The association between serum vitamin D and body composition in black South African postmenopausal HIV positive women on antiretroviral therapy

S. Mwango

orcid.org/0000-0001-8108-8806

Dissertation submitted for the Master of Science degree in Nutrition at the North-West University

Supervisor: Prof. H.S. Kruger
Co-supervisor: Ms. J Carboo

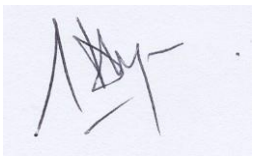
Graduation: May 2021
Student number: 33436282

PREFACE

Plagiarism Declaration

This dissertation is submitted for the Master of Science Degree in Nutrition at the North-West University. All work presented was conducted at the Center of Excellence for Nutrition (CEN) under the supervision of Prof. HS Kruger and Ms. Janet Carboo. To the best of my knowledge, unless referenced, all work in this dissertation is original and unpublished. Chapter 4 of the dissertation is an article, which will be submitted to the Southern African Journal of HIV Medicine.

Samuel Mwango 

Prof. H Salomé Kruger 

Ms. Janet Carboo 

Acknowledgements

It is with great pleasure that I express my sincere appreciation to the able hands and minds that contributed greatly to this dissertation. Without their active guidance, help, encouragement and cooperation, headway would have not happened.

Prof. H.S. Kruger (main supervisor):

Prof. H.S. Kruger, your tireless step-by-step support rendered to me throughout the Master's programme shall forever be cherished. Felt I was failing at times but in such times of despair, you offered encouragement and that gave me renewed strength. Thank you, Prof. Salomé.

Janet Adede Carboo Co-supervisor:

Janet Adede Carboo, thank you for the contribution and insights into this dissertation. Despite your tight schedule and commitment, you spared time towards this achievement.

My profound gratitude to my wife Grace you remain a pillar of great inspiration and my champion. Umunthu, my son, those video calls were not disturbances, but lightened my stressful moments to start afresh. To parents, brothers, sisters and friends, I owe you a million tons of appreciation.

To God be the glory for the sound health and renewed strength throughout the Master's programme.

But those who hope in the LORD will renew their strength. They will soar on wings like eagles; they will run and not grow weary, they will walk and not be faint.

Isaiah 40:31

ABSTRACT

Background: HIV/AIDS and highly active antiretroviral therapy (HAART) alter bodily processes, including vitamin D metabolism. Altered vitamin D metabolism is associated with adiposity and bone loss.

Objective: To determine the association between serum 25-hydroxyvitamin D (25(OH)D) and body composition in black South African postmenopausal HIV-positive women on HAART.

Methods: This two-year longitudinal study is part of a larger prospective study (n=120) in the North West Province of South Africa. Measures included serum 25(OH)D concentration, bone mineral density (BMD) at three sites, lean mass and percent body fat (%BF). Multivariable linear mixed models were used to assess the association between serum 25(OH)D and body composition over the two-year period measured at 1 year interval. Linear mixed models (LMM) were used to determine the longitudinal association between lean mass and %BF (exposures) and BMD (outcome).

Results: The 120 study participants average age was 40 years. Vitamin D deficiency and insufficiency increased from baseline (10.2% and 19.5%) to 11.5% and 37.5%, respectively, after two years. Serum 25(OH)D had no association with any BMD outcomes. Lean mass and %BF had comparable positive associations with total spine and left hip femoral neck (FN) BMD, however, lean mass proved as a stronger predictor. Serum 25(OH)D decreased significantly, however with a small effect size of 0.39 (P = 0.001), while total BMD, left hip FN BMD had significant small increases (effect size 0.03, P = 0.02 and 0.06, P = 0.0001 respectively), whereas total spine BMD did not change over the two years.

Conclusion: Serum 25(OH)D was not associated with any BMD outcomes. Though lean mass and %BF had a comparable positive association with BMD, the former exhibited a stronger association with BMD.

Keywords: Vitamin D, postmenopausal, adiposity, BMD, Africa, HIV/AIDS, HAART

LIST OF ABBREVIATIONS

ADP	Air displacement plethysmography
ART:	Antiretroviral therapy
BIA:	Bioelectrical impedance analysis
BMD:	Bone mineral density
BMI:	Body mass index
Cart:	Combination antiretroviral therapy
CEN:	Centre of Excellence for Nutrition
CT:	Computer tomography
D4T:	Stavudine
DXA:	Dual energy x-ray absorptiometry
EFV:	Efavirenz
FTC:	Emtricitabine
GPAQ:	Global physical activity questionnaire
HAART:	Highly active antiretroviral therapy
HIV:	Human immunodeficiency virus
HREC:	Health Research Ethics Committee
NRTI:	Nucleoside reverse transcriptase inhibitor
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NWU:	North West University
MRI:	Magnetic resonance imaging
RCT:	Randomised control trial
TBF:	Total body fat
TDF:	Tenofovir
WHO:	World Health Organization
PTH:	Parathyroid hormone
%BF	Percent body fat
SANHANES-1:	South African National Health and Nutrition Examination Survey-1
UWW	Underwater weighing
VDR:	Vitamin D receptor
25(OH)D	25 hydroxyvitamin D

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CHAPTER 1 INTRODUCTION

1.1 Background and study motivation

Vitamin D plays very essential roles in the human body throughout the lifecycle. Primarily, it is known to aid calcium and phosphorus homeostasis for proper bone mineralisation and bone health (Khazai *et al.*, 2008; Morris *et al.*, 2014). Presently, vitamin D is recognised as a key player in several physiological processes and clinical benefits, owing to the presence of vitamin D receptors (VDR) in a wide range of body tissues and cells (Pérez-López *et al.*, 2011). Vitamin D deficiency/insufficiency is a growing public health concern among all age groups across the world (Mansoor *et al.*, 2010; Palacios & Gonzalez, 2014). The deficiency affects approximately 50% of the worldwide population regardless of age and ethinicity (Nair & Maseeh, 2012). Low serum concentration of 25-hydroxyvitamin D [25(OH)D] is associated with some negative health outcomes besides bone-related disorders, such as neoplasia, cardiometabolic diseases including diabetes mellitus, autoimmune and infectious diseases (Hossein-nezhad & Holick, 2013).

Vitamin D intake (exogenous source) and sunlight synthesis (endogenous source) are major determinants of vitamin D status with the latter contribution at approximately 90% (Holick & Chen, 2008). However, other risk factors for vitamin D deficiency include body weight/body mass index, black skin pigmentation, old age, atmospheric pollution, gender, seasonal variations, chronic disease and drugs (Theodoratou *et al.*, 2014). HIV/AIDS is a chronic disease that has been associated with vitamin D deficiency (Lake & Adams, 2011). Approximately 40% of HIV infected patients were vitamin D deficient in population-based studies across Europe (Pinzone *et al.*, 2013; Viard *et al.*, 2011). In South Africa, vitamin D deficiency is equally present in both HIV infected and non-infected comparable groups (Martineau *et al.*, 2011). In 2018, South Africa reported an alarmingly high and the biggest HIV/AIDS pandemic profile in the world, with 7.7 million people infected and a prevalence of 20.4% among the general population. Based on sex , more women than men are infected with HIV (Avert, 2019).

Improved HIV/AIDS management strategies, coupled with the use of antiretroviral treatment (ART) have shifted HIV/AIDS trends from an inevitably fatal disease to a more chronic infection (Broder, 2010). South Africa has the largest HIV treatment programme globally, with 3.9 million people initiated on ART in 2016 in line with the UNAIDS 90 90 90 agenda (SANAC, 2017). Besides the improvement in life expectancy through the advent of ART, patients with HIV receiving ART experience unpleasant body composition changes, characterised by maldistribution of fat mass around the trunk. This is more prevalent in women than in men (Debroy *et al.*, 2019).

Furthermore, South Africa continues to experience rapid urbanisation associated with lifestyle changes and dietary habits, which have a negative bearing on body composition and general health (Kruger *et al.*, 2011). The 2012 South African National Health and Nutrition Examination Survey-1 (SANHANES-1) report indicated a national obesity prevalence of 39.2% in South African adult women. In the postmenopausal age categories of 55–64 and 65 years and older, the SANHANES-1 report of 2012 reported significantly higher mean body mass index (BMI) of 31.3 kg/m^2 and 30.0 kg/m^2 for women respectively, compared to 25.2 kg/m^2 and 25.6 kg/m^2 for men, respectively under same age category (Shisana *et al.*, 2014). Obesity has been linked to altered physiological pathways in the metabolism, production, and mobilisation of vitamin D (Tsiaras & Weinstock, 2011).

1.2 Problem statement

HIV/AIDS prevalence disparities exist by sex and ethnicity in South Africa, with black women within the age range 20-34 years ranking high compared to their male counterparts (Shisana *et al.*, 2014). The HIV prevalence trends among older South African women (post-menopausal) continue to rise, exceeding that of older men. According to the South African national HIV prevalence, incidence, behaviour and communication survey report of 2017, HIV prevalence between 2012-2017 under the following age categories 45-49, 50-54, 55-59 years increased from 19.7% to 30.3%,14.8% to 22.2% and 9.7% to 17.6% in women, compared to a lower increase in men (Simbayi *et al.*, 2019).

Globally, ART programmes are expanding, coupled with improved survival rates of HIV-infected persons comparable to that of HIV uninfected persons (Wandeler *et al.*, 2016). Presently, South Africa has the largest ART programme globally, covering 4.4 million people, approximately 61% of those infected with HIV (SANAC, 2017). The recent standard ART regimen 1 for South Africa constitutes tenofovir (TDF), emtricitabine (FTC), efavirenz (EFV) as a combination antiretroviral therapy (cART). TDF/FTC is associated with altered calcium and phosphate metabolism, raised parathyroid hormone (PTH) and consequent poor BMD (Havens *et al.*, 2012; Havens *et al.*, 2017; Rosenvinge *et al.*, 2010). The association of TDF and raised PTH is common in vitamin D deficient patients (Masiá *et al.*, 2012). Furthermore, the improved life expectancy due to ART signifies an impending aging HIV population. Thus, more women with HIV are likely to reach menopause, a state which is associated with increased risk to aging co-morbidities i.e. osteoporosis, body composition changes, and altered vitamin D metabolism (Shin *et al.*, 2014).

Vitamin D deficiency is a common disorder globally, and research shows a higher prevalence in HIV infected persons than non-infected persons. HIV/AIDS poses an additional risk factor apart from other factors than undermine vitamin D status such as black skin colour, female gender and low dietary intake (Pinzone *et al.*, 2013). HIV/AIDS and HAART, directly and indirectly, account for physiological and metabolic changes in HIV infected persons, such as dyslipidaemia, insulin resistance, lipodystrophy (alteration of body composition specifically fat distribution), poor bone health, and impaired vitamin D metabolism (Dave *et al.*, 2015; Lee *et al.*, 2006; Rodriguez *et al.*, 2009; Shiau *et al.*, 2013). Body composition alteration (lipodystrophy) is a common phenomenon in HIV infected persons (Debroy *et al.*, 2019).

Therefore, black postmenopausal HIV-positive women on HAART have increased risk factors for vitamin D deficiency and compromised bone health either directly or indirectly, through the modification of known factors. This study, therefore, is important in aggregating some information on the association between vitamin D status, bone health and the body composition of black postmenopausal HIV-positive women on HAART, which has been researched inadequately. Some studies have reported a positive association between vitamin D deficiency and overweight and obesity across all age categories (Araghi *et al.*, 2015; Bischof *et al.*, 2006; Pereira-Santos *et al.*, 2015). According to Kruger *et al.* (2011), obesity among black South African women is on the rise, amid rapid urbanisation and poor dietary practices, suggesting a potential increased risk of vitamin D deficiency. Furthermore, low serum vitamin D concentration is associated with secondary hyperparathyroidism and both contribute to increased bone turnover consequently leading to reduced BMD (Islam *et al.*, 2012).

1.3 Aim

The aim of this study is to determine the association between serum 25(OH)D concentration and body composition in black South African postmenopausal HIV-positive women on HAART. This sub-study used baseline and two-year data from the larger prospective study of black South African postmenopausal women on HAART.

1.4 Objectives

The following objectives were formulated to achieve the study aim.

- To determine the association between serum 25(OH)D and %BF, lean mass, and BMD, respectively, in black South African postmenopausal HIV-positive women on HAART.

- To determine the interdependent associations between %BF, lean mass and BMD in black South African postmenopausal HIV-positive women on HAART.

1.5 Research team

Table 1-1 below presents the research team, their roles and specific contributions made throughout the process of study preparation data collection and write-up.

Table 1-1 Research team members and their responsibilities

Role of team member	Name
Study leader/Principal investigator: The study leader gave guidance on the planning of the study, statistical analysis of the data and writing of the dissertation.	Professor H. Salomé Kruger, Professor of Nutrition, Centre of Excellence for Nutrition (CEN), NWU, Potchefstroom.
Co-supervisor: The co-supervisor provided assistance to the supervisor on the planning of the study, statistical analysis of the data and writing of the dissertation. Team member, project coordination	Ms. Janet Carboo, PhD student, CEN, NWU, Potchefstroom.
Student: Mr. Samuel Mwango completed this study (dissertation) for his Master of Nutrition (MSc) degree. The student worked on the sub-study protocol, data analysis and wrote the dissertation.	Mr. Samuel Mwango, MSc student.
Team member, recruitment of participants	Mr. Milton Semenekane, Nutrition intern, CEN, NWU, Potchefstroom.
Team member, project coordination	Ms Christa Ellis, PhD student, CEN NWU, Potchefstroom.
Trained postgraduate students assisted with data collection through questionnaires and anthropometric measurements.	Postgraduate students (Phillip Buys, Shams Bakali, Persuade Makore).
The international collaborator gave guidance on the interpretation of the results and write-up.	Professor Marlena C. Kruger, Dean, Graduate Research and Researcher Development, Massey University, New Zealand.
Supervising Nurse Practitioner.	Sr. Lessing, Registered Nursing Professional, Centre of Excellence for Nutrition, NWU, Potchefstroom.
Supervising Medical Doctor.	Dr. D Semakula, Potchefstroom Hospital.

1.6 Structure of dissertation

In this dissertation the NWU-Harvard referencing style format is used. However, the referencing style of the article in chapter 4 of this mini-dissertation is according to the Vancouver referencing style, as required by the Southern African Journal of HIV Medicine **(Annexure A)**. The dissertation is presented in the following chapters:

Chapter 1: Background and study motivation. This chapter gives an overview of the study motivation, the problem statement, main aim, objectives and the contribution of the study.

Chapter 2: Literature review. A review of literature on vitamin D and body composition is outline in this section with specific focus on postmenopausal women.

Chapter 3: Methodology. This chapter expands on specific procedures or techniques used in achieving the study aim and objectives.

Chapter 4: Article. The article follows the format of the Southern African Journal of HIV Medicine. Contents include a brief background section, methods, results and discussion.

Chapter 5: Summary. This section gives a quick take home message from the study findings and further gives recommendations for future studies to probably fill the gaps noted in this study as well as public health message.

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CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Hypovitaminosis D is considered a public-health problem due to its high prevalence as well as contribution to a number of acute and chronic diseases (Pérez-López *et al.*, 2020). The prevalence of vitamin D insufficiency/deficiency worldwide varies between 30% and 93% (Mansoor *et al.*, 2010; Palacios & Gonzalez, 2014). Durazo-Arvizu *et al.* (2014) reported a higher prevalence of vitamin D deficiency among black South African adult men and women (25-45 years) than in black populations from Ghana, Jamaica and the Seychelles. Vitamin D is primarily known for its role in bone health through calcium and phosphorus homeostasis (Carmeliet *et al.*, 2015). Vitamin D receptors (VDR) are present in multiple body cells and tissues, including skeletal muscle, immune cells, adipocytes, and pancreatic islets. This has led to the association of vitamin D with skeletal and extra-skeletal benefits (Caprio *et al.*, 2017).

Body composition is one important factor in determining vitamin D status. It either directly or indirectly influence vitamin D through interactions with other factors such as age, sex, skin pigmentation/race, chronic diseases and medications (Tsiaras & Weinstock, 2011). South Africa is experiencing an upsurge in nutrition-related non-communicable diseases due to nutrition transition and consequent body composition changes (Vorster *et al.*, 2011). The South African National Health and Nutrition Examination Survey-1 (SANHANES-1) report of 2012, indicated a national obesity prevalence of 39.2% for South African adult women (Shisana *et al.*, 2014). The 2016 South African Demographic and Health Survey affirmed the high rates of overweight and obesity in women at 27% and 41%, respectively (National Department of Health, 2016).

The subsequent sections focus on the review of literature which have reported conflicting findings on the association between vitamin D and body composition components, specifically lean mass, fat mass, and bone. The sections will add a further extention on determinants of vitamin D status and body composition, with special focus on HIV/AIDS, highly active antiretroviral drugs (HAART), gender, ethnicity/skin type, age, and menopausal status.

2.2 Body composition

Body composition research dates back to over 150 years, with emphasis on body components, their distribution, and changes (Zhu & Wang, 2011). The research on body composition is of interest to nutritionists, health professionals and sports scientists (Kuriyan, 2018). Evaluation of body composition provides insight into the developmental origins of disease, nutritional status and functional capacity of the human body across the entire lifecycle (Andreoli *et al.*, 2016; Thibault *et*

al., 2012). Body composition is widely accepted as an independent determinant of health (Francis *et al.*, 2017; Peterson & Braunschweig, 2016).

2.2.1 Levels of body composition assessment

There are five different levels of body composition assesment. These include the atomic, molecular, cellular, tissue and whole body level (Wang *et al.*, 1992). Assessment at atomic level include basic elements such as: carbon, calcium, potassium, and hydrogen (Duren *et al.*, 2008). Other elements of measurement at atomic level include oxygen, nitrate, phosphorus, sulphur, sodium, chloride and magnesium (Wang *et al.*, 2005). Molecular level measurements include amounts of water and proportions, protein, and fat, whereas at the cellular level, it includes extracellular fluid and body cell mass (Wang *et al.*, 1992).

Tissue level assesment focuses on the amounts of fat mass and fat free mass (FFM) which encompases both bone and lean mass (Duren *et al.*, 2008). A two compartment model (tissue level) of human body composition divides the body into fat mass and FFM and is the most frequently applied model to evaluate body composition (Ackland *et al.*, 2012). Fat mass is a water-free body component, whereas, FFM encompasses all body components such as body water, bone, organs and muscle content (Marra *et al.*, 2019). Excess body fat mass unlike lean mass is associated with an increased risk of chronic diseases and mortality (Bastien *et al.*, 2014). Bodyweight is an important predictor of bone mineral density BMD and is mainly determined by two compartments of body composition, namely fat mass and lean mass

As such, individuals with high weight have high BMD, strong bones, and reduced fracture risk (Ho-Pham *et al.*, 2010). Whole body level assessment is concerned with body shape, size, physical and exterior features. Measurements at whole body include breadth and circumference, skinfold, body volume, weight, body mass index (BMI) and segmental length. Whole body level assessment is simpler and useful in field work (Lohman *et al.*, 2005).

Bone tissue, fat mass and lean mass are critical body components that impact health through systemic metabolic interactions. Lean body mass is the largest tissue in the body, and constitutes the sum of body water, total body protein, carbohydrates, non-fat , and soft tissue minerals (St-Onge *et al.*, 2004). Lean mass is associated with energy regulation and bone formation, owing to its great demand for glucose and essential mechanical pulling action on bone via paracrine and endocrine signalling pathways (Hamrick, 2011).

The fat mass component encompass fat from all body parts, and exists in two categories, namely storage fat/adipose tissue, and essential fat. Adipose tissue is linked to development of metabolic disorders and is the body's largest storage site for triglycerides, and further it plays an important

role as an endocrine organ in energy homeostasis (Sethi & Vidal-Puig, 2007). Adipose tissue further provides protective function to body organs against trauma. On the other hand, essential fat is stored in small amounts in the bone marrow, kidney, heart, lungs, liver, spleen, muscles, and lipid rich tissues in the nervous system, and it is necessary for normal physiological function of the body (Mahan & Escott-Stump, 2008). Total body fat (TBF) is usually expressed as a percentage of total body weight. The TBF associated with optimal health ranges between 8% to 24% in men and 21% to 35% in women (Gallagher *et al.*, 2000).

Bone tissue continuously undergoes remodelling (bone resorption and formation), a physiological process in which old or damaged bone cells are removed by osteoclasts and replaced by new bone formed by osteoblasts (Feng & McDonald, 2011; Walsh, 2015). Osteoclasts are responsible for bone resorption, whereas osteoblasts ensure bone formation (Chapman-Novakofski, 2012). An imbalance between bone resorption and formation results in altered BMD and consequently, osteoporosis (Florencio-Silva *et al.*, 2015). For the purpose of diagnosis of osteoporosis, BMD is compared to the mean value of a young population using a T-score. A T-score is the number of standard deviations for an individual's BMD from the mean of a young reference group. According to the World Health Organization (WHO), a T-score of –2.5 and below indicates osteoporosis, and –1.0 to –2.5 indicates osteopenia, whereas a T-score of –1.0 and above represents normal BMD. These cut-offs have been applied in the white population (Bener & El Ayoubi, 2012; WHO, 1994). Fracture risk increases by 2-3 fold with any 1 SD decrease in BMD (Tsang *et al.*, 2011).

2.2.2 Body composition measurement methods

The human body comprises of more than 30 measurable components, but the most frequently applied model in evaluating body composition in clinical practice and epidemiology, splits the body into fat mass and FFM (Wang *et al.*, 1992). Prevalence of overweight and obesity continue to rise globally, and body adipose tissue accumulation and distribution in certain areas remain a health risk (Stevens *et al.*, 2012). Assessment of body composition in humans involves describing either little or an excess of a component known to be associated with increased health risks. The clinical significance of the body compartment to be measured must be determined before a method of measurement is selected, as most techniques are both sophisicated and costly (Lee & Gallagher, 2008). Apart from the cost, choice of a specific assesment method or combination of methods is based on other factors such as accuracy, precision, subject acceptability, convenience, and radiation exposure. Methods for measurement of body composition *in vivo* include computed tomography (CT), magnetic resonance imaging (MRI), bioelectrical impedance analysis (BIA), anthropometry, and dual-energy X-ray absorptiometry (DXA) (Fosbøl & Zerahn, 2015). All the available *in vivo* body composition assesment methods differ in principle, assumptions, limitations, and susceptibility to certain measurement errors (Lee & Gallagher, 2008).

2.2.2.1 Tomographic imaging

Tomographic imaging are the most accurate *in vivo* methods of body composition assesment at tissue and organ level. Examples of tomographic imaging techniques include CT and MRI. Both CT and MRI measure adipose tissue and skeletal muscle *in vivo* through high resolution images (Mazonakis & Damilakis, 2016). Computer tomography is superior to MRI, due to reliability, but both rely on high radiation dose. Exposure of healthy individuals to high radiation doses solely for the purpose of conducting body composition research may be considered unethical, hence usage is mainly in diagnostics (Lohman *et al.*, 2005).

2.2.2.2 Densitometry

Densitometry includes two methods namely underwater weighing (UWW) and air displacement pleythysmography (ADP). Both methods are based on the Archimedes' principle and uses a 2-compartment model, which divides the body into fat mass and FFM. The difference in body weight in water and air through UWW and ADP, respectively are used to compute the body's density. The Siri's or Brozek formula are then used to estimate fat mass and FFM from the body density. The Brozek equation has proven to be more accurate in the elderly than the Siri equation (Guerra *et al.*, 2010). Adjustment for air in the lungs and gastrointestinal tract is critical for accurate measurements (Going, 2005). The densitometry technique, however, does not provide any measurements of the distribution of adipose tissue or lean tissue.

2.2.2.3 Bioelectrical impedance analysis (BIA)

The BIA technique is a non-invasive, low cost, and reliable method of body composition assessment. BIA is used for both epidemiological and clinical purposes to predict body composition based on the electrical conductive properties of the body (Jackson *et al.*, 2013; Khalil *et al.*, 2014). The basic principle of the BIA technique is that the transit time of a low-voltage electric current through the body depends on body composition characteristics (Marra *et al.*, 2019). Furthermore, BIA works on the principle that lean mass consists of water and electrolytes, which are good electrical conductors, while fat mass does not have water and is thus a poor conductor. Accuracy and reliability of BIA measurements is dependent on a number of factors such as limb length, recent physical activity, nutritional status, hydration level, blood chemistry, ovulation, and placement of electrodes (Stahn *et al.*, 2012).

2.2.2.4 Anthropometry

Anthropometric measurements are simple, inexpensive, widely used, non-invasive measurements that provide information about body size, shape, and stores of fat and muscle in clinical situations and small or large epidemiological studies (Kuriyan, 2018). Measures of anthropometry include

among others body height, body weight, skinfold thickness, and circumferences of the waist, hip, arm and calf. BMI, developed by Quetelet are used to estimate overweight or obesity and is derived from anthropometric measurements of height and weight. For this index weight expressed in kg is divided by the square of height in m thus giving a ratio portraying a degree of thickness or thinness. BMI is the basis for the WHO's definition of overweight (25 ≤ BMI < 30 kg/m^2) and obesity (BMI ≥ 30 kg/m^2). The BMI directly correlates with percentage body fat in average sedentary (physically inactive) populations, with an average body composition and older adults. However, weak correlations are often seen in athletes and other physically active individuals due to a high muscle to fat mass (Staiano & Katzmarzyk, 2012). Furthermore the correlation is weakened because percentage body fat is sensitive to age, sex, height, and ethnicity, (Peterson *et al.*, 2017; Staiano & Katzmarzyk, 2012). BMI is limited in describing fat distribution and metabolic risk prediction thereby, rendering an index that only takes weight and height into consideration less desirable in (Okorodudu *et al.*, 2010; Thomas *et al.*, 2012; Tomiyama *et al.*, 2016).

Waist circumference is a measure of intra-abdominal fat, using a non-stretchable tape in a standing position at the midpoint between the lowest point of the rib and the superior iliac crest, ideally, during end-tidal expiration. Waist circumference is a good predictor of common cardio-metabolic morbidity and mortality (Ashwell *et al.*, 2012; Taylor *et al.*, 2010). The WHO cut-off points for high risk are > 102 cm for men and > 88 cm for women, however, in the elderly, propositions are that the cut-offs be raised higher (Heim *et al.*, 2011). Other circumference measurements include mid-upper arm and calf circumference. They are used as indicators of skeletal muscle mass in older adults. A low mid-upper arm circumference is a better indicator of thinness and undernutrition in adults than a low BMI (Wijnhoven *et al.*, 2012).

The skinfold technique is a measure of subcutaneous fat, assessed at the subscapular, biceps, triceps and suprailiac as well as other sites of the body (Marfell-Jones *et al.,* 2012). The skinfold thickness measurement is prone to a number of errors arising from the identification of the exact location of a measurement, the way the skinfold is picked up, the way the calipers are placed on the fold, the compression of the fold by the calipers, and the timing of the reading. The measurements are used in age and sex specific equations to arrive at values of body density and ultimately body fat percentage (Ayling, 2014).

2.2.2.5 Dual energy X-ray absorptiometry (DXA)

The DXA is a rapid non-invasive, low radiation exposure method for assessment of body fat, muscle, and BMD. DXA is regarded as a reference method in clinical research (Andreoli *et al.*, 2009). Furthermore, DXA is convenient body composition assessment method (Toombs *et al.*, 2012). The principle of the DXA is based on the use of low-radiation X-rays of two different photon

energy levels that pass through the body. These are identified by a photon detector that measures the amount of energy absorbed (attenuation) by soft tissue and bone at each pixel based on the properties of the underlying tissue (Lohman *et al.*, 2005). The variations in the attenuation of X-ray through the tissues are caused by differences in the density and chemical composition of fat, lean tissue and bone. The image splits into the components of bone and soft tissue using the two different energy levels. DXA is the gold standard for BMD measurements (Garg & Kharb, 2013). DXA is a precise method of quantifying body components in advance models, which surpasses other methods of measurements (Toombs *et al.*, 2012).

The DXA analysis assumes a constant hydration of soft tissue, however, hydration varies with age, sex, and body size, and this potentiates possible measurement errors (St-Onge *et al.*, 2004). Additionally, physical exercise has the potential to alter hydration status of lean tissue and consequently influence the accuracy of the DXA measurement (Toomey *et al.*, 2017). A comparative study between DXA and BIA by Leahy *et al.* (2012) found that BIA analysis underestimates TBF mass and overestimates FFM in healthy young adults compared to DXA.

2.2.3 Determinants of body composition

2.2.3.1 Body composition and age

Body composition play a pivotal role in the determination of health and functional capacity in the elderly (Visser & Harris, 2012). The biological aging process is characterised by increased oxidative stress, inflammatory response, and a decline in functional capacity of organs and tissues of the body (Poulose & Raju, 2014). Lean, fat and bone tissue, which are important components of the body are susceptible to changes during the normal healthy aging process (Jiang *et al.*, 2015). Age-related body composition changes are characterised by a gradual loss of lean mass and strength, termed sarcopenia, and a shift in fat redistribution from appendicular sites to accumulation around the trunk, thereby, increasing morbidity risk (Zamboni *et al.*, 2014). Sarcopenia is further associated with reduced muscle strength, functional impairment and disability (Choi, 2016).

Aging leads to a reduced production of anabolic hormones such as oestrogen, testosterone, growth hormone and insulin growth factor-1, which results in reduced capacity of the muscle to incorporate amino acids and synthesise proteins (Wang & Bai, 2012). A further increase in catabolic circulating inflammatory agents, specifically, interleukin-6 during the aging process are also related to loss of muscle mass. The decreased protein synthesis and slower protein turnover which occur during aging explains the atrophy of muscles and increased intramuscular fat (Choi, 2013; Wang & Bai, 2012). The resultant effect of the decline in muscle mass and increased adiposity around the trunk

is loss of muscle strength, functionality, and increased risk of mortality (Santanasto *et al.*, 2017; Schaap *et al.*, 2013).

2.2.3.2 Body composition and sex

Sex-specific body composition variation exists, with women having higher proportions of fat mass than lean mass compared to men who have higher lean mass than fat mass (Karastergiou *et al.*, 2012). Men accumulate adipose tissue around the trunk and abdomen and this is known as android shape, whereas women accumulate adipose tissue around the gluteal-femoral region, termed gynoid shape (Bredella *et al.*, 2010). Body weight gain during aging and in the menopause transition period in women are debated as to whether body weight change is a resultant effect of hormonal changes associated with menopause or the result of the normal aging process (Davis *et al.*, 2012). Greendale *et al.* (2019) reported that extensive gain in fat mass and decline in lean mass is a typical phenomenon during the menopause transition. The contribution of lean mass and fat mass towards BMD has been extensively researched. Kirchengast and Huber (2012) reported the sex-specific effects of lean mass on BMD, emphasising that lean mass has significant effect on BMD in men, whereas fat mass significantly predicts BMD in postmenopausal women.

2.2.3.3 Body composition and HIV/AIDS

Disease trends of HIV/AIDS have shifted from short to long-term chronic infection with the advent of antiretroviral therapy (ART) (Deeks *et al.*, 2013). The use of ART and the HIV itself is associated with increased risk of body composition changes such as peripheral fat atrophy, visceral lipohypertrophy, central fat accumulation, reduced lean mass, and bone mass loss (Brown & Glesby, 2012; Grunfeld *et al.*, 2010; Güerri-Fernandez *et al.*, 2013). The use of antiretroviral drugs (ARVs) may lead to 50% of HIV infected persons developing central fat accumulation (Grunfeld *et al.*, 2010). Protease inhibitor (PI), a class of ARV, has been associated with lipodystrophy, characterised by fat accumulation in the dorsocervical spine, the breast, and abdomen as well as fat reduction in the extremities and the face (Anuurad *et al.*, 2010). Debroy *et al.* (2019) reported a progressive fat increase around the trunk and leg among men and women with HIV on ART. Women had a higher increase in fat than men (Debroy *et al.*, 2019).

Additionally, HIV inflammatory processes directly or indirectly alter the bone metabolism and consequently lead to poor bone integrity (Ofotokun & Weitzmann, 2011; Stone *et al.*, 2010). Studies have revealed an increased incidence of low BMD, osteoporosis, and fracture risk among HIV infected persons compared to HIV negative individuals (Dave *et al.*, 2015; Prieto-Alhambra *et al.*, 2014; Sharma *et al.*, 2015). ARVs specifically, tenofovir disoproxil fumarate (TDF) under the nucleoside reverse transcriptase inhibitor (NRTI) class, is associated with bone mass loss as it is hypothesised to induce renal phosphate wasting, causing skeletal demineralisation (Stellbrink *et*

al., 2010). A case-control study, among HIV infected and non-infected South African women reported a lower BMD, percent body fat, appendicular skeletal mass, and waist circumference in the HIV infected group compared to non-infected women. Beside HIV status, age and smoking were negatively associated with BMD, whereas, calcium intake had a positive association with BMD (Ellis *et al.*, 2019). Furthermore, Han *et al.* (2020) demonstrated in adult participants the independent association between BMD decline and HIV infection as well as TDF containing HAART regimen. The five years' longitudinal study reported progressive BMD decline in HIV patients compared to non-infected as well as in TDF user versus the non-TDF user patients. Authors further reported that the initial 12-months follow-up was characterised by massive BMD decline followed by an attenuated trend in BMD loss (Han *et al.*, 2020).

2.2.4 Body composition and physical activity

Physical inactivity is associated with increased risk of mortality whereas, physical activity is also associated with the preservation of lean mass and a decrease in fat mass (Kokkinos *et al.*, 2011). Furthermore, regular physical exercise is important in bone health and contributes to improved BMD and consequently, a reduction in fracture risk (Santos *et al.*, 2017). Bone tissue undergoes continuous remodelling process, and as a dynamic tissue, it adapts and responds to various stimuli, such as physical exercise and mechanical vibration (Kelley *et al.*, 2013). Physical exercise increases the stress load on bone and acts as stimulus for bone strength and development through ground reaction forces and by the contractile activity of muscles, resulting in maintenance and gain of bone mass (Moreira *et al.*, 2014; Vieira *et al.*, 2013).

A Canadian retrospective study among postmenopausal women aged 75 years and above reported that an increased intensity in daily physical activity results in improved BMD at the hip, trochanter, Ward's triangle and total hip, but not on the lumbar spine (Muir *et al.*, 2013). Similar findings of improved BMD with physical exercise were reported in a prospective randomised controlled trial (RCT) on effect of physical exercise on BMD in postmenopausal women in India (Chowdhur, 2014). Bolam *et al.* (2013) found contrary findings to the above in two out of eight trials in a systematic review of RCTs. This systematic review investigated the effect of light physical exercise on hip and spine BMD among middle and old aged men and women. The findings confirmed that resistance and high impact loading exercise were likely to affect BMD positively, unlike light exercise, implying that the type of exercise plays a significant role.

2.3 Body composition components interaction (lean, fat and bone mass)

Studies on the interaction between fat mass, lean mass and BMD are summarised in Table 2.1. Body weight largely constituted by both lean mass and fat mass is singled out as one of the most important determinants of BMD (Gonnelli *et al.*, 2014). However, the relative contribution of each

of the two principal components of body weight, lean mass and fat mass to BMD remain a highly contentious matter (Ho-Pham *et al.*, 2014). Some studies have reported a significant positive association between lean mass and BMD, but not with fat mass (Bierhals *et al.*, 2019; Kim *et al.*, 2018; Weaver *et al.*, 2016). Nonetheless, some studies have reported fat mass or both fat and lean mass as having a positive correlation with BMD especially among women (Dytfeld *et al.*, 2011; Nur *et al.*, 2013).

Two mechanisms of action namely; increased mechanical stress mediated by muscle, and increased load placed on the skeleton, act as stimuli for osteogenesis. Lean mass is thought to employ these mechanisms to maintain a high BMD and reduced fracture risk (Zhang *et al.,* 2012). The positive effect of fat mass on bone health can be through skeletal loading effect. However, adipose tissue may also indirectly promote bone loss through the production of adipocyte hormones, and inflammatory cytokines that interfere with bone metabolism, hence the inconsistent findings on the impact of body fat on BMD (Russell *et al.*, 2010). The discrepancies in findings could be as a results of study designs, sample size or presence of confounding factors i.e. ethnicity, sex, age, and chronic diseases.

Ho-Pham *et al.* (2014) reported lean mass as having a higher significant positive correlation with BMD than fat mass among men and women. The correlation between lean mass and BMD was greater at femoral neck and whole body BMD than at lumbar spine BMD. The systematic review further reported a comparable influence of lean mass and fat mass on BMD among postmenopausal women. However, lean mass displayed a stronger significant positive correlation with BMD than fat mass in premenopausal women. This is suggestive of the fact that fat mass is an equally important predictor of BMD in postmenopausal women. The correlation between fat mass and BMD is likely site specific, and dependent on menopausal status (Ho-Pham *et al.*, 2014). Ilesanmi-Oyelere *et al.* (2018) reported lean mass as a strong significant predictor of BMD compared to fat mass at femoral neck, hip, lumbar spine, and whole body BMD. Age consistently displayed an significant inverse correlation with femoral neck and hip BMD. Aging in women is associated with a decline in oestrogen, a bone protective hormone through inhibition of accelarated bone resportion (Sànchez-Riera *et al.*, 2014). A cross-sectional study in black South African postmenopausal women also found a stronger association between lean mass with BMD than fat mass with BMD (Sotunde *et al.*, 2015). Being of the black race has been associated with better BMD outcomes compared to the white race. However, the contribution of lean and fat mass is still contentious likely because of other factors that interfere with the interaction such as lifestyles, medications, or disease i.e. HIV infection. Alcohol intake and smoking were cited as additional risk factors for low BMD among the urban black South African postmenopausal women, besides other traditional risk factors (Kruger *et al.*, 2011).

Sharma *et al.* (2012) confirmed a positive association between lean mass and BMD at all sites among HIV infected and non-infected African American adult women. The authors further reported site-specific associations between fat mass and BMD. Total fat mass had a positive association with BMD at total hip and femur neck. On regional body composition and BMD, a significant positive association between greater trunk fat (and not leg fat mass) and total hip BMD and femur neck BMD was revealed, regardless of HIV status. The finding points towards weight bearing effect on bone remodelling (Sharma *et al.*, 2012). Similarly, another longitudinal study among Chinese postmenopausal women proved lean mass unlike fat mass as the best determinant of BMD progressively over two years (Chen *et al.*, 2015). In the study, years after menopause displayed a significant inverse relationship with BMD at all sites (left hip, the lumbar spine and the total body). This can be explained by the progressive body composition changes as well as the further decline in oestrogen. Oestrogen is another critical factor in BMD determination among postmenopausal women. Throughout the two-years, fat mass progressively increased, while lean mass decreased, typical of body composition changes in aging. Being a prospective study, these findings add more weight to the hypothesis wherein lean mass is singled out as the best determinant of BMD (Chen *et al.*, 2015).

In contrast, Rodrigues Filho *et al.* (2016) reported that segmental body fat (lower limbs and trunk) positively correlated with BMD regardless of the nutritional status group by BMI in male undergraduate students. This further emphasises the site specific effect of fat mass on BMD. A sub-analysis by nutritional status groups replicated the above results, but only in the overweight group and not the normal and obese groups. This is quite unexpected, as those who are obese could be thought to have a higher positive influence on BMD, due to higher weight than other BMI groups. Being a metabolically active tissue, the influence of fat mass on BMD is not only through weight bearing, but also hormonal via production of bone-active hormones from the pancreatic β-cells and adipocytes that negatively influence BMD (Sharma *et al.*, 2012). The hormone influence of fat mass on BMD in the obese group might have outweighed the positive weight bearing effect on BMD. This probably explains the lack of positive effect in the obese participants. The relation of total and segmental fat mass and BMD was segmental and specific on nutritional status signifying the importance fat distribution in BMD determination because adipokine level differ by fat depot. Rodrigues Filho *et al.* (2016) argued on collinearity of independent variables of lean mass and fat mass as another factor for inconsistent results. The inference from this study should be made with caution, because this finding was only among young population and used body composition per segment basis, as opposed to fractional body composition. Notably also was a small sample size of 45 participants making the study underpowered. A longitudinal study in older men and women proved low fat mass as having a modest association with bone loss specifically in men and not women at lumbar spine, but not at the femur neck (Yang *et al.*, 2015).

Namwongprom *et al.* (2013) concurred with the above study results, indicating that android fat mass, gynoid fat mass, and android/gynoid (A/G ratio) fat mass ratio had a significant positive association with BMD at lumbar spine and femoral neck BMD. A/G ratio had a strong association with lumbar spine. BMD improvement at lumbar spine can be explained by the increased load due to high A/G fat ratio used the above approach examining the relationship between BMD and fat mass by both BMI and A/G fat ratio. The cross-sectional study had A/G fat ratio and BMI of postmenopausal women positively correlated with lumbar spine BMD and proximal femur BMD, respectively. The results affirmed site-specific correlation between fat mass (by body region) and BMD. Lean body mass, appendicular lean mass and isokinetic strength showed no correlation with BMD. Similarly, an earlier cross-sectional study Kapuš *et al.* (2014) revealed body fat mass as a strong predictor of proximal femur BMD than lean body mass in 11-20 and 21-30 years after menopause. However, lean body mass and not body fat mass was a strong determinant of proximal femur BMD in the 1-10 years after menopause category (Kapuš *et al.*, 2014). The explaination for these findings could be the progressive body composition changes (lean mass decline and increase in TBF and android region), and decrease in gynoid fat proportion (Fu *et al.*, 2011). This signifies that beside site specific, years after menopause add as a potential determinant to the body fat mass and BMD interaction.

A more recent cross-sectional study, A/G fat ratio which is a measure of fat distribution showed contrasting results. Significant inverse associations between A/G fat ratio and BMD at all sites (lumbar spine, femur neck and total hip) in young adult females and lumbar spine skeletal site in young adult males were reported (Xiao *et al.*, 2020). This finding stands in contradiction to the above. However, it should be noted that the above studies were in postmenopausal women and not young adults. This could potentially explain the discrepancy because body composition changes with aging. Xiao *et al.* (2020) further revealed sex differences in body compostion with men having a higher whole body lean mass than a higher fat mass in women. Weight, height, BMI, lean mass and fat mass all correlated positively with whole body BMD, lumbar spine BMD, femoral neck BMD, and total hip in both sexes, where whole-body lean mass was the strongest predictor in men.

Table 2-1 Summary of studies on interdependent interaction between body composition components (lean, fat and BMD)

Reference	Outcome (s) measured	Study design	Population/sample size	Findings
Kapuš *et al.,* 2020	A/G fat ratio and BMI and BMD	Cross-sectional	Postmenopausal (n=58)	A/G fat ratio and BMI significantly correlated positively with lumbar spine (LS) BMD and proximal femur BMD. Lean body mass and appendicular lean mass did not related to any BMD outcome.
Xiao *et al.,* 2019	Whole-body lean mass, fat mass, and BMD in the LS, femoral neck (FN), and total hip (TH) areas.	Cross-sectional	Young men and (n=786) Young women (n=825)	Women had higher FM while mens had higher lean mass. A/G fat ratio, lean mass and FM had significant positive relationships with BMD in both genders. A/G fat ratio was inversely associated with all BMD sites in women and LS BMD in men.
Namwong prom *et al.,* 2019	BMD and regional body fat	Cross-sectional	Healthy postmenopausal women (n=1448)	A/G fat mass and A/G ratio positively associated with BMD at LS and FN BMD.
Rodrigues Filho *et al.,* 2016	Total body and segmental relative body components and BMD	Cross-sectional	Young undergraduate men (n=45)	Total body and segmental relative body fat positively correlated with BMD regardless of the nutrition status. In an analysis by nutritional status the above results were replicated in the overweight group, but not in the obese or normal weight groups.
Sotunde *et al.,* 2015.	Lean mass and FM versus BMD	Cross-sectional	Postmenopausal (n=189)	Lean mass best determinant for BMD compared to FM
Yang *et al.,* 2015	Lean mass, FM, femoral neck and lumbar spine	Longitudinal study	717 individuals (204 men and 513 women)	Low FM had a modest association with LS BMD loss.
Chen *et al.,* 2015	Lean mass and FM versus BMD	Longitudinal study	Chinese postmenopausal women (n=373)	Lean mass proved as the best determinant of BMD compared to FM.
Ho-Pham *et al.,* 2014	Lean mass and FM versus BMD	Sytematic review and meta analysis	Men and women (n=20226) (4966 men and 15 260 women) 44 studies	Lean mass best determinant for BMD. Lean and FM had comparable effect on BMD among postmenopausal women
Kapus *et al.,* 2014	FM, lean mass and BMD	Cross-sectional	Postmenopausal women (n=167)	Lean mass and not FM strong determinant of femur BMD in early menopause whereas FM and not lean mass strong predictor for femur BMD in late menopause.
Sharma *et al.,* 2012	Lean mass, FM and BMD	Longitudinal study	HIV-infected and non-infected adult women (n=3,766 women (2,791 HIV-infected and 975 HIV-uninfected)	Lean mass associated with increased BMD at all sites. Total FM positively correlated with BMD at total hip and femur neck. A positive association between greater trunk fat (and not leg FM) and total hip BMD and femur neck BMD regardless of HIV status.

A/G fat ratio =; BMI = Body Mass Index; BMD = Bone Mineral Density; FM = fat mass; LS = lumbar spine

2.4 Vitamin D

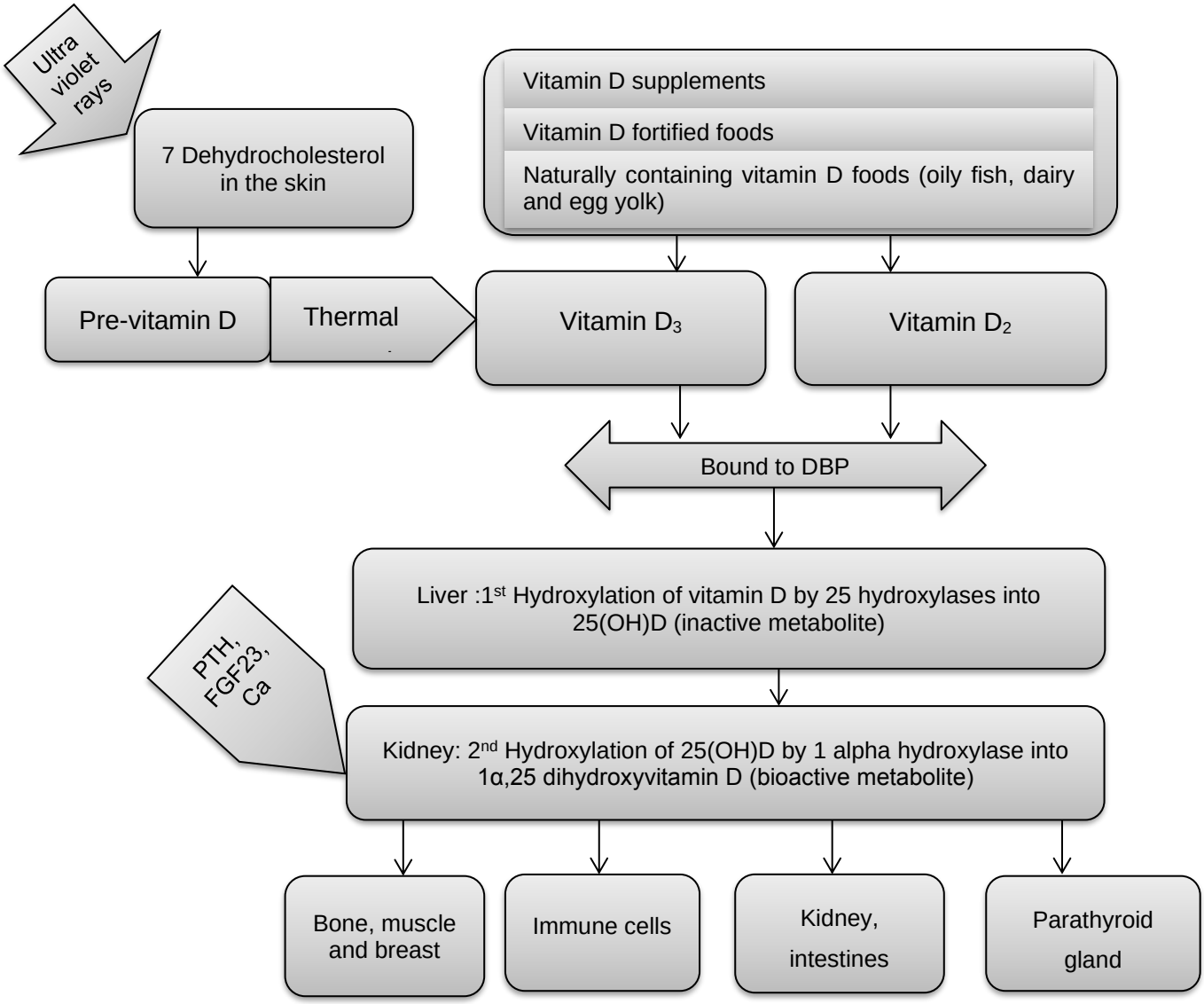
Vitamin D is a pro-hormone critically important throughout the human lifecycle. This vitamin is long known to aid the absorption of calcium in the intestines and maintain calcium homeostasis for proper bone mineralisation and consequent bone health (Khazai *et al.*, 2008; Morris *et al.*, 2014).

Presently, vitamin D is recognised to play a key role in a vast number of physiological processes, with clinical benefits, owing to the presence of VDRs in many body tissues (Pérez-López *et al.*, 2011).

2.4.1 Sources of vitamin D

Vitamin D is acquired from both dietary sources and through skin production by exposure to the sun (Bendik *et al.*, 2014; Vidailhet *et al.*, 2012). The two main forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The former is from plant sources, specifically fungi and fortified foods, and the latter is produced from 7-dehydrocholesterol in the skin, through exposure to the sun. Vitamin D_3 is also sourced from animal foods. Vitamins D_2 and D_3 differ in chemical structure in their side chains with vitamin D_2 having a methyl group at C24 and a double bond between C22 and C23 (Bikle, 2011). Food sources that naturally contain vitamin D include fish, egg yolk, and liver, and foods fortified with vitamin D, including milk products and fruit juices (Yang *et al.*, 2013). Supplements containing vitamin D also contribute to vitamin D status (Lamberg-Allardt, 2006). Cutaneous synthesis of vitamin D contributes a larger percentage than dietary intake of more than 90% of the body's requirements (Holick & Chen, 2008).

2.4.2 Vitamin D metabolism



DBP=Vitamin D binding protein; FGF23 = Fibroblast Growth Factor; PTH = Parathyroid hormone; 25(OH)D =25 Hydroxyvitamin D

Figure 2-1 Vitamin D pathway - adapted from Girgis *et al.* (2013)

Figure 2.1 portrays the vitamin D pathway, indicating two principal routes of vitamin D acquisition: synthesis in the skin and dietary intake. The ultraviolet radiation action on the skin converts 7-dehydrocholesterol present in cells of the epidermis to pre-vitamin D_3, which undergoes thermal isomerisation to vitamin D_3 (Wacker & Holick, 2013). Vitamin D_3 and D_2 are eventually bound to vitamin D binding protein (DBP) and transported in the blood to the liver and kidney for hydroxylation. The first hydroxylation in the liver is facilitated by 25-hydroxylase, which converts vitamin D into 25-hydroxyvitamin D (25(OH)D). The 25(OH)D has a long half-life of approximately

three weeks and is the most predominant metabolite of vitamin D in serum and is thus, measured for the clinical evaluation of vitamin D status. The second hydroxylation takes place in the kidney and is catalysed by 1α-hydroxylase, which converts 25(OH)D into 1 α, 25-dihydroxyvitamin D (1 α,25$(OH)_2$D) the biologically active metabolite which has a shorter half-life (Bikle, 2011). Furthermore, a similar hydroxylation process to that in the kidney takes place in other extra-renal tissues and cells, like the monocyte and macrophages (Christakos *et al.*, 2013). The rate of 1 α,25$(OH)_2$D production is regulated by plasma parathyroid hormone (PTH), serum calcium, and fibroblast growth factor (Henry, 2011). The bioactive form, 1 α, 25$(OH)_2$D acts on target cells by binding to the VDR present in multiple cells including the intestinal epithelium, renal tubules, parathyroid gland cells, skin (keratinocytes), mammary epithelium, pancreas (beta islet cells), pituitary gland, skeleton (osteoblasts and chondrocytes), immune system (monocytes, macrophages, and T-lymphocytes), and germ tissues (Deluca, 2004).

2.4.3 Functions of vitamin D

Vitamin D primary function in the absorption of calcium and phosphate in the small intestines, calcium mobilisation and reabsorption from the bones and kidneys, respectively, to maintain calcium homeostasis (Blaine *et al.*, 2015). There are two mechanisms for calcium homeostasis, namely, active transport of calcium from the intestine to the circulation through VDRs in the intestinal wall and para-cellular transport, which depends on the calcium gradient (Lips, 2012). Studies have, however, yielded mixed results on the role of vitamin D in improving BMD. Combining vitamin D with calcium is proven to be more effective in non-vertebral fracture risk reduction in old age with deficient vitamin D and calcium baseline values (Lips *et al.*, 2014).

The presence of VDR in multiple tissues such as parathyroid gland, ovarian cells, lymphocytes, colon cells, heart, liver and skin keratinocytes led to the postulation of other roles for vitamin D by binding to the VDRs (Bikle, 2014; Christakos *et al.*, 2013). In 2006, Cantorna demonstrated the role of vitamin D in the prevention of autoimmune disorders, such as type 1 diabetes mellitus which occurs as a result of autoimmune response in the islets of Langerhans cells of the pancreas by suppressing the T helper lymphocytes (Cantorna, 2006). Furthermore, vitamin D is key in the prevention of cardiovascular conditions due to its anti-atherosclerotic and anti-inflammatory benefits on cardiovascular risk factors and protecting the heart (Pilz *et al.*, 2011).

Furthermore, vitamin D sufficiency has been associated with improvement of muscle mass, muscle strength, muscle function, balance, prevention of fall, and fracture risk in older people (Bischoff-Ferrari *et al.*, 2009). Zhu *et al.* (2010) evaluated the effect of vitamin D supplementation on muscle strength and mobility in a RCT among older women with hypovitaminosis D. A positive effect of vitamin D supplementation on muscle strength at knee flexor and hip muscle strength was

demonstrated in the treatment group after 1 year of supplementation (Zhu *et al.*, 2010). However, Aloia *et al.* (2019) failed to replicate the prevention of fall benefit by maintaining serum vitamin D at 30 ng/ml in an RCT involving healthy black older women. Despite the improvement in vitamin D status to 47 ng/ml in the treatment arm as compared to 21 ng/ml in the placebo arm, 46% and 47% falls were reported, respectively (Aloia *et al.*, 2019; El Hajj *et al.*, 2019).

2.4.4 Vitamin D deficiency

Based on skeletal health outcomes, cut-off points by the Institute of Medicine (IOM) classifies 25(OH)D insufficiency levels as between 12–20 ng/mL (30–50 nmol/L) and deficiency as levels less than 12 ng/mL (30 nmol/L) (Ross *et al.*, 2011). However, the most commonly used cut-off points are the Endocrine Society and International Osteoporosis Foundation cut-off's that define vitamin D insufficiency as serum 25(OH)D concentration between 20–29 ng/mL (50–74 nmol/L), and deficiency at levels < 20 ng/mL (50 nmol/L), based on PTH rise and fall point in response to 25(OH)D status (Valcour *et al.*, 2012). Vitamin D deficiency results in low calcium absorption in the gastrointestinal tract and poor reabsorption from the kidneys. Vitamin D deficiency coupled with low calcium intake or absorption, triggers PTH production, which promotes calcium release from the kidney and the bone in order to keep calcium in circulation at optimum levels, thereby, increasing the risk of osteopenia and fractures (Wolff *et al.*, 2008). Hyperthyroidism and hypovitaminosis D have independently been found to be associated with the development of metabolic syndrome and other chronic diseases (Muscogiuri *et al.*, 2010).

2.4.5 Determinants of vitamin D status

Vitamin D intake and sun exposure are major determinants of vitamin D status. However, other risk factors leading to vitamin D deficiency include obesity, dark skin pigmentation, old age, chronic disease, atmospheric pollution, being of the female sex, and seasonal variations (Theodoratou *et al.*, 2014).

2.4.5.1 Relationship between vitamin D status and age

Advanced age is characterised by a number of bodily changes. These include increasing adiposity and alterations in vitamin D metabolism (Chan & Woo, 2011). Old age is a risk factor for decreased vitamin D production in the skin due to less sun exposure, decreased 7-dehydrocholesterol in the epidermis and poor ability of the skin to synthesise vitamin D. The vitamin D synthesis capacity of the skin in the elderly is reduced even with similar ultraviolet (UV) light exposure (Hagenau *et al.*, 2009). Furthermore, aging is characterised by a decline in the number of VDRs as well as 1α hydroxylase activity, thereby undermining vitamin D hydroxylation (de Jongh *et al.*, 2017). The Dutch Longitudinal Aging Study Amsterdam showed an increase in serum 25(OH)D concentrations in individuals aged 55–65 years old during a period of six years. In contrast, 65-88 year old

participants had their serum 25(OH)D levels decreased during a period of 13 years (Van Schoor *et al.*, 2014). Similarly, the Dallas Heart Study, a multiethnic adult population study in Dallas, Texas, USA evaluated longitudinal changes of serum 25(OH)D. The study revealed a small but significant decline in serum 25(OH)D levels over the study period between 2000-2002 and 2007-2009. Cohort aging, male sex, Hispanic ethnicity and obesity were reported as some of the factors explaining the changes (Mirfakhraee *et al.*, 2017).

2.4.5.2 Relationship between vitamin D status and sex

Sex remains one key determinant of vitamin D status, with women presenting with lower serum vitamin D levels than their male counterparts (Hussain *et al.*, 2014; Verdoia *et al.*, 2015). As previously demonstrated, women have an increased fat mass compared to men and this may partially explain the low serum vitamin D levels in women through the sequestration theory. The theory hypothesized that after absorption vitamin D as a fat soluble vitamin is sequestrated and stored in fat and released slowly into circulation (Eisner *et al.*, 2010; Wagner & Greer, 2008). However, some studies have reported conflicting results with men being more vitamin D deficient than women. Men are exposed to other factors that undermine vitamin D metabolism like smoking, central obesity and low vitamin D intake (Ardawi *et al.*, 2012).

Sex hormones play a crucial role in influencing vitamin D status (Lerchbaum & Obermayer-Pietsch, 2012). Lower serum 25(OH)D concentration has been associated with higher testosterone in both men and women. Whereas, lower oestrogen levels in females is associated with hypovitaminosis D (Zhao *et al.*, 2017). The postmenopausal state characterised by reduced oestrogen levels explains the low vitamin D status among postmenopausal women. Low serum vitamin D concentration in adults are associated with bone disorders i.e. osteoporosis (Tsiaras & Weinstock, 2011).

2.4.5.3 Relationship between vitamin D status, ethnicity and skin pigmentation

Vitamin D synthesis in the skin contributes approximately 90% of the total vitamin D status of the body. Skin pigmentation, melanin, is the most important determinant in the endogenous synthesis (Lagunova *et al.*, 2011; Wagner & Greer, 2008) of vitamin D_3 photosynthesised (Bogh *et al.*, 2011). Dark skinned people have larger amounts of the melanin pigment in the epidermal layer, which reduces the skin's ability to produce vitamin D from sunlight than fairer individuals (Nair & Maseeh, 2012). Melanin reduces vitamin D production by acting as a natural sunscreen preventing UV-B rays from reaching the layers of the epidermis that contain the highest concentrations of 7-dehydrocholesterol, the precursor to endogenous vitamin D_3 (Misra *et al.*, 2008).

A meta-analysis confirmed vitamin D deficiency among dark skinned individuals. The study further found middle east inhabitants as having a higher vitamin D deficiency, followed by Sub-Saharan

African women, as a result of the impact of dark skin than their counterparts (Martin *et al.*, 2016). However, contrary results were reported in a study by Bogh *et al.* (2010), where the baseline serum 25(OH)D and total cholesterol levels of 182 fair-skinned and dark-skinned participants were measured to determine the effect of UV radiation on their serum 25(OH)D levels. They found that the amount of serum 25(OH)D produced was influenced by the amount of cholesterol in the skin, not skin pigmentation (Bogh *et al.*, 2010).

2.4.5.4 Relationship between vitamin D and smoking

Smoking metabolites are part of heavy metals in the environment and are associated with low vitamin D status in humans (Kassi *et al.*, 2015). Heavy metals, air pollution and tobacco smoke disrupt a number of biochemical pathways including vitamin D metabolism and consequently, lower the two main metabolites of vitamin D, 25(OH)D and 1α,25$(OH)_2$D (Mousavi *et al.*, 2019). Metabolites of smoking alter the metabolism of vitamin D by inhibiting the hydroxylation processes in the liver (Aboraia *et al.*, 2010). Kassi *et al*. (2015) demonstrated an increased risk to vitamin D deficiency in smokers as compared to non-smokers. Another cross-sectional study revealed an inverse correlation between smoking and vitamin D status. The deficiency in vitamin D was also noted to be dependent on the number of cigarettes smoked (Jiang *et al.*, 2016). Further evidence suggests that smoking alters both serum vitamin D concentration and PTH (Cutillas-Marco *et al.*, 2012). Parathyroid hormone and vitamin D are both known calcium-regulating hormones and are critical in bone health.

2.4.5.5 Relationship between vitamin D status and HIV/AIDS plus ART

A number of studies have reported high prevalence of vitamin D deficiency among HIV infected patients (Pinzone *et al.*, 2013; Welz *et al.*, 2010). HIV and ART stand out as additional risk factors for hypovitaminosis D (Lake & Adams, 2011). Estimates of vitamin D deficiency in HIV-infected patients have been reported at approximately 40% in population based studies (Pinzone *et al.*, 2013; Viard *et al.*, 2011). Causes of vitamin D deficiency in those sero-positive for HIV remain multifactorial. HIV is characterised by heightened inflammatory processes and coupled with ART metabolism which alters vitamin D metabolism (Childs *et al.*, 2012; Mueller *et al.*, 2010). Protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) ARVs are associated with alteration in vitamin D metabolism. The PI and NNRTI alter metabolism via modulation of the cytocrome P450 system and inhibition of hydroxylation enzymes (Cohen *et al.*, 2014; Guidi *et al.*, 2014). Brown and McComsey (2010) demonstrated that efavirenz, a form of ARV in the NNRTI class of ARVs, is significantly more associated with decreased vitamin D deficiency than non-efavirenz regimens. A cross-sectional study in Barcelona Spain among HIV-infected patients mostly male reported hypovitaminosis D in as many as 71.2% of the infected, with 39.6% of them by having serum 25(OH)D concentration below 10 ng/mL (Lerma *et al.*, 2012). A systematic review

on the effect of vitamin D deficiency and ART on bone health in HIV-infected patients reported high vitamin D deficiency, and consequently a low BMD. Efavirenz and tenofovir were associated with reduction of vitamin D, and raised PTH, respectively (Childs *et al.*, 2012).

2.4.5.6 Relationship between vitamin D status and seasons

Endogenous synthesis of vitamin D is key to vitamin D status compared to food sources or supplementation (Bendik *et al.*, 2014). Seasonal variations as a determinant of vitamin D status is critical in both clinical and public health to aid diagnosis and guide intervention. Less sunlight during winter has been reported to extensively undermine endogenous synthesis of vitamin D due to inadequate UV radiation (Klenk *et al.*, 2013).

2.4.6 Interaction between vitamin D and body composition

The interaction between vitamin D and body composition is of interest to many researchers. In Table 2.2 studies that focused on the association between vitamin D and body composition encompassing fat mass, lean mass and bone are summarised. Obesity is a body composition change characterised by an increase in fat mass is linked to numerous poor health outcomes (Thiese *et al.*, 2015). On the other hand hypovitaminosis D is consistently associated with overweight and obesity (Araghi *et al.*, 2015). Obesity has been linked to altered physiological pathways in the metabolism, production and mobilisation of vitamin D through reductions in dermal synthesis, dermal release into circulation, and intestinal absorption (Tsiaras & Weinstock, 2011). It is hypothesised that the inverse correlation between fat mass and vitamin D status could be due to the metabolic clearance. This is through enhanced uptake of vitamin D by fat tissue and decreased bioavailability upon fat infiltration (Wortsman *et al.*, 2000). Vitamin D is a fat-soluble vitamin and evidence points towards the theory of sequestration (Rosenstreich *et al.*, 1971). Adipose tissue is the major storage of vitamin D, and the slow release of vitamin D undermines its bioavailability (Rosenstreich *et al.*, 1971). An *in vivo* study using liquid chromatography and mass spectrometry reported the presence of vitamin D in subcutaneous fat tissue and serum, but no association between tissue and serum vitamin D, probably due to the small sample of 17 in obese individuals (Blum *et al.*, 2008). Primarily, vitamin D is known for its role in bone health through the homeostasis of calcium and phosphate signifying the interaction of vitamin D and BMD in improving bone structure (Blaine *et al.*, 2015).

2.4.6.1 Postmenopausal women

Postmenopausal status is among the factors that increase the risk of hypovitaminosis D due to the associated hormonal and body composition changes (Valladares *et al.*, 2019). Vázquez-Lorente *et al.* (2020) reported an inverse correlation between serum 25(OH)D and anthropometric measurements that include mid-upper arm circumference, hip perimeter, fat mass and BMI at cut-

off of >27kg/m^2. Of note in this cross-sectional study was the small sample size of 78 women, which might have limited the control of other confounding factors that could explain the interaction. However, a similar study in India involving a fairly large sample of 250 postmenopausal women confirmed an inverse relationship between BMI and serum 25(OH)D (Joshi *et al.*, 2013). In contrast, another cross-sectional study reported no correlation between serum 25(OH)D and BMI, body weight and fat mass. The study authors argued that the above result was based on the unique body composition structure of the study participants (Chinese postmenopausal women), with a lower prevalence of obesity than in other ethnic groups (Li *et al.*, 2014). While replicating the above findings of no association between serum 25(OH)D and BMI, Andreozzi *et al.* (2016) found an association between serum 25(OH)D and A/G fat ratio. Postmenopause is characterised by unique body composition changes, with increased android fat mass. Android fat mass is postulated to have great capacity to bind vitamin D (Sharma *et al.*, 2012). This points towards fat distribution as key in the fat mass and serum 25(OH)D interaction, and probably explains the inverse correlation between A/G fat ratio and serum 25(OH)D.

Vitamin D is primarily known to influence bone health. Kruger *et al.* (2018) reported in an RCT a bone sparing effect in the vitamin D fortified supplementation group on femoral neck BMD unlike the loss in the control group. Lumbar spine BMD remained stable in all the groups over the 12 months. This signify site specific interactions of vitamin D and BMD. Both groups had adequate vitamin D status at baseline, with a significant increase in the treatment group and slight insignificant decline in the control group over time. The serum 25(OH)D change corresponded to the changes in femoral neck BMD though insignificantly (Kruger *et al.*, 2018). Another RCT confirmed a positive effect of vitamin D supplementation on BMD as a primary outcome in a 1-year RCT. Comparatively, serum 25(OH)D declined in the placebo group, where a small and big increase in serum 25(OH)D was observed in the 400 IU and 1000 IU supplemented groups, respectively. The positive effect of vitamin D supplementation on BMD was dose dependent (Macdonald *et al.*, 2013).

2.4.6.2 Premenopausal women

Hamill *et al.* (2013) investigated the association between bone mass, body composition and vitamin D status of ARV-naïve groups, in urban black South African premenopausal women without HIV and with HIV infection stratified by the number of 'helper' T-lymphocytes in a cubic mm of blood (CD4 count). The authors reported no significant difference in BMD and vitamin D status between the three groups (HIV non-infected, HIV-infected with preserved CD4 cell count, low CD4 cell counts prior to ARV initiation group). Body weight is one major determinant of BMD and might have provided a bone sparing effect. There was a high rate of overweight and obesity in all the three groups at 65% in the HIV non-infected and HIV-infected with preserved CD4 cell count and 44%

in HIV low CD4 ART eligible group. It was further argued that the participants had no huge differences clinically as the severe HIV-infected were immediately initiated on HAART as guided by the South African protocol (Hamill *et al.*, 2013).

A 12-month follow-up longitudinal study on the same cohort of participants had novel findings on body composition and serum 25(OH)D changes. At baseline, the HIV-infected group on ART was s lighter (low lean and fat mass) than the HIV non-infected and HIV infected but preserved CD4 count groups. Despite both the HIV non-infected and HIV-infected on ART group gaining weight and fat mass over 12 months, the latter remained lighter than the former. The two HIV infected groups had lower BMD at baseline, but not significantly different from the HIV non-infected group. Hamill *et al.* (2017) indicated a significant bone loss at femoral neck and lumbar spine BMD in the HIV-infected on ART group despite an increase in fat mass and body weight. The BMD of the HIV non-infected groups and HIV infected women with preserved CD4 count increased at the total hip and at the lumbar spine, but BMD was also increased in the HIV non-infected group. The changes become non-significant after size adjustment thereby suggesting a link to body weight changes. Excluding data from the ART unexposed women in the HIV-infected ART group and women who had initiated ART in the HIV infected, but not ART group was suggestive of the fact that the bone loss in HIV-infected ART group was related to ART exposure. Further evidence from the baseline BMD values showed no differences between the groups. There was no difference in 25(OH)D over time or between the groups (Hamill *et al.*, 2017).

Hamill *et al.* (2020) further followed up the cohort up to 24 months. The study participant subcategories were HIV infected on ART initiated within the first 12 months, HIV infected not on ART and HIV non-infected. A significant finding was the attenuation of bone mass loss beyond 12 months up to 24 months in the HIV infected on ART group. This signifies that bone loss in ART patients within the first 12 months could be preliminary. Despite the weight gain and fat mass increase in the HIV infected on ART group over the 24 months, they remained lighter than the other groups. The HIV non-infected group also gained a significant amount of weight over the 24 months but not those in the HIV-infected not on ART group. There was no observed bone loss in the two other groups, the HIV-infected but not on ART and the HIV negative group. Serum 25(OH)D remained at a mean concentration of greater 50 nmol/L in all the groups and there was no difference between groups over time. It was evident that the changes in body composition were not associated with serum 25(OH)D.

2.4.6.3 Vitamin D and body composition interaction in men and women

Araghi *et al.* (2015) examined the the association between obesity measured by both BMI and percentage body fat and serum 25(OH)D concentration in older men and women. BMI was

inversely associated with serum 25(OH)D concentration. The association was stronger in participants younger than 80 years than in older ones. Each unit increase in BMI equated to a 0.93 nmol/L decline in 25(OH)D concentration. A systematic review and meta-analysis on observational studies further confirmed a positive association between BMI and vitamin D deficiency regardles of age (Pereira-Santos *et al.*, 2015).

Furthermore, it was also observed that increased fat percentage was associated with low serum 25(OH)D levels among older men and women mean age 74 years (Araghi *et al.*, 2015). Similar results were reported in a cross sectional study of older men and women mean age 64 years that categorised vitamin D status into four quartiles namely 4.7–17.5 (Quartile 1), 17.6–26.0 (Quartile 2), 26.1–34.8 (Quartile 3) and 34.9–62.5 ng/mL (Quartile 4). Fat mass index of participants in the lowest serum 25(OH)D quartile was higher than those of participants in third and highest quartile 25(OH)D signifying fat mass as an important predictor of lower vitamin D status. Lean mass and quartiles of vitamin D status displayed no association (Mathieu *et al.*, 2018).

However, Aspray *et al.* (2019) on effect of 1-year vitamin D supplementation on hip BMD in older men and women (≥70 years) reported no improvement in BMD at total hip or neck of femur despite improvements in vitamin D status in the three study arms. The three-arm trial provided vitamin D supplementation once a month at 12000 IU, 24000 IU and 48000 IU. PTH decreased in all the three arms and differences were reported between the 12000 IU and the 48000 IU and not between 12000 IU and 24000 IU vitamin D supplement groups (Aspray *et al.*, 2019). Another RCT reported no positive effect of vitamin D supplementation on prevention of bone loss among 260 black elderly American men and women. The level of 25(OH)D was however improved in the treatment group with 90% of participants above 75 nmol/l (Aloia *et al.*, 2018). Similarly, a systematic review and meta-analysis of RCTs by Reid *et al.* (2014) reported no association between serum 25(OH)D and BMD. The majority (92%) of participants were women and 8% men with an average age of 59 years and mean serum vitamin D of less than 50 mmol/l (less than 20 ng/ml) was reported in eight studies (Reid *et al.*, 2014). In Saudi Arabia no correlation between spine or total femoral BMD and serum 25(OH)D was observed in both older men and women despite 61.5% of the study population being vitamin D deficient at serum 25(OH)D less than 50 nmol/l (Alkhenizan *et al.*, 2017).

2.4.6.4 HIV infected patients

HIV is reported to be one independent factor that influence hypovitaminosis D and alter body composition. Ceballos *et al.* (2019) investigated the association between vitamin D status and BMD in HIV newly diagnosed (one year before recruitment) therapy-naïve men without any secondary causes of osteoporosis. Vitamin D deficiency was found in 66% of the HIV-infected and 48% in the control group signifying an increased hypovitaminosis D in HIV-infected persons. However, the

study reported no associations between BMD, serum 25(OH)D and CD4 count in the HIV infected group (Ceballos *et al.*, 2019). Faber *et al.* (2020) examined the long-term impact of both calcium and vitamin D supplementation on BMD in male and female adult HIV-positive patients. Vitamin D and calcium status had increased from baseline up to follow-up at 70 months. The target of vitamin D level of ≥ 75 nmo/l was reached by 59% of the participants at first and second follow-up. The study results proved that long-term correction of vitamin D and calcium deficiencies improve lumbar spine BMD and tends to attenuate the cART effect. The improvement of BMD at lumbar spine was significant at first follow-up with slight improvement at last follow-up. Hip BMD had initially increased at first follow-up, but a decline preceded at last follow-up though attenuated (Faber *et al.*, 2020).

Table 2-2 Study summary on serum vitamin D and body composition

Reference	Outcome (s) measurements	Study design	Population	Findings
HIV positive participants				
Faber *et al.,* 2020	calcium and vitamin D supplementation on BMD	Retrospective single-center cohort study	Male and female adult HIV+ patients (n=268)	Improved vitamin D and calcium status. Improved lumbar spine BMD.
Ceballos *et al.,* 2019	Vitamin D status and BMD	Cross-sectional	Newly HIV diagnosed (1 year before recruitment) therapy-naïve men (n=70)	No associations between BMD, serum 25(OH)D, viral load (VL) and CD4 count in the HIV infected group.
Postmenopausal women				
Vazquez-Lorente *et al*., 2020	Vitamin D status, relationship between 25(OH)D, its metabolites and anthropometric parameters	Cross-sectional	Spanish postmenopausal women (n=78)	Inverse correlation between vitamin D and anthropometric measurements
Aloia *et al.,* 2018	Vitamin D and bone loss	RCT	Black elderly American women (n=260)	No positive effect of vitamin D supplementation on prevention of bone loss.
Kruger *et al.,* 2018	Vitamin D status after vitamin D fortified milk supplementation and BMD	RCT	Malaysian postmenopausal women (n=121)	Bone sparing effect in the vitamin D fortified supplementation group on femoral neck BMD. Bone loss at femoral neck in the control group.
Reference	Outcome (s) measurements	Study design	Population	Findings
Andreozzi *et al.,* 2016	Vitamin D and adiposity by BMI and body fat distribution	Cross-sectional	postmenopausal women (n=62)	Vitamin D deficiency positively associated with A/G fat ratio and not BMI and waist circumference.
Li *et al.,* 2014	Vitamin D and BMI, body weight and fat mass	Cross-sectional study	Chinese postmenopausal women (n=578)	No correlation between vitamin D and BMI, body weight and fat mass. But positive effect on BMD.
Joshi *et al.,* 2013	Vitamin D and BMI	Cross-sectional study	Postmenopausal women (n=250)	BMI positively associated with low vitamin D level

Table 2.2 Study summary on serum vitamin D and body composition (continued)

Macdonald *et al.,* 2013	Vitamin D supplementation on BMD	1 year RCT	Scotish postmenopausal women (n=305)	A positive effect of vitamin D supplementation on BMD.
Older men and women				
Aspray *et al.,* 2019	Vitamin D and BMD	RCT	Older men and women (n=379)	No improvement in BMD at total hip or neck of femur despite improvements in vitamin D status.
Mathieu *et al.,* 2018	Vitamin D and body composition components (lean and fat mass)	Cross-sectional	Men and women (n=271)	Fat mass index inversely correlated with serum 25(OH)D. Lean mass index not correlated with 25(OH)D.
Alkhenizan *et al.,* 2017	BMD and serum 25(OH)D	Cross-sectional	Older women and men (n=Electronic records of 1723 patients	No correlation between spine or total femoral BMD and serum 25(OH)D.
Araghi *et al.,* 2015	Obesity by both BMI and percentage body fat and serum 25(OH)D concentration	Cross-sectional	Older men and women (n=284)	BMI inversely associated with serum 25(OH)D concentration. Fat percentage associated with low serum 25(OH)D levels.
Pereira-Santos *et al.,* 2015	Obesity by BMI cut-off and vitamin D	Systematic review and meta-analysis	Men and women (23 articles included) (n=65440)	Regardless of age and gender BMI positively associated with hypovitaminosis D
Reid *et al.,* 2014	Vitamin D supplementation on BMD	Systematic review and meta-analysis of RCTs	Older men and women n=23 studies & 4082 participants	No positive effect of vitamin D supplementation on BMD.

BMD = Bone Mineral Density; BMI = Body Mass Index; RCT = Randomised Control Trial; 25(OH)D = 25-hydroxyvitamin D; VL = Viral load

Conclusion

The review shows contradictory finding on the association between serum 25(OH)D and body composition due to presence or absence of covariates. This indicates that the interaction between vitamin D and body composition is dependent on different factors known to influence the interaction. Future studies ought to pay attention to body composition measurement techniques as body composition interaction is also site-specific as demonstrated in the review.

This study focuses on HIV postmenopausal women on ART a study population with a combination of factors that potentially influence the interaction of vitamin D and body composition.

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CHAPTER 3 METHODS

The methods chapter describes in detail the study design, research setting, data collection process, ethical consideration, data management system and the statistical methods and analysis. A brief description of the methods is again described in Chapter 4 as part of the article for publication.

3.1 Study design and population

This study was part of a larger prospective bone study (NWU-00060-14-A1) at the Centre of Excellence for Nutrition (CEN) at the North-West University Potchefstroom campus, South Africa. It was a longitudinal study that involved South African black postmenopausal women with HIV on highly active antiretroviral therapy (HAART). The baseline data, which were collected in 2017 and the follow-up data of 2018 and 2019 of the larger cohort study collected were used for this sub-study. The women were recruited from an outpatient ART clinic at a local hospital and all measurements were performed at the Metabolic Unit of the North-West University. The study design was appropriate to answer the two objectives, namely: to determine the association between vitamin D and body composition (fat, lean and bone mass) and the interdependent associations between fat mass, lean mass, and bone mineral density (BMD).

3.2 Sample size calculation

A power calculation was done using Gpower version 3.1.9.2 to estimate sample size of the larger study. An estimated change in total BMD from 1.05 to 1.0 g/cm^2 in the group (difference 0.05) and a standard deviation (SD) of 0.11 g/cm^2 were used, with 80% power and a 5% level of significance. The estimated difference between the change in total body BMD and SD are based on existing data from a cross-sectional study in HIV positive black women studied in 2014 in the North West Province. The power calculation indicated that 53 women per group would be sufficient to show a difference of 0.06 g/cm^2**.**

This number is in line with the number of participants in randomised control trials (RCTs) included in the meta-analysis of Shea *et al.* (2002) ranging from 19 to 238 participants per group. The sample size in several other RCTs ranged from 20 per group Fujita *et al.* (2004) to 18 per group Jackson *et al.* (2006), but three studies included 36 to 59 participants per group (Albertazzi *et al.*, 2004; Cleghom *et al.*, 2001; Manios *et al.*, 2007).

This sub-study was performed on theparticipants from the larger study which was 120 postmenopausal women. A power calculation for the primary analysis of this study (multivariable regression analysis) indicated that a sample size of at least 262 would be necessary at a ß of 0.4

for the primary exposure, a power of 0.95 and an α of 0.05. Even at a lower ß and power a sample size of at least 220 would be required (Faul *et al.*, 2009). It was therefore possible that the sample size was too small to show an association even if one existed, but it was not possible to recruit more women, because most HIV positive women were premenopausal. We therefore analysed the available data of postmenopausal women who are at risk of low BMD.

Table 3-1 Sample size and characteristics of studies on the association between vitamin D and body composition (lean, fat and bone mass)

Authors and year of publication	Title	Sudy design	Country	Sample size
Vázquez-Lorente *et al.*, 2020	Association between body fatness and vitamin D3 status in postmenopausal women.	Cross-sectional	Spain	78
Aloia *et al.*, 2019	Vitamin D and falls in older African American women.	RCT	USA	260
Aspray *et al.*, 2019	Vitamin D supplementation in older people to optimize bone health.	RCT	England	379
Aloia *et al.*, 2018	Vitamin D supplementation in elderly black women does not prevent bone loss.	RCT	USA	260
Macdonald *et al*., 2013	Hip bone loss is attenuated with 1000 IU but not 400 IU daily vitamin D3 in postmenopausal women.	RCT	United Kingdom	305
Joshi *et al.*, 2013	Prevalence of vitamin D deficiency among postmenopausal women and associated obesity and cardiovascular risk.	Cross-sectional	India	250
Zhu et al., 2010	Effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency.	RCT	Perth, Australia	302

IU = International units; RCT =Randomised control trial

Inclusion and exclusion criteria

Inclusion criteria: HIV-positive postmenopausal women on HAART were included in the study. Menopause was defined as the absence of menses for at least six months prior to the study.
Exclusion criteria: Participants using anti-osteoporotic agents, use of treatment known to affect BMD (corticosteroids, thyroid medication initiated during past year, anti-vitamin K agents, diuretics, anti-epileptic drugs, as well as β-blockers) were excluded. Presence of secondary causes of osteoporosis (chronic liver disease, chronic obstructive pulmonary disease, chronic renal disease, immobility, rheumatoid arthritis, gastrectomy, malabsorption syndromes); diagnosed diabetes mellitus; history of metabolic bone disease; habitual use of calcium and/or vitamin D supplementation; habitual use of anti-acids containing calcium; high alcohol consumption (≥ 3 units/day); a fracture within the last six months were factors of exclusion. Women with severely low BMD (T score < –3) at baseline were not enrolled, but referred for

medical treatment. All the listed exclusion criteria above are reported to impact bone health and could have influenced the study's true result.

3.3 Recruitment, enrolment and informed consent

The women were recruited from an outpatient clinic for HIV-positive adults at the Potchefstroom Hospital where advertisements in English and Setswana were displayed in the waiting area of the wellness clinic from one week before recruitment in 2017. There were telephone contact numbers of some research team members on the advertisement. Recruitment was done by a research assistant, who was proficient in both English and Setswana, and he served as the independent person during recruitment. The assistant sat in the clinic next to the advertisement, so that the nurses at the clinic (gatekeepers) could refer those who were interested in the study to him for recruitment. He also gave study information to groups of women in the waiting room. Informed consent was obtained from all participants and they had the opportunity to ask questions **(Annexure B).** He made a list of possible participants with telephone numbers and addresses. After telephonic confirmation of participation, informed consent forms were collected from the participants.

It was expected that no more than 20 women would be recruited per week and recruitment would continue for 6-8 weeks. A screening questionnaire **(Annexure C)** was used to determine eligibility. Potential participants who could possibly meet the inclusion criteria were approached individually by the clinic nurse and the research assistant. If potential participants complied with the screening questionnaire criteria a group information session was used to discuss the study. The study procedures were explained to the potential participants by the assistant and those who gave informed consent were included. During the information session, participants were made aware that they could withdraw from the study at any time. They were also assured that non-participation in the project by the individual would be respected and would not have any negative consequences in terms of their routine treatment and care at the outpatient clinic at Potchefstroom Hospital. The potential participants had two days to decide and a follow-up phone call was made to confirm whether they wanted to participate and to collect the informed consent forms.

3.4 Study setting

The women were recruited from the outpatient ART clinic at Potchefstroom Hospital but all measurements were performed at the Metabolic Unit of the North-West University. All particiapnts were collected from their homes according to the appointment schedule to the Metabolic Unit of the North-West University for measurements.

3.5 Data collection

All measurements required and used in this sub-study were collected at three time points from each of the participants starting with the baseline data in 2017 a follow-up in 2018 and the last data collection was in 2019.

3.5.1 Social-demographic and health information

The socio-demographic and health information of the women included age, educational status, housing, occupation, smoking, alcohol consumption, chronic medication and year of HIV diagnosis and start date for ARVs. These were determined using an interviewer-administered structured questionnaire **(Annexure D and E)** validated for this population group and used in a group with a similar age range in the PURE study.

3.5.2 Anthropometric measurements

Anthropometric measurements (height, weight, waist circumference, mid-upper arm circumference, calf circumference) were performed by trained postgraduate nutrition students using standard methods, with participants wearing light underwear and gowns provided at the Metabolic Unit. Anthropometric measurements as well as CD4 count, viralload and ART regime were recorded on the case report form **(Annexure F).**

Weight and height measurements were performed using a calibrated digital scale and with stadiometer (Seca 264, Hamburg, Germany), respectively. Height (cm) was measured to the nearest 0.1 cm with the participant barefoot and with the head in the Frankfort plane. Weight was measured to the nearest 0.01 kg. BMI was calculated as weight (kg) divided by height (m) squared. After the first measurement, a second measurement was taken with the same scale or stadiometer. If the two measurements differed with 0.5 cm and 0.1 kg for height and weight respectively, a third measurement was taken and the average of the two closest measurements was used (Lee, 2013).

3.5.3 Physical activity

Physical activity was assessed at the same visits by a trained fieldworker using the Global Physical Activity Questionnaire (GPAQ) **(Annexure G)** recommended by the World Health Organisation (WHO) (WHO, 2017). The questionaire gathered information on physical activity done in the previous seven days in the following; occupation physical activity, transport-related physical activity and physical activity during leisure time. Time spent during the various physical activity domains in terms of frequency (days per week) and duration (minutes per day) were estimated.

3.5.4 Bone mineral density

BMD was measured by a registered radiographer through DXA with the default Hologic settings (Hologic Discovery W, APEX system software version 2.3.1) at total body, lumbar spine and left femoral neck of the hip in g/cm². Participants were asked to remove all jewellery and were provided with a cotton gown without any metal trimmings to wear during the DXA measurement. Each participant received verbal feedback of their DXA scans. The DXA machine is based at the metabolic unit of the North-West University, Potchefstroom campus.

3.5.5 Serum vitamin D concentration

Fasting blood samples of 5 ml were collected in serum tubes by a registered nurse from the Metabolic Unit of the North-West University. The samples were centrifuged immediately after collection. Serum was prepared and stored in a bio-freezer at –80°C until all samples of all 120 women in the study were collected. Serum 25(OH)D was measured by immunoassay (Vitamin D total Elecsys Cobas Roche Diagnostics, Johannesburg, South Africa). All samples were analysed together in one batch with the same controls.

3.5.1 Dietary assessment

Dietary intake was assessed at baseline and at one-year follow-up visits in 2018 by trained fieldworkers using a validated quantitative food frequency questionnaire **(Annexure H),** food models and a validated food picture book to estimate portion sizes (Venter *et al.*, 2000; Wentzel-Viljoen *et al.*, 2011). Portion sizes, reported in household measures, were converted to weights. Nutrient intakes were calculated using software based on the South African food composition database (Wolmarans *et al.*, 2010).

3.6 Statistical methods

The distribution of the continuous variables were assessed by histograms, q-q plots and the Kolmogorov-Smirnov test. Descriptive statistics of socio-demographic data, physical activity, BMD at the different sites, lean and fat mass, as well as serum 25(OH)D were presented as means and SD (variables following a normal distribution) or median and IQR (non-normally distributed). The percentage of participants with vitamin D deficiency and insufficiency were determined, where vitamin D deficiency was defined as serum 25(OH)D level < 20 ng/mL (50 nmol/L) and insufficiency as 20–29 ng/mL (50–74 nmol/L) according to the Endocrine Society and International Osteoporosis Foundation cut-offs (Valcour *et al.*, 2012).

Pearson's correlation coefficient (for data with a normal distribution) or Spearman's rank order correlation coefficient (for data with a non-normal distribution) was calculated to examine the correlation between serum 25(OH)D and fat mass, lean mass and whole body BMD, spine BMD and left femoral neck of the hip BMD, as well as all possible covariates (age, alcohol intake, calcium intake, physical activity level and number of years since HIV diagnosis). Partial Pearson correlation controlled for age were performed. Variables with a significant correlation were entered in the linear mixed models (LMM). Variables with a non-normal distribution were logarithmically transformed before they were entered in the LMM. Differences in exposures and outcomes between groups (smokers vs non-smokers) were assessed using the Mann-Whitney test, because most variables had a non-normal distribution.

Pearson or Spearman correlation analysis was also performed in order to examine the correlation between fat mass, lean mass and whole body BMD, spine BMD and left femoral neck of the hip BMD, as well as all possible covariates (age, alcohol intake, calcium intake, physical activity level and number of years since HIV diagnosis). Age correlated significantly with several variables, therefore partial Pearson correlation with adjustment for age was also performed. Variables with a significant correlation were entered in the linear mixed models.

LMM were used with fat mass, lean mass and BMD, respectively, as the dependent variables (outcomes) and serum 25(OH)D as the primary exposure, with adjustment for variables identified to correlate with the exposure or outcomes (age, alcohol intake, calcium intake, and physical activity level). LMMs were also used with BMD as the dependent variable (outcome) and fat mass as well as lean mass as primary exposures, with adjustment for variables identified to correlate with the exposure or outcomes (age, alcohol intake, calcium intake and physical activity level). Separate models were used for whole body BMD, spine BMD and left femoral neck of the hip BMD, respectively. Time was treated as a fixed effect and participant as random effect. The restricted maximum likelihood (REML) estimation method was used and an unstructured covariance structure was specified.

An adapted version of Cohen's d was used to indicate the practical significance of the differences between the means. The original formula for Cohen's d for ANOVA analysis is:

$$d = \frac{|\bar{x}_i - \bar{x}_j|}{\sqrt{MSE}}$$

In this formula d = effect size, ẋi and ẋj are means of two samples, MSE is mean standard error) (Cohen, 1992).

In the modified formula however the estimated marginal means, are calculated as part of the LMM analyses and the MSE was replaced by the sum of the covariance estimates. The interpretation guidelines stayed the same: an effect size of 0.2 indicates a small effect or practical non-significant difference, an effect size of 0.5 indicates a medium effect or practical visible difference and 0.8 a large effect or practical significant difference. For interpretation, the following values were used as guidelines to determine the strength of the correlations in practice, where $|r| \approx 0.1$ indicates a small practical significance, $|r| \approx 0.3$ a moderate significance, $|r| \approx 0.5$, or larger, a large practical significance. Statistical Package for the Social Sciences (SPSS) software, version 26 (IBM Company, Armonk, NY, USA) was used for all statistical analyses.

3.7 Ethical consideration

3.7.1 Permission

The Health Reasearch Ethical Committee (HREC) of the North-West University granted the prospective cohort bone study ethical approval (NWU-00060-14-A1). This sub-study forms part of the larger cohort study, a letter of permission was submitted to the HREC of the North-West University and approval was granted to conduct the study NWU-00061-17-A1-02. A goodwill permission letter to recruit patients and approval access to patients' files were obtained from the clinical manager and the nurse in charge of the HIV Outpatient Clinic at Potchefstroom Hospital. Data from the files were only obtained after the participants themselves gave consent. Approval from the North West Department of Health to conduct the study was also obtained.

3.7.2 Benefits

Participants received information regarding their BMD and other health information such as BMI and body fat percentage. The BMD values were given with an interpretation of what it meant at the study visit. If any abnormalities were detected, it was discussed with the participant and they were referred to the hospital or clinic with relevant information. This information was given to the participants in a private room by the research team.

3.7.3 Anticipated risk and precautions

Transporting participants to the Metabolic Unit could lead to stigmatisation from participants' neighbours or risk of vehicle accidents. Therefore, the participants were picked up at home early in the morning by an experienced and trusted driver in a neutral white university car without any HIV logos on it. If they had concerns about stigmatisation and preferred not to be noticed during the pick-up, they were picked up two blocks from their homes at a previously arranged place.

During anthropometry and DXA measurements, participants wore light underwear and cotton gowns provided at the metabolic unit, in order to minimise discomfort from exposing themselves. The measurements were performed in private rooms with the participant and only one or two female researchers present. The potential risk of X-ray exposure from a single DXA scan is minimal and the procedure itself does not cause any pain or discomfort. Due to regulatory requirements regarding X-ray exposure only the radiographer was present when DXA measurements were done.

Participants had a risk of feeling faint as fasted state blood samples were collected. Therefore, a snack was provided immediately after blood sample collection and anthropometry. All the measurements took about two to three hours. Lunch and tea/juice/water were provided throughout the day at the air-conditioned waiting area and TV and magazines were available to reduce the risk of boredom. Masters students in dietetics and nutrition guided the participants to get to the next measuring station. Participants could experience pain during blood sampling however, an experienced nurse took 10 ml blood using sterile syringes and needles and standard safety procedures were followed.

A medical doctor and registered nurse supervised the study. The nurse was present at all times during the study. The nurse was experienced and followed the standard procedures for possible incidents that were anticipated during the study. Both to researchers and study participants and was able to report any incidents beyond her scope to the medical doctor and/or NWU Protection Services and HREC. Furthermore, the Emergency Services of the NWU was notified of the study at the metabolic unit and was on standby if any incident occurred. All incidents were directed to the nurse and reported to the medical doctor depending on gravity of the incident. Furthermore, standard operating procedures for incidents, such as needle-prick incidents, were followed in the Metabolic Unit.

3.7.4 Anonymity, privacy and confidentiality throughout the research process

Privacy of participants was assured by using private rooms for measurements at the Metabolic Unit. Furthermore, with regards to confidentiality and anonymity, participants were only given study numbers, their names were not written on the forms or questionnaires. Identification was only done by the study leader. Participants' HIV status was not known by other persons outside the research team. All information about the participants was locked up in the study leaders' office and saved on a password protected computer.

3.8 Respect, justice and autonomy

Participants were treated with respect and had the right to exercise autonomy. No information of a participant was disclosed to anyone outside the research team. The participants were informed regarding all aspects of the study and signed a consent form after thorough understanding. They were free to withdraw at any time without any punishment or denial of general health services.

3.8.1 Management of vulnerability, beneficence/non- maleficence

Transport arrangements were made for all participants from and to their homes. They were provided with refreshments on assesment day. Referal to hospital was done for all those that had abnormal results. The potential risk of exposure to x-rays from a single DXA scan was very minimal and the assesment inflicted no pain.

3.8.2 Safety plan for participants

A medical doctor (Dr Semakula) from Potchefstroom and a registered nurse (Sr Lessing) supervised the study according to the standard safety measures set.

3.8.3 Special precautions

Laboratory work: All staff and trained postgraduate students had obtained Hepatitis A and B vaccinations as per the university requirement before commencement of the study. Appropriate personal protective equipment, including gloves and a laboratory coat were worn at all times, to ensure safe handling of samples. Torn gloves whilst collecting blood samples were immediately replaced so as to ensure safety.

Blood sampling is an invasive procedure, and was approached with the greatest care and performed by an experienced nursing professional to avoid injuries such as needle stick.. Biological sample preparation for storage was done at the CEN blood preparation laboratory. Disposal of waste produced on the site was disposed of according to the biological waste protocol of the Metabolic Unit.

Anthropometry was performed by MSc students. In order to minimise discomfort during anthropometrical measurements, all measurements were done in an allocated private space to ensure privacy and two study team members were present preferably females while the measurements were performed. During the administration of questionnaires, allocated stations were used for data collection in order to ensure privacy. The participants were allowed to rest in between in the case they needed a short break.

3.8.4 Expertise, skills and legal competencies

Study team members were experienced in their field and special training on data collection was given to all involved in data collection, i.e. anthropometry. All measurements were supervised by the principal investigator. Anthropometric measures were supervised by a researcher who is an ISAK Level 3 qualified anthropometrist. Blood samples were collected by a registered nurse, minimising risk and/or harm to the participants. Blood samples were analysed by an experienced researcher in the laboratory of the Hypertension in Africa Research Team (HART) of the NWU, following standard methods and quality control methods.

3.8.5 Token of appreciation

Participants received a token of appreciation of R50 upon completion of data collection session. Participants were transported back to their respective homes on completion of data collection.

3.9 Data management system

3.9.1 Data entry and monitoring

Data entry was conducted by the PhD student and MSc student. The post-doctoral fellow checked all data entered by checking every 10th data entry point. If any discrepancies were found, all data entry points were checked by the PhD and MSc students and corrected if necessary. Minimums and maximums were checked for correctness against hard copies. Data were entered into excel sheet on a password protected computer.

After all data was entered into the datasheets, data cleaning was conducted by the PhD student in collaboration with the postdoc and principal investigator, by checking minimums, maximums and every 10th data entry line. The study was monitored by the researchers and supervisor for progress, deviations and incidents.

3.9.2 Data archiving

All hard copies of the data collection form and other data forms used in this study are locked in the principal investigator's (Prof. H.S. Kruger) office and electronic data is archived on a password protected computer and backed up on an external hard drive which is being stored in the principal investigator's office in a locked file cabinet. Data were recorded and saved by participant number to ensure confidentiality. Data-integrity over the two years has been monitored by the study leader. All data was cleaned by the research group and the final dataset locked. Data will be shared with new postgraduate students and their study supervisors if requested. All hard copies of completed forms will be stored for seven years at least and then destroyed by shredding according to the North-West University's rules and regulations for data and record management.

3.9.3 Quality control

Before data were entered into the secure database, all questionnaires were screened for possible problematic answers. All ambiguous answers were checked with the responsible, trained post graduate students to ensure a clear answer.

All postgraduate students performing anthropometric measurements were trained according to ISAK standards by the principal investigator (Prof. H.S Kruger, a level 3 anthropometrist), who also monitored measurements weekly for accuracy. Instruments that were used (measuring scales and stadiometer was checked and calibrated before use to ensure quality of measurement). All DXA measurements were done according to standard guidelines by an experienced registered radiographer with eight years research experience and comprehensive training.

Serum samples were stored until further analyses for a specified period of time, not longer than six months before analyses. To ensure the integrity of the samples while in storage, serum samples were stored at -80 degrees centigrade in secure, lockable bio-freezers until the determination of the variables examined for the study. Access to these bio-freezers were limited to key personnel. These bio-freezers are monitored via a cellular-based monitoring system. Sample integrity was further maintained via the connection of the freezers to uninterrupted power supplies and centralised power generators. Blood samples were analysed in the Physiology laboratory at North-West University by researchers with the necessary training under supervision of the laboratory manager, Dr. C. Mels. Standard methods and quality control measures were applied.

Data was stored in the Excel file and the computer programme Statistical Package of Social Sciences (SPSS version 23). Data was cleaned and checked by the PhD student (C. Ellis) together with the supervisor (Prof. H.S. Kruger).

3.9.4 Reporting, dissemination and notification of results

Results from any measurements that were immediately available on the day of the study were verbally reported to the participants at the end of the day. This was done privately and if abnormalities in BMD, such as very low BMD were present, a referral letter **(Annexure I)** was provided to the local hospital. The principal investigator explained the results thoroughly to the participants. Feedback on the disease burden within this community is given to the Potchefstroom Hospital Patient Safety Committee after each year of the larger study. Publication of sub-study results will be done as an article in peer-reviewed academic journal and will also be presented at conferences.

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CHAPTER 4 MANUSCRIPT

Title: The association between serum vitamin D and body composition in black South African postmenopausal HIV positive women

Samuel Mwango[1], Janet Carboo[1], Christa Ellis[1], M Cockeran[2], H. Salomé Kruger[1*]

[1]Centre of Excellence for Nutrition, North-West University, Potchefstroom

[2]Statistical Consultation Service, North-West University, Potchefstroom

*Corresponding author

Centre of Excellence for Nutrition

North-West University, Potchefstroom

Tel: +27 18 299 2085

Fax: +27 18 299 2464

Email: salome.kruger@nwu.ac.za

Abstract

Background: HIV/AIDS and highly active antiretroviral therapy (HAART) alter bodily processes, including vitamin D metabolism. Altered vitamin D metabolism is associated with adiposity and bone loss.

Objective: To determine the association between serum 25-hydroxyvitamin D (25(OH)D) and body composition in black South African postmenopausal HIV-positive women on HAART.

Methods: This two-year longitudinal study is part of a larger prospective study (n=120) in the North West Province of South Africa. Measures included serum 25(OH)D concentration, bone mineral density (BMD) at three sites, lean mass and percent body fat (%BF). Multivariable linear mixed models were used to assess the association between serum 25(OH)D and body composition over the two-year period measured at 1 year interval. Linear mixed models (LMM) were used to determine the longitudinal association between lean mass and %BF (exposures) and BMD (outcome).

Results: The 120 study participants average age was 40 years. Vitamin D deficiency and insufficiency increased from baseline (10.2% and 19.5%) to 11.5% and 37.5%, respectively, after two years. Serum 25(OH)D had no association with any BMD outcomes. Lean mass and %BF had comparable positive associations with total spine and left hip femoral neck (FN) BMD, however, lean mass proved as a stronger predictor. Serum 25(OH)D decreased significantly, however with a small effect size of 0.39 (P = 0.001), while total BMD, left hip FN BMD had significant small increases (effect size 0.03, P = 0.02 and 0.06 P = 0.0001 respectively), whereas total spine did not change over the two years.

Conclusion: Serum 25(OH)D was not associated with any BMD outcomes. Though lean mass and %BF had a comparable positive association with BMD, the former exhibited a stronger association with BMD.

Keywords: Vitamin D, postmenopausal, adiposity, BMD, Africa, HIV/AIDS, HAART

4.1 Background

Vitamin D is a pro-hormone primarily known for its role in bone development through calcium and phosphorus homeostasis [1]. Epidemiological studies across the world indicate a close association between vitamin D deficiency and common chronic diseases, such as bone metabolic disorders, tumours, cardiovascular diseases and diabetes mellitus [2]. Prevalence of vitamin D insufficiency/deficiency worldwide varies between 30% and 93% and is a growing public health concern among all age categories and ethnic groups [3-5]. In low- and middle-income countries, hypovitaminosis D is also a prevalent disorder [6]. Durazo-Arvizu *et al*. [7] reported a higher prevalence of vitamin D deficiency among South Africans than blacks from Ghana, Jamaica and Seychelles in adult men and women between 25 and 45 years. HIV infected persons have a higher prevalence of vitamin D deficiency than non-infected persons owing to their additional risks aside from the common factors such as skin colour, gender and low dietary intake known to undermine vitamin D status [8-10].

In South Africa, a total of 7.7 million people were reported to be HIV infected in 2018. This makes South Africa a country with the highest HIV infection burden in the world, with a prevalence of 20.4%, and women are more infected compared than men [11]. Furthermore, South Africa has the largest HIV treatment programme, with 3.9 million people initiated on highly active antiretroviral therapy (HAART) in 2016 in line with the 90 90 90 agenda [12]. HIV/AIDS management strategies coupled with use of HAART have shifted HIV/AIDS trends from an inevitably fatal disease to a chronic infection [13]. Chronic HIV infection and exposure to HAART has been demonstrated to alter vitamin D metabolism, decrease bone mineral density (BMD) and increase fracture risk [14-16]. Body composition alteration (lipodystrophy) is a common phenomenon in HIV infected persons [17]. Furthermore, body composition is one important factor that influences vitamin D status directly or indirectly through interactions with other factors such as age, sex, skin pigmentation/ ethnicity, chronic diseases and medications [18]. An inverse correlation has been established between vitamin D status and adiposity in adults [19, 20]. Low- and middle-income countries, South Africa inclusive, are experiencing nutrition related non-communicable diseases due to the nutrition transition and consequent body composition changes towards increased adiposity [21]. The SANHANES-1 of 2012 reported a higher mean BMI of above 30 kg/m^2 for women older than 45 years, compared to 25 to 26 kg/m^2 for men of the same age [22]. The 2016 South African Demographic and Health Survey confirmed the high rates of overweight and obesity in adult women at 27% and 41% [23].

This study aimed at bringing new knowledge on the association between body composition and serum 25(OH)D among black HIV postmenopausal women. To our knowledge this was the first study to involve this targeted population in South Africa. The population under study has an

increased risk to vitamin D deficiency and poor bone health. Findings from the study can potentially inform treatment and care of the black HIV postmenopausal women.

4.2 Material and methods

4.2.1 Study design and setting

This was a longitudinal study that used data measured at baseline, 1-year and 2-year follow-up from a larger prospective bone study (NWU-00060-14-A1) at the Centre of Excellence for Nutrition (CEN) at the North-West University Potchefstroom campus, South Africa. The study was approved by the Health Research Ethical Committee (HREC) of the North-West University (NWU-00061-17-A1-02). Study participants were recruited from an outpatient ART clinic at a local hospital and all measurements were performed at the Metabolic Unit of the North-West University.

4.2.2 Study participants

The study involved black South African postmenopausal women with HIV infection on HAART. The majority of participants were on HAART regimen 1 for 2017 (tenofovir TDF, emtricitabine FTC, efavirenz EFV) as a combined antiretroviral therapy (cART). This sub-study was performed on the available participants from the larger study, which was 120 postmenopausal women.

A power calculation for the primary analysis of this study (multivariable regression analysis) indicated that a sample size of at least 262 would be necessary at a ß of 0.4 for the primary exposure, a power of 0.95 and an α of 0.05. Even at a lower ß and power, a sample size of at least 220 would be required [24]. It was therefore possible that the sample size may be too small to show an association even if there was one, but it was not possible to recruit more women, because most HIV-positive women were premenopausal. We therefore, analysed and interpreted with caution the available data of postmenopausal women who were at risk of low BMD.

Inclusion criteria included HIV-positive postmenopausal women on HAART. ‘Postmenopausal’ was defined as the absence of menses for at least six months prior to the study. Exclusion criteria included use of anti-osteoporotic agents, (corticosteroids, thyroid medication initiated during past year, anti-vitamin K agents, diuretics, anti-epileptic drugs, as well as β-blockers). Chronic liver disease, chronic obstructive pulmonary disease, chronic renal disease, immobility, rheumatoid arthritis, gastrectomy, malabsorption syndromes, diagnosed diabetes mellitus, history of metabolic bone disease, habitual use of calcium and/or vitamin D supplementation, habitual use of anti-acids containing calcium; high alcohol consumption (≥ 3 units/day), and history of a fracture within the last six months were also part of the exclusion criteria. Women with severely low BMD at the hip (T-score < –3) at baseline were not enrolled, but referred for medical treatment.

4.2.3 Measurements

Data were collected at three time points, namely September to November of 2017, 2018 and lastly 2019. Only data relevant to the substudy objectives were extracted from the larger study database.

4.2.3.1 Socio-demographic and health information

Information on participants' socio-demographic and health status was collected using an interviewer-administered structured questionnaire. The following information was captured: age, education level, housing, occupation, smoking, alcohol consumption, chronic medication use, year of first HIV diagnosis and year of HAART initiation.

4.2.3.2 Anthropometric measurements

Anthropometric measurements included height and weight and were performed by trained field workers and postgraduate Nutrition and Dietetics students. The measurements were conducted on participants without shoes and with minimal clothing. Weight and height were measured using a calibrated digital scale and a stadiometer (Seca 264, Hamburg, Germany), respectively, following standard procedures [25].

4.2.3.3 Physical activity

Physical activity was assessed by a trained fieldworker using the Global Physical Activity questionnaire (GPAQ) recommended by the World Health Organisation (WHO) [26]. The questionnaire gathered information on physical activity done in the previous seven days in the following: occupation physical activity, transport-related physical activity and physical activity during leisure time. Time spent during the various physical activity domains in terms of frequency (days per week) and duration (minutes per day) were estimated.

4.2.3.4 Bone mineral density, lean mass and fat mass

BMD, lean mass and fat mass were measured at all three time points by a registered radiographer through DXA with the default Hologic settings (Hologic Discovery W, APEX system software version 2.3.1). BMD was measured at total body, lumbar spine and left femoral neck (FN) of the hip in g/cm². Participants were asked to remove all jewellery and were provided with cotton gowns without any metal trimmings to wear during the DXA measurement.

4.2.3.5 Serum vitamin D and parathyroid hormone (PTH) concentration

Fasting blood samples of 5 ml were collected in serum tubes by a registered nurse. The samples were centrifuged immediately after collection and stored in a bio-freezer at –80°C until samples of all the study participants were collected. Serum 25(OH)D and PTH were measured by immunoassay (Vitamin D total Elecsys Cobas and PTH Elecsys Cobas, Roche Diagnostics,

Johannesburg, South Africa). Samples collected for each time point were analysed together in one batch with the same controls.

4.2.3.6 Dietary data

Dietary intakes were assessed at baseline and at one-year follow-up visits in 2018 by trained fieldworkers using a validated quantitative food frequency questionnaire, food models and a validated food picture book to estimate portion sizes [27, 28]. Portion sizes, reported in household measures, were converted to weights. Nutrient intakes were calculated using a software based on the South African food composition database [29]. There was no dietary assessment in the last year follow-up 2019.

4.3 Statistical analysis

The distribution of the continuous variables were assessed by histograms, q-q plots and the Kolmogorov-Smirnov test. Descriptive statistics of socio-demographic data, physical activity, BMD at the different sites, lean and fat mass, as well as serum 25(OH)D are presented as means and standard deviation (SD) for variables following a normal distribution or median and inter quartile range (IQR) for non-normally distributed. The percentage of participants with vitamin D deficiency and insufficiency were determined, where vitamin D deficiency was defined as serum 25(OH)D level < 20 ng/mL (50 nmol/L) and insufficiency as 20–29 ng/mL (50–74 nmol/L) according to the Endocrine Society and International Osteoporosis Foundation cut-offs [30].

Pearson's correlation coefficient (for data with a normal distribution) or Spearman's rank order correlation coefficient (for data with a non-normal distribution) was calculated to examine the correlation between serum 25(OH)D and fat mass, lean mass and whole body BMD, spine BMD and left FN of the hip BMD, as well as all possible covariates (age, alcohol intake, calcium intake, physical activity level and number of years since HIV diagnosis). Age correlated significantly with several variables, therefore partial Pearson correlation with adjustment for age was also performed. Variables with a significant correlation were entered in the linear mixed models (LMM). Variables with a non-normal distribution were logarithmically transformed before they were entered in the LMM. Differences in exposures and outcomes between groups (smokers vs non-smokers) were assessed using the Mann-Whitney test, because most variables had a non-normal distribution.

LMM were used with fat mass, lean mass and BMD, respectively, as the dependent variables (outcomes) and serum 25(OH)D as the primary exposure, with adjustment for variables identified to correlate with the exposure or outcomes (age, alcohol intake, calcium intake, and physical activity level). LMMs were also used with BMD as the dependent variable (outcome) and fat mass

as well as lean mass as primary exposures, with adjustment for variables identified to correlate with the exposure or outcomes (age, alcohol intake, calcium intake and physical activity level). Separate models were used for whole body BMD, spine BMD and left FN of the hip BMD, respectively. Time was treated as a fixed effect and participant as random effect. The restricted maximum likelihood (REML) estimation method was used and an unstructured covariance structure was specified.

An adapted version of Cohen's d is used to indicate the practical significance of the differences between the means. The original formula for Cohen's d for ANOVA analysis is:

$$d = \frac{|\bar{x}_i - \bar{x}_j|}{\sqrt{MSE}}$$

In this formula d = effect size, ẋi and ẋj are means of two samples, MSE is mean standard error)[31]. In this case, however, the estimated marginal means, as calculated as part of the LMM analyses, are used and the MSE is replaced by the sum of the covariance estimates. The interpretation guidelines stay the same: an effect size of 0.2 indicates a small effect or practical non-significant difference, an effect size of 0.5 indicates a medium effect or practical visible difference and 0.8 a large effect or practical significant difference. For interpretation the following values will be used as guidelines to determine the strength of the correlations in practice, where |r|≈0.1 indicates a small practical significance, |r|≈0.3 a moderate significance, |r|≈0.5, or larger, a large practical significance. SPSS software, version 26 (IBM Company, Armonk, NY, USA) was used for all analyses.

4.4 Results

The study participants' demographic characteristics are summarised in Table 4.1. A total of 120 postmenopausal women were included in this study at baseline. The majority of participants (approximately 85 percent) were on HAART regimen 1 (tenofovir TDF, emtricitabine FTC, efavirenz EFV) as a combined antiretroviral therapy (cART). Combined vitamin D deficiency and insufficiency was 29.7% at baseline, 42.9% at year 1 follow-up and 49.0% at year 2 follow-up. Serum PTH concentrations showed a trend of an increase over time. Only a small proportion of study participants (10%) were current smokers, with a median of 2 (IQR: 1-4) cigarettes per day. Only 5 (4.2%) of participants were previous smokers.

Table 4-1 Study participants' characteristics from baseline to 2-year follow-up

Variable		Baseline (2017) (n=120)	Year 1 (2018) (n=99)	Year 2 (2019) (n=104)	p-value
		Median (IQR) or n (%)			
Age (years)		50 (48-55)	51 (49-56)	52 (50-51)	
Weight (kg)		66.6 (54.1-80.0)	68.1 (57.9-83.0)	67.6 (54.8-83.9)	
Height (cm)		156.6 (151.5-161.0)	157.5 (152.3-161.5)	156.7 (151.8-160.4)	
BMI (kg/m^2)		27.1 (22.4-32.6)	28.4 (23.5-32.2)	28.3 (23.0-33.2)	
Underweight (BMI<18.5 kg/m^2)		10 (8.3)	4 (3.8)	7 (6.7)	
Normal BMI (18.5-24.9 kg/m^2)		37 (30.8)	33 (31.7)	29 (27.6)	
Overweight (BMI 25-29.9 kg/m^2)		27 (22.5)	24 (23.1)	27 (25.7)	
Obese (BMI > 30 kg/m^2)		46 (38.3)	42 (41.3)	42 (40.0)	
Waist circumference (cm)		85.0 (76.0-97.3)	88.6 (76.9-96.6)	90.8 (76.7-101.0)	
Calf circumference (cm)		33.9 (30.0-37.5)	34.4 (30.7-37.6)	33.9 (30.2-37.7)	
Total BMD (g/cm^2)		1.04 (0.95-1.12)	1.04 (0.98-1.14)	1.03 (0.96-1.11)	
Spine BMD (g/cm^2)		0.84 (0.75-0.95)	0.88 (0.79-0.98)	0.87 (0.78-0.97)	
Left hip BMD (g/cm^2)		0.74 (0.65-0.84)	0.73 (0.65-0.84)	0.76 (0.68-0.86)	
Lean mass (kg)		37.0 (32.9-42.6)	38.5 (33.8-45.2)	37.7 (34.0-45.6)	
Percent body fat		38.6 (33.8-43.6)	39.1 (33.3-42.8)	39.9 (33.4-43.6)	
Serum 25(OH)D (ng/ml)		36.6 (28.1-44.2)	31.5 (25.0-41.1)	30.1 (24.9-37.6)	
Parathyroid hormone (pg/ml)		41.3 (34.8-58.2)	42.5 (31.5-56.9)	47.0 (36.8-61.8)	
Ca-intake (mg/d)		668.3 (413.2-911.9)	517.6 (352.3-794.1)	-	
Energy (kJ/d))		10247 (7680-12514)	9330 (6166-11247)	-	
Fat (g/d)		76.6 (50.0-100.0)	57.1 (44.5-84.2)	-	
Protein (g/d)		75.6 (57.1-93.6)	68.9 (51.0, 96.5)	-	
Alcohol intake (g/d)		0.0 (0.0-0.86)	0.0 (0.0-1.3)	-	
PA (MET min/week)		2880 (960-7190)	2970 (840-6740)	3240 (2040-4530)	
Sedentary time(min/day)		240 (150-375)	240 (150-375)	240 (180-360)	
Vitamin D status	Deficiency	12 (10.2)	14 (14.3)	12 (11.5)	
	Insufficiency	23 (19.5)	28 (28.6)	39 (37.5)	
	Sufficiency	83 (70.3)	56 (57.1)	53 (51.0)	
Education	No school & primary	44 (36.7)	29 (29.3)	31 (29.8)	
	Grade 8-11	44 (36.7)	40 (40.4)	43 (41.3)	
	Grade 12	31 (25.8)	29 (29.3)	29 (27.9)	
	Tertiary	1 (0.8)	1 (1.0)	1 (1.0)	
Income (per month)	None	6 (5.0)	3 (3.0)	10 (9.6)	
	< R500 - R1000	31 (12.5)	21 (21.2)	14 (13.5)	
	R1001 - R6000	74 (61.7)	64 (64.6)	72 (69.2)	
	> R6000	9 (7.5)	11 (11.1)	7 (6.7)	
Hypertension		55 (45.8)	46 (46.5)	48 (46.1)	
Diabetes mellitus		10 (8.3)	9 (9.1)	8 (7.7)	

BMI = body mass index; BMD = bone mineral density, Ca = calcium, MET = Metabolic equivalent; PA = physical activity; 25(OH)D = 25 hydroxyvitamin D

Table 4.2 show correlations between continuous variables according to distribution of data. Serum 25(OH)D did not correlate with any of the BMD outcomes, or total percent fat. Total percent body fat had moderate positive correlations with total spine BMD (r =0.319; p < 0.0001) and left hip FN BMD (r = 0.408; p <0.0001), but a weak positive correlation with total BMD (r =0.239; p = 0.009). Lean mass had a weak negative correlation with age (r = –0.219; p = 0.016) and a strong positive correlation with BMI (r = 0.824; p < 0.0001). A comparison between body composition outcomes and vitamin D status of smokers and non-smokers at baseline revealed no difference in total lean mass, total spine BMD, total BMD, total left hip FN BMD, BMI or vitamin D status, the only difference was in total percent body fat (p = 0.032). There was no correlation between serum 25(OH)D and PTH at baseline (r = –0.11, p=0.24), after one year (r = –0.16, p=0.12), or after two years of follow-up (r = –0.14, p=0.17), although there was a weak trend of an increase in serum PTH concentration over time.

Variables with a non-normal distribution that showed significant correlations from the Spearman correlation were log transformed and similar correlations were found. New significant correlations that were observed included a weak negative correlation between BMI and serum 25(OH)D (r = –0.189; p = 0.041). Further, BMI had moderate positive correlations with total BMD, total spine BMD and left hip FN BMD (p < 0.0001). However, BMI had a strong correlation with total percent fat (r = 0.841; p < 0.0001). Age displayed weak inverse correlations with total spine BMD (r = –0.242; p = 0.008) and moderate inverse correlations with total BMD (r = –0.400; p < 0.0001) and left hip BMD (r = –0.346; p < 0.0001). Physical activity correlated positively though weakly with total BMD (r = 0.227; p = 0.014) and left hip FN BMD (r = 0.235; p = 0.011). Lean mass had moderate positive correlations with total spine BMD (r = 0.473; p < 0.0001), as well as total BMD (r = 0.429; p < 0.0001) and strong positive correlation with left hip FN BMD (r = 0.563; p < 0.0001). Partial Pearson correlation controlled for age did not bring any changes except between physical activity and total BMD (r = 0.177; p = 0.06).

Changes of study exposure variable and body composition outcomes over two year period

The changes were assessed over the three time points of measurements from baseline to two-year follow-up. Changes of main exposure and body composition outcomes are presented in Figure 4.1 to Figure 4.3. Serum 25(OH)D concentration declined from baseline through to year 2 follow-up (p < 0.0001) with an effect size of 0.39 indicating a practical visible difference. Bonferroni pairwise comparison showed a significant decline of serum 25(OH)D concentration from baseline to year 1 follow-up and baseline to year 2 follow-up. Total BMD increased from baseline to year 1 follow-up, but with practical non-significant difference (effect size = 0.03). Similarly, left hip FN BMD increased over two years, but the difference was practically non-significant (effect size of

0.06). Changes in left hip FN BMD over the two-years were between baseline and year 2 follow-up and year 1 and year 2 follow-up. There were no significant changes in PTH from baseline over the two years (Table 4.1). Lean mass had a practical non-significant difference between baseline and year 2 follow-up (effect size 0.02), whereas percent body fat mass had a practical visible difference over the two years (effect size 0.66).

Association between vitamin D and body composition over time

LMM were used to describe the association between serum 25(OH)D as the main exposure and body composition outcomes that included percent body fat, lean mass (logarithmically transformed), total BMD, lumbar spine BMD, and left hip FN BMD. Intermediate associations between body composition components (fat mass, lean mass and BMD) were also determined by linear mixed models. Estimates of the associations were adjusted for education level, household income, age, physical activity, duration of HAART and alcohol intake as potential confounders. Table 4.3 presents the associations. All models showed an improvement in goodness of fit after adding covariates to the crude models, as demonstrated by the Schwarz Bayesian information criterion (BIC). The model with the lowest BIC was selected as the best model.

Serum 25(OH)D was not associated with any of the three BMD outcomes, as well as percent body fat and lean mass. However, serum 25(OH)D showed a significant association with BMI in the unadjusted model ($p = 0.024$), but the association lost significance after adjusting for education level, household income, age, physical activity and alcohol intake. There was a positive association between physical activity and left hip FN BMD. Age was consistently inversely associated with all body composition outcomes. Both percent body fat mass and lean mass were associated with left hip FN BMD and total BMD, but not with total spine BMD.

Table 4-2: Correlation between serum vitamin D, lifestyle, health and body composition variables

Pearson correlation between serum vitamin D and body composition variables								
Variable	**Total spine BMD**		**Left hip BMD**		**Total BMD**		**Total percent fat**	
	r	p	r	p	r	p	r	p
Serum vitamin D	-0.003	0.970	0.001	0.990	0.000	0.999	-0.072	0.437
Total percent fat	0.319**	<0.0001	0.408**	<0.0001	0.239**	0.009	----	----

Spearman correlations between lifestyle, and demographic variables and body composition										
Variable	**Lean mass**		**Total physical activity MET min/week**		**HIV duration in years**		**Duration of ART use**		**Ca intake**	
	r	p	r	p	r	p	r	p	r	p
Age	-0.219*	0.016	-0.062	0.503	-0.042	0.648	-0.014	0.883	-0.016	0.864
BMI	0.824**	<0.0001	0.115	0.210	0.042	0.646	-0.017	0.852	-0.018	0.842
Physical activity MET (min/week)	0.147	0.108	----	----	-0.012	0.899	-0.051	0.578	0.004	0.966
HIV duration in years	0.032	0.731	-0.012	0.899	----	----	0.856**	<0.0001	-0.083	0.368
Duration of ART use	-0.038	0.684	-0.051	0.578	0.856**	<0.0001	----	----	-0.131	0.157

Abbreviations; ART = Antiretroviral therapy; BMI = Body mass index; MET = Metabolic equivalent

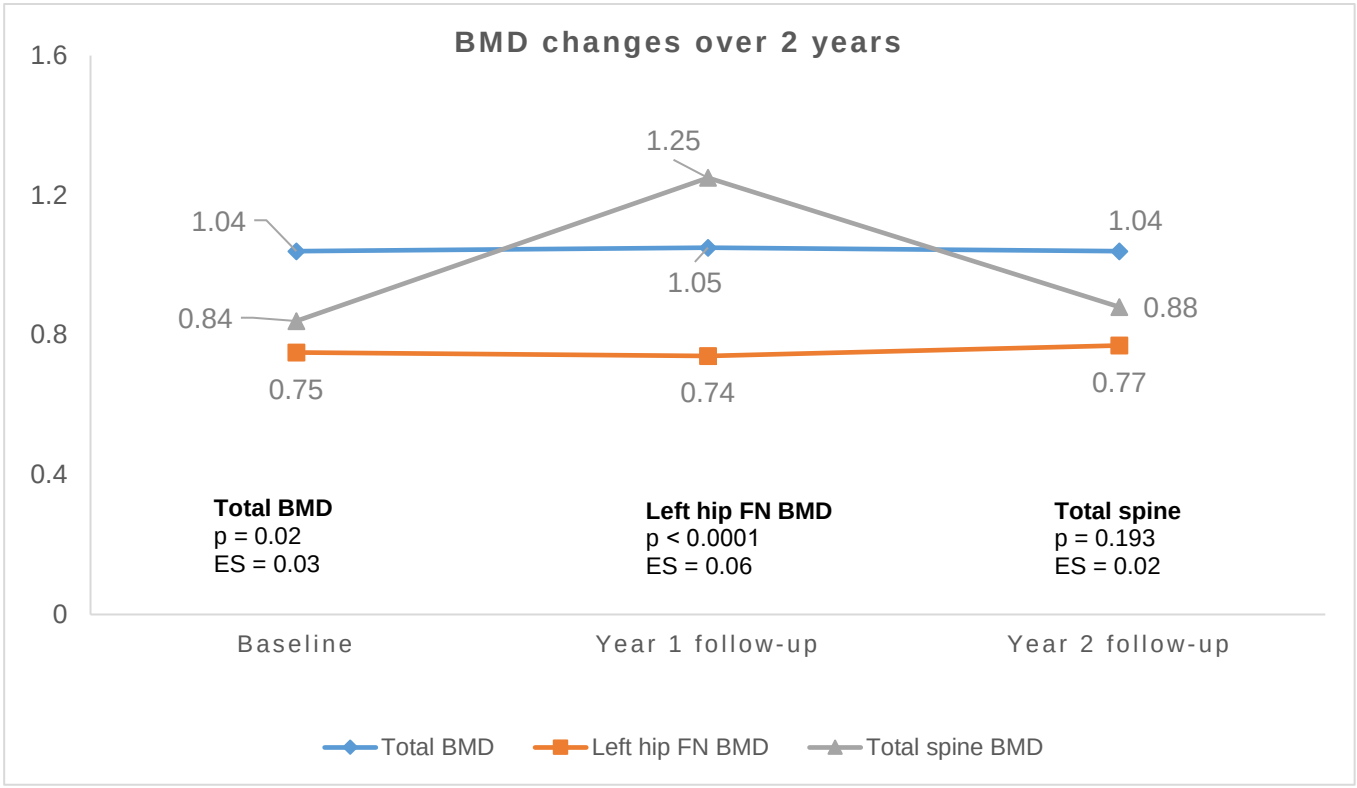

Figure 4-1 BMD changes over 2 years from baseline

BMD = Bone mineral density; ES = Effect size; FN = Femur neck

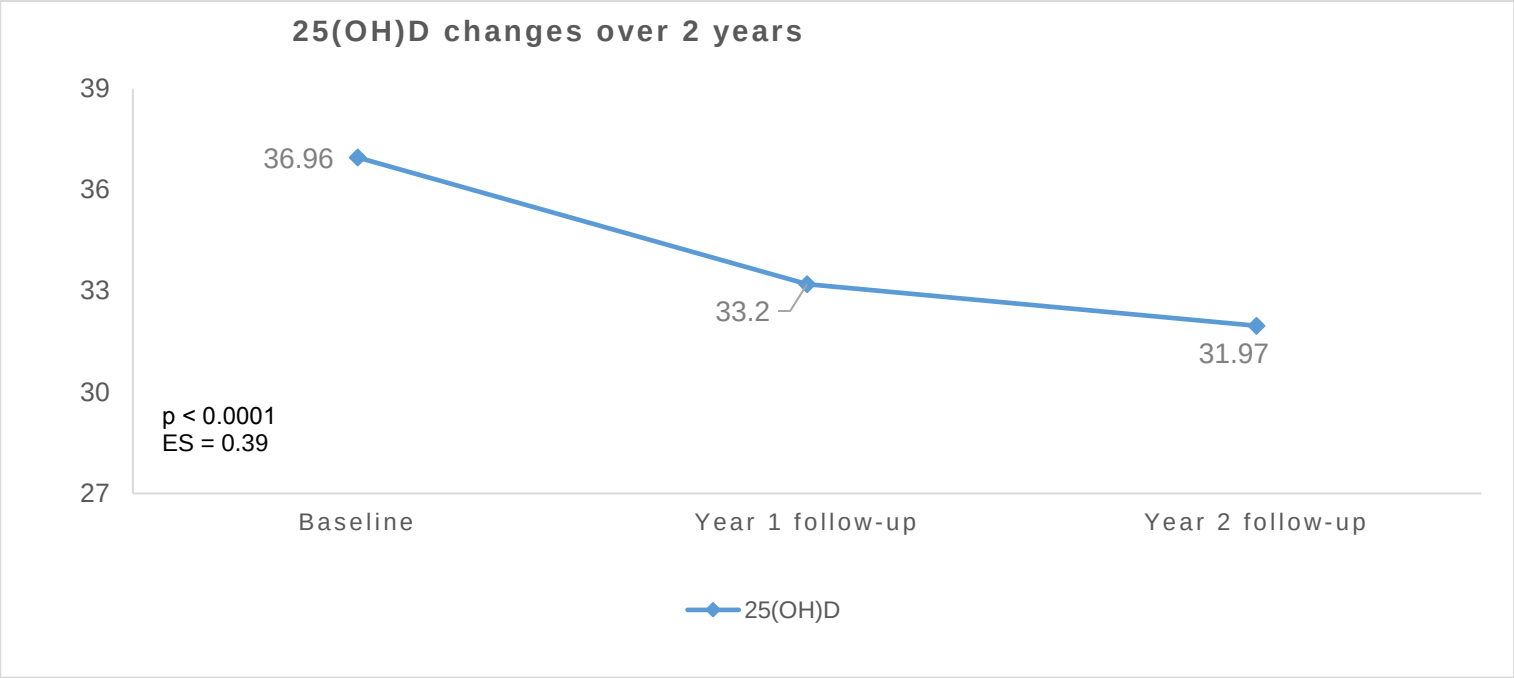

Figure 4-2 Serum 25(OH)D changes over 2 years from baseline

ES = Effect size; 25(OH)D = 25 hydroxyvitamin D

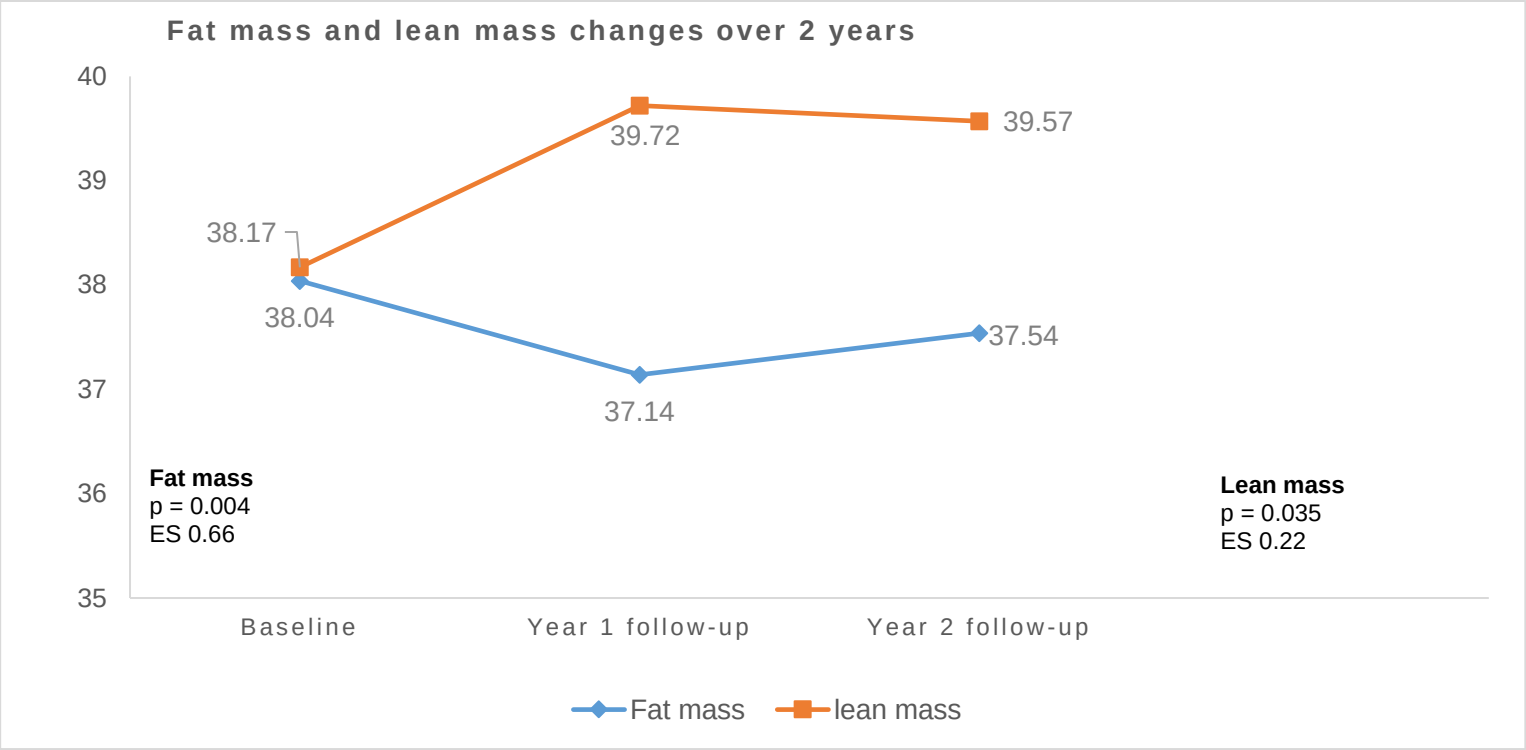

Figure 4-3 Lean and fat mass changes over 2 years from baseline

ES = Effect size

Table 4-3: Association between serum vitamin D and body composition and interdependent associations between body composition variables

	Total BMD		Total spine BMD		Left hip FN BMD		Percent body fat		Lean mass	
Variable	Mean estimate	P value	Mean estimate	P value	Mean estimate	P value	Mean estimate	P value	Mean estimate	P value
Age (years)	-0.009	<0.0001	0.068	0.031	-0.008	<0.0001	-0.105	0.406	-0.004	0.032
Education	0.021	0.836	0.137	0.467	-0.006	0.585	-	-	-	-
HH income	0.005	0.519	0.125	0.364	0.020	0.010	-	-	-	-
Duration of HAART	-0.011	0.545	0.214	0.511	-0.001	0.562	-	-	-	-
Alcohol intake (g)	0.001	0.415	-0.006	0.820	-0.0001	0.894	-0.004	0.943	0.0008	0.573
Physical Activity	0.032	0.067	-0.118	0.708	0.041	0.021	0.558	0.323	0.0001	0.994
Serum 25(OH)D	-0.951	0.886	-0.003	0.784	-8.20	0.904	-0.035	0.404	-0.001	0.131
Interdependent associations between %BF, lean mass and BMD										
	Total BMD			**Total spine BMD**			**Left hip FN BMD**			
Age	-0.009	<0.0001		0.064	0.035		-0.007	<0.0001		
Education	-0.001	0.898		0.141	0.440		-0.009	0.299		
H/H	0.005	0.496		0.125	0.353		0.021	0.003		
Duration of HAART	-0.0009	0.582		0.020	0.526		-0.001	0.482		
Alcohol	0.001	0.359		-0.007	0.772		-2.609	0.984		
Physical Activity	0.027	0.112		-0.072	0.817		0.029	0.070		
Percent body fat	0.003	0.004		-0.031	0.147		0.006	<0.0001		
	Total BMD			**Total spine BMD**			**Left hip FN BMD**			
Age	-0.008	<0.0001		0.067	0.032		-0.007	<0.0001		
Education	0.001	0.911		0.128	0.487		-0.006	0.541		
H/H	0.005	0.511		0.127	0.349		0.020	0.005		
Duration of HAART	-0.001	0.530		0.020	0.528		-0.001	0.451		
Alcohol	0.001	0.399		-0.006	0.807		-0.0002	0.857		
PA	0.033	0.051		-0.128	0.682		0.041	0.016		
Lean mass	0.256	0.001		-0.258	0.854		0.301	<0.0001		

4.5 Discussion

This two-year longitudinal study, focused on investigating the association between serum 25(OH)D and body composition (lean mass, fat mass and BMD) in black South African postmenopausal HIV positive women on HAART. According to the Endocrine Society and International Osteoporosis Foundation cut-offs for vitamin D status [30], we found serum 25(OH)D insufficiency (deficiency and insufficiency) in 29.7% of the participants at baseline and 49.0% at year 2 follow-up, respectively. There was no association between serum 25(OH)D concentration and total BMD, spine BMD, left hip FN BMD, lean mass and fat mass consistently throughout the study period. From the LMM on the interdependent associations between body composition components, lean mass and fat mass showed comparable positive associations with total BMD and left hip FN BMD, but not with total spine BMD. Despite the comparability, lean mass proved as a stronger predictor of BMD than fat mass.

Our findings on vitamin D status (insufficiency and deficiency prevalence) corresponds with those from some observational studies among black populations [32-34]. Kuchuk *et al*. [35] reported similar serum 25(OH)D deficiency levels both in summer and winter among postmenopausal women from 29 countries across the world. Serum 25(OH)D also declined over the two-year study period in the Dallas Heart Study in a multi-ethnic adult population in Dallas, Texas, USA. [36]

Vitamin D is primarily known for improving BMD via the homeostasis of two important minerals, calcium and phosphate [37]. We observed no association between serum 25(OH)D and total BMD, left hip FN BMD and total spine BMD over a follow-up period of two years. Our findings are in keeping with a number of studies, although these were RCTs with vitamin D supplementation [38,39]. A cross-sectional study by Kamineni *et al.* [40] in India among postmenopausal women had findings similar to our study where no correlation was determined between serum 25(OH)D and BMD. However, a cross-sectional study among Chinese postmenopausal women found positive correlation between serum 25(OH)D and BMD outcomes at lumbar spine, total hip and FN [41].

Study observation period is likely another plausible explanation for the lack of association between serum 25(OH) and BMD. Changes in BMD require fairly a long duration to manifest, as such the duration of our study could have been inadequate to detect noticeable significant changes. Hamill *et al.* [42] followed a cohort of urban, black HIV-infected South African premenopausal women up for 24 months. A significant finding was the attenuation of bone mass loss beyond 12 months up to 24 months in the HIV-infected group on ART. Although the participants were premenopausal women, this signifies that bone loss in HIV-positive patients within the first 12 months on ART could be transient. Our participants showed a small BMD increase at left hip FN BMD and total BMD, but no change at total spine BMD. Other authors had

suggested a longer follow-up to ascertain BMD changes [42]. The present study participants had an average HAART use duration of nine years which might explain BMD stabilisation.

Despite the lack of association between serum 25(OH)D and BMD, we reported a decline in serum 25(OH)D from baseline to two years however, the effect size was small. Very low serum 25(OH)D (deficient) trigger PTH surge, which leads to bone demineralisation as a compensatory measure in maintaining serum calcium balance [43]. The decline in serum 25(OH)D probably explains the small non-significant progressive increase in PTH, which was probably insufficient to lead to a decline in BMD. In agreement to the present study findings Kota *et al.* [44] reported no direct association between serum 25(OH)D and BMD. However, PTH showed an inverse association with serum 25(OH)D indicating that low serum 25(OH)D is indirectly associated with low BMD. Contrary findings to ours were reported in a 12-months longitudinal study among urban black South African premenopausal women. A positive change in serum 25(OH)D regardless of HIV status was reported over 12 months [45]. The obvious difference between the two studies is age, where our study participants were postmenopausal women (mean age 50 years) and not premenopausal women. Aging is a known factor for hypovitaminosis D, through decreased vitamin D receptors (VDRs) and consequent alteration of vitamin D metabolism [46]. We further reported a non-significant inverse correlation between serum 25(OH)D concentration and age. In agreement with our findings is a longitudinal study in Amsterdam that reported a small decline in serum 25(OH)D in older participants (65-88 years) over 13 years [47]. Poopedi *et al.* [48] affirmed progressive fluctuations in serum 25(OH)D in a study among healthy adolescents over 10 years. These progressive changes in serum 25(OH)D noted in longitudinal studies as well as our study signifies that single measurements as unreliable for determining associations between serum 25(OH)D and diseases.

The present study reported no association between serum 25(OH)D and percent body fat and lean mass, however, there was a weak negative correlation with BMI. In agreement to this finding, is a recent cross-sectional study among postmenopausal women that reported an inverse correlation between serum 25(OH)D and anthropometric measurements including BMI [49]. BMI is used as a proxy for adiposity and the sequestration theory provides a probable explanation for the inverse association displayed with serum 25(OH)D [50].

On the other hand, in a cross-sectional study Li *et al.* [41] reported no association between BMI and serum 25(OH)D in Chinese postmenopausal women. However, the authors argued that the above result was based on the unique body composition structure of the Chinese people characterised by a lower prevalence of obesity than in other ethnic groups. This emphasises the role of ethnicity on the interaction between serum 25(OH)D and body composition. The present study participants

had a relatively high prevalence of overweight and obesity in a range of 22.5%-25.7 and 38.3%-40.0% respectively over two years.

Previous observational studies have described other known determinants of BMD however, the relative contribution of lean mass and fat mass on BMD remains a contentious subject. A LMM of follow-up data showed that both lean mass and percent fat mass were positively associated with left hip FN BMD and total BMD, but not with total spine BMD. However, lean mass was a stronger predictor of BMD than fat mass. This result is a replication of findings from other observational studies among postmenopausal women [51-53]. Similarly, a systematic review by Ho-Pham *et al.* [54] proved lean mass to be the strongest determinant of BMD compared to fat mass in the general study group analysis. This signifies that the interdependent interactions between body composition components can be influenced by menopause status. The influence of fat mass on BMD improvement is affected by the increased load placed on the skeleton, whereas lean mass influence BMD through increased mechanical stress mediated by muscle and increased load placed on the skeleton both acting as stimuli for osteogenesis [55, 56].

An important proportion of the women in the present study were overweight (22.5% and 25.7% at baseline and 2-year follow-up respectively) and obese (38.3% at baseline and 40.0% at 2-year follow-up). We reported a quite unusual finding of a small, but significant fat mass decline and lean mass increase in the women over the two-year study period, in contrast to normal aging changes [51]. Our results indicated a significant positive association between physical activity and left hip FN BMD, the site most prone to fracture in postmenopausal women. Such finding was reported previously in a Canadian retrospective study among postmenopausal women [57].

Apart from the known serum 25(OH)D, risk factors for low BMD such as dark skin, HIV AIDS, ART and advanced age (postmenopausal) were present in the women included in our study. The participants had relatively good dietary intakes (calcium, protein and energy). Physical activity was also relatively good, signifying probable adequate exposure to sunlight and adequate stimuli to maintain bone health. We further reported low exposure to lifestyle risk activities such as smoking and alcohol. These factors may further explain the small decline in serum 25(OH)D and the preserved BMD over a follow-up period of two years.

4.6 Limitations and strengths

Limitations and strengths existed in our study that need to be acknowledged. With regards to limitations, firstly this study used an observational design thereby having limited control over other potential covariates. Secondly, participants originated from the same locality of the North West Province of South Africa. As such generalisation of the findings to a wider population across South Africa and beyond ought to be done cautiously. Thirdly, the sample size was relatively small

thereby undermining the power for subgroup analysis i.e. subgroups based on vitamin D status and its association with BMD outcomes, as well as lean mass and fat mass. It was challenging to recruit an appropriate number, because most HIV positive women were premenopausal. Some reported associations not being statistically significant could be due to type II error. A larger sample could potentially have yielded a different result.

Regarding study strengths, to the best of our knowledge this is the first longitudinal study on association between vitamin D and body composition in black South African HIV postmenopausal women on HAART in South Africa. A longitudinal design provides more reliable pattern of interactions or associations as compared to cross-sectional study design. Furthermore, use of DXA for body composition analysis was a strength of this study, as DXA is a gold standard measure of body composition. Future studies should consider all determinants of vitamin D and body composition such as duration and type of ART, as well as dietary assessment over a longer duration

4.7 Conclusion

In conclusion, this two-year longitudinal study indicates no association between serum 25(OH)D and BMD over time. However, BMI was inversely associated relationship with serum 25(OH)D concentration. In terms of relative contribution of lean mass and fat mass on BMD, there exist a comparable positive association between both lean mass and fat mass with BMD. However, lean mass is a stronger determinant of BMD than fat mass. Black South African HIV postmenopausal women on HAART maintained their BMD at all three sites over a period of two years. The study further suggests that age and physical activity are important determinants of BMD in South African postmenopausal HIV-positive women. This study can inform prevention strategies to prevent fractures in postmenopausal HIV-positive women. Increasing lean mass and reducing BMI through physical activity could be important to prevent frailty and morbidity in this vulnerable population.

Acknowledgements

We acknowledge the assistance of registered nurses, Sr CM Lessing and Sr A Anthony, who acted as study coordinators and took the blood samples. Professor C Mels supervised the laboratory assay of serum vitamin D and PTH. Ms G Wood performed language editing of the manuscript. Postdoctoral associate Dr Patricia Ukegbu and postgraduate students Shams Bakali, Phillip Buys and Persuade Makore assisted with study coordination and data collection. We acknowledge financial support from the Support for Nutrition Research in Africa (SUNReA) and the Sugar Association of South Africa.

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CHAPTER 5 SUMMARY AND CONCLUSION

5.1 Background

Vitamin D exerts multiple physiological actions on both musculoskeletal and extra-skeletal tissues in the body (Pérez-López *et al.*, 2011). Global data indicates a growing prevalence of hypovitaminosis D, hence it is becoming a public health problem (van Schoor & Lips, 2018). HIV/AIDS poses as an additional risk factor to hypovitaminosis D and South Africa has the world's highest HIV prevalence, in particular among black women (SANAC, 2017). The use of highly active antiretroviral therapy (HAART) has extended the life span of HIV-positive persons to old age, thereby increasing the likelihood of multiple health complications, including hypovitaminosis D and poor bone health further (Broder, 2010). Black HIV postmenopausal women are therefore a high-risk group to hypovitaminosis D, and impaired bone health. This study therefore, was aimed at determining the association between serum 25-hydroxyvitamin D (25(OH)D) and body composition in black South African postmenopausal HIV-positive women on HAART.

5.2 Main findings

Objective 1: To determine the association between serum vitamin D and fat mass, lean mass, and bone mineral density, respectively, in black South African postmenopausal HIV-positive women on HAART.

The prevalence of vitamin D insufficiency and deficiency, respectively, increased from 19.5% and 10.2% at baseline to 37.5% and % 11.5% after two years. Serum 25(OH)D was not associated with any bone mineral density (BMD) outcome, but had a weak significant negative correlation with body mass index (BMI) that disappeared after adjustment for covariates of age, BMI, physical activity, education level and income level. We found no association between serum 25(OH)D and percent body fat and lean mass.

Objective 2: To determine the interdependent associations between fat mass, lean mass and bone mineral density in black South African postmenopausal HIV-positive women on HAART.

Lean mass and fat mass had comparable significant positive correlations with all BMD outcomes. From the linear mixed model, lean mass and fat mass showed significant positive associations with total BMD and left hip femoral neck (FN), but not with total spine BMD. Lean mass was a stronger determinant of BMD than fat mass. Age had an inverse relationship with most body

composition outcomes (lean mass, fat mass, total and left hip FN BMD) and an unexpected positive association with spine BMD. Physical activity showed a positive association with left hip FN BMD.

Our major finding was that there was no association between serum 25(OH)D and total BMD, left hip FN and total spine BMD. This result was in concordance with some previous studies, although these studies were randomised control trials (RCTs) (Aloia *et al.*, 2018; Aspray *et al.*, 2019). However, a RCT among Malaysian postmenopausal women reported a positive correlation between serum 25(OH)D and BMD (Kruger *et al.*, 2018). The participants in the study were given vitamin D fortified milk over a 1-year period and the intervention group had increased their serum 25(OH), whereas the control group maintained their concentration of serum 25(OH)D. Similarly, a cross-sectional study Li *et al.* (2014) reported significant positive correlation between serum 25(OH) D and all BMD outcomes among Chinese postmenopausal women.

Longitudinal studies indicate massive fluctuations in serum 25(OH) measurements contrary to BMD which is an accrual over a long period of time (Jorde *et al.*, 2010; Poopedi *et al.*, 2015). This signifies that long periods of observation and multiple measurements of 25(OH)D are key in determining its associations with various health outcomes. The two-year observation time in the present study might have therefore been inadequate for noticeable BMD changes in relation to serum 25(OH)D. An Iranian study demonstrated that vitamin D supplementation improved serum 25(OH)D and a consequent positive association between serum 25(OH)D and BMD in osteoporotic participants (Shahnazari *et al.*, 2019). In the present study, participants with very low T-score (<-3) were excluded and referred to hospital for treatment. Total BMD and left hip FN BMD displayed small but significant increases over the two-year period whereas total spine BMD did not change.

Furthermore, we found no association between serum 25(OH)D and percent body fat and lean mass, however, there was a weak negative correlation between serum 25(OH)D and BMI. A more recent cross-sectional study among postmenopausal women also reported an inverse correlation between serum 25(OH)D and anthropometric measurements including BMI (Vázquez-Lorente *et al.*, 2020). Blum *et al.* (2008) demonstrated the presence of vitamin D in subcutaneous adipose tissue and serum in an *in vivo* study and this supports the serum 25(OH)D sequestration theory (Rosenstreich *et al.*, 1971). A systematic review in older men and women also confirmed a negative correlation between BMI and serum 25(OH)D (Pereira-Santos *et al.*, 2015).

The lean mass and fat mass relative contribution to BMD remains a controversial subject with conflicting findings. We demonstrated a comparable association in both lean mass and fat mass on BMD however, lean mass proved to be a stronger determinant than fat mass. A number of observational studies have had concordant results to our study (Chen *et al.*, 2015; Ho-Pham *et*

al., 2014; Ilesanmi-Oyelere *et al.*, 2018; Sotunde *et al.*, 2015). BMI is an indicator of body weight and both fat mass and lean mass constitute body weight. The mechanism of action of lean mass and fat mass on BMD is through increased mechanical stress mediated by muscle and increased load placed on the skeleton both acting as stimuli for osteogenesis (Kelley *et al.*, 2013; Zhang *et al.*, 2012). Physical activity is another modifiable factor that acts as a stimulus on bone for osteogenesis (Turner & Robling, 2004). We proved a positive association of physical activity on BMD, specifically at left hip FN BMD. Similarly, a cross-sectional study among postmenopausal women indicated a positive correlation between physical activity and BMD at trochanter and FN, but not that of lumbar spine (Ilesanmi-Oyelere *et al.*, 2019).

5.3 Limitations

Firstly, this study used an observational design thereby having limited control on other potential covariates. Secondly, participants originated from the same locality of the North West Province of South African, hence generalisation of the findings to a wider population across South Africa and beyond ought to be done cautiously. Sample size was relatively small thereby undermining the power for subgroup analysis i.e., subgroups based on vitamin D status and its association with BMD outcomes as well as lean mass and fat mass. Some reported associations as not being statistically significant could be because of type II error. A larger sample could potentially have yielded a different result.

5.4 Recommendations for future research and public health practice

For future research, it is recommended that a longer than two-year follow-up study be considered to determine long-term associations between serum 25(OH)D and BMD. Furthermore, a larger sample would be ideal for future research to probably allow subgroup analysis based on serum 25(OH)D status as associations may be dependent on specific thresholds. The study findings indicate lean mass, physical activity as best determinants for bone health. As such health care workers ought to encourage black HIV-positive women on HAART on physical exercise to maintain lean mass and control BMI.

It is evident from our findings that HIV-positive women on HAART can maintain BMD at least over the short term and may even show a small improvement on BMD and lean mass if they receive good health care and are treated with HAART and also if they practice good lifestyle habits. Very few of our participants indulged in cigarettes or alcohol. The proportion of underweight women was smaller than those who maintained normal body weight, overweight and obesity, which is another proved factor for good bone health. Dietary practices were good in terms of energy, protein and calcium intake and these probably explain the maintained bone health. The decline in serum 25(OH)D pose as risk to numerous health complications and the public ought to be

cautious on lifestyle factors that increase the risk of hypovitaminosis D. Awareness to the general public and postmenopausal women in particular, on maintaining a healthy body weight, reduced smoking and alcohol intake, confirming that good dietary intake and physical activity are therefore of paramount importance to maintain bone health. Health care providers managing HIV infected older persons should also ensure their patients receive optimal care in order to maintain healthy bodies.

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ANNEXURES

Annexure A: Journal manuscript

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Annexure C: Screening form

Annexure D: Socio-demographic questionnaire

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Annexure I: Quantitative food frequency questionnaire

Annexure I: Referral letter

Annexure A: Journal author instruction

Author Guidelines: The Southern African Journal of HIV Medicine

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Acknowledgements

The acknowledgement section follows the conclusions section and addresses formal, required statements of gratitude and required disclosures. It includes listing those who contributed to the work but did not meet authorship criteria, with the corresponding description of the contribution. Acknowledge anyone who provided intellectual assistance, technical help (including with writing and editing), or special equipment and/or materials. Authors are responsible for ensuring that anyone named in the Acknowledgements agrees to be named.

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Annexure B: Informed Consent

INFORMED CONSENT DOCUMENTATION FOR BLACK POSTMENOPAUSAL HIV-POSITIVE WOMEN ON ART REGIMEN 1

TITLE OF THE RESEARCH STUDY: The association between calcium intake, serum vitamin D concentration, respectively, and bone mineral density, lean and fat mass, as well as bone turnover markers over two years in black postmenopausal HIV-positive women on HAART: a prospective cohort study

ETHICS REFERENCE NUMBER: NWU-000061-17-S1

PRINCIPAL INVESTIGATOR: Prof H.S. Kruger

POST GRADUATE STUDENT: C. Ellis

ADDRESS: Centre of Excellence for Nutrition, NWU, Potchefstroom

CONTACT NUMBER: 018 299 2482

You are being invited to take part in a research study of the association between calcium in your diet, vitamin D in your blood and strength of your bones and muscles. The study will take two years to complete, with the first visit in 2017, followed by a second visit in 2018 and the last visit in 2019. Only black HIV-positive women on HAART who are older than 45 years will be invited to participate.

If you agree to participate, the researchers will explain the details of this study to you. Please ask the researcher or person explaining the research to you any questions about any part of this study that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research is about and how you might be involved. Also, your participation is **entirely voluntary,** and you are free to say no to participate. If you say no, this

will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part now. You will still continue with your normal clinic visits and will receive all the necessary treatment at the clinic.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-000061-17-S1) and will be conducted according to the ethical guidelines and principles of Ethics in Health Research: Principles,

Processes and Structures (DoH, 2015) and other international ethical guidelines applicable to this study. It might be necessary for the research ethics committee members or other relevant people to inspect the research records.

5.1 What is this research study all about?

- This study will be conducted at the Metabolic Unit of the North-West University and will involve the completion of questionnaires about your health and what you eat and your physical activity. Your weight, height, waist, arm and calf measurements will be taken. Your bone strength will be measured on an X-ray machine and blood will be drawn by a registered nurse. We will test the strength of your arms and legs. Health data will be collected from your hospital file. Trained researchers will perform all measurements. In total 124 women will be included in this study and you will be measured in this year, with a second measurement in 2018 and the third measurement over two years.

5.2 Why have you been invited to participate?

- You have been invited to be part of this research because you are a woman older than 45 years and attending the Wellness Clinic at Potchefstroom Hospital for treatment with chronic medication.
- You will not be able to take part in this research if you use medications from a list of medications that could make bones weak; have any disease that makes bones weak or prevent you from walking at a normal speed; using calcium and/or vitamin D tablets on a regular basis; consume more than 3 drinks of alcohol per day; or had a bone fracture within the last six months.

5.3 What will be expected of you?

- We will make an appointment with you for a study visit to the North-West University (PUKKE). We will send a taxi to pick you up at home and bring you to the university only on one day for the next three years. You must not eat on the morning of the study, but you will receive tea and bread and a cooked lunch on the day when you visit the university. You will be expected to answer questions asked by fieldworkers in a language that you can understand about your health and what you eat and your physical activity. Researchers will measure your height, weight, waist circumference,

arm and calf circumference. Health data will be collected from your hospital file. Bone strength will be measured by a radiographer. You have will have to get blood drawn for blood tests of bone strength. We will also ask questions about bone fractures and how strong you can walk. All measurements will take approximately 6-7 hours on one day in this year, again in 2018 and the last measurement over two years.

5.4 Will you gain anything from taking part in this research?

- The gains for you if you take part in this study will be that you will receive health information. The information includes if you have a healthy body fat percentage and bone strength at each visit. You will also receive a summary of your dietary intake and a pamphlet regarding healthy eating guidelines after you return for the second and third visit.
- The other gains of the study are for researchers to learn more about the long-term value of calcium in food and vitamin D in blood and the strength of bone over time. Such information may help to save health care costs and improve quality of life of ageing women on anti-retroviral medicine.

5.5 Are there risks involved in you taking part in this research and what will be done to prevent them?

- The risks to you in this study will be small: During anthropometry and DXA measurements, participants will wear gowns provided at the university. The measurements are performed in private rooms with only the participant and one or two researchers present, so that you will not feel embarrassed when you undress and wear a gown only. *You must not eat on the morning of the study, but you will receive tea and bread during the morning and a cooked lunch on the day when you visit the university.* All the measurements will take about 6-7 hours. Tea, juice and water will be provided throughout the day in the cool waiting area. When you are not in the measurement rooms, you can watch TV and read magazines in the waiting room. Research assistants will show you where to go next until all your measurements are done.
- A medical doctor (Dr. Shakung) and one registered nurse (Sr. C. Lessing) will supervise the study. The nurses will be present at all times during the study. As Sr. Lessing is the head of the Metabolic Unit, she also has standard safety plans and will help you if you do not feel well after any measurement, or we will transport you to the hospital clinic for treatment. The measurement of your bone strength with the X-ray machine is safe, because it takes only a short time and will only be done once every year. If we find that you have severely low bone strength, you will be referred for more intensive treatment at the hospital clinic.

- The benefit outweighs the risks as there are small risks to the participant

5.6 How will we protect your confidentiality and who will see your findings?

- Nobody will be able to know your results, because you will get a number and your name will not be written on any of the forms. The name list will be kept separately from project data and will only be available to the project leader. Your privacy will be respected by ensuring private rooms are used during measurements. Your results will be kept confidential by using a number of results and the name list will be known only to the project leader. Only the researchers will be able to look at your findings. Findings will be kept safe by locking paper copies in locked cupboards in the researcher's office and computer information on password protected computers. Data will be stored for 7 years.

5.7 What will happen with the findings or samples?

- The findings of this study will only be used for this study as described. All blood samples will be tested in the laboratory at the university. If we learn about new ways to test bone strength from blood samples, we will do new tests also, but all tests will only be about bone strength.

5.8 How will you know about the results of this research?

- We will give you the results that are available of this research when you have completed the assessments at baseline, 12 and 24 months. We will explain your body fat, muscle and bone tests and strength tests to you.
- Blood tests will be done later, and you will be informed of any new findings about your health if necessary, by the researcher. We will send a report for each participant to the hospital to place in your file. If there are signs of a disease, the clinic doctor will give the necessary treatment as far as possible or refer you to another hospital.

5.9 Will you be paid to take part in this study and are there any costs for you?

- This study is funded by grants from the National Research Foundation and applications have been submitted to the South African Sugar Association and the Allen Foundation. Danone will give money for your snacks and food.
- You will receive an R 50 gift voucher after you complete data collection at the first visit and on both 12 and 24 months.
- You will receive tea and sandwiches and lunch on each visit.
- There will be no costs for you if you do take part in this study.

- Is there anything else that you should know or do?
- You can contact Christa Ellis at 083 374 9477 or christae19@gmail.com, or Professor Kruger at 018 2992482 or Salome.kruger@nwu.ac.za if you have any further questions or have any problems.
- You can also contact the Health Research Ethics Committee via Mrs. Carolien van Zyl at 018 299 1206 or carolien.vanzyl@nwu.ac.za if you have any concerns that were not answered about the research or if you have complaints about the research.
- You will receive a copy of this information and consent form for your own purposes.

Declaration by participant

By signing below, I..... agree to take part in the research study titled: **The association between calcium intake, serum vitamin D concentration, respectively, and bone mineral density, lean and fat mass, as well as bone turnover markers over two years in black postmenopausal HIV-positive women on HAART: a prospective cohort study.**

I declare that:

- I have read this information/it was explained to me by a trusted person in a language with which I am fluent and comfortable.
- The research was clearly explained to me.
- I have had a chance to ask questions to both the person getting the consent from me, as well as the researcher and all my questions have been answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be handled in a negative way if I do so.
- I may be asked to leave the study before it has finished, if the researcher feels it is in the best interest, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 20....

....

Signature of participant Signature of witness

..... ……

Signature of researcher Signature of person obtaining consent

Declaration by person obtaining consent

I *(name)* ..... declare that:

- I clearly and in detail explained the information in this document to

  …….

- I did/did not use an interpreter.
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I gave him/her time to discuss it with others if he/she wished to do so.

Signed at (*place*) .....…..…… on (*date*) ……...…….20....

....

Signature of person obtaining consent Signature of witness

Annexure C: Screening Form

Screening questionnaire to determine eligibility for study:

Inclusion criteria and exclusion criteria:

Inclusion criteria	**Yes**	**No**	**Comments**
HIV-positive			
Black race			
Last menstrual period ≥ 6 months ago)			
Exclusion criteria	**Yes**	**No**	**Comments**
Use of anti-osteoporotic agents or chronic treatment known to affect BMD:			
Corticosteroids (Check patient file for Prednisone, Meticorten, Panafcort)			
Thyroid medication (initiated within the year) (Check patient file for Eltroxin, Euthyrox)			
Anti-vitamin K agents (Check patient file for warfarin)			
Diuretics> 12.5mg/day (Check patient file for Amiloretic, Adco-retic, Dyazide 25mg/day)			
Anti-epileptic drugs (Check patient file for Epanutin, Lethyl)			
β-blockers (Check patient file for Purbloka, Tenbloka)			
Secondary caused of osteoporosis (Check patient file):			
Chronic liver disease			
Chronic obstructive pulmonary disease			
Chronic renal disease			
Immobility (observe patient)			
Rheumatoid arthritis			
Gastrectomy			
Malabsorption syndrome			
Diabetes mellitus			
Metabolic bone disease			
Habitual use of calcium and/or vitamin D supplementation			
High alcohol consumption (≥ 3 units/day) (ask patient)			
Fracture within the last 6 months			
(ask patient)			
Severely low BMD (T score < -3) (measured at baseline visit): Yes: No:			

Annexure D: Socio-Demographic Questionnaire

SOCIO-DEMOGRAPHIC QUESTIONNAIRE

(All information in this questionnaire is confidential).

Interview Date:_____/_____/201_

Participant number: _____

Interviewer: _____

1. Ethnicity and language

1	Tswana		*1*		Tswana		
2	Zulu		*2*		Zulu		
3	Xhosa		*3*		Xhosa		
4	Coloured		*4*		Afrikaans		7 English
5	Sotho		*5*		Sotho		
6	Other (specify)		*6*		Other (specify)		

2. Housing

Code	*1*	*2*	*3*	*4*	*5*	*6*
Type of dwelling? You **can tick more than one** block if necessary	Brick, concrete	Traditional mud	Tin	Plank, Wood	Other (Specify)	……..
Value of the house?	Low	Middle	High			
Where do you get drinking water from most of the time? **(Tick one)**	Own tap	Communal tap	River, Dam	Borehole, Well	Other (Specify)	……..
What type of toilet does this household have? **(Tick one)**	Flush	Pit	Bucket, Pot	VIP	Other (Specify)	……..
What fuel is used for cooking most of the time (You can tick **more than one**)	Electric	Gas	Paraffin	Wood/Coal	Solar/sun	Open fire
Number of people sleeping in the house for at least 4 nights per week?						
Number of rooms in the house? (excluding bathroom, toilet and kitchen if separate)						
Number of people living/sleeping per room? **(Tick one)**	1	2	3	4	5	>5

3. Does anyone in the household own a motor **car**?

1yes	2 no

4. Number of motor cars owned in the household?

1	2	3	>3

5. Does the household have a ***working***

	Yes (1)	No (2)*	Codes
Fridge with freezer			1
Fridge only			2
Freezer only			3
Electric stove with oven			4
Gas stove with oven			5
Coal stove with oven			6
Microwave			7
Primus or Paraffin stove			8
2-plate stove/hot plate			9
Radio			10
TV			11
DSTV			12
DVD Player			13
Other electrical appliances (Specify)			14
……			

Code for "Yes" = 1, "No" = 2

6. Information with regards to own education and household income:

Codes	Education		Employment		Codes
1	None		Unemployed		1
2	Primary School		Self-employed small business		2
3	Grade 8-10		Wage earner e.g. cleaner/domestic		3
4	Grade 11-12 (matric)		Salaried trained worker		4
5	Tertiary Education		Other (specify)…….		5
6	Don't know		Pensioner		6

How many people in the household contribute to the total income (please tick one)

1	*2*	*3*	*4*	*5*	*6*
1 person	2 persons	3 persons	4 persons	More than 4	Don't know

Household income per month (including wages, rent, grants, sales of products, etc)

1	*2*	*3*	*4*	*5*	*6*	*7*
None	<R500	R500-1000	R1001-3000	R3001-6000	>R6001	Don't know

Is this the usual income of the Household?

1	*2*	*3*
Yes	No	Don't know

Was this the income you have more or less received on average over the past six months?

1	*2*	*3*
Yes	No	Don't know

Annexure E: Health Questionnaire/ Medical History

1. Has a doctor or nurse told you that you had or have any of the following:

1.2. High blood pressure

1	2	3	Duration (y)
Yes	No	Do not know	

1.3. Diabetes or high sugar levels in the blood

1	2	3	Duration (y)
Yes	No	Do not know	

1.4. Heart attack/ Angina/Chest pains

1	2	3	Duration (y)
Yes	No	Do not know	

1.5. Stroke: difficulty speaking/using hands/walking

1	2	3	Duration (y)
Yes	No	Do not know	

1.6. High blood cholesterol (fats)

1	2	3	Duration (y)
Yes	No	Do not know	

1.7. Gout

1	2	3	Duration (y)
Yes	No	Do not know	

1.8. Other (specify)…

1	2	3	Duration (y)
Yes	No	Do not know	

Annexure F: Case Report Form

Participant number: .....

Measurement	Value	Comment
Anthropometry		
Weight (kg)		
Height (cm)		
Body mass index (kg/m^2)		
Waist circumference (cm)		
Mid-upper arm circumference (MUAC) (cm)		
Calf circumference (cm)		
Blood values (from hospital file, record latest available data)		
Haemoglobin		
CD4 count		
Viral load		
Serum creatinine		
Creatinine clearance		
C-reactive protein (CRP)		
Medication		
ART regimen (standard/other)	Tenofovir TDF, Emtricitabine FTC, Efavirenz EFV (Odimune/Atripla) Other:	

Annexure G: Global Physical Activity Questionnaire

GPAQ

Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed].* In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

	Questions	Response	Code
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 4*	P1
2	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days ⌊__⌋	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 7*	P4
5	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days ⌊__⌋	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P6 (a-b)
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. [insert other examples if needed]			
7	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days ⌊__⌋	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P9 (a-b)
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), [insert relevant terms].			
10	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like [*running or football,]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 13*	P10
11	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P12 (a-b)

Continued on next page

GPAQ, Continued

Physical Activity (recreational activities) contd.			
Questions		**Response**	**Code**
13	Do you do any moderate-intensity sports, fitness or recreational (*leisure*) activities that causes a small increase in breathing or heart rate such as brisk walking,(*cycling, swimming, volleyball*)for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P16*	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational (*leisure*) activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P15 (a-b)
Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*			
16	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs min s	P16 (a-b)

Annexure H: Quantitative Food Frequency Questionnaire

Quantitative Food Frequency Questionnaire

Subject ID **Visit**

☐☐ ☐☐☐ ☐☐☐ ☐☐ B 1 2

Today's date: ☐☐☐☐ ☐☐ ☐☐

year *month* *day*

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
• *PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH*								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slap pap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____ _____ _____ _____ _____							
Do you pour milk on your porridge or cereal? Yes **1** ☐ **2** ☐ No If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								
Do you put sugar on your porridge or cereal? Yes **1** ☐ **2** ☐ No								
If yes, how much sugar								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Samp	Bought Self ground							
Samp and beans	Give ratio of samp:beans							
Samp and peanuts	Give ratio of samp:peanuts							
Rice	White							
	Brown							
	Maize Rice							
Pasta	Macaroni Spaghetti Other specify: _____ _____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pizza	Homemade: Specify topping _____ _____ _____ _____							
	Bought: Specify topping _____ _____ _____ _____							
You are being very helpful. Can I now ask you about meat? • **<u>CHICKEN, MEAT, FISH</u>** • How many times do you eat meat (beef, mutton, pork, chicken, fish) per week? _____								
Chicken (codes with skin)	Boiled							
	Fried: in batter/crumbs E.g. Kentucky							
	Fried: Not coated							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Bought: Chicken Licken							
	Bought: Nando's							
	Roasted / Grilled							
	Other: _____ _____							
Do you eat chicken skin?		**1** Always		**2** Sometimes		**3** Never		
Chicken bones stew								
Chicken feet								
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other:							
Beef Offal	Intestines: boiled nothing added							
	Stewed with vegetables							
	Liver							
	Kidney							
	Other: Specify _____ _____ _____ _____							
Goat meat	Boiled							
	Stewed with vegetables							
	Grilled / Roasted							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
• **What type of vegetables is usually put into meat stews?** _____ _____								
Wors / Sausage								
Bacon								
Cold meats	Polony							
	Ham							
	Vienna							
	Other: Specify _____ _____ _____ _____ _____ _____							
Canned meat	Bully beef							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: Specify _____ _____ _____ _____							
Meat pie	Beef							
	Steak and kidney							
	Cornish							
	Chicken							
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup							
	Salad							
Soya products e.g. Toppers	Brands at home now: _____ _____ _____ _____							
	Whole							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Pilchards in tomato/chilli/ brine	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna							
	Pickled fish							
	Other: Specify _____ _____							
Fish cakes	Bought: Fried							
	Home made with potato							
Fish fingers	Bought							
Eggs	Boiled/poached							
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit • *VEGETABLES AND FRUIT*								
Cabbage	How do you cook cabbage?							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Boiled, nothing added							
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added							
	Boiled with fat added Type of fat ……							
	With onion, tomato, potato							
	With peanuts							
	Other:							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……..							
	Without fat							
	Canned							
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added							
	Cooked in fat and sugar Fat ……							
	Boiled, little sugar and fat Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad							
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat ……							
	On cob – no fat added							
	Creamed sweet corn / canned							
	Whole kernel/canned							
Beetroot	Salad							
	Boiled, nothing added							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin							
	Boiled/baked without skin							
	Mashed							
	Roasted Fat …… ……							
	French fries (chips)							
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin							
	Boiled/baked without skin							
	Mashed							
	Other:							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Salad vegetables	Mixed salad: tomato, lettuce and cucumber							
	Raw tomato							
	Other salad vegetables:							
Other vegetables, specify + preparation	_____ _____ _____ _____ _____ _____							
Do you like fruit?		**1** Yes		**2** No				
Apples								

Pears								
Oranges								
Naartjie								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Grapes								
Peaches	Fresh							
	Canned							
Apricots	Fresh							
	Canned							
Mangoes								
Guavas	Fresh							
	Canned							
Avocado								
Wild fruit/berries	Specify type:							
Dried fruit	Types:							

Other fruit	_____ _____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
If subject eats canned fruit: Do you have custard with the canned fr Yes No 2								
Custard	Homemade: Milk							
	Commercial e.g. Ultramel							
• **BREAD AND BREAD SPREADS**								
Bread / Bread rolls	White							
	Brown							
	Whole wheat							
Do you spread anything on the bread? Always **1** Sometimes **2** Never **3**								
Margarine	What brand do you have at home now?							

	Don’t know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Peanut butter								
Jam/syrup/honey								
Marmite / Fraybentos / Oxo								
Fish/meat paste								
Cheese	Type:							
Atchar								
Other spreads	Specify:							
Dumpling								
Vetkoek	White flour							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Whole wheat flour							
Provita, crackers, etc								
Mayonnaise / salad dressing	Mayonnaise							
	Other: Specify							
• **DRINKS**								
Tea	English (normal)							
	Rooibos							
Coffee								
Sugar/cup tea or coffee	Tea:							
	Coffee:							
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full							

	Fresh/long life: 2%/low fat							
	Fresh/long life: fat free							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Whole milk powder Brand:							
	Low fat milk powder Brand:							
	Skimmed milk powder Brand:							

	Milk blend Brand:							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Whitener: type							
	Condensed milk							
	Evaporated milk							

	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full							
	Fresh/long life: 2%/low fat							
	Fresh/long life: fat free							
	Condensed milk							
	Sour/maas							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other:							
Milk drinks	Nestle:							
	Milo:							
	Flavoured milk:							
	Other:							
Yoghurt	Drinking yoghurt							
	Thick yoghurt							

	Low fat sweetened with fruit							
Squash	Sweet O							

FOOD	**DESCRIPTION**	**AMOUNT**	**TIMES EATEN**				**CODE**	**AMOUNT / DAY**
			Per day	**Per week**	**Per month**	**Seldom / Never**		
	Six O							
	Oros/Local – with sugar							
	- artificially sweetener							
	KoolAid							
	Other:							
Fruit juice	Fresh/Liqui-fruit/Ceres							
	Tropica (Dairy – fruit juice mix)							

	Other:							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Fizzy drinks	Sweetened							
Coke, Fanta, etc	Diet							
Mageu/ **Motogo**								
Home brew								
Tlokwe								
Beer								
Spirits								
Wine red								
Wine White								
Other specify	_____ _____ _____ _____ _____ _____							

• *SNACKS AND SWEETS*								
Potato crisps								
Peanuts	Raw							

FOOD	**DESCRIPTION**	**AMOUNT**	**TIMES EATEN**				**CODE**	**AMOUNT / DAY**
			Per day	**Per week**	**Per month**	**Seldom / Never**		
	Roasted							
Cheesecurls, Nik-naks, etc.								
Raisins								
Peanuts and raisins								
Chocolates	Name:							
Candies	Sugus, gums, hard sweets, etc							

Sweets	Toffees, fudge, caramels							
Biscuits/cookies	Type:							

FOOD	**DESCRIPTION**	**AMOUNT**	**TIMES EATEN**				**CODE**	**AMOUNT / DAY**
			Per day	**Per week**	**Per month**	**Seldom / Never**		
	_____ _____							

Cakes and tarts	Type:							
Scones								
Rusk's	Type:							
Savouries	Sausage rolls							
	Samoosas: Meat filling							
	Samoosas: Vegetable filling							
	Biscuits e.g. bacon kips							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other specify:							
Jelly								
Baked pudding	Type:							
Instant pudding	Milk type:							
Ice cream								
Sorbet								
Other specify	_____ _____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
• *SAUCES, GRAVIES AND CONDIMENTS*								
Tomato sauce / Worcester sauce								
Chutney								
Pickles								
Packet soups								
Other:								
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								
• *INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS* **Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts etc. to eat**								
Specify								

Annexure I: Referral Letter

Metabolic Unit Centre of Excellence for Nutrition
Tel: (018) 299 -2480/
(018) 299 2482
(Professor HS Kruger) Salome.kruger@nwu.ac.za

Dear Doctor

We are currently carrying out a research project in the North-West University on the bone health of women.

…… participated in this project.

We found the following results that may necessitate follow-up medical care:

We kindly request to please assist including further testing and treatment if applicable. Kind regards

Signature: Date:

Name
