Body composition, bone health and vitamin D status of African adults in the North West Province

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It all starts here ™

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ABSTRACT

Body composition, bone health and vitamin D status of African adults in the North West Province

Background

In South Africa, as in many other developing countries, obesity has become a major health problem causing an increase in the incidence and prevalence of various non-communicable diseases. Research has shown that excess adiposity is associated with low vitamin D status and detrimental to bone health. Low vitamin D status has been linked to various non-communicable diseases which includes osteoporosis, and also the metabolic syndrome. Information is scarce on the role of lean mass and fat mass on bone health in the black South African population. There is also a shortage of data on the association between vitamin D status and the metabolic syndrome in the South African population.

Aim

The main aim of this study was to examine factors (vitamin D status, socio-economic status [SES] and lifestyle risk factors) associated with body composition, including bone health, as well as predictors of change in body composition in African adults in the North West Province of South Africa.

Methods

The first study that forms part of this thesis was a longitudinal study aimed at examining the effects of urbanization, socio-economic status and lifestyle factors on changes in body composition over 5 years in rural and urban black South African adults. A total of 1058 men and women above age 30 years from the Prospective Urban Rural Epidemiology study were included in this study. The second study to form part of this thesis aimed to examine the association between body composition and bone health in urban black South African women. Structured questionnaires were used to collect socio-demographic and lifestyle information including medication and tobacco use. This second study is cross-sectional in design and it included 189 postmenopausal women aged > 43 years old. Dual X-ray absorptiometry was used to assess bone mineral density, lean mass and fat mass, while structured and specific questionnaires were used to assess the habitual physical activity, food frequency and fracture risk. Habitual activity energy expenditure was also measured using an accelerometer with a combined heart rate monitor. The third study aimed to examine the association of serum 25 hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) concentration, respectively, with the metabolic syndrome while controlling for adiposity in black women in the North West Province, South Africa. This third study is also cross-sectional in design and it included 209 HIV-negative urban women. Dual X-ray absorptiometry was used to assess adiposity, while habitual physical activity was accessed with questionnaire and habitual activity energy expenditure was also measured using an accelerometer with a combined heart rate monitor.

Results

Study 1: Over a 5-year period, body mass index (BMI) and waist circumference increased in both genders, but the change was significant for BMI ($P<0.01$) and waist circumference ($P<0.001$) in women only, indicating an increase in adiposity over time. Urban residency positively predicted changes in waist circumference in men ($p < 0.05$) and women ($p < 0.001$) as well as change in triceps skinfold thickness of men ($p < 0.05$). Being married positively predicted changes in BMI ($p < 0.001$) and waist circumference ($p < 0.001$) in men, while age negatively predicted changes in triceps skinfold thickness in women ($p < 0.001$).

Study 2: Fat mass and lean mass were significantly positively associated with bone mineral density (BMD) and fracture risk when adjusted for potential confounders. However, lean mass and not fat mass remained significantly associated with femoral neck BMD ($\beta = 0.49$, $p <0.001$), spine BMD ($\beta = 0.48$, $p< 0.0001$) and hip BMD ($\beta = 0.59$, $p< 0.0001$). Lean mass was also negatively associated with fracture risk ($\beta = -0.19$ $p =0.04$) when both lean and fat mass were in the same model.

Study 3: After adjusting for age, body fat, habitual physical activity, tobacco use and season, neither 25(OH)D nor PTH concentrations showed significant associations with having the metabolic syndrome. However, when body fat was replaced with waist circumference there was a weak positive association between 25(OH)D concentration and the metabolic syndrome. No significant association was found between PTH:25(OH)D ratio and the metabolic syndrome.

Conclusion

This thesis has highlighted that the prevalence of obesity among black South Africans is high particularly among women and urbanization played a significant role in the increasing adiposity of black South Africans in the North West province. Lean mass had a stronger association with bone health in comparison to fat mass in urban black South African women. Low 25(OH)D concentration was not associated with the metabolic syndrome while there was no significant association between PTH and the metabolic syndrome in our black South African women.

Keywords: Body composition, bone health, lean mass, metabolic syndrome, 25(OH)D, PTH

OPSOMMING

Liggaamsamestelling, beengesondheid en vitamien D status van swart volwassenes in die Noord-Wes provinsie

Agtergrond

In Suid-Afrika, soos in baie ander ontwikkelende lande, het obesiteit ŉ merkwaardige gesondheidsprobleem geword wat ŉ verhoogde voorkoms van verskeie nie-oordraagbare siektes tot gevolg het. Navorsing toon dat oortollige adipose weefsel geassosieer word met lae vitamien D status asook ŉ nadelige effek het op beengesondheid. Lae vitamien D status word ook verbind met verskeie nie-oordraagbare siektes soos osteoporose en die metaboliese sindroom. Inligting rakende die rol van maer liggaamsmassa en vet liggaamsmassa op beengesondheid in die swart Suid-Afrikaanse populasie is skaars. Daar is ook ŉ tekort aan data rakende die assosiasies tussen vitamien D status en die metaboliese sindroom in Suid-Afrikaners.

Doelwit

Die primêre doel van hierdie studie was om te bepaal of daar ŉ assosiasie bestaan tussen sekere faktore (vitamien D status, lewensstyl risiko faktore en sosio-ekonomiese status) en liggaamsamestelling, insluitende beengesondheid, sowel as indikators wat verandering in liggaamsamestelling voorspel in volwasse swart Suid-Afrikaners van die Noord-Wes provinsie in Suid-Afrika is.

Metodes

Die eerste studie wat deel uitmaak van hierdie tesis, was ŉ longitudinale studie wat gekyk het na die effekte van verstedeliking, sosio-ekonomiese status en lewenstyl faktore op die verandering van liggaamsamestelling oor ŉ vyf jaar tydperk in landelike en stedelike swart individue wat in Suid-Afrika woon. ŉ Totaal van 1058 swart mans en vroue bo die ouderdom van 30 jaar is ingesluit binne die Prospektiewe Stedelike en Landelike Epidemiologiese (PURE) studie. Die tweede studie wat deel vorm van die tesis het beoog om die assosiasie tussen liggaamsamestelling en beengesondheid te bepaal in swart Suid-Afrikaanse vroue wat in ŉ landelike gebied woon. Hierdie studie is ŉ dwarsdeursnitstudie wat 189 postmenopousale swart vrouens ingesluit het met ouderdomme bo 43 jaar. Gestruktureerde vraelyste was gebruik om sosio-demografiese- en lewensstyl inligting asook medikasie- en tabakgebruik in te samel. Dubbel X-straal absorptiometrie (DXA) was gebruik om beenmineraal densiteit (BMD), maer liggaamsmassa en vet liggaamsmassa te bepaal. Gestruktureerde en spesifieke vraelyste is

gebruik om hul gewoontelike fisieke aktiwiteit te bepaal, asook hul voedsel inname en fraktuur risiko. Gewoontelike energie verbruik is ook gemeet met behulp van ʼn versnellingsmeter wat gekombineer is met ʼn hartmonitor. Die derde studie wat deel vorm van die tesis, het die assosiasie tussen serum 25-hidroksi-vitamien D [25(OH)D] asook die paratiroïed hormoon (PTH), met die metaboliese sindroom ondersoek terwyl daar gekorrigeer is vir adipose weefsel. Die studie is ook ʼn dwarsdeursnit ontwerp en het 209 MIV-negatiewe swart vroue ingesluit wat in ʼn stedelike gebied woon in die Noord-Wes provinsie. DXA was gebruik om adipositeit te bepaal, terwyl gewoontelike fisieke aktiwiteit met behulp van vraelys bepaal is. Gewoontelike energieverbruik is deur ʼn versnellingsmeter wat gekombineer is met ʼn hartmonitor, bepaal.

Resultate

Studie 1: Oor ʼn vyf-jaar tydperk, het die liggaamsmassa indeks (LMI) asook die middel-omtrek in albei geslagte toegeneem, maar die verandering was slegs betekenisvol vir LMI ($p<0.01$) en middel-omtrek ($p<0.001$) in vroue, wat ʼn indikasie is van adipose weefsel toename oor tyd. Area van woning (stedelike area) het veranderinge in die middel-omtrek in mans ($p<0.05$) en vroue ($p<0.001$) positief voorspel, asook veranderinge in die dikte van die trisepsvelvou in mans ($p<0.05$). Om getroud te wees het ʼn positiewe voorspelling gehad op LMI ($p<0.001$) en middel-omtrek ($p>0.001$) in mans, terwyl ouderdom ʼn negatiewe voorspelling gehad het op die verandering in die dikte van die trisepsvelvou in vroue ($p<0.001$).

Studie 2: Vet massa en maer massa het ʼn betekenisvolle positiewe assosiasie gehad met BMD en fraktuur risiko, terwyl daar gekorrigeer was vir potensiële beperkende faktore. Maer massa en nie vet massa, het betekenisvol geassosieer met die femorale nek BMD ($\beta = 0.49$, $p<0.001$), spinale BMD ($\beta = 0.59$, $p<0.0001$) en heup BMD ($\beta = -0.19$, $p = 0.04$) wanneer maer-massa en vet massa in dieselfde model geplaas was.

Studie 3: Nadat daar gekorrigeer is vir ouderdom, liggaamsmassa, gewoontelike fisieke aktiwiteit, tabakgebruik en seisoen, was daar geen assosiasie gevind tussen 25(OH)D of PTH-konsentrasies en die metaboliese sindroom nie. Nietemin, wanneer liggaamsmassa verplaas word met middel-omtrek was daar ʼn swak positiewe assosiasie gevind tussen 25(OH)D-konsentrasies en die metaboliese sindroom. Geen betekenisvolle assosiasie was gevind tussen PTH:25(OH)D ratio en die metaboliese sindroom nie.

Gevolgtrekking

Hierdie tesis het die hoë voorkoms van obesiteit onder swart Suid-Afrikaners uitgelig, met spesifieke fokus op vroue en ook die rol wat verstedeliking speel in die toename van adipose weefsel in swart Suid-Afrikaners in die Noord-Wes provinsie. Maer massa het ʼn sterker

assosiasie met beengesondheid gewys as vet massa in verstedelikte swart Suid-Afrikaanse vrouens. Lae 25(OH)D-konsentrasies asook PTH-konsentrasies was nie geassosieer met die metaboliese sindroom in swart Suid-Afrikaanse vroue nie.

Sleutelwoorde: Liggaamsamestelling; beengesondheid; maer massa; metaboliese sindroom; 25(OH)D; PTH

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LIST OF ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
AEE	Activity Energy Expenditure
ASM	Appendicular Skeletal Muscle Mass
BIA	Bioelectrical Impedance Analysis
BMD	Bone Mineral Density
BMI	Body Mass Index
CT	Computer Tomography
CVD	Cardiovascular Disease
DALYs	Disability-Adjusted Life Years
DM	Diabetes Mellitus
DXA	Dual Energy X-ray Absorptiometry
HDL-C	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostasis Model of Assessment of Insulin Resistance
ISAK	International Society for the Advancement of Kinanthropometry
MRI	Magnetic Resonance Imaging
NHANES	National Health Nutrition Examination Survey
NWP	North West Province
NWU	North–West University
PTH	Parathyroid Hormone
PURE	Prospective Urban Rural Epidemiology
QFFQ	Quantitative Food Frequency Questionnaires

RSMI	Relative Skeletal Muscle Index
SANHANES	South African National Health and Nutrition Examination Survey
SAT	Subcutaneous Adipose Tissue
SD	Standard Deviation
SES	Socio-economic Status
SSPS	Statistical Package for Social Sciences
UBV	Ultra violet-B
VAT	Visceral Adipose Tissue
WC	Waist Circumference
WHO	World Health Organization
β	Beta
µ	Micro
ºC	Degree Celsius
r	Correlaton Coefficient
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
±	Plus or Minus
%	Percentage

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

1.1.1 Factors affecting body composition

Human body composition studies have a long history of more than 150 years and it focuses on the masses of various body components and their distribution, the measurable relations among body components, the *in vivo* quantification of body components, and the quantitative changes in these components related to various intrinsic and extrinsic factors (Zhu & Wang, 2011).

Socio-economic status (SES) which can be defined as an individual's position on a socio-economic scale is often measured by factors like education, income, occupation and place of residence. SES is identified to be associated with a variety of diseases (Adler *et al*., 1999). For many decades, a powerful association between SES and physical health has been recognized. Whether defined according to level of education, income, or occupational status, lower SES is associated with diverse disease endpoints and with premature mortality (Gallo *et al*., 2009) and it is a significant predictor of body mass index (BMI) (Jeffery *et al*., 1991). Socio-demographic characteristics are associated with long term weight gain (Lahmann *et al*., 2000). Smoking is a lifestyle risk factor which increases insulin resistance and is associated with central fat accumulation (Chiolero *et al*., 2008). Dietary intake and physical activity are major determinants of body composition (Nilas *et al*., 1987; Hui *et al*., 1988; Slemenda *et al*., 1990; Popkin *et al*., 1993; Hill et al., 2000; Vorster *et al*., 2011).

1.1.2 Body composition and health

In 1997, the World Health Organisation (WHO) emphasised that obesity is becoming a major health problem in many developing countries, particularly in adult women (WHO, 2000). A high prevalence of obesity was found in black South African women, with an increase in BMI, skinfold thicknesses, waist circumference and waist to hip ratio (WHR) with increasing age (Kruger *et al*., 2001). The recent South African National Health and Nutrition Examination Survey (SANHANES) reported a national obesity prevalence of 10.6% and 39.2% for South African adult men and women respectively (Shisana *et al*., 2013). Malnutrition manifests predominantly as overweight and high rates of abdominal obesity in adult South Africans, particularly in African women (Puoane *et al.,* 2002). Obesity (defined as a body mass index (BMI) ≥30 kg/m^2) and “normal weight adiposity” (i.e. having a BMI in the normal range of 18.5 to 24.9 kg/m^2 but having excessive body fat) are associated with a multitude of health problems (Gropper *et al*., 2012). Obesity has been linked to an increased risk of hyperlipidaemia, heart disease, hypertension, and stroke, among others, while normal weight adiposity has been linked to dyslipidaemia, hypertension, and hyperglycaemia (Daniels *et al.,* 1999; Vizcaíno *et al*., 2007; Dervaux *et al*., 2008; Romero-Corral *et al*., 2010; Gómez-Ambrosi *et al*., 2011), as well as with elevated plasma C-reactive protein concentrations, an indicator of inflammation (Ridker *et al*., 2003; Musso *et al*., 2011).

Waist circumference represents a useful marker of abdominal or central obesity (Huang *et al*., 2001), and like body adiposity, larger waist circumference measurements have been associated with multiple health conditions (Evans *et al.*, 2011; Gropper *et al*., 2012) and has also been shown to be predictive of insulin resistance (Raman *et al*., 2008; Goedecke *et al*., 2009). Waist circumference and skinfold measurements were found to be reliable substitutes for body fat mass in a cohort of Caucasian adults (Ketel, 2007). The relationship between waist circumference and body fat mass may be different for different ethnic groups (Rush *et al*., 2007). Studies have shown that for the same BMI, black South African women have lower central adiposity than white South African women (Rush *et al*., 2007; Goedecke *et al*., 2013).

Bone mineral density (BMD) is another component of body composition that also decreases with age. Under-nutrition is common among the elderly, with the potential to aggravate the physiological age-related muscle and bone mass decline (Ilich *et al.,* 2003). The effects of under-nutrition and sarcopenia, independently and in combination overlap in their contribution to loss of bone mass in the elderly (Coin *et al*., 2008). Characteristics of osteoporosis include low BMD and higher than normal incidences of fractures (ZhiMin *et al*., 2012). Fractures related to osteoporosis are associated with significantly increased risk of death and disability-adjusted life years (DALYs) lost (Melton, 2003). Since several prospective studies have clearly shown that low BMD is predictive of future fractures (Ross *et al*., 1987, Hui *et al*., 1988), it will be of substantial benefit for both individuals and society if those with a high fracture risk are detected and managed early (ZhiMin *et al*., 2012). According to Slemenda and colleagues (1990), it is important to identify low BMD in order to determine who is most likely to benefit from therapy to preserve existing bone mass. Among other factors, under-nutrition and rapid bone loss during menopause have been acknowledged as increasing the risk of osteoporosis (Prynne *et al*., 2006).

1.1.3 Strategies to improve body composition and bone health

Diet, physical activity, race and heredity are major determinants of optimal bone mass and modification of diet and physical activity may help in maintaining optimum skeletal status (Nilas *et al*., 1987; Hui *et al*., 1988; Slemenda *et al*., 1990). Studies of progressive degeneration of normal physiological functioning of bone due to aging have mostly been limited to white women as they have the highest incidence of osteoporotic fractures (Kruger *et al*. 2004; Gnudi *et al*., 2007; Navarro *et al*., 2013). Also in South Africa, osteoporosis and fractures occur more frequently in white than in black women (Kruger *et al*., 2004). It was emphasised in the past that black women are relatively protected from osteoporosis; however older black women may increasingly become more prone to the risk of osteoporosis and fractures due to changes in physical activity and the nutrition transition (Aloia, 1996; Vorster *et al*., 2002; Kruger *et al*., 2011; Vorster *et al*., 2011).

Awareness that vitamin D sufficiency is required for optimal health is on the increase (Grant & Holick, 2005). Low vitamin D status has been reported as a risk factor for increased cardiovascular events, cancer, autoimmune diseases, type 1 and type 2 diabetes mellitus, infections, cognitive decline, (Pittas *et al.*, 2006; Pittas *et al.*, 2007; Cheng *et al.,* 2010; Pearce, 2010; Hammed *et al.*, 2011) and has been associated with the metabolic syndrome (Hypponen *et al*., 2008). The relationship between low vitamin D and metabolic traits, appear to differ among different ethnicities. In America, the National Health Nutrition Examination Survey (NHANES) III data showed an inverse association between vitamin D status and insulin resistance in non-Hispanic whites and Mexican Americans, but the inverse relationship was not observed in African-Americans (Scragg *et al.*, 2004). Low serum vitamin D is also associated with elevated parathyroid hormone (PTH) secretion (Lips, 2001) and elevated PTH levels have been linked to an increased risk for the metabolic syndrome (Reis *et al*., 2007; Ahlström *et al*., 2009). Some studies also showed that PTH is associated with a higher risk of incident hypertension (Oshima & Young 1995; Taylor *et al*., 2008).

This study will provide significant and new information on the relationship between socio-economic status and lifestyle risk factors respectively, on changes in body composition; the role of body composition, particularly lean mass, fat mass and BMI on bone health; and the relationship between vitamin D status, PTH and the metabolic syndrome in black South African adults. These results could help to facilitate the development of effective public health policies in South Africa, and also enable the re-evaluation of current strategies aimed at the rising scourge of obesity and its attendant health problems in the nation.

1.2 AIMS AND OBJECTIVES

The main aim of this study is to examine factors (vitamin D status, SES and lifestyle risk factors) associated with body composition, including bone health, as well as predictors of change in body composition in African adults in the North West Province of South Africa.

Specific Objectives:

1. To examine the influence of urbanization, SES and lifestyle risk factors on changes in the body composition of black South African men and women from the North West Province between 2005 and 2010.

2. To examine the association between body composition (BMI, fat mass and lean mass) and bone health (BMD and fracture risk) in urban postmenopausal black South African women.

3. To examine the association of serum 25 hydroxyvitamin D [25(OH)D] and PTH concentration with the metabolic syndrome in urban black South African women from the North West Province.

1.3 STRUCTURE OF THIS THESIS

This thesis is presented in article format and consists of six chapters, including this introductive chapter.

Chapter 2 gives an overview of the relevant literature on body composition, bone health and vitamin D. This chapter provides a comprehensive overview of the relevant literature needed for the interpretation of the data from the articles in this thesis.

Chapter 3 is an article entitled: "Influence of urbanization, socio-economic status and lifestyle risk factors on changes in body composition among South African adults". This article has been submitted for publication to the *BMC Public Health*. It addresses the influence of urbanization, SES, physical activity, tobacco use, dietary intake and marital status on changes in BMI, waist circumference and triceps skinfold over 5 years in rural and urban black South African adults.

Chapter 4 is an article entitled: "Lean mass appears to be more strongly associated with bone health than fat mass in urban black South African women". This article has been accepted for publication in the *Journal of Nutrition Health and Aging*. It addresses the effect of fat mass, lean mass and body mass index as body composition variables on bone health in post-menopausal urban black South African women.

Chapter 5 is an article entitled: “Association of serum 25(OH)D and PTH with the metabolic syndrome in women in the North-West, South Africa.” This article will be submitted for publication to the *BMC Women’s Health*. It examines the influence of 25(OH)D and PTH respectively on the metabolic syndrome in urban black South African women.

Chapter 6 is the final chapter which comprises of a general discussion, recommendations and conclusion.

The relevant references used in Chapters 1, 2 and 6 are provided at the end of each chapter according to the required style of the North-West University. The references of Chapters 3, 4 and 5 are provided at the end of each chapter according to the required style of the respective journals.

1.4 ETHICAL CONSIDERATIONS

The study is part of the broader PURE-SA and the PURE-Bone study with ethical approval obtained from the Ethics Committee of the North–West University, Potchefstroom, South Africa - Ethics number: NWU-00016-10-A1 (Addenda A, B and C). Signed informed consent was obtained from the participants after the purpose of the study and all study procedures have been explained to them in their home language (Addendum D). Permission to conduct the study was obtained from the North West Department of Health, tribal chiefs, community leaders and employers of the participants. Participants had the choice to withdraw from the study at any time.

1.5 AUTHOR’S CONTRIBUTIONS TO THE SEPARATE PAPERS IN THIS THESIS

The contributions of the researchers involved in the studies presented in this thesis are given in Table 1

Table 1 List of members within the research team and their contributions to this study

Name and signature*	Affiliation	Role in the study
O. F Sotunde (PhD candidate)	Centre of Excellence for Nutrition, North-West University	Assisted in the data collection of the PURE study, responsible for the literature review, statistical analysis, interpretation of results and writing up of publications and thesis.
Prof HS Kruger (Promoter)	Centre of Excellence for Nutrition, North-West University	Supervised this thesis, formulated research questions, supervised the data collection of the PURE study, quality control of data, statistical analyses, interpretation of results and co-authored the 3 articles (Chapters 3, 4 & 5) in this thesis.
Dr HH Wright (Co-promoter)	School of Health and Sports Sciences, University of the Sunshine Coast, Queensland, Australia; Centre of Excellence for Nutrition, North-West University;	Co-supervised this thesis, assisted in the data collection of the PURE study, co-authored the 3 articles (Chapters 3, 4 & 5).
Dr L. Havemann-Nel (Co-promoter)	Centre of Excellence for Nutrition, North-West University	Co-supervised this thesis, assisted in the data collection of the PURE study, co-authored the 3 articles (Chapters 3, 4 & 5).
Prof A Kruger	AUTHeR, Faculty of Health Sciences, North-West University	Planning and coordinating the PURE study SA, co-authored 1 article (Chapter 4)

Name and signature*	Affiliation	Role in the study
Dr L Kruger	AUTHeR, Faculty of Health Sciences, North-West University	Co-authored 1 article (chapter 4), assisted in the data collection of the PURE study.
Prof E Wentzel-Viljoen	Centre of Excellence for Nutrition, North-West University	Responsible for the collection, coding, analysis and quality control of dietary intake data for the PURE study. Co-authored 1 article (Chapter 4)
Dr C. Nienaber-Rousseau	Centre of Excellence for Nutrition, North-West University	Co-authored 1 article (Chapter 3), assisted in the data collection of the PURE study.
Prof. M. Pieters	Centre of Excellence for Nutrition, North-West University	Co-authored 1 article (Chapter 5), assisted in the quality control of data collected in the PURE study.
Prof. S.J. Moss	Physical Activity, Sport and Recreation Research Focus Area, North-West University	Co-authored 1 article (Chapter 3), assisted in the data collection of the PURE study.
Dr M. Tieland	Division of Human Nutrition; Wageningen University; Wageningen; The Netherlands	Co-authored 1 article, (Chapter 4), expertise in sarcopenia and bone health.

Name and signature*	Affiliation	Role in the study
Dr C. Botha-Ravyse 	Centre of Excellence for Nutrition, North-West University	Co-authored 1 article (Chapter 5), assisted in the data collection of the PURE study.
Dr C.M.C. Mels 	Hypertension in Africa Research Team; North-West University	Laboratory analysis and quality control of biochemical data, co-authored 1 article (Chapter 5)
Prof. E. Feskens 	Division of Human Nutrition; Wageningen University; Wageningen; The Netherlands	Co-authored 1 article (Chapter 5), expertise in body composition and the metabolic syndrome.

* I declare with my signature that as a co-author I have approved the above-mentioned articles, that my role in the study as indicated above is representative of my actual contribution and that I hereby give consent that it may be published as part of the PhD thesis of Mrs O.F. Sotunde

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CHAPTER 2: LITERATURE REVIEW

2

2.1 INTRODUCTION

Many African countries including South Africa are in the nutrition-related non-communicable disease phase of the nutrition transition (Vorster *et al.,* 2011) with resultant unfavourable changes in body composition. The national obesity prevalence as recently reported by SANHANES was 10.6% and 39.2% for South African adult men and women respectively (Shisana *et al*., 2013).

Black South Africans have traditionally been known to have a better bone health in comparison to their white and Indian counterparts (Micklesfield *et al*., 2011). This could be explained in part by genetic and ethnic differences in lifestyle (Pollitzer & Anderson, 1989; Lei *et al*., 2006; Goedecke *et al*., 2010; Chantler *et al*., 2011; Shisana *et al*., 2013). For instance a large number of black South Africans accumulate incidental moderate –intense physical activity due to walking as a mean of transportation (Goedecke *et al*., 2009). However, due to urbanization and its attendant negative effects; there is an increasing concern about the bone health of black South Africans (Kruger *et al*., 2011).

The high prevalence of obesity among South Africans is a cause of concern as excess body weight has also been associated with vitamin D deficiency (Bischof *et al*., 2006; Reinehr *et al*., 2007; Shisana *et al*., 2013). In a recent study black South Africans had a higher prevalence of vitamin D deficiency and inadequacy in comparison to blacks from Ghana, Jamaica and Seychelles (Durazo-Arvizu *et al.,* 2014).

This chapter of the thesis will focus on the review of literature related to composition of the human body and factors that contribute to its changes, as well as bone health and factors that affect bone health. The effect of vitamin D status and parathyroid hormone on components of metabolic syndrome will also be highlighted.

2.2 BODY COMPOSITION

Studies on human body composition span over 150 years and focus on the different body components, their distribution, and measurable changes in relation to various intrinsic and extrinsic factors (Zhu & Wang, 2011). A two compartment model of human body composition divides the body into fat mass and fat free mass (Ackland *et al*., 2012). Body composition can be assessed at five levels namely; atomic level, molecular level, cellular level and tissue levels and whole body (Wang *et al*., 1992; Ackland *et al*., 2012). The sum of all components at each of the five levels is equivalent to body mass (Heymsfield, 2005). At the atomic level body mass includes 11 major elements: calcium, potassium, phosphorous, sulphur, sodium, chlorine, magnesium, oxygen, carbon, hydrogen and nitrogen, while the last four elements account for more than 96% of body mass (Heymsfield, 2005). Most of these elements can be measured in vivo by neutron activation analysis (Cohn & Dombrowski, 1971) and whole body counting (Cohn *et al*., 1969). The molecular level consists of six major components: water, lipid, protein, carbohydrates, bone minerals and soft tissue minerals. The cellular level includes three components: extracellular solids, extracellular fluid and cells, whereas the tissue-organ level consists of the adipose tissue, skeletal muscle, visceral organs, and bone (Heymsfield, 2005; Ackland *et al*., 2012). Adipose tissue components are present throughout the body and the metabolic properties of these properties differ based on their location in the anatomy (Bjorntorp, 2000; Enevoldsen *et al*., 2001; Cinti, 2012). The whole body level is divided into appendages, trunk and head regions where by trunk and appendages are usually described by anthropometric measures like skinfolds, lengths and circumferences (Heymsfield, 2005). The three specific tissues that are particularly important in body composition research are bone, adipose and muscular tissue (Ilich *et al*., 2014). They make up approximately 75% of body weight in the reference man (Snyder *et al*., 1974). Body composition has been indicated to be a primary determinant of health, and a better predictor of mortality risk than body mass index (Segal *et al*., 1987; VanItallie *et al*., 1990; Ackland *et al*., 2012). Body composition measurements like waist circumference and waist to height ratio have been shown to be significantly associated with the risk of cardiovascular events (de Koning *et al*., 2007; Evans *et al*., 2011; Goedecke & Micklesfield, 2014). A case controlled study with 27,000 participants from 52 countries found waist to hip ratio to be highly significantly associated with myocardial infaction risk world wide (Yusuf *et al*., 2005).

2.2.1 Composition and physiology of soft body tissue

2.2.1.1 Fat mass

The fat mass component of the human body is fat from all body sources and it is categorized as essential fat or storage fat (Cinti, 2012). Total body fat is usually expressed as a percentage of total body weight. According to Gallagher *et al.* (2000), 8% to 24% of total body fat in males and 21% to 35% in females are associated with optimum health. Essential fat is stored in small amounts in the bone marrow, kidney, heart, lung, liver, spleen, muscles and lipid rich tissues in the nervous system, and it is necessary for normal physiological function (Gallagher *et al*., 2000; Cinti, 2012). Storage fat accumulates under the skin and around internal organs and acts as protection for the organs against trauma (Ackland *et al*., 2012). In the human body, adipose tissue increases either in the size (hyperthrophy) of adipose cells already present, or by the number (hyperplasia) of adipose cells (Knittle *et al*., 1979). Adipose tissue is the major site for fuel storage in form of triglycerides in the body (Schuster, 2009), and it has an important effect on whole body homoeostasis (Cinti, 2012). It is also critical for thyroid function, bone health maintenance, immune response, reproduction and blood clotting (Schuster, 2009). The adipose tissue is a complex and a very active metabolic and endocrine organ (Ahima & Flier, 2000; Frühbeck *et al*., 2001; Cinti, 2012). However, important factors in determining individual risk to develop metabolic and cardiovascular co-morbidities of obesity include ectopic fat accumulation and adipose tissue dysfunction (Blüher, 2009).

2.2.1.2 Lean mass

Lean body mass is basically the part of the body that is free of adipose tissue and it is also referred to as fat-free mass. It includes muscles, bones, ligaments, tendons and internal organs. Lean body mass increases with exercise (Fielding, 1995; Morris *et al*., 1997; Ackland *et al*., 2012), it is higher in men than women (Ley *et al*., 1992), and it is lower in older adults (Kyle *et al*., 2001a; Di Iorio *et al*., 2006). According to Forbes (2003), it is predictable that older persons with weight loss will lose both lean mass and fat mass. However, if body weight remains constant, lean body mass will fall by about 1.5kg per decade (Forbes, 1999). Lean body mass is the major determinant of resting metabolic rate (Gallagher *et al*., 1998; Kim *et al*., 2014), while it also accounts for 29% of excess weight in the obese (Pierson *et al*., 1997). In the elderly, lean body mass has been linked to protection against frailty and physical dysfunction (Delmonico *et al*., 2007), and also to a favourable cardiometabolic profile mainly as a result of increased insulin sensitivity (Nam *et al*., 2001). Recently, high lean mass has been shown to have a protective effect on bone health in Korean adult men (Shin *et al.*, 2014). Chantler and colleagues (2011) found lean mass to be the strongest correlate of whole body, femoral neck and total hip BMD for white South African women and whole body BMD for black South African women. A recent study among black South African and Asian Indian South Africans concluded that lean mass was the major contributor to BMD at all skeletal sites measured for both ethnic groups (George *et al*., 2014). Lipotoxicity is the lipid induced dysfunction of lean tissue whereby fat is deposited in non-adipose tissue and it has been linked in theory to produce obesity comorbidities such as insulin resistance, type 2 diabetes mellitus and cardiovascular diseases (Zhou *et al*., 2000; Shimabukuro *et al*., 2013).

2.2.1.3 Fat distribution

A number of studies have shown that the regional distribution of fat is an important factor in the relationship between obesity, metabolism and health (Lapidus *et al*., 1984; Vague, 1985; Donahue *et al.*, 1987; Goodpaster *et al*., 2005; Britton *et al*., 2013). Vague (1956) was the first to recommend that a distinction between adiposity should be made based on the type of excess fat. He also noted that despite that body fat distribution is a sexual characteristic, men and women can differ remarkably in android or gynoid pattern (Vague, 1956). A significant association has been demonstrated between regional fat distribution and cardiovascular disease and related mortality (Lapidus *et al*., 1984; Vague, 1985; Donahue *et al.*, 1987; Goodpaster *et al*., 2005; Britton *et al*., 2013). According to Britton *et al.* (2013), visceral adiposity is associated with incident cardiovascular disease and cancer. Regional patterns of fat deposit are controlled genetically and are different between and among men and women (Rush *et al*., 2007; Nazare *et al*., 2012; Karastergiou *et al*., 2012). South African studies have also reported differences in body fat distribution across its ethnic groups (Rush *et al*., 2007; Goedecke *et al*., 2013; George *et al*., 2014). Goedecke and colleague (2009) showed that body fat distribution is differentially associated with insulin sensitivity in black and white South African women. The study showed that for the same BMI, black women were less insulin sensitive despite having less visceral adipose tissue (VAT) compared to white women (Goedecke *et al*., 2009).

In the extensive study of obese persons by Krotkiewski *et al*. (1983), it was reported that persons whose abdominal adipocytes were larger than their gluteal adipocytes had higher insulin and glucose concentrations than persons with smaller abdominal than gluteal adipocytes even at the same level of adiposity. Some other studies also confirmed that subcutaneous abdominal adipocyte size is positively associated with adverse metabolic indexes in both sexes, while femoral adipocyte size had weak or no association (Kissebah *et al*., 1982; Pouliot *et al*., 1990; Imbeault *et al*., 1999; Harwood, 2012; Rydén *et al*., 2014).

In a recent study on Ghanaian migrants, lower rates of elevated fasting glucose were observed among Ghanaian women compared to men and it may be partly due to a more favourable body fat distribution, characterized by both greater hip and smaller waist measurements amongst the women (Nicolaou *et al*., 2013). Socio-economic status (SES) also seems to contribute to body fat distribution (Lahmann *et al*., 2000). Abdominal visceral and subcutaneous fat thickness was higher in urban residents in a Kenyan study compared to their rural counterparts (Christensen *et al*., 2008). The study implies that the rural inhabitants are engaged in more physically demanding jobs in comparison to their urban counterparts, especially the females (Christensen *et al*., 2008). In a Dutch study, migrant men had a more favourable fat distribution with less abdominal fat than Dutch men (Ujcic-Voortman *et al.*, 2011). There is growing evidence that smoking affects body fat distribution and that it is associated with central obesity and insulin resistance (Eliasson, 2003; Houston *et al*., 2006; Willi *et al*., 2007; Chiolero *et al*., 2008; Clair *et al*., 2011). Visceral adipose tissue is influenced by cortisol concentrations and smokers have been shown to have higher fasting plasma cortisol concentrations compared to non smokers (Cryer *et al*., 1976; Friedman *et al*., 1987; Pasquali *et al*., 2000). Smoking induced stimulation of sympathetic nervous system activity could be the cause of higher cortisol concentrations (Williamson *et al*., 1991; Yoshida *et al*., 1999).

2.2.2 Factors affecting/influencing changes in fat-mass, lean mass and body weight

2.2.2.1 Gender

Body composition differs based on gender. For a given BMI women have been reported to have higher adiposity while men have higher lean mass (Garaulet *et al*., 2000; Geer & Shen, 2009). Men have been reported to have the tendency of storing fat centrally, while women tend to store fat peripherally (Garaulet *et al*., 2000; Machann *et al*., 2005). Also men generally have more VAT and less subcutaneous adipose tissue (SAT) when compared to women (Machann *et al*., 2005; Bray *et al*., 2008; Geer & Shen 2009). Also, changes in body composition over time have also been demonstrated to be different for males and females (Tsunenari *et al*., 1993; Sartorio *et al*., 2005; Strugnell *et al*., 2014). Gender difference in body fat distribution is largely due to differences in sex hormones between men and women (Nedungadi & Clegg, 2009; Tchernof & Depres, 2013). However, as women age and reach menopause, women accumulate more visceral fat which has been attributed to the hormonal changes experienced in women after menopause (Kotani *et al*., 1994; Tchernof & Depres, 2013).

2.2.2.2 Socio–economic status

SES which can be defined as an individual’s position on a socio-economic scale is often measured with indicators like education, income, occupation, place of residence etc. For many decades, powerful association between SES and physical health has been recognized. Whether defined according to educational attainment, income, or occupational status, lower SES is associated with diverse disease endpoints and with premature mortality (Adler *et al*., 1999; Gallo *et al*., 2009). Low SES is associated with long term weight gain and it is a significant predictor of BMI (Jeffery *et al*., 1991; Lahmann *et al*., 2000; O’Dea *et al*., 2012). This is largely explained by overcomsuption of energy dense foods which are cheaper and more easily accessible (Popkin *et al*., 1993; Hill *et al*., 2000; Abrahams *et al.*, 2011; Aounallah-Skhiri *et al*., 2011; Vorster *et al*., 2011).

2.2.2.3 Diet

Diet is a major determinant of body composition changes (Mozafarian *et al*., 2011). Dietary modification is widely used to effect changes in body composition particularly body weight (Howard *et al*., 2006; Carty *et al*., 2011; Di Daniele *et al*., 2013). High intakes of energy and fat are positively associated with increased measures of obesity (Popkin *et al*., 1993; Hill *et al*., 2000; Abrahams *et al.*, 2011). Carty and colleagues (2011) reported modest long term body composition changes of decreased percentage body fat and fat mass by a group of women on a low fat dietary pattern over a period of six years.

2.2.2.4 Physical activity

Physical activity or inactivity is a determinant of changes in body composition over time. Increase in level of physical activity is associated with higher muscle mass and lesser total body fat in adults (Raguso *et al*., 2006). Increase in physical activity over time has been demonstrated to reduce measures of obesity (Toth *et al.*, 1999; Irwin *et al*., 2003). Physical activity is one of the major determinants of energy expenditure as it accounts for 15% to 30% of total energy expenditure under normal circumstances (Ravussin *et al*., 1982; Jequier & Schutz, 1983). Decrease in physical activity could lead to weight gain as lower energy expenditure predicts increase in weight and fat mass (Mozafarian *et al*., 2011; Reddy *et al*., 2012; Piaggi *et al*., 2013). In a longitudinal study on older men and women, baseline physical activity was inversely associated with changes in fat mass in women (Hughes *et al*., 2002).

2.2.2.5 Age

Age is an established determinant of changes in body composition as changes in body composition occur with increasing age (Tsunenari *et al*., 1993; Baumgartner *et al*., 1995; Hughes *et al*., 2002; Zamboni *et al*., 2003). There is usually an increase in fat mass and a decrease in muscle mass due to aging (Evans & Campbell, 1993; Hughes *et al*., 2002; Genton *et al*., 2011). This could be as a result of decreased physical activity, hormonal changes and dietary changes (Mozafarian *et al*., 2011; Tchernof & Depres, 2013). A decrease in height also occurs as humans grow older (Chumlea *et al*., 1998). Decrease in height of an adult is shrinkage which could be due to poor posture, joint deterioration and osteoporosis (Cline *et al*., 1989; Bagga, 2013).

Other lifestyle factors that contribute to changes in body composition include smoking, alcohol use, use of specific medication, specific contraceptive usage by women and marriage for men. Smoking increases insulin resistance and is associated with central fat accumulation (Chiolero *et al*., 2008). Studies have shown that marriage for men is more associated with increased risk of obesity (Sobal *et al*., 1992; Hajian-Tilaki *et al.*, 2007).

2.2.3 BODY COMPOSITION ASSESSMENT TECHNIQUES

2.2.3.1 Surface anthropometry

Anthropometry can be defined as the science of measuring the size, weight and proportion of the human body and it is one of the basic tools to assess nutritional status (Heymsfield & Casper, 1987; Wang *et al*., 2002). Anthropometric instruments are portable and relatively inexpensive and are non-invasive procedures which make them applicable for large sample studies and can be used in rural and urban field situations (Heymsfield, 2005). Anthropometric measures can be sensitive indicators of health, development and growth in infants and children (Moore & Roche, 1983). Anthropometric measurements also predict performance and survival (De Onis & Habicht, 1996). Anthropometric variables have been recently suggested to predict regional fat tissue masses accurately (Holmes *et al*., 2005; Arthurs & Andrews, 2009; Yavari *et al.*, 2011). According to Scafoglieri *et al*. (2013), the anthropometrically derived indices of fat mass distribution demonstrate sufficient accuracy for clinical use.

2.2.3.1.1 Height, weight and body mass index

Height (or stature) and weight are useful in determining nutritional status in adults. Height is usually measured directly with a measuring rod or a stadiometer for individuals who are cooperative and able to stand without assistance, while indirect methods can be used to estimate the height of individuals who cannot stand using their knee height (Chumlea *et al*., 1985; Gordon *et al*., 1988). Body weight is one of the most important measurements in nutritional assessment. Weight is an important variable in equations predicting caloric expenditure and in indices of body composition (Mei *et al*., 2002; Lee & Nieman, 2013). Body weight is interpreted in different ways including actual weight, usual weight and ideal weight (WHO, 1986). Studies of predominantly sedentary populations show that men and women gain weight as they age (Williams & Wood, 2006). BMI, also known as the Quetelet's index (W/H^2) is the most widely used height–weight index (Lee & Nieman, 2003). BMI accounts for differences in body composition by defining the level of adiposity and relating it to height and it is often used to evaluate obesity (Stensland & Margolis, 1990; WHO, 2000). BMI however, does not differentiate between fat free mass and fat mass (Müller *et al*., 2012).

2.2.3.1.2 Skin-fold measurements

Skinfold measurement is the thickness of a double fold of skin and compressed subcutaneous adipose tissue (Muller *et al*., 2013). This is the most widely used method of indirectly estimating percentage body fat in clinical settings (Pollock & Jackson, 1984; Martin *et al*., 1985). The skinfold thickness is measured with the use of a calliper. Subcutaneous adipose tissue thickness varies largely among different skinfold sites within individuals and the same skinfold sites between individuals (Siervogel *et al*., 1982; Martin *et al*., 1985; Clarys *et al*., 1987). According to research, certain skinfold sites are highly correlated to total subcutaneous adipose tissue (Martin *et al*., 1985). There are eight most commonly used skinfold sites: chest, triceps, subscapular, midaxillary, suprailiac, abdomen, thigh and medial calf (Lee & Nieman, 2013). The most commonly used single site for assessing body composition is the triceps, while using the sum of skinfold measurements taken at various sites (multiple site skinfold measurements) has proved to be a reasonably valid and reliable indicator of body composition (Lee & Nieman, 2013). Triceps and subscapular skinfold thicknesses are positively associated with the homeostasis model of assessment of insulin resistance (HOMA-IR) and identify those at higher risk for insulin resistance (Addo *et al*., 2012)

2.2.3.1.3 Circumference measurements

Circumferential or girth measurements are used more frequently in recent times because of the growing body of evidence that body fat distribution is an indicator of risk for some non-communicable diseases (Rush *et al*., 2007; Despres, 2012; Patel & Abate, 2013). These include measures of waist circumference, mid-arm circumference, head circumference and calf circumference, using a non-stretchable tape measure (Lee & Nieman, 2013). Waist circumference is measured midway between the iliac crest and the lower margin of the last palpable rib in the mid-axillary line. Waist circumference is a sensitive measure of central fat distribution and a good predictor of abdominal obesity (Conway *et al*., 1995; Tchernof & Depres, 2013). De Koning *et al*. (2007) found a two percent increased cardiovascular disease risk for every centimetre increase in waist circumference. Mid-arm circumference is measured halfway between the acromion process of the scapula and the olecranon process at the tip of the elbow (Lee & Nieman, 2013). Mid-arm circumference is a measure of nutritional status particularly in children and the elderly (WHO, 2009). Head circumference is measured at its greater circumference which is usually above the eyebrows and pinna of the ears and around the occipital prominence at the back of the skull (Lee & Nieman, 2013). Calf measurement is used in combination with other anthropometric measures to estimate body weight gain in older adults (Lohman *et al*., 1988).

2.2.3.2 Dual energy x-ray absorptiometry

Dual energy X-ray absorptiometry (DXA) is the most widely used bone density measurement technology. It is primarily used to measure bone mineral density but has become widely used to measure fat mass and fat-free mass with a high degree of accuracy (St-Onge *et al*., 2004). The DXA energy source is an X-ray tube that contains photon energies (Lee & Nieman, 2003). The DXA machine is easy to use and emits a low level of radiation (UNSCEAR, 2004). Micklesfield *et al*. (2010), demonstrated that DXA performs as well as computer tomography for the measurement of visceral fat.

2.2.3.3 Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is a body composition analysis technique based on the principle that compared to water; lean mass has a higher electrical conductivity and lower impedance than fatty tissue (Segal *et al*., 1991; Foster & Lusaki, 1996). It is a reliable measurement of fat mass and fat free mass in comparison to anthropometric, BMI or skinfold measurements (Kyle *et al*., 2001b). BIA involves passing a small electrical current through the body by the attachment of electrodes to the right hand, wrist, ankle and foot of an individual (Segal *et al*., 1991). It is a popular means of assessment because it is non-invasive, quick, portable and safe (Segal *et al*., 1991; Foster & Lusaki, 1996). BIA may not be appropriate choice of body composition measurement in epidemiological studies with diverse ethnic population except specific calibration equations are developed for different groups participating in the study (Dehghan & Merchant, 2008). This is because BIA results are influenced by ethnicity, environment, phase of menstrual cycle and underlying medical conditions (Dehghan & Merchant, 2008).

2.2.3.4 Ultrasound

The ultrasound has been indicated as a preferable tool to skinfold callipers in measuring very obese individuals due to difficulty of accurately measuring skinfolds on them (Booth *et al*., 1966 Brodie, 1988). However, the ultrasound is more expensive and requires more training for its operation and interpretation compared to the callipers (Lukaski, 1987; Brodie, 1988).

2.2.3.5 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a technology that shows both imaging of the body and *in vivo* chemical analysis without hazard to the subject (Ellis, 2000; Ross *et al*., 2000). MRI can be used to measure the distribution and amount of intra-abdominal fat, size of visceral organs and the size of the skeleton. MRI is non-invasive and does not use ionizing radiation but it is expensive and its availability is limited (Lee & Nieman, 2003).

2.2.3.6 Underwater weighing

Underwater weighing was considered as the gold standard for measuring body composition (Ackland *et al*., 2012), but it is not always practical (Lee & Nieman, 2003). It is a direct measure of determining whole-body density based on the principle that the volume of water displaced by an object submerged in water is equal to the volume of the object (Lukaski, 1987).

2.2.3.7 Other methods

Computer tomography involves the use of ionizing radiation and is used to assess the deposition of subcutaneous and intra-abdominal fat (Lukaski, 1987; Shuster *et al*., 2012). Air displacement plethysmography relies on measurement of body density to estimate body fat and fat free mass (Lukaski, 1987). The BODPOD® is an example of an air displacement plethysmogram and is also an accurate method to measure body composition (Aleman-Mateo *et al*., 2004). Neutron activation analysis measures body calcium, iodine, hydrogen, sodium, chloride, phosphorus, carbon and nitrogen content (Lee & Nieman, 2003). Total body potassium is also used to study body composition, because more than 90% of the body's potassium is found in fat-free tissues (Lukaski, 1987).

2.2.4 Effect of aging on body composition and bone health

Body composition changes profoundly as an adult advances in age (Evans & Campbell, 1993; Liu *et al*., 2011). Aging is a normal biologic process which involves some decline in physiologic function (López-Otín *et al*., 2013) and a decrease in functional capacity which impacts on quality of life (Goodpaster, 2008). The changes in body composition due to aging are towards a decrease in skeletal muscle mass and an increase in fat mass (Evans & Campbell, 1993). Sarcopenia is also mostly a result of body composition changes with aging.

2.2.4.1 Sarcopenia and sarcopenic obesity

2.2.4.1.1 Definition and diagnosis

Sarcopenia is defined as the loss of muscle mass and strength that occurs with advancing age (Rosenberg, 1997; Cruz-Jentoff *et al*., 2010). It has also been defined as age-associated loss of skeletal muscle mass and function, a complex syndrome associated with muscle mass loss alone or in conjunction with increased fat mass (Fielding *et al*., 2011). There are possible contributory causes for sarcopenia which include age related changes in tissue secretion or responsiveness to trophic hormonal factors, changes in dietary intake and protein metabolism, and "disuse atrophy" (Bortz, 1982; Evans & Campbell, 1993; Dutta & Hardley, 1995). According to Bales and Ritchie (2002), sedentary lifestyle, nutrition and chronic diseases are factors that aggravate sarcopenia. Expected components of ageing are diminishing strength and muscle mass (Robinson *et al*., 2012). However, the rate of decline differs across the population (Syddall *et al*., 2009; Cruz-Jentoft *et al*., 2010), which suggest that modifiable behavioural factors such as diet and lifestyle may be important influences on muscle function in older age (Robinson *et al*., 2012). The total appendicular skeletal muscle mass (ASM) index assessed by DXA is one of the most commonly used indices for the definition of sarcopenia (Waters & Baumgartner, 2011). Individuals with an ASM index < 7.26 kg/m^2 (men) or < 5.45 kg/m^2 (women) are classified as sarcopenic (Baumgartner *et al*., 1998). Janssen and colleagues (2002) further classified sarcopenia using bioelectric impedance into class I and II, where skeletal muscle mass (SMM) index of 5.76 to 6.75kg/m^2 was categorized as class I sarcopenia for women and 8.51 to 10.75 kg/m^2 for men. An SMM index of 5.75 kg/m^2 or less for women and 8.50kg/m^2 or less for men is defined as class II sarcopenia. Sarcopenic obesity can be defined as a state whereby lean muscle mass is lost while fat mass is preserved or increased (Baumgartner 2000; Cruz-Jentoft *et al*., 2010). According to the reports of some studies, sarcopenic-obese persons are at particularly high risk of functional impairment and physical disability (Baumgartner 2000; Morley *et al*., 2001; Baumgartner *et al*., 2004; Rolland *et al.,* 2009). Sarcopenic obesity links adiposity and sarcopenia through inflammation derived from the adiposity particularly when the obesity is of the abdominal type (Cesari *et al*., 2005; Schrager *et al*., 2007).

2.2.4.1.2 Prevalence of sarcopenia and sarcopenic obesity

Globally, some studies have been carried out on prevalence of sarcopenia using different methods which includes the ASM index, the SMM index and the relative skeletal muscle index (RSMI) approach. A study in the older adults from California, United States reported a sarcopenia prevalence of 6.2% for men and 5.9% for women which increased with age to 16% for men and 13% for women by age 85 (Castillo *et al.*, 2003). The large United States Health, Aging and Body Composition (Health ABC) study reported different prevalence for sarcopenia based on different cut scores used (Newman *et al*., 2003). A large cohort study on more than 7000 French community dwelling women reported an increase with age in prevalence of sarcopenia from 8.9% at 76 to 80 years to 10.9% at 86 to 95 years (Gillette-Guyonnet *et al*., 2003). A cohort study on more than 1000 community dwelling older Italians reported that 20% of men were sarcopenic at 65 years and up to 70% at 85 years, and 5% of women were sarcopenic at 65 years of age and 15% at 85 years (Lauretani *et al*., 2003).

Asia and the Pacific region also have prevalence rates of sarcopenia. A Hong Kong study reported sarcopenic prevalence of 7.6% for women and 12.3% for men (Lau *et al*., 2005). A Korean study reported sarcopenia prevalence of 6.3% men and 4.1% women among a sample of 526 adults (Kim *et al*., 2009). A Taiwanese study reported a sarcopenia prevalence of 18.6% for women and 23.6% for men among their older adults (Chien *et al*., 2008). A recent Thai study reported a sarcopenia prevalence of 35.3% and 34.74% in men and women respectively (Pongchaiyakul *et al*., 2013). A small study on older European New Zealanders reported 4% of men and 12% of women as sarcopenic (Waters *et al.*, 2010).

There is a scarcity of data on prevalence of sarcopenic obesity globally and the reported prevalence of sarcopenic obesity varies significantly across study populations even when similar methods are applied. An older New Mexican cohort used the Baumgartner's cut scores to assess sarcopenic obesity and the prevalence was approximately 2% in those 60 to 69 years old increasing to approximately 10% in those above 80 (Baumgartner, 2000). Some studies reported the prevalence of sarcopenic obesity as approximately 10% in men and approximately 7% to 12% in women using a definition of the upper 2 quintiles of body fat with the lower 3 quintiles of muscle mass (Davison *et al*., 2002; Zoico *et al*., 2004; Zamboni *et al*., 2005). The United States Health ABC study reported a sarcopenic obesity prevalence rate of 8.9% in men and 7.1% in women using the RSMI method, but 15.4% men and 21.7% of women using the residual method (Newman *et al*., 2003). The Korean study by Kim *et al*. (2009), reported a prevalence rate of 1.3% and 0.8% sarcopenic obesity in men and women respectively using the RSMI and 5.1% men and 12.5% women using their own index. The lack of internationally accepted definition of sarcopenic obesity may be a reason for the wide range of prevalence reported. Presently, there is no published literature available for prevalence of sarcopenia in Africa.

2.2.4.1.3 Factors influencing sarcopenia and sarcopenic obesity

Inflammation has been linked to obesity, body composition and physical disability (Cohen *et al*., 1997; Ferrucci *et al*., 1999; Kuller, 1999; Yudkin *et al*., 2000; Roubenoff, 2003; Ryan & Nicklas, 2004). According to Cesari *et al.* (2005), obesity-associated inflammation may play an important role in the age-related process that leads to sarcopenia. Quite a large number of elderly people suffer from chronic low-grade systemic inflammation that may add to the age-related muscle weakness and wasting (Degens, 2010). According to Malafarina *et al.* (2012), loss of muscle mass and strength observed in the elderly is directly associated with inflammatory cytokines and inflammation may be a fundamental factor in the genesis of sarcopenia. Also, older people living with Human Immunodeficiency Virus [HIV] in the United States have been shown to have greater limitations in performing their physical tasks (Crystal *et al*., 2000).

There is a progressive reduction in physical activity as a result of aging (Ingram, 2000). Physical activity has been considered the major factor in slowing the age related decline in many physiological functions as physical inactivity contributes significantly to secondary aging of these functions (Booth *et al*., 2011). Physical inactivity leads to faster and greater muscle loss and from existing studies, it is clear that sarcopenia is worsened with disuse

(Roubenoff, 2001). Exercise at old age helps to reduce chronic low-grade systemic inflammation (Degens, 2010) while it has now been established that progressive resistance exercise can increase muscle mass and strength even in very elderly persons (Fiatarone *et al*., 1994; Evans, 2000; Bales & Ritchie, 2002; Tieland *et al*., 2012; Krist *et al*., 2013).

Nutrition has an important controlling influence on health and well-being in the elderly as inadequate nutrition contributes to the progression of many chronic diseases (Volkert, 2011). One of contributing factors to the development of sarcopenia is inadequate nutrition which may also worsen the age-related loss of muscle mass and function (Rolland *et al*., 2008; Boirie, 2009). According to Booth *et al.* (2011), adequate dietary intake and regular physical activity, significantly lowers the rate of muscle ageing. In an Italian study among more than 800 healthy elderly, low intakes of energy, protein, vitamins D, E, C and folate were independently associated with frailty (Bartali *et al*., 2006). Some studies have shown the relationship between sarcopenia and amount of protein intake (Castaneda *et al*., 1995; Houston *et al*., 2008), kind of protein intake (Pannemans *et al*., 1998; Lord *et al*., 2007; Paddon-Jones *et al*., 2008) and distribution of protein intake (Arnal *et al*., 1999). Recently, vitamin D has turned out to have significant importance on muscle function and physical performance (Volkert, 2011). Scott *et al*. (2010), suggest that 25 hydroxy vitamin D [25(OH)D] plays a role in the maintenance of muscle function, and higher skeletal muscle mass in older adults. In a longitudinal study on the aging in Amsterdam, lower 25(OH)D and higher PTH levels increased the risk of sarcopenia in older men and women (Visser *et al*., 2003). While another study on older Koreans, reported a strong inverse association between 25(OH)D level and sarcopenia (Kim *et al*., 2009).

2.3 BONE HEALTH

2.3.1 Bone structure and physiology

2.3.1.1 Bone structure and function

Bones give form to the body; they support tissues, permit movement by providing points of attachments for muscles and also protect many of the body’s vital organs (Huether & McCance, 2004). Bones also serve critical metabolic roles which include being an internal reservoir for calcium to ensure proper functioning of nerves and muscles (Civitelli & Ziambaras, 2011). It has been suggested recently that the skeleton also contributes to glucose homeostasis (DiGirolamo *et al*., 2012). Lee *et al*. (2007), demonstrated that the skeleton exerts an endocrine regulation of sugar homeostasis through osteocalcin, which is a bone-derived hormone. Studies have suggested that osteocalcin target β cells and insulin targeting tissues such as adipocytes, liver and muscles in order to regulate insulin secreation and insulin sensitivity (Ferron *et al*., 2008; Kanazawa & Sugimoto, 2013).

Bones are made up of an organic matrix or osteoid, primarily collagen fibres, in addition to osteocalcin, osteopontin and several other matrix proteins (Sommerfeldt & Rubin 2001). Bone cells consist of three types namely osteoblasts, osteocytes and osteoclasts (Sommerfeldt & Rubin 2001). Osteoblasts are bone forming cells with the primary function of laying down new bones; osteocytes are osteoblasts that have become imprisoned within the mineralised bone matrix with the role of maintaining the inorganic and organic elements of bone matrix, and the osteoclasts functions primarily to remove bone during the process of growth and repair (Huether & McCance, 2004). Approximately 80% of the skeleton consists of compact or cortical bone tissue while the remaining 20% of the skeleton is trabecular or cancellous bone tissue. The loss of trabecular bone tissue late in life is mostly responsible for the occurrence of fractures, particularly those of the spine (Parfitt *et al*., 1983; Parfitt, 1987; Wang *et al*., 2013). Figure 1 shows the anatomy of the bone.

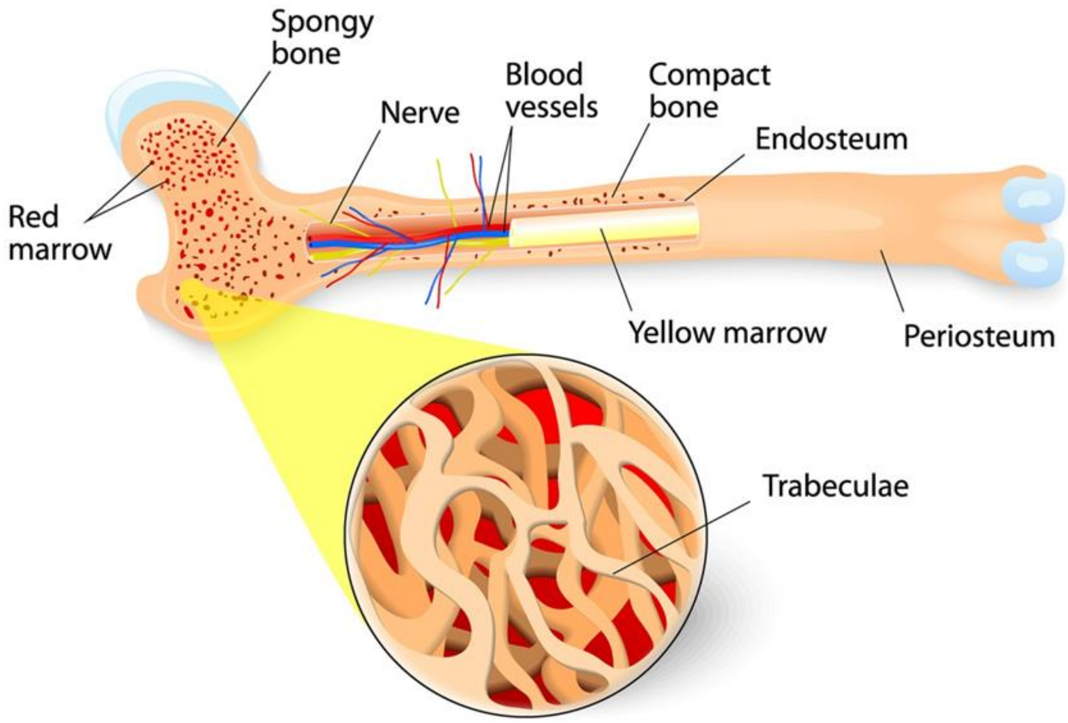

Figure 1. **Anatomy of the bone (Shutterstock.com/images/185803484 Copyright: Deignua)**

2.3.2 Bone metabolism

2.3.2.1 Bone modeling, remodeling and turnover

The skeleton is a metabolically active organ going through a constant process of remodelling throughout life (Sommerfeldt & Rubin 2001; Lee *et al*., 2007; Ferron *et al*., 2008; Kanazawa & Sugimoto, 2013). Bone modelling and remodeling are processes where bone adapts its internal structure and shape to external influences (Gerhard *et al*., 2009). Bone remodelling is essential to maintain the structural integrity of the skeleton and for the metabolic function of calcium and phosphorus storage (Feng & MacDonald, 2011). It is a physiological process in which old or damaged bone is removed by osteoclasts and replaced by new bone formed by osteoblasts (Feng & MacDonald, 2011). The process is considered to occur in three overlapping phases viz i) activation/initiation phase which occurs at a specific site leading to the formation of osteoclasts when a stimulus activates bone cell precussors; ii) the resorption phase when osteoclasts absorbs bone; iii) the formation/rebuilding phase where the osteoblasts secretes collagen and other matrix proteins for the deposition of new bone (Anderson, 2008; Feng & MacDonald, 2011). At the completion of the formation phase and at the beginning of the resorption phase, the same amount of bone tissue exists when the resorption and formation phases are in balance (Anderson, 2008). Autocrine, paracrine and endocrine factors regulate the rate and degree of coupling of bone formation to removal (resorption) during remodelling (Kular *et al*., 2012). Bone turnover is simply the total volume of bone that is both resorbed and formed over a period of time (Parfitt, 2002), and increased bone turnover persists in the elderly (Garnero *et al*., 1996a; Kruger *et al*., 2011; Hinton *et al*., 2012). Also, increased levels of biochemical markers of bone turnover, more specifically of bone resorption, have been shown to be associated with an increased risk of hip fracture independently of bone mineral density (BMD) in elderly women (Garnero *et al*., 1996b; Van Daele *et al*., 1996; Rousseau *et al*., 2014).

2.3.3 Osteopenia and osteoporosis

Osteoporosis is a multifactorial skeletal disease which is characterised by low bone mass and microarchitectural deterioration of bone tissue, with a resulting increase in bone fragility and susceptibility to fracture (CDC, 1993). Osteopenia can be defined as a condition of decreased bone mass (Sambrook & Cooper, 2006). The WHO operational definition of osteopenia is a hip or lumbar spine BMD greater than 1 standard deviation (SD) below the young adult female mean, but less than 2.5 SD below this value (T score <–1 and >-2·5) and osteoporosis as hip or lumbar spine BMD 2.5 SD or more below the young adult female mean (T score ≤–2.5). (WHO 1994; WHO 2003). The latest International Society for Clinical Densitiometry official position maintains the WHO classification for osteopenia and osteoporosis for postmenopausal women and men above the age of 50 years (Schousboe *et al*., 2013). The society also states that osteoporosis cannot be diagnosed for men under the age of 50 years on the basis of BMD alone while maintaining that the WHO diagnostic criterion may be applied for women in the menopausal transition (Schousboe *et al*., 2013). Worldwide osteoporosis is estimated to affect 200 million women (IOF, 2013). Characteristics of osteoporosis include low BMD and higher than normal incidences of fractures (ZhiMin *et al*., 2012). There are three main types of osteoporosis namely postmenopausal osteoporosis, age-related osteoporosis and secondary osteoporosis which includes glucocorticoid induced osteoporosis and immobilization induced osteoporosis (Feng & Macdonald, 2011). Postmenopausal osteoporosis is primarily caused by the decline in estrogen levels associated with menopause (Albright *et al*., 1941; Feng & Macdonald *et al*., 2011). Age related osteoporosis affects both women and men and it is centered on osteoblasts engaging a number of distinct factors associated with the aging process (Raisz, 2005; Feng & Macdonald 2011). Therapeutic use of glucocorticoids can lead to bone loss and increased fracture risk as it exerts damaging effects on the differentiation, function and survival of multiple cells types involved in the bone remodeling process (Compston, 2003; Silverman & Lane, 2009; Feng & Macdonald, 2011). Immobilization induced osteoporosis is as a result of physiological response of bone remodeling to decreased mechanical demands from paralysis or casting of a limb (Takata & Yasui, 2001; Feng & Macdonald, 2011). Locations of osteoporotic fracture occurences include the hips, the vertebral and wrists (Cummings & Melton, 2002). Fractures related to osteoporosis are associated with significantly increased risk of death and disability-adjusted life years (DALYs) lost (Melton, 2003). In 2000, fifty-six million people were estimated to have suffered a prior osteoporotic fracture worldwide and about 9 million new osteoporotic fractures each year (Johnell & Kanis, 2006). Race, heredity, physical activity and diet are major determinants of optimum bone mass and modification of physical activity and diet may help in maintaining optimal skeletal status (Nilas & Christiansen, 1987; Hui *et al*., 1988; Slemenda *et al*., 1990). Osteoporosis and fractures occur more frequently in white than in black South Africans (Kruger *et al*., 2004). According to a review by Micklesfield *et al*. (2011), black SA children and adults have greater proximal femur and femoral neck BMD, greater bone strength and a decreased hip fracture incidence compared to SA whites irrespective of adverse environmental conditions of poor nutrition low physical activity levels, as well as an unfavourable body composition. However, due to nutrition transition and changes in physical activity, older black women may increasingly represent a population at risk for osteoporosis and fractures (Aloia, 1996; Kruger *et al*., 2011).

2.3.4 Factors affecting/influencing bone health

2.3.4.1 Metabolic and hormonal control

The balance between bone resorption by osteoclasts and bone formation by osteoblasts is what regulates bone metabolism (Chen *et al*., 2009). A number of hormonal regulators of metabolism are known to influence the skeleton (Khor *et al.*, 2013). Estrogen is a major determinant of bone mass, affecting the attainment of peak bone mass during adolescence and young adult age, modulating BMD and the risk of osteoporosis later in life (Davies *et al*., 1990; Drinkwater *et al*., 1990; Fabbri *et al*., 1991). Estrogen prevents bone loss through multiple effects on bone cells and their precursors, resulting in decreased osteoclast formation and a reduced capacity of mature osteoclasts to resorb bone (Chen *et al*., 2009).

Elevated concentration of circulating cortisol retards osteoblast function and accelarates osteoclast activity (Bressot *et al*., 1979). A reduced level of circulating insulin like growth factor 1 (IGF-1) has been demonstrated to inhibit the activity of the osteoblasts and the synthesis of bone collagen (Chevalley *et al*., 1998). IGF-1 is a polypeptide synthesised primarily in the liver through the action of growth hormone and circulates to target organs like bone and cartilage (Thissen *et al*., 1994; Tahimic *et al*., 2013). IGF-1 plays a role in mediating the skeletal response after mechanical loading as IGF-1 production and responsiveness are increased in osteocytes and osteoblasts after mechanical loading (Lean *et al*., 1995; Reijnders *et al*., 2007; Klein-Nulend *et al*., 2012). Sclerostin is an osteocyte-derived inhibitor of bone formation and its absence causes scleroteosis which is a skeletal disorder characterised by high bone mass due to increased osteoblast activity (Van Bezooijen *et al*., 2004; Poole *et al*., 2005; Gaudio *et al*., 2012). A recent review reported the strong effects of gastrointestinal hormones on bone metabolism often through direct signalling pathways (Khor *et al*., 2013). For instance the growth hormone releasing peptide, ghrelin is an appetite stimulating hormone which seems to exert positive effects on the skeleton (Khor *et al.*, 2013). The gastrointestinal hormone Peptide YY (PYY) suppresses the appetite and has been shown to have inverse correlations with BMD (Wong *et al*., 2010; Wong *et al*., 2012).Some studies have shown a negative relationship between serum adiponectin levels and BMD (Richards *et al*., 2007; Peng *et al*., 2008; Kanazawa *et al*., 2009) while there have been mixed results in the association between leptin concentration and BMD showing either a positive or no association (Thomas *et al*., 2001; Lee *et al*., 2008; Ahmadi *et al*., 2013).

2.3.4.2 Dietary intake

Nutrition is one of the important modifiable factors in the development and maintenance of bone mass; and the prevention and treatment of osteoporosis (Ilich & Kerstetter, 2000). Most studies conducted so far have confirmed the positive role of a healthy diet to maintain bone health (Ilich & Kerstetter, 2000; Levis & Lagari, 2012)

2.3.4.2.1 Protein

Dietary protein is essential for bone health and osteoporosis prevention (Bonjour, 2005). In the Framingham longitudinal study, men and women with a relatively lower intake of protein had increased bone loss (Hannan *et al*., 2000). High protein intake particularly of animal source has often been alluded to as a risk factor for osteoporosis or bone fractures

(Feskanich *et al*., 1996; Barzel & Massey, 1988; Sellmeyer *et al*., 2001). However, with adequate calcium intake, higher protein diets are associated with fewer fractures and greater bone mass (Heaney & Layman, 2008). Studies point out that different source of protein might display different effects on bone metabolism (Frassetto *et al*., 2000; Heaney & Layman, 2008). A review study on the effect of dietary protein on bone health proposes that animal protein might have a greater negative effect on skeletal health than plant protein (Frassetto *et al*., 2000). However this was refuted in other studies where higher intake of animal protein was not associated with a decrease in BMD (Hannan *et al*., 2000; Dawson-Hughes & Harris, 2002). A 3 year clinical study on older adults aged 65 years and above reported greatest improvement in BMD amongst subjects that were supplemented with calcium and consumed the most protein whereby most of the protein consumed was from animal source (Dawson-Hughes & Harris, 2002). A detailed review on protein and bone balance by Kerstetter and colleagues (2007), showed that high protein consumption was associated with increased excretion of urinary calcium. This study also demonstrated that the increased urinary calcium excretion is indicative of increased intestinal absorption of calcium from the high protein consumed (Kerstetter *et al*., 2007). Kerstetter and colleagues (2007), finally concluded that a high-normal protein diet of 2.1g protein/kg body weight is not detrimental to bone health but is needed for optimal skeletal health.

2.3.4.2.2 Energy

A low energy intake usually results in low intakes of other essential nutrients (Ramakrishnan, 2002; Labadarious, 2005). Increased energy intake causes weight gain and a higher BMD (Ilich & Kerstetter, 2000). Ilich *et al*., (2003) also reported a significant relationship between BMD and energy intake. Bone loss and increased risk of developing osteoporosis has been reported for people with eating disorders particularly anorexia nervosa (Powers, 1999; Andersen *et al*., 2000). A review by Zanker and Cooke (2004) confirmed previous hypotheses that energy deficit is linked to disturbed bone turn over. Diet regulates the metabolism of bone through the provision of substrate for the synthesis of bone tissue and through an influence on the circulating levels of key hormones that regulates bone metabolism (Zanker & Cooke 2004). Prolonged energy deficit state results in reduction of body mass and change in body composition, which is accompanied by a significant reduction of bone mass largely due to multiple hormonal adaptations to undernutrition (Soyka *et al*., 1999; Hotta *et al*., 2000; Fazeli & Klibanski, 2014).

2.3.4.2.3 Minerals

An adequate supply of calcium to bone is essential at all stages of life as it is one of the main bone-forming minerals (Prentice, 2004). Earlier studies on populations with a lower average intake revealed an increasing risk of hip fracture with declining calcium intake (Lau *et al*., 1988; Holbrook *et al*., 1988; Johnell *et al*., 1995; Kanis *et al*., 1999). Studies on the relationship between calcium intake and bone mass seem to be controversial; however, a number of large studies concluded that calcium intake is a significant determinant of BMD (Cumming, 1990; Welten *et al*., 1995; Looker *et al*., 2012; Joo *et al*., 2013). High calcium intake has been shown to augment bone gain during growth, reduce osteoporotic fracture risk and retard age-related bone loss (Heaney, 2000). When calcium intake is inadequate, calcium homeostasis is almost totally dependent on the bone tissue as a source of calcium to maintain the serum calcium ion concentration (Koo & Tsang, 1994; Heaney, 2006a).

In a study conducted on the Framingham Heart Study participants, Tucker *et al*. (1999) concluded that potassium and magnesium also contribute to the maintenance of BMD. There is a concern that excessive consumption of phosphorus may be detrimental to bone (Ilich & Kerstetter, 2000). Studies have demonstrated that a high phosphorus diet leads to hyperthyroidism and reduced 25(OH)D concentrations and thus disrupts calcium homeostasis (Calvo, 1993; Calvo & Park, 1996). Zinc deficiency results in impaired DNA synthesis and protein metabolism, which leads to negative effects in bone formation (Beattie & Avenell, 1992).

2.3.4.2.4 Vitamins

Vitamin D is mainly obtained from sunlight exposure and some from dietary sources and supplements (DeLuca, 2004; Holick, 2006). Vitamin D is normally produced in the skin on exposure to ultra violet-B (UBV) sunlight through a photolytic process (DeLuca, 2004). Vitamin D obtained from sunlight, dietary sources and supplements undergoes hydroxylation in the liver to become 25(OH)D which also undergoes hydroxylation in the kidneys to become 1, 25-dihydroxyvitamin D_3 [1,25(OH)D] (DeLuca, 2004). 1, 25 (OH)D is the biological active form of vitamin D which maintains calcium and phosphorus homeostasis in the body (Chritakos *et al*., 2003). Studies are elucidating other health effects of vitamin D which includes its role in reducing risk of multiple sclerosis (Duan *et al*., 2014), hypertension (Vimaleswaran *et al*., 2014; Canale *et al*., 2014), diabetes mellitus (Nwosu & Maranda, 2014) and the metabolic syndrome (Hyppönen *et al*., 2008; Yin *et al*., 2012). It has also been indicated to reduce cancer risk (Garland & Garland 2006; Ishihara *et al*., 2008). The exact mechanisms of the protective effect of vitamin D on all these diseases are not yet clear, however, it is known that there are vitamin D receptors for 1,25 $(OH)_2D_3$ (the active form of vitamin D) in most cells and tissues in the body, including the heart, skin, stomach, pancrease, brain, gonads, and activated T and B lymphocytes (Stumpf *et al*., 1979; Manolagas *et al*., 1985; Mathieu & Adorini 2002). Thus it is not surprising that 1,25 $(OH)_2D_3$ has other noncalcemic biologic effects (Deluca & Cantorna, 2001; Holick, 2002). Also, one of the important biological function of 1,25 $(OH)_2D_3$ is its ability to control cell proliferation and differentiation (Feldman *et al*., 2000; Deluca & Cantorna, 2001; Holick, 2002; Mathieu & Adorini 2002). Figure 2 shows the metabolism of vitamin D.

Serum 25(OH)D is used to assess the vitamin D status as it reflects the combination of exposure to sunlight and diet and it is currently regarded as the best measure of vitamin D status in humans (Seamans & Cashman, 2009). There is an on-going debate in the literature about the optimum vitamin D level. However vitamin D deficiency is commonly defined as 25(OH)D less than or equal to 20 nanogram per millilitre (ng/mL), insufficiency is commonly defined as 25(OH)D of 20-29 ng/mL and a level above 30 ng/mL is considered to be sufficient (Dawson-Hughes *et al*., 2005; Holick & Chen, 2008). According to Bischoff-Ferarri *et al.* (2006), the most advantageous serum concentrations of 25(OH)D begin at 30 ng/mL, 25(OH)D levels of 30-50 ng/mL is necessary for optimal health.

Vitamin D deficiency occurs globally and it is also present among people living in countries with ample sunshine (Mithal *et al*., 2009; Lips, 2010). Countries in Africa that lie at latitude >30°N and > 30°S e.g. Tunisia, Morocco, South Africa, Libya, Algeria and Egypt would be expected to have seasonal effects on cutaneous synthesis of vitamin D (Jablonski, 2004). Seasonal variation in vitamin D status of South Africans has been documented in literature (Pettifor *et al*., 1978; Martineau *et al*., 2011). A study recently showed that black South Africans had a higher prevalence of vitamin D deficiency and inadequacy when compared to blacks from Ghana, Jamaica and Seychelles (Durazo-Arvizu *et al.,* 2014). This further confirms the seasonal effects of a country's position on the latitude on cutaneous synthesis of vitamin D (Pettifor *et al*., 1978; Jablonski, 2004; Martineau *et al*., 2011; Durazo-Arvizu *et al.,* 2014). Vitamin D deficiency in the elderly leads to secondary hyperparathyroidism, high bone turn over, bone loss, mineralization defects, hip and other fractures (Lips, 2001). Prolonged deficiency of vitamin D manifests as osteomalacia in adults and rickets in children (Prentice, 2008; Pearce & Cheetham, 2010). A study conducted on 10 years old urban South African children living in Johannesburg found vitamin D insufficiency and deficiency to be uncommon among the children despite seasonal variations in 25(OH)D levels (Poopedi *et al*., 2010).

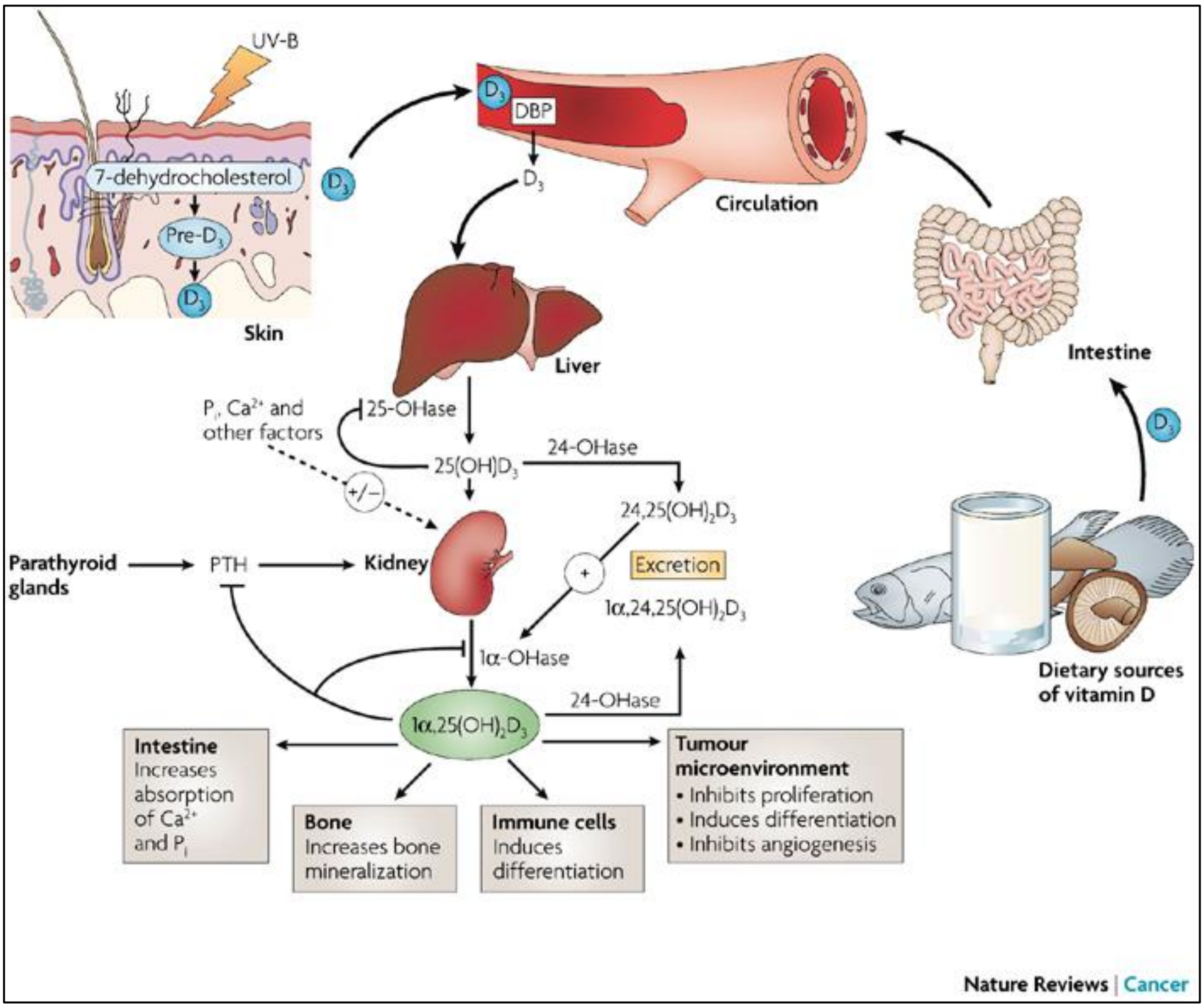

Figure 3-2. **Vitamin D metabolism reprinted from Nature Review Cancer Vol 7 no 9, Deeb et al., 2007, 684-700., Copyright (2007), with permission from Nature Publishing Groups**

Vitamin K is an important micronutrient for bone health (Weber, 2001; Nieves, 2005) and it is associated with post-menopausal bone mineral loss (Kanai *et al*., 1997). Low vitamin K intakes were associated with increased incidence of hip fractures (Booth *et al*., 2000). Osteocalcin is a bone specific protein that is dependent on vitamin K for its maturation (Weber, 2001; Neve *et al*., 2014). The exact mechanism of how osteocalcin affects bone metabolism is not well understood but it appears to be involved in prevention of overmineralisation (Yao *et al*., 2008; Woeckel *et al*., 2013). A randomised controlled trial demonstrated a synergetic effects between vitamin K1, vitamin D3 and calcium supplements in increasing the bone mineral content of the trabecular bone (Bolton-Smith *et al*., 2007).

2.3.4.2.5 Diet quality

The effect of individual nutrients on bone health can be impaired or enhanced by the overall composition of the diet (Zagarins *et al*., 2012). Complex interactions between nutrients may not be seen when looking at the effects of individual nutrients on bone health. For instance, foods containing high fibre content are likely to be poorer sources of calcium than foods that contain the same amount of calcium but less or no fibre (Bronner & Pansu, 1999). In a study by Tucker *et al*. (2002), high candy consumption was associated with low BMD in men and women and high fruit and vegetable intake appears to protect BMD in men. Studies have also reported a positive link between bone health and consumption of fruits and vegetables (New *et al*. 2000; Chen *et al*., 2006; Hamidi *et al*., 2011). A long term intake of nutrients found in large quantity in fruits and vegetables may be important for bone health (New *et al*., 1997; Hamidi *et al*., 2011). Massey and Whiting (1993), demonstrated that compared to older women, younger women seem to be able to compensate for the stresses caused by moderate caffeine consumption on calcium metabolism and thus less likely to have deleterious consequences to bone. Likewise when dietary calcium is inadequate caffeine has a harmful effect of bone health (Massey & Whiting, 1993; Barrett-Connour *et al*., 1999; Silva *et al*., 2013). According to Levis and Lagari (2012), heavy alcohol intake and a high energy diet is associated with lower bone mass, while a good general nutritional status with adequate protein, vitamin D, dairy, fruits and vegetables have positive influence on bone health. Increased dietary intake of sodium was considered as a risk factor for osteoporosis as it is associated with an alteration in calcium metabolism through increased urinary calcium excretion (Teucher *et al*., 2008). However results on effects of dietary sodium on osteoporosis are inconsistent (Greendale *et al*., 1994; Heaney, 2006b; Teucher *et al*., 2008).

2.3.4.2.6 Alcohol

Moderate alcohol consumption seems to be beneficial for bone as studies reported a positive association between moderate alcohol consumption and bone mass of different sites on the skeleton (Holbrook & Barrett-Connor, 1993; Felson *et al*., 1995; Høidrup *et al*., 1999; New *et al*., 2000; Marrone *et al*., 2012; Eleftheriou *et al*., 2013). A study from the Framingham offspring cohort examined the effect of alcohol intake on BMD at three hip sites and the lumbar spine (Tucker *et al*., 2009). The study found that compared to non-drinkers, hip BMD was greater in men who drink 1-2 drinks/day of total alcohol, while both hip and spine BMD was greater in women who drink 1-2 drinks/day of total alcohol compared to women who do not drink (Tucker *et al*., 2009). The mechanism of action of moderate alcohol consumption on BMD remains poorly established but it has been hypothysed that its protective effect is due to the effects of alcohol on androgens or estrogen concentrations (Turner & Sibonga, 2001). Also a review study suggested that the primary effect of moderate alcohol ingestion is its acute suppression of bone resorption rather than an alcohol-hormone pathway (Jugdaohsingh *et al*., 2006). Heavy consumption of alcohol (liquor intakes > 2 drinks/day) leads to increased risks of bone loss while chronic alcoholism leads to lower BMD and higher fracture risks (Ilich & Kerstetter, 2000; Tucker *et al.,* 2009). Different mechanisms for the deleterious effects of heavy consumption of alcohol on bone have been proposed (Maurel *et al*., 2012). These includes direct effects whereby ethanol has been shown to decrease indices of osteoblast activity and differenciation in human osteoblast cells (Chavassieux *et al.*, 1993) and increase bone resorption by osteoclasts (Cheung *et al*., 1995). An indirect effect of alcohol consumption on bone includes a possible decrease in calorie intake with a resultant change in body composition (Maddalozzo *et al*., 2009; Maurel *et al*., 2012).

2.3.4.3 Physical activity

Physical activity is one of the major key modifiable factors in the prevention and treatment of osteoporosis (Liu-Ambrose *et al*., 2001). Physical exercise exerts a positive effect on BMD and lowers fracture risks (Bergström *et al*., 2008). Regular high-impact and/or weight bearing activity appears to offer an osteogenic stimulus through the direct application of force to bone and a resultant development of mechanical strain (Zanker & Cooke, 2004). The relationship between lifetime physical activity and BMD was investigated in South African women of various age groups by Micklesfield *et al*. (2003). They found that physical activity between the ages of 14 and 21 years positively correlated with lumbar spine BMD. The study also found positive association between walking, impact loading exercise at a young age and increased BMD later in life (Micklesfield *et al*., 2003). Another study also found a positive association between physical activity and BMD at the lumbar spine, total hip and whole body of white South African women (Chantler *et al*., 2011). In contrast, the same study found physical activity to be negatively associated with lumbar spine BMD of black South African women and no relationship with their hip BMD (Chantler *et al*., 2011). A possible explanation for this disparity may be explained by the low intensity of the physical activity reported by the black women (Chantler *et al*., 2011). Also, it might be because physical activity was measured subjectively which may lead to underreporting of incidental physical activity which has been shown to be high among black South African women (Cook *et al*., 2010). A randomised controlled trial in Turkey showed that supervised high-impact exercise training can be effective in prevention of lumbar spine and femoral neck bone loss in postmenopausal women (Basat *et al*., 2013). In a recent study, it was reported that stepwise increase in the amount of daily activity, using simple, daily performed tasks, can help prevent decreases in post-menopausal BMD (Muir *et al.*, 2013). Apart from the direct effects of physical activity on BMD, physical activity is very effective in reducing sclerostin which inhibits bone formation and increases the levels of IGF-1, which has a very positive effect on bone formation (Boskovic *et al*., 2013). On the other hand, it is also important to note that excessive levels of physical activity has been linked to decreased BMD levels and premature bone loss particularly in active underweight women with amenorrhea (Warren *et al.*, 2002; Zanker *et al*., 2004). This is probably due to negative balance of energy as a result of increased physical activity without corresponding sufficient dietary intake.

2.3.4.4 Smoking

The negative impact of smoking on overall bone health has been previously established as it is associated with an increase in bone resorption and a decrease in BMD (Krall & Dawson-Hughes, 1999; Rapuri *et al*., 2000). The effect of smoking on bone health has been demonstrated in a number of studies and meta-analysis (Law & Hackshaw, 1997; Ward & Klesges, 2001; Yoon *et al*., 2012). Law and Hackshaw (1997) concluded that hip fracture in old age is a major adverse effect of smoking after the menopause. Ward and Klesges (2001) also showed that smoking increases the lifetime risk of developing a vertebral fracture by 13% in women and 32% in men. Also it is estimated to increase lifetime fracture risk by 31% in women and 40% in men at the hip (Ward & Klesges, 2001). The exact mechanism of the effects of smoking on bone health are not well understood but some proposed explanations include the reduction effect of smoking on calcium absorption and changes in the metabolism of adrenal cortical hormones which are precursors of estrogen (Krall & Dawson-Hughes, 1999; Rapuri *et al*., 2000; Yoon *et al*., 2012). Nicotine in tobacco products also has a toxic effect on bone collagen synthesis (Ramp *et al*., 1991; Shen *et al*., 2013; Kallala *et al*., 2013; Bender *et al*., 2014).

2.3.4.5 Body composition

Various studies have been conducted on the effect of body composition on BMD in several study populations (Felson *et al*., 1993; Ravn *et al*., 1999; Hsu *et al*., 2006; Chantler *et al*., 2011; Park *et al*., 2012; Namwongprom *et al*., 2013; Nur *et al*., 2013; Tanaka *et al*., 2013; George *et al*., 2014; Ong *et al*., 2014). One of the established risk factors for osteoporosis fracture is low BMI (Ravn *et al*., 1999). Traditionally, obesity was believed to prevent bone loss and osteoporosis due to the mechanical loading effect of body weight on bone (Felson *et al*., 1993; Ravn *et al*., 1999). Conversely, osteoporosis was recently shown to be a risk factor for fractures of some specific bone sites (Tanaka *et al*., 2013). There are differences observed in the individual effect of fat mass and lean mass respectively on bone (Felson *et al*., 1993; Ravn *et al*., 1999; Hsu *et al*., 2006; Park *et al*., 2012; Namwongprom *et al*., 2013; Nur *et al*., 2013; Ong *et al*., 2014). However, recent studies are showing a greater protective effect of lean mass on BMD in comparison to fat mass (Park *et al*., 2012; Namwongprom *et al*., 2013). A study among the Chinese showed that increased fat mass was associated with low BMD and did not protect against osteoporosis (Hsu *et al*., 2006). There are few studies carried out on the association between body composition and BMD in South Africa (Chantler *et al*., 2011; George *et al*., 2014). The two studies found lean mass to be significantly positively associated to BMD at most skeletal sites measured (Chantler *et al*., 2011; George *et al*., 2014).

2.3.4.6 Other factors

Genetics, ethnicity, hormonal influences, medication, and disease are other risk factors for osteoporosis (Daniels *et al*., 1995; Gourlay & Brown, 2004; Chantler *et al*., 2011). A study by Pocock *et al.* (1987) demonstrated the genetic contribution to bone mass at specific sites in adults. The study examined the genetic contributions to bone mass in monozygotic and dizygotic adult twins. Their results demonstrated a significant contribution to bone mass in the spine and proximal femur in adults. Compared to the lumbar spine, they found a smaller genetic determinant of bone density in the hip and forearm (Pocock *et al*., 1987). Low peak bone mass has been associated with increased risk of osteoporosis and fracture (Bachrach, 2001; Mora & Gilsanz, 2003), while the attainment of optimal peak bone mass is an important factor for the prevention of osteoporosis later in life (Zagaris *et al*., 2012). Black women have been shown to attain higher peak bone mass, have a slower subsequent rate of bone loss and a lower incidence of hip fracture than whites (Harris *et al*., 1995; Daniels *et al*., 1997). This could partly be explained by ethnic differences in lifestyle (Goedecke *et al*., 2010; Chantler *et al*., 2011; Shisana *et al*., 2013) and genetic make up (Pollitzer & Anderson, 1989; Lei *et al*., 2006). According to Micklesfield *et al*. (2011), black South African children and adults have greater proximal femur and femoral neck BMD, greater bone strength and a decreased hip fracture incidence compared to South African whites irrespective of adverse environmental conditions. Prolonged amenorrhea and estrogen deficiency can also cause bone loss while the use of oral contraceptives could have different effects on bone mass in women with low compared to women with normal bone mass (Gourlay & Brown, 2004; Cheng & Gupta 2013). The use of injectable progestin contraceptives has been associated with bone loss (Rosenberg *et al*., 2007; Walsh *et al*., 2008; Lloyd *et al*., 2010). However the result of the study on over 3,000 South African black women and women of mixed race suggests that the detrimental effect of injectable progestin contraceptive on bone is completely reversible several years after cessation of use (Rosenberg *et al*., 2007). Primary hyperparathyroidism, diabetes mellitus type I, anorexia nervosa, gastrectomy and pernicious anemia have been classified as diseases of high risk for fracture related to bone mass loss, while hyperthyroidism, diabetes mellitus type 2 and rheumatoid arthritis are moderate risk diseases (Espallargues *et al.*, 2001; Palacios *et al*., 2013). The prolonged use of corticosteroids leads to a reduction in BMD and an increase in the risk of fractures (Van Staa *et al*., 2002; Weinstein, 2012). Higher SES has also been reported to be positively associated with BMD (Wang & Dixon, 2006). A systematic review on the role of SES on BMD reported that greater educational attainment was protective against lower BMD (Brennan *et al*., 2011).

2.3.5 Parathyroid hormone

PTH plays a major role in maintaining serum calcium by stimulating the transfer of exchangeable calcium from bone into the blood when blood calcium concentration falls below normal levels (Talmage *et al*., 2000). The metabolism of PTH and 25(OH)D is related in such a way that a decrease in 25(OH)D results in an increase in PTH (Pepe *et al.*, 2005). There is a lack of consensus in the literature about if elevated PTH is a consequence of obesity or if obesity is an outcome of elevated PTH (Taniguchi *et al*., 1987; Wortsman *et al*., 2000; McCarty & Thomas 2003; Bischof *et al*., 2006; Reinehr *et al*., 2007; Valiña-Tóth *et al*., 2010). It was previously hypothesized that low 25(OH)D and reactive increases in PTH were both consequences of obesity (Wortsman *et al*., 2000; Bischof *et al*., 2006; Reinehr *et al*., 2007). Some studies on the other hand suggested that elevated PTH promote the accumulation of adipose tissue thereby postulating the possibility that elevated PTH may play a role in the development of obesity (Taniguchi *et al*., 1987; McCarty & Thomas 2003;

Valiña-Tóth *et al*., 2010). A large cohort study reported that PTH is an independent predictor of obesity (Kamycheva *et al*., 2004). The mechanisms by which overweight may be a consequence of elevated serum PTH includes how PTH stimulates the renal hydroxylation of 25(OH)D to its active form (Portale & Miller, 2000) which in turn elevates calcium influx into adipocytes (Zemel *et al*., 2000). This increased intracellular calcium enhances lipid storage in fat tissue (Shi *et al*., 2001). The cohort study, however, emphasised that serum PTH may simply be a pathophysiologically unrelated marker of obesity as they were only able to demonstrate a statistical association between serum PTH and BMI (Kamycheva *et al*., 2004).

2.4 Metabolic syndrome

The metabolic syndrome is a collection of metabolic disorders that includes at least three out of the following: hypertension, abdominal obesity, elevated fasting blood glucose, elevated serum triglycerides and low serum high density lipoprotein cholesterol (HDL-C) (Alberti *et al*., 2009). It is a complex of interelated risk factor for type 2 diabetes mellitus, cardiovascular mobidity and mortality that has become a global epidemic (Lakka *et al*., 2002; Wang *et al*., 2007). The escalating prevalence of obesity due to nutrition and epidemiological transition in Africa has greatly contributed to the increased prevalence of non-communicable diseases including the metabolic syndrome (Kruger *et al*., 2001; Abrahams *et al*., 2011; Vorster *et al*., 2011). A number of studies have been carried out in South Africa on the prevalence of the metabolic syndrome (Motala *et al*., 2011; Crowther & Norris, 2012; George *et al*., 2013). A cohort study of 1,251 black South African females reported a 42.1% prevalence of the metabolic syndrome (Crowther & Norris, 2012).

2.4.1 Relationship between vitamin D status, PTH and the metabolic syndrome

Various studies have shown associations between 25(OH)D, PTH and the metabolic syndrome (Martins *et al*., 2007; Reis *et al*., 2007; Ahlström *et al*., 2009; Hjelmesæth *et al*., 2009; Chan *et al*., 2012; George *et al*., 2013). Some studies found an inverse relationship between 25(OH)D and the metabolic syndrome independent of PTH (Lee *et al*., 2009; Brenner *et al*., 2011). Other studies found elevated PTH to be associated with an increased risk of the metabolic syndrome, but no association between 25(OH)D and the metabolic syndrome (Reis *et al*., 2007; Hjelmesæth *et al*., 2009; George *et al*., 2013). On the contrary, some studies found no association between PTH, 25(OH)D and the metabolic syndrome (Rueda *et al*., 2008; Navarro *et al*., 2013). The study that investigated the association of

25(OH)D, PTH and the metabolic syndrome in black and Asian-Indian South Africans found a significant positive association between PTH and the metabolic syndrome but no association with 25(OH)D for both ethnic groups (George *et al*., 2013). The study proposed that the impact of PTH on the metabolic syndrome was largely via its positive association with waist circumference and blood pressure (George *et al*., 2013).

2.4.2 Relationship between vitamin D status, PTH and components of metabolic syndrome

2.4.2.1 Hypertension.

25(OH)D and PTH were shown to be independently associated with blood pressure among American adults (Zhao *et al*., 2010) and elderly Germans (Jungert *et al.*, 2012). Low 25(OH)D levels were inversely and independently associated with blood pressure among young female nurses in the United States of America (Forman *et al*., 2008). Kruger *et al.* (2013), demonstrated that black South African women with insufficient or deficient 25(OH)D had significantly higher systolic blood pressure compared to women with sufficient 25(OH)D status. Chan *et al.* (2012), conducted a study on older Chinese men and found a positive association between increasing PTH level and blood pressure, but no association between 25(OH)D and blood pressure. Elevated PTH was also positively associated with both systolic and diastolic blood pressure among elderly Swedish men and women (Ahlström *et al*., 2009). One of the mechanisms that may explain the relationship of 25(OH)D, PTH and hypertention is through the direct effects of vitamin D deficiency on vascular cells or through modulating calcium metabolism and secondary hyperparathyroidism, which predisposes to hyperthropy of the left ventricle and vessel wall causing arterial hypertension (Simpson *et al*., 2007; Pilz *et al*., 2009)

2.4.2.2 Elevated fasting blood glucose/ insulin resistance

Vitamin D deficiency/insufficiency was indicated to play a possible role in development or worsening of insulin resistance among individuals with pre-diabetes in India (Dutta *et al*., 2013) and significantly associated with insulin resistance in elderly Chinese population (Lu *et al.*, 2009). Scragg *et al*. (2004) showed an inverse association between vitamin D status and diabetes which possibly included insulin resistance among non-Hispanic whites and Mexican Americans. According to Chiu *et al*. (2004), people with low vitamin D levels are at higher risk of insulin resistance. PTH correlated positively with insulin resistance among the elderly in a Swedish study (Ahlström *et al*., 2009). The mechanism of action of vitamin D on diabetes may be direct or interlinked with the actions of PTH and calcium through their actions on systemic inflammation, insulin secretion and insulin resistance (Danescu *et al*., 2009).

2.4.2.3 HDL-C

A number of studies carried out on various population groups demonstrated positive associations between 25(OH)D and HDL-C (Lu *et al*., 2009; Karhapää *et al*., 2010; Jorde *et al*., 2010). PTH correlated negatively with HDL-C among elderly Swedish men and women (Ahlström *et al*., 2009). A South African study found no significant association between 25(OH)D, PTH and HDL-C respectively (George *et al*., 2013). The mechanism of action of 25(OH)D, PTH and HDL-C metabolism is not yet clear.

2.4.2.4 Triglycerides

A number of studies carried out on various population groups showed that serum concentrations of 25(OH)D were negatively associated triglyceride levels (Lu *et al*., 2009; Karhapää *et al*., 2010; Jorde *et al*., 2010). PTH was demonstrated to be positively correlated with triglycerides among elderly Swedish men and women (Ahlström *et al*., 2009). A possible way by which vitamin D and PTH influences triglycerides is through their independent effects on lipoprotein metabolism by a direct regulation of adipocyte lipoprotein lipase production (Querfeld *et al*., 1999)

2.4.2.5 Waist circumference/abdominal obesity

Some studies have associated excess body weight with low vitamin D status (Bischof *et al*., 2006; Reinehr *et al*., 2007; Vimaleswaran *et al*., 2013). Among the Chinese elderly population vitamin D status was inversely associated with waist circumference (Lu *et al*., 2009), while PTH correlated positively with waist circumference among people of European descents (Ahlström *et al*., 2009; Kayaniyil *et al*., 2011). The mechanisms for the relationship between 25(OH)D, PTH and elevated waist circumference includes how PTH stimulates the renal hydroxylation of 25(OH)D to its active form (Portale & Miller, 2000) which in turn elevates calcium influx into adipocytes (Zemel *et al*., 2000). This increased intracellular calcium enhances lipid storage in fat tissue (Shi *et al*., 2001).

2.5 THE LINK BETWEEN BODY COMPOSITION, BONE HEALTH AND VITAMIN D STATUS

Body composition simply divided into fat mass and fat free mass is a primary determinant of health (Segal *et al*., 1987; VanItallie *et al*., 1990). The bone is part of what makes up the fat-free mass, while fat mass is critical for bone health maintenance (Schuster, 2009). Obesity and osteoporosis are complex diseases with similarities indicating some type of pathophysiological link identified between them (Rosen & Bouxsein, 2006). The global epidemic proportion of obese and osteoporotic individuals deserves detailed attention (WHO, 2000; Kanis, 2008). Traditional belief was that higher BMI is protective of bone health, however, BMI represents both lean and fat mass. Also, overweight/obesity has been recently shown to be a risk factor for osteoporotic fracture (Tanaka *et al*., 2013).

Vitamin D and PTH are important in the regulation of calcium and bone metabolism (Hunter *et al*., 2001; Joo *et al*., 2013). Associations between serum levels of vitamin D and bone mass have been shown with an inverse relation between vitamin D and risk of osteoporotic fractures (Lips, 2001; Välimäki *et al*., 2004). Excessive fat mass has been indicated to act as a sink for vitamin D, thereby reducing its bioavailability for optimal use in the body (Wortsman *et al*., 2000). It is still unclear whether low vitamin D status is a causative factor of excess adiposity in the overweight and obese individuals (Foss, 2009). However, a recent meta-analysis by Vimaleswaran *et al*. (2013), concluded that a higher BMI leads to lower 25(OH)D, while any effect of lower 25(OH)D leading to higher BMD are likely to be small.

In summary, unfavourable body composition has a negative effect on bone health and vitamin D status. Also low vitamin D status has a negative effect on bone health and might likely contribute to excessive adiposity. It is pertinent to examine the effect of body composition on bone health; the effect of vitamin D status on body composition and their effects on the metabolic syndrome which is a metabolic disorder that is fast becoming another global epidemic.

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CHAPTER 3

Influence of urbanization, socio-economic status and lifestyle risk factors on changes in body composition among South African adults

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ABSTRACT

Background: Body composition is regarded as a primary determinant of health. South Africa is in the nutrition-related non-communicable disease phase of the nutrition transition with resultant changes in body composition. The aim of this study is to investigate the effects of urbanization, socio-economic status (SES) and lifestyle factors on changes in body composition (body mass index, BMI, waist circumference and triceps skinfold) over 5 years in rural and urban black South African adults.

Methods: A total of 1058 men and women aged >30 years from the South African arm of the Prospective Urban Rural Epidemiology (PURE) study were included in this 5-year longitudinal study. Relationships were assessed using Pearson partial correlations and multiple linear regressions.

Results: The majority (80.8%) of the subjects had only primary school level education or no formal education and 88.4% were domestic or informal workers. Baseline dietary intake of energy and fat differed significantly between urban and rural residents for both genders ($P < 0.001$). Over a 5-year period, BMI and waist circumference increased in both genders, but the change was significant for BMI ($P<0.01$) and waist circumference ($P<0.001$) in women only, indicating an increase in adiposity over time. Urban residency positively predicted changes in waist circumference in men ($p < 0.05$) and women ($p < 0.001$) as well as change in triceps skinfold thickness of men ($p < 0.05$). Being married positively predicted changes in BMI ($p < 0.001$) and waist circumference ($p < 0.001$) in men, while age negatively predicted changes in triceps skinfold thickness in women ($p < 0.001$).

Conclusions: Black African adults in the North-West Province (NWP), particularly the women gained body fat over the 5-year period of this study. Urbanization played a significant role in the increasing adiposity of subjects in the present study. It is recommended that both urban and rural residents should be targeted for public health intervention programs centered on healthier lifestyle choices.

Keywords: Body composition, Urbanization, Socio-economic status, Obesity, Adiposity

BACKGROUND

Studies on human body composition span over 150 years with focus on the several body components, their distribution, and measurable changes in relation to various intrinsic and extrinsic factors [1]. Body composition which has been indicated as a primary determinant of health can be assessed in several ways, including anthropometry e.g. height, weight, body mass index (BMI), circumference measurements and skinfold thicknesses [2]. BMI as a measure of the level of adiposity in relation to height is often used to evaluate obesity [3]. Waist circumference is a simple yet sensitive measure of central fat distribution and a good predictor of abdominal obesity [4]. Skinfold measurement is an inexpensive and easily accessible method of body fat assessment which has been used to identify people at a higher risk of insulin resistance [5]. De Koning and colleagues [6] demonstrated that for every centimeter increase in waist circumference, there is a two per cent increased cardiovascular disease risk, while Nordestgaard and colleagues showed a 26-56% increased risk of heart disease for every 4 kg/m^2 increase in BMI [7].

Socio-economic status (SES) can be defined as an individual's position on a socio-economic scale and is often measured through indicators such as education, income, occupation, and place of residence. A low SES is associated with long term weight gain [8], positively predicts BMI [9-10], and may contribute to an unfavorable body fat distribution [11]. Lower educational attainment and income could lead to unhealthier lifestyle choices in comparison with higher SES [8-13]. Lifestyle risk factors also play a role in changes in body composition. For instance, physical activity is beneficial in reducing body fat [14-15] while modern inactive lifestyles play a very important role in the etiology of obesity [16]. Smoking has also been associated with markers of non-communicable diseases including central fat accumulation [17]. Previous studies had conflicting results about the effect of urban versus rural residency on body composition. In the developed countries, rural residents have higher adiposity while the opposite is reported in the developing countries [18-19].

South Africa is a country in economic and health transition and an example of a typical country in the nutrition-related non-communicable disease phase of the nutrition transition [18-19]. The South African National Health and Nutrition Examination Survey (SANHANES) recently reported a national obesity prevalence of 10.6% and 39.2% for South African adult men and women respectively with the highest prevalence of obesity in the urban areas [20]. Obesity, especially abdominal obesity was associated with an increased risk for non-communicable diseases among black South African women [21]. A number of studies have been conducted on the effects of socio-economic status on body composition in Sub-Saharan Africa [11, 22]. However, to the best of our knowledge, none has focused on the effect of urban versus rural residency, socio-economic status and lifestyle risk factors on changes in body composition over time among black South African adults. This study is part of the Prospective Urban and Rural Epidemiology (PURE) study which is aimed at tracking the effects of lifestyle and changing environment exposures on the development of non-communicable diseases in populations at different stages of epidemiologic transition [23]. Thus, the aim of the present study is to investigate the effects of urbanization, SES and lifestyle factors on changes in body composition (BMI, waist circumference and triceps skinfold thickness) over 5 years in rural and urban black South African adults.

SUBJECTS AND METHODS

Study design

The South African North-West Province (NWP) arm of the PURE (PURE-SA-NWP) study commenced with baseline data collection in 2005. Recruitment procedures, study design and methodology for PURE South Africa have been previously described in detail [24-25]. In summary, 2010 subjects, age > 30 years with no previous HIV diagnosis were recruited from 6000 randomly selected households in two urban and two rural areas of the NWP in the year 2005. Seven hundred and twenty two participants were lost to follow up of which 216 are deceased, 188 have relocated, 224 refused further contact and 94 were unable to be contacted [25]. This sub-study is a longitudinal design including men and women measured at baseline in 2005 and at 5 years follow up in 2010. We excluded 221 HIV positive participants and nine participants with missing anthropometric data. Hence a total of 1058 participants (365 men and 693 women) with complete data at baseline and at follow up were eligible for inclusion in this study. The study was approved by the Ethics committee of the North-West University (NWU), Potchefstroom Campus (NWU-00016-10-A1). All participants provided written informed consent. Additional written informed consent for HIV testing was obtained from each participant after a pre-counseling session.

Body composition measurements

All anthropometric measurements were performed according to standard methods of the International Society for the Advancement of Kinanthropometry (ISAK) [26]. Height was measured to the nearest 0.1 cm with a stadiometer (Leicester height measure, Seca, Birmingham, UK) and weight was recorded on a portable electronic scale to the nearest 0.01

kg (Precision Health Scale, A & D Company, Japan). Waist circumference was measured at the narrowest point between the lower rib border and the iliac crest and recorded to the nearest 0.1 cm with a steel tape (Lufkin, Cooper Tools, Apex NC, USA). Abdominal obesity was defined by waist circumference > 94 cm for men and > 80cm for women [27]. Triceps skinfolds measurements were performed with a Harpenden skinfold caliper (Baty International West Sussex, UK) and the average of two measurements was used for data analysis. BMI was calculated by dividing weight in kilograms by height in meter squared. Anthropometric nutritional status was determined using the WHO categories of BMI of > 18.5 as underweight, 18.5-24.99 as normal weight, 25-29.99 as overweight and ≥30 as obese [28]. Change (∆) in body composition variables was determined by subtracting body composition values of 2005 from 2010 values for each individual.

Questionnaires

Structured questionnaires were adapted and used by all countries participating in the PURE study to collect socio-demographic and lifestyle information including medication and tobacco use [23]. Questionnaires were administered by trained field workers during home visits and visits to the Metabolic Unit of the NWU in their language of choice. Validated culturally sensitive quantitative food frequency questionnaires (QFFQ) [29, 30] and modified Baecke physical activity questionnaires for this population [31] were used as previously described by Kruger and colleagues [32]. The food intake was coded and analyzed by using the South African Medical Research Council food composition database [33].

Blood collection and analysis

Registered nurses collected a fasting blood sample from the antecubital vein using a sterile winged infusion set and syringes. Participants' HIV statuses were determined using the First Response ® (PMC Medical, India) rapid HIV card test using whole blood. These tests' results were treated according to the protocol of the Department of Health of South Africa. If these tests were positive, results were confirmed with the Pareeshak card test (BHAT Bio-tech India). Participants who tested positive received counseling from registered counselors and were referred for a confirmation CD4 count and treatment at their nearest clinics.

SES index

A SES index was calculated as the sum of the graded categories for the educational level attained by the participants, type of occupation, source of household water, access to electricity and type of roofing material at baseline. The highest possible score was 14 and scores between 0-4, 5-9 and 9-14 indicate a low, moderate and high SES respectively.

Statistical analysis

Data were analyzed with IBM SPSS version 22 (IBM Company, Armonk, NY, USA). Normally distributed data are presented as means with standard deviation, non-normally distributed data as medians and interquartile range. Categorical data were analyzed using frequency tables and prevalence of specific conditions was expressed as percentages. Pearson partial correlations were used to explore the relationship between socioeconomic variables, dietary intakes, physical activity score, and changes in body composition while adjusting for age at baseline as a possible confounder. Stepwise multiple linear regressions were used to assess the association between SES index, dietary intake, tobacco use and physical activity as predictors, and changes in BMI, waist circumference, triceps over five years as the dependent variables. Potential confounders like age, marital status, rural or urban residence, and menopausal status (for women only) were included in the models. Statistical significance was set at $p < 0.05$.

RESULTS

The majority (80.8%) of all adults had only primary school level education or no formal education and 88.4% were domestic or informal workers. Women had a higher BMI, weight, waist circumference, and triceps skinfold than men ($p < 0.001$). Men had a higher energy intake and more men than women used tobacco ($p < 0.001$). Baseline characteristics (i.e. 2005 data) of the participants (365 men and 693 women) are shown in Table 1. Subsequent analyses were carried out separately for men and women due to gender differences observed. Figure 1A and 1B show nutritional status based on BMI stratified by urban versus rural residency at baseline and at follow up. A higher prevalence of obese urban women compared to rural women for baseline ($p = 0.005$) and follow up ($p = 0.01$) was observed (Figure 1B). Also, at baseline, 63.6% of urban women had abdominal obesity compared to 50.5% of rural women ($p = 0.001$), which similarly increased to 69.7% in urban women and 55.1% in rural women ($p < 0.001$) at follow up. As shown in Figure 1A, there was no significant difference between the percentages of obese men in urban versus rural areas at baseline and at follow up nor in abdominally obese men at baseline and at follow up. Over 5 years, changes were positive and significant for the women's BMI ($p = 0.007$) and waist circumference ($p < 0.001$) while the changes for the men were also positive, but not significant.

Dietary intake differed significantly between urban and rural residents for both genders at baseline. Mean energy intake for rural men was 7315.8 ± 3733.3 kJ and for urban men was 9796.2 ± 3733.3 ($p < 0.001$). Fat intake in grams was 33.9 ± 16.7 for rural men and 66.0 ± 30.8 for urban men ($p < 0.001$). Mean energy intake for rural women was 6117.0 ± 2480.2 kJ and for urban women was 9099.7 ± 3922.0 ($p < 0.001$). Fat intake in grams was 32.3 ± 16.9 for rural women and 68.6 ± 35.9 for urban women ($p < 0.001$).

Significant differences were observed for the prevalence of obesity and abdominal obesity based on marital status of men. At baseline, 6.3% of married men were obese compared to 1.3% obese single men ($p = 0.02$) and 7.7% married men and 2% single men were obese at follow up ($p = 0.02$). The same trend was observed for abdominal obesity, at baseline, 10.1% married men and 3.9% single men ($p = 0.03$) also at follow up, 13.5% married men and 4.6% single men ($p = 0.005$) were abdominally obese.

Table 1. Descriptive data for the total sample at baseline stratified according to gender

	Men (n=365*) 2005	Women (n=693*) 2005	p[a]
Age at baseline (years), mean ±SD	51.9 ± 10.1	51.8 ± 10.2	0.95
Socioe-conomic variables			
Stratum of urbanization %(n)			
Urban	49.6 (181)	42.9(297)	
Rural	50.4 (184)	57.1 (396)	0.04
Education			
None % (n)	41.5 (149)	38.4 (257)	
Low (1 to 7 years) % (n)	42.1 (151)	44.5 (298)	
Intermediate (8 to 12 years) % (n)	15.3 (55)	16.6 (111)	
High (more than 12 years) % (n)	1.1 (4)	0.6 (4)	0.59
Employed full-time % (n)	59.7 (218)	57.4 (398)	0.77
Occupation			
Domestic/informal workers % (n)	89.0 (325)	88.0 (610)	
Formally trained/skilled % (n)	4.1 (15)	2.6 (18)	
Professionals % (n)	0.8 (3)	0.6 (4)	
No answer % (n)	6.0 (22)	8.8 (61)	0.23
Type of roofing			
Tiles, slates &reinforced concrete %(n)	3.6 (13)	3.2(22)	
Galvanized iron % (n)	79.7 (291)	82.0 (568)	
Asbestos % (n)	14.2 (52)	12.4 (86)	
Scrap material % (n)	2.5 (9)	2.5 (17)	0.82
Electricity % (n)	88.5 (323)	91.3 (633)	0.23
Piped water in house % (n)	45.5 (166)	36.4 (252)	0.004
Life style			
Use of tobacco			
Tobacco use % (n)	63.2 (230)	47.2 (325)	< 0.001
Marital Status % (n)			
Living single % (n)	42.2 (152)	47.5 (317)	
Married/cohabiting % (n)	57.8 (208)	52.5 (351)	0.11
Habitual physical activity			
Physical activity score at baseline, median (interquartile range)	2.83 (2.52-3.23)	2.90 (2.57 -3.25)	0.40
Dietary intake			
Energy intake(Kcal) mean ±SD	8563.0 ±3625.4	7412.87 ± 3511.98	< 0.001
Fat intake(g)mean ± SD	50.1 ±29.5	48.0 ± 32.3	0.33
Body composition			
BMI (kg/m^2)	21.0 ± 4.32	27.6 ± 7.41	< 0.001
Weight (kg)	58.7 ± 12.7	67.9 ± 18.8	< 0.001
Waist circumference (cm)	77.1 ± 10.6	82.9 ± 13.8	< 0.001
Triceps skinfold (mm)	9.32 ± 6.09	22.3 ± 9.30	< 0.001
Obese: BMI > 30 kg/m^2, %(n)	4.1 (15)	34.9 (242)	< 0.001
Abdominal obesity: waist circumference > 80cm (women); > 94cm (men),% (n)	7.4 (27)	56.1 (389)	< 0.001

Abbreviation: BMI body mass index. *Sample size varies due to missing values. a differences between variables. Parametric data are reported as mean ± SD, non-parametric data as median and interquartile range or as percentage of the group.

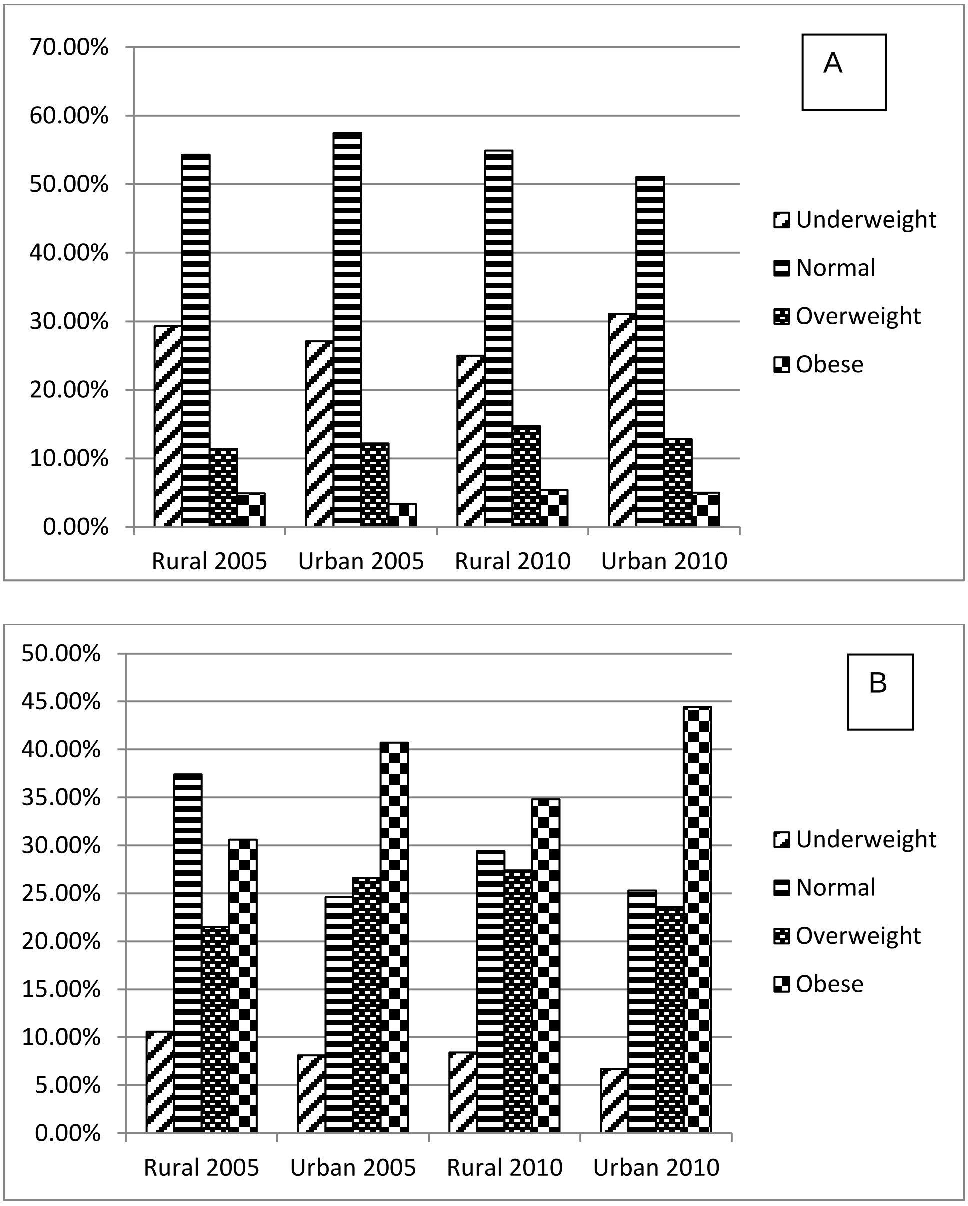

Figure 1: **Anthropometric nutritional status based on BMI of men (A) and women (B) stratified by residence in 2005 and 2010. Prevalence estimates based on WHO categories of BMI >18.5 as underweight, 18.5-24.99 as normal weight, 25-29.99 as overweight and ≥30 as obese [27].**

There was a positive correlation between SES index and change in waist circumference of men ($r = 0.12$, $p < 0.001$) and women ($r = 0.11$, $p < 0.001$) (Table 2). Level of education had positive correlations with change in waist circumference and triceps of women, while physical activity score had a positive correlation with ∆ change in BMI of men. Fat intake had positive correlations with changes in waist circumference and triceps of both men and women, while energy intake only had a positive correlation with waist circumference change of women.

Table 2: Pearson correlation between SES variable, lifestyle risk factors at baseline and changes in body composition variables

	Men			Women		
	BMI change (kg/m^2) (n=364)	WC change (cm) (n=363)	Triceps change (mm) (n=358)	BMI change (kg/m^2) (n=691)	WC change (cm) (n=685)	Triceps change (mm) (n=569)
Socio-economic variables						
Education level	0.03	0.07	0.02	0.04	**0.08***	**0.09***
Occupation (graded)	0.02	0.06	-0.03	-0.02	0.01	-0.07
SES index	0.01	**0.12****	0.06	0.01	**0.11****	0.07
Life style						
Physical activity score	**0.14***	0.07	0.02	0.06	-0.04	0.03
Dietary intake						
Energy intake (kJ)	-0.02	0.04	0.08	-0.03	**0.10***	0.04
Fat intake(g)	0.08	**0.12***	**0.14****	-0.01	**0.11****	**0.09***

Abbreviations: BMI body mass index; WC waist circumference; SES socio-economic status. Partial correlations with adjustment for age at baseline measurements. *p < 0.05; **p < 0.001

Table 3: Multiple regression analysis for the association between dependent variables and predictor variables

	ΔBMI		ΔWC		ΔTriceps skinfold	
	Men	Women	Men	Women	Men	Women
Model 1:	β	β	β	β	β	β
Age at baseline	-0.08	-0.08	-0.01	-0.08	-0.08	**-0.13***
SES index	-0.03	0.04	0.07	0.06	-0.02	0.06
Physical activity score	**0.11***	0.05	0.06	0.03	0.02	0.04
Fat intake 2005 (kJ)	0.12	-0.01	0.01	-0.00	0.06	0.01
Tobacco use	-0.05	-0.00	-0.01	0.05	0.04	-0.02
0=Never used/1=Ever used						
Marital status	**0.21****	0.03	**0.23****	-0.01	0.11	-0.06
1=single/2= married/cohabiting						
Stratum of urbanization	-0.06	-0.02	**0.17***	**0.20****	**0.17***	0.06
1=Rural/2=Urban						
Menopausal status	N/A	-0.11*	N/A	0.00	N/A	0.03
1=premenopausal/2=postmenopausal						
Adjusted R^2	**0.060**	**0.022**	**0.062**	**0.037**	**0.030**	**0.016**
Model 2						
Age	-0.07	-0.09	--	-0.08	-0.08	**-0.15****
Marital status	**0.21****	--	**0.23****	--	0.11	-0.06
1=single/2= married/cohabiting						
Stratum of urbanization	-0.07	--	**0.17***	**0.19****	**0.17***	0.05
1=Rural/2=Urban						
Physical activity score	**0.12***	0.06	0.06	--	--	--
Fat intake	0.12	--			--	--

	∆BMI		∆WC		∆Triceps skinfold	
	Men	Women	Men	Women	Men	Women
SES index	--	0.03	0.07	0.06	--	0.06
Menopausal status	N/A	**-0.11***	N/A	--	N/A	--
1=premenopausal/2=postmenopausal						
Tobacco use	--	--	--	0.06	--	--
0=Never used/1=Ever used						
Adjusted R^2	**0.063**	**0.028**	**0.071**	**0.043**	**0.038**	**0.022**

Model 1 is the full model; Model 2 is model with the best fit. Abbreviations: ∆ change, BMI body mass index, WC waist circumference, SES socioeconomic status. Data adjusted for age at baseline, SES index, physical activity score, fat intake, tobacco use, marital status, menopausal status (for women only) and stratum of urbanization. *=p < 0.05, **=p < 0.001

Table 3 shows the multiple regression analysis for the association between body composition variables as dependent variables and socio-economic variables and lifestyle risk factors as predictors. In the final model of Table 3 (model 2), only marital status and physical activity score positively predicted change in BMI for men. In women, only menopausal status negatively predicted change in BMI ($p < 0.05$).

In the final model of Table 3 (model 2), urban versus rural residency positively predicted changes in the waist circumference of both men ($p < 0.05$) and women ($p < 0.001$). Marriage/cohabitation also positively predicted change in waist circumference for men. SES index, marital status, urban versus rural and physical activity score explained 7.7% variation in waist change for men. A lesser percentage (4.3%) variations in waist change of women were explained by age, SES index, urban versus rural residence and tobacco use.

Table 3 (model 2) urban versus rural residency was the only positive predictor of change in triceps measurements of men while marital status trended. For the women, age was the only negative predictor of change in triceps, while 3.8% of variation in triceps change for men was explained by age, marital status and urban versus rural residence. A smaller percentage of variation (2.2%) in triceps change for women was explained by these same variables and SES index.

DISCUSSION

Our study clearly shows an increase in anthropometric measures of body fat in black South African women from the North-West Province over 5 years. Urbanization was a major predictor of changes in body composition of adult African men and women in our study.

Urban versus rural residence significantly predicted positive changes in waist circumference of men and women as well as triceps skinfold thickness of the men in the present study. According to Cohen (2008), food abundance, novelty and variety are some of the factors that contribute to the urban and rural environments [34]. Various studies in Sub-Saharan Africa have demonstrated higher BMI in urban compared to rural residents [19, 20, 35-38]. Christensen and colleagues found abdominal fat thickness and overall obesity to be higher amongst the urban residents of Kenya in comparison to their rural counterparts [11]. The picture is different in western countries as residents of rural areas have been reported to have higher measures of obesity compared to their urban counterparts [18, 39]. The significantly higher intake of energy and fat by urban residents compared to rural residents observed in our study could also be associated with the significantly higher measures of obesity that we observed particularly among urban women. High dietary intakes of energy and fat have been positively associated with measures of obesity [13, 40,41]. In our study fat intake (Table 2) correlated positively with changes in waist circumference and triceps measurement for both genders. There was no significant difference between the fat intake of men and women. Dietary fat was not a significant predictor in our regression models which could be an indication that our dietary assessment method was not sensitive enough to detect individual differences in fat intake. However, our results support the literature that higher intake of energy and fat were positively associated with measures of obesity. Even though measures of obesity are significantly higher in our urban women compared to their rural counterparts, we observed a similar trend of increased obesity in both urban and rural areas over the 5 years period (Figure 1B). This could be an indication of nutrition transition even in the rural areas and its resultant effects as previously observed [13].

The significantly higher prevalence of overweight, obese and abdominally obese women compared to the men of our study further corroborate results of other studies in Sub-Saharan Africa [19, 20; 35-38, 42]. The 2003 South African health survey reported a prevalence of 31% obesity among urban women compared to 21% among rural women and also 10.6% obesity among urban men and 5.1% obesity among rural men [38]. A cultural perception among black Africans where overweight or obese women are regarded to be more beautiful, symbols of happiness and higher socio-economic class among the general populace could be a reason for this continent-wide phenomenon [38, 43-46].

Marital status was a significant predictor of gains in waist circumference and BMI of African adult men, but not in the women. A study on an Iranian population showed that marriage was associated with increased risk of obesity [47]. In a cross sectional study of American men and women, married men were significantly more likely to be obese than single men, while marital status was not associated with obesity among women [48]. The significantly higher percentage of obese and abdominally obese married men compared to single obese men in our study further corroborates the observations of the aforementioned studies. The reason for this phenomenom is not very clear but a possible reason could be marriage associated lifestyle changes like reduced physical activity and increased food consumption [47-48].

SES status was not significantly associated with changes in body composition when other factors were adjusted for in our regression models. Earlier studies reported that SES was a significant predictor of BMI and that SES was inversely associated with BMI [9-10]. This is contrary to our study result where higher SES index indicated higher BMI, waist circumference and triceps skinfold thickness for both sexes ($p<0.001$ for both). This could be because only a few (6.2%) of our study participants were in the high SES range of 10-14 on the index scale. A similar trend was also demonstrated for educational status. Low educational levels were associated with higher BMI of women in various studies [10, 49, 50]. The association with educational level in our study was contradictory. However, our result is similar to what was observed by Amoah among Ghanaians [37]. The higher the educational level of the women in our study, the greater the gain in waist circumference and triceps skinfold. Only 17% of the women had a formal education above primary school level, whereas the majority of the women (83%) were educated only up to primary school level. The trend was similar for the men as only 16% of the men had been educated beyond primary school. Differences in study design, population and statistical analysis, may have contributed to the varying results observed in comparison to similar studies. We propose that the association between educational level, SES and changes in body composition variables might have been different if the majority of our participants were in the high SES index category and were educated up to at least completion of secondary school. Moreover, the relationship between measures of obesity and SES is complex with heredity factors possibly playing a role [51].

Age has been established to be a predictor of changes in body composition [52,53]. In our study, age predicted change in triceps and tended to predict change in BMI and waist circumference for women. The BMI of premenopausal women increased more over time compared to postmenopausal women (Table 3). The effect of age on changes in body composition was not apparent in the men. Gender related differences in the effect of age on body composition variables were also suggested by other studies [52-54].

Increasing physical activity has been demonstrated to reduce measures of obesity [14,15]. Earlier studies had established that habitual physical activity was low in black South African women of the NWP [32, 55]. Although physical activity at baseline positively predicted BMI change in men, change in BMI represents both lean and fat mass changes. This could mean that a higher physical activity score among males predicted a positive increase in both lean and fat mass. A reason for the relationship observed between physical activity and changes in body composition in our study could be because the majority (78.7%) of our participants were in the low physical activity index bracket (physical activity score < 3.3) [32].

Another lifestyle risk factor assocated with changes in body composition is smoking which has been linked to central fat accumulation [17] and has been shown to have strong associations with BMI of women [10]. The lack of association between tobacco use and body composition in our study could be as a result of differences in age distribution, sample size and study population.

In our study, age, marital status, urban versus rural residence, habitual physical activity and fat intake only explained 6.3% of the change in BMI variation for men. A smaller percentage of 2.8% of the change in BMI variation for women was explained by age, habitual physical activity, SES and menopausal status. Also small percentages of variations were explained for changes in waist circumference and triceps in all models. A higher variation of body composition change was explained in the WHO MONICA study [56] while a study on Polish men explained only 8% of variation in waist circumference [57]. However, when anthropometric parameters were excluded from the WHO MONICA study only 4% of variation in waist circumference were explained for men and 5% for women. The reasons for smaller variation explained in our study could be because we investigated changes in body composition variables and body composition variables at baseline were not included in our models [56]. Despite the small percentage of variation explained in our study, the study still highlights that SES, lifestyle factors and the urbanization related factors are modifiable risk factors that could be targeted to combat the increasing scourge of obesity among adults in these communities.

Our study is comparable to the general black South African population. For instance, the National Nutrition and Health Examination Survey reported an equally high national prevalence of 39.9% obesity (BMI>30 kg/m^2) among black South African women [20]. The overall prevalence of obesity in this study particularly among women further confirms the rising concern that South Africa is in the nutrition-related non-communicable disease phase of the nutrition transition [12, 13, 20, 58].

Our study has a number of potential limitations which include the wide range in age with relatively small numbers in the youngest and oldest age range. This study was performed in black men and women in one province and the results may not be generalizable to the greater black South African population. Also, we did not have information on the ownership of household assets such as cars, televisions, microwave etc. to create an asset index which could have increased the sensitivity of the SES index.

In conclusion, our 5-year longitudinal study showed that black African adults in the NWP, particularly the women are gaining body fat. Residing in an urban environment plays a significant role in the increasing adiposity of our subjects. We recommend that both urban and rural residents should be targeted for public health intervention programs centered on healthier lifestyle choices. This is important to reduce prevalence of overweight and obesity which should remain a public health priority.

AUTHORS' CONTRIBUTIONS

OFS and HSK conceptualised the study, OFS drafted the manuscript and conducted all statistical analysis. HHW, HL and HSK made substantial contributions to the drafts and were involved in critically reviewing the manuscript as well interpretation of all results. CN and HM were involved in critically reviewing the draft of the manuscript and contributed to data collection. All authors contributed to and approved the final manuscript.

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CHAPTER 4

Lean mass appears to be more strongly associated with bone health than fat mass in urban black South African women

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ABSTRACT

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ABSTRACT

Objective: To examine the association between body composition (fat mass, lean mass and body mass index, BMI) and bone health (bone mineral density, BMD and fracture risk) in urban black South African women.

Design: A cross sectional study examining associations between body composition, dietary intake (food frequency questionnaire), habitual physical activity (Activity energy expenditure (AEE) measured using an accelerometer with combined heart rate monitor and physical activity questionnaire) and bone health (BMD using dual-energy X ray absorptiometry, DXA and fracture risk).

Setting: Urban community dwellers from Ikageng in the North-West Province of South Africa

Participants: One hundred and eighty nine (189) healthy postmenopausal women aged ≥43 years.

Results: Fat mass and lean mass were significantly associated with BMD and fracture risk when adjusted for potential confounders. However, lean mass and not fat mass remained significantly associated with femoral neck BMD ($\beta = 0.49$, $p < 0.001$), spine BMD ($\beta = 0.48$, $p < 0.0001$) and hip BMD ($\beta = 0.59$, $p < 0.0001$). Lean mass was also negatively associated with fracture risk ($\beta = -0.19$ $p = 0.04$) when both lean and fat mass were in the same model.

Conclusion: Lean mass and fat mass were positively associated with femoral neck, spine and hip BMDs and negatively associated with fracture risk in urban black South African women. Our finding suggests that increasing lean mass rather than fat mass is beneficial to bone health. Our study emphasises the importance of positive lifestyle changes, intake of calcium from dairy and adequate weight to maintain and improve bone health of postmenopausal women.

Keywords Lean mass, fat mass, bone mineral density, fracture risk, African women

INTRODUCTION

Osteoporosis and obesity are two complex diseases of increasing prevalence and with great impact on mortality and morbidity. Similarities identified between these diseases indicate some type of pathophysiological link (1). Worldwide, obesity affects over 300 million women while osteoporosis affects over 200 million women (2, 3). The South African National Health and Nutrition Examination Survey (SANHANES) recently reported a national obesity prevalence of 39.2% for South African adult women (4).

Body mass index (BMI) which is an indicator of adiposity is a height-standardised measure of body weight mainly comprised of lean and fat mass. Low BMI has been established to be a risk factor for osteoporotic fracture (5–7). However, obesity was recently shown to be a risk factor for osteoporotic fracture (8). The mechanical loading of body weight on bone led to the belief that obesity may prevent bone loss and osteoporosis (5, 6). Previous studies had conflicting results about the individual effect of lean mass and fat mass on bone mineral density (BMD) (5, 6, 9–14). Recent studies are showing that lean mass has a greater protective effect on BMD in comparison to fat mass (10, 11). Indeed increased fat mass has been associated with low BMD and reported not to protect against osteoporosis in Chinese men and women (13). A number of studies have been conducted on bone health outcomes among South Africans (15–20), but to the best of our knowledge none has focused on the relationship between body composition and bone health, particularly BMD and fracture risk, among postmenopausal black South African women. Moreover, there is an increasing concern about the loss of African women's inherent advantage of higher BMD which needs further investigation (17). Consequently, this study aims to examine the association between body composition (BMI, fat mass and lean mass) and bone health (BMD and fracture risk) in urban postmenopausal black South African women.

SUBJECTS AND METHODS

Study design

The Prospective Urban and Rural Epidemiology (PURE) study is a 10 year longitudinal study aimed at tracking the effects of lifestyle and changing environment exposures on the development of non-communicable diseases in populations at different stages of epidemiologic transition (21). The South African North-West Province (NWP) arm of the PURE (PURE-SA-NWP) study commenced with baseline data collection in 2005 (17). In this sub-study we included postmenopausal women who were measured at 5 and 7 years follow up in 2010 and 2012, respectively using a cross-sectional study design. Urban black women aged ≥ 43 years from the PURE-SA-NWP study were included. Only participants who completed the quantitative food frequency questionnaires (QFFQ) and had undergone dual energy X-ray absorptiometry (DXA) measurements at follow up were eligible for inclusion in this study (n=189). We excluded women who are HIV positive in the current analysis. Blood samples and DXA measurements for each participant were done on the same day and the seasons were defined as October to December for spring (season 1) and April to June for autumn (season 2). The study was approved by the Ethics committee of the North-West University (NWU), Potchefstroom campus (NWU-00016-10-A1). All participants provided written informed consent. Another written informed consent for HIV testing was obtained from each participant after a pre-counseling session.

Body composition measurements

Height was measured to the nearest 0.1 cm with a stadiometer (Leicester height measure, Seca, Birmingham,UK) and weight was determined on a portable electronic scale to the nearest 0.01 kg (Precision Health Scale, A & D Company, Japan) by anthropometrists according to standard methods of the International Society for the Advancement of Kinanthropometry (ISAK) (22). BMI was calculated (weight in kilograms divided by height in meter squared). Women were grouped according to their BMI of either < 25 kg/m^2 or ≥ 25 kg/m^2 (overweight and obese).

Body composition (lean and fat mass) and BMD were measured by a registered radiographer with DXA (Hologic Discovery W, APEX system software version 12.7.3.1). Whole body, femoral neck (CV = 1.2%), hip (CV = 0.8%) and anterior posterior spine BMD (L1–L4, Spine, CV = 0.7%) were measured. Measurements for the non-dominant side of each participant were used for data analysis. Low bone mass (osteopenia) was defined by a femoral neck T-score between -1.0 and -2.5 standard deviations and osteoporosis was defined as a T-score ≤ −2.5 standard deviations (23, 24).

Questionnaires

Structured questionnaires were adapted and used by all countries participating in the PURE study to collect socio-demographic and lifestyle information including medication and tobacco use (21). Questionnaires were administered by trained field workers during home visits and visits to the Metabolic Unit of the NWU in their language of choice. Validated culturally sensitive QFFQ (25, 26) and modified Baecke physical activity questionnaires for this population (27) were used as previously described by Kruger and colleagues (17). The food intake were coded and analyzed by using the South African Medical Research Council database (28). Fracture risk was measured and assessed using the Black fracture risk score (29). Fracture risk questionnaires have been previously used in the black South African population (30). An index with a score from 0 to 3 was regarded as low risk; 4 to 6 as medium risk and 7 to 11 was high risk (29).

Blood collection and analysis

Registered nurses collected a fasting blood sample from the antecubital vein using a sterile winged infusion set and syringes. Serum samples were prepared and stored in aliquots in cryotubes at -80ºC. Serum 25-hydroxy vitamin D (25(OH)D) concentrations were measured using the Roche Elecsys 2010 COBAS system (Roche Diagnostics, Indianapolis, IN, USA).

Physical activity

Habitual physical activity was measured with a modified Baecke questionnaire (27) and activity energy expenditure (AEE) was measured using an accelerometer with combined heart rate monitor (ActiHeart®, Camtech, UK) for 7 days. Participants were visited by field workers on a daily basis to ensure that the ActiHeart® monitor was secure and to record possible problems with wearing the device. AEE was determined by means of 60 second epochs and data generated by the ActiHeart® were downloaded using a computer interface. Total energy expenditure and AEE were calculated (in kJ) with the ActiHeart® software. Reliability and validity of using the ActiHeart® to evaluate physical activity in sub-Saharan Africans has been previously assessed (31).

Statistical analysis

Data were analysed with IBM SPSS version 22 (IBM Company, Armonk, NY, USA). Normally distributed data are presented as means with standard deviation, non-normally distributed data as medians and interquartile range. Categorical data were analysed using frequency tables and prevalence of specific conditions was expressed as percentages. Independent t-tests were used to compare parametric variables and Mann-Whitney U-tests to compare non-parametric variables between groups. Pearson correlations were used to explore the relationship between dietary intake, physical activity, BMD, body composition and fracture risk while adjusting for possible confounders (i.e. age, height, tobacco use, contraceptive use and thiazide use). Prevalence odds ratio (OR) and 95% confidence intervals (CI) were evaluated for BMI vs. bone density categories. Separate stepwise multiple linear regressions were used to assess the association between BMI, lean mass, fat mass respectively as independent variables, and femoral neck BMD, spine BMD, hip BMD and fracture risk, respectively, as the dependent variables. Potential confounders like age, 25(OH)D, season of data collection, AEE, tobacco use, alcohol consumption, dairy food intake, contraceptive use and use of thiazide were included in the models. Another multiple regression model was used with both lean mass and fat mass as independent variables of BMD measured at the three sites and fracture risk in the same model. We based our power calculation for the appropriate sample size for multiple regression analysis based on an expected R^2 of 0.2, a maximum of 15 independent variables and a confidence level of 0.95 indicated a sample size of 150 participants (32). Statistical significance was set at $p < 0.05$. Diagnostic tests for multicollinearity were performed.

RESULTS

Demographic, body composition, health and lifestyle characteristics of the women are presented in Table 1. Using the WHO BMI classification, 7.4% of the women were underweight, 22.2% had normal weight, and 23.3% were overweight while 47.1% were obese. Women with BMI < 25 kg/m^2 had significantly lower body fat percentage, lean mass, spine BMD, femoral neck BMD, hip BMD, and whole body BMD, but had higher serum 25(OH)D, higher fracture risk, as well as a higher proportion of osteoporosis in comparison to those with a BMI ≥ 25 kg/m^2 (Table 1).

The odds of having osteopenia was not significantly different between women with BMI < 25 kg/m^2 compared to overweight and obese women (OR 1.34, 95%CI: 0.72, 2.52, p=0.37). However, the odds of having osteoporosis was seven fold higher in women with BMI < 25 kg/m^2 compared to women with BMI ≥ 25 kg/m^2 (OR 7.08, 95%CI: 2.95,16.96, p<0.001). Out of the women aged 70 years and above, 42.9% had osteoporosis while the highest percentage of osteopenia was recorded for women between the ages of 60-69 years (52.4%). Among the women aged < 60 years, 34% had osteopenia while 8.7% were already osteoporotic.

Table 1: Demographic, body composition, health and lifestyle measures of the total group as well as between women with BMI < 25 kg/m^2 and BMI ≥ 25 kg/m^2 (n=189)*

Variable	Total group (n=189) *	BMI <25 kg/m^2 (n=56)	BMI ≥25 kg/m^2 (n=133)	p#
Age(years)	61.1 (10.2)	61.0 (11.2)	61.1 (9.79)	0.951
Body fat %	40.2 (7.43)	31.7 (5.61)	43.8 (4.71)	<0.001
Fat mass (kg)	29.2 (11.9)	15.9 (4.50)	34.9 (9.21)	<0.001
Lean mass (kg)	39.0 (7.29)	31.9 (4.61)	41.9 (6.09)	<0.001
BMI (kg/m^2)	29.4 (7.57)	20.7 (2.91)	33.0 (5.68)	<0.001
Spine BMD (g/cm^2)	0.854 (0.144)	0.777 (0.123)	0.886 (0.140)	<0.001
Femoral neck BMD (g/cm^2)	0.840 (0.133)	0.649 (0.111)	0.773 (0.125)	<0.001
Hip BMD (g/cm^2)	0.840 (0.152)	0.734 (0.112)	0.882 (0.147)	<0.001
Whole body BMD (g/cm^2)	0.987 (0.124)	0.914 (0.095)	1.018 (0.122)	<0.001
Fracture risk score	1.73 (1.65)	2.31 (1.70)	1.48 (1.57)	0.002
AEE (kJ)	1160 (909)	860 (703)	1287 (957)	0.005
Physical activity score	2.93 (0.49)	2.92 (0.38)	2.93 (0.53)	0.97
25(OH)D (ng/ml)	30.2 (9.61)	32.9 (9.37)	28.9 (9.49)	0.01
Tobacco users n (%)	97 (51.3)	35 (62.5)	62 (47.3)	0.06
Contraceptive users n (%)	100 (53.8)	33 (58.9)	67 (51.5)	0.34
Thiazide users n (%)	84 (44.4%)	22 (39.3)	62 (46.6)	0.36
Osteopenic n (%)	75 (39.7)	25 (44.6)	50 (37.6)	0.37
Osteoporotic n (%)	28 (14.8)	19(33.9)	9 (6.8)	<0.001

*Sample size varies due to missing values. BMI = body mass index. BMD = Bone mineral density. AEE = activity energy expenditure, 25(OH)D = serum 25 hydroxy vitamin D. #Difference between groups with BMI </ ≥ 25 kg/m2. Data are means (SD) or frequency (%)

There was a positive correlation between body composition variables and all BMD measurements at different sites, and a negative correlation with fracture risk (Table 2). Dairy foods and dietary calcium intakes had significant positive correlations with one or more BMD measurements (Table 2).

Table 2: Pearson correlation coefficients between dietary intake, physical activity, body composition, bone markers and fracture risk for the whole group

	Spine BMD	Femoral neck BMD	Hip BMD	Whole body BMD	Fracture risk	Fat mass	Lean body mass	Body mass index
Body composition								
Body mass index (kg/m^2)	**0.40****	**0.46****	**0.55****	**0.51****	**-0.24****	**0.94****	**0.80****	**-**
Fat mass (kg)	**0.40****	**0.43****	**0.52****	**0.50****	**-0.25****	**-**	**0.79****	**0.96****
Lean mass (kg)	**0.48****	**0.48****	**0.55****	**0.54****	**-0.25****	**0.79****	**-**	**0.79****
Dietary intakes								
Energy intake (kJ)	0.06	0.09	0.02	0.07	-0.08	-0.05	-0.07	-0.04
Calcium (mg)	.068	0.14	0.09	**0.16***	-0.09	0.06	0.06	0.09
Vitamin D (µg)	-0.07	0.04	-0.02	0.02	-0.07	-0.07	-0.07	-0.10
Alcohol (g)	-0.08	-0.08	-0.06	-0.10	0.03	**-0.19***	-0.09	**-0.22****
Dairy food (g)	0.12	**0.21****	0.12	**0.20***	**-0.15***	0.10	0.10	0.12
Vitamin D status								
25(OH)D (ng/ml)	-0.07	-0.08	-0.06	**-0.15***	0.03	**-0.18***	**-0.22****	**-0.22****
Physical activity								
AEE (Kcal)	0.05	0.13	0.15	0.07	-0.14	**0.28****	**0.23****	**0.28****
Physical activity score	0.03	-0.03	0.05	0.04	-0.01	0.10	0.08	0.08

BMD =bone mineral density, AEE = activity energy expenditure, 25(OH)D = serum 25 hydroxyl vitamin D. Partial correlation with adjustment for age, tobacco use, history of contraceptive use and thiazide use. * $p<0.05$, ** $p<0.001$

Table 3 shows the multivariate regression results of the associations of body composition variables with BMD measurements. In model 2, BMI and other covariates explained 38% variation in femoral neck BMD, but when BMI was replaced with fat mass a lower percentage (35%) was explained. When fat mass was replaced by lean mass, an even greater percentage of variation (40%) in femoral neck BMD was explained.

Unadjusted beta-values showed that for each increase in one unit (1 kg) of fat mass there was an increase of 0.005 g/cm^2 in femoral neck BMD ($p<0.001$) while for an increase in each unit (1 kg) of lean mass there was an increase of 0.010 g/cm^2 in femoral neck BMD ($p<0.001$).

For spine BMD, BMI and other covariates explained 23% of the variation, changing to 25% when BMI was replaced by fat mass, while the model with lean mass also explained the highest variation of 30% (Table 3). An increase in each unit of fat mass and lean mass was associated with similar increases in spine BMD and femoral neck BMD (0.005 g/cm^2 ($p<0.001$) and 0.010 g/cm^2 ($p<0.001$), respectively).

The variation in hip BMD explained by BMI, fat mass and lean mass, respectively, and other covariates was also 38%, 36% and 40%. Unadjusted beta-values showed that the increases in each unit of fat mass and lean mass, respectively, was associated with increases in hip BMD of 0.007 g/cm^2 (p<0.001) and 0.012 g/cm^2 (p<0.001).

Table 3: Association between BMD as dependent variable and body composition parameters as independent variables

	BMI			Fat mass			Lean mass		
Femoral neck BMD	β	p	Adjusted R^2	β	p	Adjusted R^2	β	P	Adjusted R^2
Model 1	0.48	<0.001	0.23	0.46	<0.001	0.20	0.51	<0.001	0.26
Model 2	0.42	<0.001	0.38	0.39	<0.001	0.35	0.49	<0.001	0.40
Spine BMD	β	p	Adjusted R^2	β	p	Adjusted R^2	β	P	Adjusted R^2
Model 1	0.43	<0.001	0.18	0.44	<0.001	0.19	0.51	<0.001	0.26
Model 2	0.41	<0.001	0.23	0.38	<0.001	0.25	0.48	<0.001	0.30
Hip BMD	β	p	Adjusted R^2	β	p	Adjusted R^2	β	P	Adjusted R^2
Model 1	0.57	<0.001	0.32	0.54	<0.001	0.29	0.58	<0.001	0.33
Model 2	0.53	<0.001	0.38	0.50	<0.001	0.36	0.59	<0.001	0.40

BMI = body mass index Model 1: unadjusted model. Model 2: adjusted for age, height (except for BMI model), serum 25 hydroxy vitamin D, season, activity energy expenditure, dairy food intake, alcohol intake, history of contraceptive use, thiazide use and tobacco use

Table 4 summarizes the association between body composition variables and fracture risk. All body composition variables were negatively associated with fracture risk. Individual associations were β= - 0.23 (p<0.001) for BMI, β = -0.24 (p<0.001) for fat mass and β = -0.31 (p<0.001) for lean mass.

Table 4: Association between fracture risk as dependent variable and body composition parameters as independent variables

	BMI			Fat mass			Lean mass		
	β	p	Adjusted R^2	β	p	Adjusted R^2	β	P	Adjusted R^2
Model 1	-0.23	0.002	0.05	-0.24	0.001	0.05	-0.31	<0.001	0.09
Model 2	-0.18	0.03	0.10	-0.16	0.05	0.15	-0.19	0.04	0.15

BMI = body mass index. Model 1: unadjusted model. Model 2: adjusted for, height (except for BMI), 25(OH)D, season, activity energy expenditure, dairy food intake, alcohol intake, history of contraceptive use and thiazide use

In Table 5 where lean mass and fat mass were included in the same model, lean mass (β = 0.45, p<0.001) was positively associated with femoral neck BMD, while fat mass (β= 0.05 p = 0.65) was not even though it was retained in the model (Model 1).

In the final model (Model 2 of Table 5), lean mass, age, height, dairy foods, and tobacco use were the only variables associated with femoral neck BMD, while there was no association with fat mass. Lean mass, age, height, dairy foods, tobacco use and season explained 40.1% of the variation in femoral neck BMD of our participants.

Lean mass and tobacco use were the only variables associated with spine BMD (Model 2 of Table 5). Lean mass, age, tobacco use and season explained 29.7% of the variation in spine BMD of our participants. Lean mass, age, height and tobacco were also significantly associated with hip BMD and explained 39.6% of the variation in hip BMD of our participants (Model 2 of Table 5).

Body composition variables and dairy foods were negatively associated with fracture risk (Table 5). In the final model (Model 2 of table 5), lean mass and dairy foods were the only variables significantly associated with fracture risk. Lean mass, dairy foods, height, 25(OH) D, AEE, thiazide use and history of contraceptive use explained 14.8% variation in the fracture risk of our study population.

Table 5: Multiple regression analysis for the association between BMD measurements and fracture risk as dependent variables and body composition parameters as independent variables

	Femoral neck BMD		Spine BMD		Hip BMD		Fracture risk	
	β	p	β	p	β	p	β	p
Model 1:								
Fat mass	0.05	0.65	0.06	0.63	0.18	0.13	-0.07	0.62
Lean mass	0.45	<0.001	0.42	0.002	0.45	0.001	-0.14	0.36
Age	-0.32	<0.001	-0.12	0.11	-0.21	0.003	N/A	N/A
25(OH)D	0.02	0.82	-0.03	0.72	0.03	0.75	-0.15	0.09
Season	0.08	0.27	0.13	0.12	0.01	0.91	0.09	0.33
Height	-0.14	0.07	0.01	0.91	-0.12	0.11	-0.17	0.07
Tobacco use	-0.14	0.03	-0.17	0.02	-0.13	0.05	N/A	N/A
Dairy foods	0.14	0.04	0.06	0.39	0.06	0.36	-0.15	0.05
Alcohol	0.02	0.82	-0.02	0.75	0.04	0.52	-0.03	0.71
Thiazide use	0.03	0.71	0.08	0.26	-0.02	0.75	0.12	0.11
Contraceptive use	0.02	0.81	0.00	0.26	0.04	0.53	0.13	0.09
AEE	0.02	0.76	-0.03	0.97	-0.01	0.88	-0.11	0.18
Adjusted R2	**0.379**		**0.271**		**0.379**		**0.138**	
Model 2								
Lean mass	0.49	<0.001	0.48	<0.001	0.59	<0.001	-0.19	0.04
Age	-0.32	<0.001	-0.11	0.11	-0.22	0.001	N/A	N/A
Height	-0.15	0.04	--	--	-0.16	0.03	-0.16	0.06
Dairy foods	0.14	0.03	--	--	--	--	-0.15	0.05
25(OH)D	--	--	--	--	--	--	-0.11	0.14
Season	0.09	0.18	0.12	0.09	--	--	--	--
Tobacco use	-0.15	0.02	-0.19	0.007	-0.15	0.02	N/A	N/A
AEE	--	--	--	--	--	--	-0.14	0.08
Thiazide use	--	--	--	--	--	--	0.11	0.13
Contraceptive use	--	--	--	--	--	--	0.13	0.10
Adjusted R2	**0.401**		**0.297**		**0.396**		**0.148**	

BMD = bone mineral density. AEE = Activity energy expenditure. 25(OH)D = serum 25 hydroxy vitamin D. Models adjusted for age (except for fracture risk), height, serum 25 hydroxy vitamin D, season, activity energy expenditure, dairy food intake, alcohol intake, history of contraceptive use, thiazide use and tobacco use (except for fracture risk).

DISCUSSION

The results from this cross sectional study indicate that lean mass had a stronger association with bone health in comparison to fat mass in urban black South African women.

The influence of individual body composition variables to BMD remains controversial. While some studies demonstrated that lean mass exhibit a positive relationship with BMD (10, 11) another reported that lean mass does not have an impact on BMD (14). Some reported positive, negative and no association between fat mass and BMD (5, 6, 9). These conflicting findings may be due to differences in study design, study population, statistical analysis, tools used to measure body composition and skeletal sites measured.

In our participants, lean mass showed consistent stronger correlations than fat mass at all skeletal sites of BMD measurements with the highest correlation value for lean mass and hip BMD ($r = 0.55$, $p<0.001$) and lowest for fat mass and spine BMD ($r = 0.40$, $p<0.001$). These results are in agreement with the results of the large Hordaland health study who also demonstrated a stronger association between lean mass and femoral neck BMD in middle-aged and elderly men and women in comparison to fat mass (33). In our study 2.2%, 2.6% and 1.6% increase in variation in femoral neck, spine and hip BMDs were explained by lean mass and other covariates when fat mass was no longer in the model respectively. These findings are consistent with the study that demonstrated a significant beneficial effect of lean mass on BMD in both postmenopausal and perimenopausal Thai women (10). It however contradicts the result of a study, where they found lean mass not to have an impact on BMD of postmenopausal Turkish women when fat mass was taken into account (14). Also, in a similar study it was observed that lean mass plus other covariates explained the greatest variance in BMD compared to fat mass and other covariates among black premenopausal South African women (18). Fat mass and lean mass were both negatively associated with fracture risk in separate models in our study, however, only lean mass remained significantly associated with fracture risk when both variables were taken into account in the same model.

The differences in variations explained at different BMD sites in our study is an indication that body composition contributes differently at different BMD sites (19, 34). Another indication of varying contribution of body composition at different sites of our participants is that higher variations were explained by body composition variables (35% to 40%) of femoral neck and hip than of the spine BMD (23% to 30%).

Over half (51.3%) of our participants have either smoked in the past or were current smokers. Studies in the past have showed varying relationship between smoking and bone health (35-38). Moderate smoking in young women was reported not to be associated with low BMD at any site (36). Smoking's effect on bone loss has been shown to be independent, dose-dependent, cumulative and increases fracture risk significantly (35, 38). Tobacco use in our study had significant negative associations with BMD at all measured sites.

Our results indicate that BMI is associated with bone health in urban postmenopausal black South African women. The Framingham study (6) suggested that the strong effect of weight on BMD is due to load on weight-bearing bones in both men and women. The higher risk for osteopenia and osteoporosis among our women with BMI < 25 kg/m^2 is consistent with results from others (5–7, 39). In a study by Assomaning and colleagues (7), each one unit increase in BMI was associated with a significant 12% decrease in risk for osteoporosis, however, the study participants of their study were referred for a BMD examination. Such referred populations may include a large number of patients with previously recognized risk factors for osteoporosis which is a potential selection bias. The lower lean mass and habitual physical activity of our participants with BMI < 25 kg/m^2 could further explain the lower BMD and higher fracture risk observed among this group of our participants. Correspondingly, De Laet and colleagues demonstrated that the significance of BMI as a risk factor for low bone mass and osteoporosis varies based on level of BMI (40). They reported that a BMI of 20kg/m^2 when compared with BMI of 25 kg/m^2 was associated with a nearly twofold increase in risk for hip fracture. While a BMI of 30 kg/m^2, when compared with a BMI of 25 kg/m^2, was associated with only a 17% reduction in hip fracture risk (40). Furthermore, Ong and co-workers recently showed that higher BMD in obesity is not protective against fractures (9), and adiposity has been shown to be a risk factor for fractures (8).

In our study, there was no significant association between 25(OH)D and femoral neck, hip and spine BMDs similarly to another study in black South Africans (19). There were significant negative correlations between 25(OH)D and measures of adiposity. The majority (70%) of our women were overweight or obese which could explain this negative association as adipose tissue may decrease the bioavailability of vitamin D (41). Ethnicity might play a role in this observation as a negative relationship has been reported between adipose tissue and 25(OH)D concentrations in Hispanic American and African American populations (42). The unexpected negative correlation between lean mass and 25(OH)D concentrations is in contrast with what was found by Tieland and colleagues (43). This could also be as a result of the unique genetic makeup of black South Africans as genetics play an important role in determining muscle mass (44). More research is needed to further explore these findings.

Calcium has been established and extensively described in literature to play an important role in bone health (45-47). However, the protective effect of calcium on bone might not be evident in postmenopausal women with calcium intakes less than 800 mg/day (46). Total calcium intake was only related to whole body BMD in our study. Calcium intake of our study participants was low with only 19.6% having intakes higher than 800 mg/day and 9.5% having intakes higher than the estimated average requirement of 1000 mg/day (45). These low intakes of calcium could explain the high proportion of osteopenia and osteoporosis among our participants. Dairy food was associated with bone health among our study participants as previously established (47-49). Increasing dairy consumption to meet the recommended 2 cups per day (500ml) has been recently demonstrated to likely decrease the incidence of osteoporosis, fractures and the associated health care costs (49). Dietary energy, magnesium, phosphorus, zinc and vitamin D intakes were unrelated to bone health in our study, a result which is consistent with that of Coin and colleagues (50).

Varying results in the literature on the association between physical activity and bone health could be due to differences in the method of assessing physical activity and study population (51–53). In our study, reported physical activity measured with a questionnaire had no significant correlations with bone health; while physical activity measured using accelerometers had significant positive correlations with all body composition variables. This could be an indication that combined accelerometry and heart rate monitoring is a more sensitive instrument to measure physical activity than questionnaires in this population group. Habitual physical activity was not associated with bone health in our regression models irrespective of whether it was measured with accelerometer or a questionnaire. A reason could be that the majority (89%) of our participants were in the low physical activity index bracket of physical activity score at the time of the study (17). Physical activity over time may be a mediator of the effect of body composition on bone which may also impact BMD directly (54, 55). A gradual increase in the amount of physical activity can help prevent decreases in BMD even in postmenopausal women (56). Thus, increasing the habitual physical activity of our participants could still have a beneficial effect on their lean mass which could be associated with better bone health.

Use of thiazide has been demonstrated to have a protective effect on BMD (57,58). Over 44% of our study population used thiazide, however it was not significantly associated with bone health in our regression models. Use of oral contraceptives pills has also been shown to have positive effects on BMD (59) while injectable progestin contraception results in increased bone loss when compared with women using non-hormonal contraceptives (60). Positive history of contraceptives use was not associated with bone health in our regression models although 53.8% of our study participants have used contraceptives. We did not record the type of contraception used by participants. Injectable progestin contraception and oral contraceptive pills are supported by the South African National public health system and given freely at clinics (60,61). This inability to distinguish type of contraception used by our participants may be a possible explanation for the lack in association with bone health in the current study.

Our study indicates that black women seem to be losing their inherent protection against osteopenia and osteoporosis. The proportion of women with osteopenia (39.7%) and osteoporosis (14.8%) in our study was higher than previously reported for African American women (35% and 5% respectively) (62). Osteopenia was previously reported in both premenopausal white (14.4%) and black South African women (9.1%) (18). Osteoporosis was already present in women younger than 60 years in our study which further reinforces the concerns raised about the future bone health of black South Africans (17, 63).

There are similarities between the women in our study and the general population of the black South African women. For instance, a study carried out in a different South African setting reported BMD values for black women comparable to those found in our study (19). Also variances explained at the lumbar spine were lower than those explained at the femoral neck and hip (18,19). The national Nutrition and Health Examination Survey reported an equally high national prevalence of 39.9% obesity (BMI>30 kg/m^2) among black South African women (4).

Limitations of this study include its cross-sectional design, thus causal relationships cannot be identified. This study was performed in black urban women in one setting and the results may not be generalizable to the greater black South African population. Also, we did not record the type of contraception used by participants. The wide range in age with relatively small numbers in the youngest and oldest age range also made it difficult to assess the true impact of age on bone health. However, it allowed us to show that low BMD and osteoporosis were already found in black urban women younger than 60 years. Despite the limitations, our study has produced a better understanding of the relationships between body composition variables and bone health of urban postmenopausal black South African women which could be further investigated.

In conclusion, our data shows that in urban black South African women, lean mass remained a strongly associated with bone health even when adjustment for fat mass was made. Our finding proposes that increasing lean mass rather than fat mass is beneficial to bone health. Thus, meeting the recommended dietary intake for calcium obtained from dairy products and increasing habitual physical activity could have a beneficial effect on bone health. Future studies on other factors affecting lean mass and bone health of Africans are recommended. The importance of positive lifestyle changes, intake of calcium from dairy and adequate weight to maintain and improve bone health of postmenopausal women is highlighted in our study and this should be emphasised in public health intervention programmes.

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CHAPTER 5

Association of 25-hydroxyvitamin D and parathyroid hormone concentration with the metabolic syndrome in women of the North-West province, South Africa

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ABSTRACT

Background: Studies have indicated metabolic roles for 25 hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH). The relationship between low serum vitamin D, PTH and metabolic traits appear to differ among different ethnicities. The aim of our study is to examine the association of serum 25(OH)D and PTH concentration, respectively, with the metabolic syndrome while controlling for adiposity in black women in the North West Province, South Africa.

Methods: Using a cross sectional study design, urban black women aged ≥ 43 years measured at 7 years follow up from September 2012 to June 2013 of the South African arm of the Prospective Urban Rural Epidemiology study were included in this sub study. Only participants who had undergone dual energy X-ray absorptiometry (DXA) measurements at follow up and were HIV negative were eligible for inclusion in this study (n=209). Multiple regression models were used to explore the relationship between 25(OH)D, PTH and body composition variables. A separate metabolic syndrome variable was created, excluding elevated waist circumference as a diagnostic criterion for logistic regression. Logistic regression was used to examine the relationship between 25(OH)D, PTH, PTH:25(OH)D ratio, respectively and the metabolic syndrome.

Results: The prevalence of vitamin D deficiency was 15.9%, and 43.1% of the women had the metabolic syndrome. Hypertension was the most common (85.6%) while elevated triglyceride was the least common (27.8%) component of the metabolic syndrome. After adjusting for age, %body fat, habitual physical activity, tobacco use and season, neither 25(OH)D nor PTH concentrations showed significant associations with having the metabolic syndrome. However, when %body fat was replaced with waist circumference there was a weak positive association between 25(OH)D concentration and the metabolic syndrome. No significant association was found between PTH:25(OH)D ratio and the metabolic syndrome.

Conclusions: In conclusion, low serum 25(OH)D concentration was not associated with the metabolic syndrome in our black South African women. Although body composition variables were positively associated with PTH and the PTH:25(OH)D ratio, serum PTH was not associated with the metabolic syndrome. The association between 25(OH)D, PTH and the metabolic syndrome was not significantly mediated by adiposity.

Key words 25(OH)D; PTH; PTH:25(OH)D ratio; the metabolic syndrome, black South African women.

INTRODUCTION

The metabolic syndrome has become a global epidemic, which increases the risk of type 2 diabetes mellitus, cardiovascular morbidity and mortality [1,2]. It is a cluster of metabolic disorders that includes at least three out of the following five criteria: elevated fasting blood glucose, hypertension, abdominal obesity, elevated serum triglycerides and low serum high density lipoprotein cholesterol (HDL-C) [3].

A number of studies have indicated metabolic roles for 25 hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) [4,5]. A comparatively consistent relationship between low serum vitamin D and metabolic syndrome has been demonstrated [6-8]. However, the relationship between low serum vitamin D and metabolic traits, appear to differ among different ethnicities. In America, NHANES III data showed an inverse association between vitamin D status and insulin resistance in non-Hispanic whites and Mexican Americans, with no relationship observed in black Americans [9].

Some studies have associated vitamin D deficiency with excess body weight [10,11], which could be as a result of fat-soluble vitamin D getting trapped in excess body fat, thereby reducing its bioavailability [12]. Low serum vitamin D is also associated with elevated PTH secretion [13] and elevated PTH levels have been linked to obesity [14] and an increased risk of the metabolic syndrome [14,15]. The ratio of PTH:25(OH)D has recently been demonstrated to be positively associated with measures of insulin sensitivity [16] and with the metabolic syndrome [17] and showed that expressing the physiologic interaction between PTH and 25(OH)D as a ratio sheds more clarity on their associations with the metabolic syndrome [17].

South Africa is in the nutrition-related non-communicable disease phase of the nutrition transition [18] which has given rise to an epidemic of obesity. Previous studies in South Africa have shown a high prevalence of the metabolic syndrome among blacks and people of Asian-Indian origin [19,20]. To the best of our knowledge, only one study in South Africa has examined the association between 25(OH)D, PTH and the metabolic syndrome, but no adjustment was made for adiposity [20]. The study found an association between PTH and the metabolic syndrome, but no association with 25(OH)D [20]. We postulate that the association between 25(OH)D, PTH and the metabolic syndrome will be mediated by adiposity. Consequently, the aim of our study is to examine the association of serum

25(OH)D and PTH concentration, respectively, with the metabolic syndrome while controlling for adiposity in black women in the North West Province, South Africa.

SUBJECTS AND METHODS

Study design

The Prospective Urban and Rural Epidemiology (PURE) study is a 10 year longitudinal study aimed at tracking the effects of lifestyle and changing environment exposures on the development of non-communicable diseases in populations at different stages of epidemiologic transition [21]. The South African North West Province (NWP) arm of the PURE (PURE-SA-NWP) study commenced with baseline data collection in 2005 [22]. Using a cross sectional study design, urban black women aged ≥ 43 years measured at 7 years follow up from September 2012 to June 2013 from the PURE-SA-NWP study were included in this sub study. Only participants who had undergone dual energy X-ray absorptiometry (DXA) measurements at follow up and were HIV negative were eligible for inclusion in this study (n=209). Blood samples and DXA measurements for each participant were done on the same day and the seasons were defined as September to December 2012 for spring (season 1) and April to June 2013 for autumn (season 2). The study was approved by the Ethics committee of the North-West University (NWU), Potchefstroom campus (NWU-00016-10-A1). All participants provided written informed consent. A separate written informed consent for HIV testing was obtained from each participant after a pre-counselling session.

Body composition measurements

Height was measured to the nearest 0.1 cm with a stadiometer (Leicester height measure, Seca, Birmingham,UK) and weight on a portable electronic scale to the nearest 0.01 kg (Precision Health Scale, A & D Company, Japan) by certified anthropometrists according to standard methods of the International Society for the Advancement of Kinanthropometry (ISAK) [23]. Body fat percentage was measured by a registered radiographer with DXA (Hologic Discovery W, APEX system software version 12.7.3.1). Body mass index (BMI) was calculated (weight in kilograms divided by height in meter squared). Waist circumference was measured midway between the iliac crest and the lower margin of the lowest palpable rib in the mid-axillary line using a steel anthropometric tape measure (Lufkin, Apex USA). Abdominal obesity was defined as waist circumference ≥ 80cm [3]. Age-specific cut-offs for high body fat percentage were defined as ≥35.8% for women aged 43-49 years and ≥37.7% for women aged 50 years and above to indicate adiposity [24].

Biochemical analyses and blood pressure measurement

Registered nurses collected blood samples from the ante-brachial vein using a sterile winged infusion set and syringes following an overnight fast of at least eight hours. Serum samples were prepared and stored in aliquots in cryotubes at -80ºC. All samples were analysed at the same time with reagents from the same lot after all the samples were collected. Serum cholesterol, triglycerides (TG), HDL-C and plasma glucose were analysed on the Cobas Integra 400 Plus (Roche, Basel, Switzerland). Insulin, 25(OH)D concentrations and PTH were determined with an electrochemiluminescence immunoassay on the Elecsys 2010 (Roche, Basel, Switzerland). The inter- and intra assay coefficient of variation (CV) for 25(OH)D was 10.7% and 7.8% respectively. The inter- and intra assay CV for PTH was 6.5% and 4.1% respectively. The inter- and intra assay CV of insulin was 2.8% and 2.0% respectively. We defined vitamin D deficiency as 25(OH)D < 20 ng/mL, and vitamin D insufficiency as 25(OH)D between 21 and 29 ng/mL [25]. Elevated PTH was defined as PTH values > 65 pg/mL [26]. We calculated the PTH:25(OH)D ratio by dividing serum PTH in pg/mL by serum 25(OH)D in ng/mL. The homeostasis model assessment (HOMA) technique was used to calculate insulin resistance (HOMA-IR) based on fasting insulin and glucose [fasting insulin (µU/mL)] x [fasting glucose (mmol/L)] / 22.5 [27].

After a ten minutes rest, systolic and diastolic blood pressures were measured with a validated OMRON HEM-757 instrument (Omron Healthcare, Kyoto, Japan), using appropriate sized cuffs for participants. The measurements were carried out in duplicate (5 minutes apart) on the right upper arm, while the participants were seated upright with the right arm supported at heart level.

Questionnaires and physical activity

All countries participating in the PURE study used structured and adapted questionnaires to collect socio-demographic and lifestyle information including medication and tobacco use [21]. Trained field workers administered questionnaires in the language of choice of the participants. Habitual physical activity was measured with a modified Baecke questionnaire [28] and physical activity scores were obtained as previously described by Kruger and colleagues [22]. Habitual activity energy expenditure was also measured using an accelerometer with a combined heart rate monitor (ActiHeart®, Camtech, UK) for 7 days.

The monitor makes use of a statistical branch model to calculate energy expenditure both on activity counts and heart rate [29]. Participants were visited by field workers on a daily basis to ensure that the ActiHeart® monitor was secure and to record possible problems with wearing the device. The time spent in each physical activity intensity category by each individual was also recorded. Activity energy expenditure (AEE) was determined by means of 60 second epochs and data generated by the ActiHeart® were downloaded using a computer interface. Time and intensity of activity were related back to METS and classified as 1.1 – 2.9 METS as light-intensity activity, 3.0 – 5.9 as moderate-intensity activity and vigorous activity as >/= 6 METS [30]. Total energy expenditure and AEE were also calculated (in Kcal) with the ActiHeart® software. The reliability and validity of using the ActiHeart® to evaluate physical activity in Sub-Saharan Africans has been previously assessed [31].

Diagnosis of metabolic syndrome

Using the harmonized definition, participants with at least three of the following criteria were diagnosed to have the metabolic syndrome [3]: elevated waist circumference (≥ 80cm); hypertension (diagnosed hypertensive subjects on blood pressure medications and subjects with elevated blood pressure of systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg); elevated serum triglycerides (≥ 1.7 mmol/L); reduced serum HDL-C (<1.3 mmol/L) and subjects on oral hypoglycemic medications or elevated fasting blood glucose (≥ 5.6 mmol/L). None of the subjects were on hypolipidemic drugs. A separate metabolic syndrome variable was created, excluding elevated waist circumference as a diagnostic criterion, due to the strong collinear relationship between body fat percentage and waist circumference. For this variable, metabolic syndrome was defined as the presence of 3 out of 4 criteria. This modified definition of the metabolic syndrome was used for the logistic regression analysis, with presence of the metabolic syndrome as dependent variable and either body fat percentage or waist circumference as a covariate.

Statistical analyses

IBM SPSS version 22 (IBM Company, Armonk, NY, USA) was used for all analyses. Normally distributed data are presented as means ± standard deviation. Non-normally distributed data was logarithmically transformed and presented as medians and interquartile range. Categorical data were analysed using frequency tables and prevalence of specific conditions was expressed as percentages. Independent t-tests were used to compare normally distributed variables and Mann-Whitney U-tests to compare non-normally distributed variables between groups with metabolic syndrome and without metabolic syndrome. Analysis of covariance (ANCOVA) was used to adjust for body fat percentage while comparing means of 25(OH)D and PTH between groups with metabolic syndrome and without metabolic syndrome. Multiple regressions were used to explore the relationship between 25(OH)D, PTH and body composition variables while adjusting for age, physical activity score, tobacco use and season as possible confounders based on known relationships observed in the literature [4,32], as well as differences found between participants with and without the metabolic syndrome. Univariate prevalence odds ratio (OR) and 95% confidence intervals (CI) were calculated for 25(OH)D, PTH or PTH:25(OH)D ratio to determine the presence of the metabolic syndrome using the modified variable (excluding the elevated waist circumference component). Multivariate ORs were then calculated for 25(OH)D, PTH or PTH: 25(OH)D ratio, adjusting for age, tobacco use, physical activity, body fat percentage (or waist circumference) and season in logistic regression. Statistical significance was set at $p < 0.05$.

RESULTS

Demographic, body composition and metabolic characteristics of the participants are presented in Table 1. Using the World Health Organisation (WHO) body mass index (BMI) classification, 69.9% of the women were overweight or obese and 65.5% of the women had excessive adiposity using the age specific cut-off points for body fat percentage [24]. Two recent South African studies proposed using a higher cut-off value of waist circumference (> 92cm) to define abdominal obesity for black women [19,33]. A smaller percentage of 49.8% of the women were abdominally obese using this cut-off value compared to 71.8% when the waist circumference cut-off value of ≥80cm was used (Table 1). The prevalence of vitamin D deficiency (25(OH)D < 20 ng/mL) was 15.9%, while the prevalence of vitamin D insufficiency (25(OH)D 21 to 29 ng/mL) was 24.4%. The prevalence of elevated PTH (>65 pg/mL) was 17.7%.

Table 1: Demographic, body composition, health and lifestyle measures of the total group as well as between women with and without metabolic syndrome (n=209)*

Variable	**Total group (n=209)[a]**	**Women without Metabolic Syndrome (n=119)[a]**	**Women with Metabolic Syndrome (n=90)[a]**	**p[b]**
Age(years)	59.6 ± 10.6	59.8 ±10.5	59.4 ± 10.9	0.78
Body fat %	40.4 ± 7.41	38.5 ± 8.11	42.9 ± 5.51	<0.0001
BMI (kg/m^2)	29.5 ± 7.58	27.0 ± 7.64	32.8 ± 6.14	<0.0001
Waist circumference (cm)	89.8 ± 14.4	84.0 ± 14.8	97.3 ± 9.64	<0.0001
25(OH)D (ng/mL)	30.6 ± 9.52	31.3 ± 9.44	29.7 ± 9.61	0.24 (0.57[c])
PTH (pg/mL)	44.3 (34.2, 58.8)	41.9 (32.7, 54.3)	47.9 (35.1, 63.5)	0.09 (0.58[c])
HOMA-IR	2.67 (1.43, 4.99)	2.14 (1.00, 4.29)	3.33 (1.91, 7.53)	<0.0001
Glucose (mmol/L)	4.74 (4.27, 5.37)	4.48(4.10, 4.88)	5.35 (4.61, 6.37)	<0.0001
Triglycerides (mmol/L)	0.99 (0.74, 1.41)	0.91(0.70,1.22)	1.18 (0.81, 1.79)	<0.0001
HDL-C (mmol/L)	1.19 (0.98, 1.56)	1.46 (1.14, 1.82)	1.05 (0.86, 1.17)	<0.0001
Systolic BP(mmHg)	128.1 ± 22.8	125.0 ± 23.7	132.1 ± 21.1	0.03
Diastolic BP (mmHg)	81.2 ± 12.6	79.0± 13.5	84.0 ± 10.8	0.005
AEE (Kcal/day)	884.0 (521.25, 1622.0)	737.0 (522.0, 1314.0)	1073.0 (494.5, 1912.0)	0.09
Light-intensity activity/ day(1.1-2.9 METs) (min)	192.36 ± 39.1	195.6 ± 38.3	187.84 ± 40.2	0.19
Moderate-intensity activity/ day (3-5.9 METs) (min)	29.14 ± 37.3	26.1 ± 37.9	33.3 ± 36.3	0.20
Vigorous activity /day (>6 METs) (min)	0.65 ± 1.92	0.65 ± 2.2	0.65 ± 1.39	0.99
Physical activity score	2.08 (1.43, 2.64)	2.18 (1.61, 2.73)	1.90 (1.29, 2.60)	0.06
Tobacco users n (%)	100 (47.8)	66 (58.4)	34 (39.5)	0.01
Elevated fasting glucose n (%)	41 (19.6)	5 (4.7)	36 (44.4)	<0.0001
Elevated triglycerides n (%)	34 (16.3)	9 (8.0)	25 (27.8)	<0.0001
Reduced HDL-C n (%)	118 (56.5)	37 (32.7)	81 (90.0)	<0.0001
Hypertensive n (%)	151 (72.2)	74 (62.2)	77 (85.6)	<0.0001
Abdominal obesity: WC ≥ 80cm n (%)	150 (71.8)	62 (53.0)	88 (97.8)	<0.0001
Abdominal obesity: WC ≥ 92cm n (%)	104 (49.8)	40 (34.2)	64(71.1)	<0.0001
Excess adiposity [d] n (%)	137 (65.6)	66 (55.5)	71 (78.9)	<0.0001
Overweight/ obese n (%)	146 (69.9)	65 (55.1)	81 (90.0)	<0.0001
Vitamin D deficiency (<20 ng/mL)	32 (15.9)	16 (13.4)	16 (17.8)	0.44
Vitamin D insufficiency (21-29 ng/mL)	49 (24.4)	32 (28.3)	17 (19.3)	0.23
Elevated PTH (>65 pg/mL)	37 (17.7)	17(15.2)	20(22.2)	0.20

[a] Sample size varies due to missing values. BMI = body mass index. 25(OH)D = serum 25 hydroxy vitamin D, PTH= parathyroid hormone, HOMA-IR= Homeostasis model assessment Insulin resistance, HDL-C= high density lipoprotein cholesterol, BP= Blood pressure. AEE = activity energy expenditure.

[b] Difference between groups with and without the metabolic syndrome . t-test/Mann-Whitney test/chi-square test

[c] Difference between participants with and without the metabolic syndrome, adjusted for body fat percentage (ANCOVA)

[d] age specific cut off values for adiposity based on body fat percentage (≤ 35.8 - > 37.7 %).

Data presented as mean ± SD for normally distributed data and median (IQR) for non-normally distributed data

Actiheart® data for 184 (88.04%) women were available for analysis. Physical activity measured by Actiheart® indicated that the women spent on average 13.4% of their time (3.21 hours) in light-intensity activity (1.1- 2.9 METs), 2% in moderate-intensity activity (0.49 hours, 3-5.9 METs) with only 0.005% of total time (0.07 minutes) spent in activities representing ≥ 6 METs. In total 107 women (58.2%) accumulated 10 minutes or more on daily activities with intensity of 3-5.9 METs, while only one woman accumulated more than 10 minutes per day on activities representing ≥ 6 METs. Results of the physical activity questionnaire also showed a low mean physical activity score for the women, within the inactive range from 1 - 3.3 [22].

Using the harmonized definition the metabolic syndrome was diagnosed in 43.1% of the women with hypertension (85.6%) being the most common and elevated triglycerides (27.8%) being the least common component of metabolic syndrome (Table 1). Women with the metabolic syndrome had significantly higher body fat percentage, BMI, waist circumference and HOMA-IR (all $p<0.0001$). There was no difference between the mean serum 25(OH)D, PTH and age of women with the metabolic syndrome and women without the metabolic syndrome, also after adjusting for body fat. The odds of having the metabolic syndrome were not different in women who had insufficient serum vitamin D levels compared to those with sufficient vitamin D levels (OR 0.86, 95% CI 0.49, 1.52 $p=0.61$), neither was it significantly higher for women with elevated PTH compared to those in the normal range (OR 1.60, 95% CI 0.78, 3.27 $p=0.20$)

Vitamin D status of women measured in autumn was significantly higher than those measured in spring ($p<0.001$), with mean serum 25(OH)D of 36.5 (±7.30) and 27.5 (±9.23) ng/mL, respectively. Similarly more women were vitamin D insufficient in spring compared to autumn (55.4% vs. 16.9% respectively, $p<0.001$).

Table 2 shows the multiple regression results of the associations between body composition variables and 25(OH)D, PTH and PTH: 25(OH)D ratio. In the unadjusted models (model 1) all body composition variables were inversely associated with 25(OH)D and positively associated with PTH and PTH: 25(OH)D ratio. When age, physical activity score, tobacco use and season were adjusted for, the associations between body composition variables and 25(OH)D became borderline significant. On the other hand, all body composition variables remained positively associated with PTH even after adjustments for possible confounders (model 2). Similar associations were found between PTH: 25(OH)D ratio and waist circumference, as well as BMI. Body fat % was not significantly associated with 25(OH)D or the PTH: 25(OH)D ratio in the adjusted models.

Table 2: Multiple regression analysis for 25(OH)D, PTH or PTH:25(OH)D ratio on body composition variables

	25(OH)D		**PTH**		**PTH:25(OH)D**	
	B	p	β	p	β	P
Waist circumference						
Model 1-	Unadjusted model					
Waist circumference	**-0.19**	0.01	**0.19**	0.01	**0.18**	0.01
Model 2-Full model						
Waist circumference	-0.14	0.06	**0.22**	0.003	**0.18**	0.02
Age	-0.13	0.07	**0.27**	<0.001	**0.15**	0.04
Physical activity score	-0.06	0.38	-0.01	0.89	-0.1	0.44
Tobacco use	0.08	0.24	-0.00	0.97	0.03	0.71
Season (1=Spring; 2=Autumn)	**0.39**	<0.001	**-0.16**	0.03	**-0.21**	0.01
BMI						
Model 1-	Unadjusted model					
BMI	**-0.20**	0.004	**0.22**	0.002	**0.18**	0.01
Model 2	Full model					
BMI	-0.14	0.05	**0.23**	0.002	**0.15**	0.04
Age	-0.14	0.05	**0.29**	<0.001	**0.16**	0.04
Physical activity score	-0.06	0.40	0.00	0.97	-0.06	0.45
Tobacco use	0.07	0.30	0.00	1.00	0.02	0.77
Season (1=Spring; 2=Autumn)	**0.40**	<0.001	**-0.17**	0.002	**-0.22**	0.003
Body fat %						
Model 1	Unadjusted model					
Body fat %	**-0.15**	0.04	**0.27**	<0.001	0.14	0.05
Model 2	Full model					
Body fat %	-0.06	0.37	**0.26**	<0.001	0.10	0.21
Age	-0.11	0.12	**0.26**	<0.001	0.13	0.08
Physical activity score	-0.05	0.50	0.01	0.90	-0.07	0.39
Tobacco use	0.09	0.21	0.01	0.85	0.01	0.90
Season (1=Spring; 2=Autumn)	**0.41**	<0.001	**-0.17**	0.02	**-0.23**	0.002

Model 1 is unadjusted models 2 is adjusted for age, physical activity, tobacco use and season. WC is waist circumference, BMI is Body mass index

Logistic regression analysis was used to determine the association between 25(OH)D and PTH respectively, with the metabolic syndrome (excluding the elevated waist circumference component). Neither 25(OH)D nor PTH was significantly associated with the metabolic syndrome in unadjusted models (model 1). After adjusting for age, body fat, habitual physical activity, tobacco use and season, PTH concentrations were still not associated with the prevalence of the metabolic syndrome (model 2). However, in similar models, women with higher 25(OH)D concentration had a 6% (1.00, 1.11) higher odds of having the metabolic syndrome (Table 3, model 3). In these adjusted models physical activity and use of tobacco significantly influenced the odds of having the metabolic syndrome (models 2 and 3). Table 3 shows that women with higher habitual physical activity had 52% to 55% lower odds of the metabolic syndrome respectively (models 2 and 3). Tobacco use was inversely associated with the metabolic syndrome in the 25(OH)D models although no significant association was found in the PTH models.

In order to investigate the influence of body fat distribution, body fat % was replaced with waist circumference in these models and 25(OH)D was found to be significantly associated with the metabolic syndrome (Table 3 models 3).

Table 4 shows the logistic regression results of the association between PTH:25(OH)D ratio and the metabolic syndrome (excluding the elevated waist circumference component). No significant association was found between PTH:25(OH)D ratio and the metabolic syndrome.

Table 3: Multiple logistic regression analysis for the association between 25(OH)D or PTH and other covariates and the metabolic syndrome

Dependent variable	Metabolic syndrome				Metabolic syndrome		
	Odds ratios	95% CIs	p		Odds ratios	95% CIs	P
Model 1: Unadjusted model							
25(OH)D	1.03	0.98, 1.07	0.24	PTH	1.22	0.17, 8.83	0.84
Model 2 Full model with 25(OH)D				Full model with PTH			
25(OH)D	1.04	0.99, 1.10	0.10	PTH	0.41	0.03, 5.25	0.49
Age (years)	0.99	0.95, 1.03	0.52	Age (years)	0.99	0.95, 1.03	0.65
Body fat %	1.04	0.97, 1.11	0.28	Body fat %	1.04	0.98, 1.11	0.23
Physical activity score	0.42	0.20, 0.90	0.03	Physical activity score	0.47	0.23, 0.95	0.03
Tobacco use	0.35	0.14, 0.89	0.03	Tobacco use	0.45	0.19, 1.09	0.08
Season	1.54	0.56, 4.21	0.40	Season	2.00	0.79, 5.04	0.14
Model 3: Full model with25(OH)D or PTH, waist circumference instead of body fat percentage as covariate							
25(OH)D	1.06	1.00, 1.11	0.04	PTH	0.28	0.02, 3.34	0.31
Age (years)	1.00	0.96, 1.05	0.93	Age (years)	1.00	0.96, 1.05	0.82
Waist circumference	1.07	1.03, 1.11	0.002	Waist circumference	1.06	1.02, 1.10	0.002
Physical activity score	0.45	0.20, 0.98	0.04	Physical activity score	0.48	0.23, 0.99	0.05
Tobacco use	0.39	0.16, 1.00	0.05	Tobacco use	0.51	0.21, 1.23	0.14
Season	1.82	0.63, 5.24	0.27	Season	2.42	0.91, 6.41	0.08
Model 4: Full model without 25(OH)D				Full model without PTH			
Age (years)	0.98	0.95, 1.02	0.41	Age (years)	0.98	0.95, 1.02	0.41
Body fat %	1.03	0.92, 1.10	0.28	Body fat %	1.03	0.92, 1.10	0.28
Physical activity score	0.58	0.31, 1.10	0.09	Physical activity score	0.58	0.31, 1.10	0.09
Tobacco use	0.48	0.20, 1.12	0.09	Tobacco use	0.48	0.20, 1.12	0.09
Model 5: Full model, waist circumference instead of body fat percentage as covariate without 25(OH)D or PTH							
Age (years)	0.99	0.95, 1.04	0.76	Age (years)	0.99	0.95, 1.04	0.76
Waist circumference	1.05	1.02, 1.09	0.005	Waist circumference	1.05	1.02, 1.09	0.005
Physical activity score	0.63	0.33, 1.19	0.16	Physical activity score	0.63	0.33, 1.19	0.16
Tobacco use	0.54	0.23, 1.27	0.16	Tobacco use	0.54	0.23, 1.27	0.16

*Metabolic syndrome excluding the elevated waist circumference component

Table 4 Multiple logistic regression analysis for the association between PTH:25(OH)D the metabolic syndrome

Main independent variable		PTH:25(OH)D	
Dependent variable	Metabolic syndrome		
	Odds ratios	95% CIs	P
Model 1: Unadjusted model			
PTH:25(OH)D	1.00	0.83, 1.21	0.996
Model 2:	Full model		
PTH:25(OH)D	0.94	0.74, 1.20	0.62
Age (years)	0.98	0.95, 1.03	0.45
Body fat %	1.03	0.97, 1.10	0.30
Physical activity score	0.42	0.20, 0.87	0.02
Tobacco use	0.40	0.16, 0.98	0.04
Season	1.97	0.75, 5.16	0.17
Model 3: full model, waist circumference instead of body fat percentage as covariate			
PTH:25(OH)D	0.88	0.67, 1.17	0.38
Age (years)	1.00	0.96, 1.04	0.98
Waist circumference	1.06	1.02, 1.10	0.003
Physical activity score	0.44	0.20, 0.93	0.03
Tobacco use	0.45	0.18, 1.12	0.09
Season	2.36	0.86, 6.52	0.10

Model 1 is unadjusted model. All other models adjusted for age, body fat percentage or waist circumference, physical activity, tobacco use and season. *Metabolic syndrome excluding the elevated waist circumference component

DISCUSSION

In this study we investigated the relationships between 25(OH)D, PTH, PTH:25(OH)D ratio and the metabolic syndrome in black South African women and whether these relationships are influenced by adiposity. Our findings indicate a lack of significant associations between PTH, PTH:25(OH)D ratio and the metabolic syndrome. Positive associations between body composition variables and PTH were, however, found. A low 25(OH)D concentration was also not associated with the metabolic syndrome in our black South African women, although there were borderline inverse associations between 25(OH)D and waist circumference and BMI, respectively.

The relationships of 25(OH)D and PTH, respectively with the metabolic syndrome are controversial. Some studies show inverse associations between 25(OH)D and the metabolic syndrome [6-8, 34-36] and positive associations between PTH and the metabolic syndrome [14,15]. Other studies have reported no associations between 25(OH)D and/or PTH and the metabolic syndrome [14,37]. We found a weak positive association between 25(OH)D and the metabolic syndrome when we adjusted for waist circumference. The association was small and might not be clinically significant based on the confidence interval of 1.00 to 1.11 (Table 3 model 5). The lack of negative relationships between the metabolic syndrome and 25(OH)D, despite the high prevalence (43%) of the metabolic syndrome observed in our study could be explained in part by the low prevalence of vitamin D deficiency (15.9%) among our study participants. In comparison to our study, higher prevalence of vitamin D deficiency was found in studies that showed inverse associations between 25(OH)D and the metabolic syndrome [6-8, 34-36]. Another possible contributing factor for the lack in negative relationship may be an indication that black individuals are not sensitive to the metabolic effects of vitamin D as suggested by Scragg and colleagues [9]. Our results are in accordance with a recent study among black South Africans and South Africans of Asian-Indian origin where 25(OH)D was not associated with the metabolic syndrome [20]. The same study [20], however, found a positive association between PTH and the metabolic syndrome which is in contrast to the lack of association we found.

There was no association between PTH:25(OH)D ratio and the metabolic syndrome in our study. Our result is contrary to that of an European study among Flemish adults where they found the ratio of PTH:25(OH)D was positively associated with having the metabolic syndrome [17]. The relationship between the metabolic syndrome and PTH:25(OH)D ratio in their study was mainly driven by serum 25(OH)D concentrations [17]. The lack of association between PTH:25(OH)D ratio and the metabolic syndrome in our study could also be explained in part by the low prevalence of vitamin D deficiency and low prevalence of elevated PTH (17.7%) among our study participants. Waist circumference, which is an indicator of abdominal obesity was significantly associated with the PTH:25(OH)D ratio in our study. This is similar to the European study where a strong association between abdominal obesity and the PTH:25(OH)D ratio was found [17]. Adipose tissue acts as a reservoir for vitamin D in the body [12] and in addition to this, abdominal adipose tissue releases inflammatory cytokines which further decrease the amount of circulating 25(OH)D [38]. The PTH:25(OH)D ratio in our study is driven mainly by PTH as the PTH:25(OH)D ratio largely reflects the associations seen between PTH, body composition variables and the metabolic syndrome.

It has been previously postulated that low 25(OH)D and reactive increases in PTH were both consequences of obesity [10-12]. Some studies on the other hand have suggested that elevated PTH promotes the accumulation of adipose tissue, thereby suggesting the possibility that elevated PTH may play a role in the development of obesity [39-41]. Our results are consistent with previous reports on positive associations between PTH and measures of adiposity [39-42]. This might be an indication that black South African women are more sensitive to the metabolic roles of PTH compared to 25(OH)D as suggested by George et al. [20]. Body composition variables (BMI, body fat percentage and waist circumference) lost their significant inverse associations with 25(OH)D after adjusting for potential confounders. Waist circumference however, still tended to be inversely associated with 25(OH)D ($p = 0.07$), which supports the hypothesis that in comparison to subcutaneous fat, abdominal adipose tissue has a stronger inverse association with vitamin D status, as previously suggested [42-45]. Body fat percentage on the other hand, lost its significant relationship with 25(OH)D after adjusting for confounders, which is contrary to the results of a study carried out in white Europeans [32]. This lack of association between total body fat percentage and 25(OH)D in our study could be explained by the phenomenon of most black South African women having a larger percentage of their body fat distributed subcutaneously around their hips [46-47]. This could also be an indication that the effect of visceral fat on serum 25(OH)D is stronger compared to the effect of subcutaneous fat.

While 25(OH)D and PTH were not main contributors to the presence of the metabolic syndrome in African women in our study, other covariates such as smoking and physical activity seemed to make more prominent contributions. The protective effect of higher physical activity on the odds of having the metabolic syndrome in our study is in agreement with other studies [48-50]. It is not clear why objective measures of physical activity showed no association with the metabolic syndrome in our study. We also found a protective effect of the use of tobacco on the odds of having the metabolic syndrome, which is unexpected and we cannot fully explain the mechanism behind this. However, we propose that the relationship between tobacco use and the metabolic syndrome may, at least in part, be through the effect of smoking on body composition [51,52]. In addition, using the harmonised definition of the metabolic syndrome, we also found a protective effect of the use of tobacco on the odds of having the metabolic syndrome (data not shown). The women with a history of tobacco use in our study had significantly lower adiposity compared to those who never used tobacco (data not shown). The relationship between smoking and the metabolic syndrome has also been shown to be dose responsive with heavy smokers having a higher risk of developing metabolic syndrome in comparison to light or non-smokers [53,54]. Due to the relatively high cost of cigarettes and tobacco products in South Africa, it is likely that the women who used tobacco products among our study participants were light smokers/ tobacco chewers. The association observed between use of tobacco and the metabolic syndrome in our study might have been different if we had taken into account the quantity of tobacco used on a daily basis as previously demonstrated in other studies [53-56]. Therefore, the association found between use of tobacco and the metabolic syndrome in our study should be interpreted in the light of the peculiarities discussed above and needs further investigation.

Limitations of this study include its cross-sectional design, thus we cannot draw conclusions about causality. This study was performed in black urban women and the results may not be generalizable to the greater black South African population. Also we could not get accurate records from the self-report of the quantity of tobacco used, in order to separate the light users and the heavy ones. Despite the limitations, our study has further highlighted the differences between what constitutes as risk factors for the metabolic syndrome in black Africans compared to other ethnic groups.

In conclusion, low 25(OH)D concentration was not associated with the metabolic syndrome in our black South African women. Although PTH and PTH:25(OH)D ratio were significantly positively associated with body composition variables they were not associated with the metabolic syndrome. The association between 25(OH)D, PTH and the metabolic syndrome was not significantly mediated by adiposity. The relationship between PTH and measures of adiposity in black South African women needs to be further investigated.

AUTHORS' CONTRIBUTIONS

OFS and HSK conceptualised the study, OFS drafted the manuscript and conducted all statistical analysis. HHW, HL and HSK made substantial contributions to the drafts and were involved in critically reviewing the manuscript as well interpretation of all results. CB, CMCM, and MP were involved in critically reviewing the draft of the manuscript and contributed to data collection. EMJF was involved in critically reviewing the draft of the manuscript. All authors contributed to and approved the final manuscript.

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CHAPTER 6 GENERAL SUMMARY, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

This final chapter provides a summary of the main findings of the three articles that form part of this thesis. The results have already been interpreted, discussed and compared with relevant literature in Chapters 3, 4 and 5. The recommendations made in this thesis were based on the main findings. The main aim and objectives are repeated below for ease of reference, followed by the salient observations of the studies and recommendations.

Aims and objectives

The main aim of this study was to examine factors (vitamin D status, urbanization, socio-economic status (SES) and lifestyle risk factors) associated with body composition, including bone health, as well as predictors of change in body composition in African adults in the North West Province of South Africa.

The objectives of this study were to:

- Examine the influence of urbanization, SES and lifestyle risk factors on changes in the body composition of black South African men and women from the North West Province between 2005 and 2010.
- Examine the association between body composition (BMI, fat mass and lean mass) and bone health (BMD and fracture risk) in urban postmenopausal black South African women.
- Examine the association of serum 25(OH)D and PTH concentration with metabolic syndrome, respectively, while controlling for adiposity in urban black South African women from the North West Province.

6.2 The influence of SES and lifestyle risk factors on changes in the body composition of black South Africans

Changes in body composition over time are inevitable and expected. Our study revealed unfavourable changes in body adiposity measurements as indicated by the significant increase in BMI, waist circumference and triceps skinfold thickness in black South African adults over a 5-year period. Lifestyle changes associated with urbanization and their unfavourable associations with measures of obesity have been well documented in developing countries (Popkin *et al*., 1993; Hill *et al*., 2000; Vorster *et al*., 2011). Similarly, the present study demonstrated that urbanization was the major predictor of changes in body composition of adult South African men and women. At both baseline and follow up, women from the urban group had significantly higher measures of obesity compared to their rural counterparts. Urban residents also had a significantly higher mean dietary energy and fat intake compared to rural residents for both genders. It should be noted that even though our urban women gained significantly more in body adiposity measurements compared to their rural counterparts, the trend of changes in adiposity observed in both rural and urban areas over the 5-year period were similar. This could be an indication of unhealthy lifestyle transition also in the rural area.

SES has been reported in the literature to be inversely associated with BMI (Jeffery *et al*., 1991; Sundquist & Johansson, 1998). Interestingly, in contrast to the literature our study demonstrated that a higher SES was associated with higher measures of adiposity in both genders. This could, however, be due to the fact that only a few of our study participants had a relatively high SES, whereas the majority of our participants were classified with a low SES with no or limited formal education and mainly employed as domestic/informal workers. Compared to our study where over 80% of our participants attained a low educational level, only 25% of the European study participants were in the low educational level group (Sundquist & Johansson, 1998). It was interesting to see that being married was a significant predictor of gain in waist circumference and BMI in men, while it had no effect in women.

6.3 The association between body composition and bone health in urban postmenopausal black South African women.

Black African women were traditionally perceived to be protected from age-related bone loss with fewer incidences of non-traumatic fractures in comparison to white women (Aloia, 1996). Due to lifestyle changes associated with urbanization, however, there is an increasing concern about the loss of African women's inherent advantage of a higher BMD (Kruger *et al*., 2011). It was previously postulated that obesity is protective of bone health via its weight bearing effect (Felson *et al*., 1993; Ravn *et al*., 1999; Asomaning *et al*., 2006). However more recent studies have shown an inverse relationship between obesity and bone health (De Laet *et al*., 2005; Tanaka *et al*., 2013). Obesity prevalence among South African women is high with a national prevalence of 39.9% for black South African women (Shishana *et al*., 2013). The prevalence of obesity (47.1%), low bone mass (39.7%) and osteoporosis (14.8%) in the present study were also high which adds to the concerns about deteriorating bone health in black South African women. Our study further revealed that even though fat mass and lean mass were both independently associated with bone health, there was a stronger association between lean mass compared to fat mass and bone health in black South African women.

6.4 The association of serum 25(OH) D and PTH concentration with metabolic syndrome in urban black South African women

There is a scarcity of information on the association between serum 25(OH)D, PTH and the metabolic syndrome among black South Africans. A number of studies have linked low serum 25(OH)D and elevated PTH concentrations to an increased risk of the metabolic syndrome among Europeans and people of European descent (Reis *et al*., 2007; Ahlström *et al*., 2009; Lee *et al*., 2009). In our study, low 25(OH)D concentration was not associated with the metabolic syndrome in our black South African women. We found a weak positive association between 25(OH)D and the metabolic syndrome when we adjusted for waist circumference instead of body fat percentage. This could further corroborate the suggestions that abdominal adipose tissue exerts a more important effect on vitamin D status than subcutaneous fat (Snijder *et al*., 2005; Beydoun *et al*., 2010; Ding *et al*., 2010; Chacko *et al*., 2011). A similar study by George *et al*. (2013) found no association between 25(OH)D and the metabolic syndrome in black and Asian-Indian South Africans. The prevalence of vitamin D deficiency among our study participants was low compared to what is usually reported for Europeans and blacks living in the western countries. It has been suggested that blacks might not be sensitive to the metabolic effects of serum 25(OH)D (Scragg *et al*., 2004). There was no significant association between PTH and the metabolic syndrome in this study although body composition variables were positively associated with PTH, contrary to the positive association found between PTH and the metabolic syndrome in black and Asian-Indian South Africans by George and colleagues (2013).

In the present study PTH was positively associated with measures of adiposity. However, serum 25(OH)D only tended to be inversely associated with waist circumference, body fat percentage and BMI after possible confounders were adjusted for. The associations observed between PTH and all the measures of adiposity could be an indication that black South African women are more sensitive to the metabolic roles of PTH compared to that of 25(OH)D.

The ratio of PTH:25(OH)D has recently been demonstrated to be positively associated with measures of insulin sensitivity (Stanley *et al*., 2013) and with the metabolic syndrome (Richart *et al.*, 2011). Furthermore, we assessed the relationship between PTH:25(OH)D ratio and the metabolic syndrome and did not find an association. This is contrary to the result of an European study among Flemish adults (Richart *et al*., 2011). PTH:25(OH)D ratio in the Flemish study was mainly driven by serum 25(OH)D concentrations (Richart *et al*., 2011). The lack of association between PTH:25(OH)D ratio and the metabolic syndrome in our study could also be explained in part by the low prevalence of vitamin D deficiency and low prevalence of elevated PTH (17.7%) among our study participants. Waist circumference, which is an indicator of abdominal obesity was significantly associated with the PTH:25(OH)D ratio in our study. This is similar to the European study where a strong association between abdominal obesity and the PTH:25(OH)D ratio was found (Richart *et al.*, 2011). Adipose tissue acts as a reservoir for vitamin D in the body (Wortsman *et al*., 2000) in addition to this, abdominal adipose tissue releases inflammatory cytokines which further decrease the amount of circulating 25(OH)D (Blum *et al*., 2008). The PTH:25(OH)D ratio in our study is driven mainly by PTH as the PTH:25(OH)D ratio reflects the associations seen between PTH, body composition variables and the metabolic syndrome.

6.5 CONCLUSION

This thesis highlights the high prevalence of overweight and obesity among black South Africans in the North West province. Of note is also the higher than expected prevalence of low bone mass among the women from the same population. Urbanization played a significant role in the increasing adiposity of black South Africans in the North West province. Various factors like easier access to food, more food variety and novelty contribute to the urban environment and its effect on body composition. Although this thesis demonstrates that dietary energy and fat intakes were significantly higher in the urban compared to the rural residents, dietary fat intake was positively correlated with changes in waist circumference and triceps measurements in men and women, but regression analysis demonstrated that dietary fat intake was not a significant predictor of changes in body composition over five years. This thesis further demonstrates that both lean mass and fat mass were independently associated with bone health, but lean mass rather than fat mass had a stronger positive association with bone health. Dietary intake and physical inactivity also contributed to the bone health of the study participants and the high prevalence of overweight and obesity highlighted in this study. Furthermore, smoking and alcohol intake were also associated with the sub-optimal bone health and body composition of black South African adults in this study. Finally in this thesis, and contrary to literature, low 25(OH)D concentration was not associated with the metabolic syndrome, while there was no significant association between PTH and the metabolic syndrome in our black South African women.

6.6 RECOMMENDATIONS

The following conclusions and recommendations for future research were formulated from the papers included in this thesis:

1. Black adults in the North West province are gaining body fat. Scaling up of obesity intervention programmes in both urban and rural areas of the North West Province of South Africa is recommended.

2. Increasing lean mass rather than fat mass is beneficial to bone health. Further studies on other factors affecting lean mass and bone health of Africans are recommended.

3. Although 25(OH)D and PTH were not associated with the metabolic syndrome in this study, measures of adiposity were positively associated with PTH. Further studies on the causal relationship between PTH and measures of adiposity in black South Africans are, therefore, recommended.

4. The importance of positive lifestyle changes which includes increased habitual physical activity and healthy diet for maintenance of a healthy body fat percentage, optimal bone health and reduced odds of developing the metabolic syndrome was highlighted in this study. Public health measures at provincial and national levels should be taken to encourage such positive lifestyle changes.

5. Finally, attention should also be given to other influencing factors like smoking and alcohol intake when developing interventions for obesity, osteoporosis and the metabolic syndrome.

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ADDENDA

Addendum A: Ethical approval 2005

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E-Pos dnvealr@puk.ac.za

2 September 2004

Dr A Kruger
Bussie 594
Noordwes-Universiteit
(Potchefstroomkampus)

Geagte dr Kruger

GOEDKEURING VIR EKSPERIMENTERING MET MENSE

Hiermee wens ek u in kennis te stel dat u projek getiteld *"PURE study (Prospective Urban and Rural Epidemiology study)"* deur die Etiekkomitee goedgekeur is met nommer 04M10.

Gebruik asseblief die nommer genoem in paragraaf 1 in alle korrespondensie rakende bogenoemde projek en let daarop dat daar van projekleiers verwag word om jaarliks in Junie aan die Etiekkomitee verslag te doen insake etiese aspekte van hulle projekte asook van publikasies wat daaruit voortgespruit het. U sal in Mei 2005 die dokumentasie hieroor ontvang.

Goedkeuring van die Etiekkomitee is vir 'n termyn van hoogstens 5 jaar geldig (volgens Senaatsbesluit van 4 November 1992, art 9.13.2). Vir die voortsetting van projekte na verstryking van hierdie tydperk moet opnuut goedkeuring verkry word.

Die Etiekkomitee wens u alle voorspoed met u werk toe.

Vriendelike groete

N.T. Malan

PROF. NT MALAN
VOORSITTER: ETIEKKOMITEE

POTCHEFSTROOMKAMPUS
Privaatsak X6001, Potchefstroom, Suid-Afrika, 2520
Tel: (018) 299-1111 • Faks: (018) 299-2799
Internet: http://www.nwu.ac.za

Addendum B: Ethical approval 2010

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

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
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Web: http://www.nwu.ac.za

Ethics Committee
Tel +27 18 299 4850
Fax +27 18 293 5329
Email Ethics@nwu.ac.za

ETHICS APPROVAL OF PROJECT

2010-02-23

This is to certify that the next project was approved by the NWU Ethics Committee:

Project title : *PURE study (Prospective Urban and Rural Epidemiology study)*

Project leader / Student : Prof Annamarie Kruger

Ethics number: **NWU-00016-10-A1**

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Expiry date: 20/01/2015

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

The formal Ethics approval certificate will be sent to you as soon as possible.

Yours sincerely

Me.Marietjie Halgryn
NWU Ethics Secretariate

Addendum C: Ethical approval for PhD study

NORTH-WEST UNIVERSITY ®
YUNIBESITI YA BOKONE-BOPHIRIMA
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POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences Ethics Sub-committee
Tel: 018 2992092
Fax: 018 2992088
Email: Minrie.Greeff@nwu.ac.za

To whom it may concern

10 July 2013

Dear Mrs. Sotunde

Additional Request:

Ethics Application: NWU-00016-10-A1 "Prospective Urban and Rural Epidemiology Study (PURE SA Study)”

Your request to include the study, entitled “Body composition, bone health and vitamin D status of African adults in the North West Province” under the above mentioned umbrella project has been approved.

Yours sincerely

Prof. Minrie Greeff
Acting Chairperson

Original details: Prof. Minrie Greeff(10187308) C:\Users\13210572\Documents\ETIEK\2010 ETHICS\NWU-00016-10-A1 Additional Request 3.docm
10 July 2013

1

Addendum D: Informed consent form 2010

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

PURE-SA Project (Prospective Urban and Rural Epidemiology)
INFORMED CONSENT FORM (including the PRIMER-study)

I, the undersigned ....(full names and surname) read / listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project. I understand that I have the right to withdraw at any time from the study.

I agree to be tested for HIV	Yes	No
I want to know my HIV-status	Yes	No
I agree to give a blood sample	Yes	No

I hereby also declare that I am aware that:

1. This blood sample will be used for the purpose of
    a. Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
    b. DNA damage due to environmental factors such as cooking methods and smoke will be tested for.
    c. Testing for liver function by determining liver enzymes such as AST, GGT,
    d. Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_1C, Blood glucose and Insulin
    e. Analyses of the clotting profile and hypertension markers
    f. Analyses of bone health, iron and nutrition status

    And may be stored until such time as the above measurements/analyses will be done.
2. Body measurements such as height, weight, skin fold thicknesses, arm and leg circumferences will be taken
3. Vascular sonar will be done
4. Blood pressure to be taken
5. Pulse wave velocity measurements will be made
6. A Spirometer test to be performed to determine lung function
7. Bone density will be determined with an osteometer to detect possible osteoporosis

....

(Signature of the subject)

Signed at **... Potchefstroom / Ganyesa ...** (delete not applicable option) on **........./........./ 2010**

Witnesses

1. .... **2.** ....

Signed at **... Potchefstroom / Ganyesa ...** (delete not applicable option) on **........./........./ 2010**

PART 1

1. **Research Unit and Faculty:**
Africa Unit for transdisciplinary Health research (AUTHeR); Faculty of Health Sciences, North-West University

2. **Title of project/trial:**
PURE: Prospective Urban and Rural Epidemiological study

3. **Full names, surname and qualifications of project leader:**
Prof. Annamarie Kruger, Ph.D. (Nutrition)

4. **Rank/position of project leader:**
Research Director and project manager

5. **Aim of this project**
PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.

6. **Explanation of the nature of all procedures, including identification of new procedures:**
Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire, psychology questionnaire) with the help of field workers. A blood sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and bone density will be performed.

6. **Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)**
It will take each participant quite a while (about 6 [six] hours) to complete all the tests and questionnaires. Discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.

8. **Precautions taken to protect the subjects:**
The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.

9. **Description of the benefits which may be expected from this project:**
When measures with immediate results are taken, such as blood glucose, haemoglobin, HIV status and blood pressure, the information will be communicated to the individual to seek professional help during an individual feedback session. Since this study is a longitudinal study, subjects that are high at risk will be identified over time and personal feedback and counselling will be given.
On the research site each participant will receive a cooked meal, a fruit and fruit juice. Also an amount of R50 will be paid to each participant to cover transport costs and enable each person to go for follow-up at a clinic of choice.

10. **Alternative procedures which may be beneficial to the subjects:**
There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.

PART 2

To the subject signing the consent:

You are invited to participate in a research project. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:

1. **Participation in this project is voluntary.**

2. **It is possible that you personally will not derive any benefit from participation in this project, although the health knowledge obtained from the results may be beneficial to you and other people.**

3. **You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.**

4. **The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.**

5. **We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.**

6. **The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.**

7. **All information will be kept CONFIDENTIAL and no personal information will be published without my consent.**

Prof ANNAMARIE KRUGER

Contact details: 082 771 5778 / 018 299 2095(Office)

Addendum E: Adult questionnaire

PURE/South Africa

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line: (unless otherwise instructed)

X

OR

By writing number(s) in the spaces provided:

1 8

OR

By specifying the answer on the line(s) provided

April 28, 2005

Adult Questionnaire

Subject Initials- F= first letter of first name
M= first letter of middle name
L= first letter of last name

3. National I.D#

If not applicable please mark the N/A box

Ethnicity Codes

01 - South Asian (India, Sri Lanka, Pakistan, Bangladesh)

02 - Chinese (China, Hong Kong, Taiwan)

03 - Japanese

04 - Malays

05 - Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal,Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)

06 - Persian

07 - Arab

08 - Black African

09 - Coloured African (Subsaharan African only)

10 - European

11 - Native North/South American or Australian Aborigine

12 - Latin American (Latino)

13 - Bantu/Semi Bantu

14 - Hemitic/Semi Hemitic

15 - Nilotic/Hausa

16 - Pygmie

17 - Swahili

18 - Other (any other ethnoracial group not listed above)

PURE Adult Questionnaire Page 1

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

Today's date: year month day

1. **Name:** ______________ ______________
   Given name Surname

2. **Not applicable in South Africa**

3. **National identity # or equivalent:** ______________ **N/A** ☐

4. **DOB:** year month day **OR** **Age** yrs

5. **Sex:** ☐ Female ☐ Male

6. **Marital status:** (check one only)

☐ Never married ☐ Currently married ☐ Common law/Living with partner
☐ Widowed ☐ Separated ☐ Divorced

7. **Ethnicity:** → (Please refer to facing page for codes)

8. **Caste/Tribe:** ______________

9. **What level of formal education have you completed?** (check highest level only):

☐ None
☐ Primary
☐ Secondary/highschool/higher secondary
☐ Trade School
☐ College/University
☐ Unknown

Adult Questionnaire

11. Occupation

Group 1: Legislators, senior officials and managers
Legislators and senior officials
Corporate managers
General managers
Businessman

Group 2: Professionals
Physical, mathematical and engineering science professionals
Life science and health professionals
Teaching professionals
Other professionals

Group 3: Technicians and associate professionals
Physical, mathematical and engineering-science associate professionals/technicians
Life science and health associate professionals/technicians
Teaching associate professionals/technicians
Other associate professionals/technicians

Group 4: Clerks
Clerks
Customer service clerks

Group 5: Service workers and shop and market sales workers
Personal and protective services workers
Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers
Market-oriented skilled agricultural and fishery workers
Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers
Extraction and building trade workers
Metal, machinery and related trades workers
Precision, handicraft, printing and related trades workers
Other craft and related trades workers

Group 8: Plant and machine operators and assemblers
Stationary plant and related operators
Machine operators and assemblers
Drivers and mobile plant operators

Group 9: Elementary occupations
Sales and services elementary occupations
Agricultural, fishery and related labourers
Labourers in mining, construction, manufacturing and transport

Group 10: Armed forces
Armed forces

Group 11: Homemaker
Housewife/Househusband

PURE Adult Questionnaire **Page 2**

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

10. Not applicable in South Africa

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.
(Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

☐ Group 1 ☐ Group 2 ☐ Group 3 ☐ Group 4 ☐ Group 5
☐ Group 6 ☐ Group 7 ☐ Group 8 ☐ Group 9 ☐ Group 10 ☐ Group 11

c) Not applicable in South Africa

d) What is your main source of income? ______________________

If occupation is group 11 (homemaker) go to question 13

12. Are you currently employed?

☐ No → (answer 12a - 12b) ☐ Yes → Go to #13

a) Are you retired/stopped work from your primary occupation due to old age? ☐ No ☐ Yes

b) Have you stopped working due to illness? ☐ No ☐ Yes

PURE Adult Questionnaire **Page 3**

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

13. CURRENT DISABILITY:

		No	Yes
a)	Do you have any problems using your fingers to grasp or handle?	☐	☐
b)	Do you have any trouble walking about?	☐	☐
c)	Do you have any trouble bending down and picking up an object from the floor?	☐	☐
d)	Do you require a walking stick cane/walker to move about?	☐	☐
e)	Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn)	☐	☐
f)	Do you have trouble seeing a person from across the room? (12 feet/3.5 meters) (with glasses worn)	☐	☐
g)	Do you have trouble speaking and being understood?	☐	☐
h)	Do you have any trouble hearing what is said in a normal conversation?	☐	☐

Subject Medical History

14. Have you experienced any of the following in the last six months?

	No	Yes		No	Yes
a) Chest pain or tightness with usual activity	☐	☐	i) Vomiting	☐	☐
If Yes, → does the pain spread to the back, neck or inner border of arm	☐	☐	j) Loss of appetite	☐	☐
b) Breathlessness with usual activity	☐	☐	k) Painful or bleeding teeth/gums	☐	☐
c) Cough for at least 2 weeks	☐	☐	l) Jaundice	☐	☐
d) Any sputum while coughing	☐	☐	m) Burning while passing urine	☐	☐
e) Blood in sputum	☐	☐	n) Swelling of feet	☐	☐
f) Wheezing or whistling in the chest	☐	☐	o) Swelling of face	☐	☐
g) Early morning cough with chest tightness	☐	☐	p) Blood in urine	☐	☐
h) Loose stools/diarrhea for at least 3 days	☐	☐	q) Involuntary weight loss of > 3kg	☐	☐

15. Not applicable in South Africa

16a) Do you use glasses/spectacles/contact lenses at present? No ☐ Yes ☐

b) Do you use a hearing aid? No ☐ Yes ☐

Adult Questionnaire

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

Centre # Community# Household # Subject #

Subject Initials F M L

17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis
a) Diabetes	☐	☐	☐☐
b) Hypertension/ high blood pressure	☐	☐	☐☐
c) Stroke	☐	☐	☐☐
d) Angina/heart attack/ Coronary artery disease	☐	☐	☐☐
e) Heart failure	☐	☐	☐☐
f) Other heart disease	☐	☐	☐☐
h) Hepatitis/Jaundice	☐	☐	☐☐
g) Cancer	☐	☐	☐☐

Please refer to facing page for cancer sites ☐☐ site ______ other, specify

	No	Yes	#of yrs since diagnosis
i) COPD	☐	☐	☐☐
j) Asthma	☐	☐	☐☐
k) Tuberculosis	☐	☐	☐☐
l) Malaria	☐	☐	☐☐
m) Chagas	☐	☐	☐☐
n) HIV/AIDS (Not answered ☐)	☐	☐	☐☐

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? ☐ No → **go to 19** ☐ Yes

a) If yes, for what conditions:

	No	Yes
Blood pressure	☐	☐
Cholesterol lowering drugs	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Asthma	☐	☐
Chinese medicine	☐	☐
Others	☐	☐ → If Yes, specify ______
Unknown	☐	☐

Adult Questionnaire

18b) If name of medication is unknown, please list as unknown.

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

18b) List all the medications you are <u>currently</u> consuming at least once a week for the last month?

i) ______ ii) ______

iii) ______ iv) ______

v) ______ vi) ______

vii) ______ viii) ______

Men go to question #23

For Women Only (Questions 19 - 22)

19. Are you currently pregnant ? ☐ No ☐ Yes → Go to #21

20. Do you still have periods? ☐ No → (answer 20a) ☐ Yes → Go to #21

a) How many years since you stopped menstruating? ☐☐ years

21. Have you ever used an oral/ injectable contraceptive? ☐ No ☐ Yes

22a) How many live children have you given birth to? ☐☐ Boys ☐☐ Girls

b) Did you breast feed any of your children? ☐ No ☐ Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

1= Factory/industrial place
2= Office
3= Agriculture field/farm
4= Home
5= Road
6= Sport/game e.g. track, court, field, etc.
7= Public building
8= Mine/quarry
9= Construction site e.g. building, road-works, etc.
10 = Other

Type of Injury

1= Burns
2= Scalds
3= Fractures
4= Muscle and ligament sprains/tears
5= Cuts and lacerations
6= Bruises and abrasions
7= Suffocation
8= Head injury (where person did not lose consciousness)
9= Head injury (where person lost consciousness for some time)

Subject ID

☐☐ Centre # ☐☐☐ Community# ☐☐☐ Household # ☐☐ Subject #

Subject Initials ☐☐☐ F M L

23. **During the <u>past 12 months</u>, have you had any injuries that were serious enough to limit your normal activities?** (check all that apply)

☐ No → **Go to #24** ☐ Yes → **(answer 23a - 23s)**

If yes, please provide details:

Please refer to facing page for Location and Type Codes

Cause of injury	No	Yes	Location	Type	Absence from work or usual activities (Days)
a) Motor vechicle accident (as a passenger)	☐ No	☐ Yes →	☐☐	☐	☐☐☐
b) Motor vehicle accident (as a pedestrian)	☐ No	☐ Yes →	☐☐	☐	☐☐☐
c) Struck by an object	☐ No	☐ Yes →	☐☐	☐	☐☐☐
d) Explosion	☐ No	☐ Yes →	☐☐	☐	☐☐☐
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	☐ No	☐ Yes →	☐☐	☐	☐☐☐
f) Suffocation	☐ No	☐ Yes →	☐☐	☐	☐☐☐
g) Poisoning	☐ No	☐ Yes →	☐☐	☐	☐☐☐
h) Snake/scorpion bite	☐ No	☐ Yes →	☐☐	☐	☐☐☐
i) Fall	☐ No	☐ Yes →	☐☐	☐	☐☐☐
j) Fire/flames, resultant fumes	☐ No	☐ Yes →	☐☐	☐	☐☐☐
k) Physical assault (gun, kidnapping, etc.)/violent crime	☐ No	☐ Yes →	☐☐	☐	☐☐☐
l) Domestic violence (beaten by a family member)	☐ No	☐ Yes →	☐☐	☐	☐☐☐
m) Drowning/submersion	☐ No	☐ Yes →	☐☐	☐	☐☐☐
n) Hot or corrosive liquids/floods/substances	☐ No	☐ Yes →	☐☐	☐	☐☐☐
o) Crush injuries (boulders, building materials, etc.)	☐ No	☐ Yes →	☐☐	☐	☐☐☐
p) Accident caused by machinery	☐ No	☐ Yes →	☐☐	☐	☐☐☐
q) Attempted suicide	☐ No	☐ Yes →	☐☐	☐	☐☐☐
r) Armed conflict	☐ No	☐ Yes →	☐☐	☐	☐☐☐
s) Other(specify) ________	☐ No	☐ Yes →	☐☐	☐	☐☐☐

Adult Questionnaire

Location of Fractures

1= Hip/pelvis
2= Thigh
3= Leg
4= Forearm
5= Wrist
6= Hand/finger
7= Vertebrae (back)
8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter "0"

PURE **Adult Questionnaire** **Page 7**

Subject ID

Centre # ☐☐ Community# ☐☐☐ Household # ☐☐☐ Subject # ☐☐

Subject Initials ☐☐☐ F M L

24. Have you ever fractured a bone? ☐ No (go to #25) ☐ Yes (if yes, answer a),b) and c)

a) Number of fractures ☐☐

b) Years since last fracture ☐☐ (yrs)

c) Bone (s) broken in the most recent fracture(if more than 3, list most severe sites) (location) If other, specify

Please refer to facing page for fracture locations

☐ → ____________

☐ → ____________

☐ → ____________

Tobacco

25. Which best describes your history of tobacco use?

a) ☐ Formerly used tobacco products ☐ Currently use tobacco products ☐ **Never used tobacco products** → **Go to #26**

b) At what age did you start? ☐☐ yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

	Average amount/day		Duration (years)	Past users only: When Stopped (years ago)	Past users only: If less than 1 yr (months ago)
(i) Cigarettes (all kinds)	☐☐☐	number	☐☐	☐☐	☐☐
(ii) Beedies	☐☐☐	number	☐☐	☐☐	☐☐
(iii) Cigars	☐☐☐	number	☐☐	☐☐	☐☐
(iv) Pipes	☐☐☐	number	☐☐	☐☐	☐☐
(v) Sheesha/water pipe Hookah	☐☐☐	# of times	☐☐	☐☐	☐☐
(vi) Chewing tobacco	☐☐☐	# of times	☐☐	☐☐	☐☐
(vii) Snuff	☐☐☐	# of times	☐☐	☐☐	☐☐
(x) Other ________ *Specify*	☐☐☐		☐☐	☐☐	☐☐

PURE **Adult Questionnaire** **Page 8**

Subject ID

Centre # Community# Household # Subject #

Subject Initials F M L

Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

☐ No → **Go to #27** ☐ Yes → Please answer questions **26a**

a) Over the past 12 months, what has been your typical exposure to other peoples smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other peoples smoke)

Select ONE only

☐ 1-2 times/week ☐ 3-6 times/week ☐ at least once a day ☐ 2-3 times/day ☐ 4 or more times/day

27. Not applicable in South Africa

Adult Questionnaire

28c) Alcoholic Beverage: Regular use is defined as at least once a month.

Subject ID

Centre # Community# Household # Subject #

Subject Initials F M L

28. Which best describes your history of alcohol use?

a) ☐ Formerly used alcohol products ☐ Currently use alcohol products ☐ **Never used alcohol products** → **Go to #29**

b) At what age did you start? ☐☐ yrs

c) What forms of alcohol have you regularly used? (check all that apply)

Form of Alcohol	Approx. size of one "drink"	Frequency Daily	Weekly	Monthly	Average # of drinks	Duration (years)	Past users only When Stopped (years ago)
(i) Spirits(rum,whisky, gin,vodka etc)	30ml	☐	☐	☐	☐☐	☐☐	☐☐
(ii) Wine	125ml	☐	☐	☐	☐☐	☐☐	☐☐
(vi) Beer	375ml	☐	☐	☐	☐☐	☐☐	☐☐
(vii) Country liquor/arrack/ sugar cane spirit	30ml	☐	☐	☐	☐☐	☐☐	☐☐

d) **At least once a month, do you consume >5 alcoholic drinks/day?** ☐ No → **Go to #29** ☐ Yes

If yes,(i,ii)

i) How many times per month do you consume >5 alcoholic drinks in a day? ☐☐

ii) What is the average number of drinks that you consume each time? ☐☐

29 a) During your longest or nocturnal sleep period, what time do you normally go to bed? ☐☐:☐☐ (00:00-23:59)

b) During your longest or nocturnal sleep period, what time do you normally wake up? ☐☐:☐☐ (00:00-23:59)

c) Do you usually take naps/siestas? ☐ No ☐ Yes *Total nap duration* → ☐☐☐ mins

33. **Civic organization**: are defined as non-profit, voluntary organization societies, self help groups and clubs.

Religious organization: are defined as different types of formal and informal groups set up on a religious basis.

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Subject ID

Centre # | Community# | Household # | Subject #

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30. Are you a member of any of the following:

How often do you participate in the activities of this group?

	No	Yes	Per Month	OR	Per Year
(i) Self help group, Co-operative, Social club, Sports club,	☐ No	☐ Yes →	☐☐		☐☐
(ii) Religious Group (e.g. church group, etc.)	☐ No	☐ Yes →	☐☐		☐☐
(iii) Other ______ *Specify*	☐ No	☐ Yes →	☐☐		☐☐

31. Please answer the following: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
(i) People are generally honest and want to help others.	☐	☐	☐	☐
(ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them.	☐	☐	☐	☐

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree	Not Applicable
(i) Cooking oil	☐	☐	☐	☐	☐
(ii) Flour	☐	☐	☐	☐	☐
(iii) Rice/ Maize meal	☐	☐	☐	☐	☐

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree	Not Applicable
(i) Soft drinks	☐	☐	☐	☐	☐
(ii) Snacks	☐	☐	☐	☐	☐
(iii) Cigarettes	☐	☐	☐	☐	☐
(iv) Alcohol	☐	☐	☐	☐	☐

33. In a difficult situation, whose help can you count on from?(Please see facing page for definitions)

(i) Civic organizations: specify ______

☐ none ☐ little ☐ moderate/average ☐ a great deal

(ii) Religious organizations: specify ______

☐ none ☐ little ☐ moderate/average ☐ a great deal

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Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

34. Have you experienced any of the following events during the last 12 months?

	No response	No	Yes	
(i) Loss of job	☐	☐	☐	
(ii) Retirement	☐	☐	☐	
(iii) Loss of crop/business failure	☐	☐	☐	
(iv) Household break in	☐	☐	☐	
(v) Marital separation/divorce	☐	☐	☐	
(vi) Other major intra-family conflict	☐	☐	☐	→ Please specify ______
(vii) Major personal injury or illness	☐	☐	☐	
(viii) Violence	☐	☐	☐	
(ix) Armed conflict/war	☐	☐	☐	
(x) Death of a spouse	☐	☐	☐	
(xi) Death/major illness of another close family member	☐	☐	☐	
(xii) Other major stress	☐	☐	☐	→ Please specify ______
(xiii) Wedding of family member	☐	☐	☐	
(xiv) New job	☐	☐	☐	
(xv) Birth in the family	☐	☐	☐	
(xvi) Seperation from family	☐	☐	☐	
(xvii) Unavailability of food/ food insecurity	☐	☐	☐	

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Subject ID

☐☐ Centre # ☐☐☐ Community# ☐☐☐ Household # ☐☐ Subject #

Subject Initials ☐☐☐ F M L

35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

	No response	Never Experienced Stress	Some Period of Stress	Several Periods of Stress	Permanent Stress
a) How often have you felt stress at work in the last 12 months? (Mark here if not applicable: i.e. no longer working ☐)	☐	☐	☐	☐	☐
b) How often have you felt stress at home in the last 12 months?	☐	☐	☐	☐	☐

36. What level of financial stress have you felt in the last 12 months?

☐ No response ☐ Little/none ☐ Moderate ☐ High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

☐ No ☐ Yes → **If yes, during those times, did you:**

	No response	No	Yes
a) Lose interest in most things like hobbies, work or activities that usually give you pleasure?	☐	☐	☐
b) Feel tired or low on energy?	☐	☐	☐
c) Gain or lose weight?	☐	☐	☐
d) Have more trouble falling asleep than you usually do?	☐	☐	☐
e) Have more trouble concentrating than usual?	☐	☐	☐
f) Think a lot about death (either your own, someone else's, or death in general)	☐	☐	☐
g) Feel down on yourself, no good or worthless?	☐	☐	☐

PURE Adult Questionnaire Page 13

Subject ID

☐☐ Centre # ☐☐☐ Community# ☐☐☐ Household # ☐☐ Subject #

Subject Initials ☐☐☐ F M L

38. Please answer the following: (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	☐	☐	☐	☐
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	☐	☐	☐	☐
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	☐	☐	☐	☐
d) My neighbourhood streets are well lit at night.	☐	☐	☐	☐
e) I can see other people when I am walking in my neighbourhood.	☐	☐	☐	☐
f) I can speak to other people when I am walking in my neighbourhood.	☐	☐	☐	☐
g) There is a high crime rate in my neighbourhood.	☐	☐	☐	☐
h) There is a problem with unattended dogs in my neighbourhood.	☐	☐	☐	☐

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

38a) Please answer the following: (Please check all that apply)

i) Has your household been a victim of the following crime(s) in the last 12 months?

	No	Yes
1. Armed robbery	☐	☐
2. Violent attacks	☐	☐
3. Murder	☐	☐
4. Vehicle hijacking	☐	☐
5. House breaking	☐	☐
6. Theft	☐	☐
7. Rape	☐	☐
8. Women abuse eg. (beat,swear-words,sexual) please specify ______	☐	☐
9. Child abuse eg. (burn,swear-words,rejection) please specify ______	☐	☐
10. Child sexual abuse	☐	☐
11. Other, please specify ______	☐	☐

ii) Do you think that crime in your area has increased in the past 5 years? ☐ No ☐ Yes

if yes, which of the following crime(s)?

- ☐ Armed robbery
- ☐ Violent attacks
- ☐ Murder
- ☐ Vehicle hijacking
- ☐ House breaking
- ☐ Theft
- ☐ Rape
- ☐ Women abuse
- ☐ Child abuse
- ☐ Child sexual abuse
- ☐ Other, please specify ______

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? ☐ No ☐ Yes

if yes, which of these people: (please mark all that apply)

- ☐ Your children
- ☐ Your grandchildren
- ☐ Your spouse
- ☐ Your family members
- ☐ Your friends
- ☐ People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

☐ Younger than 10 years ☐ Between 11-20 years ☐ Between 21-30 years

☐ Between 31-40 years ☐ Between 41-50 years ☐ Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- ☐ Spouse
- ☐ Parents
- ☐ Family member
- ☐ Child.children
- ☐ Friends
- ☐ Volunteer

38c) Do you care for any orphans in your family? ☐ No ☐ Yes

Adult Questionnaire

40b) Health History:

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

39. How long would it take you to get from your house to the nearest facility <u>if you walked</u>?

	Minutes	Don't know		Minutes	Don't know
i) grocery/convenience store		☐	iv) video store		☐
ii) bank		☐	v) non-fast food restaurant		☐
iii) post office		☐	vi) fast food restaurant		☐

40a) **Total number of siblings**

b) **Health History:** Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes	If yes →
Diabetes	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Coronary Heart Disease	☐	☐	☐	☐	☐	☐	☐	☐	☐	
High Blood Pressure	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Stroke	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Cancer (Please refer to facing page for cancer sites)	☐	☐	☐	☐	☐	☐	☐	☐	☐	
→ if Yes, indicate site										
	Other, Specify			Other, Specify			Other, Specify			

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

Subject ID

☐☐ Centre # ☐☐☐ Community# ☐☐☐ Household # ☐☐ Subject #

Subject Initials ☐☐☐ F M L

41. **Physical Measurements**

a) Sitting Right arm blood pressure
#1 ☐☐☐ Systolic ☐☐☐ Diastolic mmHg
#2 ☐☐☐ Systolic ☐☐☐ Diastolic mmHg

b) Heart Rate
#1 ☐☐☐ beats/min
#2 ☐☐☐ beats/min

c) Waist
#1 ☐☐☐.☐ cm
#2 ☐☐☐.☐ cm
→ ☐ minimal/no clothing ☐ full clothing

d) Weight ☐☐☐.☐ kg → ☐ minimal/no clothing ☐ full clothing

e) Hip
#1 ☐☐☐.☐ cm
#2 ☐☐☐.☐ cm
→ ☐ minimal/no clothing ☐ full clothing

f) Height ☐☐☐.☐ cm (without shoes)

42a) Circumference of mid upper right arm: ☐☐.☐ cm

b) Circumference of right calf: ☐☐.☐ cm

c) Head Circumference: ☐☐☐.☐ cm

d) Upper flexed arm circumference ☐☐.☐ cm

43a) Right arm triceps skinfold:
#1 ☐☐☐.☐ mm
#2 ☐☐☐.☐ mm
#3 ☐☐☐.☐ mm

b) Right calf skinfold:
#1 ☐☐☐.☐ mm
#2 ☐☐☐.☐ mm
#3 ☐☐☐.☐ mm

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Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

c) Biceps skinfold
#1 ___ . _ mm
#2 ___ . _ mm
#3 ___ _ mm

d) Subscapular skinfold
#1 ___ . _ mm
#2 ___ . _ mm
#3 ___ _ mm

e) Supra spinal skinfolds
#1 ___ . _ mm
#2 ___ . _ mm
#3 ___ _ mm

44 a) Humerous breadth __ . _ cm

b) Femur breadth __ . _ cm

45. Grip Strength (Maximal contraction):

a) Non-dominant hand: #1 __ kg. #2 __ kg. #3 __ kg.

b) Dominant hand: #1 __ kg. #2 __ kg. #3 __ kg.

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

American Thoracic Society criteria for acceptable spirograms:
Spirograms are acceptable if they are free from:

1. Cough during exhalation
2. Early termination or cut-off
3. Variable effort
4. Leaks
5. Obstructed mouth piece

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

46. Spiromentry:

a) FEV1 (Litre): #1 ☐. ☐☐ #2 ☐. ☐☐ #3 ☐. ☐☐

b) Does FEV1 obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to **c)**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

c) FVC (Litre): #1 ☐. ☐☐ #2 ☐. ☐☐ #3 ☐. ☐☐

d) Does FVC obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to **e)**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

e) PEFR (Litre/min): #1 ☐☐☐ #2 ☐☐☐ #3 ☐☐☐

f) Does PEFR obtained meet ATS criteria?

☐ No → (answer (i) to (ii) ☐ Yes → Go to **Q#47**

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Less than 3 values ☐

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

47. Not applicable in South Africa

48. ECG obtained? No ☐ → Go to #49 Yes ☐

a) 2 0 ☐☐ ☐☐ ☐☐ → Place ECG :File Label Here
year month day

b) Please print ECG label #: ☐☐☐☐☐☐

49 a) Blood sample obtained? No ☐ → Go to #50 Yes ☐

b) ☐ Fasting sample ☐ Non-fasting sample

c) 2 0 ☐☐ ☐☐ ☐☐ Time ☐☐:☐☐ → Hours since any food/beverage consumed (excluding water) ☐☐
year month day (00:00-23:59)

d) Please print Blood label #: ☐☐☐☐☐☐ Place Blood label here

50 a) Urine sample obtained? No ☐ → Go to #51 Yes ☐

b) ☐ Fasting sample ☐ Non-fasting sample

c) Please print Urine label #: ☐☐☐☐☐☐ Place Urine label here

51. Name of Interviewer: *(please print)* ________ *First Initial* ________ *Last Name* Interviewer Code: ☐☐☐

Attitudes on HIV/AIDS

Page 13

39. Mark each answer with a X

J.1	**Imagine that a hospital has only one free bed left, and two people with pneumonia need it. The one person is infected with HIV; the other is not infected with HIV. Who should get the bed?**	1	**The HIV positive person**	
		2	**The HIV negative person**	
		3	**It depends / other**	
		9	**Don't know**	

Please respond to the following questions by answering "Yes" or "No".

If you are not sure, chose the "Probably Yes" or "Probably No" response.

If you are quite sure, Choose the "Definitely Yes" or "Definitely No" response.

		Definitely yes 1	Probably yes 2	Probably no 3	Definitely no 4	Don't Know 9
J.2	Do you think the government should provide free health care for people who need it?					
J.3	Do you think the government should provide free health care for people with AIDS?					
J.4	Would it be a good idea for the government to give job training to unemployed young people?					
J.5	Should youth who are infected with HIV get this job training?					
J.6	Should all people who are too sick to work get a welfare grant from the government?					
J.7	Should someone with AIDS who is too sick to work get a welfare grant from the government?					
J.8	Should a woman who got AIDS from sleeping around with many men get this welfare grant from the government?					
J.9	Would you be willing to look after a close family member with AIDS?					
J.10	Imagine that you find out that one of your friends is HIV infected. Would you still be friends with them?					
J.11	Would you drink from the same bottle of water as an HIV infected friend?					
J.12	If you knew that a shopkeeper had HIV/AIDS, would you buy fresh vegetables from him or her?					
J.13	Do you think it should be illegal for people with HIV/AIDS to put others at risk of infection through unprotected sex?					
J.14	Do you think people with HIV/AIDS should have to disclose their HIV status to the person they are going to have sex with *even if they use a condom*?					

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		Definitely yes 1	Probably yes 2	Probably no 3	Definitely no 4	Don't Know 9
J.15	Imagine you meet someone you really like and he/she tells you that he/she is HIV positive, would you still go out on a "date" with him/her?					
J.16	If you loved an HIV positive person, would you have sex with them using a condom?					
J.17	Would you prefer to know who has HIV/AIDS in your community so that you can be careful not to get infected by them?					
J.18	Do you worry that HIV is much easier to catch than we are told?					
J.19	Would you rather not touch someone with HIV/AIDS because you are scared of infection?					
J.20	Do you think the names of people with HIV/AIDS should be made public?					
J.21	Do you think HIV/AIDS is a punishment for sleeping around?					
J.22	Do you think that a school pupil with HIV puts other pupils in their class at risk of infection?					
J.23	Do you think a school pupil with HIV should be allowed to attend school?					
J.24	Do you think that many people who get HIV infected through sex have only themselves to blame?					
J.25	Do you think that some people with HIV/AIDS want to infect other people with the virus?					
J.26	When you hear the word "AIDS" what community or group of people first comes to mind					

		Tick only one		
J.27	Between a rich and a poor person: Who is more likely to get HIV/AIDS?	1	A rich person	
		2	A poor person	
		3	Neither / the same	
		9	Don't know	
J.28	Between a black and a white person: Who is more likely to get HIV/AIDS?	1	A black person	
		2	A white person	
		3	Neither / the same / both	
		9	Don't know	
J.29	Between a man and a woman: Who is more likely to get HIV/AIDS?	1	A man	
		2	A woman	
		3	Neither / the same / both	
		9	Don't know	

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		Tick only one		
J.30	*I am not going to ask you to tell me your result but*: Have you ever had an HIV test?	1	Yes	
		2	No	
		8	Refuse	
		9	Don't know	
J.31	Have you heard of any HIV positive people in this area?	1	Yes	
		2	No	
		9	Don't know	
J.32	Do you think people with HIV/AIDS often get treated unfairly or badly by others?	1	Yes	
		2	No	
		9	Don't know	
J.33	Have you met any HIV positive people yourself?	1	Yes	
		2	No	Skip to J.35
		9	Don't know	
J.34	If yes: What is your relationship with this person or people? **Interviewer: do not read list. You can tick more than one**	1	Partner (Husband/wife, boyfriend, girlfriend)	
		2	Sister/brother	
		3	Parent	
		4	Other relative	
		5	Friend	
		6	Neighbour	
		7	Classmate	
		8	Colleague at work/	
		9	Other / none of the above	
		10.	Refuse to answer	
J.35	If you knew you were infected with the HIV virus, would you keep it a secret from most people?	1	Yes	
		2	No	
		9	Don't know	
J.36	If you told someone, who would you tell? **Interviewer: do not read list; multi-mentions possible You can tick more than one**	1	Partner (Husband/wife, boyfriend, girlfriend)	
		2	Sister/brother	
		3	Parent	
		4	Other relative	
		5	Friend	
		6	Neighbour	
		7	Priest / someone in my church	
		8	School teacher	
		10	Anyone	
		11	Other	
		99	Don't know	
J.37	In your opinion, how at risk are *you* of HIV infection? **Interviewer: read list Tick only one**	1	No risk	
		2	Very small risk	
		3	Some risk	
		4	Great risk	
		9	Don't know	

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J.38	Imagine you had unprotected sex yesterday. Would you would be more afraid of pregnancy or of infection with HIV? **Interviewer: do not read list Tick only one**	1	More afraid of pregnancy	
		2	More afraid of HIV infection	
		3	Afraid of both pregnancy and infection	
		4.	Afraid of neither	
		9	Don't know	
J.39	Who do you think is more likely to get HIV: someone with one sexual partner who does not use condoms or someone with many partners who always uses condoms? **Interviewer: Tick only one**	1	A person with one partner who does not use condoms.	
		2	A person with many partners who always uses condoms.	
		3	They are at equal risk	
		9	Don't Know	

Adult Questionnaire

40. Cause of death

1= Heart disease
2= Stroke
3= TB
4= Cancer
5= HIV
6= Injury
7= Other
8= Unknown

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other

Date of death:

When completing the date of death, enter actual year and month of death.

Example: If a respondent indicates that the date of death was March 2004, enter as follows

Is this member alive? [X] No [ ] Yes → Year [0][4] Month [0][3]

If exact month is not known, please obtain an approximate guess

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PURE **Adult Questionnaire** **Page 17**

Subject ID

[ ][ ] Centre # [ ][ ][ ] Community# [ ][ ][ ] Household # [ ][ ] Subject #

Subject Initials [ ][ ][ ] F M L

40. Details on all members (enumerated at baseline) in the household:

Member # [ ] *Name* ____________ **Is this member a participant?** [ ] **No** [ ] **Yes**

Is this member alive? [ ] **No** → **Complete details below** [ ] **Yes** → **Go to Next Member #**

If No, indicate cause of death: [ ][ ] Date of death: [ ][ ] **YR** [ ][ ] **Mo**

If Cause of death =4, please indicate site [ ][ ] If Cause of death =7, please specify, ____________

Member # [ ] *Name* ____________ **Is this member a participant?** [ ] **No** **Yes**

Is this member alive? [ ] **No** → **Complete details below** [ ] **Yes** → **Go to Next Member #**

If No, indicate cause of death: [ ][ ] Date of death: [ ][ ] **YR** [ ][ ] **Mo**

If Cause of death =4, please indicate site [ ][ ] If Cause of death =7, please specify, ____________

Member # [ ] *Name* ____________ **Is this member a participant?** [ ] **No** **Yes**

Is this member alive? [ ] **No** → **Complete details below** [ ] **Yes** → **Go to Next Member #**

If No, indicate cause of death: [ ][ ] Date of death: [ ][ ] **YR** [ ][ ] **Mo**

If Cause of death =4, please indicate site [ ][ ] If Cause of death =7, please specify, ____________

Member # [ ] *Name* ____________ **Is this member a participant?** [ ] **No** [ ] **Yes**

Is this member alive? [ ] **No** → **Complete details below** [ ] **Yes** → **Go to Next Member #**

If No, indicate cause of death: [ ][ ] Date of death: [ ][ ] **YR** [ ][ ] **Mo**

If Cause of death =4, please indicate site [ ][ ] If Cause of death =7, please specify, ____________

Adult Questionnaire

40. Cause of death

1= Heart disease
2= Stroke
3= TB
4= Cancer
5= HIV
6= Injury
7= Other
8= Unknown

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other

Date of death:

When completing the date of death, enter actual year and month of death.

Example: If a respondent indicates that the date of death was March 2004, enter as follows

Is this member alive? [X] No [ ] Yes → Year [0][4] Month [0][3]

If exact month is not known, please obtain an approximate guess

Subject ID

Centre # [ ][ ] Community# [ ][ ][ ] Household # [ ][ ][ ] Subject # [ ][ ]

Subject Initials [ ][ ][ ] F M L

40. Details on all members (enumerated at baseline) in the household:

Member # [ ] *Name* ____________ **Is this member a participant?** [ ] **No** **Yes**

Is this member alive? [ ] **No** → **Complete details below** [ ] **Yes** → **Go to Next Member #**

If No, indicate cause of death: [ ][ ] Date of death: [ ][ ] **YR** [ ][ ] **Mo**

If Cause of death =4, please indicate site [ ][ ] If Cause of death =7, please specify, ____________

Name of Interviewer: (Please print) ____________ **Last Name** ______ **First Initial** **Date** [ ][ ][ ][ ] *year* [ ][ ] *month* [ ][ ] *day*

Addendum F: Physical activity questionnaire

YUNIBESITI YA BOKONE-BOPHIRIMA
NORTH WEST UNIVERSITY
NOORDWES UNIVERSITEIT

PURE-SA Project (Prospective Urban and Rural Epidemiology)

Physical activity questionnaire

Date: Place: Interviewer:

The information on this questionnaire is confidential

1. Subject number — (1-4)
2. Gender — Male 1 / Female 2 — (5)
3. What is your main occupation?................................

Low level: office work, housework, scholar	1	
Middle level: factory work, carpentry, farming, hospital nurse, plumber	2	(6)
High level ("sweat work"): construction work, digging, manual labour	3	

4.	At work I sit	1. never	2. seldom	3. sometimes	4.often	5.always	(7)
5.	At work I stand	1. never	2. seldom	3. sometimes	4.often	5.always	(8)
6.	At work I walk	1. never	2. seldom	3. sometimes	4.often	5.always	(9)
7.	At work I lift heavy loads	1. never	2. seldom	3. sometimes	4.often	5.always	(10)
8.	At work I am tired	1.never	2.seldom	3. sometimes	4.often	5.always	(11)
9.	At work I sweat	1.never	2.seldom	3. sometimes	4.often	5.always	(12)

10. If you work away from home, how do you get to work/school? — walk 1 / cycle 2 / car/taxi 3 — (13)
11. How long does it take you to walk/cycle to work/school? (or to the taxi rank/ bus stop/ train station) — 0-15 min 1 / 16-30 min 2 / 31-60 min 3 / 1-2 hours 4 — (14)
12. If you walk or cycle to work/school, what is your usual pace? (or to taxi rank/bus stop/ train station) — casual strolling 1 / fairly brisk 2 / brisk/fast 3 — (15)
13. Do you climb stairs often? — yes 1 / no 2 — (16)
14. If yes, how many flights of stairs do you climb each day? (1 flight = 10 steps) — (17)
15. How many days per week do you climb steps? — (18)
16. Do you play sport? — yes 1 / no 2 — (19)
17. Which sport do you play most frequently?

low level: bowling, golf, billiards	1	0.76*[1]
middle level: tennis, athletics, cycling	2	1.26
high level: soccer, rugby, netball, boxing	3	1.76(20)

18. How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4 (Write appropriate code in space) 0.5, 1.5, 2.5, 3.5, 4.5*[2] — (21-23)
19. How many months per year ? <1/ 1-3/ 4-6/ 7-9/ >9 (Write appropriate code in space) 0.04, 0.17, 0.42, 0.67, 0.92*[3] — (24-26)

*[1] intensity code of sport, *[2] time code for sport, *[3] proportion of year

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20. If you play a second sport, which is it?

low level: bowling, golf, billiards	1	0.76*[1]
middle level: tennis, athletics, cycling	2	1.26
high level: soccer, rugby, netball, boxing	3	1.76(27)

21. How many hours per week do you practice? <1/ 1-2/ 2-3/ 3-4/ >4 0.5, 1.5, 2.5, 3.5, 4.5*[2] — (28-30)
22. How many months per year? <1/ 1-3/ 4-6/ 7-9/ >9 0.04, 0.17, 0.42, 0.67, 0.92*[3] — (31-33)

23.	During leisure time I watch TV/ do sitting activities (read, needle-work, play cards)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways	(34)
24.	During leisure time I walk/ do standing activities (gardening, housework)	1.never	2.sel-dom	3.some-times	4.often	5.al-ways	(35)
25.	Other leisure-time activities:.................... (leisure-time = time off from work/. school)		2.sel-dom	3.some-times	4.often	5.al-ways	(36)

Addendum G: Bone Health Questionnaire

PURE STUDY

BONE HEALTH QUESTIONNAIRE

GENERAL QUESTIONS

QUESTION	YES	NO	COMMENTS
1. Have you fallen in the past **12 months**?			
2. If yes to above question, how many times have you fallen?			
3. Have you previously reported any falls to a health professional?			
4. If yes to above question, how many falls have you reported?			
5. Have you broken/fractured any bones **after the age of 50 yrs?**			
6. If yes to above question, which location? Hip/pelvis Thigh Leg Forearm Wrist Hand/finger Vertebrate (back) Other (specify)			
7. Have anyone in your family ever been diagnosed with osteoporosis? Mother Sister Other (Specify)			
8. Have anyone in your family **AFTER the age of 50 yrs** ever broken/fractured a bone due to a fall? Mother Sister Other (specify)			
9. If yes to above question, which location? Hip/pelvis Thigh Leg Forearm Wrist Hand/finger Vertebrate (back) Other (specify)			
10. Have anyone in your family ever developed a stooped posture (hump of the back) as they got older? Mother Sister Other (Specify)			
11. When you fall, do you need help to get back up from the ground			
12. Have you experienced a near fall? (E.g. slip, trip, stumble or bumped against a wall)			
13. Have you limited any of your activities or decreased how much you leave your home due to a fall, near fall, or fear of falling?			
14. Do you have vision problems?			
15. Is your vision blurry and not as sharp?			
16. Do you have difficulty seeing to the side or different distances?			
17. Is your vision sensitive to light or changing light?			
18. Do you have decreased feeling, numbness or tingling in your feet?			
19. Do you sometimes feel unsteady when you walk?			

20. Do you think your walking method puts you at risk for falling?			
21. Do you choose not to use a gait aid even though people tell you it is safer?			
22. Do you have problems or concerns getting in/on or out of a bed, chair, tub or toilet?			
23. Do you feel you have decreased balance?			
24. Do you sometimes feel of balance, dizzy or unsteady when you walk?			
25. Do you feel you have leg weakness or legs that tire easily when you walk?			
26. Do you have any sore joints or arthritis?			
27. Is your activity limited by pain?			

Addendum H: BLACK FRACTURE INDEX

BLACK FRACTURE INDEX

The assessment tool, called the FRACTURE Index, is comprised of a set of seven variables that include age; BMD T-score, fracture after age 50 years, maternal hip fracture after age 50, weight less than or equal to 125 pounds (56 kg), smoking status, and use of arms to stand up from a chair. The FRACTURE Index was shown to be **predictive of hip fracture, as well as vertebral and non-vertebral risk fractures**. In addition, this index was validated using the EPIDOS fracture study. The FRACTURE Index can be used either with or without BMD testing by **older postmenopausal women** or their clinicians to **assess the 5-year risk of hip and other osteoporotic fractures**, and could be useful in helping to determine the need for further evaluation and treatment of these women.

		POINT VALUE
1	What is your current age?	
	Less than 65 yrs	0
	65-69 yrs	1
	70-74 yrs	2
	75-79 yrs	3
	80-84 yrs	4
	85 yrs or older	5
2	Have you broken any bones **after age**	
	50 yrs?	1
	Yes	0
	No	
3	Has your mother had a hip fracture	
	after age 50 years?	
	Yes	1
	No	0
4	Do you weigh 56kg or less?	
	Yes	1
	No	0
5	Are you currently a smoker?	
	Yes	1
	No	0
6	Do you usually need to assist yourself in standing up from a chair?	
	Yes	2
	No	0
	TOTAL	

Score:
Low Risk = 0-3
Medium Risk = 4-6
High Risk = 7 and above

Addendum I: Copyright licence and Language editing certificate

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