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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: *Objectives:* 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.

Key words: 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

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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.

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 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

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/cm2)	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 (%)

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
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
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

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 R^2	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 R^2	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).

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

In Table 5 where lean mass and fat mass were included in the same model, lean mass ($\beta = 0.45$, $p<0.001$) was positively associated with femoral neck BMD, while fat mass ($\beta = 0.05$ $p = 0.65$) 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.

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 which 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 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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References

1. Rosen CJ, Bouxsein ML. Mechanisms of Disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheumatol 2006;2:35–43
2. Kanis JA, on behalf of the WHO scientific group. Assessment of osteoporosis at the primary health-care level. Technical report. WHO Collaborating Centre, University of Sheffield, UK 2008;1 339
3. WHO: Obesity and overweight http://www.who.int/mediacentre/factsheets/fs311/en/. Accessed 15 November 2013
4. Shisana O, Labadarios D, Rehle T, Simbayi L, Zuma K, Dhansay A, et a. South African National Health and Nutrition Examination Survey (SANHANES-1). HSRC Press; Cape Town 2013;136–140
5. Ravn P, Cizza G, Bjarnason NH, Thompson D, Daley M, Wasnich RD, McClung M, Hosking D, Yates AJ, Christiansen C. Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. J Bone Miner Res 1999;14:1622–1627
6. Felson DT, Zhang Y, Hannan MT, Anderson JJ. Effects of weight and body mass index on bone mineral density in men and women: the Framingham study. J Bone Miner Res 1993;8:567–573
7. Asomaning K, Bertone-Johnson E, Nasca PC, Hooven F, Pekow PS. The Association between Body Mass Index and Osteoporosis in Patients Referred for a Bone Mineral Density Examination. J Womens Health 2006;15:1028–1034
8. Tanaka S, Kuroda T, Saito M, Shiraki M. Overweight/obesity and underweight are both risk factors for osteoporotic fractures at different sites in Japanese postmenopausal women. Osteoporos Int 2013;24:69–76
9. Ong T, Sahota O, Tan W, Marshall L. A United Kingdom perspective on the relationship between body mass index (BMI) and bone health: A cross sectional analysis of data from the Nottingham Fracture Liaison Service. Bone 2014;59:207–210
10. Namwongprom S, Rojanasthien S, Mangklabruks A, Soontrapa S, Wongboontan C, Ongphiphadhanakul B. Effect of fat mass and lean mass on bone mineral density in postmenopausal and perimenopausal Thai women. Int J Womens health 2013;5:87–92
11. Park J, Song YM, Sung J, Kim YS, Kim T, Cho S, Lee K. The association between fat and lean mass and bone mineral density: The Healthy Twin Study. Bone 2012;50:1006–1011
12. Liu Y, Xu Y, Wen Y, Guan K, Ling W, He L, Su Y, Chen Y. Association of Weight-Adjusted Body Fat and Fat Distribution with Bone Mineral Density in Middle-Aged Chinese Adults: A Cross-Sectional Study. PLoS ONE 2013;8 (5): e63339. doi:10.1371/journal.pone.0063339
13. Hsu Y, Venners SA, Terwedow HA, Feng Y, Niu T, Li Z, Laird N, Brain JD, Cummings SR, Bouxsein ML, Rosen CJ, Xu X. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. Am J Clin Nutr 2006;83:146–154
14. Nur H, Toraman NF, Arica Z, Sarier N, Samur A. The relationship between body composition and bone mineral density in postmenopausal Turkish women. Rheumatol Int 2013;33:607–612
15. Vorster HJ, Venter CS, Kruger MC, Vorster HH, Kruger HS. Impact of urbanisation on risk factors for osteoporosis in postmenopausal black South African women. J Endocrin Metab Diabet S Afr 2002;7:92–99
16. Kruger MC, De Winter RM, Becker PJ, Vorster HH. Changes in markers of bone turnover following urbanisation of black South African women. J Endocrinol Metab Diabet S Afr 2004;9:8–14
17. Kruger MC, Kruger IM, Wentzel-Viljoen E, Kruger A. Urbanization of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. Nutr Res 2011;31:748–758
18. Chantler S, Dickie K, Goedecke JH, Levitt NS, Lambert EV, Evans J, Joffe Y, Micklesfield LK. Site-specific differences in bone mineral density in black and white premenopausal South African women. Osteoporos Int 2012;23:533–5
19. George JA., Micklesfield L, Norris S, Crowther N. The association between body composition, 25 (OH) D and PTH, and bone mineral density in black African and Asian Indian population groups. J Clin Endocrinol Metab, 2014. http://dx.doi.org/10.1210/jc.2013-3968
20. Micklesfield LK, Gray J, Taliep MS. Bone mineral density and body composition of South African cricketers. J Bone Mineral Metab 2012;30(2):232-237.
21. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S (2009) The Prospective Urban Rural Epidemiology (PURE) study: Examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. Am Heart J 2009;158:1–7
22. Marfell-Jones M, Olds T, Stewart A, Carter L. International standards for anthropometric assessment. Australia: The international society for the advancement of Kinanthropometry, 2006;
23. Kanis JA, Adachi JD, Cooper C, Harvey N, Clark P, Cummings SR, Diaz-Curiel M, Hiligsmann M, Papaioannou A, Pierroz DD, Silverman SL, Szulc P, and the Epidemiology and Quality of Life Working Group of IOF. Standardising the descriptive epidemiology of osteoporosis: Recommendations from the Epidemiology and Quality of Life Working Group of IOF. Osteoporos Int 2013;24:2763–2764
24. Hough S, Ascott-Evans B, Brown SL. NOFSA guideline for the diagnosis and management of osteoporosis: Guideline abstract. J Endocrinol, Metab Diabet S Afr 2010;15(3):107-108
25. Venter CS, MacIntyre UE & Vorster HH. The development and testing of a food portion photograph book for use in an African population. J Hum Nutr Dietet 2000;13:205–218
26. Wentzel-Viljoen E, Laubscher R, Kruger A. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. S Afr J Clin Nutr 2011;24:143–148
27. Kruger HS, Venter CS, Steyn HS. A standardised physical activity questionnaire for a population in transition: the Thusa study. Afr J Phys Health Education Recreat Dance 2000;6:54–64
28. Wolmarans P, Danster N, Dalton A, Rossouw K, Schonfeldt H (eds). Condensed food composition tables for South Africa. Medical Research Council, Parow Valley, Cape Town 2010;1–126
29. Black DM, Steinbuch M, Palermo L, Dargent-Molina P, Lindsay R, Hoseyni MS, Johnell O. An assessment tool for predicting fracture risk in postmenopausal women. Osteoporos Int 2001;12:519–528
30. Thandrayen K, Norris SA, Micklesfield, LK, & Pettifor JM. Fracture patterns and bone mass in South African adolescent- mother pairs: The birth to twenty cohort. Osteoporos Int 2014;25(2):693-700.
31. Assah FK, Ekelund U, Brage S, Wright A, Mbanya JC, Wareham NJ (2011) Accuracy and validity of a combined heart rate and motion sensor for the measurement of free-living physical activity energy expenditure in adults in Cameroon. Int J Epidemiol 2011;40(1):112–120
32. Miles J & Shevlin M (2001) Applying regression and correlation: a guide for students and researchers. Sage, London, 2001
33. Gjesdal CG, Eide GE, Tell GS, Brun JG, Halse JI (2008) Impact of lean mass and fat mass on bone mineral density: The Hordaland Health Study. Maturitas 2008;59:191–200
34. Cui LH. Shin M, Lee Y, Choi J, Kweon S, Park K, Chung E & Nam H (2007) Relative contribution of body composition to bone mineral density at different sites in men and women of South Korea. J Bone Mineral Metab 2007;25(3):165-171.
35. Law MR, Hackshaw AK. A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: Recognition of a major effect. BMJ 1997;315(7112):841-846.
36. Daniel M, Martin AD, Drinkwater DT. Cigarette smoking, steroid hormones, and bone mineral density in young women. Calcif Tissue Int 1992;50(4):300-305.
37. Bjarnason NH, Christiansen C. The influence of thinness and smoking on bone loss and response to hormone replacement therapy in early postmenopausal women. J Clin Endocrinol Metab 2000;85(2):590-596.
38. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif Tissue Int 2001;68(5):259-270.
39. Van der Voordt DJM, Dinant GJ, Geusens PP. Risk factors for osteoporosis related to their outcome: Fractures. Osteoporos Int 2001;12:630–638

40. De Laet, C ,Kanis JA, McCloskey EV, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, Mellstrom D, Melton III, L.J, Meunier PJ, Reeve J, Silman A, Tenenhouse A, Pols HAP. Body mass index as a predictor of fracture risk: A meta-analysis. Osteoporos Int 2005;16:1330–1338
41. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–693
42. Young KA, Norris JM, Engelman CD, Langefeld CD, Hairston KG, Haffner SM, Bryer-Ash M. Association of plasma vitamin D levels with adiposity in Hispanic and African Americans. J Clin Endocrinol Metab 2009;94:3306–3313
43. Tieland M, Van Loon LJC, De Groot LCPGM, Brouwer-Brolsma EM, Nienaber-Rousseau C. Low vitamin D status is associated with reduced muscle mass and impaired physical performance in frail elderly people. Eur J Clin Nutr 2013;67:1050–1055
44. Minghetti PP, Norman AW. 1,25(OH)2-vitamin D3 receptors: gene regulation and genetic circuitry. Federation of Am Soc for Experimental Biol J 1988;2:3043–3053
45. NIH. Calcium: Dietary supplements fact sheets, 2013. http://ods.od.nih.gov/factsheets/Calcium-HealthProfessional. Accessed 10th February 2014
46. Pedrera-Zamorano J, Calderon-García JF, Roncero-Martin R, Mañas-Nuñez P, Moran JM & Lavado-Garcia J. The protective effect of calcium on bone mass in postmenopausal women with high selenium intake. J Nutr Health Aging 2012;16(9):743-748.
47. Heaney RP. Dairy and bone health. J Am Coll Nutr 2009;28:82S–90S
48. Lötters FJB, Lenoir-Wijnkoop I, Rocher E, Fardellone P, Rizzoli R, Poley MJ. Dairy foods and osteoporosis: An example of assessing the health-economic impact of food products. Osteoporos Int 2013;24:139–150
49. Gunn CA, Weber JL, Kruger MC. Diet, weight, cytokines and bone health in postmenopausal women. J Nutr Health Aging 2014;18 (5):479-486
50. Coin A, Perissinotto E, Enzi G, Zamboni M, Inelmen EM, Frigo AC, Manzato E, Busetto L, Buja A, Sergi G. Predictors of low bone mineral density in the elderly: the role of dietary intake, nutritional status and sarcopenia. Eur J Clin Nutr 2008;62:802–809
51. Stewart KJ, Deregis JR, Turner KL, Bacher AC, Sung, Hees PS, Tayback M, Ouyang P. Fitness, fatness and activity as predictors of bone mineral density in older persons. J Intern Med 2002;252:381–388
52. Camhi SM, Katzmarzyk PT. Total and femoral neck bone mineral density and physical activity in a sample of men and women. Appl Physiol Nutr Metab 2012;37:947–954
53. Saraví FD, Sayegh F. Bone mineral density and body composition of adult premenopausal women with three levels of physical activity. J Osteoporos doi. org/10.1155/2013/953271, 2013
54. Morseth B, Emaus N, Wilsgaard T, Jacobsen BK, Jørgensen, L. Leisure time physical activity in adulthood is positively associated with bone mineral density 22 years later. The Tromsø study. Eur J Epidemiol 2010;25:325–331
55. Pisciottano MVC, Szejnfeld VL, Castro CHDM, Pinto SS. The relationship between lean mass, muscle strength and physical ability in independent healthy elderly women from the community. J Nutr Health Aging 2014;18 (5):554-8
56. Muir JM, Ye C, Bhandari M, Thabane L, Adachi JD. The effect of regular physical activity on bone mineral density in post-menopausal women aged 75 and over: A retrospective analysis from the Canadian multicentre osteoporosis study. BMC Musculoskelet Disord , 2013;doi: 10.1186/1471-2474-14-253
57. Ray WA, Griffin MR, Downey W, Melton LJ. Long-term use of thiazide diuretics and risk of hip fracture. Lancet 1989;1:687–690
58. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. A prospective study of thiazide use and fractures in women. Osteoporos Int 1997;7:79–84
59. Recker RR, Davies KM, Hinders SM, et al. Bone gain in young adult women. J Am Med Association 1992;268:2403–2408.
60. Lloyd R, Hind K, Carroll S, Cooke C, Micklesfield LK, Truscott JG, Parr B, Davies S. A pilot investigation of load-carrying on the head and bone mineral density in premenopausal, black African women. J Bone Miner Metab 2010;28:185–190
61. Micklesfield LK, van der Merwe L, Lambert EV. Lifestyle questionnaire to evaluate risk for reduced bone mineral density in women. Clin J Sports Med 2005;15 (5): 340–348
62. Looker AC, Orwoll ES, Johnston CCJ., Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP. Prevalence of low femoral bone density in older U.S. adults from NHANES III. J Bone Miner Res 1997;12:1761–1768
63. Micklesfield LK, Norris SA, Pettifor JM. Ethnicity and bone: A South African perspective (report). J Bone Mineral Metab 2011;(3):257–267