler et al., 2017), children with cerebral palsy (Wren et al., 2010, Ruck et al., 2010, El-Shamy, 2017), young women with low bone mass (Gilsanz et

al., 2006, Lam et al., 2013) and apparently healthy populations (Torvinen et al., 2003, Ligouri et al., 2012, Elmantaser et al., 2012, Gómez-Bruton et al., 2017).

The results of these studies are inconsistent. For instance, two trials involving postmenopausal women obtained differing results. In one, a high frequency (30 Hz), high magnitude (3.2 g) WBV protocol significantly improved lumbar spine BMD in postmenopausal women after 6 months of training (Lai et al., 2013). In the other, 18 months of high frequency (35 Hz), low magnitude (0.3 g) WBV did not lead to any significant change in BMD at lumbar spine or hip (Leung et al., 2014). Nevertheless, Zha et al. (2012a) used a higher frequency (45 – 55 Hz) and low magnitude (0.5 – 0.8 g) and found a significant increase in BMD at lumber spine and femoral neck after 6 months of WBV training. However, this study was limited due to its heterogeneous population (n= 68) of males and females across a wide age range (from 51 to 93 years) with and without osteoporosis. Due to this, the numbers within each subgroup were extremely limited. The apparently contradictory results observed thus far in studies may have been caused by several factors. These include the different populations under investigation, different durations of the intervention, the imaging technique that was used to determine the effect of intervention, the anatomical site where the effect was reported, the duration of the WBV, and the wide variation of the WBV protocols used.

While most studies in the literature have focused on the effect of WBV on areal BMD, it is clear that the full extent of such an effect has not yet been investigated. This is because BMD measured by DXA is only a surrogate measure of the amount of minerals in the bone and does not reflect the true BMD or the bone microstructure, which is overlooked when using only DXA to determine the effect

of WBV. Very few studies have used high-resolution peripheral quantitative computed tomography (HR-pQCT) to determine the full extent of the effect of WBV on bone quality. Slatkovska et al. (2011) published one of the earliest studies to use both DXA and HR-pQCT to determine the effect of WBV on bone but no significant changes were detected after 12 months of high frequency (30 to 90 Hz) and low magnitude (0.3 g) WBV on bone parameters at the distal tibia and the distal radius. In another trial that used this combination of measurement techniques, Lam et al. (2013) investigated the effect of low magnitude (0.3 g), high frequency (32–37 Hz) WBV on bone quality in osteopenic girls. Although there was a significant improvement in areal BMD, there was no significant improvement in HR-pQCT parameters after 12 months of WBV. In a recent study that used HR-pQCT, no significant changes in bone outcome in postmenopausal women with osteopenia was found after 12 months of WBV even when using higher magnitudes (2.4 –3.2 g) (Liphardt et al., 2015). However, Verschueren et al. (2011) found a significant improvement in volumetric hip BMD after 6 months of high magnitude (2.2 g) WBV in institutionalised elderly females without osteoporosis or osteopenia. Clearly, the results of these studies point to the fact that it is hard to define an effective WBV protocol or specify a target population despite the significant number of studies that have investigated the effects of WBV on the human skeleton.

Due to the great interest in WBV as evidenced by the number of studies on its effects on the skeleton, there have been several systematic reviews published of the effects of WBV on bone density as primary or secondary outcome (Merriman and Jackson, 2009, Slatkovska et al., 2010, Mikhael et al., 2010, Lau et al., 2011, Ma et al., 2016, Oliveira et al., 2016, Fratini et al., 2016, Marín-Cascales et al., 2018, Jepsen et al., 2017, Luo et al., 2017). The results of the systematic reviews are

inconclusive. Some reviewers have reported improvement at one or more anatomical sites where other reviews have reported no overall effect. Significant improvements in hip BMD were reported in three reviews (Merriman and Jackson, 2009, Slatkovska et al., 2010, Fratini et al., 2016) and significant improvements in lumber spine BMD were reported in four reviews (Fratini et al., 2016, Ma et al., 2016, Oliveira et al., 2016, Marín-Cascales et al., 2018). Additionally, (Slatkovska et al., 2010)) reported significant improvements in spine and tibia of children and adolescents. However, no significant effect of WBV on bone density was found in four systematic reviews (Mikhael et al., 2010, Lau et al., 2011, Jepsen et al., 2017, Luo et al., 2017). This contradiction in the results of these systematic reviews could have resulted from the authors of the reviews using different inclusion/exclusion criteria. For instance, Oliveira et al. (2016) reviewed effects of WBV on BMD in postmenopausal women and included 15 studies in their analysis while a later review by Luo et al. (2017) was limited to women diagnosed with postmenopausal osteoporosis and included only 9 studies.

Nonetheless, some patterns regarding the effect of WBV have started to emerge which may aid in designing more robust future trials or help in identifying an effective WBV training protocol. The lumbar spine for instance has been found to be more sensitive to WBV as significant improvement in the lumbar spine was found when analysing BMD for all WBV magnitudes (Ma et al., 2016, Marín-Cascales et al., 2018) and when analysis was repeated after excluding the low quality studies (Oliveira et al., 2016). Furthermore, side-alternating platforms seem to have had a greater impact in bone density, especially when knees were slightly bent during the vibration sessions (Oliveira et al., 2016, Fratini et al., 2016). This could be due to the side-alternating movement resembling normal gait which would

then lead to greater muscle contractions (De Zepetnek et al., 2009, Fratini et al., 2016).

Nutritional status, especially in respect of vitamin D, may alter the response to physical activity. In studying the benefits of supplementing personnel undergoing military training with calcium (2000 mg) and vitamin D (1000 IU) delivered as 2 snack bars per day, it was found that significant improvement in volumetric BMD at tibia was observed in the group who received vitamin D and calcium snack bars (Gaffney-Stomberg et al., 2014). In addition, the early animal model revealed a positive role for vitamin D status in early life in bone's response to mechanical loading (Borg et al., 2018). However, very few WBV studies had supplemented their participants with vitamin D (Slatkovska et al., 2011, Verschueren et al., 2011, Kiel et al., 2015).

These three studies are summarised in Table 1.2 (next page). There were no significant advantages for adding vitamin D to WBV in terms of improving bone density in three studies. However, there were limitations for these trials. These limitations include the absence of the baseline serum vitamin D outcomes in two trials, the absence of control groups for vitamin D in all three trials and self-reported adherence to vitamin D supplements in all three trials. Furthermore, the fact that the majority of participants were postmenopausal women with osteopenia and thus could be less responsive to mechanical stimulation because this population typically loses bone density over time (Slatkovska et al., 2010). Therefore, it is not currently clear if the positive effect of physical activity and vitamin D on bone, which was reported by (Gaffney-Stomberg et al., 2014) will be observed with regard to WBV and vitamin D.

Table 1.2: Summary of studies investigating the effect of both WBV and vitamin D on bone

| Author & Year | Population, age (range) | Duration | WBV protocol | supplements | Outcome Measures | Conclusion |
|---|---|---|---|---|---|---|
| (Slatkovska et al., 2011) | 202 females BMD T-scores between -1.0 and -2.5<br><br>60 years (44 to 79) | 12 months | G = 0.3g Frequency = 90-Hz OR 30 Hz Daily Duration = 20 minutes | 1000 IU vitamin D daily and 1200 mg of calcium from diet and supplements | HR-pQCT[1] (at the distal tibia and distal radius)<br><br>DXA[2] (areal BMD at femoral neck, total hip and spine) | WBV and daily vitamin D and calcium had no effect on BMD or bone structure of the study population after 12 months compared to vitamin D and calcium alone Change in vitamin D levels were not reported |
| (Verschueren et al., 2011) | 113 females BMD T-scores not reported<br><br>79.6 years (Not reported but all women were >70) | 6 months | G= 1.6g to 2.2g Frequency = 30 to 40 Hz 3 times / week Duration= 15 minutes | 1600 IU vitamin D daily *OR* 880 IU vitamin D daily and 1000 mg calcium daily | DXA (BMD at hip) Serum vitamin D | WBV and daily vitamin D and calcium did not improve hip BMD compared to vitamin D and calcium alone However, the high dose of vitamin D significantly improved serum vitamin D levels |
| (Kiel et al., 2015) | 174 (117 females, 57 males) BMD T scores between –1 to –2.5<br><br>82 years (62 to 102) | 24 months extend-ed to 36 months | G = 0.3g Frequency = 37 Hz Daily Duration = 10 minutes | 800 IU vitamin D daily and 1000 mg calcium daily | QCT[3] (volumetric BMD at hip and spine)<br><br>Biochemical markers of bone turnover | No statistically significant beneficial effect of WBV and daily vitamin D and calcium on the BMD compared to vitamin D and calcium alone Changes in vitamin D levels were not reported |

*1. HR-pQCT = high resolution peripheral quantitative computed tomography*
*2. DXA = dual energy x-ray absorptiometry*
*3. QCT = quantitative computed tomography*

## 1.5 Vitamin D

### 1.5.1 The role, forms and sources of vitamin D

Vitamin D is one of the most important dietary components for bone health and is a fat-soluble vitamin, vital for many metabolic processes in the body such as effective absorption of calcium, calcium and phosphate homeostasis and bone mineralisation (DeLuca, 2004, Tsiaras and Weinstock, 2011). Vitamin D deficiency has been linked to several musculoskeletal and non-musculoskeletal diseases such as osteoporosis cardiovascular disease, diabetes and cancer (Holick, 2004, Holick, 2007).

There are two forms of vitamin D: vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). These two forms have the same biological function but slightly different structures relating to their side chains. Vitamin D3 is transformed into 25 (OH) D3 and then into 1, 25 $(OH)_2D_3$, and vitamin D2 is transformed into 25 (OH) $D_2$ and then $1,25(OH)_2D_2$. Vitamin D3 is synthesised in the skin by exposure to ultraviolet B (UV-B) and found in some oily fish, such as salmon, while vitamin D2 is naturally found in some plants (Holick et al., 2011). Although the two forms have the same biological effects, it has been reported that vitamin D3 is more effective than vitamin D2 in increasing serum 25 (OH) D, and vitamin D2 may be better for reducing fracture risk (Kearns et al., 2013).

Exposure to UV-B at a wavelength of 280-320 nm is the primary source of vitamin D due to the cutaneous synthesis of vitamin D3 (Holick, 2007, Cranney et al., 2007, Lehmann and Meurer, 2010, (SACN), 2016). It has been reported that vitamin D production is more effective in a narrower wavelength band of 295- 300nm with

peak production at wavelength of 297 nm (MacLaughlin et al., 1982). However, in high latitude countries, such as in the UK, dietary products become the main source of vitamin D during the winter period due to the limited sunlight (Cranney et al., 2007, (SACN), 2016). Additionally, there are limited natural dietary sources for vitamin D (Spiro and Buttriss, 2014). Oily fish such as salmon, egg yolk and sun-exposed mushrooms are among these dietary sources (Holick, 2007, Holick et al., 2011).

In spite of the fact that sources of vitamin D have been known for several decades, there are several factors that might have been overlooked in terms of the amount of vitamin D obtained from these sources. For instance, the amount of vitamin D produced from sun exposure depends on several factors such as time of day, season, pollution level, skin pigmentation (melanin), use of sunscreen and obesity status (Cranney et al., 2007, Tsiaras and Weinstock, 2011). Moreover, it has been reported that the amount of vitamin D in some naturally rich dietary sources of vitamin D is significantly affected by the origin of these foods, as well as the way they are prepared for intake. For example, a comparison between the same amount of farmed salmon and wild caught salmon revealed that farmed salmon contains only 25% of the vitamin D that is present in wild caught salmon (Lu et al., 2007). Furthermore, the amount of vitamin D in the farmed salmon is affected by the cooking method. When the farmed salmon was baked, approximately 98% of the original vitamin D concentration was recovered compared to approximately 50% when the salmon was fried (Lu et al., 2007).

The Scientific Advisory Committee on Nutrition (SACN) recommends a 10 µg/day (400 IU) of vitamin D as a Reference Nutrient Intake (RNI) for the UK general

population during the winter months. However, some groups with limited sun exposure during the summer months should aim to achieve this RNI level year-round. Due to the limited resources of food rich with vitamin D, food fortified with vitamin D or vitamin D supplements may be the only alternatives to achieve this RNI. However, although vitamin D supplementation can be a good strategy to maintain an adequate level of vitamin D, adherence to daily supplements may not be maintained over a long period. Therefore, large doses given at regular intervals might be an alternative option to daily doses. More research regarding the effect of large doses of vitamin D is needed in order to accumulate robust evidence that can be used as a basis for future recommendations and practical advice.

## 1.5.2 The metabolism of vitamin D

Vitamin D - either from sun exposure or from diet - has to undergo several metabolic processes in order to be converted to its active biological form. When the UV-B penetrates the skin between wavelengths 280-320, it is absorbed by 7-dehydrocholesterol which leads to the production of previtamin D3 due to breaks in the chemical bonds of the 7-dehydrocholesterol molecule which are caused by the absorbed energy (Tsiaras and Weinstock, 2011). The resulting previtamin D3 is not thermodynamically stable and at body temperature it is converted to vitamin D3, enters the circulation and is transported to the liver where it is hydroxylased to 25-hydroxyvitamin D (25(OH)D), a major circulating form of vitamin D (Holick, 2007, Lehmann and Meurer, 2010). The resulting 25-hydroxyvitamin D is bound to vitamin D binding protein and transported to the kidneys where it is further metabolised by 25-hydroxyvitamin D-1α- hydroxylase and is then finally converted into its biologically active form, 1,25-dihydroxyvitamin D (Lehmann and Meurer,

2010). The production of 1,25-dihydroxyvitamin D is tightly regulated by several factors: most importantly the presence of parathyroid hormone, calcium, phosphate, calcitonin, and fibroblast growth factor 23 (Lehmann and Meurer, 2010, Allgrove and Shaw, 2015). Figure 1.6 (below) summarises the pathway for vitamin D metabolism.
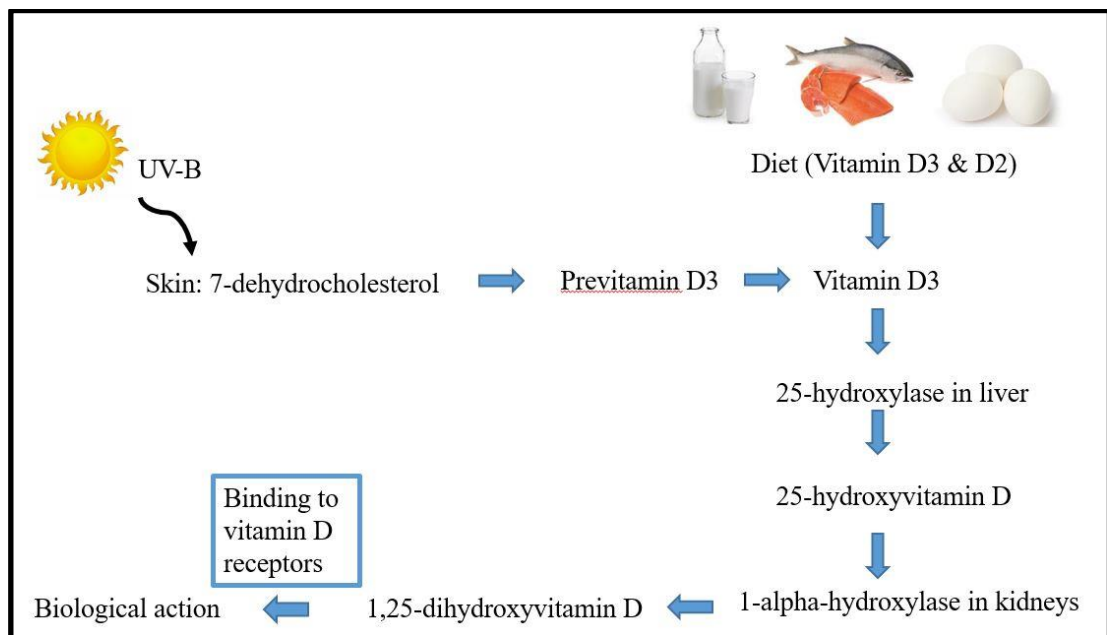


*Figure 1.6: Simplified diagram of vitamin D metabolism. Adapted from (Nair and Maseeh, 2012).*

## 1.5.3 The measurement and concentration of vitamin D

Sufficient concentration of vitamin D is important to optimise bone health over a lifespan with vitamin D deficiency being associated with a number of skeletal fragilities including rickets, osteomalacia and osteoporosis. It is highly recommended that vitamin D status is assessed by measuring the total serum 25-hydroxyvitamin D ((25(OH) D)), which is the most useful indictor for vitamin D and represents vitamin D from both diet and sun exposure (DeLuca, 2004, Calvo et al., 2005, Holick et al., 2011). Testing for 1,25-dihydroxyvitamin D, the active form

of vitamin D, can be challenging due to its relatively short half-life of around 4 hours (Holick et al., 2011) compared to the 2-3-week half-life of 25 (OH) D (Holick, 2009). In addition, 1,25-dihydroxyvitamin D circulates at an extremely low level in the blood and does not reflect the true reserve of vitamin D in the body (Holick et al., 2011). Approximately 85% of total 25(OH) D is bound to vitamin D binding protein and 10 to 15% is bound to albumin. Only a very small amount of total 25(OH)D is unbound (free 25-OH-D). The clinical importance of free 25-OH-D is yet to be established and future research should focus on determining such importance according to a recent study by (Schwartz et al., 2018).

Significant debate is evident in the literature regarding the optimal level of vitamin D, and indeed regarding the level at which vitamin D deficiency occurs. For instance, vitamin D deficiency has been defined as levels of serum 25(OH) D below 50 nmol/L and the optimal level as serum 25(OH) D above 75 nmol/L by the Task Force of the Endocrine Society in the United State (US) and Canada (Holick et al., 2011). This high level of optimal vitamin D concentration was reinforced in a review of vitamin D and bone in the following year (Bikle, 2012). Recently, a group of experts in Saudi Arabia similarly defined vitamin D deficiency as a level of serum 25 (OH)D below 50 nmol/L in an attempt to establish local guidelines (Al-Daghri et al., 2017). However, the Institute of Medicine (IOM) in the US opposes this interpretation of serum 25(OH) D levels (Rosen et al., 2012). The IOM report (based on bone health outcomes) defines vitamin D deficiency as serum 25(OH) D < 30 nmol/L, vitamin D inadequacy as serum 25(OH) D in the range of 30 to 50 nmol/L, and vitamin D sufficiency as serum 25(OH) D > 50 nmol/L (Del Valle et al., 2011, Ross et al., 2011). The UK National Osteoporosis Society supports the use of the IOM classification system for vitamin D levels and suggests that these

levels should be adopted for UK clinical purposes (Francis et al., 2013). Recently, the global consensus recommendations on prevention and management of nutritional rickets used the same levels that were used by the IOM in order to define vitamin D sufficiency, insufficiency and deficiency in their report (Munns et al., 2016).

However, the British Paediatric and Adolescent Bone Group's position statement on vitamin D deficiency defined vitamin D deficiency as levels of serum 25(OH) D below 25 nmol/, vitamin D insufficiency as levels between 25-50 nmol/L and a sufficiency level of above 50 nmol/L (Arundel et al., 2012). The latest report of The Scientific Advisory Committee on Nutrition (SACN) in the UK agrees with the British Paediatric and Adolescent Bone Group's conclusion regarding the plasma level at which vitamin D deficiency occurs. The SACN report states that vitamin D level should not fall below 25 nmol/L for the UK general population and below this level, there is an increased risk of poor musculoskeletal health ((SACN), 2016).

These variations in finding an agreed definition and interpretation for vitamin D levels have arisen for several reasons. One reason being the health outcomes that were used for defining vitamin D deficiency (i.e. osteomalacia prevention, fall prevention, fracture risk, PTH suppression or non-musculoskeletal health). It has been reported that defining vitamin D deficiency should be based on skeletal outcomes due to the limited number of studies regarding vitamin D and non-skeletal health (Arundel et al., 2012, Ross et al., 2011). Another possible reason is the absence of standardised methods for measuring serum 25 (OH) D due to the variations in the assays used by different labs. A further possible reason is the differing opinions held by experts in interpreting the data in their clinical contexts.

For instance, wide variations (up to 30 nmol/L) were reported between six experts regarding the minimum level of serum 25 (OH) D based on fracture prevention (Dawson-Hughes et al., 2005). For the purpose of vitamin D level classification in this thesis, the IOM definition of vitamin D levels, as supported by the UK Osteoporosis Society, will be used.

## 1.5.4 Prevalence of vitamin D deficiency

Low vitamin D level is very common worldwide and regarded as a common public health concern (Calvo et al., 2005, Holick, 2007, Holick and Chen, 2008, Mithal et al., 2009). In the UK, the SACN report indicated that around one quarter of the UK population aged 19 – 64 years have a level of serum 25(OH) D below 25 nmol/L based on the data of the National Diet and Nutrition Survey ((SACN), 2016). There are however seasonal variations in the level of serum vitamin D. For instance, the prevalence of vitamin D at a level below 25 (OH) D in the UK population age 19 – 64 years was 39% in the winter months compared to 8% in the summer months ((SACN), 2016). Nonetheless, certain groups among the UK population have been found to be at a greater risk of vitamin D deficiency regardless of the season of the year. These groups include pregnant and breastfeeding women, elderly people, people with limited sun exposure, such as those who cover their skin for religious or cultural purposes, and those with indoor lifestyles (Hirani et al., 2005, Ford et al., 2006, Francis et al., 2013).

Furthermore, some ethnic groups in the UK population have a greater risk of vitamin D deficiency. The UK population is a diverse population and there have been several waves of migration to the UK from South Asian, African, and Middle Eastern regions in past decades. It is well documented in the literature that South

Asians tend to have a lower concentration of vitamin D compared to Caucasians (Preece et al., 1973, Shaw and Pal, 2002, Ford et al., 2006, Farrar et al., 2011, Gopal-Kothandapani et al., 2018). However, there is a general lack of data regarding the prevalence of vitamin D deficiency among the MENA ethnic groups in the UK. Nevertheless, the high prevalence of vitamin D deficiency in the MENA region is well documented in the literature over the past 30 years, despite there being an abundance of sunshine year-round (Sedrani et al., 1983, Fuleihan and Deeb, 1999, Ghazi et al., 2004, Meddeb et al., 2005, Saadi et al., 2006, Elsammak et al., 2010, Ardawi et al., 2012, Botros et al., 2015, Chakhtoura et al., 2018). In fact, populations from the MENA region registered the lowest value in serum 25(OH) D in some of the most recent international studies (Mithal et al., 2009, Arabi et al., 2010, Hilger et al., 2014).

Due to the widespread low vitamin D levels in the MENA region as well as the limited sunshine most of the year in the UK, it could be hypothesised that the MENA population living in the UK has the same pattern as the original MENA population in terms of the prevalence of vitamin D deficiency. There are several reasons for the high prevalence of vitamin D deficiency among MENA individuals such as the conservative clothing style that covers much of the skin, limited dietary intake of calcium and vitamin D, an indoor lifestyle and absence of national regulations regarding vitamin D food fortification. It has been proposed that these factors account for around 50% of vitamin D deficiency (El-Hajj Fuleihan et al., 2015). Vitamin D deficiency is a complex issue caused by many potential factors as noted in a previous section, and tackling this issue globally is both challenging and time-consuming. In order to do so, educating healthcare professionals and populations at high risk of vitamin D deficiency, and introducing a more efficient

system for monitoring vitamin D deficiency are clearly essential steps (Ahmed et al., 2011).

### 1.5.5 Vitamin D and bone density

As mentioned earlier, most of the literature investigating the optimal level of vitamin D is based on its effects on bone health and more specifically on determining the effect of conventional and high doses of vitamin D supplementation on bone density. For instance, 400 IU of daily vitamin D has been found to increase areal BMD at the femoral neck in elderly women (Ooms et al., 1995). In another one-year trial, 400 IU of daily vitamin D did not lead to significant prevention of areal BMD loss at the hip, while a higher dose of 1000 IU of daily vitamin D led to a significant reduction in areal BMD loss at the hip in elderly women (Macdonald et al., 2013). In another two studies (Dawson-Hughes et al., 1991, Dawson-Hughes et al., 1995), various doses (100 IU to 700 IU) were associated with significant benefits in terms of bone density in postmenopausal women. Another trial reported a significant increase in total hip and femoral neck BMD following vitamin D intervention, but this result might not be generalisable as the participants were Asian women with darker skin colour and low baseline vitamin D, unlike participants in other trials (Islam et al., 2010). It has also been suggested that vitamin D is effective when combined with calcium supplements in the elderly population. In two trials which lasted three years by Dawson-Hughes et al. (1997) and Kärkkäinen et al. (2010), daily supplements of 700 IU of vitamin D plus 500 mg calcium and 800 IU of vitamin D plus 1,000 mg of calcium, respectively, led to a significant increase in total BMD in men and women over 65 years.

Several studies have investigated the effect of single high dose vitamin D on bone density. The baseline level of serum 25 (OH) D may be an important determinant for the effectiveness of vitamin D supplements in improving bone density. For instance, Harwood et al. (2004) reported a positive effect of a large dose of vitamin $D_2$ (300 000 IU administered by injection) in the neck of femur and total hip BMD BMD, in elderly women with low vitamin D at baseline. In contrast, Jorde et al. (2010) found the no positive effect with high weekly doses of vitamin $D_3$ supplementation (40,000 IU or 20,000 IU) in a population of overweight women who were vitamin D sufficient at baseline. Moreover, vitamin D3 supplementation of 100,000 IU/month for two years did not lead to significant improvement in the lumber spine and total body BMD in subjects with serum 25 (OH) D above 30 nmol/L at baseline (Reid et al., 2017). Overall though, two systematic reviews and meta-analysis of the effect of vitamin D supplements on bone density in both the elderly and in adolescents indicate that vitamin D supplementation in a population with an adequate level of vitamin D is unlikely to improve bone density (Reid et al., 2014, Winzenberg et al., 2011). Nevertheless, vitamin D combined with calcium supplements for the prevention of bone loss in elderly populations has been recommended (Tang et al., 2007).

A common issue in reporting the effect of vitamin D on bone density is the fact that most studies have relied on areal BMD (as measured by DXA) to determine the effect of the treatment. More advanced techniques in bone densitometry such as high-resolution imaging may lead to a better understanding of the relationship between vitamin D and bone structure. In a recent trial of horse jockeys, 400 IU of vitamin D supplementation and 800 mg of calcium in a population of those with sufficient vitamin D were found to significantly improve several pQCT parameters

at the proximal tibia (66%) (Silk et al., 2015) but no significant positive effect was detected at the radius in young male jockeys (Silk et al., 2017). However, the result of this trail may not be generalisable since the participants were professional jockeys who have weight restrictions plus the fact that the study contained a small sample size (n= 17).

The recent utilisation of HR-pQCT may potentially lead to an even deeper understanding of the effect of vitamin D supplementation on bone microstructure and quality. However, trials studying the effects of vitamin D using HR-pQCT are very limited in number due to its cost and very recent availability. Of those which have been conducted, Boyd et al. (2015) investigated the relationship between serum 25(OH)D and HR-pQCT parameters in three groups with different vitamin D levels (low= >75 nmol/L, medium= 75-175 nmol/L and high >175 nmol/L). The authors reported no significant relationship between serum 25(OH) D and bone microarchitecture parameters. In another cross-section study in patients with primary hyperparathyroidism, vitamin D deficiency and insufficiency was not found to significantly affect volumetric bone density, bone microarchitecture, or strength (Walker et al., 2016). However, the cross-sectional design of the study should be noted in considering the results. In another recent trial on the effect of high doses of vitamin D (50,000 IU/ week for 24 weeks) on HR-pQCT parameters in juvenile-onset systemic lupus erythematosus (JoSLE) patients who were mostly vitamin D insufficient, a significant increase in trabecular number and a significant decrease in trabecular separation was reported in the vitamin D group (Lima et al., 2018). A recent proposal by Burt et al. (2018) to administer 3 different doses of vitamin D (1000 IU/day or 4000 IU/day or 10,000 IU/day) over 3 years and to use HR-pQCT to longitudinally determine any change in bone density and

microstructure looks promising to enhance our understanding of the effect of various doses of vitamin D on bone.

## 1.6 Radiological methods for assessing bone

Radiological techniques play a pivotal role in assessing bone density. There are several techniques for assessing bone density, from basic techniques available at most hospitals, to very advanced techniques available only at limited centres worldwide. These techniques are listed in Table 1.3 (below) with their technical parameters. The following section provides explanations of the working principle of each of these methods along with a discussion of their applicability, advantages and drawbacks.

Table 1.3: Radiological methods for assessing bone

| Modality | Resolution (μm) | Time (min) | Effective Radiation Dose (μSv) |
|---|---|---|---|
| Conventional Radiography | <50 | <1 | 10 |
| DXA | 1,000–2,500 | <1 | 1 – 6 |
| QUS | 200–500 | <10 | 0 |
| High-resolution MRI | 150–200 | 10–30 | 0 |
| QCT | 250–300 | < 1 | Wrist <10 Hip, Spine 1500-2900 |
| HR-pQCT | 82 | 3 | 3-5 |

*DXA= Dual-energy x-ray absorptiometry*

*QUS= quantitative ultrasonography*

*MRI= magnetic resonance imaging*

*QCT= quantitative computed tomography*

*HR-pQCT= high resolution peripheral quantitative computed tomography*

The choice of the radiological method for assessing bone depends on several factors: the availability of the technique, the clinical or research question being asked, the radiation dose, the age of the patient and the cost of the scan. However, comprehensive assessment of bone may require combining more than one technique. The term bone strength is often used to refer to bone status. The measurement of bone strength involves an assessment of bone mineral (areal or volumetric), bone quality (including bone geometry and bone microarchitecture parameters), and bone material. Figure 1.7 (below) illustrates the different components of bone strength and imaging modalities that can be used to determine the parameters of bone strength.
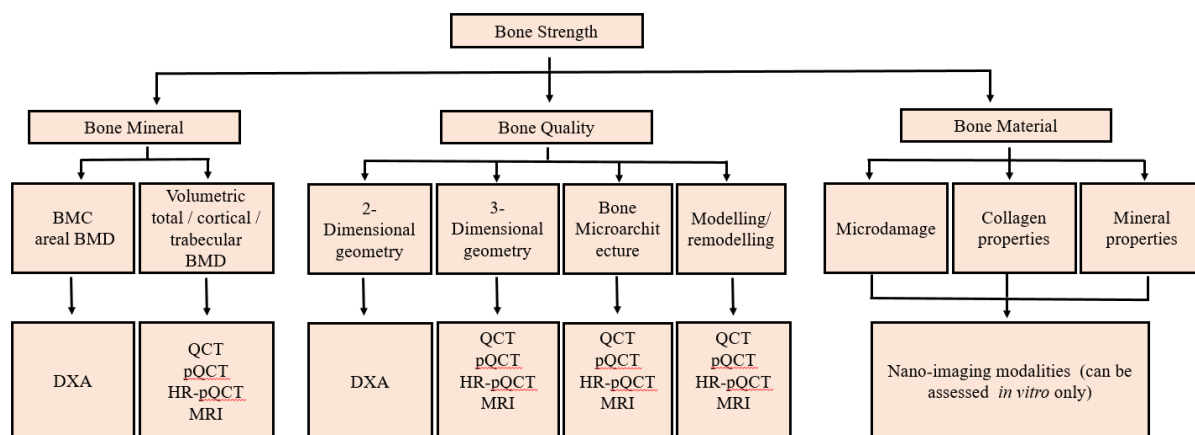


*Figure 1.7: The components of bone strength and the role of medical imaging modalities (bottom row) in assessing bone strength non-invasively. Adapted from Adams, (2013).*

*BMC= bone mineral content*

*BMD= bone mineral density*

*DXA= dual-energy x-ray absorptiometry*

*QMRI= quantitative magnetic resonance imaging*

*QCT= quantitative computed tomography*

*HR-pQCT= high resolution peripheral quantitative computed tomography*

### 1.6.1 Conventional radiography (including radiogrammetry)

Methods were developed in the 1960s to conduct quantitative assessment via conventional radiographs. These techniques are known as radiogrammetry. Radiogrammetry is mainly applied to the second metacarpal, particularly to the mid shaft of the bone. The outcome of this technique is expressed as a metacarpal index which is simply the percentage of the bone's diameter in the mid-shaft area (Adams, 2013). Although this technique is simple, quick to apply and to interpret, it has very poor precision (Adams, 2013).

Although radiogrammetry is a not a popular technique for assessing BMD, especially with the introduction of other more robust methods, there have been some attempts to modernise and digitise radiogrammetry (digital X-ray radiogrammetry (DXR)), particularly since the introduction of digital imaging technology. Automated modules have been developed in order to detect the edge of the metacarpal bones ($2^{nd}$ to $4^{th}$) and automatically produce metacarpal BMD (Jørgensen et al., 2000). Fracture risk is predicted by DXR at different anatomical sites including hip, wrist and vertebra (Bouxsein et al., 2002). More recently, an automated software called 'BoneXpert' has been developed to determine bone mass from hand radiographs. The software can be used in the clinical and research settings and the bone mass outcome are expressed as the bone health index (BHI) (Thodberg et al., 2010). The BoneXpert measurements are taken from the three middle metacarpal bones including the cortical thickness, width and length. The resulted BHI are compared with standard deviation scores (SDS), which is based on large cohort of healthy Caucasian children (Thodberg et al., 2010).

## 1.6.2 Dual-energy x-ray absorptiometry (DXA)

DXA is by far the most common imaging modality for assessing bone density and is regarded as the gold standard for bone densitometry in the clinical setting (Wren and Gilsanz, 2006). DXA was developed in the 1980s and relies on the physical principles of x-ray ionising radiation and its attenuation by the different tissues in the body (Crabtree and Ward, 2015). In DXA, two photons with different energies - one low and one high - are transmitted through the body. Soft tissues attenuate both the high and low energy photons while bone attenuates only the high energy photons. The amount of bone mineral can be calculated by subtracting soft tissue from soft tissue and bone in the whole scanned area (Ward et al., 2016). By using software with an edge-detection algorithm, pixels that represent the start and end of bone in the scan area are determined and bone density can be calculated for each pixel. Areal bone mineral density (aBMD) can be obtained for the scanned area by summing the average values of all pixels in the images, and then by comparing with a mineral phantom (Ward et al., 2016). In addition to BMD, DXA can also measure bone area (BA) by summing all pixels in the image and so bone mineral content (BMC) can be calculated by multiplying BMD by BA (Crabtree et al., 2007). DXA measurements can be taken at various anatomical sites including lumbar spine, femoral neck, hip, forearm and whole body (Wren and Gilsanz, 2006, El Maghraoui and Roux, 2008).

It has been reported that areal BMD accounts for around 60–70% of the variation in bone strength and can be used a surrogate measure for the prediction of fracture risk (Adams, 2013). The BMD generated by DXA is represented in scores of standard deviation compared to the reference data. Two scores can be generated: T-score and Z-score. The T-score is based on the BMD for a young adult

(comparing the patient's BMD with that of a healthy young adult) while the Z-score is based on the BMD for a group of people with similar age and sex (comparing the patient's BMD with that of a similar age and sex) (Cummings et al., 2002, Bachrach et al., 2011). The T-score is mainly used for osteoporosis diagnosis in adults and the Z-score for children but note that DXA is unreliable in children because it is affected by size and given the wide variation in bone size in children (Bishop et al., 2014).

Although DXA is the gold standard for bone densitometry, it has several limitations. Above all, since DXA measures BMD in two dimensions, volumetric density cannot be obtained (Binkovitz et al., 2008). Moreover, the BMD measured by DXA is a ratio between the scanned area and the bone, and therefore BMD can be underestimated in small bones and vice versa, causing it to present difficulties in accurately diagnosing osteoporosis in children (Jones et al., 2006). Figure 1.8 (next page) illustrates the effect of object size when using 2-dimensional techniques such as DXA to represent 3-dimensional objects such as bone. Furthermore, all tissues with high density in DXA scans, such as abdominal aortic calcification and surgical metal implants, are assumed to be bone and contribute to BMD, which causes potential overestimation of the bone's mineral status (Adams, 2013). Finally, DXA does not different cortical from trabecular bone. Despite these limitations, DXA has excellent reproducibility, a short examination time, low radiation dose, low cost and the ability to measure BMD at different anatomical sites (D'Elia et al., 2009).

Cube A
Mineral weight (g) = 16
Volume ($cm^3$) = 8
Projected area ($cm^2$) = 4
Volumetric BMD ($g/cm^3$) = 2
Areal BMD ($g/cm^2$) = 4

Cube B
Mineral weight (g) = 54
Volume ($cm^3$) = 27
Projected area ($cm^2$) = 9
Volumetric BMD ($g/cm^3$) = 2
Areal BMD ($g/cm^2$) = 6
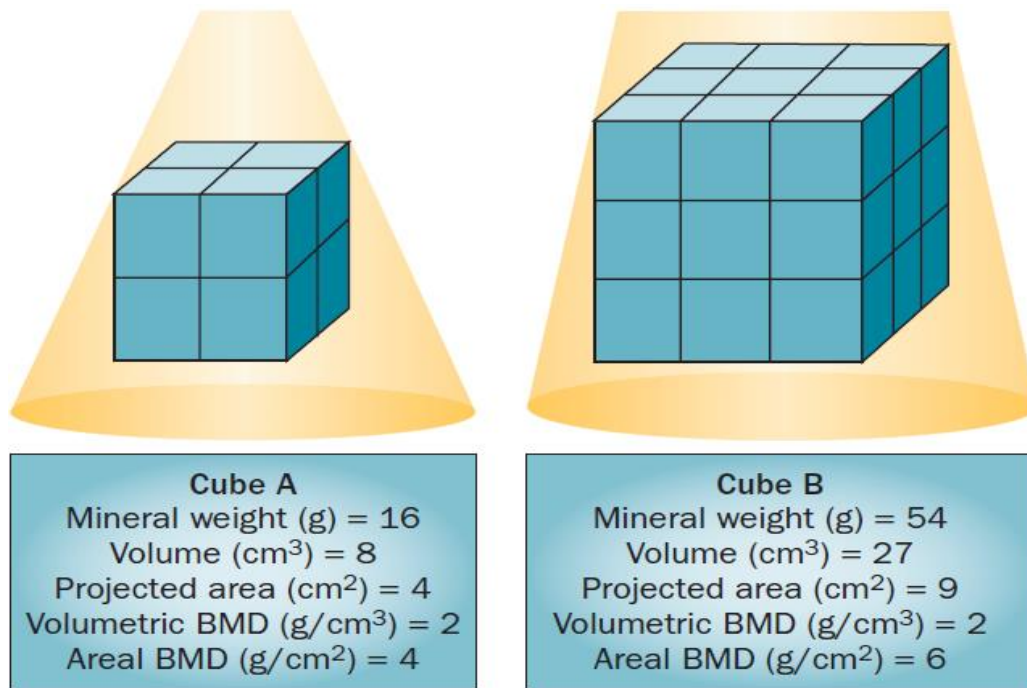
*Figure 1.8: Object size effect on DXA measurements. DXA is limited to two dimensions only and therefore the third dimension (depth) cannot be assessed. Object A has a similar volumetric BMD to Object B despite the fact that Object B has a bigger volume. From Adams, (2013) with permission.*

### 1.6.3 Quantitative computed tomography (QCT)

QCT is a cross-sectional imaging method based on the interaction between x-rays and body tissues and has the ability to visualise a range of tissue densities. In QCT, x-rays are transmitted by a rotating x-ray tube and the resulting intensities are detected by x-ray detectors on the opposite side. These signals are then converted into an attenuation profile (Donnelly, 2011). The attenuation profile contains different tissue densities gathered from individual voxels, which can then be transformed to a corresponding CT number (Hounsfield number). CT number represents a wide range of tissue densities where air is equivalent to a CT number of -1000, water is equivalent to a CT number of 0, bone has a CT number in the range of 400 to 1000 and with soft tissues having densities somewhere in between (Wren and Gilsanz, 2006).

These HU numbers are used to reconstruct a CT image where bone geometry can be obtained using special software and BMD can be obtained by transforming bone CT numbers to BMD (Donnelly, 2011). A bone equivalent phantom is used to calculate cortical and trabecular BMD and the measurements can be taken at central or peripheral sites. However, due to the trabeculae being smaller than the voxel size in QCT, in what is known as partial volume effect, the trabecular BMD obtained by QCT is a combination of bone and marrow (Crabtree and Ward, 2015).

One of the major advantages of QCT is its ability to truly measure volumetric BMD as $mg/cm^3$ (D'Elia et al., 2009). Moreover, QCT can separate the cortical and trabecular components of bone, which means cortical and trabecular BMD can be assessed separately. This is an important feature for determining the change in BMD as trabecular bone is significantly more active than cortical bone in terms of the metabolic process (Adams, 2013). Additional parameters of bone quality, such as its geometry, can be generated with QCT (Wren and Gilsanz, 2006). The vast advancement in CT technology including spiral and multi-detector CT has led to much more widespread and easy assessment of bone.

However, one of the biggest limitations of QCT is radiation dose. Although the radiation dose can be minimised to ~100 μSv by adjusting exposure factors since image quality required for quantitative assessment of skeleton is less than the image quality for standard analysis, it is still significantly greater than DXA (Adams, 2013). Moreover, access to CT may represent a drawback in some hospitals since the demand for CT scanners for other CT procedures is greater than DXA, which can lead to a long delay between scans in longitudinal assessment of BMD (Crabtree and Ward, 2015).

### 1.6.4 Peripheral quantitative computed tomography (pQCT)

pQCT is based on the same principles as QCT but uses a dedicated imaging machine for peripheral sites. pQCT can be used at anatomical sites along radius and tibia, typically at distal parts, mid-shaft and proximal parts of radius and tibia (Adams, 2013). The possible measurements that can be obtained by pQCT include trabecular BMD, cortical BMD, BMC, and bone geometrical parameters. The most important advantages of pQCT compared to standard QCT are the lower radiation dose (≈3µSv) and portability (D'Elia et al., 2009). Although pQCT is a precise technique (Swinford and Warden, 2010), it does have some limitations. The most obvious is that it is restricted to peripheral imaging (Wren and Gilsanz, 2006). In addition, pQCT operates using a "rotate translate" model which is relatively slow (Crabtree and Ward, 2015); therefore patient motion artefact can be a major problem (Adams et al., 2014). Furthermore, positioning the reference line can be technically challenging in the longitudinal assessment of bone in children and adolescents especially for those treated for metabolic bone diseases.

### 1.6.5 High resolution peripheral quantitative computed tomography (HR-pQCT)

HR-pQCT is another form of imaging based on the same physical principle as QCT but with a very high resolution that allows for the determination of bone microstructure (Nishiyama and Shane, 2013). In short, HR-pQCT is a powerful technique for assessing bone strength in a non-invasive manner using a low radiation dose. Its power lies in its ability to discriminate between two patients with identical areal BMD as measured by DXA (Figure 1.9, next page). Due to the high resolution of HR-pQCT (82 µm), it is the only method that has a spatial resolution

similar to the resolution required to assess trabecular bone *in vivo* (Geusens et al., 2014). Moreover, HRpQCT allows assessment of several parameters of bone microstructure that are not measurable by more conventional imaging modalities (Digby et al., 2016).
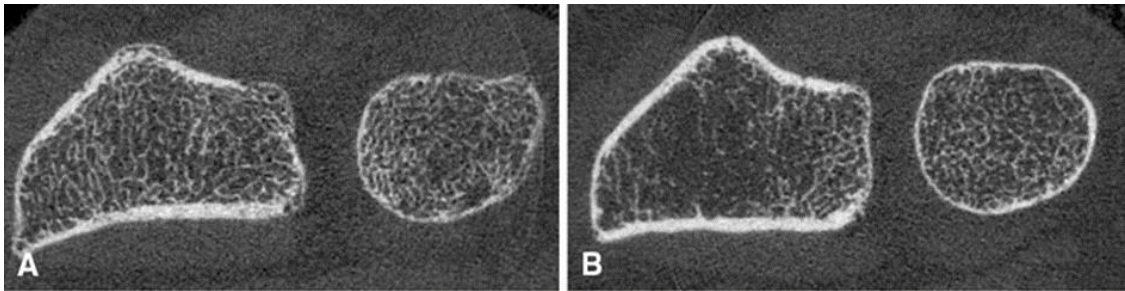


*Figure 1.9: The power of HR-pQCT as a high-resolution technique. A, B; images of radius for two individuals with identical areal BMD as measured by DXA. However, the cross-sectional HR-pQCT revealed significant differences at the trabecular and cortical levels. From (Burghardt et al., 2011) with permission.*

With HRpQCT, the patient's limb is secured inside the gantry of the machine using a special Carbone cast to minimise movement. An x-ray tube rotates around the patient's limb to accrue 110 slices in a scanning time of around 3 minutes. XtremeCT, Scanco Medical, Brüttisellen, Switzerland is the only commercial HR-pQCT scanner (Cheung et al., 2013, Nishiyama and Shane, 2013). Several parameters relating to bone quality can be assessed using HR-pQCT, which include total, cortical and trabecular volumetric BMD and various parameters to assess bone structure (Geusens et al., 2014). These parameters are explained in more detail in the relevant section of Chapter 3 (Table 3.3, Pages 126 and 127). The mechanical properties of bone can also be assessed by the application of finite elements analysis (FEA). However, this is a time consuming process (takes approximately 30 minutes to analyse one bone site) and it requires a powerful computing system (Chapurlat, 2016).

Although HRpQCT is the state-of-the-art for assessing bone quality, this imaging method suffers from some limitations. First of all, patient motion is a common issue. Using a cast and good positioning helps to overcome this issue. Furthermore, as with pQCT, it is limited to peripheral sites, thus making it impossible to directly assess other anatomical sites, such as the thoracolumbar spine, which is a common site of bone fragility (Burghardt et al., 2011). Some studies have assessed the correlation between measurements at peripheral skeleton and central skeleton and reported a strong correlation between the same skeletal sites, but weak to moderate correlation between central and peripheral sites (r = −0.27 to 070) (Cohen et al., 2010, Liu et al., 2010, Amstrup et al., 2016). Nonetheless, the radius is a common site for fracture and the tibia is a weight-bearing site which makes HR-pQCT a valuable and meaningful tool for assessing these two skeletal sites (Cheung et al., 2013). A further limitation of HRpQCT is that it cannot assess the mid-shaft of adult long bones due to the design of the scanner. Moreover, although the voxel size is 82 μm, the actual resolution of images is approximately 130 μm, meaning trabecular microarchitecture, such as trabecular thickness, has to be derived rather than directly measured (Cheung et al., 2013). However, the recent launch of a new generation HR-pQCT scanner (XtremeCTII, Scanco Medical, Brüttisellen, Switzerland) with higher resolution (61 μm) may help overcome these issues as the measurement can be taken from further proximal parts of the bone and trabecular microarchitecture can be directly measured (Manske et al., 2015).

It should be mentioned that HR-pQCT is predominantly a research tool. Many studies using HR-pQCT have focused on the age, sex and ethnic differences of bone microarchitecture. For example, a reduction in the cortical BMD has been found to occur with aging due to an increase in cortical porosity in a Canadian population-

based sample (Macdonald et al., 2011). Few other studies have investigated the related racial difference in bone microarchitecture. In one recent study, several parameters related to the radius such as cortical area, trabecular BMD and stiffness, were found to be greater in African-American girls compared to Caucasian and Asian-American girls (Misra et al., 2017). Recently, HR-pQCT is becoming increasingly used in the change in bone microarchitecture as an objective end point in the assessment of interventions, but its use is hindered by its significant cost.

## 1.6.6 High-resolution magnetic resonance imaging (QMRI)

In contrast to DXA and QCT techniques, high-resolution MRI is a non-ionising imaging modality, a feature which presents an advantage, especially in the imaging of children. A strong magnetic field is required in high-resolution MRI where sequences of radiofrequency pulses can be applied in each direction in three-dimensional space to obtain high-resolution MRI images (Link and Majumdar, 2004). High-resolution MRI can produce the trabecular structure at distal sites of the appendicular skeleton such as distal radius and distal tibia (Wren and Gilsanz, 2006).

Although trabecular bone microarchitecture can be obtained and bone strength can be estimated at high quality, the result obtained for cortical bone is less perfect (Chapurlat, 2016). In addition, MRI can be technically challenging to perform and optimise (Bauer and Link, 2009, Adams, 2013). The relatively long scanning time (around 15 minutes) can increase motion artefact due to voluntary and involuntary motion (D'Elia et al. 2009). Furthermore, claustrophobic patients might be unsuitable candidates for this technique as they are required to be stationary for this length of time in a restricted space (open-bore machines will overcome this

disadvantage). Additionally, the high costs and limited availability of this imaging modality represent further limitations.

### 1.6.7 Quantitative ultrasonography (QUS)

QUS is another non-ionising imaging modality. In this technique, high frequency ultrasound waves (200 kHz and 1.5 MHz) are transmitted through the body and then reflected by bone tissues, which can then be received and analysed by an ultrasound machine (D'Elia et al., 2009). The calcaneus is a common site for bone assessment using QUS. Ultrasound measurements using this technique include parameters that are mainly related to BMD but also reflect elements of bone structure. These parameters include speed of sound, broadband ultrasound attenuation, stiffness index and quantitative ultrasound index (Adams, 2013). QUS has some advantages over other imaging methods such as having a lack of ionising radiation, being low cost, as well as being more portable. In addition, QUS can be more convenient for scanning patients with restricted mobility and children not able to keep still during DXA, QCT or QMRI scanning (Tong et al., 2018). However, QUS can sometimes produce unreliable data if it is performed by an inexperienced operator (Wren and Gilsanz, 2006). Moreover, a recent systematic review found a non-consistent correlation between biochemical bone markers and anthropometric measurements, and QUS parameters in preterm infants (Tong et al., 2018). According to Adams (2013), the application of QUS is not clearly defined in clinical practice and fracture risk assessment using FRAX® cannot be done using QUS parameters.

## 1.7 Summary

Nutrition status especially vitamin D and calcium intake are important determinant of bone health across all life stages. The SCAN report on vitamin D intake has recently introduced a RNI of vitamin D (400 IU/day) but it is not clear if general population are achieving this RNI. The positive effect of physical activity on bone health is well documented in the literature. WBV is a relativity new training approach that may overcome some of the issues associated with conventional methods of exercising such as falls, minor injuries and stress fractures. With many WBV devices on the market and several studies on its effects in the literature, the precise role of WBV in bone health is still under investigation with many studies demonstrating conflicting results. No optimal WBV protocol or target group has been identified. The effect of such vibration may be influenced by several factors, importantly nutritional status and particularly vitamin D as demonstrated in some exercise and animal studies. The effect of WBV on bone can be measured using 2-dimentional imaging modalities such as DXA, which is the most commonly used modality to determine the effect of WBV on bone in the literature. The recent utilisation of HR-pQCT has revolutionised bone-imaging technology and allowed for a deeper assessment of bone not possible with more conventional imaging methods.

## 1.8 Rationale, aims and objectives

### 1.8.1 Rationale, aims and objectives for Section 2 (Chapter 2):

Adequate levels of vitamin D, calcium and physical activity are important modifiable factors for bone accrual in children. It is not clear if children presenting to the emergency department with wrist and ankle injuries are consuming enough amount of vitamin D, calcium and engaging in physical activities.

Aim:

1. To determine the strength of the association between injury outcome (fracture or no fracture) and vitamin D intake, calcium intake and physical activity in children aged between 6 years and 15 years presenting to the Emergency Department (ED) of Sheffield Children's Hospital following injury to their wrist or ankle

Objectives:

1. To assess vitamin D intake among children presenting to the ED of Sheffield Children's Hospital with wrist and ankle injuries
2. To assess calcium intake among children presenting to the ED of Sheffield Children's Hospital with wrist and ankle injuries
3. To assess the physical activity levels among children presenting to the ED of Sheffield Children's Hospital with wrist and ankle injuries

<u>1.8.2</u> <u>Rationale, aims and objectives for Section 3 (Chapters 3, 4, 5 and 6):</u>

While several studies have investigated the effect of WBV on bone health, the results of these studies are ambiguous and equivocal. The need to include vitamin D data when interpreting bone response to physical stimulation has recently been highlighted, particularly due to the significant issue of widespread vitamin D insufficiency. Some exercise and animal studies suggest a positive role for vitamin D in bone's response to mechanical loading, but the extent of this role has not been fully investigated.

With regard to WBV trials, there are only three – as far as we are aware – which have investigated the effect of both WBV and vitamin D on bone density and structure. Of these, in only one study was the primary outcome related to HRpQCT parameters of the distal radius and tibia. There were also several issues with this study such as not controlling for vitamin D – the entire study population received vitamin D supplements. Moreover, the vitamin D intake reported in this study was not assessed for all volunteers and more seriously, baseline serum 25 (OH) D was not known. In addition, the findings of this study might not be generalisable to the public as the study was limited to postmenopausal women with osteopenia.

Thus, there is a need for a well-designed trial to ascertain how bone responds to WBV and vitamin D. The present study aimed to ascertain the role of high dose of vitamin D, on bone's response to WBV as measured by HR-pQCT. The target population for the current study was the MENA population who live in the UK. This is due to the limited research on this ethnic group with regard to WBV and vitamin D despite the high prevalence of osteoporosis and vitamin D deficiency among this ethnic group.

The hypothesis of the proposed research was that a large oral dose of 150,000 IU of vitamin D and 12 weeks of high intensity WBV would improve the bone parameters of the distal tibia as measured by HR-pQCT beyond improvements seen with either vitamin D or vibration alone in a population of young MENA adults living in the UK. The results of this pilot study will allow us to power a larger definitive study.

Primary aim:

1- To determine the effect of a single large dose of vitamin D (150,000 IU of vitamin D3) and 12 weeks of side-alternating WBV on HR-pQCT parameters of the distal tibia

Secondary aims:

1- To determine the change in total serum 25 (OH) D 12 weeks after administering 150,000 IU of vitamin D3 as a single dose

2- To assess the impact of administering 150,000 IU of vitamin D3 on PTH level 12 weeks after administering 150,000 IU of vitamin D3 as a single dose

3- To assess the safety of a large single dose of vitamin D on a selection of the MENA population living in the UK

4- To assess the compliance and safety of side-alternating WBV on individuals from the MENA population

Primary objectives:

1. To determine baseline HR-pQCT parameters of the distal tibia in a young adult MENA population

2. To determine the effect of three months of WBV on volumetric bone density and microarchitecture of the distal tibia as measured by HR-pQCT

3. To determine the effect at three months of a single large dose of oral vitamin D on bone strength determined by FEA of the distal tibia

4. To compare the effect at three months of a combination of WBV and a single large dose of vitamin D on HR-pQCT parameters of the distal tibia

Secondary objectives:

1. To measure calcium intake at baseline

2. To determine the effect of a single large dose of vitamin D on calcium creatinine level one week after dosing

3. To measure baseline serum vitamin D, bone profile and PTH

4. To determine the effect at three months of a single large dose of oral vitamin D on vitamin D levels, bone profile and PTH

5. To compare the effect at three months of a combination of WBV and a single large dose of vitamin D on vitamin D levels, bone profile and PTH

# SECTION 2

# CHAPTER 2

# Vitamin D intake, calcium intake and physical activity among children with wrist and ankle injuries and the association with fracture risk

## 2.1 Abstract

**Objective:** To assess the strength of the association between suggested risk factors and fracture prevalence in children.

**Materials and Method:** A cross sectional observational study. Children aged 6 to 15 years and their guardians presenting to the Emergency Department of a single tertiary paediatric hospital were recruited. Self-reported data on vitamin D intake, calcium intake, and physical activity were collected. All participants had a radiograph of their injured limb reported by a consultant radiologist, on the basis of which they were classified into fracture or no fracture groups. Statistical analysis included descriptive statistics and binary logistic regression.

**Results:** Of the 130 patients recruited, 53 (41%) had sustained a fracture. The overwhelming majority of children (98%) did not consume the recommended daily dietary amount of vitamin D (400 IU/day). Low calcium intake and low levels of physical activity were also ascertained. However, there were no significant differences between fracture and no fracture groups for vitamin D intake, calcium intake or physical activity. Both site of injury (wrist) and sex (males) were associated with increased fracture risk (p=0.001 and p=0.05, respectively). Logistic regression showed a statistically significant relationship between calcium intake and fracture risk (every additional unit of calcium consumption (mg/day) decreased the likelihood of fracture by 0.002, 95% CI, 0.001 - 0.003).

**Conclusion:** Low dietary intake of calcium and vitamin D and low levels of physical activity were evident. Fracture risk was significantly associated with reduced calcium intake but showed no association with vitamin D intake or physical activity.

## 2.2 Introduction

Several epidemiological studies have revealed a substantial increase in the incidence of fractures in children in the past few decades (Cooper et al., 2004; Mathison and Agrawal, 2010; Clark, 2014). Indeed, it has been estimated that around one third of children in the United Kingdom (UK) will suffer from at least one fracture during their childhood (Cooper et al., 2004). While bone health and fracture risk are relatively well understood in the adult population, and in particular in postmenopausal women (Borges and Brandao, 2006), less is understood about this issue in children (Farr et al., 2014). There are several factors that can influence bone health in children including non-modifiable factors such as genetics, age, ethnicity and sex, and modifiable factors such as calcium and vitamin D intake, and physical activity (Golden et al., 2014). Optimising the modifiable factors protects against poor skeletal health and thus reduces fracture risk. For instance, low intake of calcium and milk was found to increase fracture risk (Wyshak and Frisch, 1994; Goulding et al., 2004), while vitamin D supplementation was found to reduce fracture risk (Anderson et al., 2017) in children. In recognition of the importance of vitamin D for skeletal health, the Scientific Advisory Committee on Nutrition (SACN) on vitamin D in the UK has introduced a Reference Nutrient Intake (RNI) of 400 International Units (IU) of vitamin D per day (equivalent to 10 micrograms) for the general UK population aged 4 years and above throughout the year ((SACN), 2016). A final key factor in determining skeletal health is level of physical activity. For example, in a long-running study, a 4-year exercise program was found to increase bone mass (Löfgren et al., 2012).

Although calcium intake, vitamin D and physical activity are important modifiable lifestyle factors for bone health in children, the strength of their impact on fracture

risk in children presenting to an Emergency Department (ED) is not clear. A fall on to the outstretched hand for example is a common occurrence; but it is not understood whether there is a difference in any of the modifiable factors between children who subsequently sustain a fracture compared to those who do not. Therefore, the purpose of this study was to determine calcium intake, vitamin D intake and physical activity among children aged between 6 and 15 years attending the Emergency Department (ED) of Sheffield Children's Hospital following injury to their wrist or ankle and to assess the strength of the association between injury outcome (fracture or no fracture) and these modifiable factors.

## 2.3 Materials and methods

This was a non-interventional, prospective, questionnaire-based observational study, with questionnaires administered independently by two researchers in the ED of Sheffield Children's Hospital between March and August 2015. Full patient and guardian assent/consent was obtained prior to enrolment and the study received Local Research Ethics Committee and NHS Research and Development approvals. The conditions of ethical approval for this study in a busy ED was that children/families would first be approached by a Triage Nurse. Only those interested were then approached by the researchers. Therefore, the number or characteristics of those approached who withheld consent was not recorded. The inclusion criteria were children aged between 6 and 15 years with no known underlying disease or long-term medication use. They had to be able to speak and read English and they were required to have undergone a wrist or ankle radiograph following acute injury to the relevant site. Patients were not eligible if they had been involved in high-energy trauma (e.g. road traffic accidents, falls from a significant

height), because in such circumstances, fracture is more likely to occur regardless of other modifiable factors (Blades et al., 2010). Patients were guided through the questionnaire. Older children (aged 10 years and above) answered the questionnaire themselves, while younger children (aged under 10 years) answered the questionnaire with the aid of their parent(s)/guardian(s). A consultant radiologist with extensive experience in musculoskeletal imaging reported the radiographs, allowing patients to be classified into two groups: "fracture" or "no fracture". The consultant radiologist had no knowledge of the patients' intake of vitamin D and calcium, nor of their level of physical activity.

The questionnaire consisted of four parts. Part 1 related to patient demographics and the mechanism of injury. Patients' age and weight in kilograms were retrieved from their ED notes. Height was recorded to the nearest 0.1cm using a wall-mounted stadiometer. Body mass index (BMI) for each patient was then calculated as weight (kg)/height (m)$^2$. An age and sex specific BMI chart provided by the Royal College of Paediatrics and Child Health (RCPCH) was used to establish BMI percentiles for children (RCPCH, 2013). Patients' ethnic group was self-reported. Socioeconomic status was determined using the English index of multiple deprivation (IMD) 2015 (Department of Communities and Local Government), extracting patients' postcodes from their ED notes to determine their IMD scores. IMD scores for England are ranked from 1 (most deprived area) to 32,844 (least deprived area). IMD scores below 10,894 are considered areas of low socioeconomic status, IMD scores between 10,895 and 21,788 are considered areas of average socioeconomic status, and IMD scores above 21,789 are considered areas of high socioeconomic status. Site and side of injury were retrieved from patients' ED notes and confirmed by inspecting the imaging request cards/images.

Pubertal stage was established in Part 2 of the questionnaire and was self-reported using the Tanner score (Carskadon and Acebo, 1993). Patients under 10 years old were assumed to have a Tanner stage of 0, because onset of puberty is not expected to occur before this age in apparently healthy girls and boys (Rubin et al., 2009; Sørensen et al., 2012). Parts 1 and 2 of the questionnaire were piloted to ensure that there were no issues with the format and that language was appropriate for children.

Part 3 used two previously validated questionnaires to assess vitamin D and calcium intake. The "validated short food frequency questionnaire" (SFFQ) - specifically designed for children - was used to assess vitamin D intake in IU (Nucci et al., 2013). The patients' daily calcium intake was assessed in milligrams (mg) using the "calcium calculator" created by the International Osteoporosis Foundation (IOF) (IOF, 2015). This calcium calculator is validated and accounts for age and sex of the child.

The final part of the questionnaire used the validated "previous day recall questionnaire" to assess physical activity (Children's Physical Activity Research Group, 2002). This generates a metabolic equivalent of task (MET) score from a table of MET scores provided by the Arnold School of Public Health (Ainsworth et al., 2000). Three variables are generated from this physical activity questionnaire: total MET score, number of 3-5 METS (moderate activity) and number of 6/6+ METS (vigorous activity).

Statistical analysis was carried out using the IBM Statistical Package for the Social Sciences (SPSS), version 23. Descriptive statistics were used to summarise the variables including means and standard deviations. To test for any association between the suggested risk factors and injury outcome, the Chi-squared test was

used for categorical variables and an independent t-test for continuous variables. If data were not normally distributed, the non-parametric Mann-Whitney test was used. Finally, binary logistic regression was carried out to calculate odds ratios (ORs) and 95% CIs to assess the impact of individual risk factors on injury outcome. The logistic regression model contained 8 independent variables (vitamin D (IU/day), calcium intake (mg/day), total MET score, number of 3-5 METS (moderate activity), number of 6/6+ METS (vigorous activity), joint affected (wrist, ankle), sex (male, female), age (years). Multicollinearity between the explanatory variables was checked including collinearity between the total MET score, the number of 3-5 METS (moderate activity) and the number of 6/6+ METS (vigorous activity). Since 85% of participants were Caucasian, ethnicity did not significantly contribute to the logistic regression model and hence was removed. Similarly, socioeconomic status did not significantly contribute to the logistic regression model and was removed.

## 2.4 Results

A total of 134 interviews (each taking approximately 30 minutes) were conducted. All interviews were carried out while the patient was still in the ED setting. 4 patients were excluded (3 because the radiographs were of sites other than wrist or ankle, 1 because this was a re-attendance for a previously sustained injury). In total then, 130 datasets were analysed. Of the 130 patients, 53 (41%) sustained a fracture. Patient demographics are summarised in Table 1. Patients' age ranged from 6 to 15 years, and 110 (85%) were Caucasian, 19 (14.5%) Asian and only one (0.5%) was Afro-Caribbean. There were no significant differences between fracture and no fracture groups in terms of age (p=0.07) or ethnicity (p=0.23). However, sex was found to be have an impact on injury outcome, with boys sustaining more fractures

than girls (p=0.05). The wrist accounted for 90 injuries (69%) and wrist injuries were significantly associated with an increased positive fracture outcome compared to ankle injuries (p <0.001). Among the 130 children recruited, 37 (28.5%) (16 in the fracture group) were from areas of high socioeconomic status, 38 (29.2%) (17 in the fracture group) were from areas of average socioeconomic status and 55 (42.3%) (20 in the fracture group) were from areas of low socioeconomic status. There was no significant difference in the socioeconomic status between the fracture and no fracture groups (p= 0.67). Moreover, there were no significant differences in vitamin D intake (p=0.77), calcium intake (p=0.59) and the level of physical activity (p=0.61) between the three socioeconomic classes.

Table 2.1: Patient demographics

|  | Fracture | No Fracture | Total | p value |
|---|---|---|---|---|
| **Number** | 53 | 77 | 130 | |
| **Number of boys** | 36 (28%) | 39 (30%) | 75 (58%) | 0.99 |
| **Number of girls** | 17 (13%) | 38 (29%) | 55 (42%) | 0.73 |
| **Mean age in years (SD)** | 10 (2.5) | 11 (2.4) | 10.6 (2.5) | 0.07 |
| **Boys mean age in years (SD)** | 10.5 (2.5) | 11.3 (2.5) | 10.9 (2.5) | 0.18 |
| **Girls mean age in years (SD)** | 9.2 (2.1) | 10.5 (2.3) | 10 (2.3) | 0.06 |

A total of 96 patients (40 in the fracture group) were in the healthy weight range (9th to 90th BMI centile), 17 patients (6 in the fracture group) were in the 91st to 97th centile, 9 patients (5 in the fracture group) were in the 0.04[th] to -8.99[th] centile, 4 patients (one in the fracture group) were in the 98th to 99.5[th] centile and 4 patients (one in the fracture group) were in the 99.6[th] centile. There was no significant difference in BMI centile between the two groups (p= 0.75).

There were 63 patients (27 in the fracture group) with a pubertal score of 0, 13 patients (8 in the fracture group) with a pubertal score of 1, 17 patients (7 in the fracture group) with a pubertal score of 2, 6 patients (2 in the fracture group) with a pubertal score of 3, 8 patients (2 in the fracture group) with a pubertal score of 4 and 23 patients (7 in the fracture group) with a pubertal score of 5. The distribution of Tanner staging was similar across the two groups (p =0.50).

Mean daily calcium intake for both groups was 713 mg (SD= 283). Overall daily calcium intake was greater in the no fracture group (Figure 1) but did not reach statistical significance (p=0.08). The overwhelming majority of children (98%) did not consume the recommended dietary amount of vitamin D (400 IU/ day), with mean daily vitamin D intake for both groups being 145 IU (SD= 201). Since dietary Vitamin D intake was negatively skewed, the Mann Whitney test was used to investigate any difference between the fracture and no fracture groups. There was no significant difference (p=0.81) in the median vitamin D intake between the two groups (Figure 2).
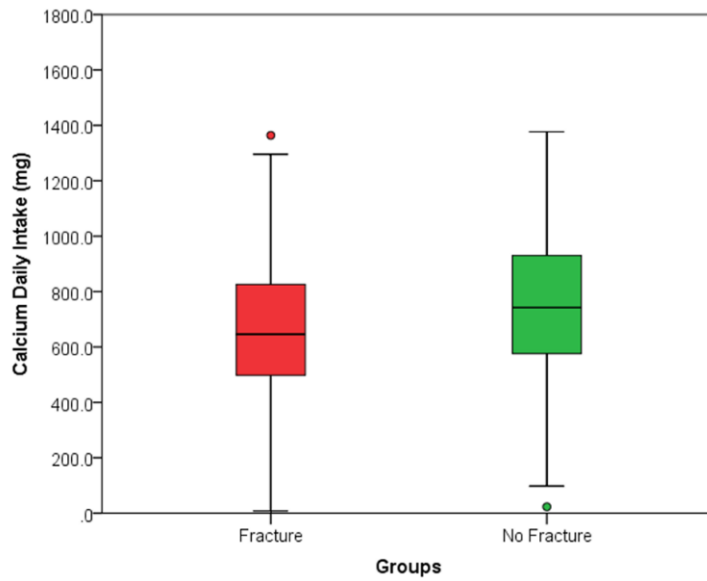
*Figure 2.1: Calcium intake by group*
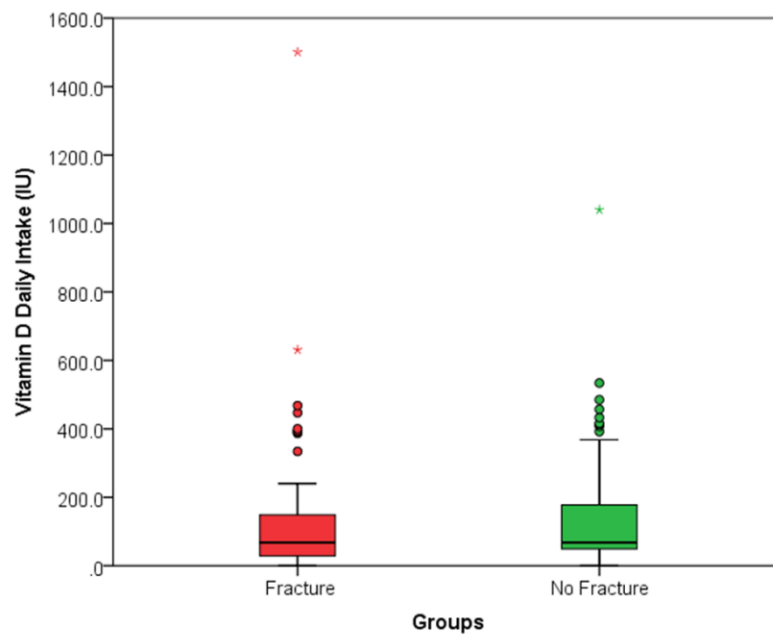


*Figure 2.2: Vitamin D intake by group*

Most children achieved a low total MET score. The distribution of MET scores was

similar between the fracture and no fracture groups (Figure 3). 72 (55%) patients

did not attain any 30-minute blocks of 3-5 MET (moderate activity), while 61 (47%) patients did not attain any 30-minute blocks of 6/6+ MET (vigorous activity) during the day before their injury. There was no significant difference between the two groups in terms of total MET (p=0.07), the number of undertaken moderate activities (p=0.80), or the number of undertaken vigorous activities (p=0.08).
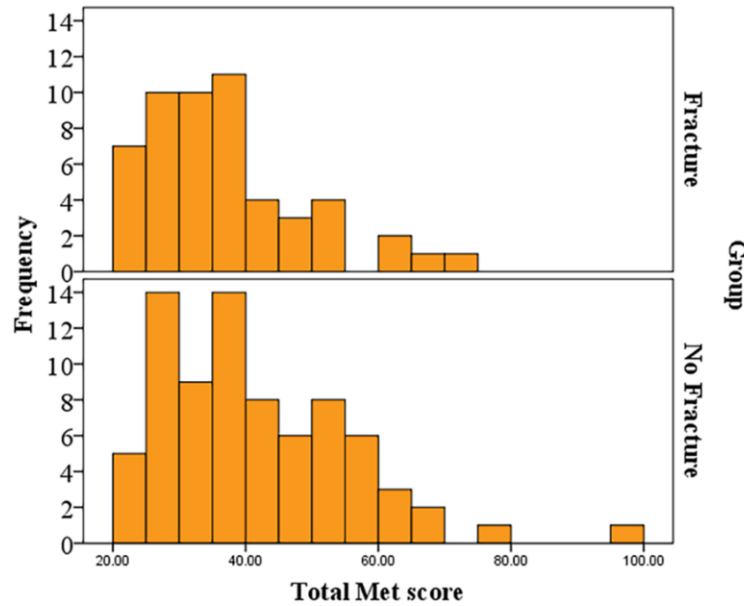


*Figure 2.3: Distribution of the Total MET's Score among the two groups*

Logistic regression was performed to determine the association between 8 explanatory variables and fracture risk. Multicollinearity between the explanatory variables was checked; high correlation was not found between any variables. For the physical activity parameters, the correlation analysis showed no high correlation between the total MET score and the number of 3-5 METS (r=-0.327), the total MET score and the number of 6/6+ METS (r=-0.655) or the number of 3-5 METS and the number 6/6+ METS (r= 0.393). The logistic regression model indicated that there was a significant relationship between 4 variables (age, sex, site of injury and calcium intake) and fracture risk (Table 2). Site of injury was the strongest predictor

of injury outcome (p=0.001), with injury to the wrist being 12 times more likely to cause fracture than ankle injuries. Sex was also a significant predictor of injury outcome (p=0.016). Males were almost 3 times more likely to fracture than females after adjusting for all other variables in the model. The regression model also showed that for every year increase in age, the likelihood of fracture decreases by 0.21 (p=0.016). Every additional unit of calcium consumption (mg/day) was found to decrease the likelihood of fracture by 0.002 (p=0.021).

Table 2.2: Logistic regression model (8 independent variables)

| Variables | p value | OR | 95% C.I. for OR | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Age (years) | 0.016* | 0.791 | 0.653 | 0.957 |
| Sex (female) | 0.032* | 2.650 | 1.087 | 6.459 |
| Joint (ankle) | 0.001* | 11.884 | 3.442 | 41.028 |
| Total MET score | 0.862 | 0.996 | 0.949 | 1.045 |
| Moderate activity | 0.994 | 0.999 | 0.799 | 1.249 |
| Vigorous activity | 0.341 | 0.864 | 0.639 | 1.168 |
| Calcium intake (mg/day) | 0.021* | 0.998 | 0.997 | 0.999 |
| Vitamin D intake (IU/day) | 0.174 | 1.002 | 0.999 | 1.004 |

Variables were considered significant predictors for fracture if p value <0.05.
* indicates a significant p value
OR = odds ratio

## 2.5 Discussion

The present study indicates that among three modifiable factors for bone health, namely vitamin D intake, calcium intake, and physical activity, only one (calcium intake) has a small but statically significant association with reduced risk of fractures. However, there were significant associations between the non-modifiable factors, sex and site of injury. We showed that girls were less prone to have an injury and, if injured, less likely to fracture (boys' relative risk of fracture was

approximately 3 times that of girls). This finding is consistent with the literature, which indicates that boys are more prone to fracture (Khosla et al., 2003; Cooper et al., 2004; Clark, 2014). While this may be due to the fact that males are more inclined towards dangerous activities which then increases their risk of injury and fractures as a result of high impact accidents (Rennie et al., 2007), we specifically excluded children who attended following major trauma, suggesting that it is the tendency towards risky behaviour rather than the severity of the trauma that is important. Socioeconomic status may have a role in determining fracture risk, as children from deprived areas potentially have an unhealthier diet and may be less engaged in sports activities. However, we found no significant differences in vitamin D intake, calcium intake and physical activity between children from the most deprived areas and children from least deprived areas neither was there an association between socioeconomic status and injury outcome.

It has previously been concluded that upper limb fractures are more prevalent than lower limb fractures in children and adolescents (Mathison and Agrawal, 2010; Rennie et al., 2007). Our study showed that injury to the wrist is 12 times more likely to be associated with fracture than injury to the ankle. This probably reflects the different mechanisms of injury of the wrist and ankle: the majority of wrist injuries occur as a result of falls onto an outstretched hand, conferring significantly more force to the wrist than that conferred to the typical ankle injury (most commonly an inversion injury).

As expected, the mean calcium intake in the fracture group was lower than in the no fracture group (Figure 1). However, this did not reach statistical significance (p=0.08), which may reflect our relatively small sample size. In the regression model, for each additional unit of calcium intake (mg/day), the likelihood of

fracture decreased by 0.002 when controlling for all other variables. This finding needs to be further investigated in a larger study. The low calcium intake may increase the risk of future fracture (Bachrach, 2001), particularly in the fracture group, given that Manias et al. (2006) found lower calcium intake among children with recurrent fractures.

Similarly, we found that children did not consume the recommended amount of dietary vitamin D, with no significant differences between children in the fracture and no fracture groups. The low dietary vitamin D intake may be partially mitigated because sunlight is a major source of vitamin D; however, vitamin D synthesis from sunlight depends on several factors including local climate, time of day, age, skin pigmentation and sunscreen use (Lips et al., 2014). The recent introduction of 400 IU of daily vitamin D as the reference nutrient intake (RNI) by SACN highlights the importance of adequate vitamin D intake. Although the SACN recommendation is 200 IU below the recommendation of the Institute of Medicine (IOM) in the United States (Prentice, 2008), the average daily vitamin D intake in both groups in our study was similar and well below even the SACN guideline levels. Based on our results, we conclude that as far as the skeletal effects of isolated low vitamin D are concerned (none of our patients showed radiographic rickets and we excluded patients with known underlying disease), increased fracture risk does not appear to be an issue.

Physical activity has been proven to increase bone mass, thus reducing fracture risk (Tan et al., 2014). Therefore, we sought to establish whether this impacts upon the outcome of injury. For all three measures of exercise within this study, (total MET score, 3-5 METS and 6/6+ METS), there were no significant differences observed between the two groups.

We have shown no major association between these modifiable factors and fracture risk, however, our results should be interpreted with caution, since any associations (particularly between calcium intake and fracture risk) may become significant in a larger sample size.

Apart from the sample size, the further limitation of this study was the questionnaire format, which may have introduced bias in terms of dietary and exercise recall, with parents/patients overestimating their consumption of healthy foods and physical activity (Cade et al., 2002). It is also possible (but less likely) that values were underestimated. However, a questionnaire design was felt to be the most pragmatic and (using the results from this study), we can now power a larger definitive study, in which food intake and exercise can be recorded prospectively in relevant diaries or perhaps even on an app. To improve the robustness of our results, all questionnaires used have previously been validated in children. Moreover, the number or characteristics of children who declined to participate in the study were not recorded due to the condition of the ethical approval. This may have produced selection bias.

## 2.6 Conclusion

To conclude, although a majority of children and adolescents were below recommended guidelines for exercise levels and vitamin D and calcium intake, only increased calcium intake showed a small but statistically significant relationship with reduced fracture risk.

# SECTION 3

# Double-blind placebo randomised controlled study of the effect of vitamin D and whole body vibration on high-resolution peripheral quantitative computed tomography (HR-pQCT) parameters of the distal tibia

# CHAPTER 3

# METHODOLOGY

## 3.1 Study design

The current study is an educational PhD project. This project was a prospective double-blind placebo pilot randomised controlled study of the effects of WBV and a large dose of vitamin D on bone density and microstructure as measured by HR-pQCT at distal tibia with four parallel study groups. There were 10 participants (5 males, 5 females) in each group. The participants in this study were apparently healthy adult volunteers who emigrated from the MENA region to the UK. They were recruited mainly from the University Of Sheffield, UK. WBV training was delivered using a Galileo platform, which is a side alternating WBV technique (Galileo Advanced platforms). The single high dose of vitamin D (150,000 IU) consisted of 3 mls of Invita D3 50,000 IU oral solution (1 ml per ampoule).

The four groups of the current study were:

1. WBV group: this group received WBV and a placebo.
2. Placebo group: this group received the placebo only and served as the control group in this study.
3. Vitamin D group: this group received one intervention only, namely the high dose of vitamin D.
4. vitamin D +WBV group: this group received both interventions, WBV and a high single dose of vitamin D

This study is a registered trial on a public database which is ClinicalTrials.gov. Figure 3.1 (next page) illustrates a consort flow-chart of the study pathway.

```
┌─────────────────────────────────┐
│   Assessed for eligibility (n=)  │
└─────────────────────────────────┘
                                    ┌──────────────────────────────────────┐
                                    │ Excluded (n= )                        │
                                    │   ❖ Not meeting inclusion criteria (n= )│
                                    │   ❖ Declined to participate (n= )      │
                                    │   ❖ Other reasons (n= )                │
                                    └──────────────────────────────────────┘

              ┌──────────────────┐
              │ Randomisation (40)│
              └──────────────────┘

                   [ Allocation ]
```

| WBV Group | Placebo Group | Vitamin D Group | Vitamin D +WBV Group |
|---|---|---|---|
| Allocated to receive WBV and placebo (n=10) | Allocated to receive placebo only (n=10) | Allocated to receive vitamin D only (n=10) | Allocated to receive WBV and Vitamin D (n=10) |
| ▪ Received the allocated intervention (n=10) | ▪ Receive the allocated intervention (n=10) | ▪ Receive the allocated intervention (n=10) | ▪ Receive the allocated intervention (n=10) |

[ Follow-Up ]

| Lost to follow-up (give reasons) (n= ) | Lost to follow-up (give reasons) (n= ) | Lost to follow-up (give reasons) (n= ) | Lost to follow-up (give reasons) (n= ) |
|---|---|---|---|

[ Analysis ]

| Analysed (n= ) Excluded from analysis (give reasons) (n= ) | Analysed (n= ) Excluded from analysis (give reasons) (n= ) | Analysed (n= ) Excluded from analysis (give reasons) (n= ) | Analysed (n= ) Excluded from analysis (give reasons) (n= ) |
|---|---|---|---|

*Figure 3.1: Consort flow-chart of the study pathway*

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The period of the intervention was 12 weeks. HR-pQCT scans of the distal tibia, vitamin D, bone profile and PTH measurements were taken at baseline (week 0) and 12 weeks after administering either vitamin D or the placebo (at week 13). Taking measurements at these points was vital. This is because establishing the baseline characteristics determines any imbalance between the different study groups while the post-intervention measurements allow for a comparison of groups' responses to the intervention.

The 12 weeks' study duration was chosen in order to strike a balance between the ability to detect changes in HR-pQCT and vitamin D parameters, while successfully recruiting the required number of participants and maintaining their interest in completing the study, since it is well known that dropout rates increase when the study duration increases. Moreover, the 12 weeks' period seems sufficient for vitamin D (in large doses) to be metabolised and any changes in its levels can be still detected. For instance, the concentration of serum 25(OH)D was found to be above 50 nmol/L at 8 weeks (Martineau et al., 2009) and at 12 weeks (Ilahi et al., 2008) after administering 1,000,000 IU of vitamin D as a single dose. However, the dose used in this study is greater by 50,000 IU and its effect is expected to last for at least 12 weeks.

In addition, from the WBV prospective, Vibration can stimulate the bone remodelling and can lead to more bone formation, perhaps through one of the suggested mechanisms, which has been previously discussed in chapter one. When the bone remodelling is activated, the resorption phase takes around 2 weeks followed by the reversal phase, which lasts for around 4 weeks. This phase is followed by the formation phase, which can last for up to 120 days (Hadjidakis and

Androulakis, 2006). However, the bone remodelling cycle is shorter in the cortical bone compared to the trabecular bone (it takes around 120 days) (Agerbaek et al., 1991). The post-intervention measurements in the current study were taken during week 13 after the bassline measurements, which is in the bone formation phase. Previous studies reported significant improvements in BMD after similar period of WBV. For instance, significant improvements were in BMD were after 12 weeks of WBV in children with haemophilia (El-Shamy, 2017), and in another study, a similar period of WBV significantly prevented bone loss in elderly women (Lee et al., 2017). In addition, another study showed significant increase in hip BMD after only 10 weeks of WBV (Prioreschi et al., 2012). It has been reported that the minimum period of WBV to have a measurable effect on bone density is 8 weeks (Mikhael et al., 2010). Therefore, it seems that the 12 weeks' intervention period is sufficient to detect any interaction between WBV and vitamin D.

## 3.2 Study procedures

The study consisted of 7 visits in total to the Clinical Research Facility (CRF) at Sheffield Children's Hospital (SCH) and to the Clinical Research Facility (CRF) at the Northern General Hospital (NGH). In addition to the 7 visits for all participants, participants randomised to WBV were informed that it was expected they would commit to the WBV protocol and undertake a total of 36 WBV sessions over 12 weeks. The vibration sessions were undertaken at the CRF- SCH. There was at least one-day rest between vibration sessions (whenever possible).

HR-pQCT scans and blood measurements were taken in the same week but not necessarily on the same day. This was due to the journey time between NGH where the HR-pQCT scanner is based, and SCH which is the primary study site (around

30 minutes by bus) as well as due to the availability of the scanning machine. Figure 3.2 (next page) illustrates the study timeline and Table 3.1 (page 106) summarises the research activities undertaken during these visits.

Due to the significant commitment that was expected form the volunteers in this study (especially those randomised to WBV), they were offered thank you vouchers of £150 each for participants randomised to WBV and £50 each for participants randomised to non WBV at the end of the study.
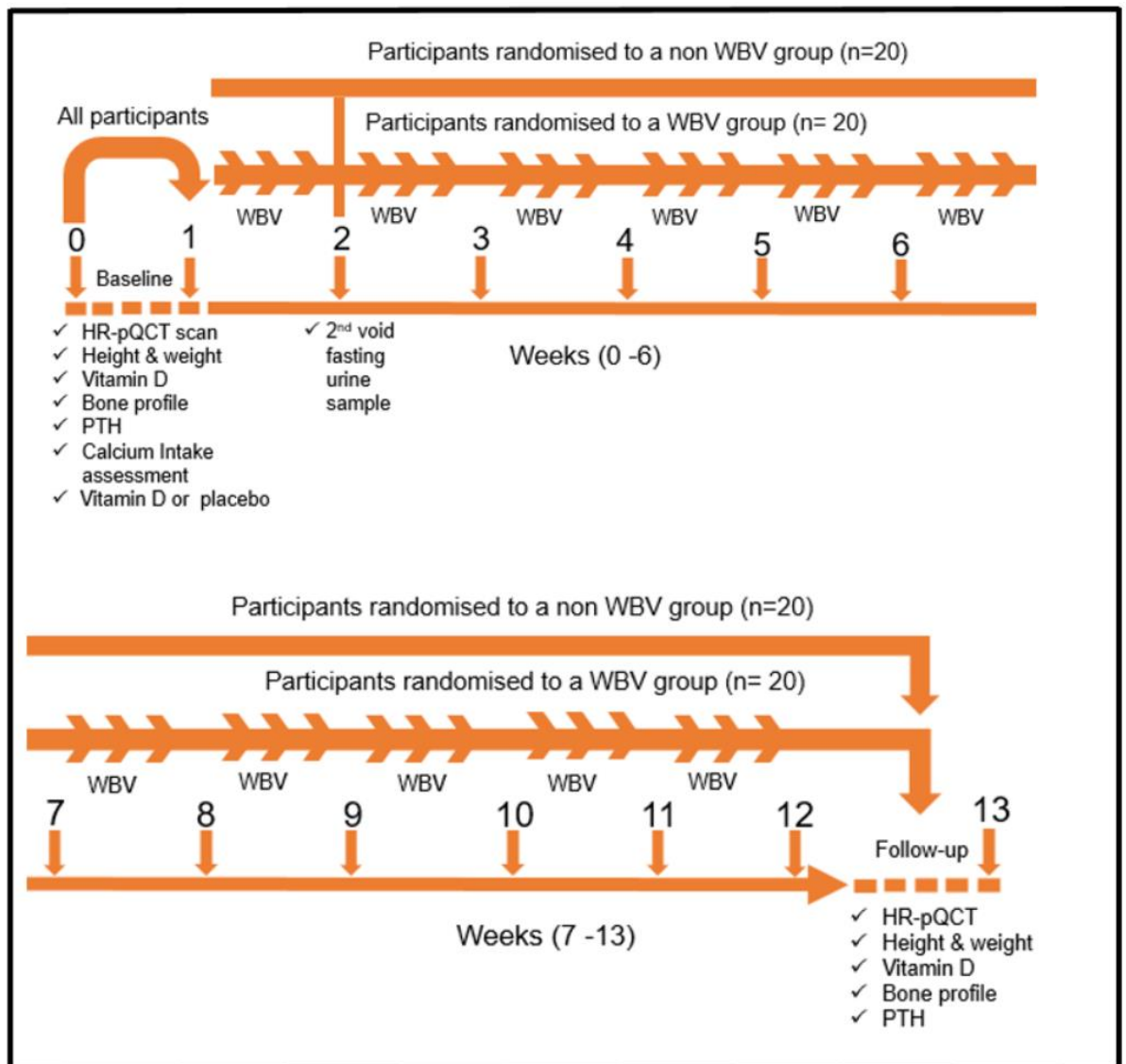
*Figure 3.2: VibeD study timeline*

Table 3.1: Summery of activities undertaken during study visits

| Visit | Location | Procedures |
|---|---|---|
| Visit 1 | SCH | 1. Explaining the study to the potential participant<br>2. Providing the information sheet (Appendix i)<br>3. Answering any questions related to the study<br>4. Assessing volunteer's eligibility using the study standard screening form<br>5. Arranging next visit to sign the informed consent form (Appendix ii) |
| Visit 2 | SCH | 1. Signing the informed consent form<br>2. Arranging the future study visits |
| Visit 3 | CRF at NGH | 1. Obtaining baseline HR-pQCT scans of the non-dominant distal tibia |
| Visit 4 | CRF at SCH | 1. Performing pregnancy test in female participants<br>2. Measuring the volunteer's baseline height and weight<br>3. Obtaining baseline blood samples for vitamin D, bone profile and PTH measurements<br>4. Providing breakfast to the participant<br>5. Administering vitamin D or placebo<br>6. Assessing dietary intake of calcium<br>7. Providing the participant with a urine bottle for $2^{nd}$ void urine sample |
| Visit 5 | CRF at SCH | 1. Participant to provide the $2^{nd}$ void fasting urine (one week after visit 4) |
| Visit 6 | CRF at NGH | 1. Obtain follow-up HR-pQCT scans of the non-dominant distal tibia |
| Visit 7 | CRF at SCH | 1. Measuring volunteer's height and weight at follow-up<br>2. Obtaining follow-up blood samples for vitamin D, bone profile and PTH measurements<br>3. Providing breakfast to the participant and a thank you voucher |

The participants were given at least 24 hours from receiving the information sheet before signing the consent form in order to give them sufficient time to think about the study and ask any further questions. Full informed consent was obtained in writing by the PhD student in accordance with good clinical practice.

Participants were asked to fast (except for water) from midnight of the night before until blood samples were taken. A urinary pregnancy test was performed by a member of the CRF nursing staff with experience of performing the test and offering support should they prove unexpectedly positive. This was performed to avoid the possibility of administering a high dose of vitamin D to a pregnant woman, which is not recommended by the manufacturer of the vitamin D used in the current study. The result of the test was documented by the research nurse.

Participants were reminded about their scheduled visits by email/text message. The clinical study visits were attended by the PhD student who recorded the visit number, date and the study activity/activities undertaken. WBV platforms were set-up for each session in the CRF-SCH by the PhD student. All WBV sessions were supervised and monitored by the PhD student.

## 3.3 Participant selection and recruitment

Inclusion criteria:

1. Males or females aged 18 to 40 years who emigrated from any of the MENA countries to Sheffield, UK (this is the ethnicity of interest for this study). The definition of the MENA countries was based upon the World Bank definition of the MENA countries which include: Algeria, Bahrain, Djibouti, Egypt, Jordan, Iran, Iraq, Kuwait, Lebanon, Libya, Malta, Morocco, Oman, Palestine/Israel, Qatar, Saudi Arabia, Syria, Tunisia, United Arab Emirates (UAE), and Yemen. This approach for defining the MENA countries was previously used in reviewing vitamin D studies in the MENA region (Chakhtoura et al., 2017a, Chakhtoura et al., 2017b) and it was felt suitable to be used for defining the MENA countries in the current study

2. Able to give full informed consent

3. Able to attend for scans, blood tests and/or vibrating sessions at various agreed points during the study

Exclusion criteria:

1. Previous fracture or surgery to any leg

2. Any allergy or hypersensitivity to vitamin D or any of the other ingredients in invitaD3

3. Known metabolic or hereditary bone disease

4. Known to have any of the following; hypercalcaemia, hypercalciuria, pseudohypoparathyroidism, hypervitaminosis D, sarcoidosis, renal calculi (kidney stones) or kidney damage or disease

5. Using, have recently used or might use any other medicines likely to interact with vitamin D

6. Taking vitamin D supplementation at the time of recruitment

7. Any chronic disorder/medication that might affect bone strength

8. Pregnant and breastfeeding women (pregnancy was excluded in all female subjects by performing urinary pregnancy test)

9. Known to have epilepsy or dizziness (in order to avoid falling while standing on the platform)

10. Serious cardiovascular or pulmonary disease or using a pacemaker

11. Restricted mobility (not able to stand on the vibration platform)

12. Allergy or intolerance to dairy products (due to the yoghurt drink which was administered with or without vitamin D)

An eligibility checklist was used for each subject to identify any of the above exclusion criteria (Appendix iii).

In order to advertise the study to potential volunteers, two recruitment strategies were used:

1. The study was advertised to University of Sheffield students and staff using the University Of Sheffield email database, asking any from MENA countries interested in receiving more information to contact either the PhD student or his main supervisor (ACO), should they wish to volunteer (Appendix iv)

2. Posters advertising the study were distributed by the PhD student across the university (Appendix v)

Although the current study required commitment over a relativity long period and involved several procedures including some minimally invasive procedures, recruitment seemed feasible based on our Department's experience from previous studies. For instance, a similar study was carried out in our Department over a 3-month period, in which participants were asked to attend 3 hammering sessions each week. The compliance of the participants was good and 16 out of 18 participants completed the study. Furthermore, a public involvement (PI) event was undertaken by the PhD student, which included 5 subjects (3 males and 2 females) to determine their opinions about attending for vibration training sessions and attending for blood and radiological procedures at various points during the study. The feedback received from the PI event was in general positive. The participants in the PI event were also given the opportunity to give their opinion about the wording of the information sheet and study documents.

From personal interaction with the student community at the University Of Sheffield, it seemed that the number of students from the MENA region would be large enough to achieve the required sample size. Nevertheless, information was sought from the University of Sheffield about precise numbers of students from the MENA region, and their number (711 students) confirmed that recruitment of the required number was feasible within the PhD timeframe.

## 3.5 Randomisation

Randomisation was performed using one of the online tools that is reported in the literature (Saghaei, 2011). A random list was generated by the PhD student's second supervisor (NJB), who was not involved in undertaking the study visits or assessing the outcome. This individual used the online tool; Randomization.com

(Randomization.com.). A block randomisation technique was used with a block size of 8. This was performed to ensure that participants were fairly distributed in the trial arms should the trial have to be terminated early for any reason. Once the eligibility of a volunteer was confirmed and consent had been obtained, ACO, who held the randomisation codes, assigned a study ID number. Randomisation codes were only to be broken in the case of an emergency.

It was explained in the advertising material that volunteers would be randomised into groups. In addition, it was explained to the volunteers in the information sheet why they could not choose their group, and why it was important to randomise them to groups by chance.

## 3.6 Blinding and other measures taken to avoid bias

In terms of allocation to receive vitamin D or the placebo, the study was double-blind. For WBV, a sham device was not used due to the difficulty in obtaining such a device and the limited funding for the current study, therefore, this aspect of the study was not blinded. Failing to obtain a sham device is a well-known limitation in trials involving the use of a device (Fregni et al., 2010).

Having randomised them to their groups, participant study ID numbers were written by ACO onto a sealed opaque envelope containing a signed and dated sheet of paper, completed by ACO, indicating whether the participant had been allocated to vitamin D or placebo. The participant's allocation into WBV or no WBV groups was written on the sealed envelopes alongside the study ID number. Sealed envelopes were handed to the PhD student. Participants' allocation into WBV or no WBV was recorded by the student then the sealed envelopes were taken to the clinical trials team at SCH pharmacy. Sealed envelopes were opened by the clinical

trials staff who communicated with the lead research nurse in this study. The unblinded research nurse in the CRF at SCH collected vitamin D (if applicable), prepared vitamin D or the placebo, checked the participant's identity against the record and administered either vitamin D or placebo. Neither the student nor the volunteer knew whether they had been given vitamin D or placebo.

## 3.7 Withdrawal of subjects

Participants were informed that they could withdraw from the study at any stage without giving a reason. However, to provide researchers with an opportunity to understand their reasons, participants who withdrew from the study were asked if they could give a reason and they were reassured that they did not have to give an answer. Data collected up to the point of their withdrawal were retained and analysed.

## 3.8 Study interventions

### 3.8.1 Vitamin D supplementation

A single dose of 150,000 IU of Vitamin D3 [3 mls of Invita D3 50,000 IU oral solution (1 ml per ampoule)] was administered to those participants randomised to receive vitamin D. The 150,000 IU of Vitamin D3 (Invita D3) was dispensed by the clinical trial team in the SCH pharmacy. The Vitamin D3 or placebo was prepared and administered by a research nurse not involved in the trial assessment within a safe clinical environment at SCH. In order to mask the taste of vitamin D3, it was mixed with a small amount (30 mls) of raspberry flavoured yoghurt drink. Participants who were randomised to receive the placebo also had the same amount of raspberry flavoured yoghurt drink, but no vitamin D was mixed within. The

raspberry flavoured yoghurt was tested by the investigators and two senior members of the clinical trial team in SCH pharmacy using a smaller volume of the same concentration (1 ml of Invita D3 50,000 IU added to 10 ml of raspberry flavoured yoghurt drink or just 10 ml of raspberry flavoured yoghurt drink), and it was agreed that the raspberry flavoured yoghurt drink tasted and looked the same with or without the added vitamin D.

The Invita D3 was supplied by the Pharmacy Department at SCH and was sourced via usual local NHS procurement arrangements. Invita D3 50,000 IU is a licenced product produced by Consilient Health Ltd. Each Invita D3 50,000 IU is a 1 ml solution (1 single-dose oral solution) and contains 1.25 mg colecalciferol, equivalent to 50,000 IU Vitamin D3. 3 ml Invita D3 were given in total per subject. Reported undesirable effects of Invita D3 include hypercalcaemia and hypercalciuria (uncommon, affect <1/100) and pruritus and urticaria (rare, affect <1/1,000). The active metabolite of vitamin D increases calcium absorption from the gut, and acts on bone cells to help maintain calcium homeostasis. In this formulation (Invita D3, Consilient), a 1 ml solution (1 single ¬dose oral solution) contains 1.25 mg colecalciferol, equivalent to 50,000 IU Vitamin D3. The excipients are tocopherol acetate, polyglyceryl oleate (E475), refined olive oil and, refined, sweet orange peel oil.

The administration of the vitamin D or placebo was documented. The advantage of using a single dose is to avoid the issue of non-compliance to the prescribed daily dose. It has been reported that a high single dose should give the same effect of weekly or daily doses over the same period of time (Ish-Shalom et al., 2008). Therefore, 150,000 IU vitamin D3 over the 12 weeks is equivalent to 1785 IU a

day. This daily dose is consistent with the conclusion of a recent systematic review which indicated that individuals from the MENA region need between 1000 IU and 2000 IU of daily vitamin D in order to achieve a concentration of serum 25(OH)D > 50 nmol/L (Chakhtoura et al., 2017b). Ideally, we would have preferred to conduct the study in winter months (October to April) to avoid sun exposure that allows skin synthesis of vitamin D. However, even in the summer months (May to September), sun screen was not used for cultural and religious reasons and because the dress code of people from the MENA region which means that skin is not exposed to much sunlight (Chakhtoura et al., 2017a).

Procedures for identifying contraindications to vitamin D, special warnings and precautions and interactions with other medicinal products are explained in Appendix vi. The procedure in case of undesirable effects and reporting via the Yellow Card Scheme is explained in Appendix vii. Safety aspects of vitamin D are also discussed in more detail in the safety section 3.13.

### 3.8.2 Whole Body Vibration (WBV)

Galileo Advanced platforms were used to deliver high intensity WBV vibration (Figure 3.3 a). The WBV regimen consisted of three supervised training sessions each week for a period of 12 weeks. Each session consisted of three bouts, and each bout lasted for three minutes with a one-minute rest between bouts. The Galileo Advanced platform is a side-alternating WBV machine. It was chosen because it has been shown that side-alternating platforms are more effective in improving BMD than vertical platforms (Fratini et al., 2016). The follow parameters for WBV were used: Frequency = 22 Hz, Peak-to-peak displacement = 4 mm, Acceleration = 3.8 g.

The selected WBV parameters were based on a study conducted by Elmantaser et al. (2012) who used a similar vibration platform in a young adult population and reported that the WBV protocol was well tolerated by participants. Moreover, the authors showed that a Galileo platform was associated with more measurable effect on the endocrine system when compared with a Juvent platform (Elmantaser et al., 2012). More recently, it has been concluded that WBV is more effective when the magnitude of the platform is higher than 3 g and / or the frequency is lower than 25 Hz (Fratini et al., 2016). Taking all of the above into consideration, it was felt that the chosen WBV platform and parameters in the current study could lead to measurable effects on HR-pQCT measurements.

However, since it was expected that the majority of participants would never have used such machines before, and in order to give them the opportunity to get used to the training regimen, the peak-to-peak displacement was increased gradually over the first three weeks. In the first week, displacement was 2 mm; 3 mm in the second week, and from week 3 to week 12, displacement was 4 mm.

Participants stood on the platform shoeless with their knees slightly flexed (Figure 3.3 b, c). This training regimen is compliant with the recommendations of the International Society of Musculoskeletal and Neuronal Interactions (Rauch et al., 2010).
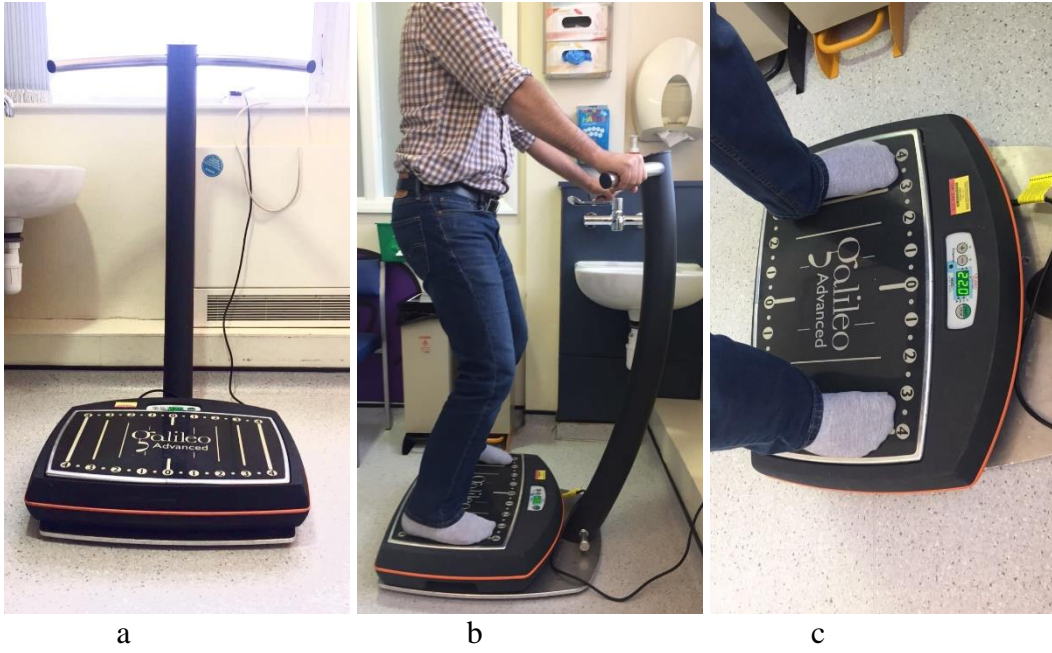
*Figure 3.3: (a); Galileo Advanced platform, (b, c); subjects' position on the platform (from week 3 to week 12).*

Each participant's adherence to the WBV protocol was calculated using the following formula to evaluate his/her compliance:

1. The percentage adherence to WBV training (%) =

   (Number of sessions performed ÷ total number of planned sessions*) ×100

   * Total number of planned sessions = 36

Safety of the WBV training is discussed later in the safety section (page 134).

## 3.9 Measurements

3.9.1 Demographic and anthropometric data

Date of birth, sex, address, country of origin and GP name and address (in order to contact participants' GP in case of abnormal results requiring their attention) were recorded at baseline. Height (m) and weight (kg) were measured at baseline (visit 4) and post–intervention (visit 7) using the same procedure and same instruments on both occasions. An electronic wall-mounted stadiometer (Seca, Birmingham, UK) was used to measure height to the nearest 0.1 cm. Participants were asked to stand with their heels against the wall-mounted stadiometer, combining their feet together and wearing no shoes. Weight was measured to the nearest 0.1 kg using an electronic scale (Seca, Birmingham, UK).

3.9.2 HR-pQCT

*3.9.2.1 Scanning procedure for the distal tibia*

HR-pQCT scans of the non-dominant distal tibia were acquired using a first generation HR-pQCT scanner (XtremeCT, SCANCO AG, Brüttisellen, Switzerland, Figure 3.4) on the high resolution mode. HR-pQCT scans were performed in accordance with the well-established standard operating procedures of the Mellanby Centre for Bone Research, University of Sheffield as previously published (Paggiosi et al., 2014). Table 3.2 (Page 119) summarises the technical scanning parameters of the HR-pQCT scanner.
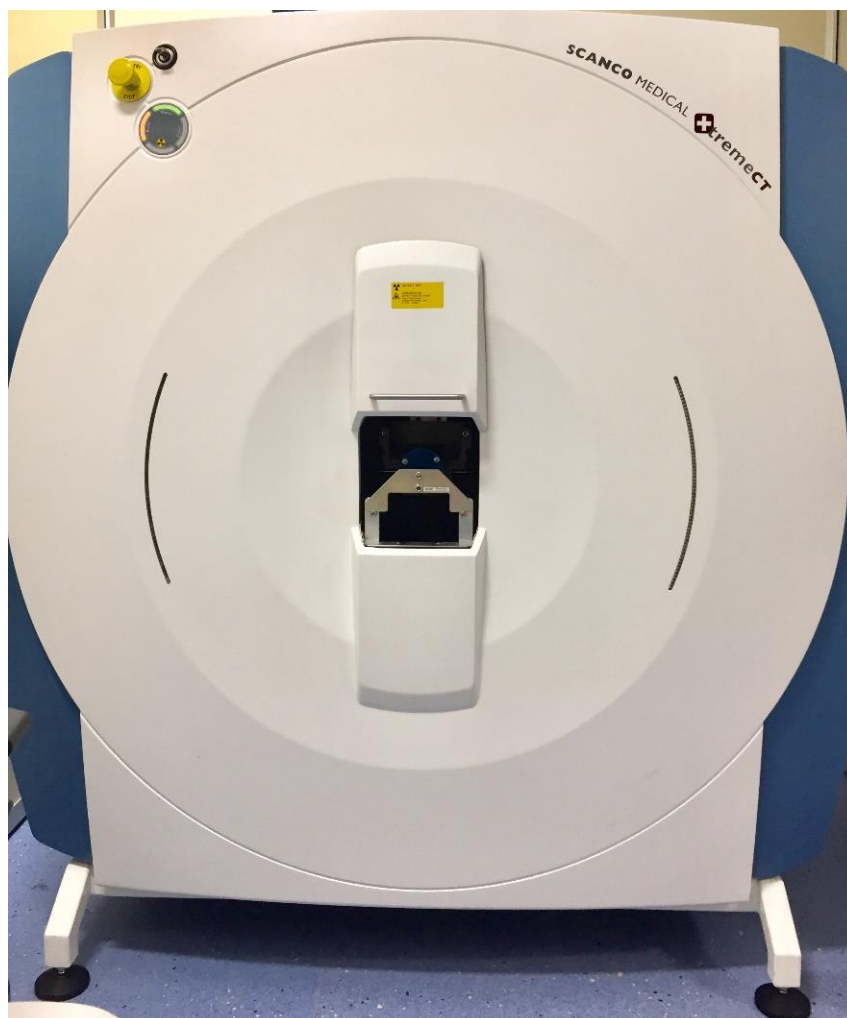
*Figure 3.4: HR-pQCT scanner (XtremeCT, Scanco Medical AG).*

Table 3.2: Technical HR-pQCT scanning parameters

| **Parameters** | **Value** |
|---|---|
| Image matrix | 1536x1536 |
| Source potential | 60 kVp |
| Tube current | 900 mA |
| Integration time | 100 ms |
| Number of slices acquired | 110 slices |
| Scanned area | 150 mm |
| Stack height | 9.02 mm |
| isotropic resolution | 82 µm |
| Total scan time | ~ 3 minutes |

A pre-calibration test was performed prior to positioning the subject's leg inside the scanner. A special leg cast (Figure 3.5 (a)) was used to position the subject's leg inside the gantry of the scanner. This is an essential part of the scanning procedures and is used to reduce movement artefact. Pads and supporting cushions were used for proper positioning when necessary in order to ensure the comfort of the subject and to eliminate movement during the scan. After immobilising the leg in the cast, the chair was raised up to the level of the scanner gantry and the leg cast was placed and secured inside the scanner. The subject was asked to keep as still as possible during the scan. Figure 3.5 (a,b,c,d) illustrate the cast and the positioning procedures.

*Figure 3.5: (a); Leg cast, (b,c,d); scanning procedures*

Before running the HR-pQCT scan, a scout view was acquired in order to identify the anatomical landmarks in the ankle joint and to place the reference line. The reference line was placed on the cortical endplate of the distal tibia (Figure 3.6). The first slice was obtained 22.5 mm from the reference line. A total region of 9.02 mm was scanned and 110 image slices were acquired.
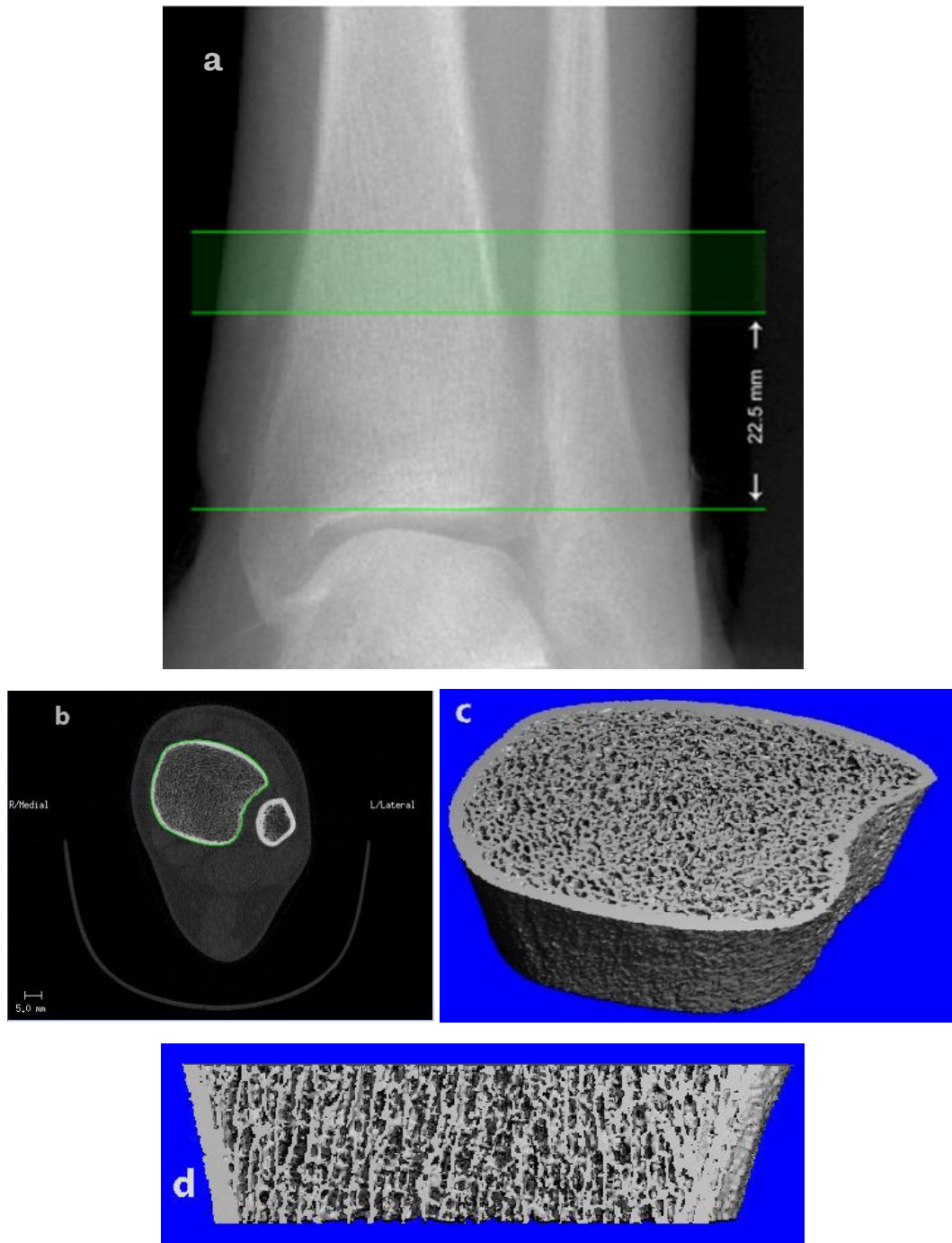
*Figure 3.6: HR-pQCT scan of the distal tibia. a; positioning the reference line on the scout view. The single green line represents the reference line. The Solid green area (9.02 mm) represents the location where 110 slices where acquired. b; axial 2D image slice, c; axial 3D image, d; coronal 3D image. (a) Taken from (Burghardt et al., 2011) with permission.*

*3.9.2.2 Image grading*

Once the scan was completed, the operator visually inspected the quality of the images acquired and validity for analysis. The scans were classified into 4 categories based on a visual grading system reported previously (Engelke et al., 2012). There are 4 grades in this classification.

(i) Grade 1 – a 'perfect' scan with no evidence of movement artefact

(ii) Grade 2 – slight movement artefact was apparent on the images

(iii) Grade 3 – more pronounced movement artefact was apparent on the images

(iv) Grade 4 – very pronounced movement artefact was apparent on the images ('unacceptable quality' as described by (Paggiosi et al., 2014).

If the quality of the scan fell into categories 1 to 3, the scan was deemed valid and the subject was released. If the quality of the scan fell into category 4, a repeated scan was obtained. If the quality of the repeated scan was still poor (grade 4), it was deemed not valid for assessment and the scan was excluded from further analyses (Figure 3.7, next page). A maximum of 2 scans were performed at any one anatomical site. Within our study, we managed to obtained acceptable scans for all subjects who underwent HR-pQCT.
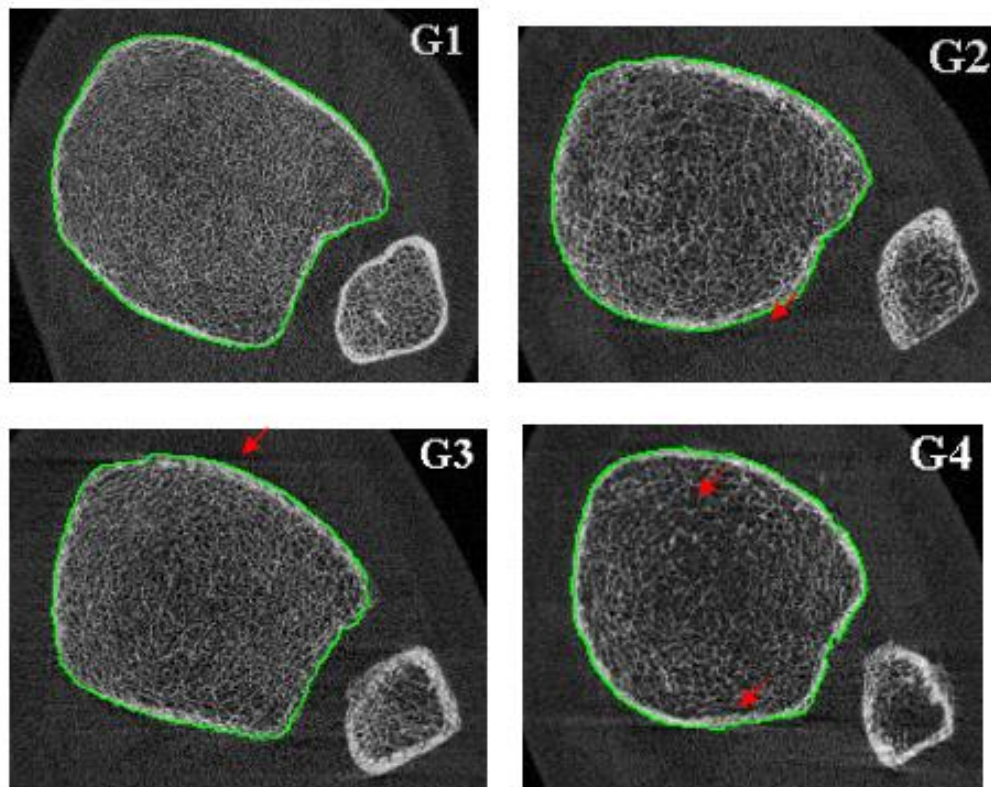
*Figure 3.7: Visual grading system for HR-pCQT scan quality (tibia). Arrows point to the motion artefact.*
*G1: Perfect scan (desirable), G2: Small degree of motion (acceptable), G3: Moderate degree of motion (acceptable), G4: Significant degree of motion (rejected)*

### 3.9.2.3 HR-pQCT Analysis

The analysis was performed by an experienced operator (Dr Margaret Paggiosi) at NGH. HR-pQCT image analysis involved the following analysis; i) standard image analysis, which provided densitometric, geometric and microstructural parameters, ii) extended cortical analysis, which provided additional cortical parameters, iii) Micro finite elements (µFE) analysis to estimate measures of bone strength.

<u>i: Standard image analysis</u>

This analysis was performed using Scanco standard software (version 6). The bone was separated from the soft tissue by drawing a contour around the periosteal region of the cortical bone in the first slice of the whole HR-pQCT stack. Contours were

applied semi-automatically for the subsequent 109 slices by the software. The HR-pQCT operator checked all the image slices manually and made any adjustments to the periosteal contours as required. Following this step, the cortical compartment was separated from the trabecular compartment by applying an automatic segmentation algorithm (Burrows et al., 2010). Trabecular and cortical densities were then determined separately by plotting the CT linear attention values against known densities obtained from a calibration phantom. In addition, trabecular bone microarchitectural properties were evaluated during the standard analysis stage.

ii: Extended cortical analysis

The software used for the extended cortical analysis was developed by Burghardt et al. (2010). The analysis consisted of three stages. In the initial stage, bone was extracted from the surrounding soft tissues using the same approach that were used in the standard analysis. The cortical bone was then automatically segmented using an automated contour algorithm. This process generated two bone compartments: the cortical compartment and the trabecular compartment. The second stage involved cortical porosity segmentation. This process was performed by using the cortical compartment (generated in stage one) to mask to the grey scale images in order to characterise the background voxels. Initially, all voids voxels that were surrounded by bone in cortical compartments were considered as intracortical pore space. A second 2D connectivity algorithm was used to enhance the ability of the software to detect the pore space that were not captured in the previous step. In the final stage, a refined image including all mineralised bone and intracortical pores in the cortical compartment was obtained by digitally combining the cortical compartment (obtained in stage one) and the intracortical porosity segmentation (obtained in stage 2) (Burghardt et al., 2010). The HR-pQCT operator checked all

the image slices manually and made any adjustments to the endosteal contours as required.

<u>iii: Micro finite elements (µFE) Analysis</u>

Micro Finite element ($\mu$FE) analysis was performed in order to estimate bone strength measures *in-vivo*. $\mu$FE modelling was performed using the extended µFE software (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland) which is fully automated and has been previously validated (MacNeil and Boyd, 2008, Nishiyama et al., 2013). The application of the $\mu$FE involved a simulation process in which the µFE model simulates a fall from standing height onto an out-stretched hand. Young's modules were applied to the cortical and trabecular bone separately (a Young's modulus of 20 GPa for cortical bone and Young's modulus of 17 GPa for trabeculae bone) (Walsh et al., 2012). A force representing 1000 N was applied to the distal part of the tibia in the axial direction while the proximal part was fixed. It was assumed that load failure occurs when 2% of the bone tissue strained beyond a critical level of 7000 µstrain based in criteria described by Pistoia et al. (2002). However, this critical level was reduced to 3500 µstrain in a modified model by Stephanie et al. (2008).

Table 3.3: Summary of the different HR-pQCT parameters that resulted from the standard analysis, extended cortical analysis and µFE Analysis outcome (Burghardt et al., 2010, Wang et al., 2010, Fuller et al., 2015)

| Parameter | Abbrev. | Unite | Description |
|---|---|---|---|
| **Bone areas** | | | |
| Total area | Tt.Ar | mm$^2$ | The average cross-sectional area of the whole bone circumscribed by the periosteal contour |
| Trabecular area | Tb.Ar | mm$^2$ | The average cross-sectional area of the trabecular compartment circumscribed by the endosteal contour |
| Cortical area | Ct.Ar | mm$^2$ | The average cross-sectional area of the cortical compartment between the periosteal and endosteal contours |
| **Bone densities** | | | |
| Total density | Tot.vBMD | mg/cm$^3$ | Mean mineralization of the total volume of interest |
| Cortical density | Ct.vBMD | mg HA/cm$^3$ | Mean mineralization of the cortical volume of interest |
| Trabecular density | Tb.vBMD | mg HA/cm3 | Mean mineralization of the trabecular volume of interest |
| **Trabecular bone microarchitecture** | | | |
| Trabecular number | Tb.N | 1/mm | Mean number of trabeculae per unit length |
| Trabecular thickness | Tb.Th | mm | Mean thickness of trabeculae |
| Trabecular separation | Tb.Sp | mm | Mean space between trabeculae |
| Trabecular inhomogeneity | Tb.N.SD | mm | SD of the intra-individual distribution of trabecular separation |
| Trabecular bone volume to tissue volume | Trabecular BV/TV | % | Trabecular bone volume fraction divide by the total volume of tissue |
| Meta/inner trabecular density | Meta/Inn | % | Ratio between outer and inner trabecular bone density |
| Meta trabecular density | Dmeta | mg/cm$^3$ | The outer 40% of the trabecular region |
| Inner trabecular density | Dinn | mg/cm$^3$ | The inner 60% of the trabecular region |

| Cortical bone microarchitecture | | | |
|---|---|---|---|
| Cortical thickness | Ct.Th | mm | Mean 3D distance from periosteal boundary to endosteal boundary, disregarding intracortical pores |
| Cortical perimeter | Ct.Pm | mm | Distance covered by the perimeter of the periosteal surface |
| Cortical tissue mineral density | Ct.TMD | mg HA/cm$^3$ | Mean mineralization of the segmented cortical bone voxels after surface partial volume suppression |
| Cortical porosity | Ct.Po | % | The volume of the intracortical pore space normalized by the sum of the pore and cortical bone volume |
| Cortical pore diameter | Ct.Po.Dm | μm | Mean 3D diameter of the intracortical pore space |
| SD Mean cortical pore diameter | Ct.Po.Dm.SD | μm | Standard deviation of the 3D diameters of the intracortical pore space |
| Connectivity density | Conn.D. | mm$^{-3}$ | Extent of trabecular connectivity normalized by tissue volume |
| μFE Analysis outcome | | | |
| Stiffness | Stiffness | kN/mm | Bone resistance to deformity when loaded |
| Estimated ultimate failure load | F.ult | kN | Maximum load applied on the bone before a fracture can occur; when 2% of the bone tissue strained beyond a critical level of 3500 μstrain |
| Distal ratio load | Tb.F/TF dist | % | Ratio of the load taken by the trabeculae in relation to the total load at distal end |
| Proximal ratio load | Tb.F/TF prox | % | Ratio of the load taken by the trabeculae in relation to the total load at proximal end |

*3.9.2.4 Precision*

The previous published work by researchers at the Mellanby Centre for Bone Research, University of Sheffield demonstrated that the XtremeCT scanner has excellent precision (Paggiosi et al., 2014). In this study, percent root mean square coefficient of variation (RMSCV) was calculated as the percent short-term root mean square coefficient of variation (%RMSCV) (Paggiosi et al., 2014). Precision (RMSCV) values for tibia measurements ranged from 0.2% for densitometric variables to 6.6% for cortical porosity (Paggiosi et al., 2014).

*3.9.2.5 Quality Assurance*

The quality assurance of the HR-pQCT scanner was checked on a daily and weekly basis. The daily calibration (QC1) was performed using the manufacturer's test object (Scanco Medical AG) in order to monitor the stability of the scanner. The weekly calibration (QC2) was performed, again using the manufacturer's test object, in order to ensure the XtremeCT's ability to measure bone microstructural properties (Figure 3.8, next page).

The manufacturer's test object consisted of five hydroxyapatite-resin compartments of different densities. The densities of these compartments mimicked different physiological bone mineral densities and started from 0 mgHA/cm$^3$, representing soft tissue, and gradually increased (100 mgHA/cm3, 200 mgHA/cm$^3$, 400 mgHA/cm$^3$ and 800 mgHA/cm$^3$). The mean attenuations (in Hounsfield units) of each of the five compartments were calculated from the images slices of the phantom. These were then converted into BMD values (mgHA/cm3). This QC step was essential and ensured that total, cortical and trabecular volumetric BMD were measured accurately by the XtremeCT scanner. This quality assurance approach is

part of the SOP procedures for Mellanby Centre for Bone Research at the University of Sheffield. and has been briefly reported in a previous publication (Paggiosi et al., 2014).
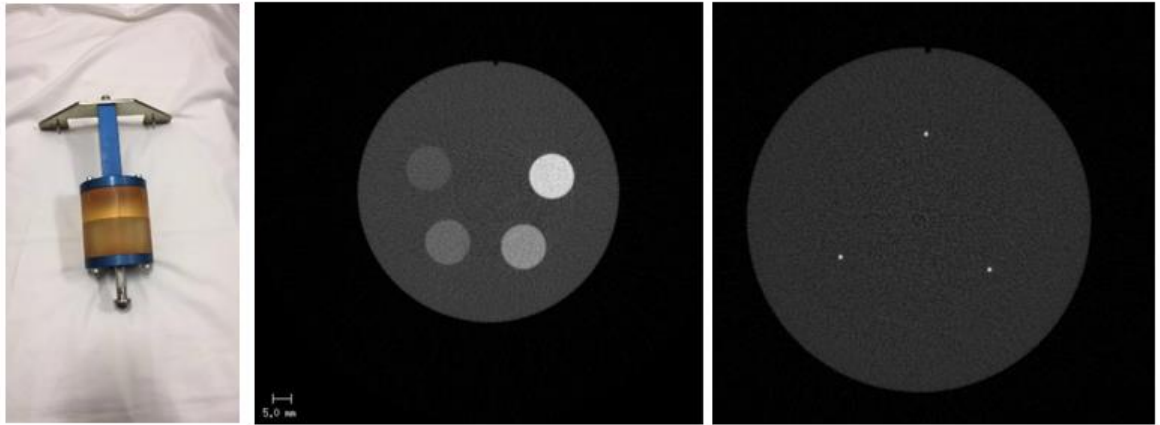


*Figure 3.8: Manufacturer's test object for the XtremeCT scanner*

### 3.9.3 Vitamin D, PTH, bone profile and 2nd void fasting urine sample measurements

Vitamin D, PTH bone profile (includes calcium, corrected calcium, phosphate, alkaline phosphate and albumin) were measured and documented at two time points during the study: at the baseline and at the end of the intervention. These elements were measured as they are strongly related to bone health. Evaluating these elements in the current study will allow for a broader evaluation of the impact of the interventions and could provide additional interpretations of the trial results. At the baseline test, blood samples were taken just before administering the vitamin D or placebo and before providing any food to the participants. All baseline measurements were taken between November 2017 and January 2018 for males, and between November 2017 and March 2018 for females. At the post-intervention

tests, the blood samples were taken within 7 days after the last WBV session and after taking HR-pQCT scans.  HR-pQCT scans and the blood samples were taken at the same day if possible or during the same week if it was not possible to take at the same day. The post-intervention samples were collected between February and April 2018 for males and between February and June 2018 for females (12 weeks after administering the vitamin D or placebo, Figure 3.2, Page 105).

The blood samples were collected following an overnight fast and the samples were collected between 8 am and 11 am. Around 8 ml of blood were withdrawn at each visit. The samples were collected by a qualified research nurse with the safe environment of the CRF at SCH. After withdrawing the samples, they were taken immediately by the PhD student to the lab. Bone profile and PTH were analysed on the same day by the Clinical Chemistry Laboratory department at SCH. Serums for vitamin D tests were frozen and then sent to Bristol for analysis where they were analysed by Biochemistry department at Bristol Royal Infirmary. This is because this centre has a well-established protocol for analysing vitamin D.

A fasting $2^{nd}$ void urine sample for spot urine calcium:creatinine ratio were collected from all participants one week after taking the baseline blood samples, regardless of the participants had taken the vitamin D or placebo. Written instructions on how to provide a second void fasting urine sample were provided to participants, and were also explained verbally. In order to provide these samples, participants were asked to avoid eating and drinking anything except water from midnight until they passed the $2^{nd}$ void urine sample. They were instructed to pass urine once shortly after they got up/woke up, and then to drink tap water before providing a second sample in the bottle provided to them (5 ml of urine).

An Acquity Ultra Performance LC/Quattro MS (Waters) analyser was used for measuring vitamin D studies using UPLC/Mass Spectrometer Semi-automated hexane extraction methods. There were three results for vitamin D analysis; 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and total 25-hydroxyvitamin D. The vitamin D analyser has good sensitivity. The lower limits of detection of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 are 6nmol/L and 3.5nmol/L, respectfully. Precisions are as following; for vitamin D2: With-in Run CV 4.4-6.7% and total CV 5.2-6.4%, for vitamin D3: With-in Run CV 4.3-4.5% and total CV 5-6.2%. PTH was measured using the 8K25 ARCHITECT intact PTH assay kit. The system was calibrated as per manufacturer's instruction. Serum sample collected from patients' blood was loaded and the sample was run on the Architect i1000 System (Abbott) analyser. Architect intact assay is a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum and plasma using Chemiluminescent Microparticle Immunoassay technology with a PTH analytical sensitivity ≤1 ng/L and PTH precision ≤ 7-9%, With-in Run CV 4.1-8.7%, and total CV 4.1-8.7%.

Bone profile (calcium, albumin, phosphate, alkaline phosphatase) and urine creatinine and calcium were analysed by Vitros 5,1 FS System (Ortho Clinical Diagnostics) analyser using Micro Slide Technology. Calcium levels were measured by the VITROS Ca Slides and the VITROS Chemistry Products Calibrator Kit 1. To measure serum albumin, the VITROS Chemistry Products Calibrator Kit 4 and VITROS albumin slide method was employed. Serum phosphate was measured using the VITROS PHOS Slides and the VITROS Chemistry Products Calibrator Kit 1. Alkaline phosphatase (AKLP) was measured using the VITROS ALKP Slides and the VITROS Chemistry Products Calibrator

Kit 3. To measure the urine creatinine level, the VITROS CREA Slides and the VITROS Chemistry Products Calibrator Kit 1 was used. All analysis was performed by qualified lab technicians following the manufacturers' instructions.

### 3.9.4 Calcium intake

The "calcium calculator" created by the International Osteoporosis Foundation (IOF) was used to assess participants' calcium intake in milligram (mg) ((IOF), 2015). This calcium calculator is a web-based food frequency questionnaire that asks how frequently certain foods are consumed per week and then combines all answers to give an estimated daily calcium intake.

## 3.10 Data handling and record keeping

Data was collected and retained in accordance with the Data Protection Act 1998 until 25th May 2018, and then with Data Protection Act 2018 from 25th May 2018 (in accordance with the change in the Data protection law in the UK). Study documents (paper and electronic) have been retained in a secure location during and after the study. All source documents will be retained for a period of 5 years following the end of the study. Where study related information is documented in the medical records – those records will be retained for 5 years after the last subject last visit.

## 3.11 Statistical analysis

CONSORT Statement guidelines were followed when analysing and presenting the study results (Moher et al., 2001). Baseline continuously distributed data was summarised by the median (25th/75th centiles); categorical data by n (%).

Percentages were rounded to the nearest whole number for ease of reading, thus giving rise to rounding errors. Given that the four treatment groups were randomised, baseline values were not compared statistically (Senn, 1989, Senn, 1994). Any between-group differences are assumed to have occurred by chance. Graphical presentation was by box and whisker plots. Missing values are given but not considered otherwise in our statistical analyses. Data was analysed by intention-to-treat. We used analysis of variance (ANOVA) to compare between-group differences from baseline to 12 weeks. Predicted means (plus 95% confidence intervals) from ANOVA were presented graphically with the controls as the reference group. A nominal level of 5% statistical significance (two-tailed) was used. We did not adjust p-values for multiple comparisons (Rothman, 1990). Given the exploratory nature of the trial statistical comparisons were kept to a minimum (Lancaster et al., 2004). Effect sizes for key outcome measures were estimated for future trial planning. The Stata statistical computer package was used to analyse the data (StataCorp, 2007).

## 3.12 Sample size calculation

The choice of the sample size is pragmatic, and not informed by power. There are no prior data on which to base a formal power calculation for changes in HR-pQCT parameters after the intervention with vibration training and a large dose of vitamin D. Therefore, this pilot study should be regarded as exploratory in nature. Different approaches to determine the sample size for a pilot clinical study have been reported in the literature. For example, 10 patients per group (40 maximum); (Birkett and Day, 1994), minimum of 20 patients in total; (Sandvik et al., 1996), 12 patients per group (Julious, 2005); or at least 9% of the main study's sample size; (Cocks and

Torgerson, 2013) were all reported. The first approach (10 per group) was chosen to determine the sample size for the current study as this approach fits best with the timescale and resources of this project. As such, 40 subjects were recruited (10 per group, 5 males and 5 females per group). Drop-out rates were monitored, and sample size will be revised accordingly for the main study.

## 3.13 Safety assessments and ethical considerations

The safety of participants in the current study was paramount. The PhD student monitored participants throughout the study and any adverse events that could be related to the study were recorded.

Many publications have shown the 150,000 IU dose of vitamin D to be safe. For instance, a single dose of vitamin D of up to 300,000 IU at intervals of 3 months or longer would not be expected to cause adverse effects in adults according to the latest report (July 2016) of SACN ((SACN), 2016). It has been reported that a single dose of 150,000 IU of vitamin D is effective in maintaining adequate blood levels of vitamin D without causing hypercalcaemia or hypercalciuria in children (Oliveri et al., 1996). In a recent systematic review of a large, single-dose of vitamin D in adults, it was concluded that the increase in 25(OH) D concentration occurred safely without adverse effects at doses less than 200,000 IU (Kearns et al., 2013). Indeed, the previous research conducted in our Department showed that a single dose of 150,000 IU of Invita D3 (the same product which was used in the current study) is safe and effective in increasing vitamin D without causing hypercalcaemia or hypercalciuria (Gopal-Kothandapani et al., 2018).

Although there was no evidence in the literature to suggested that the proposed dose may cause any harm to the participants, it was decided to perform a 2$^{nd}$ void urine sample one week after administering the vitamin D or placebo as a safety procedure. This is because there is a lack in the literature regarding the effect of high doses of vitamin D on this ethnic group (MENA ethnicity). In addition, any subject who may have contraindications for vitamin D was not recruited.

The vibrating platform has been used extensively both in clinical practice and sold 'over the counter' in countries within the EU including the UK. Minimal adverse events have been reported in the use of vibrating platforms. In a previous vibration study conducted by our research group, WBV was well tolerated with only mild effects being reported. A few of the participants experienced an itching type response in the feet and legs during the vibration (Harrison et al., 2015). In this previous vibration study in boys (aged 9 to 12 years), one subject felt dizzy whilst standing on the platform, and one felt faint after having blood taken -both issues were resolved after the subjects sat for a while (Harrison et al., 2015). However, participants in the current study are young adults who may have more ability to adapt to study procedures. It is possible that someone could fall off the platforms. Handles designed by the manufacturer for WBV platforms were used to support the participants during the WBV session (Figure 3.3 (a, b), page 116).

WBV training sessions were undertaken at the CRF at SCH, so that clinical staff could intervene in case of any emergency. A maximum of two participants were allowed to be present in the vibration room at the same time. Participants were not allowed to eat or drink while standing on the vibration platform. Participants allocated to WBV were monitored at each WBV session and were asked to report

any side effects during or after the session. Participants who reported an adverse event were followed until the adverse event was resolved

The radiation dose from HR-pQCT is negligible with an effective dose of only 3µSv per scan, which is less than the UK daily background radiation. All scans were performed by a trained operator (MAP).

Pregnant and breast-feeding women were excluded. Female participants consented to take some measures to avoid pregnancy during the study, and to have a pregnancy test as required for this study. The procedure for managing adverse events complies with the Pharmacovigilance Standard Operating Procedures of the sponsor, Sheffield Children's NHS Foundation Trust. The criteria set in advance to discontinue the trial were, if a subject experiences a serious adverse event (SAE) that it is considered at least possibly related to the study intervention. Nevertheless, no adverse events happened as a result of this study.

## 3.14 Ethical approval

The study was given a favourable ethical opinion by the South Yorkshire Research Ethics Committee (REC reference: 17/YH/0254). Health Research Authority (HRA) approval was obtain prior commencing the study (HRA reference: 225440). The local Capacity and Capability approvals were also obtained from SCH and NGH. The PhD student completed all research ethics forms and applied for all approvals with the assistance of the primary supervisor (ACO). The study was conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP), and the Research Governance Framework for Health and Social Care (2nd Edition).

# CHAPTER 4
# RESULTS

The primary aim of the current study was to determine the effects of a large single oral dose of vitamin D3 and 12 weeks of WBV on HR-pQCT parameters of the distal tibia. Participants in the current study were healthy male and female volunteers who emigrated from the MENA region to the UK. Measurement variables included volumetric bone densities, bone microarchitecture, FEA of bone strength, vitamin D, bone profile, anthropometric measurements and calcium intake at baseline.

## 4.1 Overview of recruitment, dropout and adherence

### 4.1.1 Recruitment and dropout rates

After writing the study protocol and getting it peer-reviewed, all study documents (attached as Appendices) were written and all ethics forms were completed in order to apply for the HRA approval. After gaining the HRA approval and the local research and development (R&D) approvals, recruitment began in October 2017. The target was to recruit the required sample size (n=40) within one year. In the event, recruitment was completed by March 2018, and all study visits were completed by the end of June 2018. Among the 77 subjects who were assessed for eligibility, 37 were excluded for various reasons (Figure 4.1, next page). Among the 40 participants who were enrolled and who underwent baseline assessment, two participants subsequently withdrew (5% dropout rate). Both of these participants were females, and provided no reason for not completing the study.

### 4.1.2 Adherence

All WBV sessions were supervised by the PhD student and adherence to WBV was recorded for every participant. The mean adherence to WBV training was 84% (range 66%–100%). All participants received either vitamin D or placebo. The

administration of vitamin D or placebo was witnessed and documented by the PhD

student and by a research nurse.
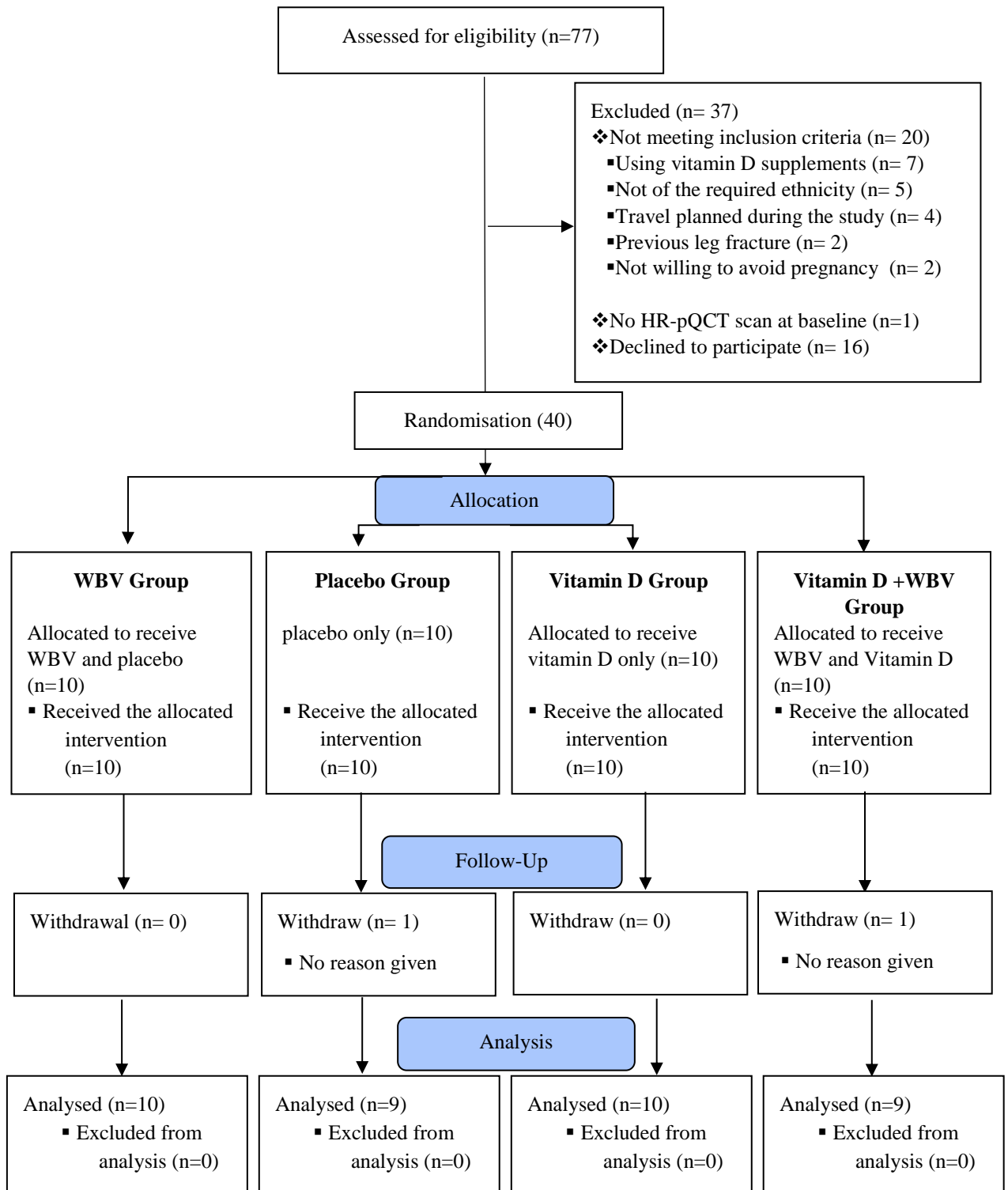


Figure 4.1: VibeD study profile

## 4.2 Baseline characteristics of participants

Participants were originally from 10 of the 20 countries identified as MENA countries, as described in Chapter 2, nearly two-thirds of whom were from just three countries (Saudi Arabia, Iraq and Libya), as illustrated in Table 4.1 (below).

Table 4.1: Participants' country of origin

| Country of origin | Number (n=40) | % |
|---|---|---|
| Saudi Arabia | 13 | 32.% % |
| Iraq | 7 | 17.5% |
| Libya | 6 | 15% |
| Egypt | 3 | 7.5% |
| Syria | 3 | 7.5% |
| United Arab Emirates (UAE) | 2 | 5% |
| Iran | 2 | 5% |
| Djibouti | 2 | 5% |
| Palestine | 1 | 2.5% |
| Algeria | 1 | 2,5% |

All baseline scans and blood samples were collected between November 2017 and March 2018. All participants had valid HR-pQCT scans at baseline and all participants who completed the study had valid HR-pQCT scans at the end of the study. Although it was possible to obtain a blood sample for one participant at the end of the study, a baseline blood sample was not obtained due to difficulty in finding a suitable site for venepuncture. PTH could not be measured for one participant due to an insufficient volume of blood.

Table 4.2 (next page) summarises the baseline variables for the four groups and for the whole sample. There were similar numbers of males and females in each group. vitamin D only had lower calcium intake compared to the other groups. However, the corrected serum calcium level for this group was similar to that of the other groups. The median baseline total 25 (OH)D levels were in the category of vitamin D deficiency for all groups. Furthermore, results for individual participants showed that the majority were vitamin D deficient (25 (OH)D < 30 nmol/L) (Table 4.3, Page 144). In addition, a slightly elevated PTH was evident in all groups. Other biochemistry data were similar for all groups and were within the normal range, except for one participant who had a slightly lowered corrected calcium level and another who had a slightly elevated phosphate level.

Table 4.2: Summary statistics are median, 25th/75th centiles, and range except for sex. Calculations subject to rounding errors

| Variable | WBV (n=10) | Placebo (n=10) | Vit D (n=10) | Vit D+WBV (n=10) | All (n=40) |
|---|---|---|---|---|---|
| **Anthropometry** | | | | | |
| **Age** **(years)** | 22.4 (18.6, 28.5) (18.5, 38.5) | 32 (30.3, 32.3) (20.1, 36.3) | 29.3 (19.1, 35.5) (18.5, 37.6) | 21.9 (18.6, 30.5) (18.2, 36) | 28.1 (19.3, 32.2) (18.2, 38.5) |
| **Sex** **(female)** | 5 (50%) | 5 (50% | 5 (50%) | 5 (50%) | 20 (50%) |
| **Height** **(M)** | 1.68 (1.62, 1.75) (1.58, 1.83) | 1.66 (1.60, 1.71) (1.57, 184) | 1.69 (1.59, 1.75) (1.51, 1.80) | 1.67 (1.63, 1.69) (1.55, 1.75) | 1.67 (1.58, 1.72) (1.51, 1.84) |
| **Weight** **(Kg)** | 65.1 (57.1, 89.1) (53, 125.3) | 78.8 (60.2, 86) (41.3, 111.3) | 66.1 (55.1, 76) (42, 116.1) | 76.1 (62.3, 94.10) (47.7, 100.2) | 73.3 (57.4, 87.5) (41.3, 125.3) |
| **BMI** **(Kg/M$^2$)** | 23.4 (20.2, 26.9) (19.2, 37.4) | 28.3 (21.6, 30.3) (16.6, 37.8) | 23.8 (19.8, 26.3) (16.6, 35.8) | 25.8 (22.1, 33.1) (19.8, 38.6) | 24.4 (21, 30.1) (16.6, 38.6) |
| **Calcium intake, vitamin D, PTH and bone profile** | | | | | |
| **Calcium intake** **(mg/day)** | 1037 (967, 1320) (800, 1533) | 1038 (893, 1093) (785, 1597) | 862 (736, 1322) (514, 1685) | 1029 (818, 1274) (753, 1350) | 1022 (821, 1254) (514, 1685) |
| **Total 25(OH)D** **nmol/L** | 15.5 (11, 25) (10, 95) | 28 (16, 38) (12, 61) | 22 (12, 42) (10, 71) | 23 (17, 32) (13, 64) | 23 (13.5, 36) (10, 95) |
| **PTH** **(ng/L)** | 73.8 (54.2, 100.9) (43.6, 119.1) | 57.8 (47.8, 72.3) (45.6, 112.8) | 63.1 (44.9, 89.6) (40.7, 199.2) | 58.9 (36.7, 76.4) (28.7, 107.9) | 61.3 (47.1,82.6) (28.7, 199.2) |
| **Corrected Calcium** **(mmol/L)** | 2.24 (2.22, 2.30) (2.21, 2.35) | 2.27 (2.22, 2.30) (2.06, 2.34) | 2.27 (2.14, 2.30) (2.13, 2.34) | 2.24 (2.17, 2.33) (2.14, 2.37) | 2.26 (2.21, 2,32) (2.06, 2.37) |
| **Phosphate** **(mmol/L)** | 1.21 (1.16, 1.40) (0.83, 1.59) | 1.07 (0.92, 1.19) (0.83, 1.23) | 1.2 (1.04, 1.27) (0.96, 1.31) | 1.16 (1.01,1.29) (0.87, 1.32) | 1.17 (1.06, 1.27) (0.83, 1.59) |
| **Alk Phos (U/L)** | 57 (44, 82) (36, 105) | 69.5 (49, 92) (46, 93) | 56 (52, 74) (41, 93) | 60.5 (49, 92) (44, 93) | 59.5 (49,87) (36, 105) |
| **Albumin (g/L)** | 44.5 (43, 46) (41, 48) | 44 (44, 47) (42, 48) | 44.5 (42, 46) (42, 47) | 45.5 (44, 48) (41, 49) | 45 (43, 47) (41, 49) |
| **HR-pQCT parameters of the distal tibia** | | | | | |
| **Total area (mm$^2$)** | 643 (553, 698) (389, 1024) | 711 (629, 766) (493, 1032) | 717 (600, 868) (536, 1054) | 690 (604, 747) (516, 882) | 679 (606, 760) (389, 1054) |
| **Cortical area (mm$^2$)** | 122.4 (113.9, 138.3) (100.3, 177.9) | 140 (117.2, 152) (90.9, 176) | 113.2 (104.6, 180.2) (82.7, 209.8) | 136.9 (113.7, 172.9) (99.9, 183.4) | 128 (112.7, 162.1) (82.7, 209.8) |
| **Trabecular area** **(mm$^2$)** | 523.4 (427, 541) (274, 885) | 539.4 (495.1, 625.8) (400.4, 882.3) | 571.9 (485.1, 685) (428.9, 852) | 554.8 (497.1, 578.4) (401.7, 698.1) | 541.4 (490.1, 639) (274, 885) |
| **Total density** **(mg/cm$^3$)** | 348.7 (310.9, 365.8) (280.2, 396.4) | 324.3 (289.4, 345.7) (285.8, 367.6) | 297.4 (274.1, 369.3) (233.2, 425.4) | 329.4 (315.1, 350.6) (249.2, 435.7) | 328 (297.4, 356.5) (233.2, 435.7) |
| **Cortical density** **(mg/cm$^3$)** | 910.8 (891.7, 951.8) (849.4, 979.2) | 919.5 (914.6, 931.7) (890.8, 981) | 912.5 (897.5, 937.5) (816.9, 950.2) | 918.9 (892.6, 950.6) (874.4, 959.1) | 916.3 (898.9, 941.7) (816.9, 981) |
| **Trabecular density** **(mg/cm$^3$)** | 183.5 (166.9, 227.2) (128.3, 262.7) | 168.8 (151.6, 194) (142.6, 218.5) | 161.3 (152.1, 227.9) (114.7, 266.7) | 190.1 (161, 196.3) (125.3, 243.1) | 180.1 (157, 217.7) (114.7, 266.7) |

| | | | | | |
|---|---|---|---|---|---|
| **Meta trabecular density (mg/cm³)** | 263.1 (239,3, 283.7) (211.1, 309) | 241.4 (223.2, 250.3) (205, 284) | 221.1 (202.9, 312.9) (162, 330.7) | 254.8 (228.8, 268.1) (196.1, 310.2) | 248.9 (221.4,273.9) (162, 330.7) |
| **Inner trabecular density (mg/cm³)** | 138.1 (117.5, 181.3) (71.7, 231.2) | 120.4 (114.5, 157.7) (96.3, 180.9) | 123.4 (113.1, 170.1) (82.5, 232.1) | 146.1 (114.7, 146.7) (77.5, 197.5) | 132.9 (114, 172.1) (71.7, 232.1) |
| **Meta inner trabecular density (no units)** | 1.81 (1.53, 2.24) (1.34, 2.95) | 1.86 (1.63, 2.19) (1.49, 2.28) | 1.80 (1.68, 1.96) (1.35, 2.23) | 1.83 (1.69, 2.03) (1.54, 2.53) | 1.83 (1.65, 2.03) (1.34, 2.95) |
| **Trabecular BV/TV (no units)** | 0.15 (0.14, 0.19) (0.10, 0.22) | 0.14 (0.13, 0.16) (0.11, 0.18) | 0.13 (0.13, 0.19) (0.09, 0.22) | 0.16 (0.13, 0.16) (0.10, 0.20) | 0.15 (0.13,0.18) (0.09, 0.22) |
| **Trabecular number (1/mm)** | 1.81 (1.46, 2.23) (1.30, 2.57) | 1.93 (1.62, 2.15) (1.53, 2.32) | 2.03 (1.67, 2.30) (1.30, 2.53) | 1.83 (1.49, 2.01) (1.13, 2.53) | 1.91 (1.6, 2.23) (1.13, 2.57) |
| **Trabecular thickness (mm)** | 0.09 (0.08, 0.10) (0.06, 0.10) | 0.08 (0.07, 0.08) (0.06, 0.09) | 0.08 (0.07, 0.09) (0.05, 0.10) | 0.08 (0.08, 0.09) (0.05, 0.11) | 0.08 (0.07, 0.09) (0.05, 0.11) |
| **Trabecular separation (mm)** | 0.47 (0.37, 0.59) (0.30, 0.66) | 0.44 (0.39, 0.54) (0.36, 0.57) | 0.42 (0.36, 0.52) (0.30, 0.68) | 0.44 (0.42, 0.59) (0.33, 0.79) | 0.44 (0.37, 0.54) (0.30, 0.79) |
| **Trabecular inhomogeneity (1/SD of trabecular number)** | 0.21 (0.16, 0.26) (0.12, 0.35) | 0.19 (0.17, 0.24) (0.15, 0.27) | 0.17 (0.15, 0.23) (0.13, 0.32) | 0.2 (0.19, 0.26) (0.12, 0.39) | 0.2 (0.16, 0.25) (0.12, 0.39) |
| **Cortical thickness (mm)** | 1.23 (1.1, 1.39) (1.05, 1.74) | 1.26 (1.15, 1.48) (1.05, 1.70) | 1.18 (1.11, 1.63) (0.77, 1.68) | 1.29 (1.21, 1.61) (1.04, 1.81) | 1.25 (1.12, 1.52) (0.77, 1.81) |
| **Cortical perimeter (mm)** | 98.3 (90.6, 102.5) (75.9, 126.7) | 104.9 (97.9, 107) (86.7, 125.4) | 105.1 (94.8, 114.5) (89.4, 129.1) | 102.3 (96, 107.9) (88.2, 118) | 102 (95.8, 108) (75.9, 129.1) |
| **Cortical porosity (no units)** | 0.02 (0.02, 0.04) (0.008, 0.05) | 0.03 (0.02, 0.04) (0.01, 0.05) | 0.02 (0.02, 0.05) (0.008, 0.06) | 0.02 (0.02, 0.03) (0.01, 0.04) | 0.02 (0.02, 0.04) (0.008, 0.06) |
| **Cortical porosity (%)** | 2.57 (1.65, 3.81) (0.89, 5.94) | 2.70 (1.82, 4.21) (1.43, 4.76) | 2.71 (1.58, 4.52) (0.84, 6.72) | 2.05 (1.79, 3.36) (1.12, 4.58) | 2.41 (1.72, 3.97) (0.84, 6.72) |
| **Mean cortical diameter (µm)** | 0.16 (0.15, 0.17) (0.14, 0.16) | 0.16 (0.15, 0.17) (0.14, 0.21) | 0.16 (0.15, 0.17) (0.14, 0.19) | 0.16 (0.15, 0.18) (0.11, 0.21) | 0.16 (0.15, 0.17) (0.11, 0.21) |
| **SD mean cortical diameter (µm)** | 0.06 (0.06, 0.07) (0.05, 0.08) | 0.07 (0.06, 0.08) (0.05, 0.11) | 0.06 (0.06, 0.08) (0.05, 0.08) | 0.07 (0.06, 0.09) (0.04, 0.14) | 0.07 (0.06, 0.08) (0.04, 0.14) |
| **Cortical tissue density (mg/ccm)** | 1034 (1019, 1041) (976, 1049) | 1036 (1021, 1048) (1004, 1075) | 1036 (1021, 1048) (955, 1068) | 1023 (1002, 1034) (970, 1066) | 1027 (1007, 1040) (955, 1075) |
| **Connectivity density (mm⁻³)** | 0.31 (0.18, 0.47) (0.11, 1.03) | 0.28 (0.16, 0.39) (0.08, 0.78) | 0.25 (0.13, 0.47) (0.10, 0.82) | 0.19 (0.15, 0.29) (0.07, 0.32) | 0.28 (0.15, 0.35) (0.07, 1.03) |
| **Stiffness (KN/mm)** | 212.1 (197.9, 290.3) (155.9, 344.8) | 241.1 (196.3, 256.3) (151.2, 336.5) | 200.1 (183.3, 312.2) (171.4, 413.7) | 248.2 (198.2, 286.9) (172.7, 307.4) | 228.6 (192.3, 288.6) (151.2, 413.7) |
| **Estimated failure load (KN)** | 10.5 (9.8, 14.2) (7.8, 17.3) | 12.1 (9.9, 12.7) (7.5, 16.9) | 10.1 (9.0, 14.6) (8.6, 20.4) | 10 (8.9, 12.4) (8.6, 15.1) | 11.6 (9.6, 14.2) (7.5, 20.4) |
| **Distal trabecular ratio load (%)** | 61.3 (52.8, 65.7) (26.2, 74.6) | 54.5 (49.9, 64.4) (46.9, 69.8) | 54.4 (52.7, 64.9) (46.1, 69.6) | 56.5 (51.6, 59.4) (47.7, 63.9) | 55.7 (52.4, 63.8) (26.2, 74.6) |
| **Proximal trabecular ratio load (%)** | 38.5 (30.4, 41.3) (14.9, 52.3) | 31.1 (28.9, 40.1) (27.4, 47.7) | 34.8 (31.2, 40.1) (25.4, 47.3) | 34.9 (30.2, 38.7) (26.1, 40.8) | 34.6 (30.3, 40.03) (14.9, 52.3) |

Table 4.3: Classification of vitamin D levels at baseline

| Category | Serum 25 (OH) Level | Number* | % |
|---|---|---|---|
| **Deficiency** | < 30 nmol/L | 26 | 65% |
| **Insufficiency** | 30 to 50 nmol/L | 7 | 17.5% |
| **Sufficiency** | > 50 nmol/L | 6 | 13% |

*Baseline vitamin D was not available for one participant*

## 4.3 Group variables at baseline and after 12 weeks

Group variables at baseline and after 12 weeks are presented as box-and-whisker plots showing the median, interquartile range and any outliers.

### 4.3.1 HR-pQCT parameters

*Cortical and trabecular areas*

Cortical area and trabecular area at baseline and post-intervention for all groups are shown in Figure 4.2 (below).
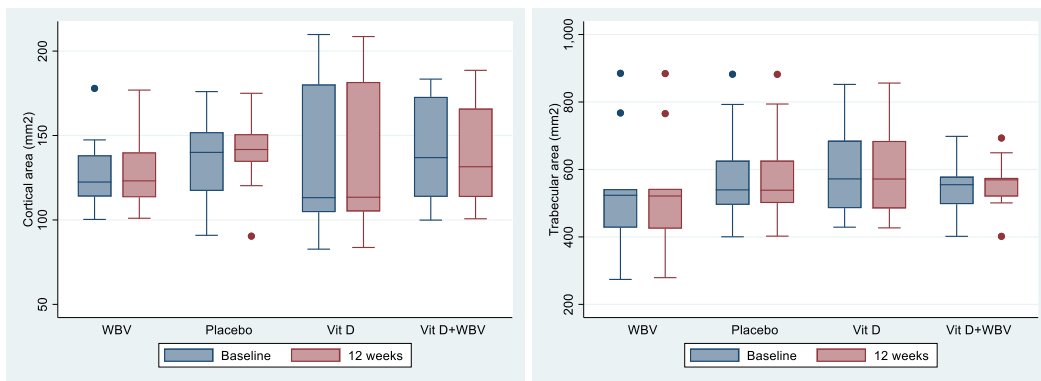


*Figure 4.2: Cortical area (left image) and trabecular area (right image) by randomization group at baseline and after 12 weeks.*

*Total, cortical and trabecular densities*

The total volumetric density at baseline and post-intervention for all groups are shown in Figure 4.3 (below). Cortical density (Figure 4.4) and trabecular density (Figure 4.5) are also shown below.
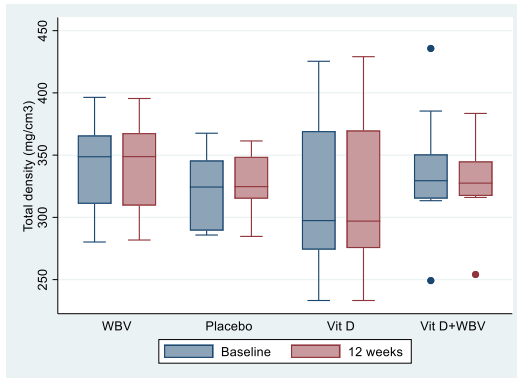


*Figure 4.3: Total density by randomization group*
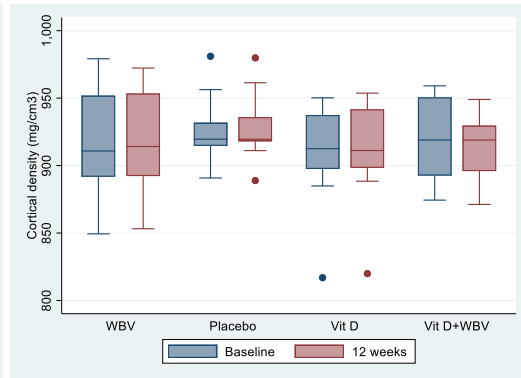


*Figure 4.4: Cortical density by randomization group*
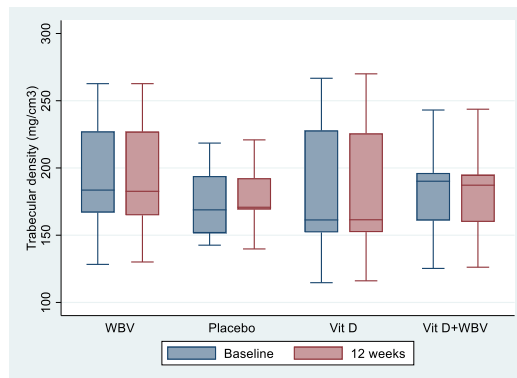


*Figure 4.5: Trabecular density by randomization group*

*Trabecular bone microarchitecture*

Trabecular bone parameters at baseline and at 12 weeks for all groups are summarised in the following figures (Figures 4.6 to 4.13). These parameters included meta trabecular density, inner meta trabecular density, meta/inner trabecular density, trabecular bone volume/tissue volume (BV/TV), trabecular number, trabecular thickness, trabecular separation and trabecular inhomogeneity.
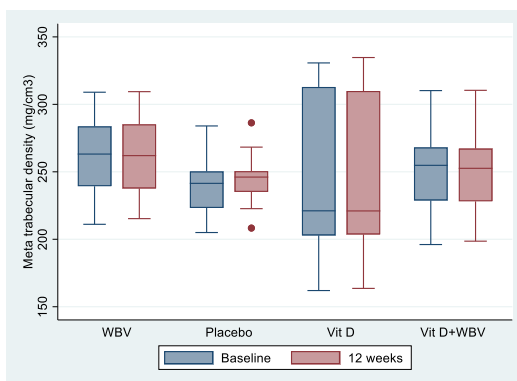


*Figure 4.6: Meta trabecular density by randomization group.*
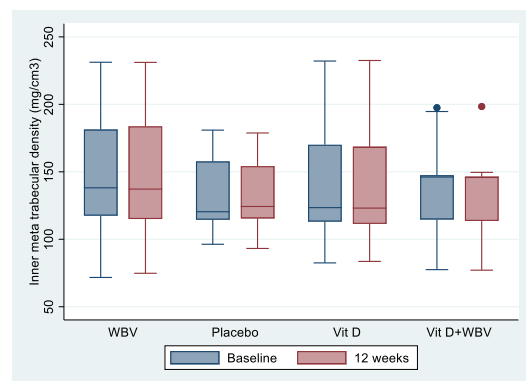


*Figure 4.7: Inner meta trabecular density by randomization group*
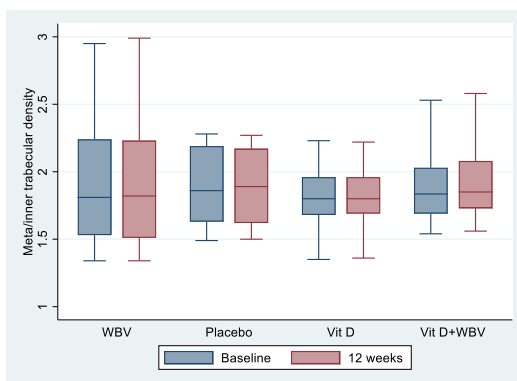


*Figure 4.8: Meta/inner trabecular density ratio by randomization group*
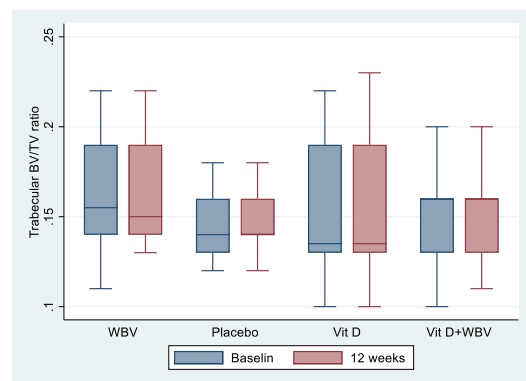


*Figure 4.9: Trabecular BV/TV ratio by randomization group*
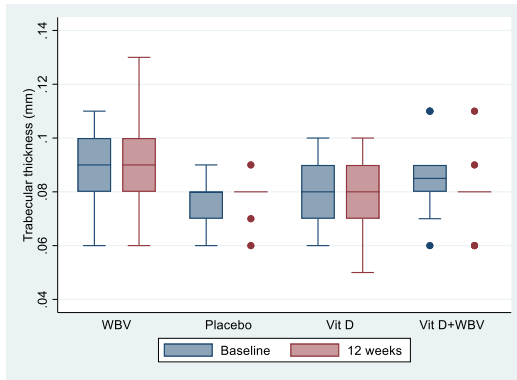
*Figure 4.10: Trabecular thickness by randomization group.*
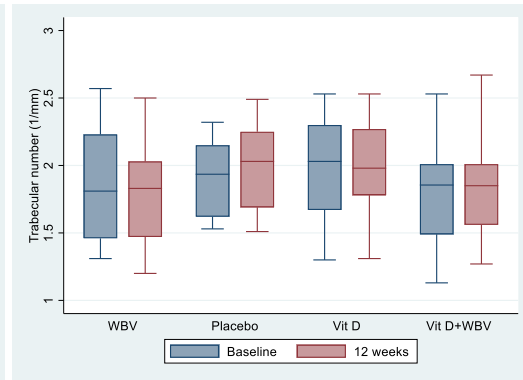


*Figure 4.11: Trabecular number by randomization group.*
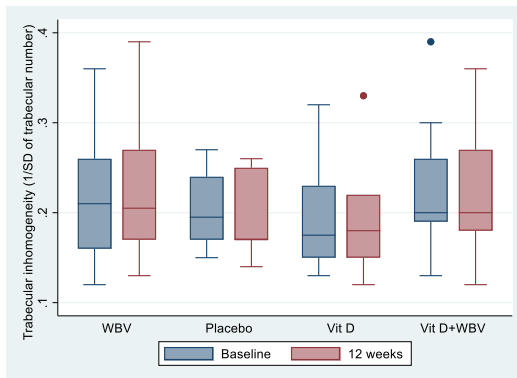


*Figure 4.12: Trabecular inhomogeneity by randomization group.*
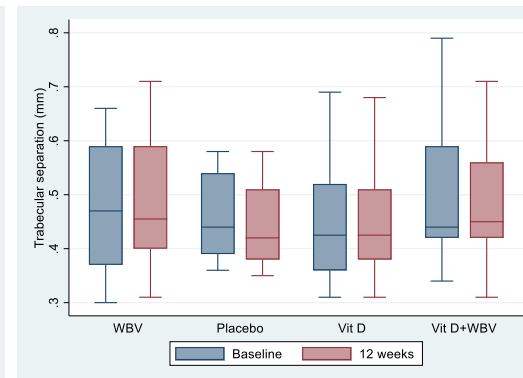


*Figure 4.13: Trabecular separation by randomization group*

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*Cortical bone microarchitecture*

Cortical bone microarchitecture at baseline and at 12 weeks for all groups are summarised in the following figures (Figures 4.14 to 4.21). These parameters included cortical thickness, cortical perimeter, cortical porosity, mean cortical diameter, SD of mean cortical diameter, cortical tissue mineral density and connectivity density.
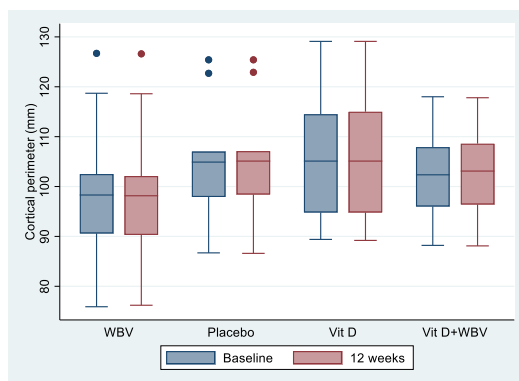


*Figure 4.14: Cortical perimeter by randomization group*
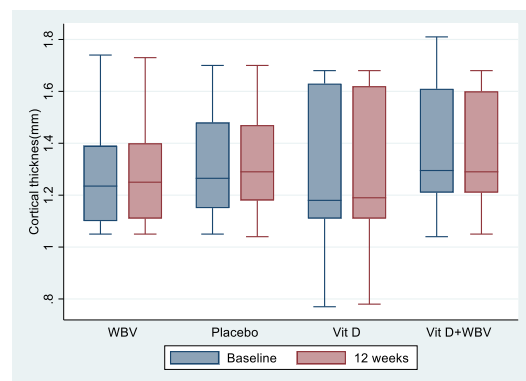


*Figure 4.15: Cortical thickness by randomization group.*
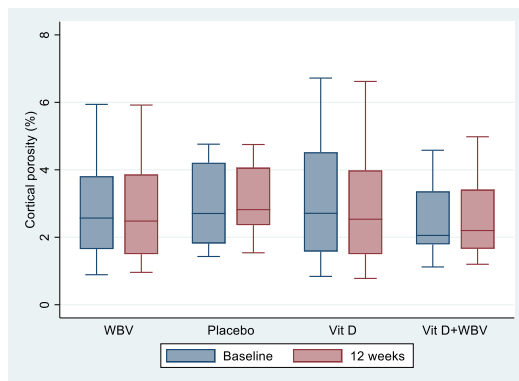


*Figure 4.16: Percentage (%) of cortical porosity by randomization group.*
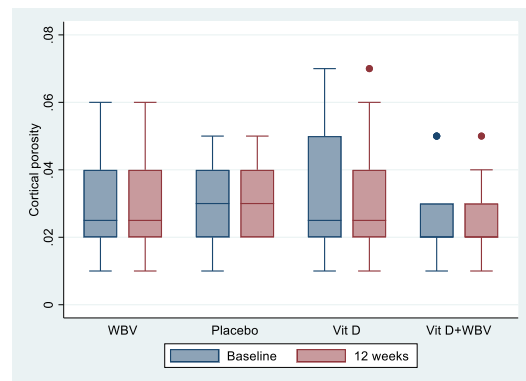


*Figure 4.17: Cortical porosity by randomization group*
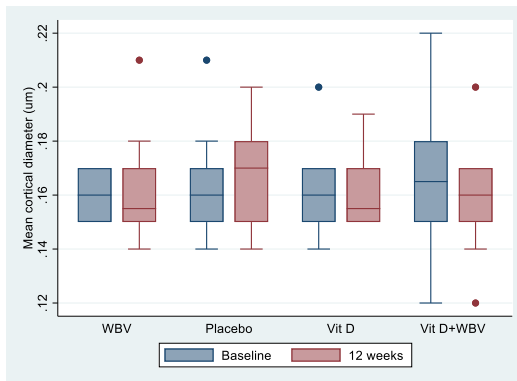
*Figure 4.18: Cortical diameter by randomization group.*
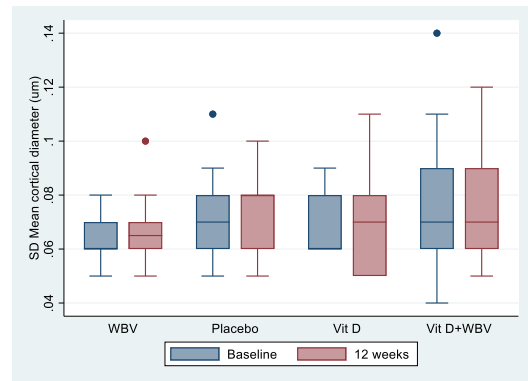


*Figure 4.19: SD of mean cortical diameter by randomization group*
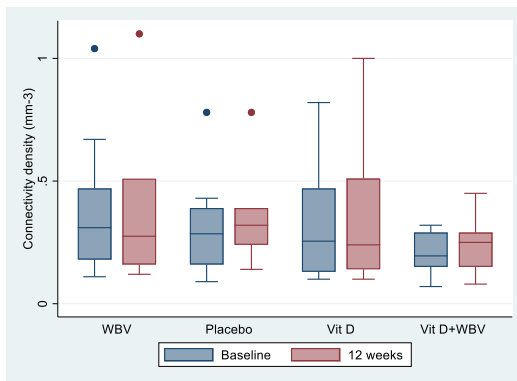


*Figure 4.20: Connectivity density by randomization group*
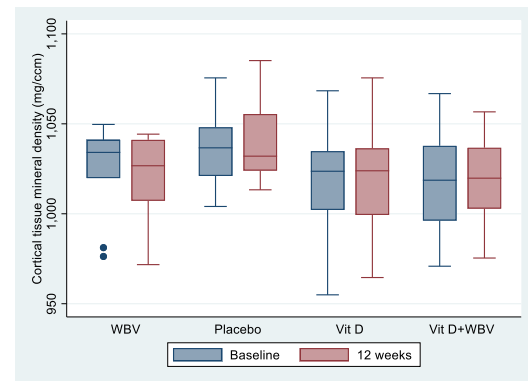


*Figure 4.21: Cortical tissue mineral density by randomization group*

FEA model was applied to estimate bone strength. FEA analysis outcome measures include stiffness, estimated failure load, distal ratio load and proximal ratio load. The changes in these parameters are summarised in Figures 4.22 to 4.25 (below).
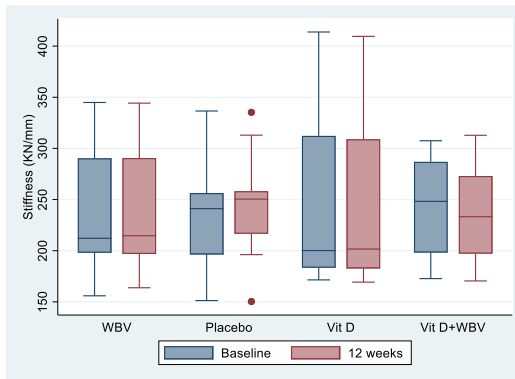


*Figure 4.22: Stiffness by randomization group.*
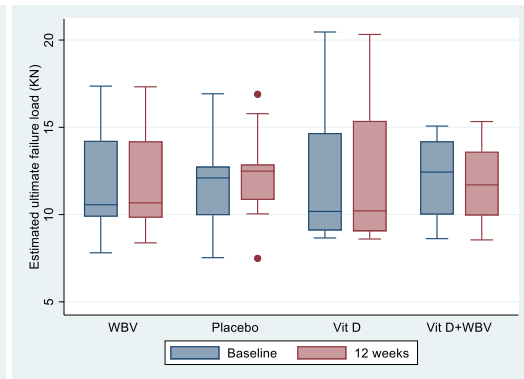


*Figure 4.23: Estimated ultimate failure load by randomization group.*
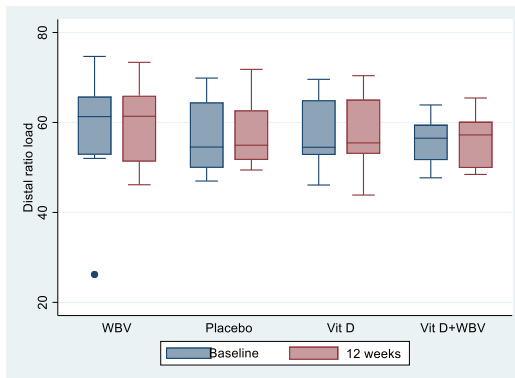


*Figure 4.24: Distal ratio of the load taken by the trabeculae in relation to the total load by randomization group.*
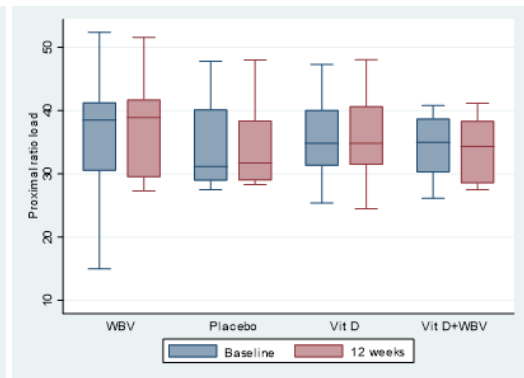


*Figure 4.25: Proximal ratio of the load taken by the trabeculae in relation to the total load by randomization group*

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## 4.3.2 Anthropometric data

Height and weight were recorded at baseline and at the final study visit in order to calculate the BMI as described in Chapter 2. Weight and BMI at baseline and at 12 weeks for all groups are summarised below (Figure 4.26 and Figure 4.27, below).
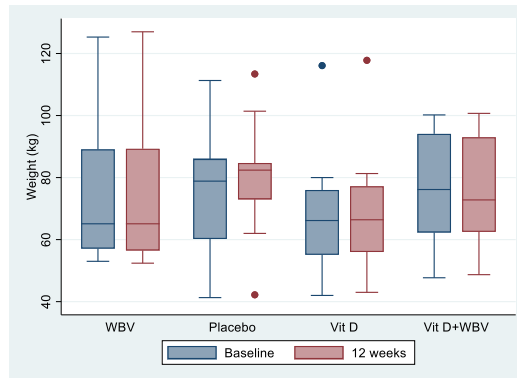


*Figure 4.26: Weight (kg) by randomization group*
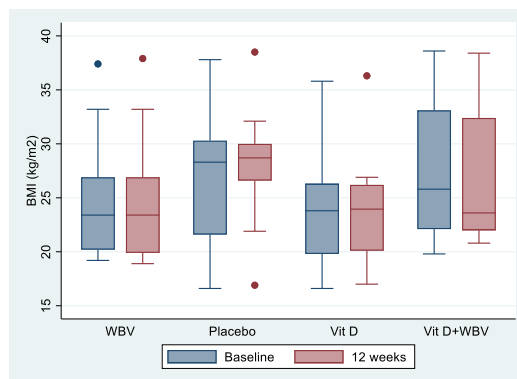


*Figure 4.27: BMI by randomization group.*

### 4.3.3 Biochemical parameters

Biochemistry included total serum 25 (OH) D (Figure 4.28, below), PTH (Figure 4.29, below) and bone profile. Bone profiles (corrected calcium, phosphate, alkaline phosphate and albumin) are summarised in Figures 4.30 to 4.33 (Next page).
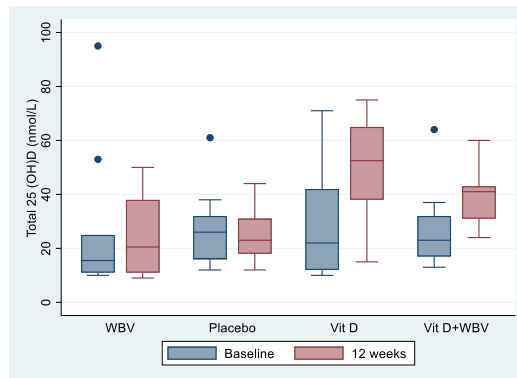


*Figure 4.28: Total 25 (OH) D by randomization group.*
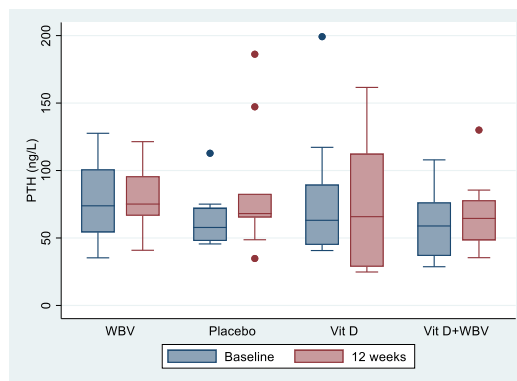


*Figure 4.29: PTH by randomization group.*
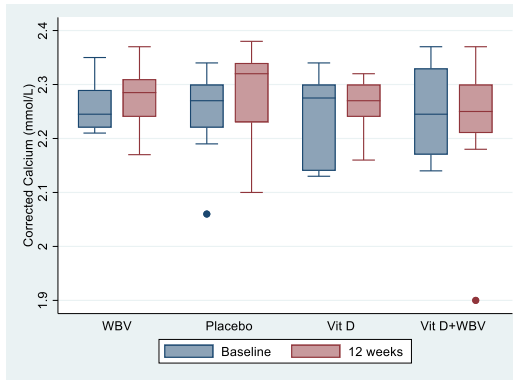
*Figure 4.30: Corrected calcium by randomization group. One subject was slightly hypocalcemic at baseline and another subject was hypocalcemic after 12 weeks. Patients' general practitioners were informed of these results.*
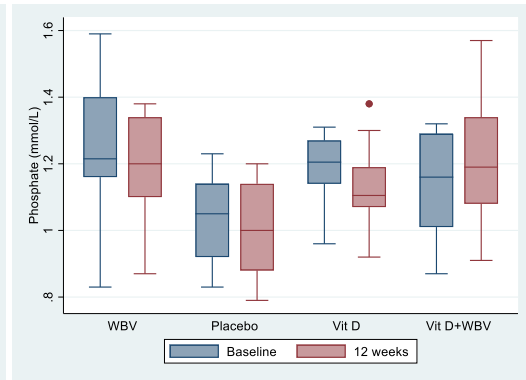


*Figure 4.31: Phosphate by randomization group.*
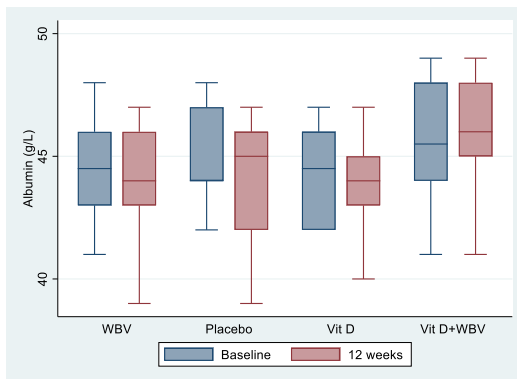


*Figure 4.32: Albumin by randomization group. Albumin levels were within the normal range (35–50 g/L) at baseline and after 12 weeks for all participants.*
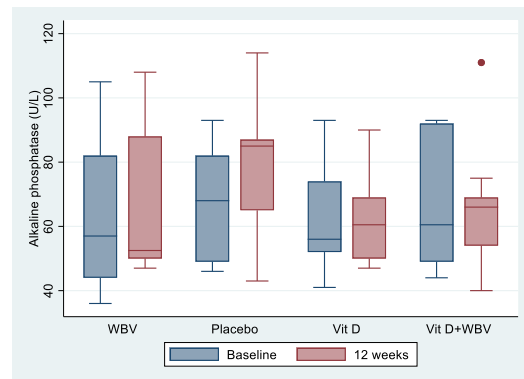


*Figure 4.33: Alkaline phosphatase by randomization group. Alkaline phosphatase levels were within the normal range (30–130 U/L) at baseline and at 12 weeks for all groups.*

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## 4.4 Change in HR-pQCT parameters over 12 weeks

*Cortical area*

No significant changes were found in any group after 12 weeks (Table. 4.4 and

Figure 4.34, below).

Table 4.4: Cortical area change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline cortical area, baseline PTH, baseline 25(OH) D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline $(mm^2)$ | Adjusted mean difference (from Placebo ) with 95% CI $(mm^2)$ | p-value |
|---|---|---|---|
| Placebo | -0.05 | Reference | |
| WBV | 0.63 | 0.68(-0.54,1.91) | 0.26 |
| Vit D | 0.81 | 0.86(-0.40,2.13) | 0.17 |
| Vit D +WBV | 0.87 | 0.92(-0.29,2.14) | 0.13 |
| F [3,28]=0.98 p=0.41 | | | |
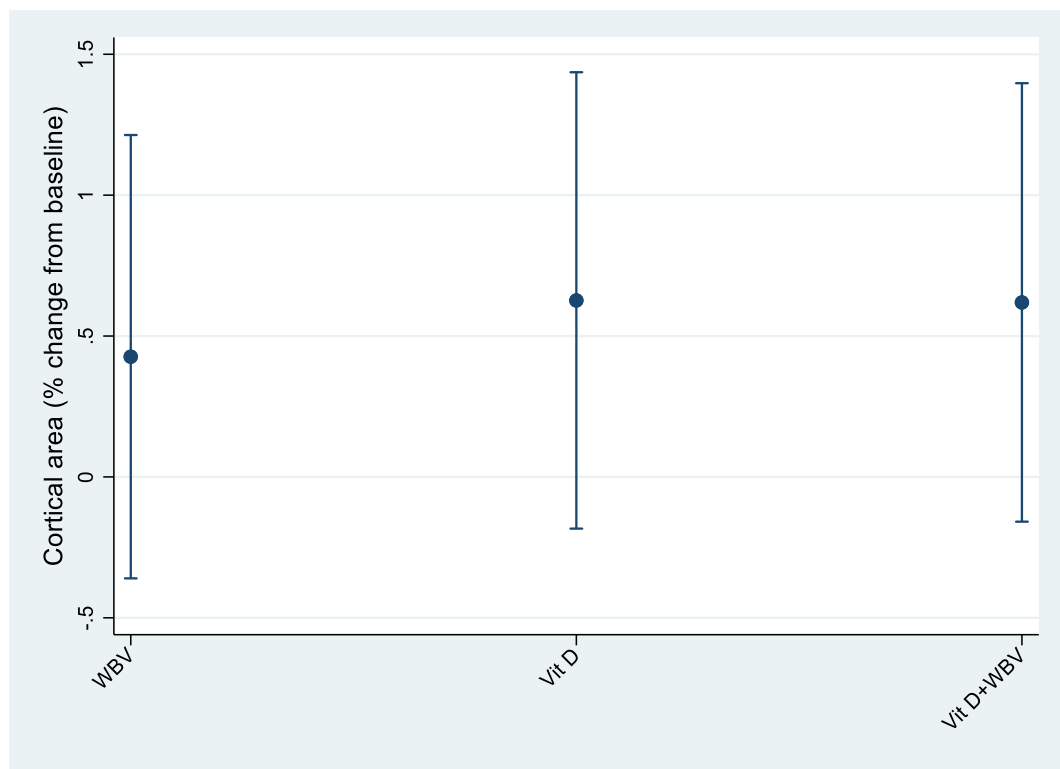


*Figure 4.34: Percentage change in cortical area from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Trabecular area*

No significant changes were found in any group after 12 weeks (Table 4.5, and Figure 4.35, below).

Table 4.5: Trabecular area change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline trabecular area, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline ($cm^2$) | Adjusted mean difference (from Placebo) with 95% CI ($cm^2$) | p-value |
|---|---|---|---|
| Placebo | -0.49 | Reference | |
| WBV | -1.04 | -0.54(-5.19,4.1) | 0.81 |
| Vit D | -1.69 | -1.2(-6.04,3.63) | 0.61 |
| Vit D +WBV | 1.35 | 1.85(-2.8,6.51) | 0.42 |
| F [3,28]=0.61, p=0.61 | | | |
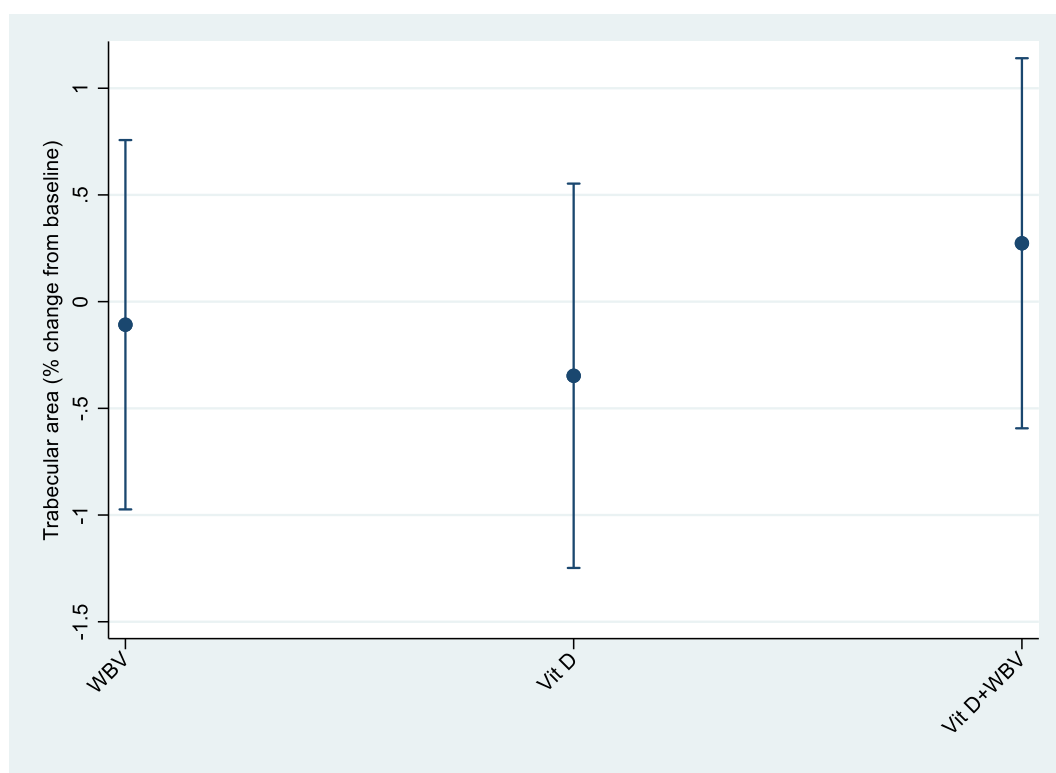


*Figure 4.35: Percentage change in trabecular area from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Total density*

A weak but significant association was found between vitamin D (Vit D group) and

total density (p = 0.05) (Table 4.6, Figure 4.36, below).

Table 4.6: Total density change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline total density, baseline PTH, baseline 25(OH) D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline $(mg/cm^3)$ | Adjusted mean difference (from Placebo) with 95% CI $(mg/cm^3)$ | p-value |
|---|---|---|---|
| Placebo | -1.15 | Reference | |
| WBV | 1.02 | 2.17(-0.45,4.8) | 0.10 |
| Vit D | 1.54 | 2.7(-0.00,5.41) | 0.05 |
| Vit D +WBV | 0.36 | 1.51(-1.08,4.12) | 0.24 |
| F [3,28]=1.54, p=0.22 | | | |
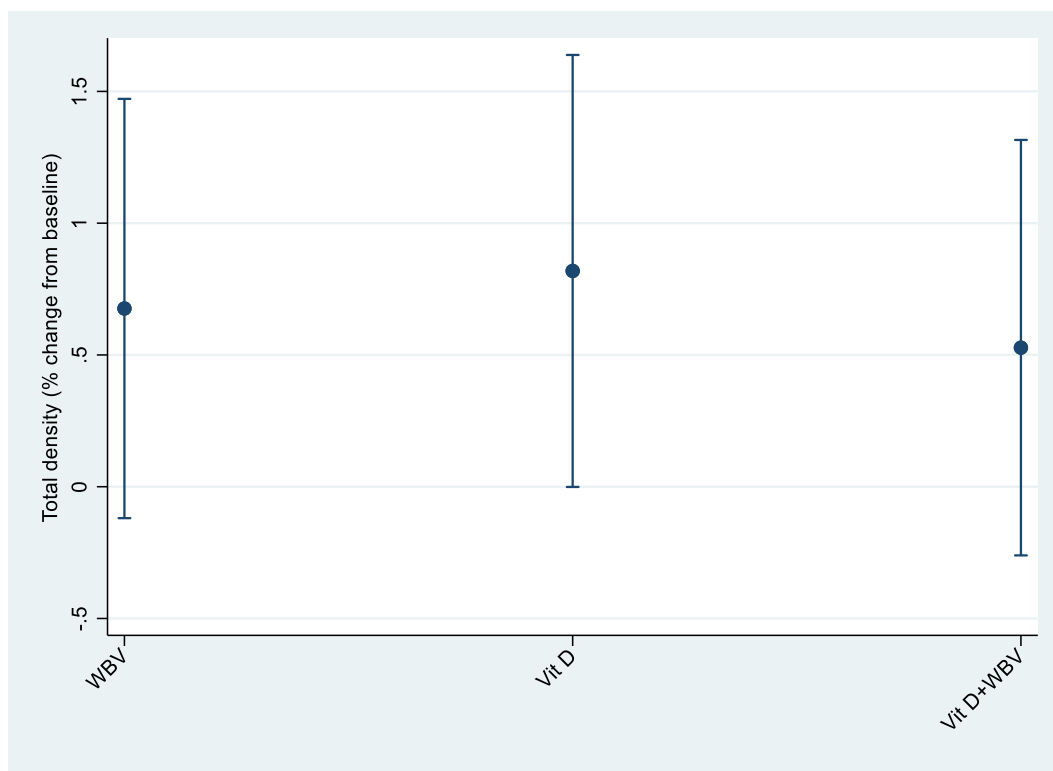


*Figure 4.36: Percentage change in total density from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Cortical density*

No significant changes were observed in any group at 12 weeks

Table 4.7: Cortical density change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline cortical density, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mg/cm$^3$) | Adjusted mean difference (from Placebo) with 95% CI (mg/cm$^3$) | p-value |
|---|---|---|---|
| Placebo | -0.36 | Reference | |
| WBV | 0.92 | 1.28(-3.01,5.59) | 0.54 |
| Vit D | 1.19 | 1.56(-2.94,6.07) | 0.48 |
| Vit D +WBV | -0.25 | 0.11(-4.14,4.37) | 0.95 |
| F [3,28]=0.25, p=0.85 | | | |
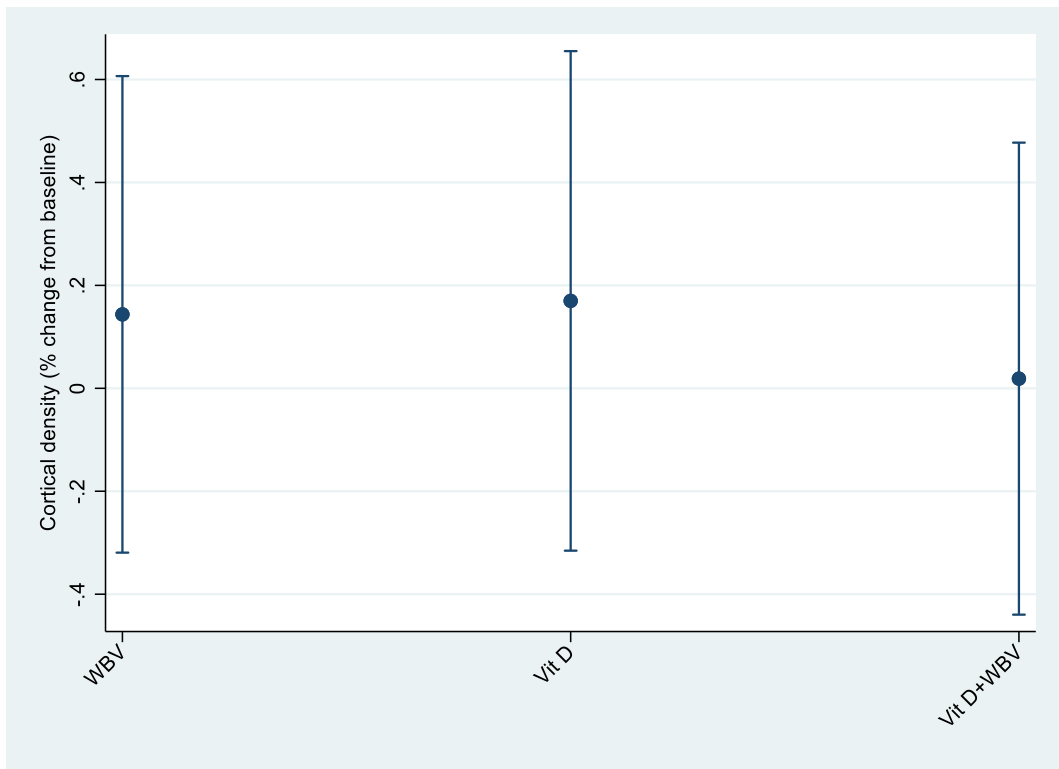


*Figure 4.37: Percentage change in cortical density from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Trabecular density*

No significant changes were observed in any group at 12 weeks.

Table 4.8: Trabecular density change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline trabecular density, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mg/cm$^3$) | Adjusted mean difference (from Placebo) with 95% CI (mg/cm$^3$) | p-value |
|---|---|---|---|
| Placebo | -0.8 | Reference | |
| WBV | -0.11 | 0.69(-1.12,2.5) | 0.44 |
| Vit D | 0.41 | 1.21(-0.65,3.08) | 0.19 |
| Vit D +WBV | 0.15 | 0.95(-0.81,2.72) | 0.27 |
| F [3,28]=0.69, p=0.85 | | | |
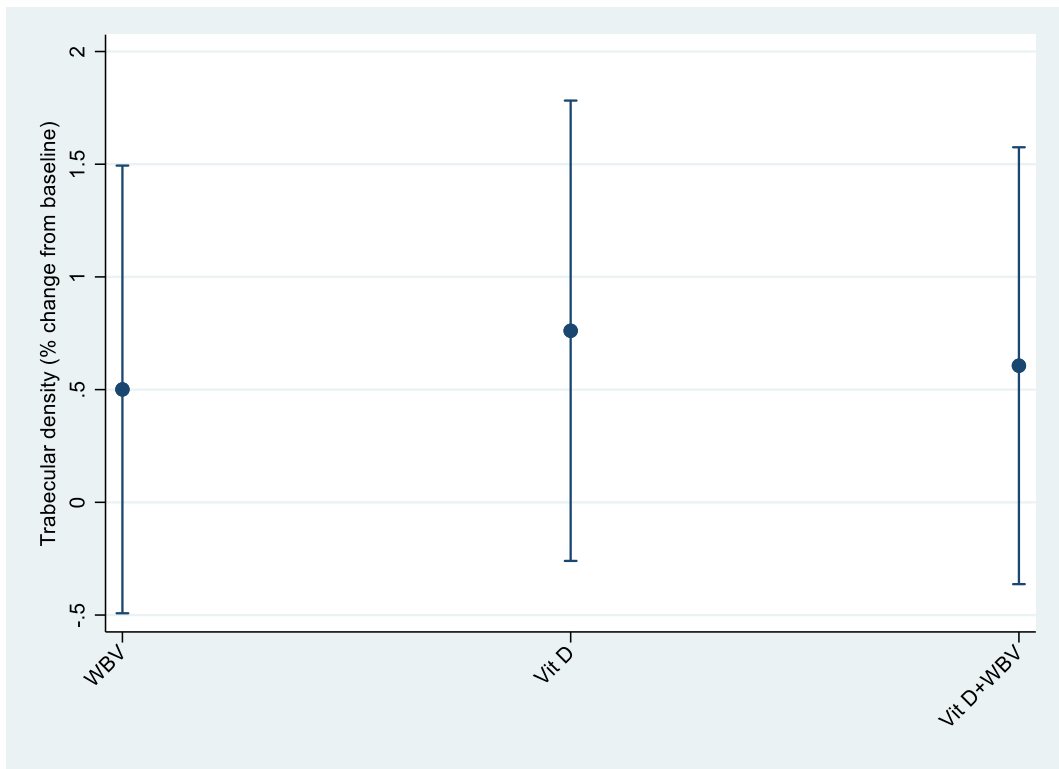


*Figure 4.38: Percentage change in trabecular density from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Trabecular number*

No significant changes in trabecular number were found at 12 weeks in any group

(Table 4.9 and Figure 4.39, below).

Table 4.9: Trabecular number change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline trabecular number, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (1/mm) | Adjusted mean difference (from Placebo) with 95% CI (1/mm) | p-value |
|---|---|---|---|
| Placebo | 0.0017 | Reference | |
| WBV | -0.035 | -0.0367(-0.158,0.085) | 0.54 |
| Vit D | 0.0298 | 0.028(-0.112,0.168) | 0.68 |
| Vit D +WBV | 0.0163 | 0.0145(-0.105,0.134) | 0.80 |
| F [3,28]=0.50, p=0.68 | | | |
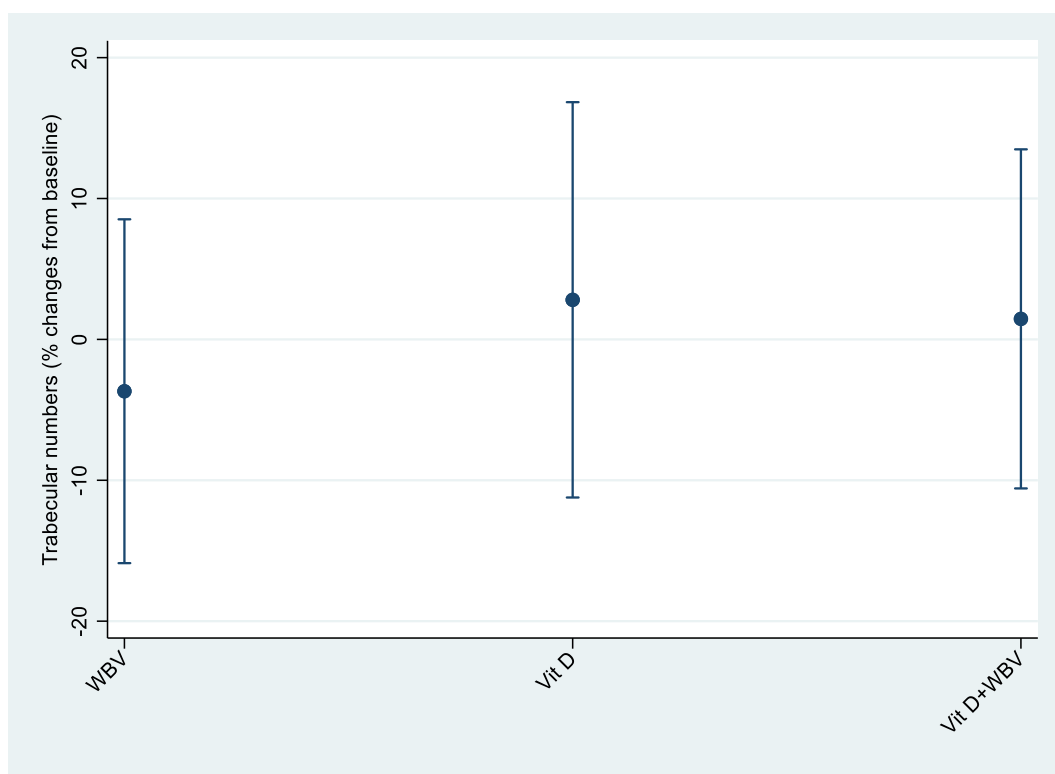


*Figure 4.39: Percentage change in trabecular number from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo.*

*Trabecular separation*

No significant changes in trabecular separation were found (Table 4.10 and Figure

4.40, below).

Table 4.10: Trabecular separation change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline trabecular separation, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mm) | Adjusted mean difference (from Placebo) with 95% CI (mm) | p-value |
|---|---|---|---|
| Placebo | 0.0001 | Reference | |
| WBV | 0.0104 | 0.0102(-0.0206,0.0412) | 0.50 |
| Vit D | -0.0122 | -0.0123(-0.0466,0.0219) | 0.46 |
| Vit D +WBV | -0.0083 | -0.0084(-0.0393,0.0223) | 0.57 |
| F [3,28]=1.00, p=0.40 | | | |
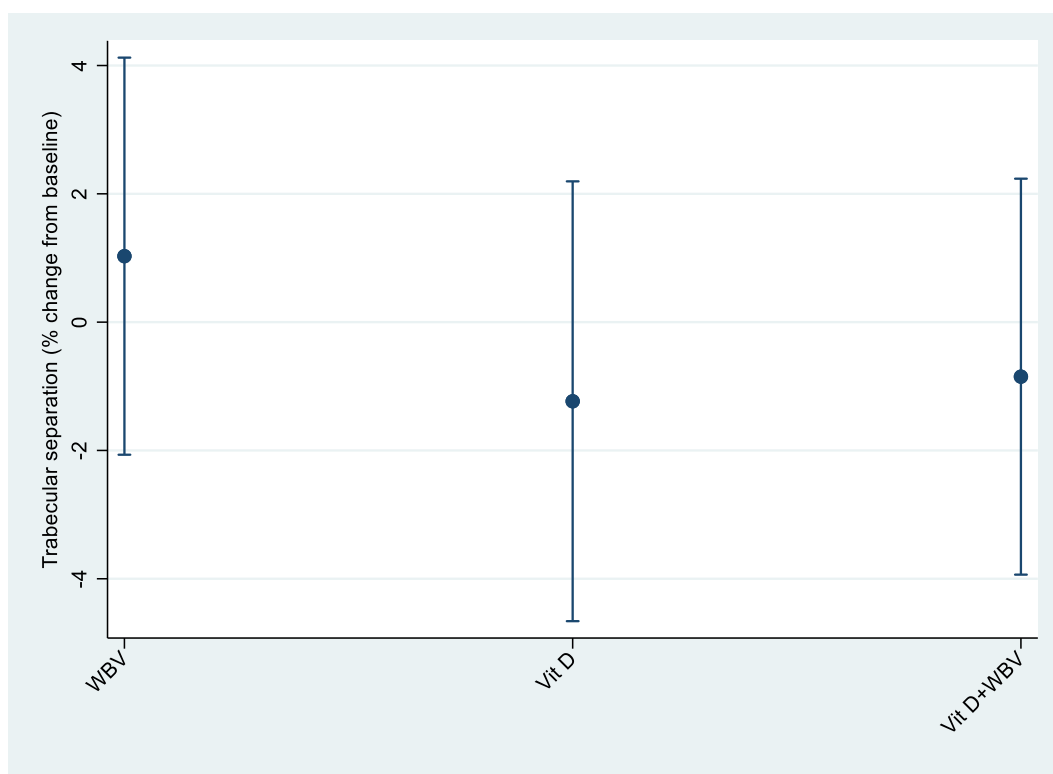


*Figure 4.40: Percentage change in trabecular separation from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Trabecular thickness*

No significant changes in trabecular thickness were found (Table 4.11 and Figure 4.41, below).

Table 4.11: Trabecular thickness change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline trabecular thickness, baseline PTH, baseline 25(OH) D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mm) | Adjusted mean difference (from Placebo ) with 95% CI (mm) | p-value |
| --- | --- | --- | --- |
| Placebo | -0.0012 | Reference | |
| WBV | 0.0008 | 0.002(-0.0045,0.0086) | 0.52 |
| Vit D | -0.0004 | 0.0007(-0.006,0.007) | 0.81 |
| Vit D +WBV | -0.0037 | -0.0025(-0.0089,0.0039) | 0.43 |
| F [3,28]=0.73, p=0.54 | | | |
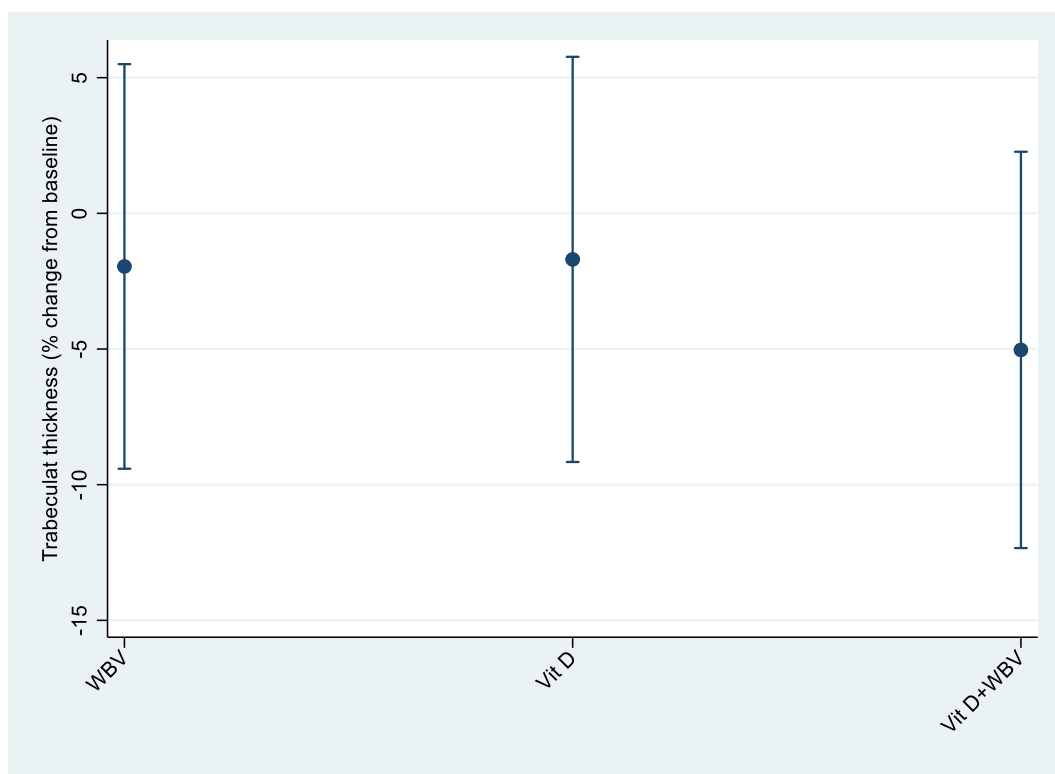


*Figure 4.41: Percentage change in trabecular thickness from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Cortical thickness*

No significant changes in cortical thickness were found (Table 4.12 and Figure
4.42, below).

Table 4.12: Cortical thickness change from baseline: regression analysis by
randomization group. Means and their differences adjusted by baseline cortical
thickness, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mm) | Adjusted mean difference (from Placebo) with 95% CI (mm) | p-value |
|---|---|---|---|
| Placebo | -0.0008 | Reference | |
| WBV | 0.0054 | 0.0063(-0.0043,0.0169) | 0.23 |
| Vit D | 0.0073 | 0.0082(-0.0028,0.0192) | 0.13 |
| Vit D +WBV | 0.0083 | 0.0091(-0.0014,0.0197) | 0.08 |
| F [3,28]=1.23, p=0.31 | | | |
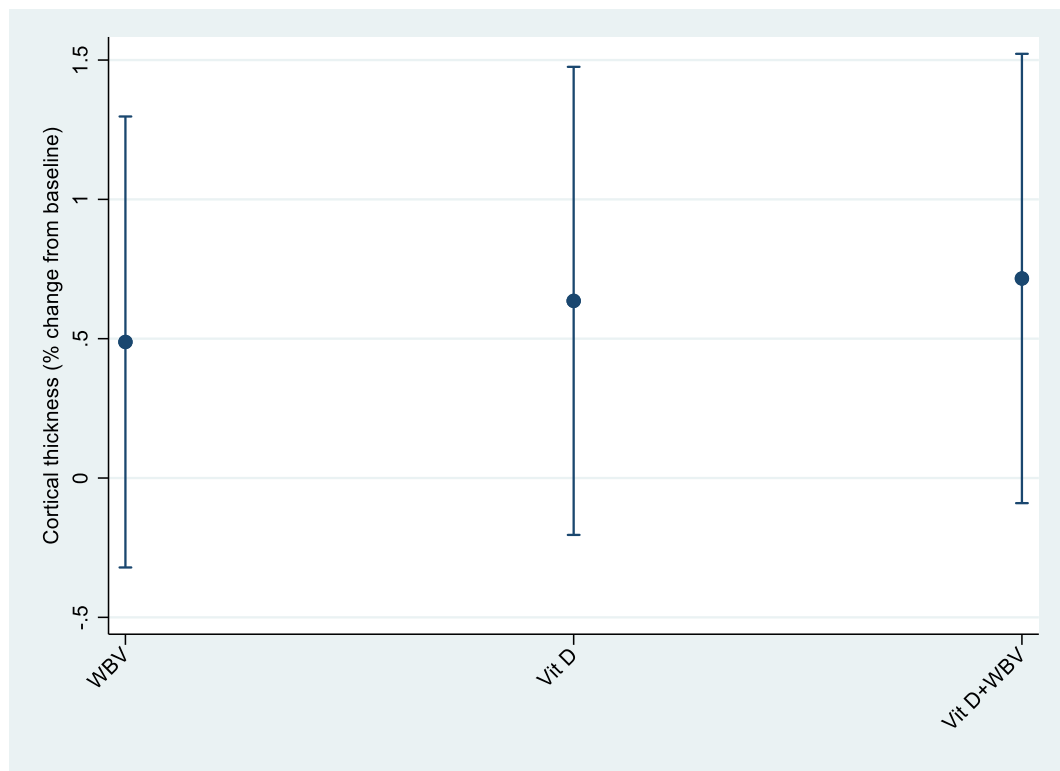


*Figure 4.42: Percentage change in cortical thickness from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Cortical tissue mineral density*

No significant changes in trabecular thickness were found (Table 4.13 and Figure 4.43, below).

Table 4.13: Cortical tissue mineral density change from baseline: regression analysis by randomization group. . Means and their differences adjusted by baseline cortical tissue mineral density, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (mg/ccm) | Adjusted mean difference (from Placebo) with 95% CI (mg/cm) | p-value |
|---|---|---|---|
| Placebo | -2.4 | Reference | |
| WBV | -2.29 | 0.10(-9.16,9.36) | 0.98 |
| Vit D | 3.21 | 5.61(-4.09,15.31) | 0.24 |
| Vit D +WBV | -0.78 | 1.61(-7.8,11.03) | 0.72 |
| F [3,28]=0.71, p=0.55 | | | |
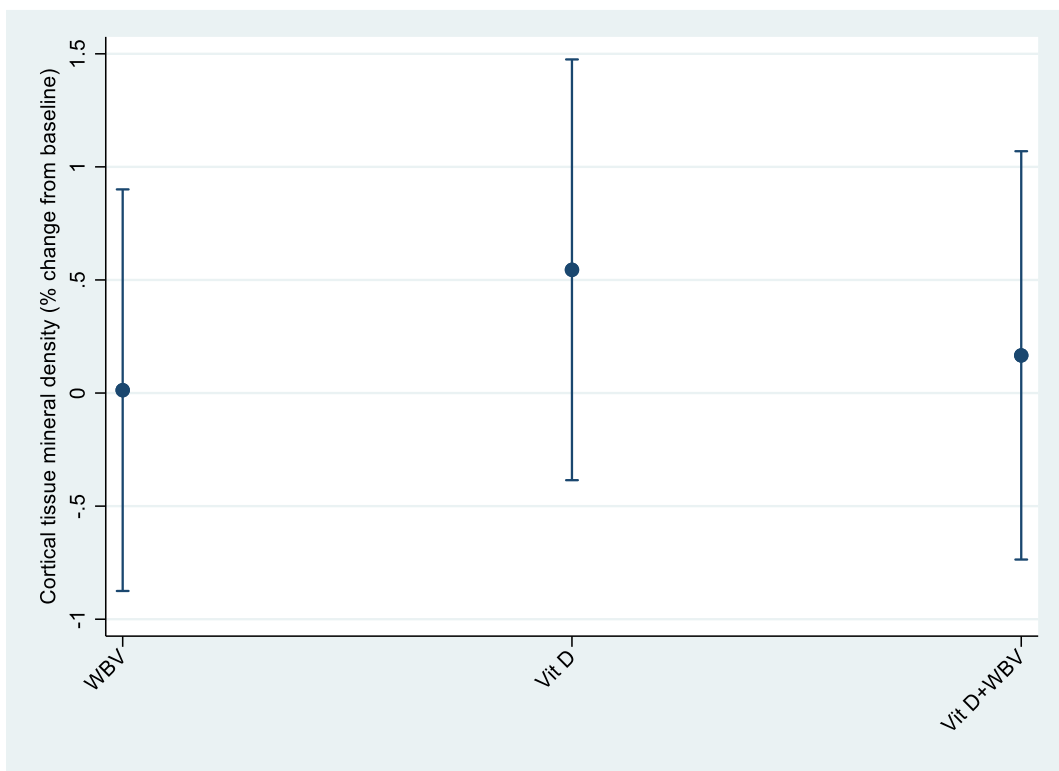


*Figure 4.43: Percentage change in cortical tissue mineral density from baseline. X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Cortical porosity* (%)

No significant changes in cortical porosity were found (Table 4.14 and Figure 4.44, below).

Table 4.14: Cortical porosity (%) change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline cortical porosity (%) baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (%) | Adjusted mean difference (from Placebo) with 95% CI (%) | p-value |
|---|---|---|---|
| Placebo | 0.0142 | Reference | |
| WBV | 0.0338 | 0.0196(-0.325,0.365) | 0.90 |
| Vit D | -0.0826 | -0.096(-0.458,0.264) | 0.58 |
| Vit D +WBV | 0.0001 | -0.014(-0.362,0.334) | 0.93 |
| F [3,28]=0.19, p=0.90 | | | |
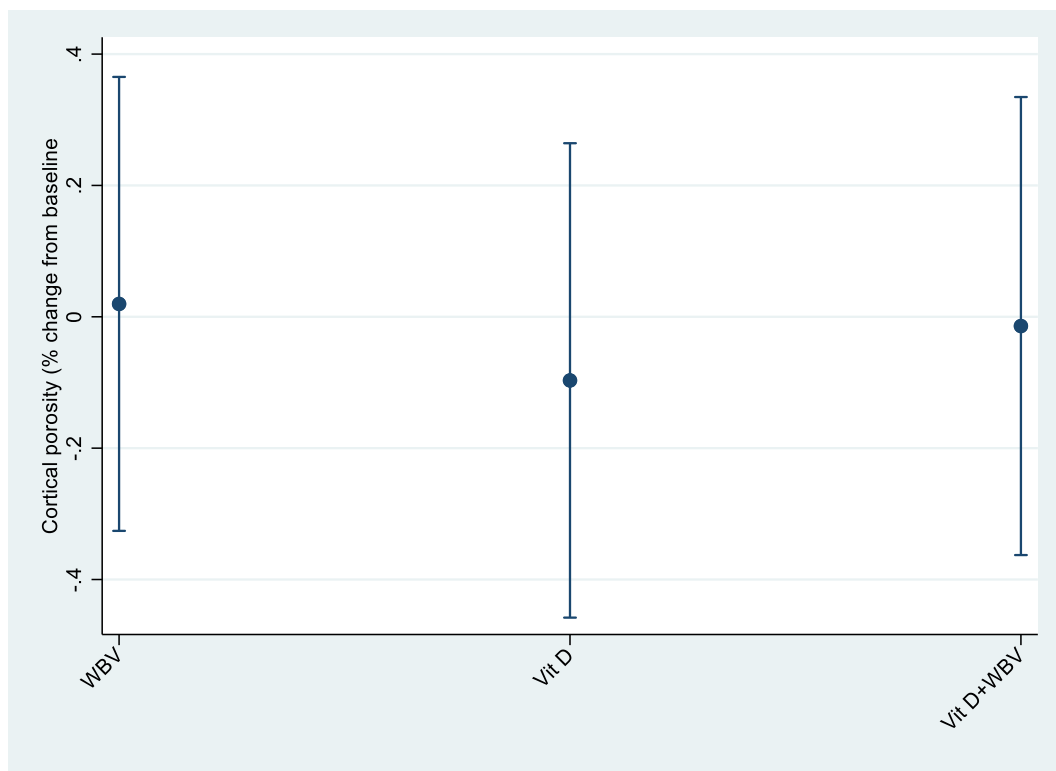


*Figure 4.44: Percentage change in cortical porosity from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Stiffness*

No significant changes in stiffness were found in any group at 12 weeks (Table

4.15 and Figure 4.45, below).

Table 4.15: Stiffness change from baseline: regression analysis by randomization group. Means and their differences adjusted by baseline stiffness, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (Kn/mm) | Adjusted mean difference (from Placebo) with 95% CI (Kn/mm) | p-value |
|---|---|---|---|
| Placebo | -0.12 | Reference | |
| WBV | 0.66 | 0.78(-2.78,4,35) | 0.65 |
| Vit D | -0.56 | -0.44(-4.15,3.27) | 0.80 |
| Vit D +WBV | -0.74 | -0.62(-4.19,2.93) | 0.72 |
| F [3,28]=0.29, p=0.83 | | | |
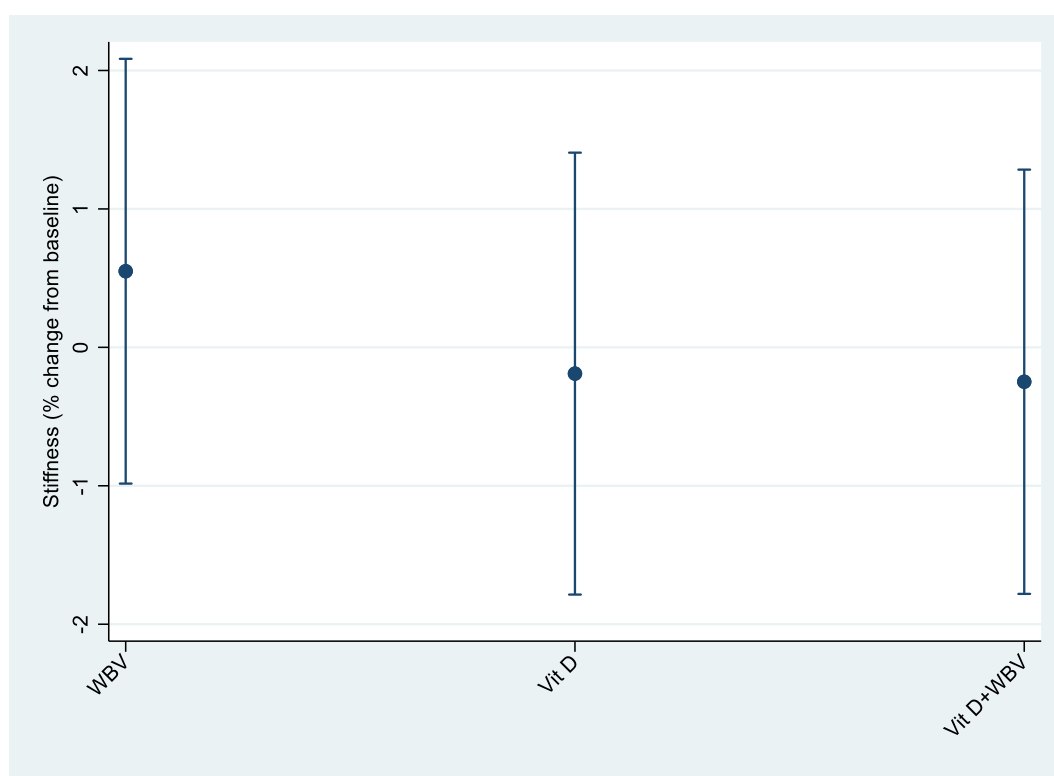


*Figure 4.45: Percentage change in stiffness from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*Estimate failure load*

No significant changes were found in any group at 12 weeks (Table 4.16 and Figure

4.46, below).

Table 4.16: Estimate failure load change from baseline: regression analysis by
randomization group. Means and their differences adjusted by baseline estimated
failure load, baseline PTH, baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (KN) | Adjusted mean difference (from Placebo) with 95% CI (KN) | p-value |
|---|---|---|---|
| Placebo | 0.030 | Reference | |
| WBV | 0.031 | 0.001(-0.27,0.27) | 0.99 |
| Vit D | 0.072 | 0.042(-0.24,0.32) | 0.76 |
| Vit D +WBV | 0.0047 | -0.025(-0.29,0.24) | 0.85 |
| F [3,28]=0.08, p=0.96 | | | |
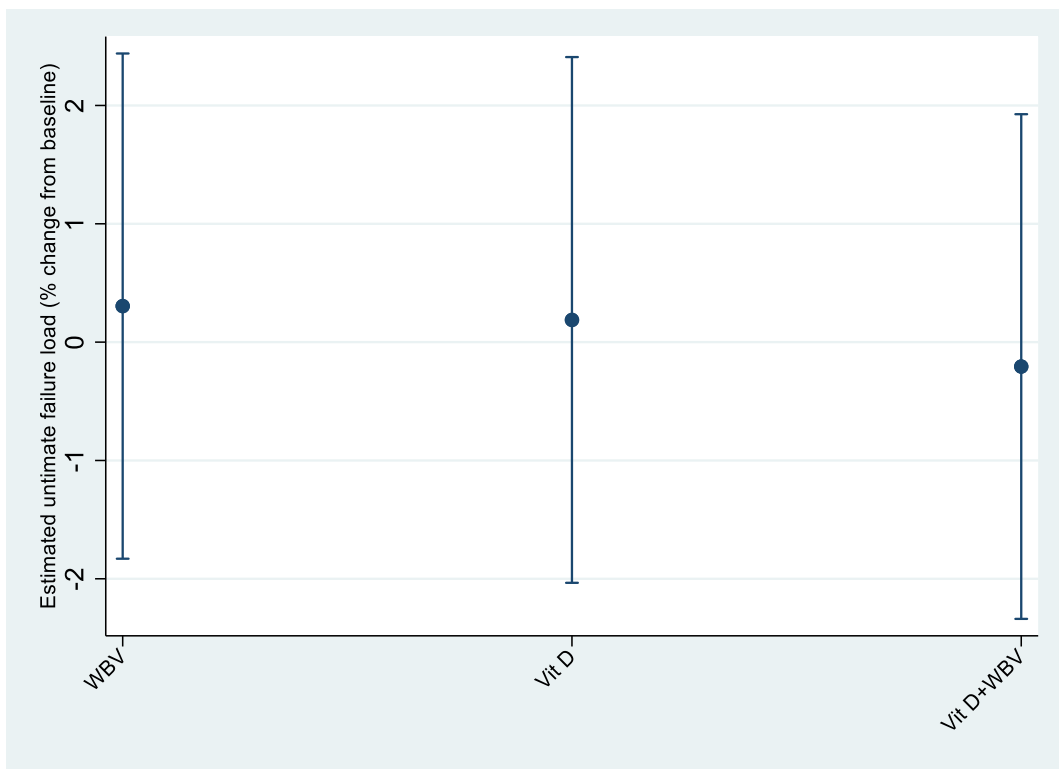


*Figure 4.46: Percentage change in estimate failure load from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

.

## 4.5 Change in serum 25(OH)D and PTH levels over 12 weeks

*Total 25(OH)D*

Significant increases were found in two groups, vitamin D group and vitamin D+

WBV group at the end of the study in comparison with the placebo group (p= 0.001

and p= 0.007, respectively) (Table 4.17 and Figure 4.47).

Table 4.17: Total 25(OH)D change from baseline: regression analysis by
randomization group. Means and their differences adjusted by baseline value
25(OH) D, baseline PTH and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (nmol/L) | Adjusted mean difference (from Placebo) with 95% CI (nmol/L) | p-value |
|---|---|---|---|
| Placebo | -3.14 | Reference | |
| WBV | -2.53 | 0.61(-9.47,10.7) | 0.90 |
| Vit D | 20.14 | 23.29(12.80,33.77) | 0.001 |
| Vit D +WBV | 10.90 | 14.05(3.98,24.13) | 0.0079 |
| F [3,29]= 11.05, p=0.0001 | | | |
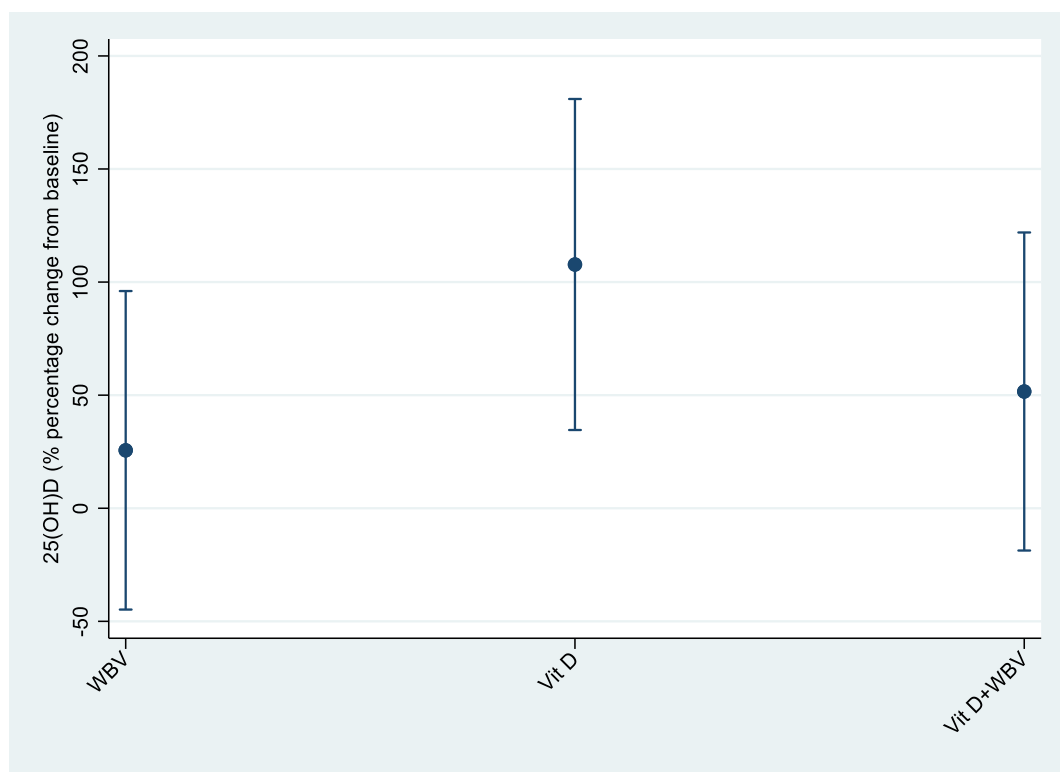


*Figure 4.47: Percentage change in serum 25(OH) D from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

*PTH*

A significant decrease in PTH level after 12 weeks was found in vitamin D only

group in response to 150,000 IU of vitamin D3 (Table 4.18, and Figure 4.48)

Table 4.18: PTH change from 12 weeks to baseline: regression analysis by
randomization group. Means and their differences adjusted by baseline PTH plus
baseline 25(OH)D and baseline BMI.

| Randomization group | Mean change 12 weeks to baseline (ng/L) | Adjusted mean difference (from Placebo) with 95% CI (ng/L) | p-value |
|---|---|---|---|
| Placebo | 27.94 | Reference | |
| WBV | 2.73 | -25.21(-54.65,4.21) | 0.09 |
| Vit D | -11.61 | -39.55(-70.14,-8.97) | 0.013 |
| Vit D +WBV | 11.21 | -16.73(-46.12,12.65) | 0.09 |
| F [3,29]=2.38, p=0.09 | | | |
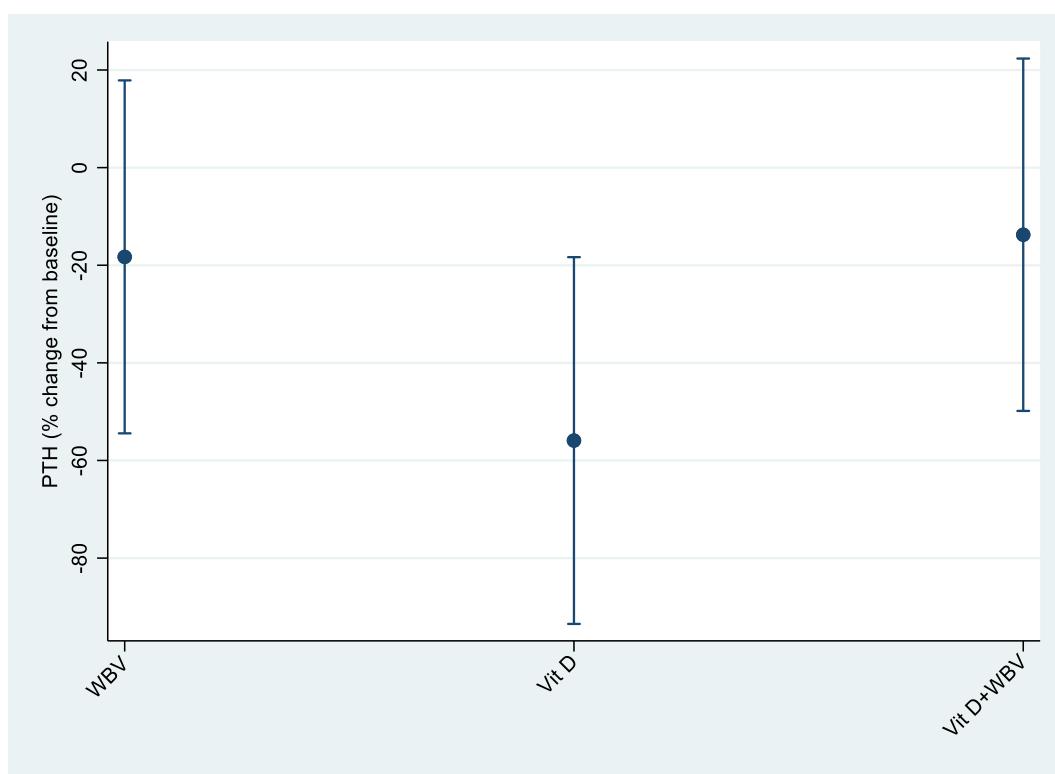


*Figure 4.48: Percentage change in serum PTH from baseline.*
*X axis; WBV, Vit D and Vit D+WBV are all difference from placebo*

## 4.6 Estimates of effect size

Table 4.19: Estimates of effect size from linear regression as described previously.

| Outcome measures 12 weeks-baseline | Effect size | n/group* |
|---|---|---|
| 25(OH)D nmol/L | 0.691 | 34 |
| PTH ng/L | 0.443 | 82 |
| Total density mg/cm$^3$ | 0.333 | 145 |
| Cortical density mg/cm$^3$ | 0.306 | 171 |
| Trabecular density mg/cm3 | 0.122 | 1075 |

*n/group makes no allowance for loss-to-follow-up. 80% power, 5% significance (2-tailed). For 90% power (5% significance, 2-tailed) multiply n/group by 1.33.*

## 4.7 Safety assessment

### 4.7.1 Vitamin D safety

Second void urine Samples were available for 37 participants. The sample for one participant was lost in the testing laboratory and one participant withdrew from the study without providing a sample. This participant was given the option to provide the sample without continuing in the study, but declined to do so. In addition, one sample was excluded because the participant did not follow the correct instructions in providing the sample (the last page in Appendix i). This participant was requested to provide another sample but declined. Therefore, 37 samples were available for analysis. No hypercalciuria was detected in any subject. The average calcium:creatinine ratio was 0.18 mmol/mmol creatinine (range 0.04-0.42), (lab reference = 0.00-0.70 mmol/mmol creatinine).

No serious adverse events were reported. However, some mild adverse events were reported in the first few weeks after starting WBV training (Table 4.20, below). The WBV protocol was well tolerated by the study population.

### 4.7.3 Mild adverse events

Table 4.20: Summary of adverse events

| Adverse event | Number of participants | Action taken |
|---|---|---|
| Fainting before/after venepuncture | 3 | Reassured the participant, gave cold water to drink, and sat the participant in a comfortable position |
| Itching of the lower legs (brief duration, during or just after WBV) | 19 | Participants were monitored at each WBV session. This symptom disappeared by the end of the second week of WBV for 6 participants, by the end of the third week for 11 participants, and by the end of the fourth week for 2 participants |

## 4.8 Examples of HR-pQCT images

Baseline:
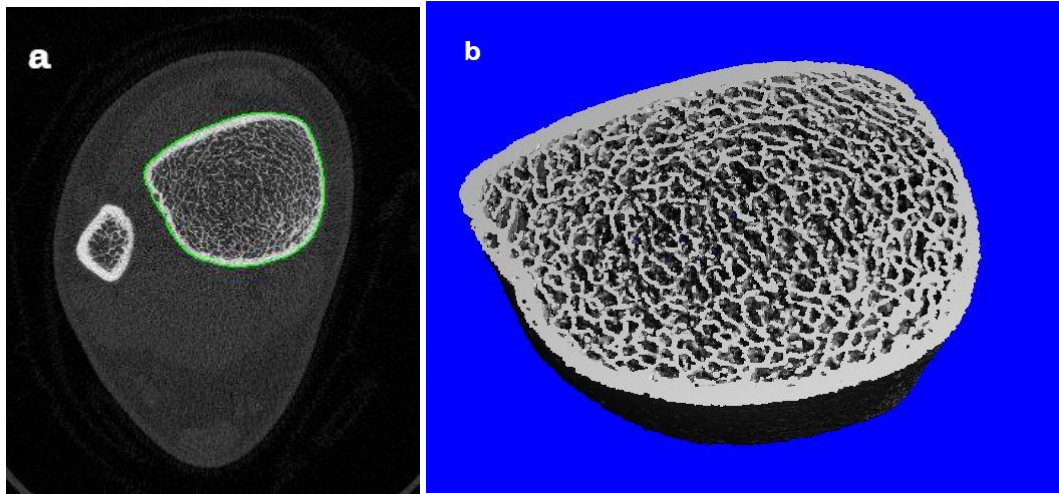


Follw-up:
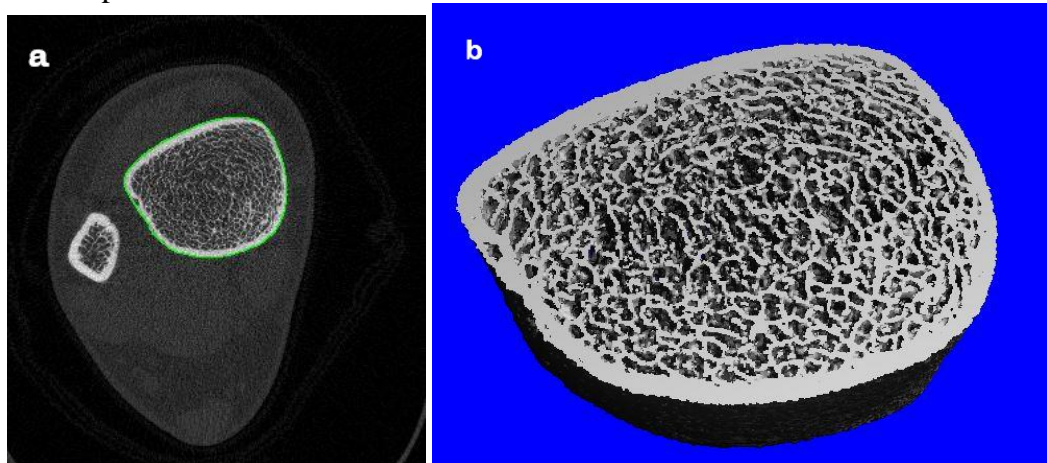


*Figure 4.49: Left tibia of a 19-year-old female volunteer who was randomised to the vitamin D and WBV group. a: axial 2D image slice, b: reconstructed 3D image.*

*Baseline: Tot.vBMD=329.6 mg/cm³, Ct.vBMD= 920.9 mg/cm³, Tb.vBMD = 161 mg/cm³*
*Follow-up: Tot.vBMD 328.6 mg/cm³, Ct.vBMD = 918.9 mg/cm³, Tb.vBMD 159.9 mg/cm³*

Baseline:
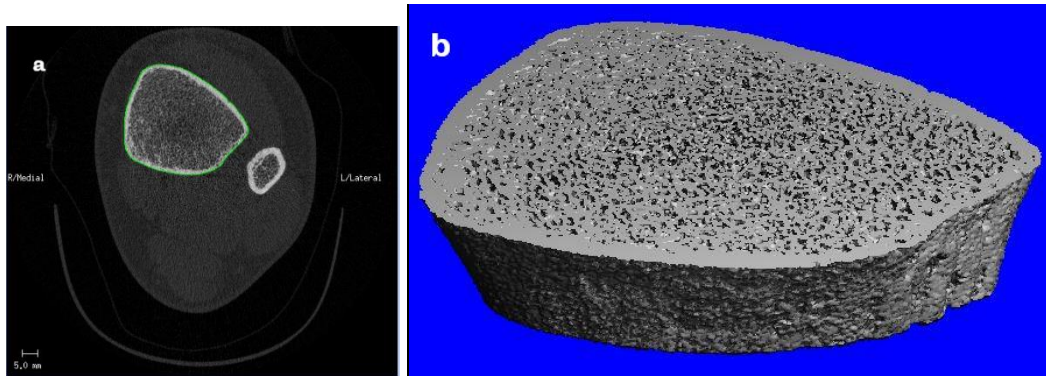


Follow-up:
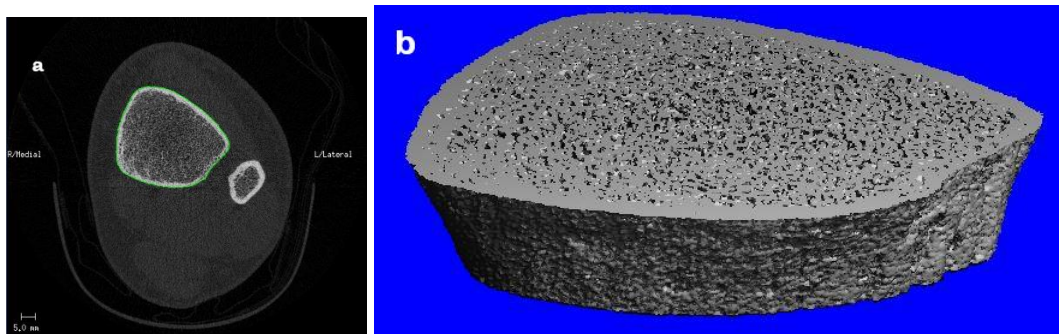


*Figure 4.50: Left tibia of a 30-year-old male volunteer who was randomised to the to the vitamin D and WBV group. a: axial 2D image slice, b: reconstructed 3D image.*

*Baseline: Tot.vBMD=343.4 mg/cm³, Ct.vBMD=907.8 mg/cm³, Tb.vBMD=194.7 mg/cm³*
*Follow-up: Tot.vBMD=350.1mg/cm³, Ct.vBMD=913.0 mg/cm³, Tb.vBMD=196.5 mg/cm³*

# CHAPTER 5

# DISCUSSION

The current study was primarily aimed at determining the effect of a large dose of vitamin D and 12 weeks of high intensity WBV on HR-pQCT parameters of the distal tibia, and to compare and contrast this to the effect of vitamin D alone, WBV alone, and no intervention. In addition, the current study aimed to determine the effect of a large dose of vitamin D and 12 weeks of high intensity WBV on the levels of serum 25 (OH)D and serum PTH, and to compare such an effect to the effect of vitamin D alone, WBV alone or no intervention. Furthermore, the current study aimed to assess the safety of both the large dose of vitamin D and the high intensity WBV on an apparently healthy adult MENA population who live in the UK. In order to achieve the study's aims and objectives, four groups of participants of equal number were created with the same number of males and females in each group. The study groups received one of the following treatments: WBV alone, placebo, vitamin D alone or vitamin D and WBV. To our knowledge, this is the first study to investigate the combined effect of vitamin D and WBV on bone density, microarchitecture and strength using this design. In addition, to the best of our knowledge, the current study is the first study to determine HR-pQCT parameters alongside serum levels of vitamin D, PTH and bone profile as part of a wider assessment of the impact of these interventions.

The main finding of this study is that there were no significant effects of WBV and vitamin D on bone density, microarchitecture or bone strength parameters after 12 weeks. However, a non-significant increase in total bone density in all treatment groups relative to controls was found. In addition, a weak correlation was found between vitamin D + WBV and an increase in cortical thickness. In contrast with three previous RCTs, which examined the effect of WBV and vitamin D on bone density, the results of the current study is consistent with two studies (Slatkovska

et al., 2011, Kiel et al., 2015), which found no effect of WBV and daily vitamin D on bone density after 12 and 24 months, respectively. However, the result of the current study is not consistent with another study, which reported a significant improvement in hip BMD after 6 months of WBV and daily vitamin D and calcium (Verschueren et al., 2011). However, in this latter study (Verschueren et al., 2011), WBV and vitamin D were not superior to vitamin D alone as significant improvements were also reported in the group that received vitamin D with no WBV. It should be noted though, that participants in Verschueren et al. (2011) performed dynamic exercises while standing on the platform, which caused it to be extremely difficult - if not impossible - to distinguish between the effect of WBV and the effect of dynamic exercises.

It should also be noted that all these previous studies (Slatkovska et al., 2011, Verschueren et al., 2011, Kiel et al., 2015) included no control group for vitamin D. Therefore, the effect of vitamin D alone on bone density was not ascertained against control group. Furthermore, these three studies used a daily vitamin D dosing regime and allowed the adherence to the regime to be self-reported. Our trial thus contains major differences from these previous studies. We have included a group with no treatment that served as a control for all study groups. Moreover, our study used one large dose administered orally under direct supervision instead of the daily dosing regimen. In addition, our study determined serum 25(OH)D, PTH and bone profile at baseline and at the end of the study alongside HR-pQCT scanning parameters. Another key difference is that our study used high intensity WBV protocol with a higher compliance (84%) than the three previous trials. Finally, the participants in our study were younger, and part of the MENA ethnic group.

In comparison with exercise trials that used vitamin D as co-intervention, the result of our study is similar to the result of another trial, which showed no effect of calcium–vitamin-D3-fortified milk plus exercise in improving bone density in older males (50 -79 years) after 12 and 18 months (Kukuljan et al., 2009, Kukuljan et al., 2011). However, our results differ from those of another study which found that military training and calcium and vitamin D supplementation significantly improved volumetric bone mineral density at the tibia compared to exercise alone in 46 military recruits (Gaffney-Stomberg et al., 2014). It may be that this difference is due to the difference between military training and the WBV protocol that was used in our study in terms of the intensity and duration of the exercise.

Although our study used the most advanced technique for high-resolution imaging of bone, namely HR-pQCT, to determine the effect of the intervention, our study did not find significant improvements in HR-pQCT parameters of the distal tibia in response to WBV and vitamin D or WBV alone. However, a weak correlation was found between the administration of 150,000 IU of vitamin D3 and an improvement in total density of the distal tibia after 12 weeks relative to control group. This increase in total density was borderline for statistical significance (p=0.05). Several previous trials have found significant effects of both conventional and large doses of vitamin D on areal bone density (Dawson-Hughes et al., 1995, Ooms et al., 1995, Islam et al., 2010, Macdonald et al., 2013, Dawson-Hughes et al., 1991). However, these trials ran over a longer period than our trial (12 months to 24 months). The current study indicates that the benefits of vitamin D supplementation might be more pronounced after only 12 weeks when using high-resolution imaging to determine the effect of vitamin D on bone density.

Our results found a weak association between administering high doses of vitamin D and an improvement in total density of the distal tibia. In contrast, Harwood et al. (2004), showed significant improvement in lumber spine and hip BMD after administering a high dose of vitamin D2. However, two previous studies showed no significant effects of large doses of vitamin D on bone density (Jorde et al., 2010, Reid et al., 2017). This variation in the outcomes may have been caused by the baseline serum 25(OH)D levels of participants in these studies. For instance, most of the participants in our study and in Harwood et al. (2004) were vitamin D deficient compared to the other two studies where most participants were vitamin D sufficient at baseline (Jorde et al., 2010, Reid et al., 2017). This finding is consistent with two previous reviews, which reported that the effect of vitamin D supplementation on bone density is determined by baseline vitamin D levels (Reid et al., 2014, Winzenberg et al., 2011). It should be noted that our study did not show any meaningful changes in other HR-pQCT parameters in response to the large dose of vitamin D. In contrast, Lima et al. (2018) reported a significant increase in the trabecular number and a decrease in trabecular separation in response to vitamin D dose of 50,000 IU/week for 24 weeks in juvenile-onset systemic lupus erythematosus (JoSLE) patients. The reason our study did not report these changes may have been due to the shorter duration of the intervention or perhaps due to the difference in vitamin D dosing strategy between the trials. This is purely conjecture however, since trials investigating the effect of vitamin D supplementation on HR-pQCT parameters are very limited.

In terms of the WBV alone intervention, this did not lead to significant improvement in bone density, strength and microarchitecture after 12 weeks. This finding is consistent with several previous studies which reported no overall

positive effect of WBV on bone density. For instance, the result of the current study is consistent with Liphardt et al. (2015), who used HR-pQCT to investigate the effect of high intensity WBV for 12 months in 42 healthy postmenopausal women and found no significant effect on bone microarchitecture and strength despite the fact that our study recruited younger population and used higher intensity and higher frequency WBV protocol. This suggests that it is not easy to define an effective WBV protocol or identify a target population that can benefit most from WBV.

In spite of the fact that our study accounted for vitamin D deficiency and included vitamin D as a co-intervention, there were still no advantages for WBV with vitamin D as an intervention. However, the current study measured the effect of the intervention at one skeletal site (the distal tibia). Some studies, which measured bone density at other skeletal sites, have shown positive effects of WBV on bone density. For instance, Lai et al. (2013) found a significant improvement in DXA lumbar spine BMD after 6 months of WBV. It possible that a significant effect might have been present in lumber spine in our cohort. However, due to the limited duration of our study, changes in areal BMD may not have had time to occur. Furthermore, due to the limited finical resources in our study, measurements were limited to one skeletal site (the distal tibia) and one modality (HRpQCT).

Apart from the correlation found between the high dose of vitamin D and the increase in total density, it is important to highlight that we did not witness significant changes in any of the other HR-pQCT parameters in any group. The small sample size of the current trial may have hindered our ability to detect statistically significant changes or it could be true a finding i.e. that there is no

significant effect of WBV and vitamin D on bone. Since there was no similar previous WBV study to our study with four arms (as far as we know), it is hard to compare these results with any from the literature. Future trials in this area based on power calculation are therefore recommended. This current study will provide a foundation to enable such larger trials.

As was predicted, the majority of participants were vitamin D deficient at baseline (serum 25(OHD) < 30 nmo/L). The median serum total 25 (OH)D was 23 nmol/L for all samples, and all four groups were considered vitamin D deficient at baseline. This finding is constant with the literature, which reports widespread vitamin D deficiency among MENA populations (Fuleihan and Deeb, 1999, Meddeb et al., 2005, Saadi et al., 2006, Elsammak et al., 2010, Botros et al., 2015, Chakhtoura et al., 2018). As a consequence of this vitamin D deficiency, the participants' serum PTH levels were slightly elevated. These are worrying findings as having a low vitamin D level and high PTH over a long period may lead to poor musculoskeletal health. What is more worrying is the fact that we recruited apparently healthy adults, most of whom were attending university and thus may be supposed as having some awareness from the media about the importance of vitamin D to their bone health. Vitamin D deficiency is therefore likely to be more common in the MENA population who may have limited access to the information about vitamin D and health. It is therefore recommended that further steps be taken to address this issue and to increase the awareness of the importance of vitamin D for the health of the MENA population living in the UK. This could be done in collaboration with GPs, community leaders' and school and university societies. Introducing screening programmes for individuals at risk of vitamin D deficiency may also be a good

strategy to tackle this issue, but such a programme would be associated with an increased financial burden to the health services.

Moving now to the results related to the use of a large dose of vitamin D and its effect on serum 25(OH) and PTH. This was effective in increasing vitamin D levels even 12 weeks after administration. Serum 25 (OH)D levels increased significantly in the two groups that received vitamin D (vitamin D only and vitamin D+WBV). The increase in total 25(OH) D was 20.14 nmol/L (p= 0.001 relative to the Placebo group) in vitamin D group and 10.90 (p=0.0079 nmol/L relative to the Placebo group) in vitamin D+WBV group. This finding is consistent with the literature, which indicates that a single large dose of vitamin D is effective in increasing vitamin D levels in individuals with low vitamin D. For instance, Gopal‑Kothandapani et al. (2018) found a single dose of vitamin D3 (150,000 IU, similar to our dose) increased 25(OH)D levels by around 56 nmol/L, 4 weeks after dosing in a cohort of Caucasian and Asian adults. Our study measured serum 25 (OH)D levels at week 13 after dosing and the effect of vitamin D was still significant at this time point. Although vitamin D rose in among the volunteers who received both WBV and vitamin D, we found that the increase in serum 25(OH)D level was lower than the increase found the volunteers who received who received vitamin D only. This difference in circulating serum 25(OH)D between the active / non-active groups who received vitamin D has been found in a previous exercise trial (Kukuljan et al., 2009). Despite the fact that the baseline levels of vitamin D in our cohort were greatly lower than the cohort recruited in this trial, (Kukuljan et al., 2009) found around 50% greater increase in the absolute change in serum 25(OH)D in the group that received vitamin D only compared to the group who received vitamin D and did undertake exercise. This suggests that WBV and vitamin D may

have the same effect of exercise and vitamin D on serum 25(OH)D status. There could therefore be an interaction between physical stimulation and vitamin D which leads to a reduction in the circulating serum 25(OH)D. However, the mechanism of such proposed interaction is not clear.

Another key finding is that PTH decreased significantly in vitamin D group. (decreased by 11.61 ng/L at week 13 after administrating the large dose of vitamin D). Due to the low vitamin D levels at baseline among our cohort, vitamin D dose used in our study might not have been high enough to cause a sharp decrease in PTH level. It has been reported that higher doses of vitamin D in the range of 200,000 to 300,000 IU may be required to effectively suppress PTH levels (Kearns et al., 2013). However, previous studies reported adverse events after administering 200,000 IU of vitamin D2 (Tellioglu et al., 2012) and 300,000 IU of vitamin D3 (Premaor et al., 2008) such as gastrointestinal complications. Thus, the current study attempted to strike a balance between the safety of vitamin D and effectively increasing serum 25(OH)D and therefore it was felt that 150,000 IU was a suitable dose on the basis of doses used in previous studies. This high dose of vitamin D was well tolerated. No adverse events were found in any participant as a result of administrating 150,000 IU of vitamin D3. Although this is consistent with some previous studies that used the same dose and reported no adverse events (Oliveri et al., 1996, Gopal‑Kothandapani et al., 2018), the ethnicity of the participants in the current study is different from the ethnicities in the two previous studies. Therefore, our study showed that the 150,000 IU dose is safe in the healthy adult MENA population.

WBV alone led to only a slight increase in PTH level. This is somewhat similar to the finding reported by (Kukuljan et al., 2011): a reduction in PTH level in response to exercise alone. However, this study lasted for 18 months while our study lasted for far less time - 3 months only. A similar effect of WBV to the effect of exercise on PTH might be observed in longer WBV trails. Since this study is the first study to determine the relation between PTH and WBV, including PTH in the future WBV is recommended. Interestingly, PTH level rose from baseline in the group that received WBV and vitamin D. This result does not agree with (Kukuljan et al., 2011) in terms of the reduction in PTH level in the group which received vitamin D and did exercise in this latter study.  This difference may have been caused by the extreme difference in the baseline level of vitamin D between our trial and Kukuljan et al's. (2011) trial.  It is therefore hypothesised that WBV and vitamin D supplements in vitamin D deficient populations increase PTH level and may indicate the need to optimise vitamin D and PTH levels before undertaking a WBV training regime but this needs to be investigated further.

Finally, the MENA population was chosen as a target population in this study due to the lack of research in this population despite the widespread vitamin D deficiency and the higher prevalence of osteoporosis among this ethnicity (Arabi et al., 2010, Hilger et al., 2014, Chakhtoura et al., 2018, Sadat-Ali et al., 2012).  This study may provide a platform for further research in the MENA population in either Europe or in the MENA countries. The recruitment rate was better than was initially expected and the study was completed earlier than had been planned. This can be attributed to several factors. First of all, the target population was involved in the study design. A public involvement (PI) event was held by the present researcher in the setting-up phase of the study to determine the convenience of the study's

location, the timing of the study visits and the timing of the WBV sessions. Accordingly, the PhD student liaised with the CRF manager to accommodate the potential participants' preferences. In addition, participants in the PI event were involved in designing the materials which were used to advertise the study and in wording the information sheet. The recruitment methods that were used (via university email database and poster distribution) may have also contributed to the effective recruitment. It is also possible that the university students are more interested in research and that recruiting from the general MENA public in the UK may not yield the same recruitment outcome.

# CHAPTER 6

# CONCLUSION, STRENGTHS AND

# LIMITATIONS,

# RECOMMENDATIONS AND FUTURE

# DIRECTIONS

## 6.1 Conclusion

The main aim of the current project was to determine the combined effect of a 150,000 IU single oral dose of vitamin D3 and 12 weeks of high intensity WBV on the HR-pQCT parameters of the distal tibia. The main findings of this work indicate that a large single oral dose of vitamin D and 12 weeks of high intensity side-alternating WBV did not improve bone density, bone microarchitecture or bone strength. Similarity, 12 weeks of high intensity WBV without vitamin D did not improve bone density, bone microarchitecture or bone strength. However, a positive association was found between administrating a large dose of vitamin D without WBV and an improvement in total volumetric BMD at the distal tibia.

The large dose of vitamin D was effective in increasing serum 25(OH)D in a healthy adult population, who were mostly vitamin D deficient and had immigrated from the MENA region to the UK. This large dose of vitamin D without WBV was found to reduce PTH. A slight increase in PTH levels was found in response to WBV without vitamin D. However, a more pronounced increase in PTH levels was found in response to WBV and a large dose of vitamin D at the end of the study.

The 150,000 dose of vitamin D3 administered to the participants was found to be safe in this MENA population. The dose was well tolerated, and there were no observed adverse effects in any participants following its administration. In addition, no hypercalciuria or hypercalcemia were found as a result of administrating 150,000 IU of vitamin D3. The high intensity side-alternating WBV was well tolerated. No serious adverse events were found in any participant as a result of undertaking the WBV training regime for 12 weeks. The compliance of the participants to 3 WBV sessions each week for 12 weeks was very good. This indicates that WBV is feasible and safe in MENA populations living in the UK.

## 6.2 Strengths and limitations

The major strength of the current study is its prospective and interventional nature, as well as its use of the randomised controlled trial design. Furthermore, the MENA population recruited in the current study is another unique point. In addition, our study included four different groups with a group without treatment/intervention, which served as a control group for the other three. Therefore, it was possible to ascertain the impact of each intervention and to compare and contrast the effect of each against the control group. We used HR-pQCT, which is the state-of-the art imaging modality for assessing bone structure non-invasively without exposing our participants to a high amount of ionising radiation. Beyond this, administering a large single dose of vitamin D under direct supervision eliminated the issue of adherence to daily doses of that vitamin. Similarly, all WBV sessions were supervised; therefore, it was possible to accurately record the participants' compliance and to ensure that the correct WBV positioning was performed, including posture and positioning of the feet on the platform.

However, the current study has some limitations that should be considered. We used strict inclusion and exclusion criteria for participation; therefore, the results may not represent the general MENA population living in the UK. For instance, it is possible that the result would have changed had we recruited children or elderly cohorts. In addition, it was not possible to obtain a sham device due to the limited funding for the current study. The advantage of double blinding is well known, and in WBV studies involves using a training device (Fregni et al., 2010). It has been suggested that a sham WBV may not totally mask the true WBV (Rubin et al., 2004). We did not have access to sham/training device, therefore double blinding was not possible for the WBV intervention used in our study.

Another limitation of our study is the fact that we did not perform additional measurements at timepoints between baseline and the end of the study interventions. This is especially correct for serum 25(OH)D and PTH, which would be expected to respond sooner than bone to a large dose of vitamin D. The effect of the large dose of vitamin D on serum 25(OH)D would be expected to be highest during the first 4 weeks following its administration (Kearns et al., 2013). We sought additional external funding in order to perform more measurements, but our application was not successful. Therefore, measurements were restricted to two time points at the beginning and at the end of the study due to the limited financial resources and the limited timescale to complete the current PhD project. Additionally, although the vast majority of data was collected during winter (including all baseline vitamin D data), some data was collected during summer months. Therefore, seasonal variations in serum 25(OH)D cannot be totally eliminated as some participants may have synthesised vitamin D by exposing themselves to the sun during the brighter months. However, this is doubtful in the MENA population due to the conservative clothing style as well as culture and religious reasons.

Measurements for bone density were taken at one skeletal site only, which represents a further limitation. Although we used the most advanced imaging modality to determine the effect of the intervention and hypothesised that the greatest effect would be observed at the distal tibia since this is the long bone closest to the platform, we cannot eliminate the possibly that a significant effect might have been detected at another skeletal site further away from the platform. However, the same limited financial reason hindered our ability to perform additional scans at various skeletal sites.

Another limitation of our study was the small sample size. This may have limited our ability to detect significant changes in HR-pQCT parameters at the distal tibia. However, there was no prior data that could be used to conduct a power calculation. Our study therefore should be regarded as a pilot trial. Among the several approaches to determine a sample size for a pilot trial, we have chosen a sample size of 40 because we felt that this approach was most suitable for our aims and available resources. The data generated from this study can be now used to power a larger definitive trial. Finally, our study only covered a relatively short period, and the results obtained were in the context of a 3 month intervention. The long-term effect of WBV in the MENA population living in the UK remains unknown. Moreover, the compliance and the dropout rate may have been higher had the WBV training protocol lasted for a longer period.

## 6.3 Recommendations and future directions

Some blood samples for our participants have been withdrawn and stored within the Sheffield Teaching Hospital (STH) biorepository, which is licenced to store human tissue for the purposes of research. The samples are stored at -80C and will be kept for a maximum period of 5 years. They include two blood samples for each participant: one at baseline and the other at the end of the study. The next step will be applying for a grant in order to carry out additional analyses on the stored samples. These analyses may include vitamin D binding protein (DBP), vitamin D binding protein (DBP) genotyping and markers of bone turnover.

Future trials examining the effect of WBV on bone should pay special attention to the vitamin D status and PTH levels. We have generated and estimates of effect size based in our data in the result chapter. This is can be used to power a larger trial

using the same design as used in our trial is recommended to understand the interaction between WBV and vitamin D and their effects on bone density, bone microarchitecture, bone strength, serum 25(OH)D level and PTH levels. In addition, future trials may consider using different WBV intensities or targeting a different age group or population. Furthermore, future studies should aim to conduct the WBV over a longer period and consider the value of taking measurements for bone density at more than one skeletal site.

The baseline serum 25(OH)D indicated that the vast majority of participants were suffering from vitamin D deficiency. Despite the issue of low levels of vitamin D among MENA populations being well known for many years, it was noticed that 12 participants (30%) had extremely low levels of serum 25(OH)D (<15 nmol/L), without any symptoms alarming them to visit their doctors. There is a need for future research in the MENA population living in the UK. The evident vitamin D deficiency among our participants indicated the fact that more research is needed to understand the causes for this phenomenon among the MENA population who live in developed country such as in the UK. This may identify some strategies to optimise vitamin D status among this population. Our study assessed the effect of 150,000 IU of vitamin D3 for a period of 3 months, and our study found it to be a safe dose. A future study is recommended to determine the safety and effectiveness of higher doses of vitamin D on vitamin D status among the MENA population. Our study did not find WBV to be effective in improving bone density among the apparently healthy MENA population who live in the UK. Therefore, based on our results, a future trial of WBV among the apparently healthy MENA population is not recommended. An alternative approach such as exercises trails aiming to improve bone density among the MENA population is recommended.

# SECTION 4

# CHAPTER 7

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## Appendices

*Appendix i: VibeD study information sheet*

Sheffield Children's **NHS**
NHS Foundation Trust

# Effect of Vitamin D and Vibration on Bone (VibeD Study)

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. **One of our team will go through the information sheet with you and answer any questions you have.** Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear.

### Part 1 – To give you first thoughts about the project

**1. What is the purpose of the study?**
The aim of this study is to find out if whole body vibration (mimicking weight-bearing exercise) 3 times every week for a period of 12 weeks and a large oral dose of vitamin D is more effective in increasing bone mass in a group of young men and women compared to either vitamin D or vibration alone. We will use a very detailed bone scanner called a High Resolution peripheral Quantitative Computed Tomography (HR-pQCT) scanner to measure the effects of the vibration and vitamin D on bone formation and strength of your ankle. This project is being undertaken as part of a PhD project.

**2. Why have I been invited?**
You have been chosen because you are a healthy adult from a Middle East or North African country who lives in Sheffield, United Kingdom, your age is between 18-40 years and you have met all of our inclusion criteria.

**3. Do I have to take part?**
No, it is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You will be given a copy of the information sheet and the signed consent form to keep for your records. You are free to withdraw at any time, without giving a reason.

**4. What will happen to me if I agree take part?**
When you consent to participate in the study, you will be allocated to one of four groups by chance (random allocation). This is because sometimes we don't know which way of treating patients is best. To find out, we need to compare different interventions. We put people into groups and give each group a different intervention. The results are compared to see if one is better. To try and make sure the groups are the same to start with, each volunteer is put into a group by chance (randomly). You have a 25% chance of being allocated to any group. This study is a double blind trial which means you will not know which group you are in (vitamin D or no vitamin D), neither will the researchers know to which group you are allocated (although if we need to find out we can do so).

According to your group allocation, we will arrange a timetable for attending research appointments that suits you. Group 1 will receive vibration training and vitamin D. Group 2 will receive vibration training and placebo (no vitamin D). Group 3 will receive vitamin D but no vibration training. Group 4 will have placebo (no vitamin D) and no vibration training.

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Neither you nor the researchers can choose which group you will be allocated to, this has to be done by random allocation. Otherwise, this will weaken the validity of the study results. By signing the consent form, you are agreeing to be randomly allocated to one of these four groups.

If your allocation falls into either one of the vibration groups, we will require you to attend for vibration training at Sheffield Children's Hospital (SCH) 3 times/week for 12 consecutive weeks. Each session will consist of three bouts and each bout will last for three minutes with a one-minute rest between bouts (12 minutes in total for each session). Vibration training will require you to stand on a platform with your knees slightly bent.

Some individuals in the study will be taking a dose of 150,000 IU vitamin D. All participants will have some x-ray exposure. We wish to avoid any risk to an unborn child that might result from this. **If you think that you are or might become pregnant during this study, we would ask you not to take part.**

All participants in all study groups are required to make five visits to Sheffield Children's Hospital (SCH) and two visits to the Northern General Hospital (NGH). There is a free shuttle bus for university staff and students, which runs daily, every 30 minutes, between the university campus and NGH. The journey between SCH and NGH takes approximately 25 minutes by bus. Details of these visits are summarised below:

Visit 1, SCH: time estimate 20-30 min

During this visit we will answer any questions you may have in relation to the study, provide information sheets and assess your eligibility to participate in the study.

Visit 2 (SCH): time estimate 15-20 min

At this visit we will obtain signed informed consent and confirm future study visits.

Visit (NGH): time estimate 10 min (males), 15 min (females)

At this visit, you will have a pregnancy test if you are female. We will only recruit you to the study and do the scan if the result of the pregnancy test is negative. A high resolution scan of your non-dominant ankle will be acquired. It is a very quick scan which looks at the microstructure of your bone. You will be asked to stay as still as possible during the scan. The actual scan will take about 3 minutes. We will supply you with a map illustrating the location where you will have your scan at NGH.

Visit 4 (SCH): time estimate 45-60 min

For this visit, we will ask you to not have anything to eat or drink except tap water from midnight before the day of your visit. Breakfast will be provided towards the end of your visit. We will do the following during your visit:

1. Measure your height and weight.

2. Take a blood sample from you (10 ml, approximately 2 teaspoons) to measure various parameters including vitamin D and calcium and parathyroid hormone. After your blood sample has been taken, breakfast will be provided to you and you will be free to eat and drink after that.

3. We will give you a small amount of yoghurt to take– this may contain 150,000 IU of vitamin D in it or no vitamin D in it (placebo). **If you are allergic/intolerant to dairy products, then you cannot take part in this study.** You will take the yoghurt (with or without vitamin D) under our direct supervision. For female participants, we will

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repeat the pregnancy test if the gap between visit 3 and this visit is more than one week. We will only administer vitamin D when pregnancy is excluded.

4. We will provide you with a urine bottle and ask you to provide a 2nd void fasting urine sample (instructions are on the last page of this document).
5. Finally, we will assess your dietary intake of calcium by asking you a set of standardised questions.

Visit 5 (SCH): time estimate 10 min

This visit is one week after visit 4. During this visit, we will ask you to not eat or drink anything except water from midnight until you do your 2nd void fasting urine sample.

Visit 6 (NGH): time estimate 10 min (males), 15 min (females)

This visit is 12 weeks after visit 4. The HR-pQCT scan of your non-dominant ankle will be repeated. If you are female, we will repeat the pregnancy test and scan you once we confirm that the test result is negative.

Visit 7 (SCH): time estimate 15-20 min

This visit is 12 weeks after visit 4; it will be in the same week as visit 6, but not necessarily on the same day. All blood samples and height and weight will be repeated.

## 5. Expenses and payments

You will be given a £150 or a £50 voucher according to the nature of your involvement in the study (paid on completion of the study) in recognition of your time and dedication. Those randomised to vibration will receive a £150 voucher; those not randomised to vibration will receive a £50 voucher. Breakfast will be provided at visit 4 and visit 7 (after taking blood samples). There is a free shuttle bus for university students and staff which travels daily, every 30 minutes between Sheffield University Campus and NGH.

## 6. What will I have to do?

You are expected to attend all scheduled visits at SCH and NGH and to attend for your vibration training if your allocation falls in one of the vibration groups. You will be requested not to make any plans to travel abroad during the study period. If you are planning to go abroad, please tell us so we can schedule your participation for a later date. You will also be asked to refrain from taking vitamin supplements during the study period and to take measures to avoid pregnancy during the study if you are a female.

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

You will receive a small radiation dose if you choose to participate in the study. Each HR-pQCT scan is equivalent to a half day's naturally occurring background radiation. If we need to repeat the scan due to poor quality of the image, we will do this only once. Participation in the study will therefore expose you to a maximum of few days of additional background radiation. As a result of this we must exclude pregnancy and all women will have a pregnancy test just before each scan.

There is a risk, as with many other clinical research projects, of discovering previously unknown or unexpected clinical findings in you. With your permission, we will write to your general practitioner (GP) to let them know about your participation and we will communicate any clinical findings to your GP for further action. We will also contact you to tell you what is happening.

## 8. What are the side effects of any treatment received when taking part?

You might feel itching in your legs after your vibration session which should improve after the first few sessions. The manufacturer of the platform has said that the person standing on the

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platform may feel slightly sick or dizzy, get blisters on the bottom of his/her feet, or feel itchy in his/her legs, if the platform is used for a long time. There is no evidence in the literature to suggest that vibration (for the duration planned in this study) will cause any serious adverse effects. We will monitor you during and after every vibration session to make sure you are comfortable with the vibration training.

Developing an adverse reaction to vitamin D is uncommon. Taking a single large dose of vitamin D might lead to an increase in vitamin D levels in the blood. This might then lead onto an increased level of calcium in the blood and its excretion in the urine. The common symptoms and signs related to high levels of calcium in the blood are nausea, vomiting, loss of appetite, flushing, muscle weakness, excessive thirst, increased urine output, constipation, abdominal pain and inflammation of the pancreas. Excessive amounts of calcium in the urine over the long term might lead to kidney failure.

However, there is no evidence in the literature suggesting a rise in calcium levels in blood or urine following a single dose of 150,000 IU of vitamin D3 in otherwise normal individuals. To ensure your safety we will check for calcium levels in the urine with the spot urine test that is scheduled for visit 5.

The figures for side effects quoted by the manufacturer of the Invita D3 solution are – hypercalcaemia and hypercalciuria occurring in less than 1 in 100 individuals; rash, pruritus (itching) and urticaria (allergic rash, hives) occurring in less than 1 in 1,000 individuals. These figures may represent a broader (in terms of both age and other illnesses or ill-health) population who may therefore be more at risk of such complications than in healthy young adults.

9.     **What are the possible benefits of taking part?**
There is no expected direct benefit for you but we hope the research will increase our understanding of how bones respond to vibration and vitamin D, provide us with a means of assessing that response and help us to optimise bone accrual in children and prevent osteoporosis in adults. If your vitamin D levels are low before taking the vitamin D dose, taking 150,000 IU of vitamin D will help you increase your body vitamin D stores. With your consent, we will inform your GP if your vitamin D level is low.

10.     **What happens when the research study stops?**
We will collect all the information together and we will decide if it is useful in telling us if a combination of a large dose of vitamin D and vibration training had any effects on your ankle that were detectable by HR-pQCT.

11.     **What if there is a problem?**
We do not anticipate any problems. However, any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

12.     **Will my taking part in the study be kept confidential?**
Yes. We will follow standard ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

**This completes Part 1.**

**If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.**

**Part 2 of the information sheet**

### 13. What if relevant new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, someone from the research team will tell you and discuss whether you should continue in the study. If you decide not to carry on, arrangements will be made for your care to continue. If you decide to continue in the study you may be asked to sign an agreement outlining the discussion.

### 14. What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will use the data collected up to the time of your withdrawal. We will ask why you have withdrawn, however you may choose NOT to answer if you don't wish to. Any stored samples that can still be identified as yours will be destroyed if you wish.

### 15. What if there is a problem?
**Complaints**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

Dr Amaka C Offiah , Reader in Paediatric Musculoskeletal Imaging

Sheffield Children's Hospital

Tel: 01142260716        Email: a.offiah@sheffield.ac.uk

If you remain unhappy and wish to complain formally, you can do this by contacting:

Patient Advice & Liaison Co-ordinator, Sheffield Children's NHS Foundation Trust

Tel: 0114 271 7594

**Harm**

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation, but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

### 16. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. Once the study is complete, all study data will be retained for a period of 5 years following the end of the study.

Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 1998.

### 17. What will happen to any samples I give?

Once tested for your vitamin D levels along with the other related metabolites, the residual blood (serum/plasma) samples not used in the analyses will be transferred to an appropriate Biobank to be saved for up to 5 years with your consent. If you have given consent the sample will be used for future research. This would be in relation to future studies of vitamin D and/or bone health. Dr Amaka C Offiah will act as custodian and control access to the samples.

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Participant Information Sheet
Version 6
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**18. Will any genetic tests be done?**
Genetic testing relating to vitamin D research may be carried out on the saved blood samples in the future for a period of up to 5 years after storing samples.

**19. What will happen to the results of the research study?**
When the study has finished we will present our findings to other researchers, and we will put the results in medical magazines and websites that researchers read. We would also like to put a brief summary on the hospital research website so that you will be able to read about our results too. This will be available at the end of the study, on: www.sheffieldchildrens.nhs.uk/research-and-innovation.htm

The results will also be included as part of a PhD thesis and made available on White Rose eTheses Online - White Rose University Consortium, where you can read it and download it free of charge. Results will be anonymous, which means that you will not be able to be identified from them.

**20. Who is organising and funding the research?**
Researchers at Children's NHS Foundation Trust are organising this study. They will not get any extra money for doing this research.

**21. Who has reviewed the study?**
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by Yorkshire and the Humber – South Yorkshire Research Ethics Committee.

It has also been given approval to run at this hospital by the Research Department.

**22. How can I find out more?**
If you would like to find out more about research in general, the Clinical Research Facility at this hospital has an **Information for families** section on its website
www.sheffieldchildrens.nhs.uk/research-and-innovation.htm or you can contact the hospital
Clinical Research Facility:
Ms Wendy Swann,
R&D Manager, Sheffield Children's NHS Foundation Trust
Tel: 0114 3053478


If you would like to know more specific information about this research project, please contact the PhD student:

Mr Hassan Alshamrani
PhD Student, Department of Oncology & Metabolism, University of Sheffield
Tel: 01142717228      Email: haalshamrani1@sheffield.ac.uk
If you have any concerns during the study, you should contact the project team.

**If you decide to take part in this study, you will be given this information sheet and signed consent form to keep.**

**Thank you for taking the time to read this information sheet.**


**(Please see next page for instructions on how to provide a 2nd void urine sample)**

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**\* Instructions for providing a 2nd void fasting urine sample**

We kindly ask you not eat or drink anything except water from midnight until you pass your 2nd void urine sample. To provide this sample, you pass urine once shortly after you get up/wake up, drink tap water and then provide a second sample in the bottle provided to you – this second sample is called a "2nd void urine sample". If you haven't had anything to eat or drink from midnight except water, then it is a "fasting 2nd void urine sample". After you have passed the fasting 2nd void urine sample, you will be free to eat and drink. Please hand the urine sample either to the researcher or to a receptionist in the Clinical Research Facility at SCH.

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**Sheffield Children's** *NHS*
NHS Foundation Trust

## PARTICIPANT CONSENT FORM

### Title of project: Effect of Vitamin D and Vibration on Bone (VibeD Study)

Name of researchers: Mr H A Alshamrani, Dr M Paggiosi, Prof NJ Bishop, Dr AC Offiah

Participant study number: _____          **Please initial box**

1. I confirm that I have read and understand the information sheet dated 14th August 2017 (Version 6) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical or legal rights being affected.

3. I understand that data collected during the study, may be looked at by researchers and those involved in the running and supervision of the study from Sheffield Children's NHS Foundation Trust or from regulatory authorities, where it is relevant to my taking part in research. I give permission for these individuals to have access to my data.

4. I agree to my GP being informed of my participation in the study.

5. I agree not get pregnant during the 12 weeks of the study, and to have a pregnancy test as required for this study (for females only).

6. I agree for my blood samples to be stored and used for future research for up to 5 years.

7. I agree to take part in the study.

_____        _____        _____
Name of Participant                   Signature                        Date

_____        _____        _____
Name of Person taking consent      Signature                        Date

When completed: 1 for participant; 1 (original) for researcher site file

**Sheffield Children's NHS**
NHS Foundation Trust

**Study title: Effect of Vitamin D and Vibration on Bone (VibeD Study)**

**Volunteer Screening Form**

| | | |
|---|---|---|
| 1- Aged 18 to 40 years? | Yes | No |
| 2- Originally from Middle East or North Africa? | Yes | No |
| 3- Willing to have pregnancy tests as required? | Yes | No |
| 4- Willing to take measures to avoid pregnancy during the study period? | Yes | No |
| 5- No previous fracture/surgery to or metal work in either leg? | Yes | No |
| 6- Is apparently healthy and NOT on long term medication? | Yes | No |
| 7- Is NOT allergic to vitamin D and/or dairy products? | Yes | No |
| 8- Willing to refrain from vitamin D supplementation during the study? | Yes | No |
| 9- Willing to avoid travel to sunny places during the study? | Yes | No |
| 10- Willing to commit to exercise protocol? | Yes | No |

**If any answer 1 to 10 above is NO, then ineligible for recruitment.**

Eligible [ ]          Not Eligible [ ]

**Recruited to the study:**

Yes [ ]          No [ ]

..........................................................................................................................

**This section is only to be completed for those subjects recruited to the study:**

Volunteer Name:

Date of Birth:

Sex:

Volunteer E-mail:

Volunteer telephone (optional):

GP name and address:

Study ID:

Preferred contact method: Calls [ ]     Texts [ ]     Emails [ ]

Name of person completing this form

_____

Signature                                    Date ____/____/_____  |

*Appendix iv: Advertising email*

Invitation Email to a Research Study

**Subject: [student & staff volunteer]** Effect of Vitamin D and Vibration on Bone, healthy volunteers form Middle East or North Africa needed

We are looking for male and female volunteers from any of the following countries: Algeria, Bahrain, Djibouti, Egypt, Jordan, Iran, Iraq, Kuwait, Lebanon, Libya, Malta, Morocco, Oman, Palestine/Israel, Qatar, Saudi Arabia, Syria, Tunisia, United Arab Emirates (UAE), and Yemen who live in Sheffield (aged 18 to 40 years) to take part in a PhD research project. This study aims to assess the response of the ankle bone to vitamin D and 12 weeks' vibration training (3 times/ week), as measured by high-resolution peripheral quantitative computed tomography (HR-pQCT). The research study will consists of four groups. Group 1 will receive vibration and vitamin D, Group 2 will receive vibration only, Group 3 will receive vitamin D only, Group 4 will not receive vibration or vitamin D. The allocation to groups has to be made by chance (random allocation).

Vitamin D will be given by mouth. Vibration training mimics weight-bearing exercise. The amount of radiation from the HR-pQCT scans is equivalent to few days of natural background radiation. Participants will have between 2 and 4 ankle scans.

Ethical approval has been granted and participants will receive either a £150 voucher or a £50 voucher (paid on completion of the study) according to the nature of their involvement.

**Interested? Then for more details please contact:**

Mr Hassan Alshamrani (PhD student)

haalshamrani1@sheffield.ac.uk

Or

Dr Amaka C Offiah (Supervisor)

a.offiah@sheffield.ac.uk

## VibeD Study: Effect of Vitamin D and Vibration on Bone

We are looking for male and female healthy volunteers from the Middle East or North Africa who live in Sheffield (aged 18 to 40 years) to take part in a PhD research project. This project aims to assess the response of the ankle bone to vitamin D and 12 weeks' vibration training, as measured by high-resolution peripheral quantitative computed tomography (HR-pQCT). The research study will consist of four groups. Group 1: vibration and vitamin D, Group 2; vibration only, Group 3; vitamin D only, Group 4: control group (no vibration or vitamin D). The allocation to groups has to be made by chance (random allocation).

Vitamin D will be given by mouth. Vibration training mimics weight-bearing exercise. The amount of radiation from the HR-pQCT scans is equivalent **to few days** of natural background radiation. Study participants will have between 2 and 4 ankle scans.

Ethical approval has been granted and participants will receive either a £**150** voucher for those in the vibration groups or a £50 voucher for those in the non-vibration groups (paid on completion of the study).

**Interested? Then for more details please contact:**
**Mr Hassan Alshamrani (PhD student)** haalshamrani1@sheffield.ac.uk
**Or**
**Dr Amaka C Offiah (Supervisor)** a.offiah@sheffield.ac.uk

Effect of Vitamin D and Vibration on Bone (VibeD study)
Invitation Poster
Version 3
Date: 11th August 2017
IRAS 225440

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*Appendix vi: Procedures for identifying contraindications to vitamin D*

Appendix 2: Procedures for contraindications for vitamin D, special warnings and precautions, interaction with other medicinal products as well as in case of undesirable effects

Table 1: Procedures for contraindications for vitamin D

| Contraindications | Procedure |
|---|---|
| Hypersensitivity to the active substance(s) or to any of the excipients | Volunteers will be screened prior to recruitment using eligibility questionnaire. Any volunteer with allergy to (vitamin D, Colecalciferol, tocopherol acetate, polyglyceryl oleate (E475), olive oil, refined and sweet orange peel oil) will be excluded. |
| Hypercalcaemia and/or hypercalciuria | Volunteer will be excluded |
| Nephrolithiasis and/or nephrocalcinosis | Volunteer will be excluded |
| Serious renal impairment | Volunteer will be excluded |
| Hypervitaminosis D | Volunteer will be excluded |
| Pseudohypoparathyroidism | Volunteer will be excluded |

Table 2: Procedures for special warnings and precautions

| Special warnings and precautions for use | Procedure |
|---|---|
| Impairment of renal function | Volunteer will be excluded |
| Cardiovascular disease | Volunteer will be excluded |
| Sarcoidosis | Volunteer will be excluded |
| Taking treatment for vitamin D at the time of recruitment | Volunteer will be excluded |
| Consuming foods or milk enriched with vitamin D | Volunteer will be recruited but will be advised to reduce the amount of food enriched with vitamin D |
| Renal stones | Volunteer will be excluded |
| Oral administration of high dose vitamin D (500,000 IU by single annual bolus) was reported to result in an increased risk of fractures in elderly subjects | No elderly subjects will be recruited (study age group between 18 and 40 years). The proposed dose is 150,000 IU (much less than 500,000 IU) |
| Pregnancy and breast-feeding | Volunteer will be excluded |

Table 3: Procedures for interaction with other medicinal products and other forms of interaction

| Interaction with other medicinal products | Procedure |
|---|---|
| Medicines that act on the heart or kidneys, such as cardiac glycosides (e.g. digoxin) or diuretics | Volunteers who are using, have recently used or might use any other medicines will be excluded |
| Medicines containing vitamin D | Volunteers will be excluded |
| Actinomycin (a medicine used to treat some forms of cancer) and imidazole antifungals | Volunteers who are using, have recently used or might use any other medicines will be excluded |
| Antiepileptic medicines, glucocorticoids, medicines that lower the level of cholesterol in the blood, medicines for weight loss, laxatives | Volunteers who are using, have recently used or might use any other medicines will be excluded |

*Appendix vii: The procedure in case of undesirable effects and reporting via the Yellow Card Scheme*

**Undesirable effects**

Adverse reactions are listed below, by system organ class and frequency. Frequencies are defined as: uncommon (>1/1,000, <1/100) or rare (>1/10,000, <1/1,000).

**Table 4: Procedures in case of undesirable effects**

| Undesirable effects | Procedure |
|---|---|
| *Metabolism and nutrition disorders*<br>Uncommon: Hypercalcaemia and hypercalciuria | We will perform a second void fasting urine 1 week after dosing to reassure that no hypercalciuria has occurred in any subject. |
| *Skin and subcutaneous disorders:*<br>Rare: pruritus, rash, and urticaria. | We will ask the volunteers to immediately report to us or to their GP. In addition, at their visit for the second void fasting urine we will ensure they do not have any undesirable effects. Should there be any undesirable effects, we will write to their GPs to inform them and arrange for their care. |
| Reporting of suspected adverse reactions:<br>Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the Yellow Card Scheme at: www.mhra.gov.uk/yellowcard. | We will ask volunteers to immediately report to us so we can report it via the Yellow Card Scheme. |

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