The relationship of bone health to vitamin D status and body composition in pre-adolescent children (Pretoria, South Africa)

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ABSTRACT

Introduction: Bone health development and maintenance is important in children to reduce the risk for osteoporosis later in life. Knowledge on the vitamin D and bone health status of preadolescent children in South Africa is limited. Vitamin D and body composition both play important roles in bone health, but the relationship between adiposity and bone mass in children has been debated. The objective of this study was firstly, to describe the bone health status, body composition and vitamin D status of preadolescent children in Pretoria, South Africa. Secondly, the study examined bone health in relation to body composition and vitamin D status.

Methods: A cross-sectional study, using conveniently sampled preadolescent black children aged 5-10, was conducted. Body weight was measured with the Seca medical body composition analyser and height using the Seca 274 stadiometer. Dual x-ray absorptiometry (DXA) was used for bone health (bone mineral content (BMC), areal bone mineral density (BMD) and bone area at the total body less the head (TBLH) and lumbar spine (LS) sites) and body composition (body fat percentage, fat mass and lean mass) assessments (n = 84). Vitamin D status (25(OH)D2 and 25(OH)D3) was determined from blood spot analysis (n = 59). To compare bone health means between vitamin D status groups, children were grouped as sufficient (25(OH)D ≥ 30 ng/ml), insufficient (25(OH)D = 21-29 ng/ml) or deficient (25(OH)D ≤ 20 ng/ml) accordingly. To compare bone health means between body composition groups, children were grouped as normal (BMI-for-age Z-score ≤ 1) or over-nourished (BMI-for-age Z-score > 1).

Simple linear regression models were used in defining the relationship between bone health parameters and body composition components. Adjustments of bone health parameters for height-for-age, gender, age and body composition components was done using multiple linear regression. Comparison between adjusted bone health parameters of normal and over-nourished were made using the student's two sample t-test.

Results: The 59 children in the vitamin D study groups had a 24% prevalence of low BMD for chronological age and 7% presented with a low BMC for chronological age. A peculiar finding was that LS-BMAD differed significantly between the vitamin D insufficient and deficient groups. There was no relationship between any bone health parameters at all sites measured and serum levels of 25(OH)D ($p > 0.05$).

Fat mass (FM) and body fat percentage least explained the observed variation in bone health parameters, whereas lean mass (LM) was the most important body composition component in explaining the variations observed in bone health parameters. The relationship between LS bone health parameters and body composition components was weaker than the relationship between TBLH bone health parameters and body composition components.

Summary and / or Conclusion: In this population, 66% of preadolescents were vitamin D insufficient or deficient, but with a healthy bone health status and 40% of the preadolescents were over-nourished with greater crude BMD than those with healthy BMI Z-scores. Vitamin D status does not appear to be associated with parameters of bone health. Lean mass was the greatest body compositional determinant for variations observed in bone health parameters. Bone health parameters of healthy and over-nourished children did not differ after adjusting for body composition.

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ABBREVIATIONS

Table 1: List of abbreviations

Abbreviation	Meaning
25(OH)D	Calcidiol / 25-hydroxyvitamin D (Collective for all precursors)
25(OH)D3	Vitamin D3 / 25-hydroxycholecalciferol / 25-hydroxyvitamin D3
25(OH)D2	Vitamin D2 / 25-hydroxyergocalciferol / 25-hydroxyvitamin D2
$1,25(OH)_2D$	Calcitriol
aBMD	Areal Bone Mineral Density
AIDS	Acquired Immune Deficiency Syndrome
ALP	Serum Alkaline Phosphatase
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMAD	Bone Mineral Apparent Density
BMD	Bone Mineral Density
DXA	Dual-energy X-ray Absorptiometry
DBP	Vitamin-D Binding Protein
DRI	Dietary Reference Intakes
FM	Fat Mass
GIT	Gastrointestinal
HIV	Human Immunodeficiency Virus
IFNuW	Institute for Food, Nutrition and Well-being
IGF-I	Insulin-like Growth Factor I
IOM	Institute of Medicine
ISCD	International Society for Clinical Densitometry
IU	International Units
LM	Lean Mass
LS	Lumbar Spine
MCS^2	Multivariate Semi-metric Smoothing Algorithm
MRI	Magnetic Resonance Imaging
PBM	Peak Bone Mass

Abbreviation	Meaning
PTH	Parathyroid Hormone
QCT	Quantitative Computed Tomography
QUS	Quantitative Ultrasound
RANKL	Receptor Activator of Nuclear Factor (NF)-kB Ligand
SANHANES	South African National Health and Nutrition Examination Survey
TBLH	Total Body Less Head
UVB	Ultraviolet B-Rays
vBMD	Volumetric Bone Mineral Density
VDR	Vitamin D Receptor
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Bone health is important throughout the lifecycle and maintenance thereof should begin from childhood. Bones have an important role in the body above and beyond the structural functions. They are organs and like other organs of our body, they are vital for bodily functions such as storage of minerals and blood cell production, and thus continuous bone health maintenance is essential.[1, 2] Maintaining bone health throughout life aids in the prevention of rickets, osteoporosis or osteopenia. Bone health is particularly important during childhood and adolescence as bone formation should exceed bone resorption to achieve a peak bone mass (PMB) for maintenance in adulthood. Peak bone mass accumulated by about age 30, at the end of the growth stages of life, determines the risk for osteoporosis. Later in adulthood, as a function of aging, bone mineral density (BMD) slowly declines as resorption exceeds formation.[2]

Worldwide, the increasing prevalence of poor bone health status in children has highlighted the need for focus on bone health in children and improved interventions.[3] Bone mass is a measure of bone health that can be measured by dual energy X-ray absorptiometry (DXA) scans. Dual-energy X-ray absorptiometry is also used for the assessment of body composition and is accurate, precise, and suitable for children due to minimal radiation exposure.[4]

There is a known positive correlation between weight bearing exercise and bone mass. This is due to the force exerted by muscles on the bones, stimulating osteoblast to increase bone formation. The bone cells trapped in the bone matrix then differentiate in to osteocytes, which control various processes by releasing molecular signals.[5] The relationship between adiposity and bone mass has however been debated. While some studies show increased forces, like with muscle force, increasing bone mass, other studies show a higher fracture risk in overweight/obese children, or no difference in bone mass between overweight/obese and normal weight children.[6-9]

Vitamin D also plays an important role in bone health and bone mass accretion. It is an essential fat-soluble micronutrient that functions as a hormone to balance calcium and phosphorus levels in the blood. Vitamin D also has a non-calcaemic role in bone health as it modulates osteoclast functioning.[10] Vitamin D deficiency or insufficiency in children have been associated with increased PTH activity and decreased rates of bone mass accumulation.[11,12] The potential for vitamin D insufficiency has been an increasing concern in the general population, and its potential impact on bone health and other health consequences have

emphasised the need to update our current scientific knowledge on the topic.[3, 10] There is a concerning gap in knowledge on the vitamin D status of children in South Africa, a country and age-group prone to nutrient deficiencies. The biomarker used to quantitatively assess vitamin D status is 25(OH)D, which can be analysed using liquid chromatography tandem mass spectroscopy (LC-MS/MS).[2, 13] While results from studies on the topic are conflicting, they have generally pointed towards a possible concern that vitamin D deficiency in South African children may exist. Conflicting results may be due to the lack of research or due to the vast array of factors that can affect vitamin D intake and status within and between population groups.[3, 14] Some of these factors include geographical location, skin pigmentation and nutrient intake.[15] Further studies are necessary for definitive data on the vitamin D status of South African children.[14]

This research study aims to address the knowledge gaps and provide insight in to the vitamin D status and bone health of preadolescent South African children. There are conflicting results found in the literature regarding body composition, vitamin D and bone health status among children, specifically within the South African context. The purpose of this study is to add data to support the current findings for more clarity and evidence.

1.2. PROBLEM STATEMENT

Bone health maintenance and assessment is important in children to reduce the risk for osteoporosis later in life.[2] The increasing prevalence of poor bone health status has highlighted the need to focus on bone health in children and for improved interventions.[3] Preadolescence is an important time in the development of the skeleton as it is a period of growth that precedes the attainment of PBM. Body composition is vital in bone health as bones adapt to external forces exerted on them however the relative contributions of lean and fat mass in this adaptation have been disputed.[6, 7, 16] The body composition of South African children is increasingly leaning towards a greater body fat percentage and by race and gender, black girls have the highest body fat percentage.[17] Vitamin D is vital in bone health due to its role in calcium and phosphorus homeostasis but the relationship between bone health and vitamin D status needs to be further examined for validation particularly in younger age groups.[3, 10, 18] The vitamin D status of South Africans generally indicates insufficiency. Of the younger preadolescent population, data on vitamin D status is inconclusive and additional studies are required to clarify whether vitamin D deficiency in South African children is a public health concern that needs to be addressed.[14, 19, 20] Cornish *et al*.[21] observed a difference between the vitamin D status of normally pigmented black and albino black children thus indicating that further studies are needed, with the inclusion of skin pigmentation as a contributing factor, to conclude on the vitamin D status of South African children.

1.3. STUDY AIM AND OBJECTIVES

AIM

To describe bone health, body composition and vitamin D status, and their relationship in preadolescent school children in Pretoria, South Africa.

OBJECTIVES

- To describe the bone health status (Z-scores), vitamin D status and body composition of preadolescent school children in Pretoria, South Africa.
- To examine the relationship between bone health parameters (bone area, BMD and BMC) and vitamin D status.
- To examine the relationship between bone health parameters (bone area, BMD and BMC)and body composition.

1.4. IMPORTANCE AND BENEFITS OF THE PROPOSED STUDY

Nutrition as exposure or “intermediary” outcome is one of the research focus areas of the Institute for Food, Nutrition and Well-being (IFNuW) overarching the nutrition research project. Furthermore, school children in early adolescence are proposed as the focus population of IFNuW, as this group in South Africa is under-researched. This research project is a sub-study of the study entitled “Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DXA) and relationship to vitamin D status in children”. The sub-study will involve preadolescent school children as study participants to address the knowledge gaps and conflicting results found in the literature regarding body composition, vitamin D and bone health status among children, specifically within the South African context.

1.5. DELIMITATIONS AND ASSUMPTIONS

1.5.1. Delimitations

- In this study, sampling was done at two after-care facilities delimiting the area from which data was collected.
- Bone health and body composition are variable in people of different growth stages. Participants included in this study were preadolescent children between the ages of five and ten.

- This study aimed to examine the relationship between bone health and vitamin D, and bone health and body composition. The relationship between vitamin D and body composition was not addressed.
- Data collection was done at a single point in time during the South African summer months and data from winter months was not made available for comparison specifically relating to vitamin D status due to sun exposure.

1.5.2. Assumptions

- An assumption was made that all female preadolescents were premenarcheal.
- It was assumed that all participants were hydrated and their bladders were voided.
- All participants of the study were assumed to be South African.

1.6. OPERATIONALIZATION

In this study, anthropometric measurements done by registered dietitian, Amanda Jansen van Rensburg, included height (measured by the Seca 274 wireless stadiometer) and weight which were taken at a single point in time at the Netcare Femina Hospital and used to calculate BMI. I, Samantha White, calculated and converted body mass index to Z-scores and used this to categorize participants as healthy or over-nourished (overweight and obese). Bone health was also measured at the Netcare Femina Hospital as bone mass (BMD and BMC) and bone area by a trained radiographer using DXA scans with the Hologic Discovery W densitometer. I interpreted bone mass as healthy or low-for-age using Z-scores. Body composition data was also obtained from the DXA scans and included lean mass and fat mass, which was used to calculated body fat as a percentage of the body weight. Blood spot collection cards were used for blood spot collection at the aftercare facilities. These were sent to ZRT laboratories (Oregon, USA) for LC-MS/MS analysis of serum 25(OH)D which is the gold standard for vitamin D analysis.

1.7. DEFINITION OF KEY TERMS

The following key terms are used throughout this dissertation.

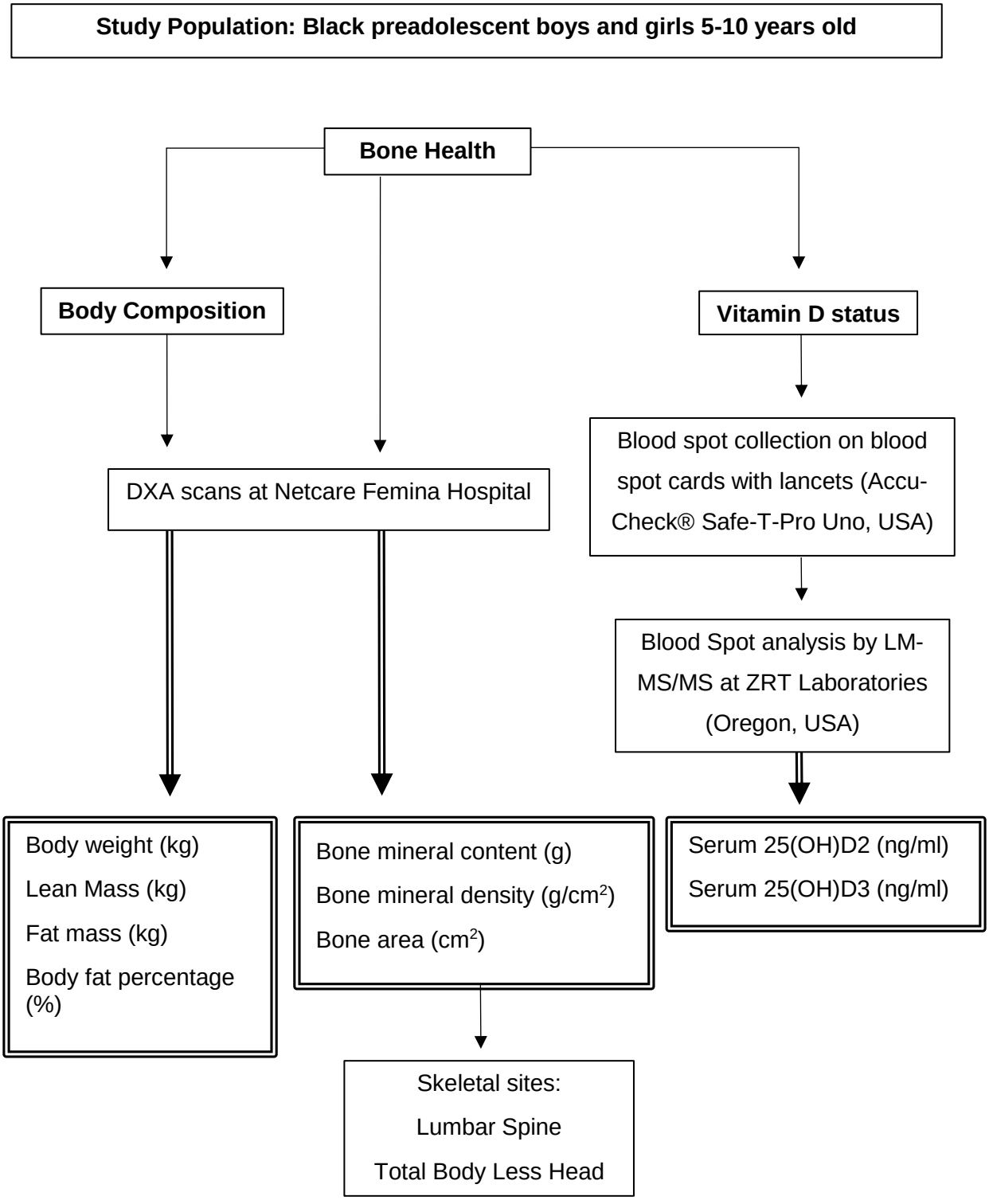
Table 2: Definitions of key terms

Concept	Definition
Bone Health	Bone health refers to the formation and maintenance of the skeleton in an aim to prevent rickets, osteopenia or osteoporosis.[2] Bone health is measured using bone imaging techniques which provide

Concept	Definition
	quantitative data for bone mineral content (BMC), bone mineral density (BMD) and bone area.[22]
Bone Mass	Bone mass refers to the BMC which is measured in grams of bone mineral. BMD is thus the bone mass per unit of area.[2]
Bone Health Status	Bone mineral content and BMD are converted to Z-scores for children and then used for comparison to a healthy age-matched reference group. Bone health status referred to in this study denotes bone mass for chronological age interpreted from bone mass Z-scores.[23]
Body Composition	Body composition is a description of the proportions of fat mass and fat-free mass in the body. Fat mass includes all adipose tissue in the body including the brain and fat stored in the bones. Fat-free mass encompasses water, mineral (bone) and protein (muscle) components of the body.[24] For the purpose of this study, body composition will include total body weight as a parameter.
Vitamin D	A fat-soluble vitamin, also known as calciferol, is a collective term for all precursors/metabolites with vitamin D activity. Vitamin D status is determined by the level of serum 25(OH)D classified by the Endocrine Society as sufficient, insufficient or deficient.[10]
BMI	Body mass index is a measure of weight per height which is an indirect measure of weight class but is not an indicator of body fat percentage.[25] It is expressed as a Z-score based on BMI-for-age in this study.
Body weight classification	Body weight classifications referred to in this dissertation are as it is referred to by the authors from which they are cited. When referring to the population group included in this study, participants are classed as “healthy” or “over-nourished” based on BMI Z-score.
Preadolescent children	Children beyond infancy and early childhood that are of the ages preceding this life stage.[26]
DXA	A technique used to assess bone tissues with the added function on providing body compositional data. Bone density is expressed as g/cm^2.[4]

Concept	Definition
Peak Bone Mass	The maximum attainable bone mineral mass accrued during childhood and maintained during early adulthood which sets a benchmark for bone health during adulthood.[27]
Race/Ethnicity	Race/Ethnicity referred to in this dissertation is as it is referred to by the authors from which it is cited. When referring to the population group included in this study, race/ethnicity is reported by the parent or guardian of the participants involved and referred as “self-reported”.

1.8. OPERATIONAL FRAMEWORK



1.9. LAYOUT OF THE DISSERTATION

This dissertation is structured according to the guidelines followed at the Department of Human Nutrition, University of Pretoria. It consists of six chapters, references and appendices.

Chapter one is an introductory chapter which provides a basis for understanding why and how this study was performed. The second chapter is a detailed literature review for a background in to bone health, vitamin D status and body composition. This chapter outlines the importance of these topics and addresses the gaps in knowledge. Chapter three is a methodology chapter which follows the introductory chapter with a more in depth look at how the study was conducted. It provides details on the selected study population, data collection from these individuals, and analyses of that data. Ethical considerations are addressed with reference to some the appendices provided.

The fourth and fifth chapters are the results and discussion chapters respectively. The demographic, anthropometric, bone health, vitamin D status and body composition characteristics are provided in great details. Results from regression models are provided to view a possible relationship between bone health to vitamin D status and body composition. The results in chapter four are delineated in the discussion chapter and then limitations are addressed. From the previous chapters, conclusions are drawn in the final chapter, chapter six. Recommendations are then finally made for possible future research.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

Bone health maintenance is particularly important during childhood to assess the adequacy of bone development and metabolism. It is important during this phase to ensure optimal PBM is achieved thus reducing the risk for developing osteoporosis later in life. Bone formation or modelling occurs during childhood whereas remodelling, bone metabolism, continues throughout the life cycle. This is controlled genetically and can be mediated through the force of external loading and through nutrition.[28] DXA is considered the gold standard for bone health assessments in children due to its safety for use with minimal radiation exposure and it also provides body compositional data.[29]

The dual-burden of disease, rife in underdeveloped countries such as South Africa, means that over- and undernutrition coexist in these populations. Clinical signs of both overweight or obesity and stunting appear collectively as energy-dense and nutrient deprived foods form the staple of most South African's diets.[30] With the rise of body fat percentage due to inactivity and the increasing prevalence of vitamin deficiencies in preadolescent South African children, bone health status is a concern.[17] While external load bearing on bones by added body weight has been found to be associated with increases in bone mass, and lean mass has shown to be positively associated with bone mass, the contribution of fat mass to this phenomenon has been disputed.[6, 9, 16] Not only is bone health affected by body composition, but nutrients, namely, calcium and vitamin D, are vital role players in this context. The vitamin D status of South African children cannot be described as more data is needed to define the vitamin D status of this multi-cultural population. It has however been postulated that vitamin D is lacking, putting strain on the bone health of these children.[12, 14]

In this literature review, bone health, body composition and vitamin D in preadolescent children will be discussed in terms of physiology and assessment methods. The functions of body lean mass or fat mass and vitamin D in relation to bone health will be addressed. The prevalence and consequences of vitamin D deficiency will be considered relative to children in South Africa. Current data available on the body composition, vitamin D and bone health status of South African children will be reviewed and the effects in relation to bone mass accretion will be discussed.

2.2. BONE PHYSIOLOGY

The human body is comprised of different types of tissues that form various components such as the organs, muscles and bones. Bone tissue is essentially connective tissue or osseous tissue that consists of extracellular matrix and bone cells.[2]

There are different types of bones in the body namely, long, short, flat and irregular bones. Bones have specific functions in the body as they are important not only for structure and protection of our organs, but they also serve as storage sites for triglycerides, minerals and growth factors. Blood cells are formed from the red marrow of the bones, and non-collagenousproteins such as osteocalcin are produced by bone cells.[1]

2.2.1. Bone Tissue

Macroscopically, there are two types of bone tissues, cortical bone also known as the compact bone, and trabecular bone which is sometimes referred to as cancellous bone or spongy bone.[2] Cortical bone envelopes trabecular bone as it forms a hard shell that protects and provides strength to the bone. All bones contain a periosteum membrane and an endosteum membrane. The periosteum completely covers the outside of the bone and is therefore in contact with the cortical bone. The endosteum layers the inside of the bone where trabecular bone is present.[1]

Cortical bone is made up of functional units called osteons, also referred to as Haversian systems. Osteons are cylindrical, pillar-like structures that run parallel to the long axis of the bone. Each osteon is comprised of a few cylindrical tubes somewhat like a tree trunk, and these are called lamellae.[2] Within the lamellae there are collagen fibres and mineral crystals that run transversely to the fibres and crystals of the adjoining lamellae. This juxtaposition serves the purpose of improving mechanical bone strength. In the centre of the lamellae of the osteon, there is a Haversian canal housing the blood and nerve fibres for the osteons. Perforating canals, also known as Volkmann's canals, are perpendicular to the Haversian canals. They link the blood and nerve fibres from the periosteum to those in the Haversian canals. Circumferential lamellae are the lamellae layered around the osteons that have a periosteum layer and endosteum layer on either side.[31]

Trabecular bone is known as such due to the beams of bone (trabeculae) that connect and form large spaces in between. Trabecular bone strength is relative to the orientation of the

trabeculae but, spongy bone is porous and is therefore of less mechanical importance than cortical bone, and is more metabolically important.[31,32]

2.2.2. Bone cells

The different types of bone cells include osteogenic cells, osteoblasts, bone lining cells, osteocytes, and osteoclasts. Osteogenic cells are the stem cells found in periosteum and endosteum membranes that produce other bone cell types.[33]

Osteoblasts are cells that play an important role in the formation of bone as they secrete organic unmineralized bone matrix. This bone matrix (known as the osteoid) is a proteinous matrix consisting of approximately 90% collagen and the remaining 10% is calcium-binding proteins. Because this is an unmineralized matrix, it is very soft. Calcium and phosphate salts along with hydroxyl ions bind to form crystals known as hydroxyapatite, which act as "concrete" by binding to calcium-binding proteins of the osteoid. Hydroxyapatite combined within the osteoid gives rise to a tensile matrix of great strength. Bone lining cells are inactive osteoblasts.[2, 34] They are flat cells that line the bone underneath the membranous layers. The function of these cells is unclear but it is known that bone lining cells prevent contact between the bone matrix and osteoclasts when bone resorption should not be taking place.[33]

Osteocytes are the most abundant bone cells, making up 90% of the bone cells in the skeleton.[35] They are mature osteoblasts that maintain and uphold the bone matrix. These cells occupy shallow spaces known as lacunae which are resorption bays. These lacunae are situated between the lamellae of both the cortical and trabecular bone. Osteocytes are unique in their dendritic morphology which allows them to cross-communicate with each other. They form a network between the lacunae through canals called canaliculi. They are able to sense stress and strain within the bone through mechanosensors and thereby communicate this to other cells thus enabling the maintenance of the bone matrix. This communication allows osteocytes to communicate to osteoblasts or osteoclasts when the bone matrix needs to be made or degraded and this is beneficial in maintaining calcium homeostasis.[36]

Osteoclasts break down bone during bone resorption and these cells are not produced from osteogenic stem cells as are all the other bone cells but, like macrophages, are formed from hematopoetic stem cells in the red marrow tissue. These are large cells with multiple nuclei and they are located specifically at sites where bone resorption is taking place. Osteoclasts have a ruffled border (Figure 1), which has direct contact with the bone. The reason for this distinctive morphology is that it creates increased surface area for increased production of the necessary enzymes needed to break down bone. Sealing zones, or clear zones surround the

osteoclast and bone as a tight attachment is formed to seal off the site of bone resorption, specifically isolating the area where resorption should take place.[33,37]

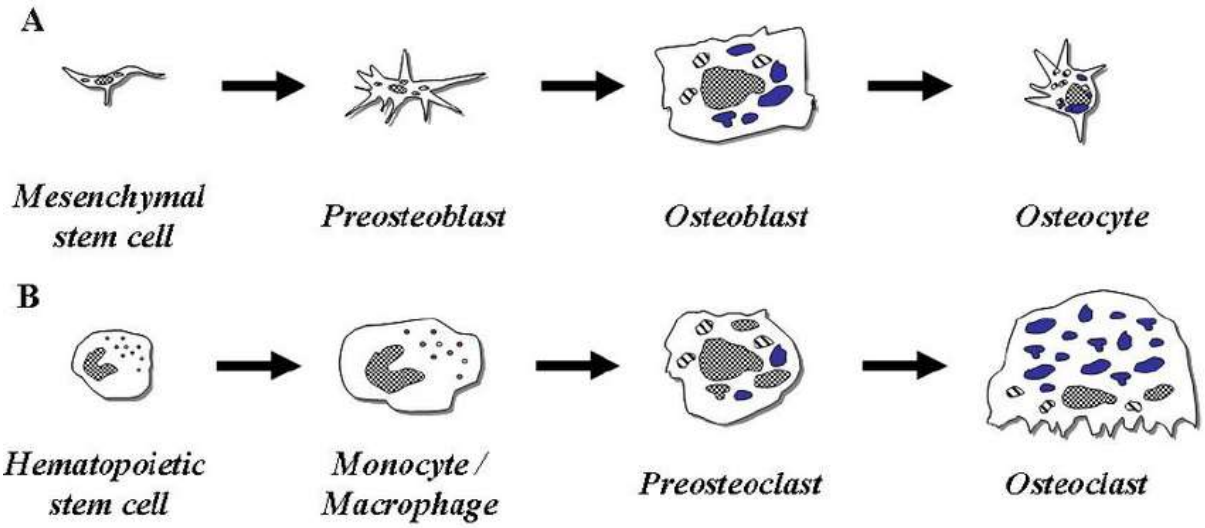


Figure 1. Formation of bone cells from mesenchymal stem cells (A) and hematopoetic stem cells (B)[32]

2.2.3. Bone modelling

Bone modelling is the process of bone formation that occurs during skeletal development or fracture healing. The process of bone modelling is called “ossification”, of which there are two types, endochondral ossification and intramembranous ossification. The sections of skeleton below the skull, the vertebrate appendicular and axial skeleton are formed through endochondral ossification whereas the craniofacial skeleton is formed through intramembranous ossification.[38]

During the development of a foetus, there is initially no bone and cartilage acts as a mould by filling the space where bone development will take place. The cartilage mould is developed through chondrocytes which are differentiated from mesenchymal cells. Bone tissue eventually replaces almost all the growth cartilage and this is specifically in the case of endochondral ossification. For bone to replace the cartilage, the cartilage must be broken down first.[34] Endochondral ossification therefore occurs through various stages as summarized by Mackie *et al*.[34] By the final stage of endochondral ossification, the diaphysis or shaft of the infant’s bone is comprised of cortical and trabecular bone with a cavity, while the epiphyses or ends consist of trabecular bone and a cartilage plate, or growth plate. Growth of bones continue until the end of adolescence, entering early adulthood, when the growth plate disappears and is replaced by bone. Cartilage that remains is not involved in bone growth but is a flexible support.[2] When a foetus is developing, apart from cartilage, there is fibrous

tissue developed from mesenchymal cells, and this too is replaced by bone.[39] This type of ossification is the intramembranous ossification that occurs in the craniofacial skeleton. Bone modelling progresses in females until about 16 to 18 years of age and in males until about 18 to 20. The bones then stop growing in length but bone mass accretion continually increases thereafter.[2] From a longitudinal study by Bailey *et al*.[40] it was estimated that peak bone mineral velocity is at approximately 12.5 years old for girls and 14.1 years old for boys. Bone mass accretion rates then decline but continue to increase until about the age of 30 where it plateaus. From about 40 years of age, age-related and menopausal-related bone losses commence.[2]

2.2.4. Bone remodelling

Bone remodelling is a three-stage process of bone resorption and formation that enables old bone to be replaced by healthy bone tissue.[33] During childhood and adolescence, formation of bone exceeds bone resorption until about 30 years old. Peak bone mass is the maximum bone mass which peaks at the end of adolescence where approximately 85-90% of the final bone mass that will be reached is achieved.[27] Peak bone mass accumulated at the end of the growth stages of life is of importance as it determines the risk for osteoporosis. Once the PBM is achieved, the bone mass remains constant as the rate of formation and resorption are equal. From about the age of 40, bone mineral density (BMD), which is the BMC within a specific unit of area, slowly declines as resorption exceeds reformation. The losses of bone mass are age-related, but women are at a greater risk of osteoporosis at this age due to the drop in oestrogen levels associated with menopause.[2]

The principle cells involved in bone remodelling are osteoclasts and osteoblasts due to their resorptive and reformative abilities. The first stage starts with activation of preosteoclasts within the bone marrow which then migrate to the surface of the bone tissue and differentiate from mononuclear preosteoclasts in to multinucleated osteoclasts. The osteoclasts resorb or break down the old bone tissue. Bone resorption by osteoclasts occurs through their release of acids and proteolytic enzymes which act against the bone matrix and hydroxyapatites. Resorption is a process that is completed within two weeks. This is followed by the second stage which is referred to as the reversal phase. During the reversal phase, resting osteoblasts on the bone surface prepare the bone for action of activated osteoblasts. The active osteoblasts dominate the third and final phase of bone remodelling. They begin secreting new bone matrix proteins and collagen, filling the cavities formed by the osteoclasts, which is eventually hardened with the binding of calcium phosphate to form hydroxyapatites.[2,41] The entire surface is then lined with resting osteoblasts or bone lining cells which lay flat on the bone surface during the resting period when no resorption takes place. The second and third

phases of bone remodelling are lengthier processes than resorption. The reversal phase takes about a month, but the formation of new bone can take up to 4-6 months. Although the phases of bone remodelling vary in duration, resorption and formation are in balance and the same amount of bone tissue is present throughout remodelling.[41]

2.3. FACTORS AFFECTING BONE HEALTH

The eventual percentile (or Z-score) of PBM and height reached compared to the mean is determined genetically however evidence shows that non-genetic factors such as physical exercise and nutrient intake may shift these to a different Z-score.[28] Overall energy intake from food consumed does not directly impact bone health, but it impacts body weight which in turn affects bone health. The intake of macro- and micronutrients are vital and nutrient deficiencies may directly or indirectly impact bone health status.[2] Nutrient intake has been shown to influence bone growth as well as bone loss and is therefore an important determinant of bone mass not only in the initial growth of the skeleton but at all stages of life.[28]

Bioavailability refers to how much of a nutrient that is ingested, is absorbed and available for use by the body. This depends on digestion and liberation from the food matrix as well as absorption and transportation mechanisms of the somatic cells.[42] Factors that may influence the bioavailability of nutrients include, the chemical form of the nutrient, nature of the food matrix and interactions between the nutrient and other substances also ingested. Demographic and lifestyle factors affect bone health some of which may be controlled for optimal bone health which may offer an advantage over the bone losses that may occur by factors that are uncontrollable.[43]

2.3.1. Dietary factors affecting bone health

While calcium has been the primary focus of many studies regarding the influence of nutrition in bone health, bone growth and maintenance is affected by many other dietary factors some of which will be discussed further.[2] The dietary reference intakes (DRI's) of the nutrients involved in bone health that will be discussed are summarized in Table 3.

Table 3. Dietary Reference Intakes (DRI's) of nutrients that play a role in bone health[2]

	Children 4 – 8 years	**Males and Females 9 – 13 years**
Calcium (mg/day)	1000	1300

Protein (g/day)	19	34
Phosphorus (mg/day)	500	1250
Magnesium (mg/day)	110	350
Zinc (mg/day)	12	23
Vitamin A (µg/day)	400	600
Vitamin K (µg/day)	55	60
Vitamin D (IU/day)	600	600

* Vitamin D is expressed as cholecalciferol where 1 µg cholecalciferol = 40 IU vitamin D

2.3.1.1. Calcium

Calcium has a direct effect on bone health as bone tissue stores calcium. Serum calcium remains constant and therefore calcium homeostasis is essential so that the body is provided with calcium as needed without altering the serum calcium ion concentration. Calcium homeostasis is achieved by a complex process balancing calcium intake with serum calcium and calcium urinary excretion. This process is balanced because calcium ions are released and replaced daily by continuous bone turnover (resorption and formation). Continuous bone turnover allows for calcium serum levels to remain constant while supporting the dynamic nature of bone tissue. Calcium intake is thus vital as low intake levels result in additional calcium ions released from bone to balance serum calcium levels and increase calcium absorption which is then not replaced by the calcium intake.[2]

Food sources of calcium include milk, milk products such as cheese and yoghurt, sardines with bones, legumes, broccoli and kale.[2] Calcium supplementation has been proven to increase bone mineral mass accumulated during childhood. The forms of supplements in several trials has varied from calcium carbonate, calcium citrate malate and calcium phosphate extracts from milk to supplementation in the form of dairy products[44-47]. The trials have all shown the same positive association of calcium and bone mass however there are discrepancies as to whether the bone mass accumulated can be maintained. A study was done by Bonjour *et al*.[45], where three and a half years after the supplementation trial using calcium phosphate extracts from milk, a follow-up assessment was performed. It was

determined that the bone mass accumulated from the 1-year trial was maintained for more than three years afterwards. However, in the study by Lambert *et al*.[44], a two-year post-trial follow-up was done and it was noted that the bone mass accumulation had not been maintained and had been reversed upon withdrawal of the calcium citrate malate. While both studies used Caucasian prepubertal female participants, one aspect of the participants differed. In the study by Bonjour *et al*.[45] the participants were described as healthy whereas Lambert *et al*.[44] screened participants for having a habitually low-calcium intake diet.

Calcium is predominantly but not solely a reason that dairy intake is recommended for improved bone health development and the modern diet requires three servings of dairy per day for this. Dairy consumers have been found to have lower fracture risk with a positive impact on bone mineralization than those that do not consume dairy. Reasons for not consuming dairy may be due to intolerances or dietary patterns. Dairy products are sources of a mixture of nutrients including protein, calcium, sodium, phosphorus and vitamin D in the case of fortified milk.[46-48]

Dairy products are a relatively inexpensive means of obtaining calcium and they provide an abundance of other nutrients. Some of these nutrients support bone health but dairy also provides some nutrients that negate the effects of calcium and thus a large body of evidence exists that goes against guidelines of including dairy in the diet to support bone health.[48, 49]

2.3.1.2. Protein

While some studies have identified protein as adversely associated to bone health due to increased calcium excretion as acid load in the body rises, the results are controversial.[2, 50] In a study by Alexy *et al*.[51], Caucasian children between the ages of 6 and 18 years old were included in a four-year study to observe the long-term effect that protein in the diet has on bone variables reflecting bone modelling and remodelling. Their findings indicated that protein intake had a positive association with the bone variables measured and thus that adequate protein intake improves bone strength during bone growth in children. A possible scientific explanation for this positive association can be attributed to the fact that dietary protein has been found to stimulate insulin-like growth factor I (IGF-I) secretion.[52] Insulin-like growth factor I is a hormone that plays a key role in bone growth and mineral content. Adequate protein intake is generally important in prepubescent children for optimal growth, but based on these findings this macronutrient is also essential at this life stage for its impact on bone growth and bone mass.[51] Additionally, it has been shown that inadequate protein intake, in instances where energy intake is controlled, negatively impacts bone health as there is an imbalance in bone resorption and formation.[53] Contrary to the belief that high protein intake results in urinary

calcium excretion, protein and calcium have been found to have a beneficial interrelationship.[50] Adequate calcium intake is believed to potentiate the effects of protein on the bone strength during growth and protein is believed to reduce calcium excretion.[54]

Protein intake and physical activity levels also appear to be complimentary in the roles they play in bone health particularly in prepubescent children. A study was done by Chevalley *et al*.[55] observing the interactions of protein intake, physical activity and calcium intake and the effects thereof on the BMC of prepubescent males. They found that while protein intake, calcium intake and physical activity were significantly associated with BMC, the positive association of calcium intake was not statistically significant when protein intake was high. This suggests that, while it has been undoubtedly proven that calcium is an important nutrient in bone health, the requirements may be lower when protein intake is high. In Chevalley's study, the subjects were males between 6 and 8 years old where the DRI for protein is 34 g/day (Table 3). High protein intakes were those above the median at approximately 56.5 g/day and below the median were 38.5 g/day.

Protein in the diet may come from sole-sources or a mixture of foods as protein intake is required to meet the amino acid demands. Some protein sources, namely animal sources such as poultry, meat, eggs, milk and fish are considered high quality due their score based on amino acid profiling. Other protein sources, those from plant sources in particular, may have a lower amino acid score due to their lack in certain amino acids.[56] For example legumes and cereals when eaten alone lack in methionine and lysine respectively. When eaten together, the protein quality is improved as what one lacks, the other makes up for.[57]

2.3.1.3. Phosphorus

Hydroxyapatite crystals which contribute to the strength of bones are formed by the complexing of calcium with phosphorus. Phosphorus is distributed throughout the body as phosphate and has multiple functions, but the majority is found within the teeth and bones which is indicative of the importance that it serves in bone health. Because phosphorus is so vital in bone mineralisation and adequate functioning of the body, homeostasis of the mineral is important.[58]

Dietary intake of phosphorus recommended for infants is 100 mg/day and increases to 1250 mg/day by the age of 9 (Table 3) which can be obtained from food sources such as yoghurt, liver and sunflower seeds.[2] Phosphorus homeostasis is maintained through complex systems involved within the gut, bones and kidneys. While adequate intake of the nutrient is important to maintain this homeostasis, its absorption and thus availability is vital, and this occurs

passively and actively. Vitamin D is believed to play a role in the active diffusion of phosphorus.[58]

2.3.1.4. Magnesium

Magnesium plays vital role in ATP functioning for glucose metabolism, cytoskeleton contraction of cells, muscle contraction, and is also important in bone health. More than half of the magnesium body stores are found in bones as this acts as a reservoir in ensuring normal blood concentrations of magnesium. The rest, approximately 33%, is stored in the muscles and soft tissue. Magnesium is thus an important structural component of bones and its role in cell functioning includes that of bone cells such as osteoblasts and osteoclasts. Magnesium is required for adequate bone metabolism and its requirements are summarised in Table 3. Deficiencies of this nutrient have been associated with inactivity of PTH which can be avoided by obtaining sufficient amounts from food sources such as nuts, legumes, dark green leafy vegetables and seafood.[2, 59]

2.3.1.5. Zinc

Zinc deficiency poses a growing global burden on childhood morbidity and mortality, with Southern Africa being one of the most at-risk regions in terms of insufficient zinc intake and deficiency.[60, 61] Zinc exists in all body tissues including bone tissue. Sever zinc deficiencies present with delayed bone development and mild deficiencies have been associated with retarded growth rates. This is due to the rapid turnover rate of plasma zinc which is replenished by increased bone resorption when levels are depleted.[62] The greater prevalence of zinc deficiency in developing countries can be attributed to a low intake of zinc-rich foods, namely animal products.[63] Animal food sources are expensive and are not within the budgets of individuals with a low socioeconomic status. There is a high intake of plant-based food sources, such as cereals or grains, as they are more affordable and available. Plant-based diets are high in anti-nutritional factors like fibre or phytates that form insoluble complexes in the gut with minerals such as zinc, which the human digestive system is not able to degrade.[64] As a source of zinc, they may provide adequate amounts to meet the daily requirements of an individual however the large amounts of inhibitors present reduce the bioavailability of the zinc that is present.

2.3.1.6. Vitamin A

Vitamin A has been known to play a vital role in the body for vision, cell differentiation, cell recognition at the surface of the cell, reproduction and growth and development but evidence shows that vitamin A also has a role in bone health.[2, 65] Hypervitaminosis A is as a result of

excessive intake of vitamin A which leads to multiple adverse effects, one of which is reduced bone density. Excess retinol is believed to reduce bone mass, increase fracture risk and cause osteoporosis. The scientific reasoning behind this is unclear but is believed to be linked to poor regulation of cells involved in bone remodelling.[65]

Preformed vitamin A, or retinol, can only be found in animal products such as liver, cheese and eggs. Plant food sources such as carrots and green leafy vegetables contain compounds which are vitamin A precursors present as yellow or orange pigments collectively known as provitamin A or carotenoids. Carotenoids can be metabolized by the body to form retinoids and thereby have vitamin A activity in the body.[2,66] Carotenoids are believed to be superior to preformed vitamin A in bone health. Because of the lower vitamin A activity of carotenoids, an excessive intake to the point of hypervitaminosis A is difficult to achieve and therefore they are not linked to the adverse effects on bone health. Carotenoids are in fact believed to have benefits in bone health as antioxidants however more research is required on the topic of vitamin A and bone health.[65]

2.3.1.7. Vitamin K

Vitamin K is an essential vitamin known for its primary role in blood coagulation but also plays a role in bone metabolism. Vitamin K_1 obtained from food sources such as green leafy vegetables, avocado, kiwis and some vegetable oils and vitamin K_2 produced by gut bacteria have both been found to have a role in bone health.[2, 67] The role of vitamin K_2 has however been disputed and requires further investigation for evidence.[68] During bone mineralization as bone formation takes place, osteocalcin, produced by osteoblasts, modulates the formation of hydroxyapatite crystals. Osteocalcin has three glutamate (Glu) residues bound to it and for osteocalcin to be functional, these Glu residues must be gammacarboxylated in to gamm-carboxy-glutamate residues (Gla). Osteocalcin depends on vitamin K to be activated and bind minerals to form hydroxyapatite crystals as vitamin K is the cofactor for the gammacarboxylation enzyme. While vitamin K is involved in bone metabolism, little evidence shows the effects of inadequate intake on bone health.[69] According to Booth *et* al.[70, 71], vitamin K_1 has no significant effect on BMD. Supplementation of vitamin K_1 in children is not necessary to obtain optimal BMD particularly as adequate intakes are easily achievable through dietary sources such as green leafy vegetables. However, supplementation in the elderly and postmenopausal women may be beneficial due to its observed reduction in risk of osteoporosis and fracture risk but more definitive research is required to confirm this.

2.3.1.8. Vitamin D

Vitamin D is believed to play a very important role in bone health and is a focus of this study and will therefore be discussed in detail further on.

2.3.1.9. Inhibitors/Antinutrients

Intake of nutrients is important in ensuring bone health. More importantly is the absorption of these essential nutrients because while intake might meet the daily requirements, the absorption of calcium for example may be inhibited by other food components such as oxalic acid and phytate. Inhibitors or antinutrients reduce the bioavailability of calcium by binding to it to form insoluble complexes.[72] Antinutrients are often found in plant food sources and through certain cooking techniques calcium may be liberated from them or the antinutrients may be reduced.[43]

High sodium intake is linked to excess excretion of calcium in urine and thereby may adversely affect bone.[2] However, Ilich and Kerstetter [73] noted in their study involving postmenopausal woman that sodium was not detrimental when calcium and vitamin D intake met the daily requirements. Thus, data on sodium and bone health is conflicting and additional research is required.

2.3.2. Physical activity

Overweight or obesity is defined as an excess of fat accumulation.[74] The incidence of overweight and obesity in South African school children appears to be increasing which is concerning due to the health implications associated with a high body fat percentage. Body fat percentage is a measure of the fat mass to body mass and the increased incidence thereof may be associated with decreased physical activity levels.[17, 75]

Physical activity and nutrition go together in their roles of gaining bone mass during bone growth for adequate bone mass accretion. Physical activity is beneficial to bone health in the prevention and treatment of osteoporosis.[2] The mechanostat theory was first proposed by Harold Frost in 1987 suggesting that bone growth or loss is stimulated proportionally by forces exerted on bone by muscles resulting in local mechanical elastic deformation of the bone. By this understanding, it's clear that as muscle force increases due to exercise, the skeleton adapts proportionally and increases in strength. Excessive exercise is not recommended for children due to the counteractive effect it plays on the skeleton.[5] Weight training and resistance training such as jogging or dancing, for approximately 20-30 minutes at moderate intensity multiple times a week is recommended for optimum bone mass accumulation in children.[27]

Lack of physical exercise has been linked to bone loss possibly due to inadequate bone mass accretion.[2]

Hind and Burrows[76] reviewed the data from controlled trials researching the effects of weight bearing activities on the bone mineral accretion in children. They found a general positive trend between exercise and bone mass. Laing *et al*.[77] studied girls between four and eight years old for two years and concluded that girls enrolled in gymnastics had a greater rate of bone mass accrual than those that were not enrolled in gymnastics and doing either no activity or non-gymnastics activities. Additionally, Laing *et al*.[77] noted that the bone mass gains increased with the level of gymnastics performance although lower level gymnastics training was sufficient stimulus for bone mass increases.

The time at which exercise is initiated is also a contributing factor which may greatly impact bone mineral accrual. During preadolescence and adolescence, bone mass accrual is crucial and initiating physical exercise at this age is greatly beneficial in achieving an optimal PBM. A cross-sectional study by Kannus *et al*.[78] observed that the increase in bone mass in adult squash players that had begun playing the sport during pre-menarche was double that of those who had only started playing post-menarche. This suggests that initiating physical activity during preadolescence may be advantageous in attaining adequate bone mass and preventing osteoporosis. The reason for this is possibly due to low oestrogen production pre-menarche which has been postulated to make bones more adaptive to mechanical stresses.[79] Not only have girls been found to benefit from initiation of physical activity during preadolescence, but evidence also shows that this is an opportune time for boys too due to the responsiveness of their immature bones.[80, 81]

2.3.3. Demographic factors affecting bone health

Peak bone mass is genetically preconceived and thus varies and is unique to everyone. Bone mineral density varies based on demographic factors beyond which nutrition can influence it. Likewise, the risk for osteoporosis is different for each individual. Gender and age affect risk for osteoporosis development as this relates to hormonal differences and changes that are unique to women and men of different ages.[82] Ethnic variances in BMD are also genetically related with Asian individuals found to have the weakest bones and black individuals the strongest.[83, 84]

2.3.3.1. Gender

Skeletal development and attainment of PBM is regulated by sex hormones, namely, oestrogen and testosterone as these hormones increase bone mass. Oestrogen is the key

role player in skeletal development in both sexes, but the greater bone mass of males is attributed to the added testosterone in male subjects.[85] Oestrogen is believed to modulate bone remodelling and make mineral deposits in to the bone. Increased oestrogen increases the deposits and as this would be greater in females, it is believed that female bones are more responsive to hormones than to mechanical adaptations.[79] Only during puberty is a clear difference in bone mass achieved as bone mineral stores increase with the skeletal growth. Bone size increases more in males during puberty and the duration of puberty is longer in males as compared to females which means that males reach PBM later than females and have a greater final PBM.[28] During preadolescence sex hormones are lower in both males and females making their bones more responsive to mechanical loading during this age.[80] During puberty, oestrogen, growth hormones and IGF-I levels increase as skeletal growth accelerates and these hormones then decrease again after the pubertal phase. While gradual age-related bone loss is observed in males and females, females are at a greater risk of developing osteoporosis due to rapid bone loss during menopause as sex hormones levels decrease. Both oestrogen and testosterone levels decline in the aging male however males typically do not experience rapid bone loss as oestrogen is the major role player in bone loss and men do not go through menopause.[85]

2.3.3.2. Age

During childhood, bone growth increases longitudinally as cartilage at the ends of bones differentiates in to bone. By bone modelling, bone width also increases. Bone modelling is accelerated during childhood but the rate at which bone area increases is genetically predetermined and is also a response to the increasing load due to the increase in body weight. As height velocity is rapid during preadolescence and adolescence, BMD is expected to decrease. This is because bone area at this age increases more rapidly than the increase in weight of body composition components. Because these forces are exerted on a larger bone surface area, the external pressure is reduced and there is a lag in bone accretion. A lower BMD during the growth phase means that fracture risk is greater and nutrition and physical exercise are vital at this age.[86] Peak bone mass is reached around 30 years of age however the exact age is determined genetically.[2]

Age-related bone loss occurs in both males and females universally and is a gradual loss unlike the rapid losses that may be experienced in menopausal females.[85] Age is considered a risk factor for developing osteoporosis as from about the age of 40, bone resorption by osteoclasts exceeds bone formation by osteoblasts due to the decline in oestrogen that occurs as a function of aging as previously discussed. This imbalance of bone cell activity is called “uncoupling” and results in increased bone loss as bone mass gradually begins to diminish.

Its effects are more prominent in females than in males due to the rapid decline in oestrogen associated with menopause until about the age of 70 where bone loss rates are equal in both genders.[2]

2.3.3.3. Ethnicity

Differences in bone health status between ethnic groups has been the focus of many studies and clear variations have been observed. It has been noted that the differences in bone health are due to varying PBM and BMD declines between ethnic groups due to genetic and environmental factors.[84] The BMD of black subjects has proven to be higher than white, Hispanic and Asian subjects due to larger bone size and a greater amount of cortical bone present at the same age and weight.[87, 88] Because many studies are limited to different ethnic groups living in the same geographic location, Nam *et al*.[84] and Nam *et al*.[82] did studies using subjects of different ethnic groups in different regions and still interethnic variations were observed. Nam *et al*.[84] noted that a limitation to their study was the fact that serum vitamin D levels were not assessed which could account for differences across the geographical regions.

2.3.3.4. Socioeconomic status

Socioeconomic status indirectly affects bone health as nutrient status is heavily influenced by socioeconomic factors. Socioeconomic status relates to the prevalence of stunting in children which is an indication of bone health because stunting is a sign of nutritional status which as discussed, directly or indirectly affects bone health (Figure 2[89]). Even in countries with well-established health care systems, a health gradient persists in association with socioeconomic status. During times of economic hardship, budget shortfalls hinder the fulfilment of interventions that are the most resource intensive. In most cases, the lowest socioeconomic groups of the population become even more susceptible to ill health.[90]

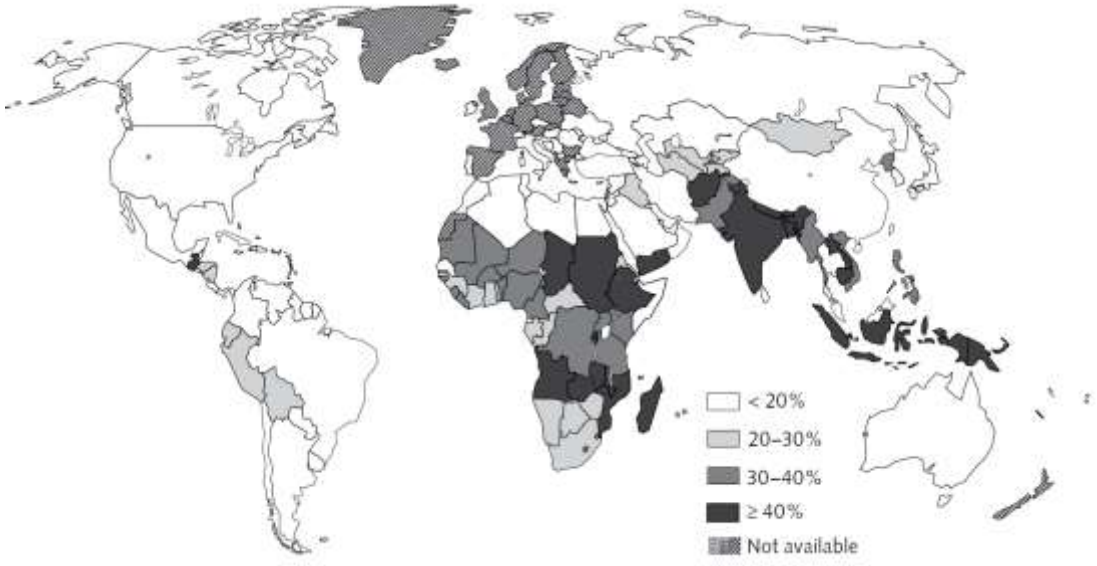


Figure 2. Estimated prevalence of stunting in children under 5 years of age.[89]

2.4. ASSESSING BONE HEALTH

Bone health assessments are important throughout the life cycle for the prevention or treatment of osteoporosis and to achieve optimal PBM. Because the skeleton is an organ, bone health is important and plays a major role in adequate bodily functioning. The fact that the skeleton is continuously remodelling itself can be utilized in these assessments by using markers of the rate of bone turnover.[2]

2.4.1. Anthropometry

Anthropometry is a physical measurement to assess gross body composition. This non-invasive technique is used to measure factors such as height, weight, skin-folds and circumferences at different areas of the body. This type of measurement requires minimal training, can be done in various settings due to the ease of transport and provides results of high accuracy and precision. Anthropometric measures are low-cost measures and are particularly valuable when used to assess children over time as they thereby demonstrate growth or nutritional sufficiency. If done once-off in children, it does not allow for the evaluation of growth trends.[91]

Anthropometry alone is inadequate for determining bone health status and should be used in conjunction with other nutritional assessment methods before finalising nutritional diagnoses. Although it is not adequate alone, this method plays a vital role in nutritional assessments.[2]

2.4.2. Dietary assessments

Dietary assessments offer value in assessing bone health as nutrient intake is such a vital aspect of good bone health and a clear idea of the types of foods eaten by an individual can be established.[28] Dietary assessments may be used as an initial screening to assess whether further testing is required or whether poor bone health relates to inadequate nutrition. This method of assessment is non-invasive and affordable however it cannot be used alone as it is inaccurate as it relies on memory and cannot be reproduced. Types of dietary assessments include a diet history, food diary, food frequency questionnaire and a 24-hour recall.[44]

2.4.3. Biochemical assessments

Biochemical assessments may be done as an indication of bone metabolism and thus bone health, but biomarkers should be measured in combinations rather than as single entities and are best done in combination with DXA scans for diagnosis of osteoporosis. As these are not diagnostic indicators of osteoporosis, they are best suited to monitor the treatment thereof. Indicators of the resorption include degradation products. Bone formation is assessed by measurement of enzymes and proteins involved in this step of bone turnover.[92] In Table 4 the biochemical assessments used to analyse bone health are described as well as the disadvantages and advantages of each. Serum vitamin D in the form 25(OH)D is useful in providing causality of poor bone health and will be discussed in greater detail further on.

2.4.4. Clinical assessments

Clinical assessments are also physical assessments or examinations performed on a subject and this can be done to determine bone health.[91] Clinical assessments differ from anthropometric assessments as they should be performed by a trained health professional. Table 4 describes the densitometric techniques used to analyse bone density as a function of bone health as well as the disadvantages and advantages of each. The techniques described are *in vivo* techniques that are commonly practiced, providing information on bone densitometry of living humans. Dual-energy X-ray absorptiometry is the most commonly used imaging technique for assessing the bone health of preadolescent children because of its safety in low radiation exposure and for clinical studies it is particularly advantageous as it is cost effective. Errors in processing of the information are however likely as density is a volumetric measurement but BMD is not expressed volumetrically by DXA and thus bones of a larger area may appear to have a greater density. Because of the widespread use of DXA in clinical research, using DXA makes data comparable to that of other studies and reference data from DXA is extensive.[86]

Clinical measures of bone physiology from bone imaging include BMC, aBMD (areal BMD), vBMD (volumetric BMD), and bone area. Bone mineral content and bone area are measured using specialized machines in clinical assessments of bone health. Areal BMD (g/cm^2) can be calculated by dividing BMC by bone area whereas vBMD (g/cm^3) requires 3D analysis of the bone which is not practical for research purposes due to the high costs involved.[22] Bone mass measurements (BMC and BMD) are converted in to T-scores for adults and Z-scores for children and then used for comparison to a healthy age-matched reference group. T-scores and Z-scores serve as an indication of the standard deviation (SDs) from the healthy references. Children and adults do not use the same scoring system due to the curvilinear relationship between bone mass and age.[23] Z-scores below -2 are an indication of low bone mass for age.[93]

To compare BMD measurements of children to a reference group it is necessary to take factors such as height, which is dynamic at this age, in to account which may distort measurements. This can be done by calculating bone mineral apparent density (BMAD) which is bone area squared divided by height and BMC is then divided by that. Alternatively, Z-scores can be adjusted for height using height-for-age Z-scores.[94, 95]

Table 4. Clinical and biochemical methods of assessing bone health and their strengths and limitations.

Assessment	Assessment Details	Strengths	Limitations	References
CLINICAL				
DXA: Dual-energy X-ray absorptiometry	An imaging technique that measures bone mass and area. Uses two x ray beams. Results expressed as areal density (aBMD) (g/cm^2). Scans include the whole body, but isolated skeletal sites are expressed individually too.	Radiation dose is minimal; Fast; Provides info on body composition over and above BMD (Body fat, lean body mass and bone mineral mass)	Provides info on the based on the bone's cross-sectional area; Cannot distinguish between cortical and trabecular bone	Li *et al*.[96] Baroncelli[4] Binkley *et al*.[86]
QCT: Quantitative computed tomography	Measures vBMD using 3D x-ray scanning at the peripheral sites (calcaneus, phalanges of the hand, and tibia). A source and detector rotate around the area of interest and a 3D image is reproduced. Results expressed as g/cm^3. HR-pQCT (High resolution peripheral QCT) and pQCT provide images at the peripheral sites.	Offers 3D (geometric) information about the bones and differentiates cortical and trabecular bone	Costly; Delivers ionizing radiation to patients (Higher radiation dose than DXA) and is not therefore used in clinical assessments of children	Donnelly[97] Li *et al*.[96] Genant *et al*.[98] Baroncelli[4]
QUS: Quantitative Ultrasound	A scanning assessment that measures vBMD by emitting pulses of acoustic waves between two probes, one emits and the other receives the waves. Results are given as a reading not an image.	Affordable method of analysis; differentiates cortical and trabecular bone; Radiation-free. Ease of transport. Requires little training.	Measures BMD at small focussed skeletal sites only (e.g. heel, tibia or proximal phalnges); Results not comparable as there is little data available; QUS devices are diverse and there are many variables used to assess BMD	Baroncelli[4]
MRI: Magnetic Resonance Imaging	A magnetic field and radiofrequency pulses are applied to the body to produce 3D images reflecting water with trabeculae seen as dark space. Bone densitometry is expressed volumetrically	Does not emit radiation; Produces 3D images for bone geometry	Costly; Time intensive; Large equipment required means it is not easily transported	Donnelly[97]

BIOCHEMICAL				
C-Terminal Telopeptides Type I Collagen (CTX)	CTX is Type-1 collagen broken down by osteoclasts and is therefore a measure of bone resorption. A recommended marker of choice.	The sample remains stable in EDTA for about 48 hours; Low variability between samples	Varies at different times of the day and must therefore be sampled at morning fasting;	Wheater *et al*.[99, 99, 99]
Procollagen type 1 N-terminal Propeptide (P1NP)	Procollagen such as P1NP are collagen precursors synthesized by osteoblasts in bone formation. High levels of circulation P1NP indicate increased bone formation. A recommended marker of choice.	Low variability between individuals; High precision; Remains stable at room temperature;	Varies at different times of the day; Costly	Wheater *et al*.[99] Mayo Clinic[100]
Osteocalcin	Osteocalcin, produced by osteoblasts, is a non-collagenous protein that bind to hydroxyapatite to form the bone matrix. Osteocalcin is liberated during bone resorption. Thus, osteocalcin indicates both processes of bone formation and bone resorption.	Stable in EDTA; Useful in monitoring bone health when therapy with antiresorptive agents is necessary (e.g. hormone replacement therapy)	Indicating bone turnover means it cannot diagnose osteoporosis; Varies between laboratory assessments	Wheater *et al*.[99] Mayo Clinic[100]
Bone-specific alkaline phosphatase (ALP)	Increased ALP activity may be associated with poor bone health status however ALP is associated with the liver and intestines too. Bone-specific ALP is an isoenzyme involved in bone metabolism.	Highly specific; A single serum sample can be used to measure other biomarkers for validity	Cannot predict short-term variations in bone mass; Recommended be used in combination with other biochemical assessments	Backstrom *et al*.[92] Betto *et al*.[101]
Parathyroid Hormone (PTH)	PTH is a hormone involved in the regulation of calcium homeostasis. It responds to low calcium serum levels and promotes calcium release from the bones. PTH serum levels >65 pg/ml may indicate high bone turnover and poor bone health.	PTH serum levels remain stable at room temperature for up to 6 hours	No standard method of measurement; Different methods are greatly variable; Cannot be used alone to assess bone health	Souberbielle *et al*.[102]
Inorganic phosphate	Phosphate plays a key role in the bone matrix along with calcium. Low levels of urinary or serum phosphate is an indication of bone disease.	High specificity in diagnosing poor bone health	Must be used in combination with other biochemical assessments to be valid	Catache and Leone[103] Backstrom *et al*.[92]

2.5. VITAMIN D PHYSIOLOGY

Vitamin D is a unique essential nutrient because the active form of the nutrient cannot be obtained from the diet like other micronutrients. Vitamin D, or calciferol, is a term used to collectively describe the different types of vitamin D precursors/metabolites. For this reason, vitamin D content is recorded as units of calciferol in international units (IU) and 1 µg of the precursor is equivalent to 40 IU calciferol.[10] Vitamin D precursors are obtained from the food and UV sources and are converted by the liver and kidneys in to the active form (Figure 3). Vitamin D3, also known as cholecalciferol, is the main contributor in terms of vitamin D precursors/previtamins as it has been shown to most effectively increase 25(OH)D levels. The primary source of vitamin D, particularly in South Africa, can be obtained through sunlight exposure as UVB rays convert 7-dehydrocholesterol, a precursor involved in cholesterol synthesis, in to vitamin D3.[59] Vitamin D3 can also be obtained naturally from food sources including fatty fish, egg yolks, beef liver and cheese. Vitamin D2, also known as ergocalciferol, is a precursor found in plant foods such as mushrooms and yeasts, and is believed to be a lot less effective than vitamin D3 in raising 25(OH)D levels. Because so few food sources contain vitamin D, certain foods are fortified with the nutrient or supplementation may be necessary. The form of vitamin D, hydroxylated from precursors in the liver, is calcidiol or 25(OH)D which is then hydroxylated by the kidneys in to the active form of vitamin D, calcitriol or $1,25(OH)_2D$ (Figure 5).[2, 104]

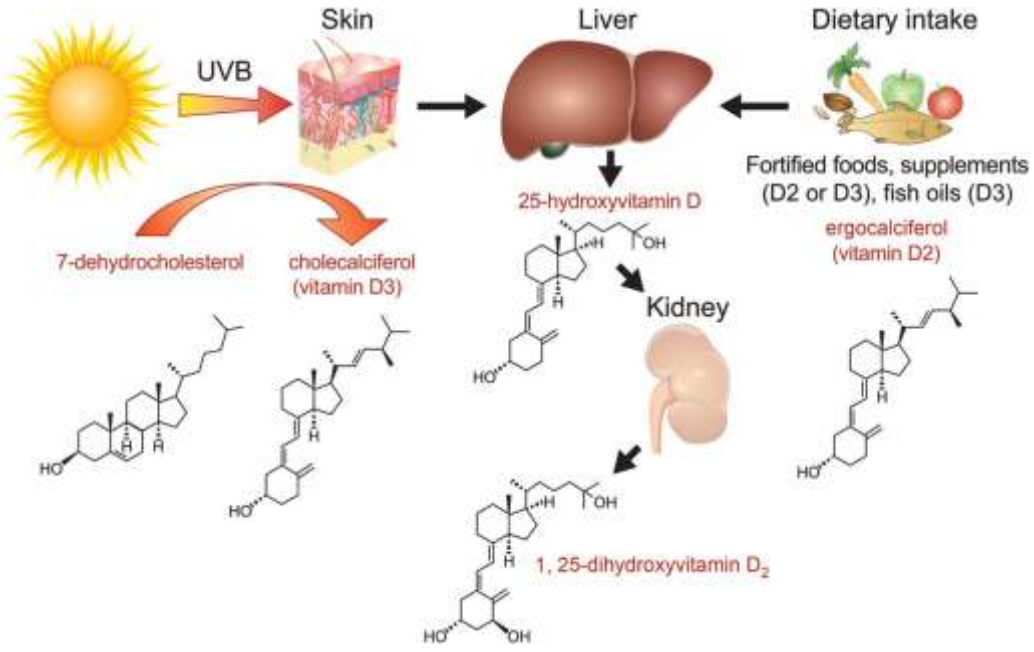


Figure 3. Vitamin D synthesis from precursors and metabolism within the human body.[104]

2.5.1. Vitamin D absorption, transportation and storage

Because vitamin D is a fat-soluble nutrient, its absorption is dependent on the presence of lipids and micellerisation must take place where the nutrient is taken up in to a micelle to be transported. A micelle is a lipid-containing structure formed during digestion within which the vitamin D is incorporated to diffuse in to the enterocytes. For vitamin D to function it requires two proteins, vitamin-D binding protein (VDBP) and vitamin D receptors (VDR). Vitamin D is ingested and then absorbed by the intestines and as it enters the blood, it is bound and transported to the various sites by VDBP. Vitamin D becomes biologically active as it binds to the VDR in the target cell nucleus and by this binding, regulates gene expression. Vitamin D is stored both in the liver and in adipose tissue but is not readily available from the adipose tissue stores.[10,107,105]

2.5.2. Daily requirements of vitamin D intake

Because so few foods contain adequate amounts of vitamin D, food fortification and biofortification strategies become necessary to address nutrient deficiencies. Not many foods are fortified with vitamin D in South Africa, thus new fortification strategies need to be implemented. In South Africa, the typical diet contains approximately 200 IU vitamin D per day, which is well below the requirement of 600 IU per day, which emphasises the need for improved fortification and biofortification strategies.[106, 107]

2.5.2.1. Daily intake requirements for Vitamin D

The dietary reference intakes updated and published by the IOM in 2011 provides an outline of the dietary requirements for vitamin D ingested with the assumption of minimal cutaneous vitamin D synthesis (Table 5). This is as such since multiple factors influence the amount of vitamin D synthesis by UV radiation. Despite having lower circulating 25(OH)D and greater BMD, the black population is still highly prone to fracture risk and osteoporosis and thus recommended vitamin D intakes are not varied between ethnic groups.[83]

Table 5. Dietary reference intakes for vitamin D in children.[10]

Life stage	Adequate Intake (IU)	Estimated Average Requirement (IU)	Recommended Dietary Allowance (IU)	Tolerable Upper Intake Level (IU)
Children 4 – 8 years	-	400	600	3000
Individuals > 9 years	-	400	600	4000

2.5.2.2. Vitamin D supplementation and fortification in South Africa

Depending on the severity of vitamin D deficiency or the underlying cause, different intervention methods will be more and/or less successful in addressing this micronutrient deficiency. Generally, food fortification can improve vitamin D status where foods are consumed that are not rich in vitamin D. As very little foods contain adequate amounts of vitamin D, food fortification as a permanent intervention method should be considered globally. Various countries fortify certain foods, but the high prevalence of vitamin D deficiency shows that this may be inadequate. Supplementation is an appropriate intervention method where there is a need for it due to severe deficiencies in certain populations or where access to food is limited.[106, 108]

Supplements are administered directly either in a syrup or through pills through primary healthcare systems or healthcare delivery systems. Supplementation is generally considered to be a short-term solution to be replaced at a later stage by more sustainable and long-term approach such as food-based interventions.[109,111,115]

Food fortification regulations are classified as either voluntary or mandatory, where voluntary fortification is performed at the discretion of the manufacturer and mandatory fortification is required by law. In South Africa, fortification of margarine with vitamin D is voluntary and no form of vitamin D fortification is mandatory. Improving the diet through fortification is a widespread, inexpensive and sustainable method to increase baseline vitamin D levels.[109]

2.5.3. Factors affecting vitamin D absorption

Bioaccessibility and micellerisation are terms used to refer to how much of a fat-soluble nutrient that is ingested has the potential to be accessible for absorption. This depends on liberation from the food matrix within which the nutrient is embedded and then digestion processes before absorption. Bioavailability is a term that refers to how much of a nutrient is ingested, but differs from bioaccessibility in that it also refers to what is both absorbed and

available for use by the body. Bioavailability depends on digestion and liberation from the food matrix as well as absorption and transportation mechanisms of the somatic cells.[42] The bioavailability or bioaccessibility of vitamin D may be enhanced or inhibited and this depends on a host of factors such as the chemical form; amount ingested; food matrix within which it is embedded; presence of other nutrients; and host-related factors.[15]

2.5.3.1. Chemical form of vitamin D

Firstly, the chemical form of the nutrient is thought to affect the bioavailability as many studies have shown the greater potency of vitamin D3 as compared to vitamin D2 in increasing levels of circulating calcidiol in the blood.[110, 111] However, the biological efficacy of vitamin D2 as compared to vitamin D3 is still under scrutiny. In a study by Holick *et al*.[112], subjects were supplemented daily with either 1000 IU vitamin D2, 1000 IU vitamin D3, 500 IU vitamin D2 in conjunction with 500 IU vitamin D3, or a placebo. They found that all the forms of supplementation had similar effects on levels of circulating 25(OH)D. These results aligned with the results of an earlier study done by Armas *et al*.[110]. Contrarily, Heaney *et al*.[111] found that when supplemented with approximately 50 000 IU per week of vitamin D2 or vitamin D3, vitamin D3 was more effective in increasing serum 25(OH)D as well storage of the vitamin. Heaney *et al*.[111] measured storage at baseline and again 12 weeks later by fat biopsies obtained surgically and analysed using HPLC.

Another approach towards this is that fat-soluble vitamins, such as vitamins D2 and D3, out-compete each other as they follow the same mechanism of absorption. The results and theories of multiple studies are conflicting and the subject of bioavailability of the chemical forms of vitamin D needs to be further investigated.[113, 114]

2.5.3.2. Dietary intake

The amount of vitamin D ingested is also a determinant of the nutrient's bioavailability. Doses of vitamin D given to vitamin D deficient rats in a study by Hohman *et al*.[115] were directly proportional to improvements in overall bone health irrespective of the chemical form and source from which the vitamin D was provided. Various studies performed have shown that there is a linear relationship between the amount of vitamin D absorbed and the absorption efficiency which goes against the assumption that absorption efficacy decreases with an increase in dose.[15]

2.5.3.3. Food matrix

The food matrix has been proven in multiple studies to have little or no effect on the bioavailability of vitamin D.[116-118] Due to the limited amount of vitamin D2 present in plant sources and the effect of UV irradiation on vitamin D, UV-rays have been applied to foods such as mushrooms and yeasts in an effort to enhance the vitamin D2 present in the fungi.[115, 119] Ko *et al*.[119] proved that UV irradiation is an effective method of increasing vitamin D concentration in plant food sources and this may prove to be particularly valuable in rural populations where animal sources and supplementation are not affordable.

2.5.3.4. Other nutritional factors

Other nutritional factors incorporated within the same meal or present in the food source of vitamin D may either inhibit or enhance the absorption of vitamin D depending on the interaction of the component and the fat-soluble vitamin. Dietary fibre present in the diet is one such example as it reduces fat absorption, a key component necessary for micelle formation in the absorption of fat soluble vitamin D. Vitamin D absorption is therefore inhibited with increased fibre content. Fat substitutes included in the diet in the aim of reducing fat intake for example if cholesterol levels are elevated have limited or no absorbability. They have shown to decrease the amount of circulating serum vitamin D due to the impaired incorporation in to micelles and are thus, like dietary fibre, an inhibitor of vitamin D absorption.[15,43,41]

Lipids are an essential vehicle for the absorption of vitamin D which allows for the assumption that incorporation of dietary lipids may enhance the absorption and increase the bioavailability of vitamin D. While the exclusion of lipids from the diet hinders its absorption, studies performed have generally found that the absorption of vitamin D is not affected by the amount of dietary lipids incorporated. Rather, it has been identified that the type of lipids incorporated are what affect vitamin D bioavailability. A difference has been seen in studies comparing the effects of medium versus long-chain fatty acids involved in vitamin D absorption. The results are conflicting and further studies are required to confirm or explain this.[15]

2.5.3.5. Host-related factors

Vitamin D insufficiency is often prevalent in obese individuals and can be attributed to a lower vitamin D bioavailability due to a higher fat deposition (Figure 4).[120] Wortsman *et al*.[120] found that the bioavailability of vitamin D in obese subjects ingested orally or obtained cutaneously by UV exposure was both far less than that of the matched lean control subjects. Vitamin D3 obtained from UV exposure was produced at the same concentration from equal concentrations of the 7-dehydrocholesterol precursor. This indicated that bioavailability

differences were due to a larger amount of vitamin D3 sequestration by subcutaneous fat in obese individuals and thus a reduced amount of circulating vitamin D3. By the same principle, vitamin D2 orally supplemented was less bioavailable in obese individuals. Vitamin D status has been found to normalise following weight loss interventions where dietary vitamin D intake was not increased.[121]

Although gastric bypass surgery promotes weight loss thus reducing sequestration of vitamin D in the adipose tissue, poor absorption and reduced intakes of the vitamin predispose gastric bypass surgery patients to vitamin D deficiency. Likewise, gastrointestinal (GIT) disorders are frequently paired with nutrient deficiencies namely, hypovitaminosis D. The primary underlying cause of GIT disorder-related vitamin D deficiencies being impaired absorption in the gut. Various GIT disorders, such as inflammatory bowel disease or coeliac disease, hinder absorption in the gut and malabsorption of fat directly influences the absorption of all fat-soluble vitamins as is the case with vitamin D (Figure 4).[105] gastrointestinal disorders are ubiquitous in elderly individuals which are a major cause of vitamin D deficiency at this life stage. Not only are GIT disorders associated with vitamin D deficiency in the elderly, but absorption is impaired by other age-associated factors including reduced liver functioning, efficiency of vitamin D synthesis in the skin, sunlight exposure and dietary intake of the nutrient.[15]

In sunny regions, such as South Africa, vitamin D3 from 7-dehydocholesterol is the primary source of vitamin D synthesis.[20] The epidermis consists of five layers where the precursor 7-dehydocholesterol is found at its highest concentrations in the bottom two layers, the stratum basale being the inner most layer and the stratum spinosum above that. This is thus where most of the vitamin D3 is synthesised from UV-rays. Also found in the stratum basale, are melanocytes. Melanocytes are skin cells responsible for the production of melanin which gradually migrate to all the layers of the epidermis and are responsible for pigmentation of the skin. Melanin has a protective function in the body to filter the amount of UV-rays that enter the skin. Melanin absorbs the UV-B rays thus reducing the amount of UV that reaches the stratus spinosum and basale where 7-dehydocholesterol is present. The potential for vitamin D3 synthesis is therefore dependent on concentrations of melanin in the skin and the larger the concentration of melanin is, the darker the skin tone is and the lower the potential for cutaneous vitamin D3 synthesis is (Figure 4).[122] Harris and Dawson-Hughes (1998) according to Norman *et al*.[122] confirmed this when seasonal variations of serum 25(OH)D3 were greater in white American women than black American women. Not only does pigmentation affect the synthesis of this vitamin, but use of sunblock or sunscreens, less exposure to sunshine by

staying indoors and skin cover with certain clothing reduce the cutaneous synthesis of vitamin D3 dramatically.[20]

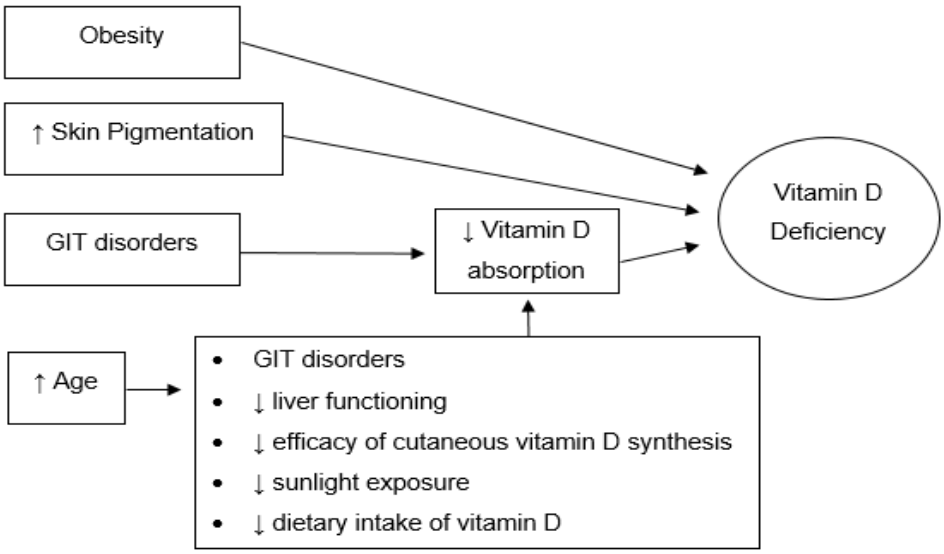


Figure 4. Possible host-related causes of vitamin D deficiency.

2.5.3.6. Environmental and Geographical factors

Vitamin D, commonly referred to as “the sunshine vitamin”, is synthesised in the skin upon exposure to UV-B rays.[14] For the conversion of 7-dehydocholesterol to form previtamin D, a certain amount of UV radiation is required. The zenith angle of the sun is the angle at which the sun hits the earth which changes based on the latitude, season and the time of the day. UV radiation is reduced when the zenith angle of the sun is reduced, and this results in reduced formation of vitamin D from 7-dehydocholesterol (Figure 5[123]). At higher latitudes, the zenith angle reduces and in winter months at a latitude above 70º, the zenith degree is likely to decrease to a point that UV radiation is not adequate to synthesise vitamin D3 at all.[124] The amount of vitamin D synthesised in the skin annually of individuals in America at 52º latitude is half of that synthesised by those at 40º latitude.[125]

Hypovitaminosis D is prevalent in African countries which is a concern as there is year-round sunshine and optimal geographical placement. While host-related factors may affect the cutaneous vitamin D3 synthesis, there are other environmental factors that may add to the limitation of its synthesis.[20] For example, cloud cover absorbs and reflects UV-B rays reducing radiation by up to 30%. Altitude differences also affect UV radiation with a reduction of between 2% and 4% per 300m descent in elevation.[126] Cloud cover, latitude and altitude differences may explain the impaired cutaneous vitamin D3 synthesis in some regions of Africa like Cape Town as compared to Johannesburg reported by Pettifor *et al*.[127]

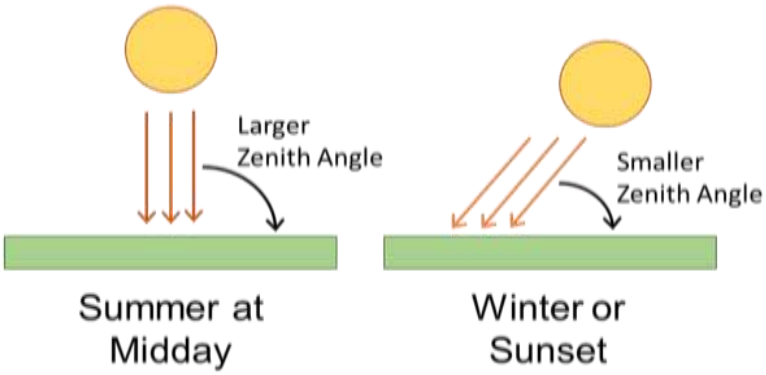


Figure 5. The solar zenith angle is the angle at which the sun hits the earth.[123]

Some reasons for the prevalence of hypovitaminosis D in preadolescent South African children may include the inconsistent diets of children, the prevalence of malnutrition in South Africa, and the reduced exposure to sunlight due to increasing urbanization.[19, 20]

2.6. THE ROLE OF VITAMIN D IN BONE HEALTH

Vitamin D is important in the body as it regulates genes which encode proteins responsible for controlling intestinal calcium absorption, bone growth and remodelling and phosphate homeostasis. While the primary role of vitamin D is its role in the calcium-phosphorus-vitamin D homeostatic system, it is believed to have various other non-calcium related roles too.[106] Vitamin D is believed to be important in reducing the risk for cancer development; in the prevention and treatment of people suffering from HIV and tuberculosis; and in modulating the immune system and muscle metabolism.[128] In a report on vitamin D and calcium dietary reference intakes by the Institute of Medicine, it was stated that recommended values are based on dose-response relationships for bone health only.[10]

When calcium serum levels are depleted, hormones act to achieve homeostasis through resorption, absorption and reabsorption. The three organs targeted in the process of calcium and bone homeostasis are the kidneys, bones and intestines. Firstly, the parathyroid gland is stimulated by the low serum calcium and parathyroid hormone (PTH) is released. The kidneys are targeted by PTH where an enzyme in the kidneys, 1-α-hydroxylase, is stimulated to convert 25(OH)D (calcidiol) in to active vitamin D, $1,25(OH)_2D$ (calcitriol). $1,25(OH)_2D$ in turn targets the osteoclasts in the bone, stimulating bone resorption for an increase of serum calcium and phosphate levels.[129] Parathyroid hormone in conjunction with active vitamin D targets the bone. Parathyroid hormone stimulates bone resorption, releasing calcium and phosphate from the bone thus elevating the serum calcium and phosphate levels. There is a negative feedback by $1,25(OH)_2D$ inhibiting PTH and this controls the amount of bone resorption. Reabsorption of calcium is an additional process mediated by both $1,25(OH)_2D$

and PTH to achieve calcium homeostasis, targeting the kidneys to prevent urinary calcium losses. 1,25$(OH)_2$D without the involvement of PTH, targets the intestines for increased absorption of calcium thus increasing the calcium in the blood and in turn reducing bone resorption.[59]

Though the active form of vitamin D plays a role at these various sites, the primary function of vitamin D in calcium homeostasis is to increase its absorption in the intestine. Through genomic effects, vitamin D stimulates the active uptake of calcium. It is understood that this genomic effect occurs whereby the active form of vitamin D, 1,25$(OH)_2D_3$, binds with the VDR. This promotes dimerisation and the VDR in turn binds with a nuclear receptor known as retinoid X receptor (RXR).[130] The VDR/RXR dimer modulates gene transcription by binding 1,25$(OH)_2D_3$ to the DNA sequence known as vitamin D response elements (VDRE), increasing the expression of the calcium transport proteins. These proteins in the intestine that are enhanced include, transient receptor potential 5 and 6 (TRPV5, TRPV6), calbindin (CB9K), Ca-ATPase (PMCA), Na+-Ca- (NCX1). With the increased expression, the intestine is able to absorb more calcium.[131]

Vitamin D deficiencies are associated with poor bone health throughout the lifecycle and its supplementation reverses these effects. Active vitamin D compounds have been used in treatments for improved bone density as 1,25$(OH)_2$D increases the intestinal absorption of calcium increasing bone mineralization. It has been postulated that 1,25$(OH)_2$D may additionally add to bone homeostasis by a direct effect on bone cells.[132] *In vitro* it was observed that supplementing with 1,25$(OH)_2$D promoted osteoclast formation but contrarily *in vivo* 1,25$(OH)_2$D has been given to improve BMD possibly believed to be by preventing bone resorption. Receptor activator of nuclear factor (NF)-kB ligand (RANKL) is expressed on osteoblasts and RANK, expressed on osteoclast precursors must interact with RANKL for differentiation in to osteoclasts.[133] The action of 1,25$(OH)_2$D in response to PTH when calcium levels are low is to promote osteoclast differentiation by acting on osteoblasts to stimulate this through the RANK-RANKL interaction. The inhibition of bone resorption observed *in vivo* can be explained by two possible mechanisms. The first mechanism proposed is that RANKL is supressed by a shift in the calcium endocrine system from prolonged exposure to 1,25$(OH)_2$D and osteoclasts do not differentiate thus inhibiting resorption. The second possible mechanism is that RANKL is supressed as osteoblast formation is altered by prolonged exposure to 1,25$(OH)_2$D. These mechanisms have not been validated and further studies are required for this.[132]

Serum 25(OH)D is considered the gold standard for assessing vitamin D status. The role of vitamin D in regulating bone metabolism has been well established and poor vitamin D status

has been linked to poor bone health, however, the relationship between serum 25(OH)D levels and bone mass in children remains unclear. The optimal serum 25(OH)D levels for adequate bone mass to ensure peak bone mass is reached in children has not been defined.[13] A challenge in defining the recommended vitamin D dietary intake has been defining what an adequate amount of circulating 25(OH)D is for optimal functioning of the human body and optimal bone health. Furthermore, 25(OH)D levels are known to reflect bone health differently in different ethnicities as despite generally lower 25(OH)D levels, BMD remains greater in Africans as compared to all other ethnic groups.[61] While BMD has been used as a biomarker of circulating serum 25(OH)D, and a threshold for serum 25(OH)D at which bone loss due to elevated PTH occurs has been determined, 25(OH)D has not been identified as a concrete biomarker in determining bone mass. This may be due to the fact that bone mass parameters (BMD and BMC) indicate prolonged exposure whereas 25(OH)D levels indicate recent exposure.[83, 134]

2.7. ASSESSING VITAMIN D STATUS

Vitamin D status assessments are important in ensuring optimum 25(OH)D levels for optimum functioning of the human body due to the vital roles that vitamin D plays. Vitamin D status may be described as deficient, insufficient, sufficient or intoxicated.[13]

2.7.1. Anthropometry

Anthropometric measurements are not a direct measure of vitamin D status but rather an indirect measure by bone health which without other analyses is not relevant. Charts are available reflecting the height and weight measurements of children of a certain age and gender within a population. On the charts are percentiles where the subject child's weight or height can be mapped out and compared for interpretation of nutritional status.[2]

2.7.2. Dietary assessments

Because vitamin D status relies on sun exposure and so few foods naturally contain or are fortified with vitamin D, performing dietary assessments does not give a good indication of vitamin D status.[91]

2.7.3. Biochemical assessments

While the levels of serum 25(OH)D that define deficiency, insufficiency and sufficiency have not been clearly defined, certain ranges have been proposed. There has been much debate on what the suitable level of serum 25(OH)D is for adequate bone mass maintenance. While the cut-off point set by the IOM in 2010 was 20 ng/ml of circulating 25(OH)D, there has been

much deliberation on the findings used to set this limit, so the Endocrine Society conducted studies on the topic to draw their own inferences. In 2011 the Endocrine Society countered the IOM's recommendations and suggested that a minimum concentration of 30 ng/ml of circulating 25(OH)D should be maintained.[10, 108] Boschoff-Ferrari (2014) according to Mahan and Raymond[2] made recommendations of higher optimal values of 36-40 ng/ml. In an article authored by Holick[13], it was concluded that in accordance with the views of multiple experts, 25(OH)D levels below 20 ng/ml indicate deficiency and more than 30 ng/ml defines a healthy vitamin D status. For this biochemical measurement of vitamin D status to serve as the gold standard of analyses, standardization of the methods and ranges are required.

Biochemical markers of vitamin D status are described in Table 6. Using the biologically active form of vitamin D, $1,25(OH)_2D$, as a biochemical marker of vitamin D status is not recommended as it provides for false interpretation. This is because at the onset of hypovitaminosis D, catabolism of 25(OH)D stored in the liver increases, raising $1,25(OH)_2D$ levels; $1,25(OH)_2D$ has a short half-life; $1,25(OH)_2D$ is present in very low concentrations making the analysis more difficult.[13]

2.7.4. Clinical assessments

Clinical indicators of vitamin D deficiency are signs and symptoms associated with osteomalacia (bone softening) such as bone pain and with rickets including bone deformation.[135] These indicators can be assessed using the clinical analyses such as x-rays and those described in the bone health section (Table 6) as they pertain to bone health status. Bone deformities as in the case of rickets are the only clinical observational signs of poor vitamin D status and therefore screening for poor vitamin D status is difficult particularly in population studies.

Table 6. Biochemical methods of assessing vitamin D status and their strengths and limitations.

Assessment	Assessment Details	Strengths	Limitations	References
Serum 25(OH)D	Methods of measuring vitamin D status have included competitive binding-protein assays (CBPA), enzyme-linked immunoassays (ELISAs) and chemiluminescent immunoassays (CLIA) but LC-MS/MS (liquid chromatography with mass spectrometry) is considered superior as it measures 25(OH)D_2 and 25(OH)D_3 separately.	Definitive thus does not require additional measurements; LC-MS/MS is accurate and Vitamin D metabolites can be measured separately	Lacks standardization or gold standard; Human error may result in shortcomings	Holick[13] Carter[136] Le Goff *et al*.[137] He *et al*.[138]
Serum PTH (Parathyroid Hormone)	PTH forms a part of a calcium-phosphorus-vitamin D homeostatic system with an inverse relationship to vitamin D. PTH serum levels should not exceed 65 pg/ml and these levels are reduced with vitamin D supplementation.	PTH serum levels remain stable at room temperature for up to 6 hours	The relationship between PTH levels and vitamin D status at different life stages and populations must be better understood to be used as a marker	Prentice *et al*.[139] Souberbielle *et al*.[102]
Serum VDBP (Vitamin D-Binding Protein)	Serum 25(OH)D is a measure of vitamin D status without consideration of the bioavailability. VDBP is a measure of bioavailable 25(OH)D done in cases when VDBP may be suppressed. VDBP is measured using ELISA (enzyme-linked immunosorbent assay).	Using bioavailable 25(OH)D provides a greater accuracy for assessing vitamin D status	VDBP affinity for 25(OH)D is genetically determined; There is no reference range for bioavailable 25(OH)D	Kim *et al*.[140]

2.8. CURRENT KNOWLEDGE ON THE BONE HEALTH, VITAMIN D STATUS AND BODY COMPOSITION OF SOUTH AFRICAN CHILDREN

2.8.1. Bone Health

South Africa is stricken with diseases such as HIV and Aids, and the high prevalence of morbidity and mortality due to these diseases have allowed concerns such as osteoporosis to be set aside. With the increase in HIV prevalence, there is increased risk of osteoporosis due to decreased bone mineral density.[141] Little is known about the bone health status of South Africans throughout the life cycle. In an audit by the International Osteoporosis Foundation (IOF) it was estimated that around 180 DXA machines are available in South Africa of which 30 000 scans are done annually at the cost of the state.[142] Data exists suggesting that the prevalence of osteoporosis in mixed-race, white and Asian people living in South Africa mimics that of developed countries. The evidence is however unreliable due to poor accuracy. Black South Africans are known to have a lower fracture risk compared to other ethnic groups, but further research is required to assess incidence in the South African population of osteoporosis.[143]

2.8.2. Vitamin D status

Nutritional rickets and other bone disorders that relate to nutrition remain prevalent in developing countries such as South Africa. Vitamin D and calcium are pivotal in the pathogenesis of these bone diseases and this is a public health concern in South Africa. The relationship between vitamin D status and bone health in children beyond infancy has yet to be clarified due the inconsistencies of the findings of multiple studies conducted.[12, 18, 135, 144] Currently, there are no mandatory requirements for the fortification of products with vitamin D and this is because the vitamin D status of South Africans is unknown. This is concerning as there is a lack of awareness as to whether this is a health concern that needs to be addressed or not.[14] Pettifor *et al*.[127] concluded in a study comparing seasonal variations of vitamin D3 synthesis in Johannesburg and Cape Town, that Johannesburg has sufficient UV radiation and latitude for adequate vitamin D3 levels throughout the year. It was stated that the impact that this may have on the vitamin D status in Cape Town residents was not known due to the limited information on this topic.

According to Naude *et al*.[14], there is limited knowledge on the vitamin D status of children in South Africa, a country and age-group prone to nutrient deficiencies. In the study done by Naude *et al*.[14], adolescents in the Western Cape with and without alcohol use disorders were assessed. Although the study group was non-representative of the whole population, an observed vitamin D insufficiency and deficiency in both groups and poor dietary intake of the vitamin pointed towards a possible concern that may exist in many individuals of the same age group. In a study done in Johannesburg by Poopedi *et al*.[19], 74% of the 475 10-year old children assessed, presented a

sufficient vitamin D status (≥ 75 nmol/L or 30 ng/mL 25(OH)D) and vitamin D deficiency was concluded to not be a public health concern in Johannesburg. There was a significant difference observed in the seasonal changes in serum 25(OH)D between black and white children. It was also stated by Poopedi *et al*.[19] that further studies are necessary for definitive data on the vitamin D status of South African children. Results of the few studies done differ and this may be for a number of environmental and host-related factors that impact the populations from various regions of the country.

The burden of disease in Africa needs to be considered when vitamin D studies are done as this affects vitamin D status and a representative group of the whole population needs to be assessed for conclusive evidence. Not only is South Africa burdened with both under- and overnutrition as well as HIV/AIDS, but non-communicable diseases are ever prevalent due to the increasing urbanization. All of these impact vitamin D status as an add-on to the host-related factors that may hinder vitamin D synthesis or intake.[135]

2.8.3. Body Composition

Developing countries tend to have higher rates of malnutrition than developed countries, confirming that a low socioeconomic status is a precursor for malnutrition. Food insecurity leads to undernutrition in developing countries as nutrient-rich foods may not be accessible or, if accessible may not be affordable. There is the dual-burden of disease that largely affects the nutritional status in developing countries. Poor living standards increase the prevalence of communicable diseases. Undernutrition in pregnant mothers leading to low birth weights and malnutrition in infants and children leading to stunting on the other hand, is believed to increase the risk of non-communicable diseases.[91] The dual-burden of disease is rife in South Africa also due to the increase in urbanization, lack of nutrition knowledge and low physical activity levels whilst energy-dense foods form a staple in the South African diet.[74] Obesity and overweight is on the rise and it has been noted that research is needed on the effects of this on South African children.[30]

2.9. THE RELATIONSHIP BETWEEN BONE HEALTH AND VITAMIN D IN PREADOLESCENT CHILDREN

The importance of vitamin D in the bone health at all stages of life is well documented and in children particularly vitamin D status has been emphasized due to the importance of bone mass accretion for optimal PBM. Yet, the exact effects of vitamin D and the levels of serum 25(OH)D necessary for bone mass accrual and maintenance is not well understood.[134, 145]

Two studies were conducted in an attempt to provide clarity on the relationship between vitamin D status and bone mass. Both studies involved participants from preadolescence to early

adolescence, but one was cross-sectional and the other prospective. In both studies, participants were grouped in to categories of high, middle and low vitamin D status. In the 3-year prospective study, baseline serum 25(OH)D had a fair positive correlation with change in BMD at the femoral neck and lumbar spine. The adverse effects of low 25(OH)D levels were prominent in the growth spurt during puberty, thus confirming the pre-established notion that poor vitamin D status adversely affects bone mass during puberty without identifying a clear relationship between the two variables.[145] For the cross-sectional study, greater forearm BMD was observed in the girls categorized as high vitamin D status compared to low vitamin D status but no difference was observed for forearm nor heel BMD for boys. This inconclusiveness impeded a definitive conclusion and no consensus was made on the optimal serum 25(OH)D level for healthy bone mass.[146]

Although only serum 25(OH)D levels below 20 ng/ml may be associated with clinical signs of poor bone health, the serum levels required to achieve PBM may be greater in growing children. Children with limited access to sunshine in particular have been identified as at risk of vitamin D deficiency and those with greater pigmentation and thus a reduced ability to synthesize vitamin D cutaneously should be considered for added requirements of the micronutrient.[147] Chapuy *et al*.[148] found that serum 25(OH)D levels below 30 ng/ml had increased iPTH activity in healthy adults which affects bone mass negatively. Heaney[149] reviewed the literature and noted that calcium absorption, vital for bone health maintenance, does not depend on vitamin D alone. Calcium absorption is regulated by $1,25(OH)_2D$ which is regulated by iPTH. Heaney[149] pointed out that at 25(OH)D levels of 30 ng/ml, calcium absorption plateaus and at levels below this, the supply of 25(OH)D limits the rate at which calcium is absorbed which may affect the ability to reach the maximum genetically obtainable PBM. While the debate continues, the general consensus is in favour of the Endocrine Society guidelines and for preadolescent children, for whom vitamin D deficiency is a higher risk, the minimum of 30 ng/ml is less risk in ensuring requirements are met to obtain adequate PBM.[10, 108]

2.10. THE RELATIONSHIP BETWEEN BONE HEALTH AND BODY COMPOSITION IN PREADOLESCENT CHILDREN

Excessive fat mass has shown to have a similar effect to increased muscle force as the application of this additional body weight to the bone, stimulates bone cells and bone mineralization is increased. The bones of obese children are believed to be greater in mass and size due to this phenomenon. Several studies have agreed with this phenomenon, however the positive relationship between bone mass and fat mass has not been confirmed as the results of studies contradict. While some have shown that obese/overweight children have a greater bone mass[6, 8, 16] or no significant difference between the bone mass of obese/overweight and normal-weight children[6, 9], when adjusted for body weight, fat mass and lean mass, the results of other

studies show low bone mass and area for weight.[7, 150] Where greater bone mass has been observed in obese/overweight children, adiposity is believed to have a significant effect on the bone mass due to the increase in anabolic agents such as oestrogen, adiponectin or insulin linked to increased adipose tissue.[151] The mechanical loads exerted by fat mass are however static, unlike lean mass which is dynamic due to the continual contraction and relaxation of the muscles. Fat mass is thus believed to be less effective in stimulating bone cell differentiation from mechanical sensing and is the rationale for an observed low bone mass for weight in over-nourished children.[152, 153]

While the relationship of body composition and bone mass remains uncertain, Goulding *et al*.[154] undertook to identify the potential risk factors for bone fractures present in children with repeated fractures at the forearm. Goulding *et al*.[154] identified that compared to age and gender-matched fracture-free children, children with repeated forearm fractures had a significantly greater adiposity and body weight confirming that over-nourished children have a higher risk of bone fractures. This may be due to inadequate adaptation of the skeleton for body weight due to the static load of fat mass or due to the greater force at which a child that is over-nourished falls.[154]

Attempts at finding a definitive relationship between FM and bone health have included observing the distribution of fat and bone mass. Some studies have shown that abdominal fat in and bone mass are strongly related and that while increased abdominal fat is associated with metabolic disease, it may prove to protect against osteoporosis.[155] Other studies have shown that in preadolescent children, increased abdominal fat is inversely related to bone mass.[156] When separated in to visceral fat and subcutaneous fat however, visceral fat has been found to be the contributing factor to an increased BMD. This has been postulated to be due to the secretion of BMD protectants, leptin and aromatase.[157]

Body mass index is interpreted as weight-for-height and does not differentiate between lean mass (LM) and fat mass (FM). Body mass index status is classified by BMI-for-age Z-score which indicates whether individuals are under-nourished, normal or over-nourished compared to children of the same age and sex. It may increase or decrease due to either of the body composition compartments. Thus, BMI is not a true reflection of body fatness, body fat percentage is a measure of fatness and is calculated as the percentage that FM contributes to the total body weight. While some studies have shown a positive relationship between body fat percentage and other have shown a negative or no association.[157-159] These inconclusive findings may be attributed to the use of different population groups studied. Additionally, body fat percentage does not reflect the load bearing on the bones as it does not adjust for the body size. While two children with equal body fat percentages may have different FM contributions to a different total body weight which would lead to variations in bone mass measurements even though body fat percentage was equal (Figure 6). Contrarily, two children with different body fat percentages may

have different FM contributions but equal total body weight and still bone mass measurements may vary due to variances in LM contributions.[160]

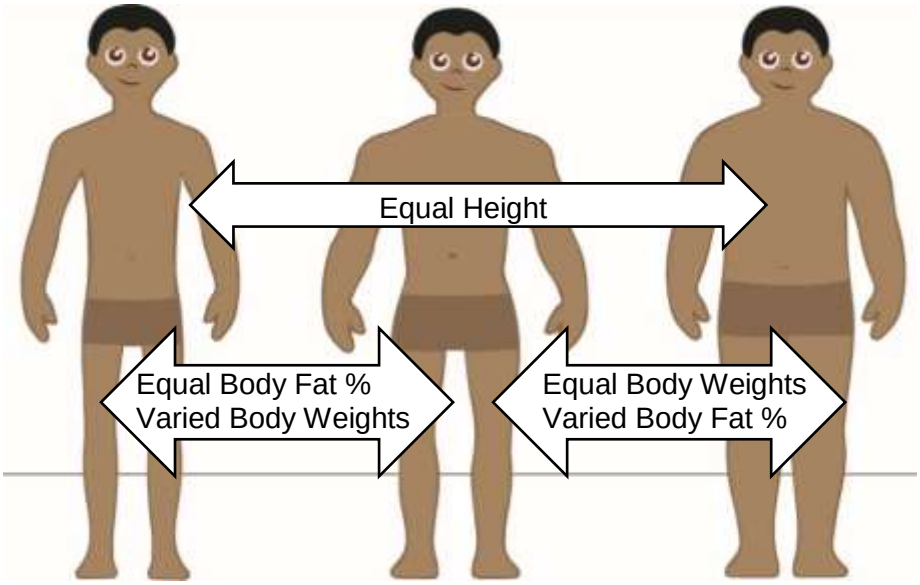


Figure 6. Children of equal body fatness may vary in BMI or with equal BMI may vary in body fatness

2.11. CONCLUSION

Bone mineral accrual and maintenance is important in children to reduce the risk for osteoporosis and prevent the irreparable bone disease later in life. Vitamin D and body composition both play important roles in bone health. Poor bone health has been linked to poor vitamin D status but the relationship between outcome measures of vitamin D and bone health has not been confirmed. Black South African children are at risk of over- and under-nutrition, but their vitamin D status remains unknown. Body fat percentage in black South African children is rising and the relationship between adiposity and bone mass in children has been debated. The implications of the concurrence of under- and overnutrition on bone health has not yet been determined while nutritional bone diseases continue to be of public health concern in South Africa. There is a need for bone health and its relationship to vitamin D and body composition in South African preadolescent school children to be clarified.

CHAPTER 3: METHODOLOGY

Dr Zelda White was responsible for the study concept and design, and overall management of the study. Amanda Jansen van Rensburg coordinated and managed all data collection procedures. Samantha White, Amanda Jansen van Rensburg, and Dr Zelda White collected data. Samantha White analysed the data with the help of Prof Marlena Kruger and Prof Piet Becker. Prof Piet Becker assisted in interpreting the data using statistical analysis. Samantha White wrote this dissertation overseen by Dr Zelda White and Prof Marlena Kruger.

3.1. STUDY DESIGN

For this project, data was collected by means of an analytical, observational cross-sectional design to observe the relationship of bone health to vitamin D and body composition in preadolescent children in Pretoria, South Africa.

3.2. STUDY SETTING

The data collection took place from the end of September to mid-November at Netcare Femina Hospital, and at the aftercare facilities on the premises of Arcadia Primary School and at Little Tubbies day care situated in Arcadia, Pretoria.

3.3. STUDY POPULATION AND SAMPLING

The target population for this study included primary school children attending aftercare facilities on the premises of Arcadia Primary School and at Little Tubbies day care. The little Tubbies aftercare facility is independently owned and Arcadia Primary School aftercare is managed by the governing body of the primary school. The study population was selected based on the following criteria:

3.3.1. Inclusion Criteria

- Boys and girls
- Children in grades R-4 that are 10 years old and younger were included in the analysis of the data obtained

3.3.2. Exclusion Criteria

- Children with moderate or severe physical disabilities (e.g. amputees)
- Children with electrical and metallic implants (e.g. Pacemakers)

3.3.3. Sampling method

For this study, non-random, convenience sampling was used as the sampling method. Children attending two aftercare facilities in close vicinity to the Prinshof campus of the University of Pretoria were recruited for participation. Children who were willing to participate, and met the inclusion and exclusion criteria, and those that received consent from their parents, were included in the study.

3.3.4. Sample size

Of the 91 participants that were recruited for the study, two did not participate in the data collection due to lack of attendance. One child had incomplete data collection and five children that were not of black race/ethnicity were excluded from the analysis of the data. None of the exclusion criteria were applied in this study. Therefore, 84 children (44 girls, 40 boys) participated in the bone health assessment and 59 children (32 girls, 27 boys) participated in the vitamin D assessment.

3.4. DATA COLLECTION AND MANAGEMENT

Two weeks prior to the study, the aim of the study and data collection process was explained to the children attending the after-care facilities with use of visual aids and illustrations. Letters and consent forms for the parents to read and complete were handed to children on two non-consecutive days (two weeks apart) to ensure that all children had the opportunity to take part in the study. Parents and guardians were then provided with a detailed description of the study along with demographic data forms to be completed (Appendix A). Date of birth, race/ethnicity, gender, medication/supplement use were provided by the parents and guardians.

The data collection of the dual x-ray absorptiometry (DXA) scan and the anthropometric measurements took place at Netcare Femina Hospital, thereafter Vitamin D analysis was done at the aftercare facilities on the premises of Arcadia Primary School and at Little Tubbies day care. All data collection was done by trained personnel or researchers. All children were given a standard drinking yoghurt 30 minutes prior to DXA and BIA assessments and were not allowed to eat or drink until all their data was collected (approximately 30-60 minutes). They were requested to void their bladders before measurements. Children of self-reported black ethnicity/race were included in the analysis of the data obtained.

3.4.1. Anthropometric measurements

Anthropometric measurements were performed by Amanda Jansen van Rensburg, a registered dietitian. Standing height was measured using standardised procedures.[161] Standing height was measured (to the nearest 0.1cm) using the seca 274 wireless stadiometer. Each child was positioned to stand upright, with their heels, buttocks, scapula and back of the head against the vertical surface of the stadiometer. Jerseys, jackets, shoes and socks had been removed for height measurements.

For the measurement of body weight, the children wore minimal clothing. Jerseys, jackets, shoes and socks were removed. The children stood upright with weight distributed evenly on both feet. Weight was recorded to the nearest 100g, using the seca mBCA. Weight measured by the seca mBCA was used in making comparisons by population characteristics. Body weight was also estimated by the DXA (dual X-ray absorptiometry) on a Hologic Discovery W densitometer (Hologic, Madison WI, USA), and this estimation was used in adjusting bone measurements

BMI-for-age was expressed as Z-scores which were calculated by the seca mBCA software applying the WHO Reference for ages 5-19 years as a measure of weight-for-age to categorise children as healthy or over-nourished (overweight and/or obese).[162] Children with BMI-for-age Z-scores > 1 were classified as “over-nourished” and children with BMI-for-age Z-scores ≤ 1 were classified as “healthy”, and none of the children in this study had BMI-for-age Z-scores indicating that they were under-nourished.

3.4.2. Bone Health

Bone mineral density (BMD), bone mineral content (BMC) and bone area were measured by the DXA (dual X-ray absorptiometry) performed by a trained radiographer on a Hologic Discovery W densitometer (Hologic, Madison WI, USA). The Hologic system has continuous calibration provided by the system’s patented Automatic Internal Reference System.[163] The use of DXA in children has been proven to be a suitable method due to its minimal radiation exposure.[164] Dual X-ray absorptiometry systems expose subjects to a small dose of radiation. This dose can be quantified to the amount of energy that will be absorbed by one kilogram of body tissue. The effective dose is measured in Sievert (Sv). This measurement takes the type of radiation as well as the amount of energy absorbed by the tissue into account. On a daily basis, humans are exposed to natural sources of radiation from radioactive substances in food, soil, water and air as well as cosmic rays. The average annual natural radiation dose is approximately 2400µSv. One of the benefits of the Hologic Discovery W densitometer is its very low dose of radiation of less than 10µSv. The total dose of radiation to which the children were exposed to is 3µSv, which is much lower than the range normally used in medical diagnostics. In comparison to the natural

sources of radiation, the doses from DXA are very low.[29] Children were provided with a clinic gown to wear for the duration of the scan to ensure that nothing (E.g. zips or buckles) interfered with the imaging and provide false results.

The total body less head (TBLH) and lumbar spine (LS) bone mass parameters are commonly used in reporting of paediatric data due to the highly reproducible nature of these parameters. BMC has also been considered an ideal method of assessing bone status as areal density-related errors are omitted.[165]

Bone mineral density is lower during the growth stages as the growing child has a rapid increase in bone area during this time due to increased bone modelling. Bone modelling is genetically predetermined, and the height velocity is variable between children. Thus, BMD in preadolescent children gives a false interpretation of bone mass.[166] Additionally, BMD is not measured by DXA, but is rather calculated from BMC and bone area measured by DXA, providing a 2-dimensional interpretation (g/cm^2) from the areal measurement. Density is a volumetric parameter and aBMD (g/cm^2) is thus a flawed interpretation of bone density.[95]

Raw mean BMC was expressed in grams and BMD as grams/centimeter^2. The current method of reporting TBLH-BMD DXA results in children is as a Z-score which is adjusted for height-for-age to override areal measurement flaws whereas LS-BMD is recommended to be converted to BMAD (bone mineral apparent density) which is a volumetric adjustment to aBMD. Bone mineral apparent density (g/cm^3) at the LS and TBLH reflect vBMD for greater accuracy by eliminating aBMD errors.[95]
Bone mineral apparent density was calculated using the following equations:[95, 167]

$$LS - BMAD = \frac{BMC}{Bone\ Area^{1.5}}$$

$$TBLH - BMAD = \frac{(BMC)}{(\frac{Bone\ Area^2}{height})}$$

For TBLH bone mass, the Z-score is recommended to be adjusted by the height-for-age Z-score also to prevent size-related errors. Dual X-ray absorptiometry measured TBLH-BMD and TBLH-BMC were converted to Z-scores to provide an estimated standard deviation of the bone health from the population mean for chronological age.[165] Z-scores were standardly reported using BMD making them comparable to other studies, but BMC Z-scores were included as BMD is highly variable in the growing child. When not adjusted for height, the Z-score could lead to a misinterpretation of the results. For example, a person with a short stature for age, may have a lower Z-score due to the lower bone mass observed in smaller bones. The official paediatric

position of the International Society for Densitometry (ISCD) states that Z-score values below -2 standard deviations from the mean should be construed as low bone mass for chronological age.[168] It has been suggested that when body fat percentage is available, it should be considered in the calculation of bone mass Z-scores as this has been found to impact bone mass considerably.[93] Because the DXA software database did not include children below the age of 8 years old and thus could not calculated Z-scores for some of the sample population, the entire population's Z-scores were calculated manually.[94]

Z-scores without height adjustments were calculated and adjusted for age, gender and ethnicity:[94]

$$\boldsymbol{A}: Bone\ mass\ Z-score = \frac{\left(\frac{X}{M}\right)^{L}-1}{LS}$$

Where variables specific to age, gender and ethnicity created using LMS curves by Kalkwarf *et al*.[169] include:

- X = DXA BMD/BMC measurement
- L = Power in the Box-Cox transformation
- M = Median (given as a function of different age percentiles)
- S = Standard Deviation

The importance of stature was considered and the bone mass Z-score ($\boldsymbol{A}$) was then adjusted using the height-for-age Z-score (HAZ). Height-for-age Z-scores were provided by the seca mBCA based on the WHO Reference for ages 5-19 years, and applied in a bone mass prediction equation. The height-adjusted bone mass prediction equation was determined by Zemel *et al*.[94] using revised reference curves:

$$\boldsymbol{B}: HAZ\ bone\ mass\ prediction\ equation = \ intercept + (HAZ\ x\ \beta)$$

$$\boldsymbol{C}: HAZ\ adjusted\ Z-score = \ Bone\ mass\ Z\ (\boldsymbol{A}) - HAZ\ predicted\ bone\ mass(\boldsymbol{B})$$

Where:

- L; M; S; intercept and β = For specific age, ethnicity and gender (Appendix B) obtained from Zemel *et al*.[94]
- HAZ = Height-for-age Z-score

3.4.3. Vitamin D status

Vitamin D status was measured as concentrations of serum 25-hydroxyvitamin D_2 ($25(OH)D_2$) and 25-hydroxyvitamin D_3 ($25(OH)D_3$). The $25(OH)D_3$ and $25(OH)D_2$ levels were measured by collecting a drop of blood from the finger onto the blood spot cards (Figure 7) using the OneTouch® lancing device (LifeScan Inc, USA). This was done by two trained researchers including myself, Samantha White, and Dr Zelda White. The spot cards were dried, sealed, labelled with the participant number and sent to ZRT laboratories (Beaverton, Oregon, USA) for analysis. At ZRT laboratories, a 3mm disk (containing 3uL whole blood) was punched from each of the dried blood spots into glass tubes. The $25(OH)D_2$ and $25(OH)D_3$ concentrations were determined according to the methodology of Newman *et al*.[170] by ZRT laboratories (Beaverton, OR, USA)[11]. In short, the spots were reconstituted in 600uL distilled water, 600uL of methanol containing internal standard (D4-25-hydroxyvitamin D) was added and the samples were vortexed

and centrifuged. 900uL of the supernatant was then extracted with C18 solid phase extraction. The extracted samples were derivatised with 200uL of 0.1mg/mL PTAD (4-phenyl-1,2,4-triazoline-3,5-dione) blown to dryness with nitrogen and reconstituted with 50uL of methanol and 20uL injected into the LC-MS/MS system (Applied Biosystems, USA). The level of detection using this method is 1nmol/L. The results were captured in ng/ml.

Serum 25(OH)D2 and 25(OH)D3 were combined and expressed as total 25(OH)D in ng/ml. In accordance with the views of multiple experts, 25(OH)D levels ≤ 20 ng/ml (n = 4) was construed as vitamin D deficient and ≥ 30 ng/ml (n = 20) as vitamin D sufficient. Vitamin D insufficiency was defined by 25(OH)D levels between 21 and 29 ng/ml (n = 35). With concern to bone health, there is general consensus in agreement with the vitamin D classification described by the Endocrine Society due to the fact that PTH is elevated at serum 25(OH)D concentrations below 20 ng/ml.[12,13,108]

Figure 7. Blood spot collection procedure and collection card.[171]

3.4.4. Body Composition

Body weight, lean mass and fat mass were measured by DXA scans performed by a trained radiographer on a Hologic Discovery W densitometer (Hologic, Madison WI, USA). Body composition factors were expressed in kilograms. Body fat percentage was a body composition factor calculated as the fat mass per the total body weight and expressed as a percentage.

3.5. STATISTICAL ANALYSES

Seven participants were excluded from the DXA scan data analysis process, and three from the vitamin D data analysis process due to absenteeism and to retain homogeneity in the ethnicity of the sample. Data was analysed using STATA version 14 software. Population descriptive characteristics were expressed as means with standard deviations. To compare the mean

descriptive characteristics and the calculated BMD and BMC Z-scores between healthy and over-nourished children categorised by BMI-for-age Z-scores, the student's two sample t-test was used. To compare the mean descriptive characteristics and the calculated BMD and BMC Z-scores of the children categorised by vitamin D status one-way ANOVA was applied. The mean differences were evaluated at the 5% significance level ($p \leq 0.05$). Tukey's post-hoc tests were applied where significant differences using one-way ANOVA were found. Normal distribution was tested for using the Shapiro-Wilk normality test.

Inferential statistics in the form of simple linear regression models were used in studying the relationship between bone health parameters and vitamin D or body composition components. Adjustments of raw bone health parameters for height, gender, age and body composition factors was done using multiple linear regression. Age as well as age^2 were included in the regression models because of the non-linear relationship between bone health parameters and age. To adjust for body weight using multiple linear regression models, the body weight used was that which was estimated by DXA from the measured bone mass, lean mass and fat mass. Comparisons between adjusted bone health parameters of healthy and over-nourished were made using the student's two sample t-test.

3.6. ETHICAL AND LEGAL CONSIDERATIONS

This study was reviewed and ethically approved by the Faculty of Health Sciences Research Ethics Committee at the University of Pretoria: Application 73/2016 (Appendix C). Parents were informed of the proposed study (Appendix A) and signed consent forms (Appendix D) as well as assent forms signed by the participating children (Appendix E) were received before the start of data collection. Transport indemnity for transporting of the children to the Netcare Femina hospital for data collection were signed by each participant's parent (Appendix F) and an approval letter for data collection at the aftercare facilities was received from Arcadia Primary School and Little Tubbies (Appendix G). Participation in the research study was voluntary and the confidentiality of each participant was maintained using an identification code assigned to each child. Funding for this study was provided by the Institute of Food, Nutrition and Well-being at the University of Pretoria, South Africa.

CHAPTER 4: RESULTS

The aim of this study was firstly, to describe the bone health, vitamin D status and body composition of preadolescent children in Pretoria, South Africa. Secondly, the study aimed to provide insight in to the relationship between bone health and vitamin D status, and between bone health and body composition. The demographic and anthropometric characteristics are outlined for the whole study population and for the boys and girls separately to describe the population. Bone health, vitamin D status and body composition characteristics are described for the total population and also separately for the two study groups: body composition study group (n=84) and the vitamin D study group (n=59). The relationship between bone health and vitamin D status is observed. The relationship between bone health and body composition is addressed and adjustments are also made to the bone health parameters to compare these parameters between BMI status categories.

4.1. DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

All participants were conveniently sampled black children between 5 and 10 years of age. The age (mean (SD)) of the preadolescents was 8.6 years (± 1.4). Of the 91 participants recruited for the study, 84 (44 girls, 40 boys) met the inclusion criteria and their data measurements collected were statistically analysed (Figure 8). Of the 91 recruited, two children were absent at data collection. Five children that underwent DXA scans and three that had finger pricks for vitamin D analysis did not report themselves to be black race/ethnicity. These children were thus not included in the data analysis (Figure 8). Fifty-nine (70%) of the 84 children assessed provided consent and assent to be included in the vitamin D analysis of which 31 were girls and 28 were boys (Figure 8).

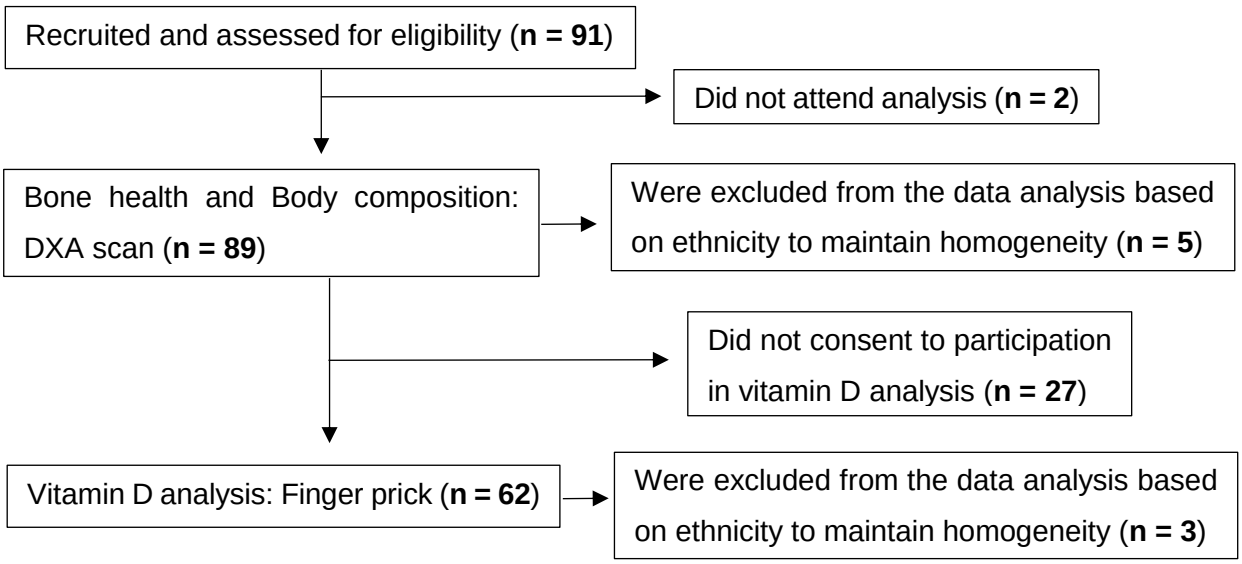


Figure 8. Sample size flow diagram

4.2. ANTHROPOMETRIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

Anthropometric characteristics of the children of this study population are summarized in Table 7 and expressed as mean(SD). Standing height and height-for-age Z-scores were provided by the Seca software for all children in the study participants. All 84 children had a height-for-age Z-score above – 2 which is indicative of a normal height-for-age and no stunting was observed in this study population. The mean height-for-age Z-score was 0.37 (± 0.94) for the 84 children. BMI-for-age Z-score were also provided by the Seca software for interpretation of BMI status. The mean BMI-for-age for the study population was 1.02 (1.63) which is above one and thus indicates over-nourishment for the total population. Of these 84 children assessed using the DXA, 60% have a BMI in the healthy range with a Z-score ≤ 1 and 40% are over-nourished (Z-score > 1).

Table 7. Anthropometric characteristics of the study population.

Anthropometric Characteristics	**Mean (SD)**			*p*
	Total Population (n = 84)	Boys (n = 40)	Girls (n = 44)	
Height (m)	1.32 (0.10)	1.32 (0.10)	1.32 (0.09)	0.94
Height-for-age (Z-score)	0.37 (0.94)	0.34 (0.95)	0.40 (0.95)	0.76
Weight (kg)	33.5 (10.9)	33.4 (11.1)	33.7 (11.0)	0.91
BMI (kg/m^2)	18.9 (4.7)	18.9 (4.9)	19.01 (4.6)	0.88
BMI-for-age (Z-score)	1.02 (1.63)	1.05 (1.82)	0.99 (1.47)	0.87

4.3. BODY COMPOSITION OF THE STUDY PARTICIPANTS

The total body weight reported in Table 7, for all the participants included in the study was 33.5 kg (± 11.0). The mean body weight of the over-nourished (high BMI-for-age) group is significantly greater than the healthy group ($p < 0.05$) (not tabulated). The mean LM and FM for this study population is 21.1 (4.7) kg and 12.6 (7.3) kg respectively. Body fat percentage for this study population is 34.0 (9.8) %. Body fat percentage, LM and FM body compositional components are all higher in the over-nourished children as compared to the children in the healthy group. Fat mass of children in the over-nourished group however is considerably higher than the FM of the children in the healthy group (Figure 9). The mean FM of the over-nourished children is 18.1 kg (6.7) and the mean FM of the healthy children is 7.3 kg (2.3). The mean LM of children in the over-nourished group is 22.9 kg (4.4), 3.9 kg more than the mean LM of the children in the healthy

group.

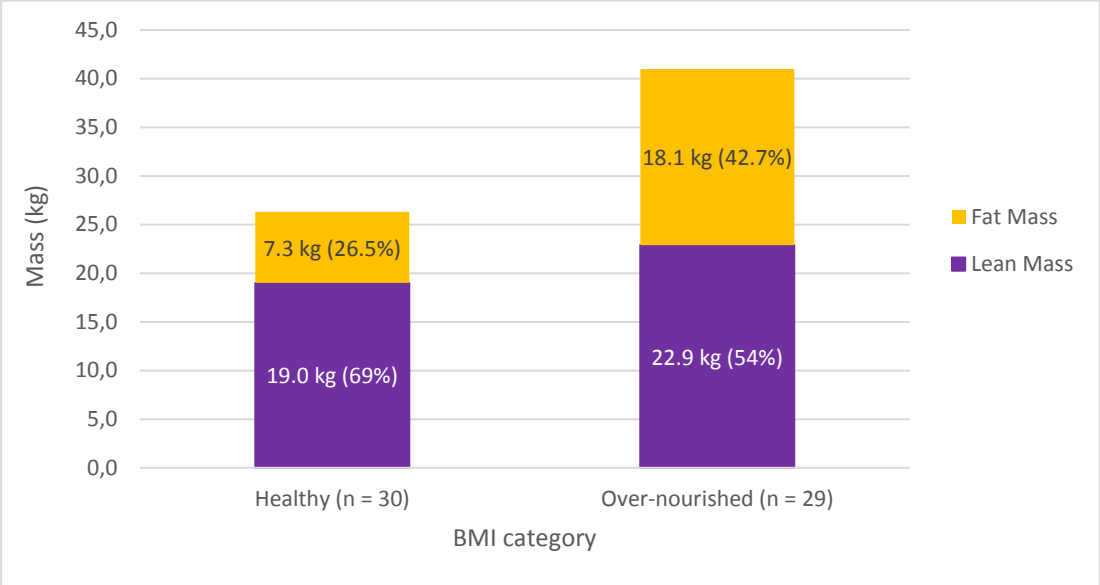


Figure 9. Mean lean mass and fat mass in children grouped according to BMI status.

4.4. VITAMIN D STATUS OF THE STUDY PARTICIPANTS

The total population of children assessed by blood spot analysis had mean serum 25(OH)D concentrations of 27.3 ng/ml (± 5.3), The serum 25(OH)D concentrations differ significantly between the children of varying vitamin D statuses ($p < 0.05$) but does not differ significantly between the over-nourished and healthy children (Table 8). A Tukey post-hoc test revealed that serum 25(OH)D concentrations are statistically significantly higher in the sufficient group compared to the insufficient ($p < 0.001$) and deficient ($p < 0.001$) groups. The insufficient group also has statistically significantly higher serum 25(OH)D compared to the deficient group ($p = 0.001$).

Table 8. Mean serum 25(OH)D concentrations in children categorized by vitamin D and BMI status.

<table>
<tr><th></th><th colspan="2">Vitamin D status categories</th><th colspan="2">BMI status categories</th></tr>
<tr><td rowspan="4">Mean (SD) serum 25(OH)D (ng/ml)</td><td rowspan="2">Sufficient (n = 20)</td><td rowspan="2">33.4 (3.1)</td><td rowspan="2">Healthy (n = 30)</td><td rowspan="2">27.4 (5.6)</td></tr>
<tr></tr>
<tr><td rowspan="2">Insufficient (n = 35)</td><td rowspan="2">24.7 (2.5)</td><td rowspan="2">Over-nourished (n = 29)</td><td rowspan="2">27.2 (4.9)</td></tr>
<tr></tr>
<tr><td>Deficient (n = 4)</td><td>19.5 (1.0)</td><td></td><td></td></tr>
<tr><td>p</td><td colspan="2">< 0.001</td><td colspan="2">0.89</td></tr>
</table>

4.5. BONE HEALTH OF THE STUDY PARTICIPANTS

Raw bone health parameters include BMC, BMD and bone area measured directly with the DXA machine. Bone health status is the interpretation of bone mass for chronological age from bone mass Z-scores. Z-scores outline the status of bone health as they are used for comparison to a healthy age-matched reference group

4.5.1. Raw bone health parameter

One-way ANOVA was conducted to determine if bone health parameters at the LS and TBLH sites differed between the vitamin D status categories. There are no significant differences in bone health parameters between children with vitamin deficiency, insufficiency or sufficiency (Table 9). The BMADs at the LS site are significantly different between the children of varying vitamin D statuses ($p < 0.05$) but at the TBLH, no significant difference is observed ($p > 0.05$). From a Tukey's post-hoc test it was determined that the insufficient group has a significantly higher LS-BMAD than the deficient group ($p = 0.003$) but is not significantly higher than the sufficient group's LS-BMAD ($p = 0.098$). The vitamin D deficient and sufficient groups do not differ significantly in terms of LS-BMAD ($p = 0.924$).

Using the student's two-sample t-test, bone health parameters, namely BMC, BMD and bone area were considered statistically significant at $p < 0.05$. Statistically significant differences were found in LS-BMD, TBLH-BMC and TBLH-BMD raw values with over-nourished children having greater raw bone health values than healthy children in all three measures (Table 9). The BMAD at the LS and TBLH were calculated to reflect vBMD for better interpretation of the raw BMD measured by DXA. BMAD values reflect whether the significantly greater measures of BMD are due to size related differences although bone area between healthy and over-nourished children did not differ significantly. Total body less head-BMAD, like with the raw BMD values, is significantly greater in over-nourished children than in healthy children ($p < 0.001$). Over-nourished children also have a significantly greater LS-BMAD compared to healthy children ($p < 0.001$).

4.5.2. Bone health status

The TBLH DXA results were converted in to Z-scores and adjusted for height using height Z-scores to provide an estimated standard deviation of the child's bone health from the population mean for chronological age, summarised in Table 10. Although the raw bone health data provides means for comparison between categories, this does not indicate bone health status. Factors including age, race, gender and height are considered in the calculation to ensure an accurate interpretation of the Z-score. A person with a short stature for age, may have a lower Z-score due to the lower bone mass observed in smaller bones which is why inclusion of height-for-age Z-scores has been advised.

Table 9. Bone health characteristics of participants categorized by vitamin D status and BMI status.

Bone Health Characteristics	Vitamin D status categories				*p*	BMI status categories			*p*
	Mean (SD)					Mean (SD)			
	Total Population (n = 59)	Sufficient (n = 20)	Insufficient (n = 35)	Deficient (n = 4)		Total Population (n = 84)	Healthy (n = 50)	Over-nourished (n = 34)	
LS-BMC (g)	18.5 (4.0)	18.7 (3.5)	18.4 (4.4)	17.5 (3.4)	0.86	17.8 (4.1)	17.2 (3.9)	18.8 (4.2)	0.06
LS-BMD (g/cm^2)	0.686 (0.080)	0.665 (0.077)	0.704 (0.077)	0.640 (0.080)	0.10	0.674 (0.082)	0.647 (0.083)	0.712 (0.066)	< 0.001
LS-BMAD (g/cm^3)	0.133 (0.016)	0.126 (0.013)^{ab}	0.139 (0.016)^{a}	0.123 (0.013)^{b}	0.0033	0.132 (0.016)	0.127 (0.016)	0.140 (0.013)	< 0.001
LS-area (cm^2)	26.8 (3.9)	28.0 (3.1)	26.0 (4.2)	27.3 (3.0)	0.18	26.3 (4.1)	26.4 (4.1)	26.3 (4.1)	0.91
TBLH-BMC (g)	657.0 (153.9)	651.3 (148.3)	664.0 (160.9)	623.4 (150.5)	0.87	646.1 (157.5)	607.4 (152.0)	702.9 (149.8)	< 0.05
TBLH-BMD (g/cm^2)	0.637 (0.076)	0.631 (0.075)	0.643 (0.077)	0.612 (0.076)	0.68	0.629 (0.079)	0.602 (0.080)	0.667 (0.060)	< 0.001
TBLH-BMAD (g/cm^3)	0.083 (0.008)	0.082 (0.008)	0.084 (0.008)	0.078 (0.005)	0.3533	0.082 (0.008)	0.079 (0.009)	0.086 (0.006)	< 0.001
TBLH-area (cm^2)	1019.9 (127.6)	1021.0 (120.5)	1020.9 (134.0)	1066.1 (137.6)	0.98	1014.3 (128.2)	993.9 (119.8)	1044.3 (135.9)	0.08

* LS = lumbar spine; TBLH = total body less head; BMC = bone mineral content; BMD = bone mineral density; BMAD = bone mineral apparent density

A Z-score value below -2 standard deviations from the mean is construed as low bone mass for chronological age. Total body bone mass parameters are reported excluding the head as the skull is not as responsive to external loads like from body composition impacting accuracy of the results for this study.[95, 172]

Z-scores are reported using BMD for comparison to other studies as well as BMC for greater precision as BMD is highly variable in the growing child. The 59 children in the vitamin D study groups had a 24% prevalence of low BMD for chronological age and 7% presented with a BMC Z-score below -2. Of the 84 children in the body composition study group, 25% had a BMD Z-score below -2 indicating low bone mass density for chronological age and 7% percent of children presented with low BMC-for-age (not tabulated).

In this study population, vitamin D sufficient and vitamin D deficient girls have low BMD for chronological age (Table 10). All other categories have mean TBLH-BMD Z-scores above -2 and thus have healthy TBLH-BMD for age. All mean TBLH-BMC Z-scores in all categories are above -2 and are thus considered as adequate bone health status (Table 10).

Table 10. Mean TBLH-BMC and TBLH-BMD Z-scores adjusted for height, race, gender and age.

		TBLH-BMC Z-score	**TBLH-BMD Z-score**
GIRLS			
BMI status categories (n = 44)	Healthy (n = 25)	- 0.746	- 1.995
	Over-nourished (n = 19)	- 0.392	- 1.117
Vitamin D status categories (n = 31)	Vitamin D Sufficient (n = 9)	- 0.746	- 2.055
	Vitamin D Insufficient (n = 20)	- 0.479	- 1.376
	Vitamin D Deficient (n = 2)	- 1.088	- 2.182
BOYS			
BMI status categories (n = 40)	Healthy (n = 25)	- 1.436	- 1.830
	Over-nourished (n = 15)	- 0.902	- 0.880
Vitamin D status categories (n = 28)	Vitamin D Sufficient (n = 11)	- 1.113	- 1.320
	Vitamin D Insufficient (n = 15)	- 1.260	- 1.259
	Vitamin D Deficient (n = 2)	- 0.747	- 1.052

4.6. RELATIONSHIP BETWEEN BONE HEALTH AND BODY COMPOSITION

The relationship between bone health and body composition is addressed by observing the direct relationship between the bone health parameters and body weight, FM, LM and body fat percentage. It is also studied by adjusting and comparing bone health parameters between healthy and over-nourished children.

4.6.1. Relationship between bone health parameters and body compositional components

Linear regression models of individual bone parameters against total body weight, and body compositional components including, lean mass (LM), fat mass (FM) and body fat percentage were used to examine the relationship between bone health and body composition. Lean mass is strongly associated with TBLH bone mass parameters and weakly associated with LS bone mass parameters (Table 11). Lean mass has a strong and statistically significant positive association with TBLH bone parameters and a weak positive association with LS bone parameters ($p < 0.05$). It explains 81%, 79% and 71% of the variations observed in TBLH-BMD, TBLH-BMC, and TBLH-area respectively. Thirty two percent of the variation in LS-BMC and LS-BMD, and 16% of the variation in LS-area is explained by LM. While a 1 kg increase in total body weight may result in 11.25 g ($\beta_1 = 11.25$) of TBLH-BMC gained, as LM increases by 1 kg, TBLH-BMC increases by 29.83 g ($\beta_1 = 29.83$). Bone measure increases per increase in one unit of the dependent variable, as indicated by β_1, are the largest when age is the dependent variable, followed by LM.

An increase of FM by 1 kg, results in a 13.11 g increase in TBLH-BMC. FM has a weak but positive association with all bone mass parameters for TBLH and LS, and the association with FM LS-area, unlike all other bone health parameters, is not statistically significant ($p = 0.53$). FM explains 34%, 11%, 37%, 27% and 26% of the variation in TBLH-BMC, LS-BMC, TBLH-BMD, LS-BMD and TBLH-area respectively.

Body fat percentage is very weakly associated with TBLH-BMC, TBLH-BMD, LS-BMD and TBLH-area, explaining 9%, 12%, 15% and 5% of the variation in these bone health parameters respectively. Body fat percentage does not describe variability of LS-BMC ($p = 0.162$), nor LS-area ($p = 0.44$) due to their non-significance.

Due to the importance of height and age in bone development and the roles they play on bone measures, they were also regressed individually (not displayed in table). The coefficient of determination (R^2), indicates that height is a strong significant determinant of TBLH bone mass parameters. In this population, it explains 79%, 75% and 74% of the variability in TBLH-BMC, TBLH-BMD and TBLH-area respectively ($p < 0.05$).

Table 11. Association between bone health parameters and body composition.

	Body weight (kg)	**Lean mass (kg)**	**Fat mass (kg)**	**Body Fat (%)**
TBLH-BMC (g)				
β_1 (SE)	11.25 (1.04)	29.83 (1.58)	13.11 (2.01)	4.76 (1.72)
p	< 0.001	< 0.001	< 0.001	0.007
R^2	0.59	0.81	0.34	0.09
LS-BMC (g)				
β_1 (SE)	0.18 (0.04)	0.48 (0.08)	0.19 (0.06)	0.06 (0.05)
p	< 0.001	< 0.001	0.002	0.162
R^2	0.22	0.32	0.11	0.02
TBLH-BMD (g/cm^2)				
β_1 (SE)	0.006 (0.0005)	0.014 (0.0008)	0.007 (0.001)	0.003 (0.0008)
p	< 0.001	< 0.001	< 0.001	0.001
R^2	0.59	0.79	0.37	0.12
LS-BMD (g/cm^2)				
β_1 (SE)	0.004 (0.0007)	0.01 (0.002)	0.006 (0.001)	0.003 (0.0009)
p	< 0.001	< 0.001	< 0.001	< 0.001
R^2	0.33	0.32	0.27	0.15
TBLH-Area (cm^2)				
β_1 (SE)	8.35 (0.94)	22.68 (1.60)	9.31 (1.74)	2.91 (1.43)
p	< 0.001	< 0.001	< 0.001	0.045
R^2	0.49	0.71	0.26	0.05
LS-Area (cm^2)				
β_1 (SE)	0.09 (0.04)	0.34 (0.09)	0.04 (0.06)	-0.04 (-0.05)
p	0.035	< 0.001	0.53	0.44
R^2	0.05	0.16	0.005	0.007

* LS = lumbar spine; TBLH = total body less head; BMC = bone mineral content; BMD = bone mineral density

Height has a fair and statistically significant association with LS bone mass parameters as it explains 44%, 34% and 29% of the variability in LS-BMC, LS-BMD and LS-area respectively ($p < 0.05$). Age in this study population, is also positively associated with bone parameters however the association is not strong. All associations between age and TBLH and LS bone parameters are statistically significant ($p < 0.05$). Age explains 43%, 36% and 47% of the variability in TBLH-BMC, TBLH-BMD and TBLH-area respectively. Age has a weak association at the LS site, explaining 24%, 10% and 25% of the variability in LS-BMC, LS-BMD and LS-area respectively.

4.6.2. Unadjusted and adjusted bone health parameters

Multiple linear regression models are used to adjust for body composition factors so that over-nourished and healthy children can be compared while observing the effects of body composition on the bone health of these children. Adjustments treat the body composition factor as a confounder so that the over-nourished and healthy children can be compared while taking the body weight, LM or FM into consideration. Because of the marginal or non-significant associations between body fat percentage and bone mass parameters, body fat percentage was not included in adjustments made on bone mass parameters in comparing healthy and over-nourished children. The raw BMC was adjusted for height, gender and age as well as age^2 to account for any effect this had on the BMC and ensure that the adjustments for body compositional factors were observed independently of the effects that height or age may have on bone mass parameters. Age^2 was included in the regression model due to the non-linear relationship of age and bone mass accumulation. Observing LM and FM in separate regression models and together in one single model, allowed for the independent and individual effects of LM and FM to be examined.

After adjusting for height, gender and age, the TBLH-BMC for children with healthy BMIs increases from the raw unadjusted value of 607.4 g to 625.3 g (Table 12). The TBLH-BMC for over-nourished children decrease from 702.9 g to 676.6 g. The greater TBLH-BMC of the over-nourished children remains statistically significant even after this adjustment ($p < 0.05$). After adjusting for height, gender and age, and including body weight in the model, healthy children have an adjusted TBLH-BMC of 656.0 g and 631.5 g is observed in the over-nourished children. This means that when treating body weight as a confounder, the TBLH-BMC increases for healthy and decreases for over-nourished children to a TBLH-BMC that does not have a statistically significant difference between the two groups ($p > 0.05$). This is consistent for adjustments made for LM, FM and LM and FM combined ($p > 0.05$).

After adjusting for height, gender and age, as well as body weight, BMC at the LS, like the TBLH-BMC, for children with healthy BMI increases and the LS-BMC for over-nourished children decreases. This is consistent for all body compositional adjustments made. The raw unadjusted

mean values do not have a statistically significant difference and the adjusted LS-BMC mean values do not differ between the two groups of children either ($p > 0.05$).

Table 12. Unadjusted and adjusted bone health parameter in relation to body weight, lean mass and fat mass.

Adjustment Variable	Total Body Less Head		Lumbar Spine	
	Healthy (n = 50)	Over-nourished (n = 34)	Healthy (n = 50)	Over-nourished (n = 34)
BMC (g)				
Unadjusted	607.4	702.9 *	17.2	18.8
Height, age and gender[1]	625.3	676.6 *	17.6	18.1
Body weight[1]	656.0	631.5	17.6	18.2
Lean mass[1]	645.0	647.6	17.8	17.9
Fat mass[1]	656.2	631.2	17.3	18.5
Lean mass and fat mass[1]	655.2	632.6	17.3	18.6
BMD (g/cm^2)				
Unadjusted	0.602	0.667 *	0.647	0.712 *
Height, age and gender[1]	0.612	0.653 *	0.658	0.697 *
Body weight[1]	0.621	0.640	0.663	0.690
Lean mass[1]	0.619	0.643 *	0.656	0.699 *
Fat mass[1]	0.622	0.638	0.660	0.693
Lean mass and fat mass[1]	0.622	0.638	0.660	0.694
Area (cm^2)				
Unadjusted	993.9	1044.3	26.4	26.3
Height, age and gender[1]	1007.7	1024.0	26.7	25.8
Body weight[1]	1033.9	985.5 *	26.3	26.3
Lean mass[1]	1020.7	1004.8	26.6	25.9
Fat mass[1]	1033.7	985.8 *	26.0	26.7
Lean mass and fat mass[1]	1032.8	987.0 *	26.0	26.7

[1]Adjusted for height, age and gender

* Significantly different to the bone mass parameter of the children with healthy BMI-for-age after adjusting for the same body compositional measure ($p < 0.05$)

As with the BMC adjusted values, the TBLH-BMD and LS-BMD raw unadjusted mean values increases, but stays statistically significantly greater ($p < 0.05$) in the over-nourished children after adjusting for height, age and gender (Table 12). In the healthy children, after adjusting for body weight, LM and FM, TBLH-BMD and LS-BMD increases and in the over-nourished children they decrease.

The raw unadjusted mean TBLH-BMD ($p < 0.0001$) and LS-BMD ($p = 0.007$) differs significantly between the two groups of children. This statistically significant difference remains after adjusting TBLH-BMD for LM ($p = 0.006$). When adjusted for body weight, FM and both LM and FM together there is no significant difference between the two groups of children in terms of TBLH-BMD ($p > 0.05$). The LS-BMD does not differ between the over-nourished and healthy children after all of the body compositional adjustments ($p > 0.05$) except for LM.

Bone area is a measure of bone size and the raw unadjusted TBLH-area and LS-area of the children does not differ significantly ($p > 0.05$) between the groups (Table 12). When adjusted for height, age and gender, there is still no statistically significant difference between the bone areas of the two groups of children at both measurement sites ($p > 0.05$). After adjusting for body compositional factors, the TBLH-area differs significantly between the two groups when adjusted for total body weight, FM and both LM and FM together ($p < 0.05$). TBLH-area increases in healthy children and decreases in over-nourished children after making body composition adjustments that include FM. Lumbar spine-area decreases from the raw unadjusted measurement or stays the same after adjustments in the over-nourished group.

4.7. RELATIONSHIP BETWEEN BONE HEALTH AND VITAMIN D STATUS

Linear regression models revealed that there is no association found between all bone parameters at all sites measured and serum levels of 25(OH)D or vitamin D status (Table 13). The associations are not only extremely weak ($R^2 < 0.25$) but they are also not statistically significant ($p > 0.05$). The TBLH-BMC, TBLH-BMD, TBLH-area, LS-BMC and LS-area values were log transformed to better fit the regression model and even under this robust option, the outcome remained the same.

Table 13. Association between bone health parameters and vitamin D status.

	TBLH-BMC	**LS-BMC**	**TBLH-BMD**	**LS-BMD**	**TBLH-Area**	**LS-Area**
R^2	0.0003	0.0009	0.0002	0.0305	0.0005	0.0299
p	0.89	0.82	0.92	0.19	0.87	0.19

CHAPTER 5: DISCUSSION

Optimal bone health maintenance and adequate attainment of PBM is affected by body composition and nutritional status. In this cross-sectional study, DXA was used to measure and describe bone health and body composition for identifying a relationship between the two. Vitamin D status measured by 25(OH)D serum concentrations was analysed to describe the vitamin D status and relate that to bone health too. For this reason, the study population was viewed as two sub-sets, the body composition study group and the vitamin D study group.

5.1. BODY COMPOSITION AND BMI STATUS OF THE STUDY PARTICIPANTS

Most of the children that participated in this study were considered over-nourished due to a high BMI-for-age. The over-nourished children had a greater LM, and FM was considerably greater in these participants compared to their healthy counterparts. There was affirmation of this finding by the NFCS (National Food Consumption Survey) which found that most overweight/obese children between 1-9 years old in South Africa reside in urban areas.[173] The prevalence of overweight and obesity amongst children in Africa doubled between the years 1990 and 2010, and the cause of the increasing prevalence is multifaceted. The increasing prevalence of overweight and obesity can be linked to cultural beliefs or to the transition of the South African population from rural to urban. South Africa suffers the dual burden of disease whereby malnutrition in the form of undernutrition as well as overnutrition is present. The increased consumption of energy-dense foods due to increasing trends of urbanization are creating this problem of over-nourished children with micronutrient deficiencies which is what was seen in this study where the majority of the children were over-nourished and vitamin D insufficient.[30] Children in urban areas have been shown to spend a larger amount of time indoors than children in rural communities which can be linked to a reduced amount of physical activity. This implicates urbanization in the greater FM and body fat percentage of over-nourished children in this study group.[174] Results from the most recent data from the South African Health and Nutrition Examination Survey (SANHANES) disclosed that 13.2% of boys and 21% of girls between two and fourteen years old were overweight or obese. We found a greater prevalence with 42% of the children in our study being over-nourished. The childhood over-nourishment statistics described by the SANHANES was however not separated by ethnic groups and socioeconomic status. The data provided by the SANHANES for adults showed that obesity was greatest amongst African and urban populations which pertained to our study population.[175]

5.2. VITAMIN D STATUS OF THE STUDY PARTICIPANTS

The majority of the children in this study did not have sufficient serum 25(OH)D when grouped according to the classification described by the Endocrine Society. This was the chosen method

of classification due to the elevation of PTH at serum 25(OH)D concentrations above 20 ng/ml which is specifically vital with reference to bone remodelling. Only 34% of the children involved in our study had sufficient serum 25(OH)D ≥ 30 ng/ml. Seven percent were vitamin D deficient (serum 25(OH)D ≤ 20 ng/ml) and 59% were vitamin D insufficient (25(OH)D = 21-29 ng/ml). Little is known about the vitamin D status of the black South African preadolescent population who although have adequate sunshine available, are prone to Vitamin D deficiencies due to the increase in urbanization as well as skin pigmentation.[21, 176] Poopedi *et al*.[19] found that 22% of the 295 black children involved in their study based in urban areas of Johannesburg, South Africa, were vitamin D insufficient. Vitamin D deficiency was presented in 8% of these children. With the majority being sufficient in vitamin D, they did not find vitamin D deficiency to be a public health issue that needed to be addressed. An earlier study by Pettifor *et al*.[177] supported this finding as none of the black South African children involved in his study from either urban or rural areas were vitamin D deficient. Contrary to these findings, Naude *et al*.[14] observed a large proportion of vitamin D insufficiency and deficiency in their study participants consisting of multiple ethnic groups in the Western Cape. Naude *et al*.[14] noted poor dietary intake of vitamin D and concluded that this may be an issue that exists in children in other parts of South Africa.

The vitamin D assessment for this study was done in the South African summer and although South Africa has an abundance of sunshine and vitamin D status of South African children is expected to be adequate, this expectation was not met by most of our study participants. Skin pigmentation of black children hinders their ability to synthesize vitamin D through the skin even when adequate sunshine is available. This was confirmed when a biochemical comparison was made between black and albino children in South Africa. The melanin in the skin of the black children acted as a sunscreen and thus children with darker pigmentation required more time in the sun compared to those with lighter skin to produce adequate amounts of vitamin D.[21]

5.3. BONE HEALTH OF THE STUDY PARTICIPANTS

In studying the bone health status of this study population, mean bone mass Z-scores were calculated using standard procedures recommended by the ISCD. The procedures recommended using the TBLH as the skeletal site and adjusting for height with height-for-age Z-scores. The BMC Z-scores indicated that the total population had healthy BMC for chronological age. The Z-scores were calculated using BMC as BMC is more accurate in DXA scans as is does not rely on bone area to be measured. The BMD Z-scores indicated healthy BMD for chronological age of the total population. The Z-scores of the healthy girls however were bordering the cut-off value of what is considered adequate bone mass for age. The BMD of the vitamin D sufficient and deficient girls were low for age. The same trend was not found in the boys and thus cannot be attributed to vitamin D status. Because BMD relies on bone area for its estimation, and bone area is highly variable among children, the interpretation of Z-scores using BMD has been questioned.

Therefore, BMC Z-scores were calculated and could explain the varied findings between BMC and BMD Z-scores. Our study population consisted of black South African preadolescents in an urban setting whom are prone to undernutrition and underactivity, but the majority were found nonetheless to have healthy bone status. Black South Africans have a lower fracture risk and greater bone mass than white South Africans despite adverse bone health attributers which can be accredited to genetic contributions.[167]

The raw BMD values measured by DXA differed between the over-nourished and healthy children in this study. Over-nourished children had significantly denser bones at both the TBLH and LS sites. The BMC at the TBLH was also greater in over-nourished children. These findings are in agreement with the theory that increased weight bearing on the bone increases bone mineralization due to the mechano-responsive reactions of osteocytes and osteoblasts.[178, 179] Where TBLH-BMC was greater in over-nourished children, LS-BMC was not different between the two groups of children. This suggests that the effect of added weight on bones in the over-nourished children is site-specific. In an article by Skerry[180] the complexity of mechanical loading is described, outlining that the effects of loading are not only magnitude-related, but create stimuli signalling biochemical signals, related to the rate, frequency, periods and durations of loading as well. It was noted that these stimuli respond differently at different sites of the skeleton. The varied effect of mechanical loading can be seen in different types of bone tissue too. The greater TBLH-BMC but not LS-BMC may be explained by the differences in bone tissue composition. LS consists predominantly of trabecular bone as it is functions to withstand stresses whereas the total skeleton is mostly cortical bone tissue. The differences in responses of trabecular and cortical bone to mechanical loading is postulated to be due to differences in rates of bone turnover and bone metabolism.[95, 178, 181] Other reasons for differences have been linked to osteocytes and osteoblasts, the mechanosensing bone cells, responding differently depending on the bone tissue type. It has been proposed that osteoblasts from the axial and appendicular skeletons respond differently to mechanical and biochemical signals as they are of different lineages.[178] Additionally, osteocyte morphology differs at the trabecular and cortical bone tissue where at the trabecular tissue they are rounded and at the cortical bone they are more elongated causing their mechanosensations to differ. However, mechanosensing is not completely understood and thus the reason for varied responses to mechanical loading at different skeletal sites have not yet been confirmed.[33, 182]

Although it is plausible for over-nourished children to have greater bone densities as compared to healthy children, inaccuracies in aBMD are probable as DXA is unable to measure bone density volumetrically. There are two x-ray beams projected during a DXA scan and thus thee bone is examined in two dimensions. The vBMD (g/cm^3) is measured directly by 3-D x-ray scanning but aBMD (g/cm^2) is calculated from DXA 2-D scans affecting the accuracy. With the BMC and bone

area measured by DXA, aBMD is calculated by dividing BMC by bone area.[86, 96] Bone mineral apparent density was calculated as an attempt to reflect vBMD as areal measurements of BMD over-estimate the BMD of larger bones and under-estimate in smaller bones thus falsely reporting BMD in children who have varied rates of growth.[166] The TBLH-BMAD and LS-BMAD of the over-nourished children were, like TBLH-BMD and LS-BMD, significantly greater than the children in the healthy group, confirming that the over-nourished children had denser bones. The bone area of the over-nourished children did not indicate a significantly larger bone size than that of the healthy children as modelling of the bones is a response to not only weight but also to genetics during childhood growth. The greater TBLH-BMAD and LS-BMAD of the over-nourished children is an adaptation due to the greater mechanical loading on the bones as body weight increases more rapidly than bone area.[86]

The raw bone values of the children compared by vitamin D classification did not differ. Thus, in this population, adequate bone health was not reliant on sufficient vitamin D status. The bone health values when calculated as BMAD at the LS site, did however differ significantly between the insufficient and deficient groups. This was a peculiar finding as the adjusted and raw bone health measures did not have the same outcome. This may be attributed to the low power of this study of 7% which affects the interpretation of the results and is further discussed in the limitations. While vitamin D is essential and plays a vital role in bone metabolism, poor bone health status did not pertain to vitamin D insufficiency or deficiency. These findings are supported by the lack of efficiency of vitamin D supplementation in multiple bone health intervention studies.[183] This finding should however not downplay the role of vitamin D in bone health as nutritional rickets is known to be caused by both vitamin D and calcium deficiencies individually and together.[12] Additionally, findings of the positive association between vitamin D and PTH levels are ubiquitous in literature.[146, 184-187]

5.4. RELATIONSHIP BETWEEN BONE HEALTH AND BODY COMPOSITION

The forces exerted on bones by the added body weight of over-nourished children increase bone mass. The LM and FM of the over-nourished children were both significantly greater than those in the healthy group. It was therefore necessary to determine the relative contributions of factors that affect bone mass. The results of linear regression models assessing the relationship between the varying factors influencing bone health indicated that height, age, body weight, LM and FM were positively associated with all TBLH and LS bone parameters (Table 11). Lean mass was the major body compositional determinant for variations observed in TBLH bone parameters as it had the strongest associations. This means that according to these findings, LM is the largest contributing body compositional factor for ensuring adequate bone mass accretion in preadolescent children.[188] This supports the mechanostat theory that bone mass proportionally adapts with muscle strength as increased LM results in increases in BMC and BMD. This theory

has been confirmed by the findings of multiple studies.[158, 189-192] Petit *et al*.[189] compared the bone geometry of overweight and healthy weight participants and found LM to be the main contributor of differences between the two groups. Similar results were found when Jeddi *et al*.[158] evaluated the effects of body composition on BMD and concluded that LM had the greatest influence thereon. Arabi *et al*.[190] studied the relative impact of LM and FM on bone mass variables for different sex categories and the findings remained that LM made the largest contribution to bone mass accumulation. In this study of participants between 10 and 17 years old however, the results showed that FM also greatly contributed to bone mass of girls at the postmenarcheal stage. A longitudinal study of overweight compared to healthy weight children aged 9-11 validated our findings. The results of the longitudinal study indicated that changes in bone strength over 16 months calculated using a strength index were associated with changes LM and not FM.[192]

Fat mass in this study, had a weaker positive relationship with all bone health parameters than LM at all sites. The load exerted by LM is a dynamic load as muscles contract and relax continuously, whereas FM is static. The weaker association of FM on parameters of bone mass may be because static loads exerted by FM do not stimulate bone cells involved in the remodelling process as effectively as dynamic loads.[152, 153] This suggests that the greater bone mass parameters observed in over-nourished children is largely attributed to the greater LM and is less likely due to the higher overall FM. This finding contradicts that of Arabi *et al*.[190] which involved children and adolescents of a different age group. When comparing the bone geometry of children and adolescents between 4 and 20 years old of different weight categories, Petit *et al*.[189] found that the additional weight contributed by FM of the overweight participants did not positively affect bone strength.

Children in this study were categorised by their BMI-for-age Z-score which indicated whether they were under-nourished, healthy or over-nourished compared to children of the same age and sex. Body mass index is not a true reflection of body fatness, and body fat percentage may have been high in a child with a healthy BMI-for-age due to high FM and low LM. A child that had the same healthy BMI-for-age may have had a lower FM to LM ratio in comparison and thus a low body fat percentage.[160] Nonetheless, body fat percentage had weak associations or no association at all with bone mass and bone area which is in agreement with the associations measured by Zagarin *et al*.[188] Although there was an association between FM and bone parameters, this association did not remain when regressed against body fat percentage. This is because body fat percentage does not reflect the load bearing on the bones as it does not adjust for the body size (Figure 6).[160] The child with a greater total body weight or LM is likely to have greater BMC, BMD and bone area as they are largely associated with each other.

The relationship between LM and TBLH bone parameters was far greater than that of LM and LS bone parameters. This was also seen in the relationship between body weight and FM with TBLH

and LS bone parameters. The findings of this greater association to TBLH bone parameters agree with the findings of Rocher *et al*.[7] The weaker association validates that the magnitude of the effect of mechanical loading on bone measurements is site-specific.

5.4.1. Bone health parameters after body compositional adjustments

Because the raw TBLH-BMC, BMD and calculated BMAD at both measurement sites of the over-nourished children were greater than that of the children in the healthy BMI category, it may be thought that over-nourished children have stronger bones. Although the bones of over-nourished children should be stronger to support the additional weight, this cannot be confirmed without adjusting for confounders. Unadjusted values may lead to a false interpretation of bone mass and area resulting in an under- or overinterpretation of the bone health status. The bone mass to total body weight ratio of the over-nourished children (1.7% ± 0.26%) was lower than the healthy children (2.2% ± 0.26%). This warranted further statistical investigation by making adjustments to the bone mass of the children to determine the effects that the relative body composition compartments had on the skeleton.[7]

To study the effect of body composition independently, non-body compositional adjustments needed to be made first. Due to the importance of height, gender and age in bone mass accretion, adjustments for these needed to be made to ensure that the effects of body composition observed were independent of those non-body compositional contributing factors.[165]

After adjusting for non-body compositional factors including height, age and gender, the difference in TBLH-BMC between the groups of children remained suggesting that over-nourished children had greater TBLH-BMC to support a greater weight. When adjusted for FM though, there was no difference between the TBLH-BMC of healthy and over-nourished children. This is in accordance with the findings by Rocher *et al*.[7] who found that the significantly larger TBLH-BMC of obese children did not differ from the control group after adjusting for FM. Rocher *et al*.[7] found that after adjusting for LM however, obese children had a significantly lower TBLH-BMC than the lean controls. In our study though, after adjusting for LM, like with the FM adjustment, over-nourished and healthy children did not differ significantly in terms of TBLH-BMC. Other findings have been contradictory, reporting an increased raw bone mass in over-nourished individuals, which was greater or equal even after adjusting for body composition and body size.[16, 150] Zagarins *et al*.[188] proposed that conflicting results between studies may be due to differences between population groups whereas Leonard *et al*.[16] and Manzoni *et al*.[9] suggested that discrepancies may have arisen due to different approaches used to analyse the DXA data. In multiple studies comparing the bone mass of over-nourished and healthy children, similar age groups were used, all using DXA to obtain raw data, but conflicting results were found.[6, 7, 9, 16, 150] Additionally, these studies used different methods of categorising the children. While Ellis *et al*.[6] categorised children

by adiposity, Rocher *et al*.[7] used BMI cut-off values and Manzoni *et al*.[9] classified children by relative body weight using Tanner growth charts. Leonard *et al*.[16] and Goulding *et al*.[150] used the same classification system of grouping children in to BMI centiles yet still found contrasting results possibly due to the fact that Leonard *et al*. included children from the 5th to 85th centile in the non-obese group and Goulding *et al*.[150] had three groupings of <85th centile, 85-94 centile and >95th centile. Leonard *et al*.[16] and Goulding *et al*.[150] also differed in methods as Leonard *et al*.[16] excluded the head in total body measurements whereas Goulding *et al*.[150] did not.

Lumbar spine-BMC in this study did not differ at the raw measurements nor after adjustments were made. This is because as previously discussed, the mechanical loading and the responses to these forces are site-specific and are not a result of direct forces but are a biochemical response.[180]

When adjusted for LM, the two groups did not differ in terms of TBLH bone area. While the bone area of a heavier person is expected to be larger than that of a person with a smaller total body weight due to the increased weight bearing increasing bone cell differentiation, this was not true in this study.[178, 179] The raw mean TBLH bone area of the two groups of children did not differ and this remained the same when adjusted for height, age and gender. After adjusting for body weight however, the two differed with the bone area of the over-nourished children being significantly smaller than that of the healthy group. This difference can be attributed to differences in FM as the bone area of over-nourished children remained smaller when adjusted for FM but when adjusted for LM, the TBLH bone area of the two groups of children did not differ significantly. The TBLH bone area of over-nourished children was smaller than bone area of children with a healthy BMI relative to FM and did not differ between the groups relative to LM. There is concordance between these findings and that of Rocher *et al*.[7] who observed an augmented raw bone area of obese children which did not differ from non-obese children when adjusted for FM and LM. Contrarily, Leonard *et al*.[16] observed greater bone areas of obese children compared to their non-obese counterparts both before and after adjusting for body composition.

A possible reason for the difference in bone area between the two groups not being statistically significant when adjusted for LM even though LM was greatly associated with TBLH bone area is because the difference in LM between the two groups was not as prominent as the difference in FM. The FM of the over-nourished group was more than double the FM of the healthy group. Bone area at the LS did not differ between the two groups before nor after adjustments and this too is related to the site-specific effects of load bearing on the bones.[180] While TBLH bone area of the over-nourished children was low for body weight, it was also low for fat mass. But the TBLH bone area was not low for the load that the LM of the over-nourished children exerts.

The BMC and bone area are important in estimating aBMD therefore significant differences in aBMD reflect the differences in BMC and bone area. When adjusted for LM, the TBLH-BMD of the two groups differed significantly and did not differ when adjusted for FM. The same trend was seen in the FM adjustment made to whole body aBMD of the 9-12-year olds compared in the study by Rocher *et al*.[7] however our results differed in that the TBLH-BMD of the over-nourished children was still greater than the TBLH-BMD of the healthy children after adjusting for LM. This is because, as discussed, the bone area of the healthy group was significantly larger than the over-nourished group bone area when adjusting for body compositional components that included FM but when adjusted for LM, the bone area of the two groups did not differ. Thus, using the LM adjusted bone area as a denominator when it was not significantly larger results in a significantly smaller TBLH-BMD for the healthy group children. The mean raw aBMD at the LS site differed significantly with over-nourished children displaying greater LS-BMD values than healthy children. This is possibly due to errors related to areal measurements of BMD nonetheless after adjusting for body composition, the two groups did not have differences in LS-BMD.[193]

5.5. RELATIONSHIP BETWEEN BONE HEALTH AND VITAMIN D STATUS

Previously it has been found that children with low 25(OH)D have poorer bone health status as compared to children with high 25(OH)D levels, and contradictions remain in the correlation between BMD at various skeletal sites and 25(OH)D.[145, 146, 185] In this study, a trend was not established between serum 25(OH)D and bone health parameters.

Studies on the association between vitamin D and BMD are sparse particularly those relative to the population group used in this study. While some studies have indicated a possible association, the results have indicated a moderate relationship or are inconclusive in the findings. Two studies involving Chinese children up to 7 years of age, confirmed a linear relationship between 25(OH)D levels and BMD however the relationship was very weak in both cases.[194, 195] While Yu *et al.* suggested that 20 ng/ml 25(OH)D as a threshold for adequate BMD, the finding is not supported by any other literature. The inconclusive results found by Lehtonen-Veromaa *et al*.[145] and Cashman *et al*.[146] as discussed in Chapter 2 are evidence of the inconsistencies that prove to be a limitation in providing concrete evidence to support the relationship observed between 25(OH)D and BMD. In this study, only 7% of the children had serum 25(OH)D at or below 20 ng/ml. This is the limit described by the Endocrine society as deficient and by the IOM as being at risk for inadequacy. It has been found that at or below 20 ng/ml, PTH levels are mildly elevated and when 25(OH)D concentrations drop further below 16 ng/ml, PTH levels increase further and calcium homeostasis is disrupted.[12] The lowest serum 25(OH)D in this study population was only 18 ng/ml in one child and three children had 20 ng/ml serum 25(OH)D, which could explain why differences in bone mass were not found to be associated with vitamin D.

Although adequate serum 25(OH)D levels based on bone mass in children have not yet been confirmed, the relationship between PTH, bone health and 25(OH)D also presents limitations in providing insight to establish a threshold for the vitamin D status biomarker. The inverse relationship between 25(OH)D levels and PTH concentration has been confirmed in multiple studies involving preadolescents and adolescents.[146, 184, 185, 187, 196] Because of this, it has been suggested that the level of 25(OH)D at which PTH is suppressed could provide insight in to what the 25(OH)D threshold for vitamin D deficiency may be. However, the relationship between PTH and vitamin D in bone metabolism is also complicated. Parathyroid hormone concentrations, like 25(OH)D depends on ethnicity. Parathyroid hormone has been found to be significantly higher in black populations in comparison to other ethnic groups and thus using this as an indication for a 25(OH)D threshold would provide inaccuracies. Changing concentrations of PTH due to pubertal factors is another reason this biomarker would be a false indicator of the relation of 25(OH)D and bone mass.[83, 186]

5.6. STRENGTHS AND LIMITATIONS OF THE STUDY

Bone mass, serum 25(OH)D and the response of bone mass to serum 25(OH)D is unique to different ethnic groups. Using a single ethnic group for this study thus proved to be a limitation in the findings. This is particularly relevant in the South African context due to the cultural diversity of this country. Additionally, the assumption that the children were pre-menarche, particularly the over-nourished 9-10 year old girls, may have proven to be a limitation in this study.

Calcium and PTH were not assessed in this study, proving to be a limitation to the evidence. Due to the importance of calcium in bone health and the interrelationship of calcium, PTH and serum 25(OH)D, assessing calcium and PTH in black South African preadolescent children would have shed light on the African paradox of lower fracture risk despite lower serum 25(OH)D.

Bone health was assessed by DXA and while this is the gold standard for assessing bone health, it does not provide qualitative information. Therefore, although the bone health of over-nourished children in this study were found to be comparable to healthy children, the bone quality could not be addressed. Whether the bone mass was sufficiently adapted to the weight of the over-nourished children could not be determined. Additionally, DXA provides BMD as an areal measurement rather than volumetric. While calculations of height adjusted Z-scores and BMAD have been used in an attempt to accurately present BMD, this is a limitation that cannot be avoided when using DXA for bone health assessments.

This study adds to the limited knowledge on the vitamin D status and bone health status of children in South Africa. Using the preadolescent age group strengthened the study due to the desperate need for data on this age group.

The strengths of this cross-sectional study lie in the methods used to do the assessments. Both DXA and LC-MS/MS are considered the gold standards for the relative data collections. These methods allow for accuracy, precision and reliability in the study.

The power of this study is both a strength and a weakness. While the study group used to define the relationship between bone health and body composition allowed for a study with 83% power, the power of the study defining the relationship between bone health and vitamin D was merely 7%. This limitation may be attributed to the fact that participants were not screened based on vitamin D status.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The maximum bone density of the adult skeleton and throughout life is established at the peak bone mass achieved during childhood. Heredity sets limits as to the maximum attainable peak bone mass, but this can be modulated by external factors such as exercise or body composition, and nutrition. Preadolescence is a period of rapid growth and an important time in which optimal peak bone mass can be controlled for future benefits by reducing the risk for osteoporosis later in life. The bone density of blacks is generally higher but, bone health status has not been summarised in the South African preadolescent context, a population prone to nutrient deficiencies. This study aimed to define the vitamin D and bone health status of South African children while investigating the relationship between bone health and the external factors affecting bone health, including body composition and vitamin D status.

In this study, most of the children, classified by vitamin D status according to the Endocrine Society guidelines, were vitamin D insufficient but with healthy bone mass for age. A direct relationship could not be established between 25(OH)D and bone mass in this study. Low serum 25(OH)D is common in black South African children but this does not negatively impact the bone mass of this population.

The majority of the children in this study had adequate bone health status according to their Z-scores. The results of this study suggest that both lean and fat mass are positively associated with bone mass due to the mechanical loading of the added weight applied to the skeleton. Lean mass however, has a strong relationship with bone mass and fat mass has a weaker association. This positive relationship between body composition and bone health is site specific, having a greater influence on the appendicular skeleton than on the axial skeleton. In this study, almost half of the preadolescents were over-nourished and had a greater raw bone mass at the appendicular skeleton than their healthy counterparts. After adjustment for non-body compositional factors, height, age and gender, the bone mass of the over-nourished children remains greater. When adjusted for body weight, lean mass or fat mass though, the bone mass of over-nourished children is comparable to children with a healthy BMI-for-age.

6.2. RECOMMENDATIONS FOR FUTURE RESEARCH

Further research is required on the topic of bone health and the relationship to body composition and vitamin D. This topic would benefit from longitudinal studies which would confirm and quantify the positive relationship found between bone health and body composition, particularly with respect to fat mass. Longitudinal studies would further add to this study by assessing seasonal

variations in vitamin D status of black South African children and the effects thereof on bone mass.

The greater raw bone mass in over-nourished children may indicate increased bone strength but this is believed to be inadequate to overcome the greater impact should the child fall. Strength and fracture risk was however not an outcome measured in this study and should be considered for further research.

Fat mass measured by the DXA machine in this study was a measurement of the total body fat rather than the relative distributions of body fat. The body of knowledge on bone mass relative to body composition may benefit by further studies addressing the effect of FM distributions on bone mass. This is particularly relevant in understanding the ethnic differences in bone mass as body composition also varies between ethnic groups.

REFERENCES

1. Starr C, Taggart R, Evers C, Starr L. Biology: The unity and diversity of life. Animal structure and function. 14th ed. Boston, MA: Cengage Learning; 2014;5:612-614.
2. Mahan LK, editor, Raymond, JL, editor. Krause's food & the nutrition care process. 14th ed. St. Louis, Missouri: Elsevier; 2016;456-466.
3. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: Systematic review and meta-analysis. BMJ. 2011 Jan 25;342:c7254.
4. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: Technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220-8.
5. Bass S, Eser P, Daly R. The effect of exercise and nutrition on the mechanostat. Journal of musculoskeletal and neuronal interactions. 2005;5(3):239-54.
6. Ellis K, Shypailo R, Wong W, Abrams S. Bone mineral mass in overweight and obese children: Diminished or enhanced? Acta Diabetol. 2003;40:s274-7.
7. Rocher E, Chappard C, Jaffre C, Benhamou C, Courteix D. Bone mineral density in prepubertal obese and control children: Relation to body weight, lean mass, and fat mass. J Bone Miner Metab. 2008;26(1):73-8.
8. Ducher G, Bass SL, Naughton GA, Eser P, Telford RD, Daly RM. Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: Implications for bone strength at the distal forearm. Am J Clin Nutr. 2009 Oct;90(4):1104-11.
9. Manzoni P, Brambilla P, Pietrobelli A, Beccaria L, Bianchessi A, Mora S, et al. Influence of body composition on bone mineral content in children and adolescents. Am J Clin Nutr. 1996 Oct;64(4):603-7.
10. IOM (Institute of Medicine). Dietary reference intakes for calcium and vitamin D. 2011.
11. Foo L, Zhang Q, Zhu K, Ma G, Trube A, Greenfield H, et al. Relationship between vitamin D status, body composition and physical exercise of adolescent girls in Beijing. Osteoporosis Int. 2009;20(3):417-25.
12. Pettifor JM. Nutritional rickets: Deficiency of vitamin D, calcium, or both? Am J Clin Nutr. 2004 Dec;80(6 Suppl):1725S-9S.
13. Holick MF. Vitamin D status: Measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73-8.
14. Naude CE, Carey PD, Laubscher R, Fein G, Senekal M. Vitamin D and calcium status in south African adolescents with alcohol use disorders. Nutrients. 2012;4(8):1076-94.
15. Borel P, Caillaud D, Cano N. Vitamin D bioavailability: State of the art. Crit Rev Food Sci Nutr. 2015;55(9):1193-205.

16. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr. 2004 Aug;80(2):514-23.
17. Goon D, Toriola A, Shaw B, Amusa L, Khoza L, Shaw I. Body fat percentage of urban south African children: Implications for health and fitness. West Indian Med J. 2013;62(7):582-8.
18. Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. Best Practice & Research Clinical Endocrinology & Metabolism. 2011;25(4):573-84.
19. Poopedi MA, Norris SA, Pettifor JM. Factors influencing the vitamin D status of 10-year-old urban south African children. Public Health Nutr. 2011;14(02):334-9.
20. Green RJ, Samy G, Miqdady M, El-Hodhod M, Akinyinka O, Saleh G, et al. Vitamin D deficiency and insufficiency in africa and the middle east, despite year-round sunny days. SAMJ: South African Medical Journal. 2015;105(7):603-5.
21. Cornish DA, Maluleke V, Mhlanga T. An investigation into a possible relationship between vitamin D, parathyroid hormone, calcium and magnesium in a normally pigmented and an albino rural black population in the northern province of south Africa. Biofactors. 2000;11(1, 2):35-8.
22. Bachrach LK, Gordon CM, SECTION ON ENDOCRINOLOGY. Bone densitometry in children and adolescents. Pediatrics. 2016 Oct;138(4):e20162398.
23. Bogunovic L, Doyle SM, Vogiatzi MG. Measurement of bone density in the pediatric population. Curr Opin Pediatr. 2009 Feb;21(1):77-82.
24. Shah AH, Bilal R. Body composition, its significance and models for assessment. Pakistan Journal of Nutrition. 2009;2:198-202.
25. Rothman KJ. BMI-related errors in the measurement of obesity. Int J Obes. 2008;32(S3):S56.
26. Merriam-Webster [homepage on the Internet]. . 2017 [cited 14 March 2017]. Available from: https://www.merriam-webster.com/.
27. Heaney R, Abrams S, Dawson-Hughes B, Looker A, Looker A, Marcus R, et al. Peak bone mass. Osteoporosis Int. 2000;11(12):985-1009.
28. Rizzoli R. Nutrition: Its role in bone health. Best Practice & Research Clinical Endocrinology & Metabolism. 2008;22(5):813-29.
29. Dual energy X-ray absorptiometry - bone mineral densitometry [homepage on the Internet]. . 2013. Available from: https://rpop.iaea.org/RPOP/RPoP/Content/InformationFor/HealthProfessionals/6_OtherClinicalSpecialities/DEXA/.
30. Rossouw HA, Grant CC, Viljoen M. Overweight and obesity in children and adolescents: The south african problem. S Afr J Sci. 2012;108(5-6):1-7.
31. Boskey A, Wright T, Blank R. Collagen and bone strength. Journal of Bone and Mineral Research. 1999;14(3):330-5.

32. Marquis M, Lord E, Bergeron E, Drevelle O, Park H, Cabana F, et al. Bone cells - biomaterials interactions. Frontiers in Bioscience. 2009;14:1023-67.
33. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simoes MJ, Cerri PS. Biology of bone tissue: Structure, function, and factors that influence bone cells. Biomed Res Int. 2015;2015:421746.
34. Mackie E, Ahmed Y, Tatarczuch L, Chen K, Mirams M. Endochondral ossification: How cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol. 2008;40(1):46-62.
35. Boskey AL, Coleman R. Aging and bone. J Dent Res. 2010;89(12):1333-48.
36. Verbruggen SW, Vaughan TJ, McNamara LM. Strain amplification in bone mechanobiology: A computational investigation of the in vivo mechanics of osteocytes. J R Soc Interface. 2012 Oct 7;9(75):2735-44.
37. Stenbeck G. Formation and function of the ruffled border in osteoclasts. 2002;13(4):285-92.
38. Ortega N, Behonick DJ, Werb Z. Matrix remodeling during endochondral ossification. Trends Cell Biol. 2004;14(2):86-93.
39. Thompson Z, Miclau T, Hu D, Helms JA. A model for intramembranous ossification during fracture healing. Journal of Orthopaedic Research. 2002;20(5):1091-8.
40. Bailey D, McKay H, Mirwald R, Crocker P, Faulkner R. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan bone mineral accrual study. Journal of Bone and Mineral Research. 1999;14(10):1672-9.
41. Hadjidakis DJ, Androulakis II. Bone remodeling. Ann N Y Acad Sci. 2006;1092(1):385-96.
42. Etcheverry P, Grusak MA, Fleige LE. Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B(6), B(12), D, and E. Front Physiol. 2012 Aug 6;3:317.
43. Gibson RS, Perlas L, Hotz C. Improving the bioavailability of nutrients in plant foods at the household level. Proc Nutr Soc. 2006;65(02):160-8.
44. Lambert HL, Eastell R, Karnik K, Russell JM, Barker ME. Calcium supplementation and bone mineral accretion in adolescent girls: An 18-mo randomized controlled trial with 2-y follow-up. Am J Clin Nutr. 2008 Feb;87(2):455-62.
45. Bonjour J, Chevalley T, Ammann P, Slosman D, Rizzoli R. Gain in bone mineral mass in prepubertal girls 3–5 years after discontinuation of calcium supplementation: A follow-up study. The Lancet. 2001;358(9289):1208-12.
46. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. Am J Clin Nutr. 2000 Feb;71(2):544-9.
47. Matkovic V, Landoll JD, Badenhop-Stevens NE, Ha EY, Crncevic-Orlic Z, Li B, et al. Nutrition influences skeletal development from childhood to adulthood: A study of hip, spine, and forearm in adolescent females. J Nutr. 2004 Mar;134(3):701S-5S.

48. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: A reevaluation of the evidence. Pediatrics. 2005 Mar;115(3):736-43.
49. Lanou AJ. Should dairy be recommended as part of a healthy vegetarian diet? counterpoint. Am J Clin Nutr. 2009 May;89(5):1638S-42S.
50. Vatanparast H, Bailey DA, Baxter-Jones AD, Whiting SJ. The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. J Nutr. 2007 Dec;137(12):2674-9.
51. Alexy U, Remer T, Manz F, Neu CM, Schoenau E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. Am J Clin Nutr. 2005 Nov;82(5):1107-14.
52. Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. Am J Clin Nutr. 2004 Aug;80(2):447-52.
53. Bourrin S, Ammann P, Bonjour J, Rizzoli R. Dietary protein restriction lowers plasma insulin-like growth factor I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats 1. Endocrinology. 2000;141(9):3149-55.
54. Kerstetter JE, O'brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. The Journal of Clinical Endocrinology & Metabolism. 2005;90(1):26-31.
55. Chevalley T, Bonjour J, Ferrari S, Rizzoli R. High-Protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. Journal of Bone and Mineral Research. 2008;23(1):131-42.
56. Consultation R. Dietary protein quality evaluation in human nutrition. FAO Food Nutr Pap. 2011;92.
57. Shewry PR. Improving the protein content and composition of cereal grain. J Cereal Sci. 2007;46(3):239-50.
58. Penido MGM, Alon US. Phosphate homeostasis and its role in bone health. Pediatric nephrology. 2012;27(11):2039-48.
59. Moe SM. Disorders involving calcium, phosphorus, and magnesium. Primary Care: Clinics in Office Practice. 2008;35(2):215-37.
60. Walker CF, Ezzati M, Black R. Global and regional child mortality and burden of disease attributable to zinc deficiency. Eur J Clin Nutr. 2009;63(5):591.
61. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. PloS one. 2012;7(11):e50568.
62. Joint F, World Health Organization. Vitamin and mineral requirements in human nutrition. 2005.
63. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: A review of the evidence. Br J Nutr. 2008;99(S3):S14-23.

64. Lonnerdal B. Dietary factors influencing zinc absorption. J Nutr. 2000 May;130(5S Suppl):1378S-83S.
65. Tanumihardjo SA. Vitamin A and bone health: The balancing act. Journal of Clinical Densitometry. 2013;16(4):414-9.
66. Kean EG, Hamaker BR, Ferruzzi MG. Carotenoid bioaccessibility from whole grain and degermed maize meal products. J Agric Food Chem. 2008;56(21):9918-26.
67. Plaza SM, Lamson DW. Vitamin K2 in bone metabolism and osteoporosis. Altern Med Rev. 2005;10(1):24-35.
68. Apalset EM, Gjesdal CG, Eide GE, Tell GS. Intake of vitamin K1 and K2 and risk of hip fractures: The Hordaland health study. Bone. 2011;49(5):990-995.
69. Hamidi MS, Gajic-Veljanoski O, Cheung AM. Vitamin K and bone health. Journal of Clinical Densitometry. 2013;16(4):409-13.
70. Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama, Sabine MPF. Bone mineral density in children and adolescents: Relation to puberty, calcium intake, and physical activity 1. The Journal of Clinical Endocrinology & Metabolism. 1997;82(1):57-62.
71. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr. 2000 May;71(5):1201-8.
72. Weaver CM. Should dairy be recommended as part of a healthy vegetarian diet? point. Am J Clin Nutr. 2009 May;89(5):1634S-7S.
73. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: A story beyond calcium. J Am Coll Nutr. 2000;19(6):715-37.
74. James PT. Obesity: The worldwide epidemic. Clin Dermatol. 2004;22(4):276-80.
75. McVeigh J, Meiring R. Physical activity and sedentary behavior in an ethnically diverse group of South African school children. J Sports Sci Med. 2014;13(2):371-8.
76. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: A review of controlled trials. Bone. 2007;40(1):14-27.
77. Laing EM, Wilson AR, Modlesky CM, O'Connor PJ, Hall DB, Lewis RD. Initial years of recreational artistic gymnastics training improves lumbar spine bone mineral accrual in 4-to 8-year-old females. Journal of bone and mineral research. 2005;20(3):509-19.
78. Kannus P, Haapasalo H, Sankelo M, Sievanen H, Pasanen M, Heinonen A, et al. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med. 1995;123(1):27-31.
79. Järvinen T, Kannus P, Pajamäki I, Vuohelainen T, Tuukkanen J, Järvinen M, et al. Estrogen deposits extra mineral into bones of female rats in puberty, but simultaneously seems to suppress the responsiveness of female skeleton to mechanical loading. Bone. 2003;32(6):642-51.

80. Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez J, Dorado C, Calbet J. Enhanced bone mass and physical fitness in prepubescent footballers. Bone. 2003;33(5):853-9.
81. MacKelvie KJ, Petit MA, Khan KM, Beck TJ, McKay HA. Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. Bone. 2004;34(4):755-64.
82. Nam H, Kweon S, Choi J, Zmuda JM, Leung P, Lui L, et al. Racial/ethnic differences in bone mineral density among older women. J Bone Miner Metab. 2013;31(2):190-8.
83. Aloia JF. African Americans, 25-hydroxyvitamin D, and osteoporosis: A paradox. Am J Clin Nutr. 2008 Aug;88(2):545S-50S.
84. Nam H, Shin M, Zmuda J, Leung P, Barrett-Connor E, Orwoll E, et al. Race/ethnic differences in bone mineral densities in older men. Osteoporosis Int. 2010;21(12):2115-23.
85. Riggs BL, Khosla S, Melton III LJ. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev. 2002;23(3):279-302.
86. Binkley TL, Berry R, Specker BL. Methods for measurement of pediatric bone. Reviews in Endocrine and Metabolic Disorders. 2008;9(2):95-106.
87. Wilkin LD, Jackson MC, Sims TD, Haddock BL. Racial/Ethnic differences in bone mineral density of young adults. International journal of exercise science. 2010;3(4):197.
88. Zengin A, Pye S, Cook M, Adams J, Wu F, O'Neill T, et al. Ethnic differences in bone geometry between white, black and south Asian men in the UK. Bone. 2016;91:180-5.
89. Hess SY, Lönnerdal B, Hotz C, Rivera JA, Brown KH. Recent advances in knowledge of zinc nutrition and human health. Food and nutrition bulletin. 2009;30(1_suppl1):S5-S11.
90. Pesce JE, Kpaduwa CS, Danis M. Deliberation to enhance awareness of and prioritize socioeconomic interventions for health. Soc Sci Med. 2011;72(5):789-97.
91. Covic N, Dhansay A, Gevers W, Kruger S, Mbhenyane X, Mendelow B, et al. Improved nutritional assessment of micronutrients. Consensus Study. ASSAf; 2013.
92. Backström M, Kouri T, Kuusela A, Sievänen H, Koivisto A, Ikonen R, et al. Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. Acta paediatrica. 2000;89(7):867-73.
93. Short DF, Gilsanz V, Kalkwarf HJ, Lappe JM, Oberfield S, Shepherd JA, et al. Anthropometric models of bone mineral content and areal bone mineral density based on the bone mineral density in childhood study. Osteoporosis Int. 2015;26(3):1099-108.
94. Zemel BS, Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: Results of the bone mineral density in childhood study. The Journal of Clinical Endocrinology & Metabolism. 2011;96(10):3160-9.
95. Estrada A, Ramnitz MS, Gafni RI. Bone densitometry in children and adolescents. Curr Opin Obstet Gynecol. 2014 Oct;26(5):339-46.

96. Li N, Li XM, Xu L, Sun WJ, Cheng XG, Tian W. Comparison of QCT and DXA: Osteoporosis detection rates in postmenopausal women. Int J Endocrinol. 2013;2013:895474.
97. Donnelly E. Methods for assessing bone quality: A review. Clinical Orthopaedics and Related Research®. 2011;469(8):2128-38.
98. Genant HK, Engelke K, Prevrhal S. Advanced CT bone imaging in osteoporosis. Rheumatology (Oxford). 2008 Jul;47 Suppl 4:iv9-16.
99. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM. The clinical utility of bone marker measurements in osteoporosis. Journal of translational medicine. 2013;11(1):201.
100. Test ID [homepage on the Internet]. . 2017. Available from: http://www.mayomedicallaboratories.com/test-catalog/search.php?search=test+id.
101. Betto M, Gaio P, Ferrini I, De Terlizzi F, Zambolin M, Scattolin S, et al. Assessment of bone health in preterm infants through quantitative ultrasound and biochemical markers. The journal of maternal-fetal & neonatal medicine. 2014;27(13):1343-7.
102. Souberbielle JP, Roth H, Fouque DP. Parathyroid hormone measurement in CKD. Kidney Int. 2010;77(2):93-100.
103. Catache M, Leone C. Role of plasma and urinary calcium and phosphorus measurements in early detection of phosphorus deficiency in very low birthweight infants. Acta Paediatrica. 2003;92(1):76-80.
104. Al Mheid I, Patel RS, Tangpricha V, Quyyumi AA. Vitamin D and cardiovascular disease: Is the evidence solid? Eur Heart J. 2013 Dec;34(48):3691-8.
105. Javorsky BR, Maybee N, Padia SH, Dalkin AC, Maybee B. Vitamin D deficiency in gastrointestinal disease. Pract Gastroenterol. 2006;36:52-72.
106. American Dietetic Association. Practice paper of the American Dietetic Association: Using the dietary reference intakes. J Am Diet Assoc. 2011;111(5):762-70.
107. Pearce S, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ. 2010;340(jan11 1):b5664-.
108. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96(7):1911-30.
109. Kennedy G, Nantel G, Shetty P. The scourge of" hidden hunger": Global dimensions of micronutrient deficiencies. Food Nutrition and Agriculture. 2003(32):8-16.
110. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. The Journal of Clinical Endocrinology & Metabolism. 2004;89(11):5387-91.
111. Heaney RP, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D3 is more potent than vitamin D2 in humans. The Journal of Clinical Endocrinology & Metabolism. 2010;96(3):E447-52.

112. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. The Journal of Clinical Endocrinology & Metabolism. 2008;93(3):677-81.
113. Hymøller L, Jensen S. Vitamin D 2 impairs utilization of vitamin D 3 in high-yielding dairy cows in a cross-over supplementation regimen. J Dairy Sci. 2011;94(7):3462-6.
114. Reboul E, Borel P. Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. Prog Lipid Res. 2011;50(4):388-402.
115. Hohman EE, Martin BR, Lachcik PJ, Gordon DT, Fleet JC, Weaver CM. Bioavailability and efficacy of vitamin D2 from UV-irradiated yeast in growing, vitamin D-deficient rats. J Agric Food Chem. 2011;59(6):2341-6.
116. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK, et al. Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. Am J Clin Nutr. 2010 Jun;91(6):1621-6.
117. Natri AM, Salo P, Vikstedt T, Palssa A, Huttunen M, Karkkainen MU, et al. Bread fortified with cholecalciferol increases the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplement. J Nutr. 2006 Jan;136(1):123-7.
118. Wagner D, Sidhom G, Whiting SJ, Rousseau D, Vieth R. The bioavailability of vitamin D from fortified cheeses and supplements is equivalent in adults. J Nutr. 2008 Jul;138(7):1365-71.
119. Ko J, Lee B, Lee J, Park HJ. Effect of UV-B exposure on the concentration of vitamin D2 in sliced shiitake mushroom (lentinus edodes) and white button mushroom (agaricus bisporus). J Agric Food Chem. 2008;56(10):3671-4.
120. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000 Sep;72(3):690-3.
121. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. Vitamin D status and parathyroid hormone in obese children before and after weight loss. Eur J Endocrinol. 2007 Aug;157(2):225-32.
122. Norman AW. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: Integral components of the vitamin D endocrine system. Am J Clin Nutr. 1998;67:1108-10.
123. Dunne S, Bell JA. Vitamin D's role in health - deterministic or indeterminate? July 2014;16(7):48.
124. Engelsen O, Brustad M, Aksnes L, Lund E. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. Photochem Photobiol. 2005;81(6):1287-90.
125. Godar DE, Pope SJ, Grant WB, Holick MF. Solar UV doses of adult Americans and vitamin D3 production. Dermato-endocrinology. 2011;3(4):243-50.
126. Godar DE. UV doses worldwide. Photochem Photobiol. 2005;81(4):736-49.

127. Pettifor JM, Moodley GP, Hough FS, Koch H, Chen T, Lu Z, et al. The effect of season and latitude on in vitro vitamin D formation by sunlight in South Africa. S Afr Med J. 1996 Oct;86(10):1270-2.
128. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: Form, function, and metabolism. Endocr Rev. 2012;34(1):33-83.
129. Norman AW. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008 Aug;88(2):491S-9S.
130. St-Arnaud R. The direct role of vitamin D on bone homeostasis. Archives of Biochemistry and Biophysics. 2008 15 May 2008;473(2):225-30.
131. Song Y, Peng X, Porta A, Takanaga H, Peng J, Hediger MA, et al. Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1, 25 dihydroxyvitamin D3 in the intestine and kidney of mice. Endocrinology. 2003;144(9):3885-94.
132. Takahashi N, Udagawa N, Suda T. Vitamin D endocrine system and osteoclasts. BoneKey reports. 2014;3.
133. Baldock PA, Thomas GP, Hodge JM, Baker SU, Dressel U, O'Loughlin PD, et al. Vitamin D action and regulation of bone remodeling: Suppression of osteoclastogenesis by the mature osteoblast. Journal of Bone and Mineral Research. 2006;21(10):1618-26.
134. Brannon PM, Yetley EA, Bailey RL, Picciano MF. Overview of the conference "vitamin D and health in the 21st century: An update". Am J Clin Nutr. 2008 Aug;88(2):483S-90S.
135. Prentice A, Schoenmakers I, Jones KS, Jarjou LM, Goldberg GR. Vitamin D deficiency and its health consequences in Africa. Clinical reviews in bone and mineral metabolism. 2009;7(1):94-106.
136. Carter GD. 25-hydroxyvitamin D assays: The quest for accuracy. Clin Chem. 2009 Jul;55(7):1300-2.
137. Le Goff C, Cavalier E, Souberbielle J, González-Antuña A, Delvin E. Measurement of circulating 25-hydroxyvitamin D: A historical review. Practical Laboratory Medicine. 2015;2:1-14.
138. He C, Gleeson M, Fraser WD. Measurement of circulating 25-hydroxy vitamin D using three commercial enzyme-linked immunosorbent assay kits with comparison to liquid chromatography: Tandem mass spectrometry method. ISRN nutrition. 2013;2013.
139. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: Physiology and biomarkers. Am J Clin Nutr. 2008 Aug;88(2):500S-6S.
140. Kim HJ, Ji M, Song J, Moon HW, Hur M, Yun YM. Clinical utility of measurement of vitamin D-binding protein and calculation of bioavailable vitamin D in assessment of vitamin D status. Annals of laboratory medicine. 2017;37(1):34-38.
141. Mirani G, Williams PL, Chernoff M, Abzug MJ, Levin MJ, Seage III GR, et al. Changing trends in complications and mortality rates among US youth and young adults with HIV infection in the era of combination antiretroviral therapy. Clinical Infectious Diseases. 2015;61(12):1850-61.

142. El-Hajj Fuleihan G, Adib G, Nauroy L. The middle east & Africa regional audit, epidemiology, costs & burden of osteoporosis in 2011. International Osteoporosis Foundation. 2011:102011-5000.
143. Micklesfield LK, Norris SA, Nelson DA, Lambert EV, Van der Merwe L, Pettifor JM. Comparisons of body size, composition, and whole body bone mass between north American and South African children. Journal of Bone and Mineral Research. 2007;22(12):1869-77.
144. Norval M, Coussens AK, Wilkinson RJ, Bornman L, Lucas RM, Wright CY. Vitamin D status and its consequences for health in South Africa. International journal of environmental research and public health. 2016;13(10):1019.
145. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: A 3-y prospective study. Am J Clin Nutr. 2002 Dec;76(6):1446-53.
146. Cashman KD, Kiely M. Towards prevention of vitamin D deficiency and beyond: Knowledge gaps and research needs in vitamin D nutrition and public health. Br J Nutr. 2011;106(11):1617-27.
147. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.
148. Chapuy M, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis Int. 1997;7(5):439-43.
149. Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol. 2008 Sep;3(5):1535-41.
150. Goulding A, Taylor R, Jones I, McAuley K, Manning P, Williams S. Overweight and obese children have low bone mass and area for their weight. Int J Obes. 2000;24(5):627.
151. Goulding A, Taylor RW, Grant AM, Murdoch L, Williams SM, Taylor BJ. Relationship of total body fat mass to bone area in New Zealand five-year-olds. Calcif Tissue Int. 2008;82(4):293-9.
152. Lanyon LE, Rubin C. Static vs dynamic loads as an influence on bone remodelling. J Biomech. 1984;17(12):897-905.
153. Turner C. Three rules for bone adaptation to mechanical stimuli. Bone. 1998;23(5):399-407.
154. Goulding A, Grant AM, Williams SM. Bone and body composition of children and adolescents with repeated forearm fractures. Journal of Bone and Mineral Research. 2005;20(12):2090-6.
155. Tarquini B, Navari N, Perfetto F, Piluso A, Romano S, Tarquini R. Evidence for bone mass and body fat distribution relationship in postmenopausal obese women. Arch Gerontol Geriatr. 1997;24(1):15-21.
156. Afghani A, Goran M. Racial differences in the association of subcutaneous and visceral fat on bone mineral content in prepubertal children. Calcif Tissue Int. 2006;79(6):383-8.
157. Lu H, Fu X, Ma X, Wu Z, He W, Wang Z, et al. Relationships of percent body fat and percent trunk fat with bone mineral density among Chinese, black, and white subjects. Osteoporosis Int. 2011;22(12):3029-35.

158. Jeddi M, Dabbaghmanesh MH, Ranjbar Omrani G, Ayatollahi SM, Bagheri Z, Bakhshayeshkaram M. Relative importance of lean and fat mass on bone mineral density in Iranian children and adolescents. Int J Endocrinol Metab. 2015 Jul 1;13(3):e25542.
159. Jeon H, Lee K, Kim J, Park T, Kang D, Park D. The relationship between body fat percent and bone mineral density in Korean adolescents: The fifth Korea national health and nutrition examination survey (KNHANES V-1), 2010. Korean journal of family medicine. 2014;35(6):303-8.
160. Liu P, Ma F, Lou H, Liu Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. BMC Public Health. 2013;13(1):629.
161. de Onis M, Onyango AW, Van den Broeck J, Chumlea CW, Martorell R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. Food and nutrition bulletin. 2004;25(1_suppl1):S27-36.
162. Onis Md, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85(9):660-7.
163. Wilson KE. Practical considerations when replacing a DXA system. Spine. 2011;1:1.2.
164. Hosking J, Metcalf BS, Jeffery AN, Voss LD, Wilkin TJ. Validation of foot-to-foot bioelectrical impedance analysis with dual-energy X-ray absorptiometry in the assessment of body composition in young children: The EarlyBird cohort. Br J Nutr. 2006;96(06):1163-8.
165. Crabtree NJ, Arabi A, Bachrach LK, Fewtrell M, Fuleihan GE, Kecskemethy HH, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: The revised 2013 ISCD pediatric official positions. Journal of Clinical Densitometry. 2014;17(2):225-42.
166. Bianchi ML, Baim S, Bishop NJ, Gordon CM, Hans DB, Langman CB, et al. Official positions of the international society for clinical densitometry (ISCD) on DXA evaluation in children and adolescents. Pediatric nephrology. 2010;25(1):37-47.
167. Vidulich L, Norris S, Cameron N, Pettifor J. Bone mass and bone size in pre-or early pubertal 10-year-old black and white South African children and their parents. Calcif Tissue Int. 2011;88(4):281-93.
168. Gordon CM, Leonard MB, Zemel BS. 2013 pediatric position development conference: Executive summary and reflections. Journal of Clinical Densitometry. 2014;17(2):219-24.
169. Kalkwarf HJ, Zemel BS, Gilsanz V, Lappe JM, Horlick M, Oberfield S, et al. The bone mineral density in childhood study: Bone mineral content and density according to age, sex, and race. The Journal of Clinical Endocrinology & Metabolism. 2007;92(6):2087-99.
170. Newman MS, Brandon TR, Groves MN, Gregory WL, Kapur S, Zava DT. A liquid chromatography/tandem mass spectrometry method for determination of 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 in dried blood spots: A potential adjunct to diabetes and cardiometabolic risk screening. Journal of diabetes science and technology. 2009;3(1):156-62.

171. Whole blood collection procedure (blood spot collection card) [homepage on the Internet]. 16020 Linden Ave North, Shoreline, WA 98133 USA: . 2017 [cited 14/03/2017]. Available from: http://www.usbiotek.com/.
172. Brismar T, Ringertz H. Effect of bone density of the head on total body DEXA measurements in 100 healthy Swedish women. Acta Radiol. 1996;37(1P1):101-6.
173. Labadarios D, Steyn N, Maunder E, MacIntryre U, Gericke G, Swart R, et al. The national food consumption survey (NFCS): South Africa, 1999. Public Health Nutr. 2005;8(05):533-43.
174. Matz CJ, Stieb DM, Brion O. Urban-rural differences in daily time-activity patterns, occupational activity and housing characteristics. Environ Health. 2015;14(1):88.
175. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, et al. The South African national health and nutrition examination survey, 2012: SANHANES-1: The health and nutritional status of the nation. 2014.
176. Kruger MC, Kruger IM, Wentzel-Viljoen E, Kruger A. Urbanization of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. Nutr Res. 2011;31(10):748-58.
177. Pettifor JM, Ross P, Wang J, Moodley G, Couper-Smith J. Rickets in children of rural origin in South Africa: Is low dietary calcium a factor? J Pediatr. 1978;92(2):320-4.
178. Clarke B. Normal bone anatomy and physiology. Clin J Am Soc Nephrol. 2008 Nov;3 Suppl 3:S131-9.
179. Ehrlich P, Lanyon L. Mechanical strain and bone cell function: A review. Osteoporosis Int. 2002;13(9):688-700.
180. Skerry T. One mechanostat or many? modifications of the site-specific response of bone to mechanical loading by nature and nurture. Journal of Musculoskeletal and Neuronal Interactions. 2006;6(2):122.
181. Bono CM, Einhorn TA. Overview of osteoporosis: Pathophysiology and determinants of bone strength. European Spine Journal. 2003;12(2):S90-6.
182. van Oers RF, Wang H, Bacabac RG. Osteocyte shape and mechanical loading. Current osteoporosis reports. 2015;13(2):61-6.
183. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: A systematic review and meta-analysis. The Lancet. 2014;383(9912):146-55.
184. Hollis BW. Editorial: the determination of circulating 25-hydroxyvitamin D: no easy task. 2004.
185. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr. 2005 Aug;82(2):477-82.
186. Jemielita T, Leonard M, Baker J, Sayed S, Zemel B, Shults J, et al. Association of 25-hydroxyvitamin D with areal and volumetric measures of bone mineral density and parathyroid

hormone: Impact of vitamin D-binding protein and its assays. Osteoporosis Int. 2016;27(2):617-26.

187. Cheng S, Tylavsky F, Kroger H, Karkkainen M, Lyytikainen A, Koistinen A, et al. Association of low 25-hydroxyvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. Am J Clin Nutr. 2003 Sep;78(3):485-92.
188. Zagarins SE, Ronnenberg AG, Gehlbach SH, Lin R, Bertone-Johnson ER. The association of lean mass and fat mass with peak bone mass in young premenopausal women. Journal of Clinical Densitometry. 2010;13(4):392-8.
189. Petit MA, Beck TJ, Shults J, Zemel BS, Foster BJ, Leonard MB. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. Bone. 2005;36(3):568-76.
190. Arabi A, Tamim H, Nabulsi M, Maalouf J, Khalife H, Choucair M, et al. Sex differences in the effect of body-composition variables on bone mass in healthy children and adolescents. Am J Clin Nutr. 2004 Nov;80(5):1428-35.
191. Ho-Pham LT, Nguyen UD, Nguyen TV. Association between lean mass, fat mass, and bone mineral density: A meta-analysis. The Journal of Clinical Endocrinology & Metabolism. 2014;99(1):30-8.
192. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: A longitudinal study. Journal of Bone and Mineral Research. 2008;23(12):1946-53.
193. Binkovitz LA, Henwood MJ. Pediatric DXA: Technique and interpretation. Pediatr Radiol. 2007;37(1):21-31.
194. Fu Y, Hu Y, Qin Z, Zhao Y, Yang Z, Li Y, et al. Association of serum 25-hydroxyvitamin D status with bone mineral density in 0-7 year old children. Oncotarget. 2016 Dec 6;7(49):80811-9.
195. Yu X, Zhang J, Yan C, Shen X. Relationships between serum 25-hydroxyvitamin D and quantitative ultrasound bone mineral density in 0–6year old children. Bone. 2013;53(1):306-10.
196. Outila TA, Karkkainen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: Associations with forearm bone mineral density. Am J Clin Nutr. 2001 Aug;74(2):206-10.

Appendix A: Cover letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Department Human Nutrition

[insert date here]

Dear Parent/guardian

The department of Human Nutrition, University of Pretoria, is inviting your child to participate in a nutrition research study. The principal of Arcadia Primary a School and Arcadia Aftercare has agreed to assist us in our research study.

The aim of this study is to assess your child's body composition (weight, height, body fat and bone health) and if you agree, his/her vitamin D status (amount of vitamin D in their blood). Please see the consent form for more detail on the study.

In order for your child to participate we do, however, need a signed informed consent form from you. Please read through the attached **PARENT OR LEGAL GUARDIAN INFORMATION & INFORMED CONSENT DOCUMENT,** and if you agree please complete page 2 of the consent form and return all the completed and signed forms back to the aftercare school by 10 October 2016 in order for your child to participate. ***Please note that only the first 50 boys and 50 girls will be included in our study.***

Your child will be collected (at 14h00) from Arcadia aftercare during one specific day, and transported by research staff from Department of Human Nutrition (University of Pretoria), to Netcare Femina Hospital (460 Belvedere Street, Arcadia) where the assessments will be performed. He/she will be back at Arcadia Primary Aftercare at 16:30. Your child will receive refreshments on the day of the data collection.

If you agree for your child to participate, and signed the consent form, please select ONE day of the week that best suits you and your child. We will select only one date between 10-28 October 2016 and will contact you to confirm the exact date and send you a reminder on the day before the study so that you can collect them at Arcadia Aftercare **after** 16:30 on that day. You are welcome to send a sibling or other family member with if they are between the ages of 5-10 years. (please let your child request an additional form to complete).

Please do not hesitate to contact us if you have any questions with regard to the project.

Yours sincerely

Dr Zelda White

Tel: 012 356 3209

Cell: 082 738 2916

E-mail: zelda.white@up.ac.za

PLEASE INDICATE YOUR PREFERRED DAY

☐ Monday

☐ Tuesday

☐ Wednesday

☐ Friday

☐ Thursday

Please also complete the Demographic Information of your child below. This information will be kept confidential and is needed for the analysis on the machines we use:

Child's Demographic Information		
Child's name and surname:		
Child's date of birth:	☐☐ ☐☐ ☐☐☐☐ dd mm year	
Gender of participant	☐ Male	☐ Female
Race/Ethnicity	☐ Caucasian ☐ Asian ☐ African ☐ South & Central ☐ Other: Please specify _____	
Does your child take any vitamin/mineral supplements	☐ Yes ☐ No	If yes, please indicate name/brand: _____

Parent/Guardian contact details

Primary contact number

Name: _____

_____

Number: _____

_____

Secondary contact number

Name:

Number:

Appendix B: Reference percentiles for TBLH BMD Z-score calculation

TABLE 1. Age- and sex-specific reference percentiles for total body less head bone mineral content for Black children

	TBLH BMC (g)															
	Black females								Black males							
			M								M					
Age (yr)	L	S	3rd	10th	50th	90th	97th	HZ prediction equation	L	S	3rd	10th	50th	90th	97th	HZ prediction equation
5	−0.051	0.154	324	355	432	527	579	−0.042 + (HZ × 0.911)	1.209	0.155	309	353	443	529	569	−0.188 + (HZ × 0.733)
6	−0.051	0.154	395	432	526	641	704	0.094 + (HZ × 0.524)	1.032	0.154	380	430	536	642	691	−0.165 + (HZ × 0.830)
7	−0.051	0.153	469	514	625	761	835	0.267 + (HZ × 0.511)	0.852	0.153	458	514	637	763	824	−0.122 + (HZ × 0.689)
8	−0.051	0.153	532	583	708	863	947	0.158 + (HZ × 0.525)	0.686	0.153	534	597	736	884	956	−0.050 + (HZ × 0.692)
9	−0.051	0.154	596	653	794	967	1062	0.053 + (HZ × 0.495)	0.539	0.154	605	673	829	1000	1085	−0.090 + (HZ × 0.607)
10	−0.051	0.155	678	743	905	1104	1213	−0.229 + (HZ × 0.596)	0.395	0.158	677	753	929	1128	1230	−0.186 + (HZ × 0.727)
11	−0.051	0.156	796	873	1065	1302	1432	−0.384 + (HZ × 0.832)	0.241	0.165	757	842	1045	1283	1409	−0.318 + (HZ × 0.729)
12	−0.051	0.155	942	1033	1260	1539	1691	−0.356 + (HZ × 0.910)	0.069	0.176	857	954	1197	1497	1660	−0.411 + (HZ × 0.710)
13	−0.051	0.151	1085	1187	1439	1749	1917	−0.192 + (HZ × 0.873)	−0.090	0.188	996	1112	1411	1800	2022	−0.557 + (HZ × 0.877)
14	−0.051	0.146	1203	1313	1582	1910	2088	−0.079 + (HZ × 0.794)	−0.177	0.191	1184	1320	1677	2152	2429	−0.402 + (HZ × 0.742)
15	−0.051	0.143	1286	1400	1679	2016	2199	−0.108 + (HZ × 0.797)	−0.192	0.180	1381	1531	1918	2429	2724	−0.329 + (HZ × 0.786)
16	−0.051	0.140	1335	1451	1735	2077	2262	−0.143 + (HZ × 0.817)	−0.177	0.164	1568	1723	2118	2625	2911	−0.276 + (HZ × 0.730)
17	−0.051	0.139	1360	1477	1763	2108	2294	−0.258 + (HZ × 0.939)	−0.158	0.150	1717	1873	2264	2752	3023	−0.283 + (HZ × 0.754)
18	−0.051	0.138	1373	1491	1778	2124	2311	−0.365 + (HZ × 0.794)	−0.145	0.141	1815	1971	2355	2827	3086	−0.204 + (HZ × 0.796)
19	−0.051	0.138	1385	1503	1791	2139	2325	−0.331 + (HZ × 0.827)	−0.140	0.137	1855	2010	2390	2856	3109	−0.291 + (HZ × 0.946)
20	−0.051	0.137	1398	1517	1807	2155	2342	−0.417 + (HZ × 0.938)	−0.139	0.136	1866	2020	2400	2863	3115	−0.108 + (HZ × 0.881)

L, M, and S values to calculate Z-scores and HZ prediction equations to calculate height adjusted Z-scores are also shown. This measure excludes the BMC of the head from the total body measurement. HZ, Ht-Z.

Appendix C: Ethical Approval

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

30/06/2016

Approval Certificate
New Application

Ethics Reference No.: 73/2016

Title: Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children

Dear Dr Zelda White

The **New Application** as supported by documents specified in your cover letter dated 29/02/2016 for your research received on the 29/02/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29/06/2016.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**73/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Level 4-59*

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

☎ 012 356 3085 fhsethics@up.ac.za http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4-59, Gezina, Pretoria

Appendix D: Consent Form

PARENT OR GUARDIAN INFORMATION & INFORMED CONSENT DOCUMENT

TITLE OF STUDY: **Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children.**

Dear Parent/guardian

1) INTRODUCTION

We invite your child to participate in a research study. This information leaflet will help you to decide if you want your child to participate. Before you agree for your child to take part you should fully understand what is involved. If you have any questions that this leaflet does not fully explain, please do not hesitate to ask the investigator.

2) THE NATURE AND PURPOSE OF THIS STUDY

The research study is co-ordinated by Dr. Zelda White from the Faculty of Health Sciences, Department of Human Nutrition, University of Pretoria. The study consists of two sections *(section A and Section B).* The aim of *section A* of this study is to assess your child's body composition (including percentage body fat, fat free mass and bone health). This study will help us as researchers to compare the results from two different machines/scales. In *section B* of this study we will investigate your child's vitamin D status, in order to see how his/her body composition will influence his/her vitamin D status.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study involves that we will collect information and measurements from your child on one specific day during October-November 2016. The two sections of the study are explained as follows:

SECTION A:

We will measure his/her weight and height. We will also use an X-ray machine to measure your child's body composition (including body fat, muscle and bone health). The measurement on the X-ray machine will take about ten minutes. Your child will not experience any pain or discomfort during this measurement. The measurements will take place at Steve Biko Academic Hospital. Children will be transported to the hospital by the researchers on the day of the data collection, and will be returned to their aftercare facility on the same day. Another body composition measurement will be taken at the university of Pretoria, faculty of health sciences, on a special scale. Your child will be expected to stand on this scale for about 75 seconds. No discomfort will be experienced during this measurement. The results from the X-ray machine and the scale will be compared for us to see how accurate the scale is. An example of the X-ray machine (Figure 1) and the scale (Figure 2) can be seen on the next page:

Figure 1: DEXA (X-ray machine)

Figure 2: Seca mBCA

SECTION B:

For the second part of the study, blood samples will be collected from the fingers' tiny blood vessels (capillary) by a finger prick done by an experienced nurse and will be used to determine Vitamin D status of your child.

4) RISK AND DISCOMFORT INVOLVED

Your child will need to take off their shoes and excess clothing to be weighed and for their height to be taken accurately. This may cause some discomfort. Your child may experience some discomfort during the taking of blood from the finger by means of a finger prick. In order to protect your child, this procedure will be performed under sterile conditions by experienced personnel. The data collection session will take about 120 minutes of your child's time.

If your child experiences any discomfort, we will speak to your child and make sure he/she understands what is going on and still feels comfortable to continue with the project. The information obtained during the study will be kept private

5) POSSIBLE BENEFITS OF THIS STUDY

Although your child will not benefit directly from the study, the results from the measurements will be made available to you upon your request. The results of the study will enable researchers to use the scale on children in clinical settings and plan future studies for determining body composition in children. The results of the vitamin D and body fat will give an indication of the general health and bone status of your child. If your child is identified with low levels of vitamin D, they will be referred to a clinic

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Your child's participation in this study is entirely voluntary. Your child can refuse to participate or stop at any time during the study without giving any reason. Your child's withdrawal will not affect his/her treatment in the class room or school in any way.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This study will only be implemented after it has received written approval from the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria, telephone numbers **012 356 3085.**

8) INFORMATION AND CONTACT PERSON

The contact person for the study is Zelda White. If you have any questions about the study please contact her at the following telephone numbers: **012 354 1993 / 082 738 2916.**

9) COMPENSATION

Your participation is voluntary. No compensation will be given for your child's participation.

10) CONFIDENTIALITY

All information that your child will give will be kept strictly confidential. Once we have analysed the information no one will be able to identify your child. Research reports and articles in scientific journals will not include any information that may identify your child.

CONSENT TO PARTICIPATE IN THIS STUDY

I confirm that the person asking my consent for my child to take part in this study has told me about nature, process, risks, discomforts and benefits of the study. I have also received, read and understood the above written information (Information Leaflet and Informed Consent) regarding the study. I am aware that the results of the study, including personal details, will be anonymously processed into research reports. My child is participating willingly. I have had time to ask questions and have no objection for my child to participate in the study. I understand that there is no penalty should my child wish to discontinue with the study and his/her withdrawal will not affect any treatment at school in any way.

If you sign at 'Section A', your child will only be included in the first part of the study, if you sign at 'Section B', your child will only be included in the second part of the study. You are welcome to sign at both sections to be included in the whole study.

SECTION A: BODY COMPOSITION

Child name and surname:..... (Please print)

Parents/guardian's name: .....(Please print)

Parents/guardian's signature: .....Date:….....

Investigator's name:.....(Please print)

Investigator's signature .....Date.….....

Witness's Name: .....(Please print)

Witness's signature ..... Date.….....

SECTION B: VITAMIN D STATUS

Child name and surname:..... (Please print)

Parents/guardian's name: .....(Please print)

Parents/guardian's signature: .....Date:….....

Investigator's name:.....(Please print)

Investigator's signature .....Date.….....

Witness's Name: .....(Please print)

Witness's signature ..... Date.….....

Appendix E: Assent Form

ASSENT FORM FOR 7-10 YEARS

Assent form for Protocol Title: Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children.

We wish to know if you would like to volunteer to be part of a research study in which we would like to gather information on your body composition (the amount of fat and muscle in your body) and vitamin D status (the amount of vitamin D in your blood that helps calcium to build your bones).

About 100 children are going to take part in this study, and we will only be collecting information from you on one specific day during October-November 2016. There will be two parts/sections of information and measurements needed from you.

PART A:

During the study we will collect information and measurements from you. We will measure your height and weight. When we are measuring your weight and height, we will ask you to remove heavy clothing, shoes and socks. You will not have to remove your uniform. We will also use two different methods to test your body fat and muscle. This will be done with an X-ray machine at the Steve Biko Academic hospital, and a scale at the department of human nutrition, University of Pretoria. An example of the X-ray machine (Figure 1) and the scale (Figure 2) can be seen in the following figures:

Figure 1: DEXA (X-ray machine)

Figure 2: Seca mBCA

PART B

For the second part of the study, you will be asked to give a small amount of your blood from your finger, this may hurt, but it will take no longer than one minute. We will use this blood to test the amount of vitamin D in your body.

If you sign at the bottom of this form, it will mean that you have read this paper, and that you agree to take part in one or both parts of the study.

All these measurements and tests will take about 120 minutes of your time.

If you do not want to take part any more you may decide at any time during the study, not to carry on. No-one will force you to carry on. No-one will be cross or upset with you if you don't want to. You don't have to give us your answer now, take your time and read the rest of this form before you decide.

If you sign at 'PART A', you will only be included in the first part of the study (PART A), if you sign at 'PART B', you will only be included in the second part of the study (PART B). You are welcome to sign at both parts to be included in the whole study.

PART A: BODY COMPOSITION

	Your Name	**Person Obtaining Consent**	**Parent / Guardian / Nurse As Witness**
Name Please Print			
Signature			
Date			

PART B: VITAMIN D STATUS

	Your Name	**Person Obtaining Consent**	**Parent / Guardian / Nurse As Witness**
Name Please Print			
Signature			
Date			

Appendix F: Transport Indemnity Form

INDEMNITY: TRANSPORT PROVIDED BY THE UNIVERSITY FOR PURPOSES OF RESEARCH PROJECT

(Children between the ages of 7-14)

Parent/guardian: _____ (full names and surname)

of

Child/ward: _____(full names and surname)

hereby declare and agree as follows towards the University of Pretoria:

1. My child/ward will be involved in a research project ('Body Composition by multi-frequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children') conducted by the University of Pretoria's Faculty of Health Sciences, for purposes of which my child/ward will have to be transported by the University from the Arcadia primary school to the Netcare Femina Hospital during the period October-November 2016.

2. I hereby grant permission that my child/ward be transported as indicated in paragraph 1 above.

3. I am aware that as a result of or arising from the transport of my child/ward he/she will be exposed to situations that put him/her at risk. I am fully aware of the risks involved and acknowledge that my child/ward is being transported at his/her own risk.

4. I acknowledge and agree that neither the University nor its employees, representatives, agents, contractors and/or students shall be liable for any injury (including death), illness, damages or loss of whatever nature that my child/ ward or our property may sustain as a direct or indirect result from the transport provided by the University to my child/ward, whether arising from any act or omission, negligent or otherwise, on the part of the University, its employees, representatives, agents, contractors and/or students, or from any other cause whatsoever.

5. I hereby defend, indemnify and hold harmless the University its employees, representatives, agents, contractors and students from all claims instituted against any of them as a result of any injury (including death), illness, damages or loss of any nature caused by any act or omission on the part of my child/ward.

6. I acknowledge and agree that I have read this indemnity form in its entirety, that I fully understand the nature, content and implications hereof and agree hereto, and that I/we shall be fully bound hereto.

To be completed and signed by learner's Parent/Guardian

Full Names and
surname…….

Identity number
…….……

Physical Address
……..

……
…..

Signed at ……..... the ..... day of …..... 20…..

WITNESSES:

1. ....
PARENT/GUARDIAN

2. ……..... ***[By signing this document the parent/guardian declares that he/she is concluding this contract on behalf of the child/ward]***

Appendix G: Approval Letters

Established 1903

Arcadia School
School of Excellence

Parenden Street, Arcadia, Pretoria, 0083
Tel: (012) 344-2249 • Fax: (012) 343-0235
E-mail: aps@arcadiaps.org.za / finance@arcadiaps.org.za
www.arcadiaprimary.co.za

PERMISSION LETTER FOR RESEARCH STUDY TO BE CONDUCTED AT ARCADIA PRIMARY SCHOOL

23 February 2016

Dear Zelda White

Re: Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children.

We hereby confirm knowledge of the above named research study and give permission for the study to be conducted at the after-care school facility of Arcadia Primary school.

Yours faithfully

S. VILJOEN - Principal

Name and signature

(Specify designation)

Date 01/03/2016

GAUTENG DEPARTMENT OF EDUCATION
ARCADIA PRIMARY SCHOOL
01-03-2016
TEL: 012-344-2249
FAX: 012 343-0235
PARENDEN ST, ARCADIA, 0083

13 October 2016

PERMISSION LETTER FOR RESEARCH STUDY TO BE CONDUCTED AT
Little Tubbles Day Care Center [265 Beckett St, Pretoria, 0007]

Dear Zelda White

Re: Body composition by multifrequency bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DEXA) and relationship to vitamin D status in children.

We hereby confirm knowledge of the above named research study and give permission for the study to be conducted at our facility.

Yours faithfully

CARMENITA MUOJEKWU

Name and signature

Principal

(Specify designation)

Date 13-10-2016