The association between fracture risk and bone mineral density in black postmenopausal HIV-positive women on HAART

C van der Merwe

orcid.org/ 0000-0003-0364-1113

Mini-dissertation submitted in partial fulfilment of the requirements for the degree Master of Science in Dietetics at the North-West University

Supervisor: Prof HS Kruger

Co-supervisor: Dr PO Ukegbu

Graduation: May 2019

Student number: 23498889

Plagiarism Declaration

I, Carlien van der Merwe 23498889, hereby declare that this mini-dissertation is my own work. I further declare that:

1. the text and bibliography reflect the sources I have consulted, and
2. where I have made reproductions of any literary or graphic work(s) from someone else, I have obtained the necessary prior written approval of the relevant author(s)/publisher(s)/creator(s) of such works and/or, where applicable, from the Dramatic, Artistic and Literary Rights Organisation (DALRO).
3. sections with no source referrals are my own ideas, arguments and/or conclusions.

Signature: 

Student number: 23498889

Date: 29-10-2018

Acknowledgements

I hereby express my sincere appreciation to those who have contributed to this dissertation and have supported me, without them, this dissertation would not have been possible.

To my supervisor Prof H.S. Kruger and co-supervisor Dr P.O. Ukegbu:

I would like to express my sincere thanks to Prof. H.S. Kruger for providing me with the oppurtunity to completed my Masters degree. I would also like acknowledge my co-supervisor Dr P.O. Ukegbu for her contribution and insights into this dissertation.

To my parents:

Thank you for your encouragement, support and patience during this process.

To the Lord:

I express my gratitude for giving me wisdom and strengthen my heart in times of doubt.

“If any of you lack wisdom, you should ask God, who gives generously to all without finding fault, and it will be given to you”. James 1:5.

Abstract

Background: Osteoporosis affects millions of people, especially postmenopausal women, worldwide. Osteoporosis and associated fractures are also becomming a concern in the HIV-positive population as a result of higher life expectancy and possible fragility fractures due to the advancement in antiretroviral therapy (ART); increased prevalence of low bone mineral density (BMD) as a result of ART-induced bone demineralization as well as increased bone loss due the HIV-infection it self.

Urbanisation also places the urban HIV-positive postmenopausal women at risk for the development of low BMD. In South Africa rapid urbanisation is associated with dietary and lifestyle changes that negatively influence BMD. Thus, the effects of long term use of ART in combination with other factors associated with the aging body as well as urbanisation are a concern.

Objectives: This study aimed to determine the number of fracture risk factors and the association with BMD in black postmenopausal HIV-positive women on highly active antiretroviral therapy (HAART).

Methods: This study was a cross-sectional analysis and baseline data from 120 HIV-positive black post-menopausal women in a prospective cohort study in the North West Province of South Africa was used. Bone mineral density (at the spine, left femoral neck and total body) was measured by dual X-ray absorptiometry (DXA). The number of fracture risk was determined using a checklist. Multivariate linear regression models were applied to assess associations of fracture risk score with site specific BMDs, adjusting for age, calcium intake, serum vitamin D, duration of HIV infection, duration of HAART and physical activity.

Results: All participants had the age (>40 years) and female sex risk factors, with 39.2% having only two and 37.5% having three risk factors. The maximum number of risk factors was five. Age and underweight were the only individual risk factors significantly associated with BMD. In adjusted models, only age was significantly associated with BMD, but fracture risk was included in the final model for spine BMD and left femoral neck BMD. No significant association between fracture risk score and BMD was found.

Conclusions: A maximum of five fracture risk factors were found, but fracture risk score was not significantly associated with BMD in this group of HIV-positive women.

Keywords: HIV, postmenopausal, BMD, osteoporosis, fracture risk, Africa.

List of abbreviations

ART:	Antiretroviral therapy
BMC:	Bone mineral content
BMD:	Bone mineral density
BMI:	Body mass index
CT:	Computed tomography
D4T:	Stavudine
DXA:	Dual energy x-ray absorptiometry
FFQ:	Food frequency questionnaire
FRAX:	The Fracture Risk Assessment Tool
GH:	Growth hormone
GPAQ:	Global Physical Activity questionnaire
HAART:	Highly active antiretroviral therapy
HIC:	High income country
HIV:	Human immunodeficiency virus
HREC:	Health Research Ethical Committee
LBM:	Lean body mass
MET:	Metabolic equivalent of task
NRTI:	Nucleoside reverse transcriptase inhibitors
NNRTI:	Non-nucleoside reverse transcriptase inhibitors
NWU:	North-West University
MI:	Myocardial infarction
PA:	Physical activity
PBM:	Peak bone mass
PPI:	Proton pump inhibitor
PI:	Protease inhibitors
PTH:	Parathyroid hormone
RCT:	Randomized control trials
RNA:	Ribonucleic acid
TDF:	Tenofovir
WHO:	World Health Organization

Content

1 Chapter 1: Introduction 1
1.1 Background to the problem 1
1.2 Problem statement 1
1.3 Aim 3
1.4 Objectives 3
1.5 Study design 4
1.6 Research team 5
1.7 References 6
2 Chapter 2: Literature review 10
2.1 Introduction 10
2.2 Normal bone physiology, remodelling and its hormonal regulation 11
2.3 Bone mass 15
2.4 Role of nutrients in bone health 18
2.5 Lifestyle and behavioural factors 22
2.6 Bone disease 23
2.7 HIV infection 26
2.8 Bone health in the South African context 28
2.9 Conclusion 30
2.10 References 32
3 Chapter 3: Methodology 41
3.1 Study design and population 41
3.2 Sampling 41
3.3 Recruitment of participants 43
3.4 Setting 44
3.5 Data collection 44
3.6 Statistical methods 48
3.7 Ethical considerations 48
3.8 Data management plan 52
3.9 References 55
4 Chapter 4: Article 58
5 Chapter 5: Summary and conclusion 77

Figures

Figure 1: Calcium Homeostasis. 15

Tables

Table 3.1: Characteristics and sample size of studies on the association between fractue risk and BMD. 42
Table 3.2: Units of alcoholic drinks according to the United Kingdom National Health Service guidelines 46
Table 4.1: Baseline characteristics of the study participants 64
Table 4.2: Fracture risk factors identified among women. 66
Table 4.3: Multiple regression models for the association between number of risk factors and BMD with site-specific BMD as the dependant variable (N=120). 67

Annexures

Annexure A: Screening Form 82
Annexure B: Socio-Demographic Questionnaire 83
Annexure C: Health Questionnaire/ Medical History 86
Annexure D: Case Report Form 87
Annexure E: Quantitative Food Frequency Questionnaire 88
Annexure F: Global Physical Activity Questionnaire 116
Annexure G: Fracture Risk 118
Annexure H: Informed Consent 119
Annexure I: Referral Letter 124

1 Chapter 1: Introduction

1.1 Background to the problem

Osteoporosis is a multifactorial skeletal disease affecting millions of people, especially postmenopausal women, worldwide (Eastell, 2013; Cano *et al*., 2018; Castiglioni, 2013). Osteoporosis and associated fractures are not only a concern in postmenopausal women but also in the HIV-positive population (Piso *et al*., 2013). Due to the advancement in antiretroviral therapy (ART) HIV-positive patients have a higher life expectancy (Piso *et al.,* 2013). However, with higher life expectancy the prevalence of fragility fractures may also increase (Triant *et al.,* 2008; Young *et al*., 2011; Cortés *et al.,* 2015).

Various studies have shown a marked increase in the prevalence of bone demineralization, low bone mineral density (BMD) and fracture incidences in HIV-positive individuals (Compston, 2016; Tebas *et al.,* 2000; Bruera *et al*., 2003; Landonio *et al.,* 2004; Dave *et al*., 2015; Cotter & Powderly, 2011; Cortés *et al.,* 2015). HIV-infection is not the only factor that leads to increased bone loss. ART is also known to decrease BMD by various mechanisms (McComsey *et al.,* 2010; Dave *et al.,* 2015). Thus, the effects of long term use of ART in combination with other factors associated with the aging body are a concern (Piso *et al.,* 2013).

Urbanisation is another factor that increases postmenopausal women's risk for the development of low BMD and consequently osteoporosis and associated fractures (Kruger *et al.,* 2011). South Africa is experiencing rapid urbanisation that is associated with dietary and lifestyle changes that negatively influence BMD (Kruger *et al.,* 2011; Vorster *et al.,* 2002). HIV-positive urban women experiencing menopause and associated bone loss are especially a concern (Cortés *et al.,* 2015). Urbanisation combined with increasing prevalence of HIV and ART use, have detrimental effects on the bone health of South Africans (Cortés *et al*., 2015; Kruger *et al*., 2011; Vorster *et al*., 2002).

1.2 Problem statement

Osteoporosis is a common chronic disorder that has become a global epidemic with more than 8.9 million fractures annually (International Osteoporosis Foundation [IOF], 2017). Bone is constantly being repaired and renewed through the process of bone remodelling (Castiglioni, 2013). However, when the rate of bone resorption exceeds the rate of bone formation, bone loss will occur (Rachner *et al*., 2011; Ralston, 2013). In South Africa, the

incidence of osteoporosis is similar to that of high income countries (HIC), however; limited fracture data exist (IOF, 2017).

South Africa is experiencing rapid urbanisation with associated lower BMD that may increase the risk for the development of osteoporosis (Kruger *et al.*, 2011; Vorster *et al.,* 2002). Changes in diet and physical activity as a result of urbanisation may influence the risk for lower BMD (Kruger *et al.*, 2004). A shift from traditional foods consumed in rural areas towards consumption of western foods is associated with urbanisation (MacIntyre *et al*., 2002). These western food items are often low in nutrients that are essential for bone health such as calcium and vitamin D (Steyn & Mchiza, 2014; MacIntyre et al., 2002). Low calcium intakes are associated with lower BMD (Kruger & Wolber, 2016), while vitamin D deficiency is associated with muscle weakness, low bone mass and an increased susceptibility to osteoporosis, fractures (Borji & Nasri, 2017:29) as well as fall risk in the elderly (Bischoff-Ferrari *et al*., 2004).

Urbanisation is not the only factor that influences the risk for osteoporosis and associated fractures in South Africa. HIV and ART use also have an influence on BMD and the risk for osteoporosis and associated fractures. South Africa has the highest prevalence of HIV-infection in the world with 7.06 million people living with HIV in 2016 (UNAIDS, 2016a). In spite of the high prevalence of HIV, 6.19 million people in South Africa had access to highly active antiretroviral therapy (HAART) in 2015 (UNAIDS, 2017). The estimated life expectancy of patients living with HIV has increased significantly as a result of a decline in the prevalence of opportunistic infections (Piso *et al*., 2013). However, with higher life expectancy it can be expected that the prevalence of fragility fractures will also increase (Triant *et al*., 2008; Young *et al*., 2011; Cortés *et al*., 2015).

Various studies showed a marked increase in the prevalence of bone demineralization in HIV-positive individuals (Compston, 2016; Tebas *et al*., 2000; Bruera *et al*., 2003; Landonio *et al*., 2004). However, it should be noted that most research regarding fracture risk and HIV was conducted in Europe, Australia and North America (Compston, 2016).

The causes of low BMD in patients with HIV is multifactorial and includes traditional and HIV-associated factors (Dave *et al*., 2015). Both ART use and HIV-infection lead to increased bone loss (McComsey *et al*., 2010). Traditional factors include: low weight, older age, smoking and female gender. HIV-associated factors include: period of HIV-infection, stavudine (D4T) use, HIV ribonucleic acid (RNA), tenofovir (TDF) use, protease inhibitors (PI) use and duration of nucleoside reverse transcriptase inhibitors (NRTI) use (Dave *et al.*, 2015). ARTs decreases BMD by various mechanisms and have also been associated

with poor vitamin D status (Hamill *et al*., 2013; Dave *et al*., 2015). Race plays a role in modifying the relationship between HIV and fracture risk. Previously, studies reported that black populations have a reduced risk for the development of osteoporosis due to their enhanced BMDs, however; these studies were conducted in America and Europe and may not be relevant for South African's black population (Aloia *et al*., 1996; Handa *et al*., 2008). In fact, very little data on BMD is available on non-white population groups living in low- to middle income countries such as South Africa (George *et al*., 2014).

Urbanisation combined with increasing prevalence of HIV and ARV use have detrimental effects on the bone health of South Africans and is a growing concern. This study will contribute information on the association between fracture risk and BMD in black postmenopausal HIV-positive women on HAART. HIV-positive women experiencing menopauseand associated bone loss are especially a vulnerable group (Cortés *et al*., 2015).

1.3 Aim

This study is a sub-study of a larger prospective cohort study with HIV-positive postmenopausal women on HAART from the North West Province and has a cross-sectional study design. The aim of the larger study is to assess the association between calcium and vitamin D status and bone health in adult black HIV-positive and HIV-negative postmenopausal women. For the purpose of this study only the baseline data was used.

The aim of this sub-study was to investigate the association between fracture risk (number of risk factors) and BMD in black postmenopausal HIV-positive women on HAART.

1.4 Objectives

To accomplish the aim of this study, the following objectives were determined:

- The number of fracture risk factors of black postmenopausal HIV-positive women on HAART.
- The association between number of risk factors and BMD of the whole body, left femoral neck and spine, respectively, in black postmenopausal HIV-positive women on HAART, after adjustment for possible covariates.

1.5 Study design

This study was a cross-sectional analysis of baseline data from the prospective cohort study with HIV-positive women on HAART. The methodology is described in detail in Chapter 3.

1.6 Research team

Role of team member	Name
Study leader/Principal investigator: The study leader gave guidance on the planning of the dissertation, statistical analysis, and writing up of the data.	Prof H. Salome Kruger, Professor of Nutrition, Centre of Excellence for Nutrition, NWU, Potchefstroom
Co-supervisor: The co-supervisor gave assistance to the supervisor on the planning of the dissertation, statistical analysis and writing up of the data.	Dr Patricia O. Ukegbu, Postdoctoral Fellow, Centre of Excellence for Nutrition, NWU, Potchefstroom
Student: Ms Carlien van der Merwe completed this study (mini-dissertation) for her MSc degree in Dietetics. The student participated in collection, computerisation and cleaning of data.	Ms Carlien van der Merwe, MSc student and registered dietitian.
Blood sampling, coordination of measurements in Metabolic Unit.	Sr. Chrissie Lessing, Registered Nursing Professional, Centre of Excellence for Nutrition, NWU, Potchefstroom.
Collaborator: Team member, support with data analysis	Dr Cristian Ricci, Postdoctoral Fellow, Centre of Excellence for Nutrition, NWU, Potchefstroom.
Team member, recruitment of participants.	Mr Milton Semenekane, Nutrition intern, Centre of Excellence for Nutrition, NWU, Potchefstroom.
Trained post graduate students and research assistants.	Post graduate students were trained and assisted with data collection, through conducting interviewer administered questionnaires and conducting anthropometric measurements. C. Ellis, I. Jacobs, K. Bengis, N.M. Mate, E. Strydom, B. Olifant, K. Lee, M. Britz, M. Jansen, H. Asare, P. Molefi.
Supervising Medical Doctor.	Dr Semakula, Potchefstroom hospital.

1.7 References

Aloia, J.F., Vaswani, A., Yeh, J.K. & Flaster, E. 1996. Risk for osteoporosis in black women. *Calcified tissue international*, 59(6):415-423.

Bischoff-Ferrari, H., Dawson-Hughes, B., Willet, W.C., Staehelin, H.B., Bazemore M.G., Zee R.Y. & Wong, J.B. 2004. Effect of vitamin D on falls: a meta-analysis. *Journal of the American medical association*, 291:1999-2006.

Borji, S. & Nasri, H. 2017. An update on prevention and treatment of osteoporosis. *Journal of parathyroid disease*, 5(2):29.

Bruera, D., Luna, N., David, DO., Bergoglio, L.M. & Zamudio, J. 2003. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS*, 17:1919-1922.

Cano, A., Chedrauib, P., Goulisc, D.G., Lopesd, P., Mishrae, G., Mueckf, A., Senturkg, L.M., Simoncinih, T., Stevensoni, J.C., Stutej, P., Tuomikoskik, P., Reesl & Lambrinoudakim, M.I. 2018. Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. Maturitas*,* 107:7-12.

Castiglioni, S., Albisetti, W. & Maier, J.A.M. 2013. Magnesium and Osteoporosis: Current State of Knowledge and Future Research Directions. *Nutrients*, 5:3022-3023.

Compston, J. 2016. HIV infection and bone disease. *Journal of internal medicine*, 280:350-358.

Cortés, Y.I., Yin, M.T. & Reame, N.K. 2015. Bone Density and Fractures in HIV-infected Postmenopausal Women: A Systematic Review. *Journal of the Association of Nurses in AIDS care,* 26(4):387.

Cotter, A.G. & Powderly, W.G. 2011. Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity. *Best practice & research clinical endocrinology & metabolism,* 25:501-515.

Dave, J.A., Cohen, K., Micklesfield, L.K., Maartens, G. & Levitt, N.S. 2015. Antiretroviral Therapy, especially Efavirenz, is associated with low bone mineral density in HIV-infected South Africans. *PLoS ONE*, 10(12). https://doi.org/10.1371/journal.pone.0144286 Date of access: 14 July 2017.

Eastell, R. 2013. Osteoporosis. *Medicine,* 41(10):586-587.

Farsinejad-Marj, M., Saneei, P. & Esmaillzadeh, A. 2015. Dietary magnesium intake, bone mineral density and risk of fracture: a systematic review and meta-analysis. *Osteoporosis international*, 27:1389-1394.

Focà, E., Motta, D., Borderi, M., Gotti, D., Albini, L., Calabresi, A., Izzo, I., Bellagamba, R., Narciso, P., Sighinolfi, L., Clò, A., Gibellini, D., Quiros-Roldan, E., Brianese, N., Cesana, B.M., Re, M.C. & Torti, C. 2012. Prospective evaluation of bone markers, parathormone and 1,25-(OH)2 vitamin D in HIV positive patients after the initiation of Tenofovir/ Emtricitabine with Atazanavir/Ritonavir or Efavirenz. *BioMed central*, 12:38.

George, J.A., Micklesfield, L.M., Norris, S.A. & Crowther, N.J. 2014. The Association between body composition, 25(OH)D, and PTH and bone mineral density in black African and Asian Indian population groups. *The journal of clinical endocrinology & metabolism*, 99(6):2146-2154.

Hamill, M.M., Ward, K.A., Pettifor, J.M., Norris, S.A. & Prentice, A. 2013. Bone mass, body composition and vitamin D status of ARV-naive, urban, black South African women with HIV infection, stratified by CD count. *Osteoporosis international*, 24:2855-2861.

Handa, R., Kalla, A.A. & Maalouf, G. 2008. Osteoporosis in developing countries. *Best practice & research clinical rheumatology*, 22(4):693-708.

International Osteoporosis Foundation. 2017. *Facts and Statistics: osteoporosis - incidence and burden.* Retrieved from https://www.iofbonehealth.org/facts-statistics#category-14 Date of access: 23 October 2017 .

Joint United Nations Programme on HIV and AIDS. 2016a. Country factsheet South Africa 2016. www.unaids.org/en/regionscountries/countries/southafrica Date of access: 23 October 2017.

Joint United Nations Programme on HIV and AIDS. 2017. Global HIV statistics. Geneva. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf Date of access: 23 October 2017.

Kauta, N., Held, M., Dlamini, S., Kalula, S., Ross, I., Kalla, G. & Maqungo, S. 2017. The management of fragility fractures of the hip: a quality assessment project. *South African orthopaedic journal,* 16(3):41-45.

Kruger, M.C., Dewinter, R.M., Becker, P.J. & Vorster, H.H. 2004. Changes in markers of bone turnover following urbanisation of black South African women. *Journal of endocrinology, metabolism and diabetes of South Africa,* 9(1):103-108.

Kruger, M.C., Kruger, I.M., Wentzel-Viljoen, E. & Kruger, A. 2011. Urbanisation of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition research*, 31:748-758.

Kruger, M.C. & Wolber, F.M. 2016. Osteoporosis: modern paradigms for last century's bones. *Nutrients*, 8:6.

Kryst, J., Kawalec, P. & Pilc, A. 2014. Efavirenz-based regimens in antiretroviral-naive HIV-infected patients: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*, 10(5):e0124279.

Landonio, S., Quirino, T., Bonfanti, P., Gabris, A., Boccassini, L., Gulisano, C., Vulpio, L., Ricci, E., Carrabba, M. & Vigevani, G.M. 2004. Osteopenia and osteoporosis in HIV+ patients, untreated or receiving HAART. *Biomedicine & pharmacotherapy,* 58:506-507.

MacIntyre, U.E., Kruger, H.S., Venter, C.S. & Vorster, H.H. 2002. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutrition research*, 22:239-256.

McComsey, G.A., Tebas, P., Shane, E., Yin, M.T., Overton, E.T., Haung, J.S., Aldrovandi, G.M., Cardoso, S.W., Santana, J.L. & Brown, T.T. 2010. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. *Clinical infectious disease*, 51:937-943.

Piso, R.J., Rothen, M., Rothen, J.P., Stahl, M. & Fux, C. 2013. Per oral substitution with 300000 IU Vitamin D (Cholecalciferol) reduces bone turnover markers in HIV-infected patients. *BMC infectious diseases*, 13:577.

Rachner, T.D., Khosla, S. & Hofbauer, L.C. 2011. Osteoporosis: now and the future. *Lancet,* 377:1276-1277.

Ralston, S.H. 2013. Bone structure and metabolism. *Medicine,* 41(10):581-582.

Shiau, S., Broun, E.C., Arpadi, S.M. & Yin, M.T. 2013. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. *AIDS*, 27:1949-1957.

Steyn, N.P. & Mchiza, Z.J. 2014. Obesity and the nutrition transition in Sub-Saharan Africa. *The annals of the New York academy of sciences*, 1311:88-101.

Tebas, P., Powderly, W.G., Claxton, S., Marin, D., Tantisiriwat, W., Teitelbaum, S.L. & Yarasheski, K.E. 2000. Accelerated bone mineral loss in HIV infected patients receiving potent antiretroviral therapy. *AIDS,* 14:64-67.

Triant, V.A., Brown, T.T., Lee, H. & Grinspoon, S.K. 2008.Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non–HIV-infected patients in a large U.S. healthcare system. *Journal of clinical endocrinology metabolism*, 93:3499-504.

Young, B., Dao, C.N., Buchacz, K., Baker, R., Broks, J.T., 2011. HIV outpatientstudy (HOPS) investigators. Increased rates of bone fracture among HIV- infectedpersons in the HIV outpatientstudy (HOPS) comparedwith the U.S. general population. 2000-2006. *Clinical infectious disease*, 52:1061-1068.

Vorster, H.H., Oosthuizen, W., Jerling, J.C., Voldman, F.J. & Burger, H.M. 1997. The nutritional status of South Africans: A Review of literature form 1975-1996. Durban: *Health systems trust,* 1-122.

Vorster, JNR. H.H., Venter, C.S., Kruger, M.C., Vorster, H.H. & Kruger, H.S. 2002. The impact of urbanisation on risk factors for osteoporosis in black postmenopausal South African women. *Journal of endocrinology, metabolism and diabetes of South Africa,* 7(3):92-99.

2 Chapter 2: Literature review

2.1 Introduction

Bones of the skeleton provide support for tendons, joints and ligaments (Ralston, 2013). It also provides organ protection, assures normal mineral homeostasis by providing a reservoir for phosphate and calcium and provides an environment for bone marrow (Herman, 2016:251). Bone is metabolically active tissue that undergoes remodelling throughout the adult life (Walsh *et al*., 2014). When abnormalities in the remodelling process occurs, the architecture, structure or strength of the bone can be affected, which leads to clinical symptoms, such as deformity, pain, fracture and abnormalities in the calcium and phosphate homeostasis (Ralston, 2013).

Adequate nutrition is of vital importance for bone health and a decreased risk of developing osteoporosis and associated fractures (Mangels, 2014). Various nutrients are needed for growth, development, formation of collagen and cartilage as well as calcium and phosphate homeostasis. Nutrients that especially play a role include: calcium, vitamin D, protein, phosphorus, magnesium, zinc, copper, manganese, vitamin C, vitamin B12, vitamin K and potassium (Castiglioni, 2013).

Osteoporosis has become a global epidemic with more than 8.9 million fractures annually. Fractures associated with osteoporosis have a significant impact on morbidity and mortality (IOF, 2017). Osteoporosis and associated fractures are also becoming a concern in the HIV-positive population (Piso *et al*., 2013). Low BMD and fractures incidences are higher in patients with HIV in comparison with the general population (Dave *et al*., 2015). Due to the advancement in ART the life expectancy of HIV-positive patients has increased (Cortés *et al*., 2015). However, concerns related to long term use of ART in combination with other factors associated with the aging body is becoming more prevalent (Piso *et al*., 2013).

This literature review will focus on normal bone physiology, remodelling and its hormonal regulation, nutrition and lifestyle factors in bone health, and especially on the current bone health situation in South Africa, including the effects of HIV and HIV treatment and bone health.

2.2 Normal bone physiology, remodelling and its hormonal regulation

2.2.1 Bone anatomy

Bone contains different types of tissue and may therefore be regarded as an organ (Marieb & Hoehn, 2016:197). The two main bone tissues are trabecular and cortical bone that undergo bone remodelling throughout the human lifespan (Stagi *et al*., 2013). The skeleton consists of approximately 80% of cortical bone and is found primarily in the shafts of long bones, whilst the remaining 20% consists of trabecular bone (Rolfes *et al*., 2012:389).

The cortical bone is the outer, hard compartment of bone and surrounds the trabecular bone (Bayliss *et al*., 2011). Cortical bone may appear to be a solid structure but in fact it contains passageways for nerves and blood vessels (Marieb & Hoehn, 2016:200).The cortical bone consists of an outer periosteal surface and inner endosteal surface. The periosteum contains nerve fibres, osteoblasts, osteoclasts and blood vessels. It provides nourishment, protection and plays a role in bone formation and fracture repair. The functional unit of the cortical bone is called the Haversian system which contains lamellae (Ralston, 2013:581; Marieb & Hoehn, 2016:200).

The trabecular bone is characterised as the inner, lacy matrix of the bone (Rolfes *et al*., 2012:389). It consists of a meshwork of interconnecting bony spicules, called trabeculae (Stagi *et al*., 2013; Ralston, 2013). The pressure applied on the bones during development is a determining factor of the position of the trabeculae (Stagi *et al*., 2013). The spaces between the trabeculae contain marrow (Marieb & Hoehn, 2016:197). This meshwork gives the bone a spongy appearance and makes trabecular bone less dense than cortical bone (Chapman-Novakofski, 2012:532). The trabecular tissue provides support to the cortical bone shell in long bones as well as a large surface area that is exposed to circulating substances from the marrow (Chapman-Novakofski, 2012:532). Because trabecular bone has a larger surface area than cortical bone, it is more metabolically active and responsive to changes in mineral homeostasis (Uusi-Rasi *et al*., 2013).

2.2.2 Chemical composition of bone

Bone contains both organic and inorganic substances. Organic substances include an organic matrix, called the osteoid, and bone cells. The inorganic substances are mineral salts. When the organic and inorganic substances are present in the right proportions the bone is extremely strong and durable (Marieb & Hoehn, 2016:203).

The organic component of bone includes bone cells (osteoclasts, osteocytes, osteoblasts, bone-lining cells and osteogenic cells) and the osteoid or organic matrix. The organic matrix consists of ground substance, composed of proteoglycans and glycoproteins, as well as collagen fibres (Walsh *et al*., 2014). Other components of the matrix include osteocalcin, osteopontin and other proteins (Bayliss *et al*., 2011). The inorganic component of bone includes hydroxyapatites, or mineral salts (Walsh *et al*., 2014). These salts, of which mainly calcium and phosphate salts, are deposited with hydroxyl ions in crystals of hydroxyapatite (Chapman-Novakofski, 2012:532).

2.2.3 Bone modelling

Bone modelling refers to the growth of the skeleton in response to mechanical stimuli until mature height is reached (Sims & Vahnas, 2014). During childhood and adolescence, bones will increase in size and become mineralized (Walsh *et al*., 2014:1). In females, bone modelling is usually completed by ages 16 to 18 and by 18 to 20 years of age in males (Chapman-Novakofski, 2012:533). During bone modelling the growing bones widen as they increase in length (Marieb & Hoehn, 2016:207) and bone mass, size and geometry are influenced during this process (Walsh *et al*., 2014). During the bone modelling process bone formation occurs before bone resorption (Chapman-Novakofski, 2012:533). Typically there is more bone formation than resorption going on which produces a thicker, stronger bone (Marieb & Hoehn, 2016:207). Beneath the periosteum, bone matrix is secreted by the osteoblasts on the external bone surface as the osteoclasts remove bone on the endosteal surface (Sims & Vahnas, 2014). Growth in long bones occurs both at the terminal epiphyses and in the lamellae (Chapman-Novakofski, 2012:533).

2.2.4 Hormonal regulation of bone modelling

Bone modelling or growth is regulated by various hormones until mature height is achieved (Stagi *et al*., 2013). Growth hormone (GH) is secreted by the anterior pituitary gland and serves during infancy and childhood as the stimulus of bone growth (Walsh *et al*., 2014:5). The activity of GH is regulated by thyroid hormones, enabling the skeleton to grow within proper proportions (Marieb & Hoehn, 2016:207). During puberty the sex hormones is responsible for growth spurts as well as feminization or masculinizing of specific parts of the skeleton (Stagi *et al*., 2013). Later during puberty, the secretion of sex hormones stimulates the closure of growth plates, ending longitudinal bone growth (Marieb & Hoehn, 2016:207).

2.2.5 Bone remodelling

The integrity of the human skeleton is assured through the continuous process of breakdown and repair, throughout the adult life, and is called remodelling (Marcus, 2012:862; Ralston, 2013). This remodelling process replaces old and micro damaged bone with new bone that preserve bone strength and integrity. Bone remodelling also plays a role in the maintenance of calcium homeostasis (Walsh *et al*., 2014). The process occurs in an estimated 10% of the adult human skeleton at a given time (Ralston, 2013). Trabecular bone is replaced every three to four years, whereas cortical bone is only replaced every 10 years. This is an essential process, as bone that is not replaced becomes brittle and prone to fractures due to the crystalizing of calcium salts (Marieb & Hoehn, 2016:207).

The two bone cells responsible for remodelling include osteoblasts and osteoclasts (Marcus, 2012:860). Osteoblasts are responsible for the formation of bone tissue and osteoclasts are responsible for the degradation thereof (Chapman-Novakofski, 2012:532). The remodelling process takes place in two stages. The first stage is resorption by the osteoclasts and the second stage is replacement by the osteoblasts. The osteoclasts secrete protons and lysosomal enzymes as they move along the bone surface, digesting the organic matrix (Marieb & Hoehn, 2016:207). During the resorption stage old, micro-damaged bone is removed and replaced by new bone during the replacement stage (Borji & Nasri, 2017).

2.2.6 Regulation of bone remodelling

Bone remodelling is regulated by genetic factors and two sets of controls. The first is a negative feedback hormonal loop that is responsible for maintaining constant serum calcium and the second involves mechanical and gravitational forces (Marieb & Hoehn, 2016:208).

2.2.6.1 Hormonal regulation and calcium homeostasis

Calcium is a mineral most often associated with bone integrity. The body contains 1200–1400 g of calcium of which 99% is present in the bones, where it forms an integral part of the bone structure and serves as a calcium reservoir to ensure calcium homeostasis (Marieb & Hoehn, 2016:208). The remaining 1% of the calcium is present in the extracellular and intracellular fluids and is vital for a variety of life processes (Rolfes *et al*., 2012:378), such as normal functioning of the nervous system, blood clotting and muscle contraction (Uusi-Rasi *et al*., 2013). Intracellular and extracellular

calcium concentration is closely regulated to ensure homeostasis. The skeleton system, gut, kidneys and 1,25-dihydroxyvitamin D3 (calcitriol) play a role in calcium homeostasis as well as two hormones namely, parathyroid hormone (PTH) and calcitonin (Chapman-Novakofski, 2012:533). A decline in serum calcium concentrations stimulates the parathyroid glands to secrete PTH. In turn PTH activates vitamin D. Vitamin D and PTH activates calcium resorption in the kidneys as well as stimulates the osteoclasts to resorb bone, releasing calcium into the blood (Marieb & Hoehn, 2016:208; Rolfes *et al*., 2012:379; Chapman-Novakofski, 2012:533). Vitamin D also works in on the intestines resulting in an increased calcium absorption (Chapman-Novakofski, 2012:533). These mechanisms result in an increase in calcium released into the bloodstream and restoration of adequate serum calcium levels (Rolfes *et al*., 2012:379). As serum calcium levels increase the parathyroid glands are stimulated to secrete less PTH. When serum calcium levels increase the thyroid glands are stimulated to secrete calcitonin (Marieb & Hoehn, 2016:208). The secretion of calcitonin inhibits the activation of vitamin D, inhibits calcium resorption in the kidneys, decreases calcium absorption in the intestines as well as inhibits osteoclast resorption in the bone. These mechanisms result in a decrease in calcium released into the bloodstream and inhibits calcitonin secretion by the thyroid glands (Rolfes *et al*., 2012:379). Figure 1 depicts the roles of the skeleton system, gut and kidneys in calcium homeostasis (adapted from Rolfes *et al*., 2012:379).

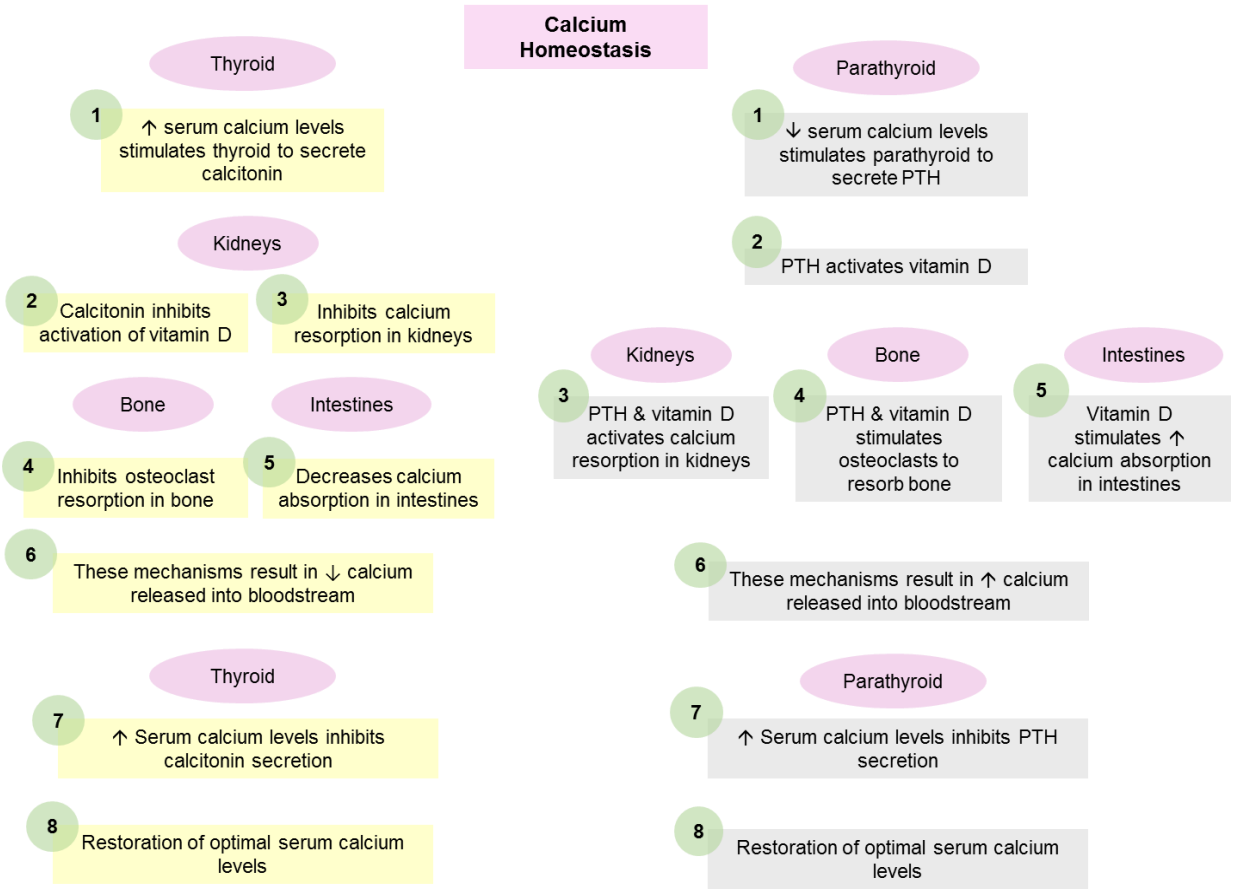

Figure 1: Calcium Homeostasis (adapted from Rolfes et al., 2012:379).

2.2.6.2 Response to mechanical and gravitational forces

Wolff's law depicts that the remodelling of bones is dependent on the everyday demands placed on it (Bayliss *et al*., 2011). Thus, the anatomy of bone reflects the stressors placed on it. Where hormonal controls determine when and if remodelling takes places, mechanical stress determines where remodelling occurs (Marieb & Hoehn, 2016:209). Loading is thus an import stimulus for the maintenance of bone mass. In the absence of reduction of mechanical stress, decreased bone formation and increased resorption will occur. (Walsh *et al*., 2014). The bone will become weaker and less metabolically demanding (Bayliss *et al*., 2011).

2.3 Bone mass

Bone mass is the general term used to refer to bone mineral content (BMC) but not to bone mineral density (BMD). BMC is used to describe the amount of bone accumulated before maturity of the skeleton is reached. BMD is the term used to refer to bone after maturity is reached. BMD measurements are used to monitor changes in the adult bone (Chapman-Novakofski, 2012:535). An individual's bone mass later in life is determined by

two factors namely, peak bone mass acquired and the rate of bone loss (Fausto *et al*., 2006).

2.3.1 Accumulation of bone mass and peak bone mass

Peak bone mass (PBM) can be described as the amount of bone attained after growth is completed and bone accumulation ceases (Gordon *et al*., 2017; Carsote & Valea, 2016). PBM is typically reached by the third decade (Lu *et al*., 2016). The long bones will cease growing in length at about 18 years of age in females and 20 years of age in males. However, bone mass will continue to accumulate for a few more years (Chapman-Novakofski, 2012:535). Various factors have an influence on bone mass accumulation including genetic factors and modifiable factors such as physical activity, hormonal factors, smoking, alcohol use and diet (Lu *et al*., 2016). A recent review concluded that lifestyle factors may have up to 30-60% influence of PBM attained (Carsote & Valea, 2016).

In males, PBM is generally greater than in females due to their larger frame size (Chapman-Novakofski, 2012:536; Lu *et al*., 2016). However, the skeleton at birth shows no difference in sex-related bone mass (Rizzoli, 2014). The variance in timing of bone mass accumulation between females and males are related to sex-specific patterns of pubertal development (Gordon *et al*., 2017; Rizzoli, 2014). The highest increases in bone mass follow closely after the adolescent growth spurt and continue for years afterwards (Gordon *et al*., 2017). The PBM accumulated during growth and development determines the bone mass later in life (Lu *et al*., 2016). Research suggests that a 10% increase in PBM may reduce the risk of osteoporotic fractures by as much as 50% later in life (Lu *et al*., 2016). A higher PBM accumulated during young adulthood is thus a protective factor against osteoporotic fractures during old age (Stagi *et al*., 2013).

2.3.2 Loss of bone mass

Age is a significant contributing factor of BMD. BMD starts to reduce at the age of 40 years in both male and female as a result of imbalances between bone formation and bone resorption (Chapman-Novakofski, 2012:536; Borji & Nasri, 2017). This age-related bone loss is associated with a decline in sex steroids, other endocrine alterations such as a decline in insulin-like growth factor-1, increased PTH secretion and a reduced ability of the kidneys to reabsorb calcium (Borji & Nasri, 2017; Rizzoli, 2014; Walsh *et al*., 2014). Contributing factors to the increased bone turnover may be a decrease in lean body mass and loading (Walsh *et al*., 2014).

Loss in bone mass increases significantly in women after menopause (Chapman-Novakofski, 2012:536). The rate of bone formation and resorption increases in both pre- and postmenopausal women (Rizzoli, 2014). The decline in ovarian activity in the premenopausal women and in oestrogen levels in menopausal women results in changes in the rates of bone formation and bone resorption (Borji & Nasri, 2017). Bone resorption rates by the osteoclasts increase whereas bone formation rates by the osteoblasts decrease. This imbalance between bone formation and bone resorption results in lower BMD and promotes osteoporosis in menopausal women (Borji & Nasri, 2017). In addition to a decline in oestrogen levels, age-related body composition changes, such as a decrease in lean body mass contributes to the onset of postmenopausal osteoporosis. Osteoporotic fractures occur most often in the hip and wrist regions of postmenopausal women (Motyl *et al*., 2017). Males have a much lower bone loss rate than females of the same age. However, at the age of 70 years, bone loss rates will be approximately the same in both sexes. Causes of bone loss in males are similar to those of females and include: aging, idiopathic or secondary to an underlying disease or medication use (Chapman-Novakofski, 2012:536).

2.3.3 Measurement of bone mineral density

BMD can be measured using dual-energy X-ray absorptiometry (DXA). The method involves exposure to low doses of X-rays (Eastell, 2013). However, DXA has some limitations as it does not allow for differentiation between different compartments of bone such as the cortical bone, trabecular bone, the spongy inner part or the hard outer shell, nor does it allow for the study of the bone geometry (Malgo *et al*., 2015). It is important to distinguish between different compartments of bone for assessment of bone strength and bone loss rates at the various compartments (Rachner *et al*., 2011). It has been suggested that the use of BMD measurements with DXA may give an incomplete fracture risk assessment as altered microarchitecture and bone material properties also contribute to fracture (Malgo *et al*., 2015).

Computed tomography (CT) allows for assessment of volumetric bone-density and aids in improved prediction of fracture risk and bone strength information (Rachner *et al*., 2011). With this technique three dimensional measurements of BMD of the lumber spine as well as the measurement of only the trabecular bone can be taken (Eastell, 2013). Despite the benefits of CT, DXA remains the preferred screening tool due to the fact that it involves lower exposure to radiation and is lower in cost (Eastell, 2013).

2.4 Role of nutrients in bone health

Nutrient intake plays a vital role in bone health and is largely modifiable. Adequate nutrient intake is of vital importance during growth and development, before skeletal maturity is reached (Mangels, 2014). Important nutrients include not only calcium and vitamin D but a variety of others such as vitamins A, B, C, E, folate, phytoestrogens, flavonoids and copper, zinc, selenium, magnesium, iron and fluoride (Castiglioni, 2013).

2.4.1 Calcium

An estimated 99% of the body's calcium is found in bones where it plays a vital role in the structural integrity, forming part of the hydroxyapatite crystals that provide the rigidity of the collagen network (Mangels, 2014; Cano *et al.,* 2018). Adequate calcium intake is an important factor that influences the development of PBM (Balk *et al*., 2017). Sub-optimal calcium intakes during growth and development lead to poor bone mass accumulation and low bone mineralization which in turn results in a higher risk for the development of osteoporosis and related fractures (Cano *et al.,* 2018). The serum calcium concentration is effected by daily dietary calcium intake, absorption, urinary and respiratory excretion and losses through sweat (Rolfes *et al*., 2012:380). An estimated 35% of dietary calcium intake is absorbed by the intestines through passive diffusion and active absorption mechanisms. Passive diffusion of calcium takes place in the event of adequate luminal calcium concentration whereas active absorption involving vitamin D receptors, takes place during low calcium concentrations (Cano *et al.,* 2018).

The most readily available form of dietary calcium is found in dairy products. Other dietary sources of calcium include: salmon, almonds and leafy green vegetables (Price *et al*., 2012). The lower the calcium absorption, the higher the risk for fractures (Borji & Nasri, 2017). Various physiological and dietary factors influence calcium absorption and excretion (Borji & Nasri, 2017). Physiological factors include: age, gender, life stage such as, pregnancy, lactation and menopause and hormones such as oestrogen and thyroid hormones (Rolfes *et al*., 2012:380). Dietary factors that decrease calcium absorption include: phytates, fibre, oxalates and iron, and high sodium and protein intakes, coupled with low calcium intake, result in increased calcium urinary excretion (Borji & Nasri, 2017).

Calcium deficiency is associated with increased risk of osteoporosis. The majority of the world's population fail to meet the recommended intake of 800-1000 mg/day as a

result of a suboptimal diet, food intolerances and impaired absorption (Kruger & Wolber, 2016). Another factor contributing to low calcium absorption is vitamin D deficiency. Adequate sunlight exposure is vital for an optimal vitamin D status as vitamin D is primarily obtained from cutaneous synthesis and secondary from dietary intake (Chapman-Novakofski, 2012:533; Borji & Nasri, 2017). Adequate intake is vital for optimal calcium absorption (Kruger & Wolber, 2016). Due to the synergistic relationship, calcium and vitamin D supplementation are often prescribed as a baseline treatment in most patients with osteoporosis (Rachner *et al*., 2011).

A calcium intake of 1000-1500 mg/day is recommended for postmenopausal women to replace daily calcium losses and to protect against osteoporosis (Bolland *et al*., 2015). However, a systematic review and meta-analysis conducted by Tai and colleagues found only small increases in BMD from dietary sources and supplements of calcium and concluded that it was unlikely to result in a lower fracture risk (Tai *et al.,* 2015).

2.4.2 Vitamin D

Vitamin D plays an important role in the mineralization of the skeleton by maximizing the intestinal absorption of calcium (Mangels, 2014). This results in normal mineralization of the osteoid and calcification of the growth plate (Stagi *et al*., 2013). A suboptimal serum vitamin D concentration results in secondary hyperparathyroidism which leads to increased bone loss (Reid *et al*., 2014). There exists controversy on what serum level of 25(OH)D is considered to be deficient and what serum level is considered sufficient (Holick, 2017). The National Osteoporosis Society as well as the Institute of Medicine considers a serum level of <30 nmol/l (< 12 ng/ml) as deficient, 30-50 nmol/l as inadequate (12-20 ng/ml) and >50 nmol/l (20 ng/ml) to be sufficient (Aspray *et al*., 2014).

Symptoms of vitamin D deficiency include muscle weakness, low bone mass and an increased susceptibility to osteoporosis and fractures in the elderly. Vitamin D status depends primarily on exposure to sunlight and secondary on dietary intake (Borji & Nasri, 2017). Few foods naturally contain vitamin D (Holick, 2017) and include: salmon, swordfish and tuna (Price *et al*., 2012:143). Vitamin D is important to maintain normal serum calcium and phosphate levels, ensuring normal bone mineralization (Wang *et al*., 2017). Various factors affect vitamin D production via sunlight and include use of sunscreen, skin pigmentation, and skin exposure to sun, season and age (Mangels, 2014). In the human body vitamin D is activated by two sequential

hydroxylations (Borji & Nasri, 2017). The first hydroxylation of vitamin D takes place in the liver and produces 25-hydroxyvitamin D3. The second hydroxylation takes place in the kidneys and produces 1,25(OH)2 D3 (Chapman-Novakofski, 2012:535).

Vitamin D has long been viewed as an important part of osteoporosis treatment and prevention. However, research has shown controversial results with regards to the benefits of vitamin D on bone health. Observational studies found varying results regarding the associations between BMD and vitamin D status and meta-analysis of vitamin D trials showed no associations between only vitamin D supplementation and protection against fractures (Reid *et al*., 2014). Reid and colleagues conducted a systematic review on the effects of vitamin D supplements on BMD and found little data on the overall improvement of BMD with vitamin D supplementation or protection against fractures (Reid *et al*., 2014). A recent meta-analysis and systematic review on the effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power found a positive influence of vitamin D on muscle strength (Beaudart *et al*., 2014). However, a meta-analysis of genome wide association studies concluded that vitamin D levels had no effect on fracture risk (Trajanoska *et al*., 2018).

2.4.3 Phosphate

Phosphate is found abundantly in the human body. An estimated 85% of the body's phosphate is found with calcium in the form of hydroxyapatite crystals in the bone (Takeda *et al*., 2014). Remaining phosphate is found as phosphoproteins, esters and free ions in soft tissues and cell membranes. Serum phosphate concentrations are tightly regulated to ensure that homeostasis is maintained through various processes in the gut, kidney and skeleton. Collaborations between vitamin D from the kidneys, PTH and fibroblast growth factor-23 regulate phosphate homeostasis (Penido & Alon, 2012).

Phosphates play an important role in various biological processes including: cell membrane integrity, cell structures, cellular metabolism, regulating acid-base homeostasis, bone growth and mineralization (Penido & Alon, 2012; Takeda *et al*., 2014). Calcium and phosphate are needed for proper bone mineralization in the ratio of 1:1. High phosphorus intakes alter the calcium/phosphate ratio (Chapman-Novakofski, 2012:538). Excessive phosphorus intake leads to decreased calcium absorption and thus a lower serum calcium concentration (Takeda *et al*., 2014). The drop in serum calcium stimulates PTH secretion and bone resorption. This may result in chronic bone loss (Chapman-Novakofski, 2012:538; Takeda *et al*., 2014). A review

conducted by Takeda and colleagues (2014) found an association between high phosphate intakes and lower hip BMD suggesting that excessive phosphate intakes may have a negative effect on bone health.

2.4.4 Magnesium

Magnesium plays an important role in various life processes in the body, including bone health (Castiglioni, 2013). Magnesium also plays a role in bone development and the bone mineral matrix (Price *et al*., 2012). The majority, approximately 60%, of magnesium in the body is stored in the bone where it serves as a reservoir that is used to maintain magnesium homeostasis (Castiglioni, 2013). Magnesium can be obtained from dietary sources such as nuts, cereals, lentils, potato skins, shellfish and green leafy vegetables (Price *et al*., 2012).

Results from a meta-analysis and systematic review by the International Osteoporosis Foundation and National Osteoporosis Foundation showed that high levels of magnesium intake were not significantly associated with the risk of hip and total fractures. A significant correlation between magnesium intake and BMD in femoral neck and total hip was found. However, no association between magnesium intake and BMD in lumbar spine was reported (Farsinejad-Marj *et al*., 2015).

2.4.5 Vitamin K

Vitamin K is a fat soluble vitamin and is found in two forms namely, vitamin K1 and vitamin K2 (Hamidi & Cheung, 2014). Vitamin K1 can be obtained from plants and green vegetables, while, vitamin K2 is synthetized by intestinal bacteria in the human body (Palermoa *et al*., 2017). A vitamin K deficiency may develop due to low dietary intake; insufficient or interference of intestinal production by antibiotics use or fat malabsorption (Gallieni *et al*., 2013). Vitamin K plays an important role in bone health and is involved in post-translational activation of various matrix proteins, including osteocalcin (Vermeer, 2012). Osteocalcin plays a role in bone mineralization (Gallieni *et al*., 2013) and is released in the bloodstream after bone resorption and serves as a serum bone marker used in predicting fracture risk (Chapman-Novakofski, 2012:540). A recent systematic review by Palermoa *et al*. (2017) concluded that low vitamin K intake in elderly and young women is associated with bone demineralization. However due to contrasting results from randomised controlled trials and cross-sectional studies, routine supplementation in postmenopausal women is not recommended.

2.4.6 Vitamin C

Vitamin C (ascorbic acid) is an essential water-soluble vitamin that is involved in development, function, and maintenance of various tissues in the body (Stunes *et al*., 2017). Vitamin C is known for its antioxidant properties and role in collagen synthesis (Aghajanian *et al*., 2015). Vitamin C also plays a role in osteoblast differentiation (Segawa *et al*., 2016). Ascorbic acid levels are often low in older adults due to reduced intestinal absorption and renal reabsorption (Segawa *et al*., 2016).

Rat studies found that vitamin C has a regulating role in gene transcription in bone (Aghajanian *et al*., 2015) and results from epidemiology studies demonstrated increased risk of osteoporosis and associated fractures as a result of a vitamin C deficiency (Aghajanian *et al*., 2015). Adequate vitamin C intake has been associated with less hip fractures. This protective mechanism may be attributed to antioxidant properties of vitamin C and its role in collagen synthesis (Mangels, 2014).

2.5 Lifestyle and behavioural factors

2.5.1 Physical activity

Bone is a metabolically active tissue that responds to mechanical stress such as physical activity and gravitational forces (Marieb & Hoehn, 2016:208). As a result of mechanical stress the trabeculae is constantly undergoing remodelling, ensuring that the bone adapt as needed (Stagi *et al*., 2013). The anatomy of bone will reflect the daily stressors it undergoes, making the process vital for normal bone mass (Marieb & Hoehn, 2016:209; Stagi *et al*., 2013). Inactivity leads to loss of bone mass commonly seen in bedridden patients. The loss of gravitational pull on bones also leads to bone mass loss and is observed in astronauts (Stagi *et al*., 2013). Physical activity is thus an important modifiable factor in the accumulation of bone mass. Bone accumulation during childhood and adolescense can be positively influenced by regular physical activity. Increased physical activity is especially important during adolescence to maximize bone mass (Gunter *et al*., 2008; Stagi *et al*., 2013). Postmenopausal women can also benefit from physical activity. Resistance training in combination with high impact weight bearing exercise has been known to increase bone formation and improve bone structure and maintain BMD in postmenopausal women (Bilek *et al*., 2016; Van Schoor, 2011).

2.5.2 Alcohol

The effects of alcohol consumption on bone is dose related with some studies finding an increase of BMD with low alcohol intakes (Borji & Nasri, 2017) and high alcohol intake of ≥ three drinks per day associated with decreased osteoblastic replacement during the remodelling phase and increased risk for the development of osteoporosis (Van Schoor, 2011).

2.5.3 Smoking

Smoking is a risk factor for the development of osteoporosis (Van Schoor, 2011), and smokers typically have a low bone mass and thus a high risk of fracture (Borji & Nasri, 2017). This may be attributed to the effect of smoking on osteoblast malfunctioning (Borji & Nasri, 2017), as well as reduced intestinal absorption of calcium (Van Schoor, 2011).

2.6 Bone disease

Imbalances between bone resorption and bone formation leads to changes in the architecture, structure or strength of bone and result in deformity, pain or fracture (Ralston, 2013). For the purpose of this study osteoporosis and fracture healing will be discussed.

2.6.1 Osteoporosis and fractures

Osteoporosis is a multifactorial skeletal disease presenting most often in postmenopausal women (Eastell, 2013). Worldwide, a vast number of people are affected by osteoporosis and the growing prevalence of this chronic disease is attributed to a higher life expectancy (Cano *et al*., 2018; Castiglioni, 2013). Osteoporosis is a public health concern and a major burden on healthcare systems (Motyl *et al*., 2017). Adequate nutrition, especially calcium intake as well as life style factors such as regular physical activity is of vital importance for a decreased risk of developing osteoporosis and associated fractures later in life (Mangels, 2014; Cano *et al*., 2018). Osteoporosis is characterized by reduced bone mass and deterioration of the micro-architecture of the bone (Castiglioni, 2013; Motyl *et al*., 2017). As a result, bone strength is reduced and vulnerability to fractures increases (Eastell, 2013). Osteoporotic fractures lead to a substantial increase in morbidity and mortality (Farsinejad-Marj *et al*., 2015). Loss of mobility often results in a decreased quality of life for patients (Rachner *et al*., 2011).

Bone continuously undergoes remodelling, a process that involves the synchronised interactions between osteoclasts and osteoblasts (Castiglioni, 2013). However, bone loss occurs when the rates of bone resorption by the osteoclast exceeds the rate of bone formation by the osteoblasts (Rachner *et al*., 2011; Ralston, 2013). Genetic and environmental factors contribute to this increased rate of bone resorption (Ralston, 2013). Lifestyle factors that contribute to low BMD include alcohol use, smoking, nutrient intakes, especially inadequate calcium intake, and reduced physical activity (Farsinejad-Marj *et al*., 2015).

Risk reduction is a preferred strategy to protect against the development of osteoporosis. Adequate calcium intake during all life stages starting from an early age is considered a vital risk reduction strategy (Cano *et al*., 2018). Fractures caused by osteoporosis tend to occur more in the trabecular bone. Loss of bone results in thinning and often perforation of the trabecular plates (Eastell, 2013).

2.6.2 Treatment

Treatment aims of diagnosed osteoporosis are management of symptoms and the decrease of further fracture risk (Eastell, 2013). Osteoporosis medications can be divided into two categories namely anabolic drugs and anti-resorptive drugs. Anabolic drugs work to increase bone formation, where anti-resorptive drugs hinder bone resorption (Castiglioni, 2013). The use of drugs may prevent further bone loss and reduce the risk of further fractures. BMD of patients using these drugs should be monitored as some do not respond to certain drugs (Eastell, 2013). Calcium and vitamin D supplementation is prescribed as a baseline treatment in most patients (Rachner *et al*., 2011). However, it has been suggested by a meta-analysis by Bolland and colleagues (2010) that calcium supplementation may increase the risk of myocardial infarction (MI). Another meta-analysis concluded that calcium together with vitamin D supplementation may also modestly increase the risk of MI (Bolland *et al*., 2011).

Lifestyle and dietary changes such as regular physical activity, cessation of tobacco and alcohol use and the intake of a balanced diet are also recommended for patients with osteoporosis. These recommendations should also be seen as preventative strategies to be implemented earlier in life as bone mass is determined by factors before skeletal maturity is reached (Castiglioni, 2013).

2.6.3 Fracture healing

Fracture healing involves four stages namely, hematoma formation, fibrocartilaginous callus formation, bony callus formation and lastly bone remodelling (Marieb & Hoehn, 2016:210).

After a fracture occurs a hematoma forms at the fracture site (Marieb & Hoehn, 2016:210). The inflammatory phase is immediately initiated and may continue for three to four days or until the bone or cartilage is formed. As a result of the fracture, blood supply to the bone is decreased causing necrosis (Bigham-Sadegh & Oryan, 2014).

During the second phase, capillaries grow into the hematoma, allowing phagocytic cells to enter the fracture site (Bigham-Sadegh & Oryan, 2014). Granulation tissue slowly replaces the haematoma (Bayliss *et al*., 2011). While the haematoma is replaced, nearby fibroblast, osteogenic cells and cartilage enter the fracture site and begin reconstructing the bone (Marieb & Hoehn, 2016:210). The ends of the broken bone are reabsorbed by osteoclasts (Bayliss *et al*., 2011). Collagen fibres are synthesised by the fibroblasts and connects the ends of the broken bones. Precursor cells differentiate into chondroblasts and produce cartilage matrix, while the osteoblasts forms spongy bone. Cartilaginous matrix that calcifies is secreted by the cartilage cells (Marieb & Hoehn, 2016:210). The calcified tissue is later replaced with new bone by the osteoblasts. This replacement with new bone is endosteal bone formation. The whole repair tissue is called the fibrocartilaginous callus (Bayliss *et al*., 2011).

Within a week or two, the bony callus forms (Bayliss *et al*., 2011). New trabeculae appear in the fibrocartilaginous callus and form into the bony callus (Marieb & Hoehn, 2016:210). The last phase involves bone remodelling of the bony callus. During this phase, fracture repair can be described as the adaption of the bone to regain optimal strength and function (Bigham-Sadegh & Oryan, 2014).

2.6.4 Fracture risk assessment

The WHO introduced the Fracture Risk Assessment Tool (FRAX®) in 2008. This tool is used to identify patients who are at risk of fracture by using several clinical factors including age, sex, height, weight, parental hip fracture, previous fracture, smoking, glucocorticoids use, rheumatoid arthritis, secondary osteoporosis and alcohol intake of ≥ 3 units/day with or without information on BMD. Data from population-based

cohorts from Europe, Australia, Japan, and Canada were used to develop FRAX (Kanis *et al*., 2009).

A fracture risk assessment tool for South Africa is currently being developed by a team coordinated by Professor Bilkish Cassim of the University of KwaZulu-Natal and is planned to be available by 2019.

2.7 HIV infection

It is estimated that globally 36.9 million people are HIV-positive. The highest prevalence of HIV-infection is in Sub-Saharan Africa. South Africa has the highest prevalence in the world with 7.06 million people living with HIV in 2016 (UNAIDS, 2016a). HIV cannot be cured, however the viral load can be suppress by using ART and thus prolonging life expectancy. A combination of three antiretroviral drugs are referred to as HAART. The number of people in South Africa having access to ART in 2015 was estimated at 6.19 million (UNAIDS, 2017).

As a result of a decline in the prevalence of opportunistic infections, the estimated life expectancy of patients living with HIV has increased significantly and HIV infection is now considered a treatable, chronic illness (Piso *et al*., 2013). It is estimated that due to the advancement in ART, HIV-positive patients will be expected by 2020 to reach the age of 50 years and beyond (Cortés *et al*., 2015). However, concerns related to long term use of ART in combination with other factors associated with the aging body is becoming more prevalent (Piso *et al*., 2013). HIV-positive women experiencing menopause and associated bone loss are especially of concern (Cortés *et al*., 2015). Concerns regarding the effects of ART and bone metabolism is rising (Sharma *et al*., 2015). Various studies showed a marked increase in the prevalence of bone demineralization in HIV-positive individuals (Compston, 2016; Tebas *et al*., 2000; Bruera *et al*., 2003; Landonio *et al*., 2004). It is evident that both ART use as well as HIV-infection lead to increased bone loss (McComsey *et al*., 2010).

Low BMD and fractures incidences are higher in patients with HIV in comparison with the general population (Dave *et al*., 2015). In comparison with non-HIV-positive individuals, HIV-positive individuals were 6.4 times more likely to develop low BMD (Cotter & Powderly, 2011). Research has shown that patients living with HIV may have a fracture risk as high as 58% in comparison with the general population (Cortés *et al*., 2015). It should be noted that most data regarding fracture risk and HIV was conducted in Europe, Australia and North America (Compston, 2016). Osteoporosis has long been established as a growing

epidemic in the older non HIV-positive population and is now becoming a concern in the HIV-positive population as well (Piso *et al*., 2013). It is estimated that 15% of patients living with HIV will be diagnosed with osteoporosis (Cortés *et al*., 2015). Other bone diseases associated with HIV includes osteonecrosis and osteopenia. The prevalence of osteonecrosis per year has been reported to be higher than in the general population (Cotter & Powderly, 2011). The sites most often affected include the single and bilateral femoral heads, bilateral knees, and multiple other sites such as hips and humerus. The prevalence of osteopenia range between 22% to 71% (Cotter & Powderly, 2011). The underlying mechanisms in the changes of bone metabolism are unclear (Fausto *et al*., 2006).

The causes of low BMD in patients with HIV is multifactorial and includes traditional and HIV-associated factors (Dave *et al*., 2015; Mulubwa *et al*., 2017). The most cited risk factors for low BMD in HIV-positive patients include:

- Low weight,
- length of HIV infection,
- older age,
- smoking,
- non-black/white race,
- female sex,
- HIV RNA,
- stavudine,
- tenofovir,
- protease inhibitors, and
- duration of NRTI use (Cotter & Powderly, 2011).

Research found that ARTs decrease BMD by various mechanisms (Dave *et al*., 2015). The use of ARTs has also been associated with poor vitamin D status (Hamill *et al*., 2013). Highly active ART has been proved to meaningfully improve survival and quality of life (Kryst *et al*., 2014). However, osteoporosis and associated fractures have become a significant concern (Focà *et al*., 2012). HAART consists of two nucleoside reverse transcriptase inhibitors (NRTI) and either a protease inhibitor (PI) or a non- nucleoside reverse transcriptase inhibitor (NNRTI) (Focà *et al*., 2012). Both NRTI and PIs are associated with low BMD in exposed patients (Mulubwa *et al*., 2017).

Efavirenz (a NNRTI) is recommended as part of the current first-line ART regime by the WHO (Kryst *et al*., 2014; NDoH, 2015). However, efavirenz has been shown to interfere

with vitamin D metabolism and consequently reduce the serum concentration. Stavudine formed part of the South African first line regime until 2013 (NDoH, 2015) and was known to reduce BMD. Due to various side-effects, stavudine was later replaced with TDF which was proven to reduce BMD to a greater degree than stavudine (Haskelberg *et al*., 2012). It is recommended by the WHO that ritonavir-boosted PI forms part of second line ART regimen. Lopinavir is most often used but has also been associated with decreased BMD and associated increased fracture risk. Research found that patients treated with PIs had a 1.5 and 1.6 chance of developing low BMD and osteoporosis (Cotter & Powderly, 2011). It is important to note that most data concerning HIV, bone health and BMD is derived from studies conducted in high-income countries and might not be relevant for the South African setting (Dave *et al*., 2015).

A meta-analysis and systematic review by Shiau and colleagues (2013) concluded that race plays a role in modifying the relationship between HIV and fracture risk, finding non-black population groups to be the highest at risk for fractures. Very little data on BMD is available on non-white population groups living in low- to middle income countries such as South Africa (George *et al*., 2014).

2.8 Bone health in the South African context

South Africa is experiencing rapid urbanisation; the driving force being increased work opportunities and improved living conditions in cities (Kruger *et al*., 2011). In fact 65.8% of South Africa's population are living in cities (Statssa, 2017). However, research has shown that despite the perceived higher availability of work opportunities and improved living conditions, urban populations are growing poorer with detrimental effects on food security, nutritional status and bone health (Frayne *et al*., 2014; Kruger *et al.,* 2011).

Various health implications such as lower BMD which may increase the risk for the development of osteoporosis has been associated with urbanisation (Kruger *et al.,* 2011; Vorster *et al.,* 2002). Research indicates that South African urban postmenopausal women have an increased risk for the development of low BMD and consequently osteoporosis and associated fractures (Kruger *et al.,* 2011; Vorster *et al.,* 2002). Factors influencing the risk for lower BMD in the South African population may be changes in diet and PA as a result of urbanisation (Kruger *et al*., 2004). Other lifestyle factors that contribute to increased risk for bone disease in black urban postmenopausal South African women include alcohol abuse and smoking (Kruger *et al.,* 2011).

A shift from traditional foods consumed in rural areas towards consumption of western foods is associated with urbanisation. For many South Africans western food items are considered desirable and is widely consumed as a status symbol. However, these food items are often low in essential nutrients such as calcium and vitamin D (Steyn & Mchiza, 2014; MacIntyre *et al*., 2002).

Low calcium intakes are associated with lower BMD (Kruger & Wolber, 2016). Populations in Africa generally consume inadequate amounts of calcium due to limited financial resources, cultural preferences and taboos and high prevalence of lactose intolerance (Vorster *et al*., 1997). According to MacIntyre *et al*. (2002) a decreased intake of milk and milk products are associated with urbanisation and results in a decreased intake of calcium. Research found that urban black postmenopausal women have a low calcium intake of 200-450 mg/d which is lower than the recommended daily intake of 1000-1200 mg/d (Kruger *et al.,* 2011).

Vitamin D deficiency is associated with muscle weakness, low bone mass and an increased susceptibility to osteoporosis, fractures (Borji & Nasri, 2017) and fall risk in the elderly (Bischoff-Ferrari *et al.,* 2004). South Africans generally have a low intake of vitamin D (Norval *et al*., 2016). Due to the limited food sources fortified with vitamin D, South Africans are mainly dependent on cutaneous synthesis of vitamin D (Hamill *et al*., 2013). Kruger and colleagues (2011) reported low serum 25(OH)D levels in black urban postmenopausal women.

A recent review by Norval and colleagues (2016) reported contradictory results on whether urbanisation may lead to an increased or decreased vitamin D status, suggesting that various factors play a role. Some of the factors include a lower exposure to sunlight in urban areas compared to rural areas, however a higher standard of living in urban areas might lead to higher intake of foods containing vitamin D. A recent study found more urban black women to be vitamin D insufficient during spring time (after winter) than during autumn (after summer) (Sotunde *et al*., 2016).

Physical inactivity is associated with lower BMD (Farsinejad-Marj *et al*., 2015). Postmenopausal women can benefit from regular physical activity as it may contribute towards increasing bone formation, improving bone structure and maintaining BMD (Bilek *et al*., 2016; Van Schoor, 2011; Stagi *et al*., 2013). Kruger and colleagues found that black urban women have low physical activity levels, especially women older than 70 years (2011).

Previous studies reported that black populations have a greater protection against the development of osteoporosis due to their enhanced BMDs, however; these studies were conducted in America and Europe and may not be relevant for the South African black population (Aloia *et al.,* 1996; Handa *et al*., 2008). Urbanisation combined with increasing prevalence of HIV infection and ART thus have detrimental effects on the bone health of South Africans and is a growing concern.

2.9 Conclusion

Worldwide millions of people are affected by osteoporosis and the growing prevalence of this chronic disease is partly attributed to a higher life expectancy (Cano *et al*., 2018; Castiglioni, 2013). Adequate nutrition, especially calcium intake, as well as life style factors such as regular physical activity, are of vital importance for a decreased risk of developing osteoporosis and associated fractures later in life (Mangels, 2014; Cano *et al*., 2018).

South Africa is experiencing rapid urbanisation; the driving force being increased work opportunities and improved living conditions in cities (Kruger *et al*., 2011). Research suggests that South African urban postmenopausal women have an increased risk for the development of low bone mineral density (BMD) and consequently osteoporosis and associated fractures (Kruger *et al.,* 2011; Vorster *et al.,* 2002). A shift from traditional foods consumed in rural areas towards consumption of western foods, is associated with urbanisation. These food items are often low in essential nutrients such as calcium and vitamin D (Steyn & Mchiza, 2014; MacIntyre *et al*., 2002). South Africans generally have a low intake of vitamin D (Norval *et al*., 2016). Due to the limited food sources fortified with vitamin D, South Africans are mainly dependent on cutaneous synthesis of vitamin D (Hamill *et al*., 2013). Populations in Africa generally consume inadequate amounts of calcium due to limited financial resources, cultural preferences and taboos and high prevalence of lactose intolerance (Vorster *et al*., 1997).

Osteoporosis and associated fractures are also becoming a concern in the HIV-positive population (Piso *et al*., 2013). Low BMD and fractures incidences are higher in patients with HIV in comparison with the general population (Dave *et al*., 2015). Due to the advancement in antiretroviral therapy (ART), the life expectancy of HIV-positive patients have increased (Cortés *et al*., 2015). However, concerns related to long term use of ART in combination with other factors associated with the aging body are becoming more prevalent (Piso *et al*., 2013). Research found that ARTs decrease BMD by various mechanisms (Dave *et al*., 2015). The use of ARTs has also been associated with poor vitamin D status (Hamill *et al*., 2013). HIV-positive urban women experiencing menopause

and associated bone loss are especially a concern (Cortés *et al*., 2015). Urbanisation combined with increasing prevalence of HIV and ART use thus have detrimental effects on the bone health of South Africans and therefore it will be important to assess the fracture risk of HIV-positive women (Cortés *et al*., 2015; Kruger *et al.,* 2011; Vorster *et al.,* 2002).

2.10 References

Aghajanian, P., Hall, S., Wongworawat, M.D. & Mohan, S. 2015. The roles and mechanisms of actions of vitamin C in bone: new developments. *Journal of bone and mineral Research,* 30(11):1945-1955.

Aloia, J.F., Vaswani, A., Yeh, J.K. & Flaster, E. 1996. Risk for osteoporosis in black women. *Calcified tissue international*, 59(6):415-423.

Aspray, T.J., Bowring, C., Fraser, W., Gittoes, N., Javaid, M.K., Macdonald, H., Patel, S., Selby, P., Tanna, N. & Francis, R.M. 2014. National Osteoporosis Society Vitamin D Guideline Summary. *Age and ageing*; 43(5):592-595.

Balk, E. M., Adam, G.P., Langberg, V.N., Earley, A., Clark, P., Ebeling, P.R., Mithal, A., Rizzoli, R., Zerbini, C.A.F., Pierroz, D.D. & Dawson-Hughes, B. 2017. Global dietary calcium intake among adults: a systematic review. *Osteoporosis international*, DOI 10.1007/s00198-017-4230-x.

Bayliss, L., Mahoney, D.J. & Monk, P. 2011. Normal bone physiology, remodeling and its hormonal regulation. *Surgery,* 30(2):47-53.

Beaudart, C., Buckinx, F., Rabenda, V., Gillain, S., Cavalier, E., Slomian, J., Petermans, J., Reginster, Y. & Bruyère, O. 2014. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *The journal of clinical endocrinology & metabolism*, 99(11):4336-4345.

Bigham-Sadegh, A. & Oryan, A. 2014. Basic concepts regarding fracture healing and the current options and future directions in managing bone fractures. *International wound journal,* 12(3):238-247.

Bilek, L.D., Waltman, N.L., Lappe, J.M., Kupzyk, K.A., Mack, L.R., Cullen, D.M., Berg, K., Langel, M., Meisinger, M., Portelli-Trinidad, A & Lang, M. 2016. Protocol for a randomized controlled trial to compare bone-loading exercises with risedronate for preventing bone loss in osteopenic postmenopausal women. *BioMed central women's health*, 16:59.

Bischoff-Ferrari, H., Dawson-Hughes, B., Willet, W.C., Staehelin, H.B., Bazemore M.G., Zee R.Y. & Wong, J.B. 2004. Effect of vitamin D on falls: a meta-analysis. *Journal of the American medical association*, 291:1999-2006.

Bolland, M.J., Avenell, A., Baron, J.A., Grey, A., MacLennan, G.S., Gamble, G.D. & Reid, I.R. 2010. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *British medical journal,* 341:c3691.

Bolland, M.J., Grey, A., Avenell, A., Gamble, G.D. & Reid, I.R. 2011. Calcium supplements with or without vitamin D and risk of cardiovascular events: re-analysis of the Women's Health Initiative limited access dataset and meta-analysis. *British medical journal,* 342:d2040.

Borji, S. & Nasri, H. 2017. An update on prevention and treatment of osteoporosis. *Journal of parathyroid disease*, 5(2):29.

Bruera, D., Luna, N., David, DO., Bergoglio, L.M. & Zamudio, J. 2003. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS*, 17:1919-1922.

Cano, A., Chedrauib, P., Goulisc, D.G., Lopesd, P., Mishrae, G., Mueckf, A., Senturkg, L.M., Simoncinih, T., Stevensoni, J.C., Stutej, P., Tuomikoskik, P., Reesl & Lambrinoudakim, M.I. 2018. Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. *Maturitas, 107:7-12.*

Carsote, M. & Valea, A. 2016. Insights of Peak Bone Mass. *Atmospheric measurement techniques*, 21(2):82-84.

Castiglioni, S., Albisetti, W. & Maier, J.A.M. 2013. Magnesium and Osteoporosis: Current State of Knowledge and Future Research Directions. *Nutrients*, 5:3022-3023.

Chapman-Novakofski, K. 2012. Nutrition and Bone Health. (*In Mahan, L.K., Escott-Stump, S. & Raymond, J.L. Krause's Food and the Nutrition Care Process. United States of America: Elsevier. p. 532-540).*

Compston, J. 2016. HIV infection and bone disease. *Journal of internal medicine*, 280:350-358.

Cortés, Y.I., Yin, M.T. & Reame, N.K. 2015. Bone Density and Fractures in HIV-infected Postmenopausal Women: A Systematic Review. *Journal of the Association of Nurses in AIDS care,* 26(4):387.

Cotter, A.G. & Powderly, W.G. 2011. Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity. *Best practice & research clinical endocrinology & metabolism,* 25:501-515.

Dave, J.A., Cohen, K., Micklesfield, L.K., Maartens, G. & Levitt, N.S. 2015. Antiretroviral Therapy, especially Efavirenz, is associated with low bone mineral density in HIV-infected South Africans. *PLoS ONE*, 10(12). https://doi.org/10.1371/journal.pone.0144286 Date of access: 14 July 2017.

Eastell, R. 2013. Osteoporosis. *Medicine,* 41(10):586-587.

Farsinejad-Marj, M., Saneei, P. & Esmaillzadeh, A. 2015. Dietary magnesium intake, bone mineral density and risk of fracture: a systematic review and meta-analysis. *Osteoporosis international,* 27:1389-1394.

Fausto, A., Bongiovanni, M., Cicconi, P., Menicagli, L., Ligabò, E.V., Melzi, S., Bini, T., Sardanelli, F., Cornalba, G. & Monforte, A. 2006. Potential predictive factors of osteoporosis in HIV-positive subjects. *Bone*, 38:893-897.

Focà, E., Motta, D., Borderi, M., Gotti, D., Albini, L., Calabresi, A., Izzo, I., Bellagamba, R., Narciso, P., Sighinolfi, L., Clò, A., Gibellini, D., Quiros-Roldan, E., Brianese, N., Cesana, B.M., Re, M.C. & Torti, C. 2012. Prospective evaluation of bone markers, parathormone and 1,25-(OH)2 vitamin D in HIV positive patients after the initiation of Tenofovir/ Emtricitabine with Atazanavir/Ritonavir or Efavirenz. *BioMed central*, 12:38.

Frayne, B., Crush, J. & McLachlan, M. 2014. Urbanisation, nutrition and development in Southern African cities. *Springer food security*, 6:101-112.

Gallieni, M., Rizzo, M.A., Maggi, S., Regalia, A., Molfino, I., Crepaldi, G. & Fusaro, M. 2013. Vitamin K and bone metabolism in the elderly with normal and reduced kidney function. *European geriatric medicine,* 4(1):32-38.

George, J.A., Micklesfield, L.M., Norris, S.A. & Crowther, N.J. 2014. The Association between body composition, 25(OH)D, and PTH and bone mineral density in black African and Asian Indian population groups. *The journal of clinical endocrinology & metabolism*, 99(6):2146-2154.

Gordon, M.C., Zemel, B.S., Wren, T.A.L., Leonard, M.B., Bachrach, L.K., Rauch, F., Gilsanz, V., Rosen, C.J. & Winer, J.K. 2017. The determinants of peak bone mass. *The journal of pediatrics,* 180:261.

Gunter, K., Baxter-Jones, A.D., Mirwald, R.L., Almstedt, H., Fuchs, R.K., Durski, S. & Snow, C. 2008. Impact exercise increases BMC during growth: an 8-year longitudinal study. *Journal of bone and mineral research*, 23:986-993.

Hamidi, M.S. & Cheung, A.M. 2014. Vitamin K and musculoskeletal health in postmenopausal women. *Molecular nutrition & food research journal*, *58*:1647-1657.

Hamill, M.M., Ward, K.A., Pettifor, J.M., Norris, S.A. & Prentice, A. 2013. Bone mass, body composition and vitamin D status of ARV-naive, urban, black South African women with HIV infection, stratified by CD count. *Osteoporosis international*, 24:2855-2861.

Handa, R., Kalla, A.A. & Maalouf, G. 2008. Osteoporosis in developing countries. *Best practice & research clinical rheumatology*, 22(4):693-708.

Haskelberg, H., Hoy, J.F., Amin, J., Ebeling, P.R., Emery, S. & Carr, A. 2012. Changes in bone turnover and bone loss in HIV-infected patients changing treatment to tenofovir-emtricitabine or abacavir-lamivudine. *PLoS ONE*, 7:38377.

Herman, I.P. 2016. Physics of the Human Body. In Biological and Medical Physics, Biomedical Engineering. Switzerland: Springer.

Holick, M.F. 2017. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Reviews in endocrine and metabolic disorders*, 18:153-165.

International Osteoporosis Foundation. 2017. *Facts and Statistics: osteoporosis - incidence and burden.* Retrieved from https://www.iofbonehealth.org/facts-statistics#category-14 Date of access: 23 October 2017 .

Joint United Nations Programme on HIV and AIDS. 2016a. Country factsheet South Africa 2016. www.unaids.org/en/regionscountries/countries/southafrica Date of access: 23 October 2017.

Joint United Nations Programme on HIV and AIDS. 2017. Global HIV statistics. Geneva. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf Date of access: 23 October 2017.

Kanis, J.A., Oden, A., Johansson, H., Borgstrom, F., Strom, O. & McCloskey, E. 2009. FRAX and its applications to clinical practice. *Bone,* 44:734-43.

Kruger, M.C., Dewinter, R.M., Becker, P.J. & Vorster, H.H. 2004. Changes in markers of bone turnover following urbanisation of black South African women. *Journal of endocrinology, metabolism and diabetes of South Africa,* 9(1):103-108.

Kruger, M.C., Kruger, I.M., Wentzel-Viljoen, E. & Kruger, A. 2011. Urbanisation of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition research*, 31:748-758.

Kruger, M.C. & Wolber, F.M. 2016. Osteoporosis: modern paradigms for last century's bones. *Nutrients*, 8:6.

Kryst, J., Kawalec, P. & Pilc, A. 2014. Efavirenz-based regimens in antiretroviral-naive HIV-infected patients: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*, 10(5):e0124279.

Landonio, S., Quirino, T., Bonfanti, P., Gabris, A., Boccassini, L., Gulisano, C., Vulpio, L., Ricci, E., Carrabba, M. & Vigevani, G.M. 2004. Osteopenia and osteoporosis in HIV+ patients, untreated or receiving HAART. *Biomedicine & pharmacotherapy,* 58:506-507.

Lu, J., Shin, Y., Yen, M. & Sun, S.S. 2016. Peak bone mass and patterns of change in total bone mineral density and bone mineral contents from childhood into young adulthood. *Journal of clinical densitometry*, 19(2):180-191.

MacIntyre, U.E., Kruger, H.S., Venter, C.S. & Vorster, H.H. 2002. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutrition research*, 22:239-256.

Malgo, F., Hamdy, N.A.T., Papapoulos, S.E. & Appelman-Dijkstra, N.M. 2015. Bone material strength as measured by microindentation in vivo is decreased in patients with fragility fractures independently of bone mineral density. *Journal of clinical endocrinology & metabolism*, 100(5):2039-2045.

Mangels, A.R. 2014. Bone nutrients for vegetarians. *American journal of clinical nutrition*, 100(1):469-473.

Marcus, R. 2012. Osteoporosis in adults. *(In Coulston, A.M., Boushey, C.J. & Ferruzzi, M. Nutrition in the prevention and treatment of disease. California: Academic Press. p. 860-862).*

Marieb, E.N. & Hoehn, K. 2016. Human anatomy & physiology. 10th edition. England: Pearson.

McComsey, G.A., Tebas, P., Shane, E., Yin, M.T., Overton, E.T., Haung, J.S., Aldrovandi, G.M., Cardoso, S.W., Santana, J.L. & Brown, T.T. 2010. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. *Clinical infectious disease*, 51:937-943.

Motyl, K.J., Guntur, A.R., Carvalho, A.L. & Rosen, C.J. 2017. Energy metabolism of bone. *Toxicologic pathology,* 45(7):1-7.

Mulubwa, M., Viljoen, M., Kruger, I.M., Kruger, H.S. & Rheeders, M. 2017. Bone turnover markers in HIV-infected women on tenofovir-based antiretroviral therapy. *Southern African journal of HIV medicine,* 18(1):a739. https://doi.org/10.4102/sajhivmed.v18i1.739 Date of access: 24 October 2017.

Norval, M., Coussens, M.K., Wilkinson, R.J., Bornman, L., Lucas, R.M. & Wright, C.J. 2016. Vitamin D status and its consequences for health in South Africa. *International journal of environmental research and public health*, 13:1019.

Palermoa, A., Tuccinardi, D., D'Onofrio, L., Watanabec, M., Maggia, D., Maurizi, A.R., Greto, V., Buzzetti, R.,Napoli, N., Pozzilli, P. & Manfrini, S. 2017. Vitamin K and osteoporosis: myth or reality? *Elsevier*, 70(2017):57-71.

Penido, M.G.M.G. & Alon, U.S. 2012. Phosphate homeostasis and its role in bone health. *Paediatric nephrology*, 27:2039-2048.

Piso, R.J., Rothen, M., Rothen, J.P., Stahl, M. & Fux, C. 2013. Per oral substitution with 300000 IU Vitamin D (Cholecalciferol) reduces bone turnover markers in HIV-infected patients. *BMC infectious diseases*, 13:577.

Price, C.T., Langford, J.R. & Liporace, F.A. 2012. Essential nutrients for bone health and a review of their availability in the average North American diet. *The open orthopaedics journal*, 6:143.

Rachner, T.D., Khosla, S. & Hofbauer, L.C. 2011. Osteoporosis: now and the future. *Lancet,* 377:1276-1277.

Ralston, S.H. 2013. Bone structure and metabolism. *Medicine,* 41(10):581-582.

Reid, I.R., Bolland, M.J. & Grey, A. 2014. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet,* 383(9912):146-155.

Rizzoli, R. 2014. Nutritional aspects of bone health. *Best practice & research clinical endocrinology & metabolism,* 28:796.

Rolfes, S.R., Pinna, K. & Whitney, E. 2012. Normal and Clinical Nutrition. 9th edition. United States: Cengage Learning.

Segawa, T., Miyakoshi, N., Kasukawa, Y., Aonuma, H., Tsuchie, H. & Shimada, Y. 2016. Combined treatment with minodronate and vitamin C increases bone mineral density and strength in vitamin C-deficient rats. *Elsevier*, 2:30-37.

Shiau, S., Broun, E.C., Arpadi, S.M. & Yin, M.T. 2013. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. *AIDS*, 27:1949-1957.

Sharma, A., Flom, P.L., Clifford, J.R. & Schoenbaum, E.E. 2015. Racial differences in bone loss and relation to menopause among HIV-infected and uninfected women. *Bone*, 77:24.

Sims, N.A. & Vahnas, C. 2014. Regulation of cortical and trabecular bone mass by communication between osteoblasts, osteocytes and osteoclasts. *Archives of biochemistry and biophysics*, 561:22-23.

Sotunde, O.F., Kruger, H.S., Wright, H.H., Havemann-Nel, L., Mels, C.M.C., Ravyse, C. & Pieters, M. 2016. Association of 25-hydroxyvitamin D and parathyroid hormone with the metabolic syndrome in black South African women. Applied physiology, nutrition, and metabolism. www.nrcresearchpress.com/doi/abs/10.1139/apnm-2016-0257 Date of access: 24 October 2017.

South Africa. National Department of Health. 2015. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults 2015. Pretoria.

Stagi, S., Cavalli, L., Lurato, C., Seminara, S., Brandi, M.L. & De Martino, M. 2013. Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. *Clinical cases in mineral and bone metabolism*, 10(3):172-176.

Statista the Statistics Portal. South Africa: Urbanisation from 2007 to 2017. https://www.statista.com/statistics/455931/urbanisation-in-south-africa/ Date of access: 31 January 2018.

Steyn, N.P. & Mchiza, Z.J. 2014. Obesity and the nutrition transition in Sub-Saharan Africa. *The annals of the New York academy of sciences*, 1311:88-101.

Stunes, A.K., Syversen, U., Berntsen, S., Paulsen, G., Stea, T.H., Hetlelid, K.J., Lohne-Seiler, H., Mosti, M.P., Bjørnsen, T., Raastad, T. & Haugeberg, G. 2017. High doses of vitamin C plus E reduce strength training-induced improvements in areal bone mineral density in elderly men. *European journal of applied physiology,* 117:1073-1084.

Tai, V., Leung, W., Grey, A., Reid, I.R. & Bolland, M.J. 2015. Calcium intake and bone mineral density: systematic review and meta-analysis. *The British Medical Journal*. http://dx.doi.org/10.1136/bmj.h4183 Date of access: 16 July 2017.

Takeda, E., Yamamoto, H., Yamanaka-Okumura, H. & Taketani, Y. 2014. Increasing dietary phosphorus intake from food additives: potential for negative impact on bone health. *Advances in nutrition,* 5:92-97.

Tebas, P., Powderly, W.G., Claxton, S., Marin, D., Tantisiriwat, W., Teitelbaum, S.L. & Yarasheski, K.E. 2000. Accelerated bone mineral loss in HIV infected patients receiving potent antiretroviral therapy. *AIDS,* 14:64-67.

Trajanoska, K., Morris, J.A., Oei, L., Zheng, H., Evans, D.M., Kiel, D.P., Ohlsson, C., Richards, J.B. & Rivadeneira, F. 2018. Assessment of the genetic and clinical determinants of fracture risk: genome wide association and mendelian randomisation study. *British medical journal.* https://doi:10.1136/bmj.k3225 Date of access: 23 October 2017.

Uusi-Rasi, K., Kärkkäinen M.U.M. and Lamberg-Allardt, C.J.E. 2013. Calcium intake in health maintenance – a systematic review. *Food & nutrition research*, 57:21082. http://dx.doi.org/10.3402/fnr.v57i0.21082 Date of access: 11 July 2017.

Van Schoor, J. 2011. National Osteoporosis Foundation of South Africa Osteoporosis Guideline. *South African family practice,* 53(5):413.

Vermeer, C. 2012. Vitamin K: the effect on health beyond coagulation - an overview. *Food & nutrition research*, 56:5329.

Vorster, H.H., Oosthuizen, W., Jerling, J.C., Voldman, F.J. & Burger, H.M. 1997. The nutritional status of South Africans: A Review of literature form 1975-1996. Durban: *Health systems trust,* 1-122.

Vorster, JNR. H.H., Venter, C.S., Kruger, M.C., Vorster, H.H. & Kruger, H.S. 2002. The impact of urbanisation on risk factors for osteoporosis in black postmenopausal South African women. *Journal of endocrinology, metabolism and diabetes of South Africa,* 7(3):92-99.

Walsh, J.S. 2014. Normal bone physiology, remodelling and its hormonal regulation. *Basic Science: surgery*, 33:1.

Wang, Y., Wactawski-Wende, Y., Sucheston-Campbell, L.E., Preus, L., Hovey, K.M., Nie, J., Jackson, R.D., Handelman, S.K., Nassir, R., Crandall, J.C. & Ochs-Balcom, H.M. 2017. The influence of genetic susceptibility and calcium plus vitamin D supplementation on fracture risk. *American journal of clinical nutrition*, 105:971.

3 Chapter 3: Methodology

This chapter describes the study design, population, sampling, research setting, data collection process, statistical methods, ethical consideration and the data management plan of the research study in detail. The methodology is again described briefly in chapter 4 and will be presented as a publication with a word limit.

3.1 Study design and population

This study is a sub-study of a larger prospective cohort study with HIV-positive women on HAART from the North West Province and has a cross-sectional study design. Black postmenopausal HIV-positive women older than 45 years on HAART were studied, because little data on bone health in the southern African HIV-positive population exists.

3.2 Sampling

A power calculation was done to estimate sample size for the larger study, a comparison of BMD and bone turnover markers of women with calcium intakes below or above the median intake. An estimated change in total body BMD from 1.05 to 1.0 in the low intake group over two years versus no change in the high intake group (difference 0.05) g/cm^2 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 0.05 g/cm^2 between the group with lowest and the group with highest calcium intakes. A different power calculation based on a generalized linear model was conducted to take into account the use of multivariate models adjusted for up to 10 confounders. According to this analysis a sample size of 55-60 subjects per group is sufficient to obtain a power >80% (type-II error ≤20%) for an effect size of 0.4 F when type-I error was set to 5% (Dunlap *et al.*, 2004).

This number is in line with the number of participants in randomized 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 18 176 per group (Jackson *et al*., 2006), but three studies included 36 to 59 participants per group (Cleghorn *et al*., 2001; Albertazzi *et al*., 2004; Manios *et al*., 2007). In a meta-analysis the drop-out rates ranged from 8.6% to 25% in most studies, except for a dropout rate of 57.4% in one study (Shea *et al*., 2002). The mean drop-out rate was

16.8%. To allow for a 17% loss to follow-up, the sample size of the present study was increased to n=124 to allow for comparison of women with calcium intakes below or above the median intake (62 per group). The same participants will be part of the current descriptive observational sub-study to determine the association between number of risk factors and BMD. Published studies of the association between fracture risk score and BMD had sample sizes of 129-728 (Table 3.1). Only one study included HIV-positive participants (n = 138) (Prior *et al*., 2007). The available sample size for our study is 120 women. Although, this study may be underpowered to assess the association between the number of risk factors and BMD, it should be taken into account that the number of postmenopausal HIV-positive women are limited and in our experience it is not feasible to recruit large numbers of HIV-positive women older than 45 years, because most HIV-positive women are younger than 45 years. This study will be considered as an exploratory study and the results will be interpreted with caution.

Table 3.1: Characteristics and sample size of studies on the association between fractue risk and BMD.

Author	Journal	Participants	Country	Sample size
Bastos-Silva *et al.*	Archives of osteoporosis	Postmenopausal women	Brazil	402
Olmez Sarikaya *et al.*	Clinical rheumatology	Women >40 years old with osteopenia	Turkey	129
Chao *et al.*	Taiwanese Journal of obstetrics and gynecology	Postmenopausal women	Taiwan	231
Prior *et al.*	Osteoporosis international	Adult women, mean age 37.7 years	Canada	138 HIV-positive, 402 controls
Cano et al.	Maturitas	Postmenopausal women with osteopenia	Spain	728

Inclusion and exclusion criteria

HIV-positive postmenopausal women on HAART have been identified as a group at risk for suboptimal bone health. This study adds information about the potential to identify black HIV-positive older women that have an increased fracture risk. 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. The women were expected to have a BMI between 17 and 32 kg/m^2, but body mass index (BMI) was not an inclusion criterion. Table 3.1 justifies the inclusion criteria used in this study.

Exclusion criteria include use of anti-osteoporotic agents; chronic 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); 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. Women with severely low BMD (T score <-3) at baseline were not enrolled, but referred for medical treatment. This information was available after baseline measurement and was not applied as a screening exclusion criterion. All of these factors are known to have an impact on bone health. It was important to recruit a homogeneous group of women without additional risk factors for poor bone health.

3.3 Recruitment of participants

An advertisement was displayed in the waiting area of the clinic from one week before recruitment started, up to the end of 2017 to introduce the project to potential participants. Participants were recruited by an assistant that served as the independent person to recruit study participants. Participants were recruited from an outpatient clinic for HIV-positive adults at a local hospital. Patients return to the clinic every 60 days to collect their HAART, or more often if they are sick. It was expected that no more than 20 women would be recruited per week and recruitment continued for three months.

The research assistants explained the study procedures to the participants, and those who gave informed consent were included. Only women who were postmenopausal for at least six months were recruited.

Participants who were likely to meet the inclusion criteria were approached individually by the assistant and informed about the project. The research assistants conducted a screening questionnaire to determine eligibility for the study (Annexure A). If participants complied with the screening questionnaire criteria, an information session was held to discuss the study and obtain informed consent. Participants had the opportunity to ask any questions during the information session. During the information session participants were made aware that they could withdraw from the study at any time.

Dates were arranged with participants for the commencement of the study. Participants were collected from their homes and brought to the metabolic unit at the North-West University. A maximum of 12 participants were scheduled per day.

Participants had the right to choose not to participate in the study without negative consequences in terms of their routine treatment at the outpatient clinic at Potchefstroom Hospital.

3.4 Setting

Although, the participants were recruited from an outpatient clinic for HIV-positive adults at a local hospital, all measurements were performed at the Metabolic Unit of the North-West University in Potchefstroom. Participants were collected at their homes and transported to the Metabolic Unit according to appointments scheduled at the clinic for research visits.

3.5 Data collection

Data collected at baseline in the larger study were used for the purpose of this sub-study. Baseline visits were scheduled over time until all measurements were completed.

3.5.1 Socio-demographic and health information

The socio-demographic and health information of the participants were determined by an interviewer-administered structured questionnaire (Annexure B and C). Information that was collected included: age, educational status, housing, occupation, smoking, alcohol consumption, chronic medication use and year of first diagnosis of HIV infection as well as year of initiation of HAART.

3.5.2 Anthropometric measurements

Anthropometric measurements were performed by trained fieldworkers, postgraduate students and registered dieticians using standard methods with participants wearing light clothing. Measurements were recorded on the case report form (Annexure D). Anthropometric measurements included height and weight.

Height was measured using a stadiometer (Seca 264, Hamburg, Germany). Height of participants was measured without shoes and participants wore light clothing. Fieldworkers ensured that participants stood with, arms relaxed at their sides, shoulders relaxed and head in the Frankfort horizontal plane. The heels, buttocks, shoulder blades and back of the head were touching the vertical measuring surface. The measurement was taken after the participant inhaled. The measurement was taken to the nearest 0.1 cm. After the first measurement, a second measurement was taken with the same stadiometer. If the measurements differed with more than 0.5 cm, a third measurement was taken and an average of the measurements was used (Lee & Nieman, 2013).

The weight was measured using a calibrated digital scale with stadiometer (Seca 264, Hamburg, Germany). Participants were weighed without shoes and wore light clothing. The measurement was taken to the nearest 0.1 kg. After the first measurement, a second measurement was taken with the same scale. If the measurements differed with more than 0.1 kg, a third measurement was taken and the average of the measurements was used (Lee & Nieman, 2013: 170).

3.5.3 Dietary intakes

Dietary intakes were assessed by trained fieldworkers, postgraduate students and registered dietitians. Dietary intakes over the peroid of three months were assessed using a standardised quantitative food frequency questionnaire (QFFQ) that was previously validated in this population (Wentzel-Viljoen *et al*., 2011; Venter *et al*., 2000). The QFFO gathered information on the dietary intake of the previous month. The QFFQ was administered in a language that the participant understood, namely English, Afrikaans, Tswana or Xhosa. Food models and photographs were used to estimate portion sizes (Annexure E). Photo books with 3 portion sizes of the most commonly eaten foods were used to estimate portion sizes (Wentzel-Viljoen *et al*., 2011; Venter *et al*., 2000). The average daily intake from the QFFQ was used. The amount of each food item consumed was determined in gram per day. Dietary energy,

macronutrients and micronutrients were analysed using the South African Medical Research Council Food Finder software®, which is based on South African food composition tables (Wolmarans *et al*., 2010).

3.5.4 Alcohol intake

Alcohol intake was assessed by trained fieldworkers, postgraduate students and registered dietitians using the standarised (QFFQ). The average alcohol intakes were converted to gram/day and alcohol units/day were calculated according to the United Kingdom National Health Service guidelines (Table 3.2) (NHS, 2018).

Table 3.2: Units of alcoholic drinks according to the United Kingdom National Health Service guidelines

Type of drink	Volume	Number of alcohol units
Spirits	Single small shot, 25ml	1 unit
Alcopop	275 ml	1.5 units
Red/white/rosé wine	Small glass, 125 ml	1.5 units
Lager/beer/cider	Bottle, 330ml	1.7 units
Lager/beer/cider	Can,440ml	2 units
Lower-strength lager/beer/cider	Pint, 473-560ml	2 units
Red/white/rosé wine	Standard glass, 175ml	2.1 units
Higher-strength lager/beer/cider	Pint, 473-560ml	3 units
Red/white/rosé wine	Large glass, 250 ml	3 units

3.5.5 Physical activity

Physical activity (PA) was assessed at the same visits by trained fieldworkers using the Global Physical Activity questionnaire (GPAQ) recommended by the WHO (Annexure F). The questionnaire gathers information on physical activity done in the previous seven days in the following domains: occupational physical activity, transport-related physical activity and physical activity during leisure time (WHO, 2017). The times spent during the various physical activity domains in terms of

frequency (days per week) and duration (minutes per day) were estimated. The total physical activity level of each individual was calculated in metabolic equivalent of task-minutes (MET-minutes) per week by multiplying the MET intensity for each activity domain by minutes per week spent during each activity and according to reported day(s). The MET intensities used to score GPAQ were vigorous (8METs), moderate (4METs) and leisure time (4METs) PA as defined in the WHO guidelines (WHO, 2008). Participants were classified into GPAQ active or inactive categories according to the WHO cut-point of <600 METs of inadequate vs. ≥600 for adequate (WHO, 2008).

3.5.6 Health data

Health data was collected from routine follow-up data in patient files of the participants (haemoglobin, CD4 count, viral load, ART regimen) and recorded in the case report form (Annexure G). Data collection occurred in a closed office.

3.5.7 Bone mineral density

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): total body, lumbar spine and left femoral neck of the hip in g/cm^2, at baseline. The DXA machine is based at the Metabolic Unit at the North-West University, Potchefstroom campus. Participants were asked to change into gowns and asked to remove all jewellery and other personal effect that could interfere with the DXA exam, before lying down on the scan table. Verbal feedback was given to each participant.

3.5.8 Fracture risk

Risk for fractures and fall risk were assessed using a checklist developed from information in the literature (Compston, 2016; Vivrette *et al.,* 2011) (Annexure H). The checklist was used to gather information on fracture risk factors such as: older age, female sex, underweight, previous fractures, parent fractured hip, current smoking, glucocorticoid therapy during the last six months, proton pump inhibitor use during the last six months, rheumatoid arthritis, secondary osteoporosis and alcohol consumption of ≥ 3 units per day. Currently, there is no validated fracture risk instrument available for South African populations, therefore a fracture risk was not calculated and a cut-point was not applied. The number of risk factors was recorded. This checklist was compiled based on available literature (Compston, 2016; Vivrette *et al.,* 2011) and after consultation with South African scientists working on bone

health and fracture risk (Professor J. Pettifor from the University of the Witwatersrand and Professor B. Cassim from the University of KwaZulu-Natal).

3.5.9 Serum vitamin D concentration

Fasting blood samples of 5 ml were collected in serum tubes by a registered nurse and centrifuged as soon as possible after sample collection. Serum was prepared and stored in three Eppendorf tubes of 1 ml each in a bio-freezer in the Metabolic Unit at the North-West University at -80°C until all samples were collected. Fasting serum concentrations of vitamin D (25OHD3) was measured by an electrochemiluminescence immunoassay on the Elecsys 2010 (Roche, Basel, Switzerland). All samples collected was analysed in one run with the same controls.

3.6 Statistical methods

The distribution of the different variables was assessed using the Kolmogorov-Smirnov test and QQ plots. Descriptive statistics of socio-demographic data, dietary intakes, physical activity, BMD at the different sites, as well as fracture risk scores are presented as means and standard deviation (data with normal distribution) or median and interquartile range (non-normal distribution).

To determine the association between number of risk factors and BMD of the whole body, left femoral neck and spine, respectively, multivariable regression models were used with site-specific BMD as the dependent variable for each model, with adjustment for age, smoking, alcohol intake, calcium intake, serum vitamin D concentration, duration of HIV infection and physical activity level.

Statistical analysis was performed by researchers and the student in consultation with a statistician from the Centre of Excellence for Nutrition at North-West University.

3.7 Ethical considerations

3.7.1 Permission

The Health Research Ethical Committee (HREC) of the North-West University granted the larger prospective cohort study ethical approval (project number NWU-00061-1-17-A1). As 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.

Participants were recruited by an assistant that served as the independent person to recruit study participants (See 3.1. for the recruitment process of participants). Informed consent was obtained from all participants and they had the opportunity to ask questions (Annexure I). During the information session, participants were made aware that they could withdraw from the study at any time. Informed consent forms were collected on the day the participants reported for measurements after the forms had been signed by the participants and the person obtaining consent.

A goodwill permission letter to recruit patients and approval to access patient files was obtained from the Clinical Manager and the nursing professional in charge of the HIV outpatient clinic at Potchefstroom Hospital. Data were only obtained from files after the participants themselves also gave consent to access their files. Approval from the North West Department of Health to conduct the study was also obtained.

3.7.2 Benefits

Participants directly benefited from the study through receiving information regarding BMD with an explanation of these measurements by the researchers. The information participants received did not necessarily directly benefit their overall health status. The participants received a small reimbursement of R50 for participation, but this cannot be regarded as a direct benefit.

If any abnormalities were detected, it was discussed with the participants and they were referred to the hospital clinic with a report to the general practitioner (Annexure J). The researchers, the PhD student or the postdoc fellow gave this information to the participants in a private room.

3.7.3 Anticipated risks and precautions

Transporting participants to the Metabolic Unit could have led to stigmatisation or risk for vehicle accidents. Therefore, the participants were collected 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 stigmatization they were picked up two blocks from their homes at a previously arranged spot.

During anthropometry and DXA measurements, participants wore cotton gowns provided at the Metabolic Unit. In order to minimize discomfort, the measurements were performed in private rooms with only the participant and 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 blood samples were collected. A snack was provided directly after blood sampling and anthropometry. Concluding all the measurements took about 3-5 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. Research assistants guided the participants to the next measuring station.

Participants who took part in the study directly benefited only in the sense that they received results regarding their own BMD, which would not have been accessible to them as routine measurement at the hospital. All results from this study, including BMD results were provided as a report to the head of the clinic for consideration and action. Women with severely low BMD (T score <-3) were not enrolled, but referred for more intensive treatment, and women with low BMD (T score < -2) were enrolled, but received a referral for attention of the general practitioner at the hospital clinic. Osteoporosis was defined as a T-score at the femoral neck of -2.5 SD below the young white female adult mean, but a reference for black African women is not currently available. The data will provide information for future studies and health programmes on the association between calcium and vitamin D status and bone health.

3.7.4 Risk associated to researchers

A medical doctor from Potchefstroom Hospital and one registered nurse supervised the study. The nurse was experienced and followed standard procedures for possible incidents that were anticipated during the study. The nurse was present at all times during the study and had standard safety plans/measures (e.g. oxygen) in place to handle incidents, both to researchers and participants and helped to report them as well, to the medical doctor and/or NWU Protection Services and HREC. Furthermore, the Emergency Services of the NWU was notified of the study and was given dates and times of the study. They were on standby if any incident should have occurred. Standard operating procedures for incidents were followed for all procedures in the Metabolic Unit. The unit (G17) is equipped with a number of rooms for all assessments.

3.7.5 Risk/benefit ratio analysis

The benefits outweighed the risks as there were minimal risks to the participants.

3.7.6 Anonymity, privacy and confidentiality throughout the research process

Privacy of participants was assured by using private rooms for measurements and by performing measurements in a Metabolic Unit. The HIV status of the participants was not known to persons outside the research team. The identifier list to the coded data was kept separate from project data and was available to the project leader only. Confidentiality was ensured by assigning each participant a unique participant number during the baseline data collection. Upon arrival on the day of the study, participants showed their SA identity document in order to verify the national identification number against their unique participant number. Identification was done only by the project coordinator.

Anonymity and privacy of participants were ensured during data collection for the case report form. The postdoc was responsible for supervision of data collection. Data collection occurred in closed rooms. Confidentiality was ensured by only using participant numbers.

3.7.7 Respect, justice and autonomy

Participants were treated with respect and had the right to exercise autonomy. Participant information was not disclosed to anyone outside the research team. The participants were informed regarding all aspects of the study and signed a consent form, after having the opportunity to ask study related questions. Participants had the right to withdraw from the study at any time.

3.7.8 Management of vulnerability, beneficence/non-maleficence

Transport arrangements were made for all participants to and from the research facility, refreshments were provided to participants on the day of the study. They were referred to the hospital or clinic when necessary based on study results.

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.

3.7.9 Safety plan for participants

A medical doctor from Potchefstroom Hospital and one registered nurse supervised the study according to standard safety measures described under 3.7.4.

3.7.10 Specific precautions

Anthropometry was performed by trained researchers and 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 while the measurements were performed.

During the administration of questionnaires, allocated stations were used for data collection in order to ensure privacy. The questionnaires were administered to the participants and guidance and assistance were given to estimate food portion sizes to reduce participant burden. The participants were allowed to rest in between in the case they needed a short break.

3.7.11 Expertise, skills and legal competencies

All of the researchers that were part of this study are experienced in their fields. All involved with data collection received training that ensured standardized data collection (e.g. anthropometry) and completion of questionnaires. All measurements were supervised by the principal investigator and postdoc.

3.7.12 Token of appreciation

Participants received a token of appreciation following the completion of data collection to a total value of R50 to thank them for participating in the study. They were transported to the university and did not have travel expenses.

3.8 Data management plan

3.8.1 Data entry and monitoring

Data entry was conducted by the PhD and MSc student. The postdoc 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 student and corrected if necessary. Minimums and maximums were checked for correctness against hard copies. Data were entered onto an Excel worksheet on a password protected computer.

After all data have been entered into the datasheets, data cleaning were conducted by the PhD and MSc student in collaboration with the postdoc and principal investigator, by checking minimums, maximums and every 10th data entry line.

3.8.2 Data archiving

All hard copies of the data collection forms and other data forms used in this study is locked in the principal investigator's office and electronic data is archived on a password protected computer and backed up on an external hard drive which is 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 was monitored by the study leader. All data were cleaned by the research group and the final dataset is locked. Only the study leader, postdoc, PhD and MSc student had access to the data. All hard copies of completed forms will be stored for seven (7) years at least and then destroyed by shredding according to the North-West University's rules and regulations for data and record management.

3.8.3 Quality control

Before data were entered into the secure database, all questionnaires were screened for possible problematic answers by the researchers and the principal investigator. 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 (a level 3 anthropometrist). Anthropometric measurements were taken following standard procedures as described under 3.5.2. All DXA measurements were done according to standard guidelines by an experienced registered radiographer as described under 3.5.6.

Serum samples were stored until further analyses for a specified period of time, probably never longer than six (6) months before analyses. To ensure the integrity of the samples while in storage, serum samples were stored at -80°C in secure lockable bio-freezers until the determination of the variables examined for the study. These bio-freezers are monitored via a cellular-based monitoring system. Sample integrity was 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. Standard methods and quality control measures were applied.

Captured data were checked and cleaned to ensure accuracy. Data were stored in the Excel file and the computer programme Statistical Package of Social Sciences (SPSS version 23 program). Data were cleaned and checked by the PhD student and MSc student together with the supervisors.

3.8.4 Reporting, dissemination and notification of results

Feedback on the disease burden within this community was also being given to the Potchefstroom Hospital Patient Safety Committee after conclusion of the study.

Results from any measurements which 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 were present a referral letter was provided to the local hospital. The research leader thoroughly explained the results to the participants. Feedback was also given to the Potchefstroom hospital and North West Department of Health in the form of an official report regarding the disease burden within these communities after conclusion of the study.

3.9 References

Albertazzi, P., Steel, S.A., Howart, E.M. & Purdie, D.W. 2004. Comparison of the effects of two different types of calcium supplementation on markers of bone metabolism in a postmenopausal osteopenic population with low calcium intake: a double-blind placebo-controlled trial. *Climacteric*, 7:33-40.

Bastos-Silva, Y., Aguiar, L.B., Pinto-Neto, A.M., Baccaro, L.F. & Costa-Paiva, L. 2016. Correlation between osteoporotic fracture risk in Brazilian postmenopausal women calculated using the FRAX with and without the inclusion of bone densitometry data. *Archives of osteoporosis*, 11:16. https://www.ncbi.nlm.nih.gov/pubmed/27067597 Date of access: 16 July 2017.

Cano, A., Baró, F., Fernández, C., Inaraja, V. & García-Domínguez, C.A. 2016. Evaluation of the risk factors of asymptomatic vertebral fractures in postmenopausal women with osteopenia at the femoral neck. *Maturitas,* 87:95-101. doi: 10.1016/j.maturitas.2016.02.014 Date of access: 16 July 2017.

Chao, A.S., Chen, F.P., Lin, Y.C., Huang, T.S., Fan, C.M., & Yu, Y.W. 2015. Application of the World Health Organization fracture risk Assessment tool to predict need for dual-energy X-ray absorptiometry scanning in postmenopausal women. *Taiwanese journal of obstetrics and gynecology*, 54(6):722-5. doi: 10.1016/j.tjog.2015.10.005. Date of access: 16 July 2017.

Cleghorn, D.B., O'loughlin, P.D., Schroeder, B.J. & Nordin, B.E. 2001. An open, crossover trial of calcium-fortified milk in prevention of early postmenopausal bone loss. *Medical journal of Australia*, 175:242-5.

Compston, J. 2016. HIV infection and bone disease. *Journal of internal medicine*, 280:350-358.

Dunlap, W.P., Xin, X. & Myers, L. 2004. Computing aspects of power for multiple regression. *Behavior research methods, instruments and computer*, 36:695-701.

Fujita, T, Ohue, M, Fujii, Y, Miyauchi, A. & Takagi, Y. 2004. Reappraisal of Katsuragi calcium study, a prospective, double-blind, placebo-controlled study of the effect of active absorbable algal calcium (AAACa) on vertebral deformity and fracture. *Journal of bone and mineral metabolism*, 22:32-8.

International osteoporosis foundation. 2017. *Facts and statistics: osteoporosis - incidence and burden.* Retrieved from https://www.iofbonehealth.org/facts-statistics#category-14 Date of access: 17 July 2017.

Jackson, R.D., LaCroix, A.Z., Gass, M., Wallace, R.B., Robbins, J., Lewis, C.E., Bassford, T., Beresford, S.A.A., Black, H.R., Blanchette, P., Bonds, .E., Brunner, R.L., Brzyski, R.G., Caan, B., Cauley, J.A., Chlebowski, R.T., Cummings, S.R., Granek, I., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Johnson, K.C., Judd, H., Kotchen, J.M., Kuller, L.H., Langer, R .D., Lasser, N.L., Limacher, M.C., Ludlam, S., Manson, J.E., Margolis, K.L., McGowan, J., Ockene, J.K., Jo O'Sullivan, M., Phillips, L., Prentice, R.L., Sarto, G.E., Stefanick, M.L., Van Horn, L., Wactawski-Wende, J., Whitlock, E., Anderson, G.L., Assaf, A.R. & Barad, D. 2006. Calcium and Vitamin D supplementation and the risk of fractures. *The New England journal of medicine*, 354(7):669-683.

Lee, R.D., & Nieman, D.C. 2013. Nutritional assessment. 6th Edition. New York: McGraw-Hill. 167-170.

Manios, Y., Moschonis, G., Trovas, G. & Lyritis, G.P. 2007. Changes in biochemical indexes of bone metabolism and bone mineral density after a 12-month dietary intervention program: the Postmenopausal Health Study. *American journal of clinical nutrition*, 86:781-9.

National Health Service. 2018. *Alcohol support – alcohol units.* Retrieved from https://www.nhs.uk/live-well/alcohol-support/calculating-alcohol-units/ Date of access: 3 February 2018.

Prior, J., Burdge, D., Maan, E., Milner, R., Hankins, C., Klein, M. & Walmsley, S. 2007. Fragility fractures and bone mineral density in HIV positive women: a case-control population-based study. *Osteoporosis international,* 18(10):1345-1353.

Sarikaya, O.N., Yavasi, S.K., Tan, G., Satiroglu, S., Yildiz, A.H., Oz, B., Yoleri, O. & Memis, A. 2014. Agreement between FRAX scores calculated with and without bone mineral density in women with osteopenia in Turkey. *Clinical rheumatology*, 33(12):1785-1789. https://doi.org/10.1007/s10067-014-2491-8 Date of access: 17 July 2017.

Shea, B., Wells, G., Cranney, A., Zytaruk, N., Robinson, V., Griffith, L., Ortiz, Z., Peterson, J., Adachi, J., Tugwell, P. & Guyatt, G. 2002. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocrine reviews*, 23:552-559.

Venter, C.S., MacIntyre, U.E. & Vorster, H.H. 2000. The development and testing of a food portion photograph book for use in an African population. *Journal of human nutrition and dietetics*, 13:205-218.

Vivrette, R.L., Rubenstein, L.Z., Martin, J.L., Jesephson, K.R. & Kramer, B.J. 2011. Development of a fall-risk self-assessment for community-dwelling seniors. *Journal of aging and physical activity*, 19:16-29.

Wentzel-Viljoen, E., Laubscher, R. & Kruger, A. 2011. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. *South African journal of clinical nutrition,* 24(3):143-148.

Wolmarans, P., Danster, N., Dalton, A., Rossouw, K. & Schönfeldt, H. 2010. Condensed food composition tables for South Africa. Cape Town: *Medical Research Council.*

World Health Organization. 2008. Global Physical Activity Questionnaire (GPAQ) Analysis Guide. Geneva.

World Health Organization. 2017. Global Physical Activity Questionnaire (GPAQ). http://www.who.int/ncds/surveillance/steps/GPAQ_EN.pdf. Date of access: 23 October 2017.

4 Chapter 4: Article

Title: The association between fracture risk and BMD in black postmenopausal HIV-positive women on HAART.

Carlien van der Merwe[1], H. Salome Kruger[1], P.O. Ukegbu[1].
[1]Centre of Excellence for Nutrition, North-West University, Potchefstroom

Correspondence:
Centre of Excellence for Nutrition
North-West University, Potchefstroom campus
Tel: +27 18 299 2085
Fax: +27 18 299 2464
Email: salome.kruger@nwu.ac.za

Abstract

Background: South African urban postmenopausal women have an increased risk for the development of low bone mineral density (BMD) and consequently osteoporosis. Osteoporosis and fractures are a concern in the HIV-positive population as a result of higher life expectancy and possible fragility fractures due to the advancement in antiretroviral therapy (ART); increased prevalence of low bone mineral density (BMD) as a result of ART-induced bone demineralization as well as increased bone loss due the HIV-infection it self.

Objectives: This study aimed to determine the number of fracture risk factors and the association with BMD in black postmenopausal HIV-positive women on highly active antiretroviral therapy (HAART).

Methods: This study is a cross-sectional analysis that form part of a prospective cohort study in the North West Province of South Africa. Baseline data from 120 HIV-positive black post-menopausal women were used. Bone mineral density (at the spine, left femoral neck and total body) was measured by dual X-ray absorptiometry (DXA). The number of fracture risk was determined using a checklist and scored. Multivariate linear regression models were applied to assess associations of fracture risk score with site specific BMDs, adjusting for age, calcium intake, serum vitamin D, duration of HIV infection, duration of HAART and physical activity (PA).

Results: All participants had the age (>40 years) and female sex risk factors, with 39.2% having only two and 37.5% having three risk factors. The maximum number of risk factors was five. Age and underweight were the only individual risk factors significantly associated with BMD. In adjusted models, only age was significantly associated with BMD, but fracture risk was included in the final model for spine BMD and left femoral neck BMD. No significant association between fracture risk score and BMD was found.

Conclusions: A maximum of five fracture risk factors were found, but fracture risk score was not significantly associated with BMD in this group of HIV-positive women.

Keywords: HIV, postmenopausal, BMD, osteoporosis, fracture risk, Africa.

Background

Osteoporosis is a multifactorial skeletal disease presenting most often in postmenopausal women (Eastell, 2013). Osteoporosis is characterized by reduced bone mass and deterioration of the micro-architecture of the bone (Castiglioni *et al*., 2013; Motyl *et al*., 2017). As a result, bone strength is reduced and vulnerability to fractures increase (Eastell, 2013). This disease has become a global epidemic with more than 8.9 million fractures annually (IOF, 2017).

In South Africa, the incidence of osteoporosis is similar to that of high-income countries, however; fracture data is limited (IOF, 2017). A recent retrospective review reported 113 cases of fragility fractures of the hip at Groote Schuur Hospital, Cape Town (Kauta *et al*., 2017). Currently, South Africa is experiencing rapid urbanisation, the driving force being increased work opportunities and improved living conditions in cities (Kruger *et al*., 2011). Environmental factors influencing the risk for lower bone mineral density (BMD) may be attributed to changes in diet and physical activity (PA) as a result of urbanisation (Kruger *et al*., 2004). Dietary changes associated with urbanisation such as a shift from traditional foods consumed in rural areas towards consumption of western foods low in calcium and vitamin D negatively influence BMD (Kruger *et al*., 2011; Kruger *et al*., 2004; Steyn & Mchiza, 2014; MacIntyre *et al*., 2002). Postmenopausal women can benefit from regular PA as it may contribute towards increasing bone formation, improving bone structure and maintaining BMD (Bilek *et al*., 2016). Kruger and colleagues found that black urban women have low PA levels, especially women older than 70 years (Kruger *et al*., 2011).

Osteoporosis and associated fractures are also becoming a concern in the HIV-positive population (Piso *et al*., 2013). It is estimated that 15% of patients living with HIV will be diagnosed with osteoporosis (Cortés *et al*., 2015). Various studies have shown a marked increase in the prevalence of bone demineralization in HIV-positive individuals (Compston, 2016; Tebas *et al*., 2000; Bruera *et al*., 2003; Landonio *et al*., 2004). In comparison with the general population, HIV-positive individuals are 6.4 times more likely to develop low BMD and have a fracture risk as high as 58% (Cotter & Powderly, 2011; Cortés *et al*., 2015). The highest prevalence of HIV-infection is in Sub-Saharan Africa, where South Africa reports the highest prevalence in the world with 7.06 million people living with HIV in 2016 (UNAIDS, 2016a). HIV-infection is not the only factor that leads to increased bone loss. Antiretroviral therapy (ART) is also known to decrease BMD by various mechanisms and have also been associated with poor vitamin D status (Hamill *et al*., 2013; Dave *et al*., 2015; McComsey *et al*., 2010). Despite the fact that highly active antiretroviral therapy (HAART) has been proved to meaningfully improve survival and quality of life, the higher incidence of osteoporosis and associated

fractures have become a significant concern (Focà *et al*., 2012; Kryst *et al*., 2014). Other risk factors for low BMD in patients with HIV includes low weight, older age, smoking and female gender (Hamill *et al*., 2013; Dave *et al*., 2015).

Urbanisation combined with the increasing prevalence of HIV and ART use has detrimental effects on the bone health of South Africans (Kruger *et al*., 2011; Cortés *et al*., 2015; Gandy, 2014). The objectives of this study were to determine the number of fracture risk factors of black postmenopausal HIV-positive women on HAART and the association between number of risk factors and BMD of the total body, spine and left femoral neck, respectively.

Method

Study design and setting

This study was a cross-sectional analysis using baseline results from a prospective cohort study from the North West Province with black postmenopausal HIV-positive women on HAART. Participants were recruited from an outpatient clinic for HIV-positive adults at a local hospital. All measurements were performed at the Metabolic Unit of the North-West University (NWU).

Study population

Black postmenopausal HIV-positive women older than 45 years on HAART were studied, because little data on bone health in the southern African HIV-positive population exists. The HAART consisted of tenofovir, emtricitabine (FTC) and efavirenz combined in a fixed dose tablet.

The available sample size for the study was 120 women. Although, this study may be underpowered to assess the association between number of risk factors and BMD, it should be taken into account that the number of postmenopausal HIV-positive women is limited and in our experience it is not feasible to recruit large numbers of older HIV-positive women, because most HIV-positive women are younger than 45 years. This study will be considered as an exploratory study and the results will be interpreted with caution. Informed consent was obtained from all participants. Approval from the Human Research Ethical Committee (HREC) of the North-West University (NWU-00061-17-A1-01) and the Department of Health to conduct the study was also obtained.

Inclusion criteria included HIV-positive postmenopausal women on HAART. Exclusion criteria included use of anti-osteoporotic agents; chronic 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); 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. Women with severely low BMD (T score <-3) at baseline was not enrolled, but referred for medical treatment.

Measures

Socio-demographic and health information

The socio-demographic and health information of the participants were determined by an interviewer-administered structured questionnaire. Information that was collected included: age, educational status, housing, occupation, smoking, alcohol consumption, chronic medication use, year of first diagnosis of HIV infection and year of initiation of HAART.

Anthropometric measurements

Anthropometric measurements were performed by trained fieldworkers, postgraduate students and registered dietitians. Anthropometric measurements included height and weight. Participants were weighed without shoes and wore minimal clothing. Height and weight were measured using a calibrated digital scale with stadiometer (Seca 264, Hamburg, Germany) following standard procedures (Lee & Nieman, 2013).

Dietary intakes

Dietary intakes over the period of a month were assessed using a standardised quantitative food frequency questionaire QFFQ that was previously validated in this population (Wentzel-Viljoen *et al*., 2011; Venter *et al*., 2000). The QFFQ was administered in a language that the participant understood, namely English, Afrikaans, Tswana or Xhosa. Food models and photographs in photo books with three portion sizes of the most commonly eaten foods were used to estimate portion sizes population (Wentzel-Viljoen *et al*., 2011; Venter *et al*., 2000). Dietary energy, macronutrients and micronutrients were analysed using the South African Medical Research Council Food Finder software®, which is based on South African food composition tables (Wolmarans *et al*., 2010).

Physical activity

PA was assessed using the Global Physical Activity questionnaire (GPAQ) recommended by the WHO (WHO, 2017). The questionnaire gathers information on PA done in the previous seven days in the following domains: occupational PA, transport-related PA and PA during leisure time (WHO, 2017). The times spent during the various PA domains in terms of frequency (days per week) and duration (minutes per day) were estimated. The total PA level of each individual was calculated in metabolic equivalent-minutes (MET-minutes) per week by

multiplying the MET intensity for each activity domain by minutes per week spent during each activity and according to reported day(s). The MET intensities used to score GPAQ were vigorous (8METs), moderate (4METs) and leisure time (4METs) PA as defined in the WHO guidelines. Participants were classified into GPAQ active or inactive categories according to the WHO cut-point of <600 METs of inadequate vs. >600 for adequate (WHO, 2008).

Bone mineral density

Bone mineral density (BMD) at baseline 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/cm^2. Participants were asked to change into cotton gowns with no metal buttons or zipper pull fasteners and asked to remove all jewellery and other personal item(s) that could interfere with the DXA scan.

Fracture risk factors

Risk for fractures was assessed using a checklist developed from information in the literature (Vivrette *et al*., 2011; Compston, 2016). The checklist included eleven fracture risk factors such as: age >40 years, female sex, underweight (BMI <18.5 kg/m^2), previous fractures, parent fractured hip, current smoking, glucocorticoid therapy during the last six months, proton pump inhibitor (PPI) use during the last six months, rheumatoid arthritis, secondary osteoporosis and alcohol consumption of ≥3 units per day. Currently no validated fracture risk instrument is available for South African populations, therefore fracture risk was not calculated and a cut-point was not applied. The number of risk factors represents the sum of the conditions present in each participant and this was recorded as fracture risk score.

Serum vitamin D concentration

Fasting blood samples of 5 ml were collected by a registered nurse in serum tubes and centrifuged as soon as possible after sample collection. Serum was prepared and stored in a bio-freezer at -80°C until analyses were performed. Fasting serum concentrations of vitamin D (25OHD3) was measured by an electrochemiluminescence immunoassay on the Elecsys 2010 (Roche, Basel, Switzerland).

Statistical analysis

The distribution of the different variables was assessed using the Kolmogorov-Smirnov test and QQ plots. Descriptive statistics of socio-demographic data, dietary intakes, PA, BMD at the different sites, as well as fracture risk scores are presented as means and standard deviation (data with normal distribution) or median and interquartile range (non-normal distribution). To determine the association between number of risk factors and BMD of the total body, left femoral neck and spine, respectively, multivariable regression models were

used with site-specific BMD as the dependent variable for each model, with adjustment for age, smoking, alcohol intake, calcium intake, serum vitamin D concentration, duration of HIV infection and PA. The Statistical Package of Social Sciences (SPSS version 23 program) was used to perform all statistical analyses and a p-value of ≤ 0.05 was accepted as statistically significant.

Results

Participant characteristics are summarized in Table 4.1. A small proportion of the participants (5%) had PA level of >600 METs (adequate PA), 38% were obese, 3.8% were underweight and almost half of the total group (45.8%) were hypertensive.

Table 4.1: Baseline characteristics of the study participants.

Characteristics	n (%)	Median	Interquartile range
Age (years)		50	48; 55
Number of people living per household		4	3; 5
Number of household electric appliances:			
1 – 6 Appliances	50 (42%)		
7 – 13 Appliances	70 (59%)		
Education:			
No school and primary	45 (37.5%)		
Grade 8 - 11	43 (35.8%)		
Grade 12	30 (25.0%)		
Tertiary	2 (1.7%)		
Employment:			
Unemployed	53 (44.2%)		
Employed	54 (45%)		
Pensioner	13 (10.8%)		
Income (per month):			
None	6 (5.0%)		
< R500 - R1000	31 (25.8%)		
R1001 - R6000	74 (61.7%)		
> R6000	9 (7.5%)		
Hypertension	55 (45.8%)		
Diabetes	10 (8.3%)		

Table 4.1: Baseline characteristics of the study participants (continued)

Characteristics	n (%)	Median	Interquartile range
Smokers:			
Current smokers	12 (10%)		
Previous smokers	5 (4.2%)		
Alcohol intake (g/day)		0.0	0, 1
Weight (kg)		66.7	54.1, 80.0
Height (cm)		156.0	151.5, 161.1
BMI (kg/m^2)*		27.1	22.4, 32.6
Total body BMD (g/cm^2)		1.04	0.95, 1.12
Total spine BMD (g/cm^2)		0.82	0.73, 0.94
Left femoral neck BMD (g/cm^2)		0.74	0.65, 0.84
Lean mass (kg)		36.2	32.5, 42.1
Body fat (%)		38.6	33.8, 43.6
Calcium intake (mg)		724	462-1040
Physical activity moderate level (min/d)		145.0	45, 300
Physical activity vigorous level (min/d)		0.0	0, 0
Sedentary time (min/d)		240.0	120, 360
Duration of HIV infection (years)		10	7, 14
Duration of HAART (years)		9	5, 13

*BMI = body mass index; BMD = bone mineral density

The summary of number of fracture risk factors is given in Table 4.2. The most common fracture risk factors were age and female sex (100%), followed by previous fracture (19.2%). Most (39.2% and 37.5%) had only two or three fracture risk factors, respectively. The highest number of risk factors was five (5%).

Table 3.2: Fracture risk factors identified among women.

Risk factors	N (%)
Female	120 (100%)
Age >40y	120 (100%)
Underweight	10 (8.3%)
Smoking current	17 (14.2%)
Alcohol consumption >3 units per day	6 (5.0%)
Previous fracture	23 (19.2%)
Parent fracture hip	14 (11.7%)
Glucocorticoid therapy	4 (3.3%)
Rheumatoid Arthritis	4 (3.3%)
Proton pump inhibitor	8 (6.7%)
Secondary osteoporosis	4 (3.3%)

The association between fracture risk and BMD was assessed in two ways. First, the association between individual fracture risk variables and total, spine and left femur neck of the hip BMD, were assessed in multivariable linear regression models. Participant age, number of cigarettes smoked per day and alcohol intake per day were entered as continuous variables, while the other risk factors were entered as dichotomous categorical variables (1 = risk factor present versus 0). In an additional model the association between fracture risk score and BMD was assessed with further adjustment for age (continuous variable), serum vitamin D concentration, calcium intake, duration of HIV infection and PA as additional covariates. Results of fully adjusted models are shown in Table 4.3. Final models with only fracture risk variables and with additional adjustment are reported.

Among the individual risk factors age (marginal association with spine BMD) and underweight were significantly associated with total, spine and left femoral neck BMD. Although, fracture risk was included in the final model for spine BMD and left femoral neck BMD, no significant association with BMD was found. Fracture risk was not included in the final model for total BMD.

Table 4.3: Multiple regression models for the association between number of risk factors and BMD with site-specific BMD as the dependant variable (N=120).

Variables	Standardised ß coefficient*	P
Total BMD: Final model with fracture risk variables only		
Age (years)	-0.34	<0.0001
Underweight (BMI <18.5 kg/m^2)	-0.20	0.03
Adjusted R^2	0.16	
Total BMD: Final model with fracture risk score		
Age (years)	-0.513	<0.0001
Adjusted R^2	0.253	
Spine BMD: Final model with fracture risk variables only		
Age (years)	-0.174	0.055
Underweight (BMI <18.5 kg/m^2)	-0.21	0.02
Adjusted R^2	0.069	
Spine BMD: Final model with fracture risk score		
Age (years)	-0.30	0.007
Duration of HIV (years)	-0.20	0.07
Adjusted R^2	0.118	
Left Femoral neck BMD: Final model with fracture risk variables only		
Age (years)	-0.29	0.001
Underweight (BMI <18.5 kg/m^2)	-0.27	0.001
Glucocorticoid therapy	-0.16	0.053
Adjusted R^2	0.194	
Left Femoral neck BMD: Final model with fracture risk score		
Age (years)	-0.40	<0.0001
Calcium intake (mg)	-0.168	0.113
Fracture risk score	-0.11	0.314
Adjusted R^2	0.161	

*Regression models adjusted for age, smoking, alcohol intake (g/day), calcium intake (mg/day)), duration of HIV infection and PA level.

Fracture risk together with age and calcium intake explained 16.1% of the variance in left femoral neck BMD. Only age was significantly associated with total BMD, while age and duration of HIV explained 11.8% of the variance in spine BMD. Although, fracture risk explained some of the variance in left femoral neck BMD no association with BMD was found

(P = 0.31). Age was negatively associated with spine, left femoral neck and total BMD. In separate models where duration of HIV infection was replaced by duration of HAART, similar results were found (data not shown).

Discussion

The main findings of this study was that fracture risk explained some of the variance in left femoral neck BMD, but no association with BMD was found. Age and underweight were the only variables consistantly and significantly associated with BMD. In the adjusted models including fracture risk score only, age was significantly associated with BMD, but fracture risk was included in the final model for left femoral neck BMD.

Race plays a role in modifying the relationship between HIV and fracture risk. A meta-analysis and systematic review concluded that non-black population groups have the highest risk for fractures (Shiau *et al*., 2013). Previously, studies reported that black populations have a reduced risk for the development of osteoporosis due to their enhanced BMDs, however; these studies were conducted in America and Europe and may not be relevant to South Africa's black population (Aloia *et al.*, 1996; Handa *et al*., 2008). In fact, very little data on BMD is available on non-white population groups living in low- to middle-income countries such as South Africa (George *et al*., 2014). There is some evidence from a study among South African black women that changes in diet and PA as a result of urbanisation, influence the risk for lower BMD (Kruger *et al*., 2004).

HIV and HAART use have been shown to decrease BMD and consequently increase the risk for osteoporosis and associated fractures by various mechanisms (Cotter & Powderly, 2011; Cortés *et al*., 2015; Hamill *et al*., 2013; Dave *et al*., 2015; McComsey *et al.,* 2010; Focà *et al*., 2012; Kryst *et al*., 2014). The South African study by Dave *et al.* (2015) demonstrated that exposure to antiretroviral therapy is associated with lower total hip BMD in a young group of HIV-positive men and women. Other factors for fracture risk in the HIV population include older age, female sex, underweight, previous fractures, parent fractured hip, smoking, glucocorticoid therapy, PPI use, rheumatoid arthritis, secondary osteoporosis and alcohol consumption of ≥3 units per day (Vivrette *et al*., 2011; Compston, 2016). The present study confirms the negative association between older age and total, spine and left femoral neck BMD, as well as the negative association between duration of HIV infection and spine BMD.

More than a third (38%) of the participants was obese, while 3.8% of the participants were underweight. The results also confirm the association between underweight and BMD reported in other studies (De Laet *et al*., 2005). BMI was not included in the regression models

as underweight was included as a fracture risk factor. Low BMI is a known risk factor for osteoporosis and fracture, whereas a high BMI has a strong positive association with BMD (Compston, 2016; Vivrette *et al*., 2011). Previously, a larger body size was suggested as a protective factor against low bone mass attributed to the mechanical loading effects on the bone (Tremollieres *et al*., 1993). However, a study by Zhao and colleagues have suggested a negative correlation between bone mass and fat mass after adjusting for mechanical loading effects (Zhao *et al.*, 2007). Similarly, Compston *et al.* (2011) concluded that obesity was not a protective factor against fracture in postmenopausal women.

In this study, 19.2% of the study participants reported a previous fracture, while 11.7% reported a parent hip fracture. This is noteworthy as previous research has suggested an association between increased risk for another fracture and previous fracture history (Kanis *et al*., 2004). Similarly, Kanis and colleagues demonstrated a modest increase in the risk of any fracture, osteoporotic fracture and hip if there is a parental history of fracture in both men and women combined. Previous or parent fractures were not associated with BMD in this study (Kanis *et al*., 2004).

Smoking is a known risk factor for the development of osteoporosis. Smokers typically have low bone mass and thus a high risk of fracture. This may be attributed to the effect of smoking on osteoblast malfunctioning as well as reduced intestinal absorption of calcium (Melhus, 1999). A meta-analysis study concluded that smoking increases the risk of developing a vertebral fracture by 13% and a hip fracture by 31% in women (Ward & Klesges, 2001). Kruger *et al.* (2011) found that smoking contributed to increased risk for bone disease in black urban postmenopausal women. In this study 14.2% of the participants smoked, but those who smoked reported only smoking one to 11 cigarettes per day. However, no significant association was observed between smoking and BMD. Possible reasons for this include that the effect of smoking is dose dependant, as well as that smoking during young adulthood may increase the risk for developing low BMD later in life (Ward & Klesges, 2001). In this study the number of cigarettes smoked per day was very low, ranging from one to 11 cigarettes per day and number of years smoked was not investigated. Given the implication of smoking on bone health, it is important that education regarding this subject be given.

The prolonged use of glucocorticoid drugs can have adverse effects on bone by decreasing intestinal calcium absorption and reabsorption in the renal tubule as well as inhibiting osteoblast function. Research has demonstrated that fracture incidence can be as high as 30% if these drugs are taken for an average of five years (Reid, 1997). In this study medical records showed that only 3.3% of the study participants have used intermittent glucocorticoid therapy. Use of glucocorticoid drugs were inversely associated with left femoral neck of the

hip BMD in this study (marginal association), despite the small number of participants who have used glucocorticoids. Previous research has reported an association between PPI therapy and the risk of an osteoporosis-related fracture. PPI may increase the risk for fractures by reducing intestinal calcium absorption (Targownik *et al*., 2008). A recent meta-analysis concluded that PPI use was associated with a moderately increased risk of spine, hip and any-site fracture (Zhou *et al.*, 2016). In our study, 6.7% of the participants received PPI therapy, but PPI use was not associated with BMD. Participants could not recall the duration of PPI use, but the dose and duration was probably not sufficient to show an association with BMD.

Alcohol intake of ≥2 drinks per day is associated with an increased risk for the development of osteoporotic fractures in a white population (Kanis *et al*., 2005). Chronic alcohol intake may result in increased bone resorption due to increased parathyroid hormone levels. The outcome is bone loss and an increased incidence of fractures (Sampson, 2003). Other studies have demonstrated that alcohol abuse is a risk factor for bone disease in black urban postmenopausal women (Kruger *et al*., 2011). In our study 5% of the study participants reported that they consumed >3 units of alcohol per day, but alcohol intake was not associated with BMD.

Postmenopausal women can benefit from regular PA as it may contribute towards increasing bone formation, improving bone structure and maintaining BMD (Bilek *et al.*, 2016). In this study only a small proportion of the participants had an adequate PA level of >600 METS/day. Participants did not partake in vigorous activity, had a median moderate activity level of 145 min/day and spent a median of 240 min/day in sedentary activities. Similarly, Kruger and colleagues found that black urban women have low PA levels, especially women older than 70 years (Kruger *et al*., 2011). PA across the life cycle is an important factor for developing peak BMD and is a protective factor against osteoporotic fractures during old age (Lu *et al*., 2016). In this study only the current PA levels were measured which is thus not reflective of the effects of PA during young adulthood on BMD.

In this study, a negative correlation between calcium intake and total BMD, spine BMD and left femoral neck BMD was demonstrated. The median calcium intake of the women was 724 mg which is higher than what was previously reported for women living in the same province by other researchers (Kruger *et al*., 2011). Kruger and colleagues reported a low calcium intake (200-450 mg/d) in both rural and urban black women older than 45 years. In this study no association between calcium intake and total BMD, spine BMD and left femoral neck BMD was demonstrated, but the trend was in the negative direction. The high self-reported calcium intake may be explained by possible over-reporting by the participants and/or the influence of

previous nutrition counselling which have resulted in participants consuming more dairy foods than previously during early life.

Limitations and strengths

To our knowledge this study is the first to investigate the association between fracture risk and BMD in black postmenopausal HIV-positive women on HAART.

The study had some limitations. The study had a relatively small sample size and may be underpowered to assess the association between number of risk factors and BMD. It should be taken into account that the number of postmenopausal HIV-positive women i limited and in our experience it is not feasible to recruit large numbers of HIV-positive women older than 45 years, because most HIV-positive women are younger than 45 years. Other limitations include a possible over-reporting of calcium intake and the prevalence of rheumatoid arthritis diagnosis was not confirmed but self-reported. It is likely that the participants had other forms of arthritis, such as osteoarthritis.

Conclusions

Among black postmenopausal HIV-positive women, age and underweight showed significant associations with BMD. Although, fracture risk score was not associated with BMD it was included in the final model with an indication of a negative association and together with age and calcium intake explained 16.1% of the variance in left femoral neck BMD.

Urbanisation combined with increasing prevalence of HIV and ARV use have detrimental effects on the bone health of South Africans and is a growing concern. This study contributed information on the association between fracture risk and BMD in black postmenopausal HIV-positive women on HAART, but more research in prospective studies and larger study samples are necessary to develop a fracture risk tool for HIV-positive and HIV-negative South African women.

References

Aloia, J.F., Vaswani, A., Yeh, J.K. & Flaster, E. 1996. Risk for osteoporosis in black women. *Calcified tissue international*, 59(6):415-423.

Bilek, L.D., Waltman, N.L., Lappe, J.M., Kupzyk, K.A., Mack, L.R., Cullen, D.M., Berg, K., Langel, M., Meisinger, M., Portelli-Trinidad, A & Lang, M. 2016. Protocol for a randomized controlled trial to compare bone-loading exercises with risedronate for preventing bone loss in osteopenic postmenopausal women. *BioMed central women's health*, 16:59.

Bruera, D., Luna, N., David, D.O., Bergoglio, L.M. & Zamudio, J. 2003. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS*, 17:1919-1922.

Castiglioni, S., Albisetti, W. & Maier, J.A.M. 2013. Magnesium and Osteoporosis: Current State of Knowledge and Future Research Directions. *Nutrients*, 5:3022-3023.

Compston, J., Watts, N.B., Chapurlat, R., Cooper, C., Boonen, S. 2011. Obesity is not protective against fracture in postmenopausal women: GLOW. *American journal of medicine*, 124(11):1043-1050.

Compston, J. 2016. HIV infection and bone disease. *Journal of internal medicine*, 280:350-358.

Cortés, Y.I., Yin, M.T. & Reame, N.K. 2015. Bone density and fractures in HIV-infected postmenopausal women: a systematic review. *Journal of the Association of Nurses in AIDS care,* 26(4):387.

Cotter, A.G. & Powderly, W.G. 2011. Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity. *Best practice & research clinical endocrinology & metabolism,* 25: 501-515.

Dave, J.A., Cohen, K., Micklesfield, L.K., Maartens, G. & Levitt, N.S. 2015. Antiretroviral therapy, especially efavirenz, is associated with low bone mineral density in HIV-infected South Africans. *PLoS ONE*, 10(12):e0144286. https://doi.org/10.1371/journal.pone.0144286 Date of access: 17 July 2017 .

De Laet, C., Kanis, J.A., Odén, A., Johanson, H., Johnell, O., Delmas, P., Eisman, J.A., Kroger, H., Fujiwara, S., Garnero, P., McCloskey, E.V., Mellstrom, D., Melton, L.J., Meunier, P.J., Pols, H.A.P., Reeve, J., Silman, A. & Tenenhouse, A. 2005. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporosis international*, 16:1330-1338.

Eastell, R. 2013. Osteoporosis. *Medicine,* 41 (10):586-587.

Focà, E., Motta, D., Borderi, M., Gotti, D., Albini, L., Calabresi, A., Izzo, I., Bellagamba, R., Narciso, P., Sighinolfi, L., Clò, A., Gibellini, D., Quiros-Roldan, E., Brianese, N., Cesana, B.M., Re, M.C. & Torti, C. 2012. Prospective evaluation of bone markers, parathormone and 1, 25-(OH) 2 vitamin D in HIV positive patients after the initiation of tenofovir/ emtricitabine with atazanavir/ritonavir or efavirenz. *BioMed central*, 12:38.

George, J.A., Micklesfield, L.M., Norris, S.A. & Crowther, N.J. 2014. The Association between body composition, 25(OH)D, and PTH and bone mineral density in black African and Asian Indian population groups. *The journal of clinical endocrinology & metabolism*, 99(6):2146-2154.

Hamill, M.M., Ward, K.A., Pettifor, J.M., Norris, S.A. & Prentice, A. 2013. Bone mass, body composition and vitamin D status of ARV-naive, urban, black South African women with HIV infection, stratified by CD count. *Osteoporosis international*, 24:2855-2861.

Handa, R., Kalla, A.A. & Maalouf, G. 2008. Osteoporosis in developing countries. *Best practice & research clinical rheumatology*, 22(4):693-708.

International osteoporosis foundation. 2017. *Facts and statistics: osteoporosis - incidence and burden.* Retrieved from https://www.iofbonehealth.org/facts-statistics#category-14 Date of access: 23 October 2017 .

Joint united nations programme on HIV and AIDS. 2016a. Country factsheet South Africa 2016. www.unaids.org/en/regionscountries/countries/southafrica Date of access: 23 October 2017.

Kanis, J.A., Johansson, H., Oden, A., Johnell, O., De Laet, C., Eismane, J.A., McCloskeya, E.V., Mellstromf, D., Melton, L.J., Polsh, H.A.P., Reevei, J., Silmanj, A.J. & Tenenhouse, A. 2004. A family history of fracture and fracture risk: a meta-analysis. *Bone*, 35:1029-1037.

Kanis, J.A., Johnell, O., De Laet, C., Johansson, H., Oden, A., Delmas, P., Eisman, J., Fujiwara, S., Garnero, P., Kroger, H., McCloskey, E.V., Mellstrom, D., Melton, L.J., Pols, H., Reeve, J., Silman, A. & Tenenhouse A. 2004. A meta-analysis of previous fracture and subsequent fracture risk. *Bone*, 375382.

Kanis, J.A., Johansson, H., Johnell, O., Oden, A. & De Laet, C. 2005. Alcohol intake as a risk factor for fracture. *Osteoporosis international*, 16(7):734-42.

Kauta, N., Held, M., Dlamini, S., Kalula, S., Ross, I., Kalla, G. & Maqungo, S. 2017. The management of fragility fractures of the hip: a quality assessment project. *South African orthopaedic journal,* 16(3):41-45.

Kruger, M.C., Dewinter, R.M., Becker, P.J. & Vorster, H.H. 2004. Changes in markers of bone turnover following urbanisation of black South African women. *Journal of endocrinology, metabolism and diabetes of South Africa,* 9(1):103-108.

Kruger, M.C., Kruger, I.M., Wentzel-Viljoen, E. & Kruger, A. 2011. Urbanisation of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition research*, 31:748-758.

Kryst, J., Kawalec, P. & Pilc, A. 2014. Efavirenz-based regimens in antiretroviral-naive HIV-infected patients: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*, 10(5):e0124279.

Landonio, S., Quirino, T., Bonfanti, P., Gabris, A., Boccassini, L., Gulisano, C., Vulpio, L., Ricci, E., Carrabba, M. & Vigevani, G.M. 2004. Osteopenia and osteoporosis in HIV+ patients, untreated or receiving HAART. *Biomedicine & pharmacotherapy,* 58:506-507.

Lee, R.D., & Nieman, D.C. 2013. Nutritional assessment. 6th edition. New York: McGraw-Hill. 167-170.

Lu, J., Shin, Y., Yen, M. & Sun, S.S. 2016. Peak bone mass and patterns of change in total bone mineral density and bone mineral contents from childhood into young adulthood. *Journal of clinical densitometry*, 19(2):180-191.

McComsey, G.A., Tebas, P., Shane, E., Yin, M.T., Overton, E.T., Haung, J.S., Aldrovandi, G.M., Cardoso, S.W., Santana, J.L. & Brown, T.T. 2010. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. *Clinical infectious disease*, 51:937-943.

MacIntyre, U.E., Kruger, H.S., Venter, C.S. & Vorster, H.H. 2002. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutrition research*, 22:239-256.

Melhus, H., Michaelsson, K., Holmberg, L., Wolk, A. & Ljunghall, S. 1999. Smoking, antioxidant vitamins and the risk of hip fracture. *Journal of bone and mineral research,* 14(1):129-35.

Motyl, K.J., Guntur, A.R., Carvalho, A.L. & Rosen, C.J. 2017. Energy metabolism of bone. *Toxicologic pathology,* 45(7):1-7.

Piso, R.J., Rothen, M., Rothen, J.P., Stahl, M. & Fux, C. 2013. Per oral substitution with 30 000 IU vitamin D (cholecalciferol) reduces bone turnover markers in HIV-infected patients. *BMC infectious diseases*, 13:577.

Reid, I.R. 1997. Glucocorticoid osteoporosis – mechanisms and management. *European journal of endocrinology,* 137:209-217.

Sampson, H. 2003. Alcohol and other factors affecting osteoporosis risk in women. *Alcohol research and health*, 26(4):292-296.

Shiau, S., Broun, E.C., Arpadi, S.M. & Yin, M.T. 2013. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. *AIDS*, 27:1949-1957.

Steyn, N.P. & Mchiza, Z.J. 2014. Obesity and the nutrition transition in Sub-Saharan Africa. *The annals of the New York Academy of Sciences*, 1311:88-101.

Targownik, L.E., Lix, L.M., Metge, C.J., Prior, H.J., Leung, S. & Leslie, W.D. 2008. Use of proton pump inhibitors and risk of osteoporosis related fractures. *Canadian Medical Association journal,* 179(4):319-26.

Tebas, P., Powderly, W.G., Claxton, S., Marin D., Tantisiriwat, W., Teitelbaum, S.L. & Yarasheski, K.E. 2000. Accelerated bone mineral loss in HIV- infected patients receiving potent antiretroviral therapy. *AIDS,* 14:64-67.

Tremollieres, F.A., Pouilles, J.M. & Ribot, C. 1993. Vertebral postmenopausal bone loss is reduced in overweight women: a longitudinal study in 155 early postmenopausal women. *Journal of clinical endocrinology and metabolism,* 77:683-6.

Venter, C.S., MacIntyre, U.E. & Vorster, H.H. 2000. The development and testing of a food portion photograph book for use in an African population. *Journal of human nutrition and dietetics*, 13:205-218.

Vivrette, R.L., Rubenstein, L.Z., Martin, J.L., Jesephson, K.R. & Kramer, B.J. 2011. Development of a fall-risk self-assessment for community-dwelling seniors. *Journal of aging and physical activity*, 19:16-29.

Ward, K. & Klesges, R.C. 2001. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcified tissue international*, 68(5):259-270.

Wentzel-Viljoen, E., Laubscher, R. & Kruger, A. 2011. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. *South African journal of clinical nutrition,* 24(3):143-148.

Wolmarans, P., Danster, N., Dalton, A., Rossouw, K. & Schönfeldt, H. 2010. Condensed food composition tables for South Africa. Cape Town: *Medical Research Council.*

World Health Organization. 2008. Global Physical Activity Questionnaire (GPAQ) Analysis Guide. Geneva.

Zhao, L., Liu, Y., Liu, P., Hamilton, J., Recker, R.R. & Deng, H. 2007. Relationship of obesity with osteoporosis. *Journal of clinical endocrinology & metabolism*, 92(5):1640-1646.

Zhou, B., Huang, Y., Li, H., Sun, W. & Liu, J. 2016. Proton-pump inhibitors and risk of fractures: an update meta-analysis. *Osteoporosis international*, 27:339-347.

5 Chapter 5: Summary and conclusion

The aim of this study was to investigate the association between fracture risk (number of risk factors) and BMD in black postmenopausal HIV-positive women on HAART. For each objective a summary of the discussion and conclusion follow below.

Objective 1: To determine the number of fracture risk factors of black postmenopausal HIV-positive women on HAART.

The most common fracture risk factors were age and female sex (100%), followed by previous fracture (19.2%). Most (39.2% and 37.5%) had only two or three fracture risk factors, respectively. The highest number of risk factors was five (5%).

Objective 2: To determine the association between number of risk factors and BMD of the whole body, left femoral neck and spine, respectively, in black postmenopausal HIV-positive women on HAART, after adjustment for possible covariates.

The main findings of this study was that fracture risk together with age and calcium intake explained 16% of the variance in left femoral neck BMD, but no significant association with BMD was found.

The results from this study confirm the association between underweight and BMD reported in other studies (De Laet *et al.*, 2005). More than a third (38%) of the participants were obese, while 3.8% of the participants were underweight. BMI as a continuous variable was not included in the regression models, because underweight was included as a fracture risk factor.

Previous or parent fractures were not associated with BMD as shown in previous studies (Kanis *et al.*, 2004). In this study, 19.2% of the study participants reported a previous fracture, while 11.7% reported a parent hip fracture.

There was no significant association between smoking and BMD. In this study, 14.2% of the participants smoked. Possible reasons for the lack of association is that the effect of smoking is dose dependent, as well as that smoking during young adulthood may increase the risk for developing low BMD later in life (Ward & Klesges, 2001). In this study, the number of cigarettes smoked per day was very low, ranging from one to 11 cigarettes per day and number of years smoked was difficult for the participants to recall. Therefore no accurate data about duration of smoking was available.

There was a marginal association between the use of glucocorticoid drugs and the left femoral neck of the hip BMD. In this study, medical records showed that only 3.3% of the study participants have used intermittent glucocorticoid therapy. In our study, 6.7% of the participants received PPI therapy, however; PPI use was not associated with BMD. Previous research has demonstrated an association between PPI use and a moderately increased risk of spine, hip and any-site fracture (Zhou *et al.*, 2016). In this study, the participants could not recall the duration of PPI use, but the dose and duration was probably not sufficient to show an association with BMD.

Other studies have demonstrated that alcohol abuse is a risk factor for bone disease in black urban postmenopausal women (Kruger *et al.*, 2011). However in this study, alcohol intake, derived from quantitative food frequency questionnaire data was not associated with BMD and 5% of the study participants reported that they consumed >3 units of alcohol per day. A possible reason may also be that cumulative alcohol use over years could not be recorded accurately. Recent alcohol intakes, based on quantified frequency recall of the past 30 days may not be an adequate indicator of alcohol abuse.

No association between PA and BMD was confirmed in this study. A reason for the lack of association may be that only the current PA levels were measured which is thus not reflective of the effects of PA during young adulthood on BMD. The direction of the association was positive, but the overall PA was probably too low to show a significant association with BMD. Other research also found that black urban women have low PA levels, especially women older than 70 years (Kruger *et al.*, 2011).

No association between calcium intake and total BMD, spine BMD and left femoral neck BMD was demonstrated, but the trend was in the negative direction. The median calcium intake of the women was 724 mg which is higher than what was previously reported for women living in the same province by other researchers (Kruger *et al.*, 2011). The high self-reported calcium intake may be explained by possible over-reporting by the participants and/or the influence of previous nutrition counselling which have resulted in these HIV positive women who receive comprehensive treatment at outpatient clinics consuming more dairy foods than previously during early life.

Limitations

The study had some limitations. The study had a relatively small sample size and may be underpowered to assess the association between number of risk factors and BMD. It should be taken into account that the number of postmenopausal HIV-positive women are limited and

it is not feasible to recruit large numbers of HIV-positive women older than 45 years, because most HIV-positive women are younger than 45 years. Other limitations include a possible over-reporting of calcium intake and the prevalence of rheumatoid arthritis diagnosis was not confirmed but self-reported. It is likely that the participants had other forms of arthritis, such as osteoarthritis.

Recommendations for future research

This study contributed information on the association between fracture risk and BMD in black postmenopausal HIV-positive women on HAART, but more research in prospective studies and larger study samples are necessary to develop a fracture risk tool for HIV-positive and HIV-negative South African women. For future research, it is recommended to determine the number of years that participants have been receiving HAART as accurately as possible, as well as to confirm the diagnosis of rheumatoid arthritis.

References

De Laet, C., Kanis, J.A., Odén, A., Johanson, H., Johnell, O., Delmas, P., Eisman, J.A., Kroger, H., Fujiwara, S., Garnero, P., McCloskey, E.V., Mellstrom, D., Melton, L.J., Meunier, P.J., Pols, H.A.P., Reeve, J., Silman, A. & Tenenhouse, A. 2005. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos international*, 16:1330-1338.

Kanis, J.A., Johansson, H., Oden, A., Johnell, O., De Laet, C., Eismane, J.A., McCloskeya, E.V., Mellstromf, D., Melton, L.J., Polsh, H.A.P., Reevei, J., Silmanj, A.J. & Tenenhouse, A. 2004. A family history of fracture and fracture risk: a meta-analysis. *Bone*, 35:1029-1037.

Kruger, M.C., Kruger, I.M., Wentzel-Viljoen, E. & Kruger, A. 2011. Urbanisation of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition research*, 31:748-758.

Ward, K. & Klesges, R.C. 2001. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcified tissue international*, 68(5):259-270.

Zhou, B., Huang, Y., Li, H., Sun, W. & Liu, J. 2016. Proton-pump inhibitors and risk of fractures: an update meta-analysis. *Osteoporosis international*, 27:339-347.

Annexures

Annexure A: 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 B: 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 C: 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 D: 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 E: 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? **1** Yes **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? **1** Yes **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 fru 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 / Fray bentos / 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:	_____ _____ _____ _____							
<u>WILD BIRDS, ANIMALS OR INCECTS</u> (hunted in rural areas or on farms)								
Wild fruit								

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

Thank you very much for your cooperation and patience.

Good-bye!

Annexure F: 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 G: Fracture Risk

Participant number: ..... Visit: Baseline/1y/2y

Fracture risk

Risk factor tick list

Fracture risk	**YES**	**NO**
Age > 40 years		
Sex: Female		
Weight: underweight (BMI < 18.5 kg/m^2)		
Previous fracture		
Parent fractured hip		
Current smoking		
Glucocorticoid therapy (Last 6 months)		
Proton pump inhibitors (Last 6 months)		
Rheumatoid arthritis		
Secondary osteoporosis		
Alcohol consumption ≥ 3 units per day		
Total number of risk factors		

Annexure H: Informed Consent

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

HREC Stamp

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 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

Referral Letter : Calcium and Bone study

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

_____