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ORIGINAL ARTICLE

Association between bone characteristics and cardiovascular risk factors among adults in selected urban areas in Selangor, Malaysia

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Abstract

Introduction: Cardiovascular disease and osteoporosis (OP) have been shown to have similar risk factors but studies have demonstrated contradictory results with regards to their associations. This study evaluated relationships between bone characteristics and cardiovascular risk factors among adults in selected urban areas in Malaysia. *Materials and Methods:* A cross-sectional study was performed involving 331 subjects between 45-90 years recruited at a health screening programme. Sociodemographic and clinical characteristics were recorded. Biochemical analyses on fasting blood samples and dual energy X-ray absorptiometry scan to determine bone mineral density (BMD) were performed. *Results:* Increased waist circumference (WC) was protective for abnormal BMD status (osteopenia and OP). Males with increased high-density lipoprotein cholesterol (HDL) were more likely to be osteoporotic. WC, fasting blood glucose (FBG) and triglyceride (TG) were positively associated with BMD at all sites but was gender specific. In contrast, WC was negatively associated with trabecular bone score (TBS) for females but this association became attenuated when adjusted for fat percentage. HDL and MetS were negatively and positively associated with BMD, respectively in males. *Conclusion:* The cardiovascular risk factors of raised WC, FBG, TG and low HDL were significantly associated with increased BMD with skeletal site and gender specific differences after adjusting for confounders. However, a higher WC was associated with a weaker skeletal microstructure reflected by lower TBS in females driven by fat percentage. A higher BMD was demonstrated among MetS individuals. These findings suggest that adiposity may have a protective effect on BMD.

Keywords: adiposity, bone density, cardiovascular risk factors, Metabolic Syndrome, osteopenia, osteoporosis

INTRODUCTION

The world's population aged 60 years and older is estimated to be 2 billion by 2050[1] and 15% of Malaysia's population will be more than 60 years old by 2030.[2] Cardiovascular disease (CVD) and osteoporosis (OP) are two significant public healthcare issues with increased morbidity and mortality.[3] The rising proportion of the ageing population globally indicates that urgent action is required to tackle the projected burden of these chronic diseases. In Malaysia, ischaemic heart disease is the major cause of mortality; in both genders as well as in the three major ethnicities (Malays, Chinese and Indians), contributing to 17% of all deaths in 2020.[4] OP, on the other hand, is a silent disease and the health and economic impact of the disease results from fractures. Due to an ageing population, it is estimated that over 1 million people in Malaysia are at risk from OP.[5]

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Although CVD and OP are considered independent non-communicable diseases, a direct relationship between these chronic diseases is suggested.[6] Both conditions are affected by hormonal, nutritional, genetic, lifestyle and metabolic factors. Several studies have demonstrated that OP shares similar risk factors and pathogenesis with CVD including sedentary lifestyle, raised oxidative stress, inflammation, sex hormone deficiency and smoking. *In vivo* animal studies have demonstrated that each component of Metabolic Syndrome (MetS) including abdominal obesity, dyslipidaemia, hypertension and hyperglycaemia has a distinct impact on bone health.[7,8] In contrast, findings from epidemiological research are inconclusive on the association between MetS and OP.[7] A few studies showed that an atherogenic lipid profile is associated with a lower bone mineral density (BMD), the possible contributing factor being lipids affecting bone and blood vessels concurrently.[9,10] On the other hand, no relationship between lipid profile and BMD could be demonstrated by others.[11] Understanding this link in pathophysiology is important for the prevention and treatment of these disorders. Common biomarkers can be used as tools for early identification of individuals at higher risk for both CVD and OP.

The escalating prevalence of OP and CVD globally with its increased morbidity and mortality notwithstanding the contradictory results on their association stresses the need for further research on this topic. Hence, this study evaluated the associations between bone characteristics such as BMD, trabecular bone score (TBS), and biochemical parameters of bone homeostasis with cardiovascular (CV) risk factors in Malaysian adults.

MATERIALS AND METHODS

Subjects

This cross-sectional study involved 331 subjects between 45-90 years who attended health screening at a specialist clinic in Malaysia, as previously described in a study by Yeap *et al*.[12] Subjects on lipid-lowering or anti-diabetic medications were also excluded in addition to what was already stated in the exclusion criteria.[12] The study was approved by The Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia [FPSK (FR16) P002].

Data collection

The subjects were invited via brochures, and given a detailed information sheet together with a verbal explanation about the study. Those who agreed to participate signed a standardised consent form. Each subject was interviewed by the researcher and sociodemographic factors and clinical characteristics were recorded in the pro-forma. Anthropometric measurements, namely weight, height, waist circumference (WC) and blood pressure (BP) measurement were taken. Body mass index (BMI) was calculated using the formula: weight /height squared and categorised as underweight/normal (<18.5-24.9kg/m^2) and overweight/obese (≥25 kg/m^2).[13] MetS was defined based on the criteria by the Joint Interim Statement as the presence of at least three out of five risk factors: 1) central obesity (WC: males ≥90cm and females ≥80cm (Asian cut-off); 2) Elevated TG >1.7mmol/L; 3) Low high-density lipoprotein cholesterol (HDL): males <1.0mmol/L and females <1.3mmol/L; 4) Elevated BP: systolic BP (SBP) ≥130mmHg or diastolic BP (DBP) ≥85mmHg; and 5) Disorders of glycaemia that include type 2 DM, impaired glucose tolerance or impaired fasting glucose.[14] Dyslipidaemia was defined as at least one lipid parameter [total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL) or HDL] outside the reference range.[15]

Biochemical analysis

10mls of fasting blood was drawn from each subject between 8am to 9am for laboratory investigations that included 25-hydroxyvitamin D [25(OH)D], and parathyroid hormone (PTH) analysed on ADVIA Centaur (Siemens Healthcare Diagnostics Inc., Germany) while fasting serum lipid (TC, TG and HDL), fasting blood glucose (FBG), alkaline phosphatase (ALP), total calcium (Ca), phosphate (PO_4) and magnesium (Mg) were measured on Roche Cobas® c311 and procollagen type I N propeptide (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX) on Roche Cobas® e411 (Roche Diagnostics, Germany). Adjusted calcium, LDL (Friedwald formula) and Non-HDL (TC minus HDL) are calculated values. Calibration and quality control for all assays were performed in conjunction with normal laboratory operations.

Assessment of BMD, TBS and whole body composition

A dual-energy X-ray absorptiometry (DXA)

scan was done to measure BMD (g/cm^2), TBS as well as fat and lean body mass, using a HOLOGIC Discovery W densitometer (Hologic Corporation, Bedford, MA, USA). BMD for lumbar spine (LS), left femoral neck (L-FN), left total hip (L-TH) and total body (TB) were measured and categorised as normal, osteopenia (OPe) and OP based on T-scores using the World Health Organization classification. A T-score greater than -1.0, between -1 and -2.5 and less than -2.5 was classified as normal, OPe, and OP, respectively.[16] Although DXA is the gold-standard for OP diagnosis, BMD alone is not adequate for assessment of bone strength. Hence, TBS, an indirect textural index of trabecular microarchitecture, which assesses pixel gray-level variations in the LS DXA image[17] was assessed using TBS iNsight® software (v.2.1, Med-Imaps, Merignac, France) directly computed from the same spine DXA image. A ratio of fat mass over TB mass was used to estimate fat mass percentage (%). The precision of the machine is ± 2%. The reference population used was the machine manufacturer's Asian population database. All scans were performed in the same densitometer, operated by the same person and analysed by a trained radiographer.

Statistical analysis

Analysis was performed using the statistical software package, IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp). For normally distributed continuous variables, mean and standard deviation (SD) were reported and bivariate analysis was done by using independent sample t-test. Median and interquartile range (IQR) were reported for not normally distributed continuous variables while Mann-Whitney U and Chi-Square analyses were used for bivariate testing. ANOVA and Kruskal Wallis Test was used for comparison of normally distributed and skewed data between the three BMD groups: normal, OPe and OP. Multiple linear regression was used to analyse the relationship between BMD at all skeletal sites (LS, L-TH, L-FN, TB) and TBS with CV risk factors [MetS status and its components (WC, SBP, DBP, FBG, TG, HDL) and dyslipidaemia status]. Binary logistic regression was used to analyse the association between the occurrence of abnormal BMD status (OPe + OP) with CV risk factors as well as the occurrence of MetS with bone parameters (adjusted Ca, PO_4, Mg, PTH, 25(OH)D, ALP, CTX, P1NP, TBS, BMD at all skeletal sites and BMD status). For all subjects, analysis was adjusted to the following confounders: (i) Model 1: age, gender, race; and (ii) Model 2: Model 1 + fat percentage. For males: (i) Model 1a: age, race; and (ii) Model 2a: Model 1a + fat percentage. For females: Model 1a, Model 2a with an additional Model 3: Model 2a + menopausal status. P-value of < 0.05 was considered statistically significant.

RESULTS

The mean age of subjects was 61.6 ± 9.7 years. Majority were females (66.2%: post-menopausal women, 58.3%, pre-menopausal, 7.9%) and Chinese (51.4%), followed by Indians (29.9%) and Malays (18.7%). Most of them were in the underweight/normal category (53.8%) but had a raised WC (68.3%). Majority did not smoke (96.4%) or consume alcohol (84.9%). More than half were hypertensive (60.1%) and dyslipidaemic (83.1%). Subjects were of equivalent proportion for normal (41.4%) and OPe (41.4%), whilst 17.2% were in the OP category. Prevalence of MetS was 39.9%. Medians (IQR) of biochemical parameters were within normal range except for TC [5.5 (1.09) mmol/L], LDL [3.35 (0.95) mmol/L] and Non-HDL [3.92 (1.01) mmol/L].

In Table 1, there were significant difference in age, gender, race and menopausal status between the BMD groups. BMI, WC, LS-BMD, L-FN-BMD, L-TH-BMD, TB-BMD, TBS, FBG showed a significant decreasing trend across the normal to OP groups, whereas bone turnover markers (ALP, CTX and P1NP) showed a significant increasing trend. There was also a significant difference in Mg, TG and HDL between BMD groups.

In Table 2, MetS status was significantly associated only with race. Subjects with MetS had significantly higher median values of BMI, WC, SBP, DBP, FBG, TG and mean values of LS-BMD, L-FN-BMD, L-TH-BMD and fat percentage than subjects without MetS. Whereas, mean values of 25(OH)D and median values of Mg and HDL were significantly lower in the MetS group compared with non-MetS group.

Among CV risk factors, only WC and HDL remained significant for abnormal BMD status (OPe and OP) after adjusting for confounders in Table 3. Increased WC was protective for abnormal BMD status in all subjects as well as in males and females after adjustment with the strongest association seen in females (highest OR). HDL was only significant in males. Males

Table 1: Sociodemographic factors, clinical characteristics and biochemical parameters according to BMD status

Sociodemographic factors	**Normal n=137 n (%)**	**Osteopenia n=137 n (%)**	**Osteoporosis n=57 n (%)**	**χ^2**	**p value**
Age (years old)					
< 61	89 (65.0)	65 (47.4)	14 (24.6)	27.31	<0.001*
≥ 61	48 (35.0)	72 (52.6)	43 (75.4)		
Gender					
Male	67 (48.9)	35 (25.5)	10 (17.5)	24.86	<0.001*
Female	70 (51.1)	102 (74.5)	47 (82.5)		
Menopausal status (n=219)					
Pre-menopause	16 (11.7)	9 (6.6)	1 (1.8)	13.245	0.001*
Post-menopause	54 (39.4)	93 (67.9)	46 (80.7)		
Race					
Malay	33 (24.1)	22 (16.1)	7 (12.3)	29.03	<0.001*
Chinese	50 (36.5)	76 (55.5)	44 (77.2)		
Indian	54 (39.4)	39 (28.5)	6 (10.5)		
MetS status					
Present	63 (46.0)	50 (36.5)	19 (33.3)	3.80	0.149
Absent	74 (54.0)	87 (63.5)	38 (66.7)		
	Median (IQR)	Median (IQR)	Median (IQR)	H	p value
BMI (kg/m^2)	26.10 (5.53)	24.20 (6.23)	21.43 (4.86)	51.15	<0.001*
WC (cm)	92.00 (10.75)	87.00 (14.65)	80.00 (11.00)	42.91	<0.001*
SBP (mmHg)	130.0 (17.0)	131.0 (20.0)	132.0 (21.0)	0.732	0.482
DBP (mmHg)	80.0 (15.0)	80.0 (15.0)	82.0 (19.0)	0.369	0.692

DXA measurements	**Normal n=137 Mean ± SD Median (IQR)**	**Osteopenia n=137 Mean ± SD Median (IQR)**	**Osteoporosis n=57 Mean ± SD Median (IQR)**	**F**	**p value**
LS BMD (g/cm^2)	1.08 ±0.14	0.89 ±0.10	0.74 ± 0.13	183.6	<0.001*
LFN BMD (g/cm^2)	0.83± 0.10	0.64± 0.07	0.52 ± 0.08	242.31	<0.001*
LTH BMD (g/cm^2)	0.97±0.11	0.77±0.08[a]	0.64±0.09	267.91	<0.001*
TB BMD (g/cm^2)	1.12±0.10	0.98±0.09[a]	0.87±0.11	143.34	<0.001*
TBS (L1 – L4)	1.40±0.09	1.32±0.08	1.25±.0.08	74.45	<0.001*
Total fat (%)	32.90±8.24	34.68±7.61	33.22±7.61	3.70	0.16

Biochemical variables	**Normal n=137 Mean ± SD Median (IQR)**	**Osteopenia n=137 Mean ± SD Median (IQR)**	**Osteoporosis n=57 Mean ± SD Median (IQR)**	**H/F†**	**p-value**	**Ref. range**
Adjusted Ca (mmol/L)	2.32±0.07	2.31±0.07	2.325±0.07	0.652†	0.522	2.12-2.52
PO4 (mmol/L)	1.15±0.17	1.18±0.20	1.19±0.18	4.64†	0.10	0.80-1.60

Mg (mmol/L)	0.87(0.10)	0.90 (0.10)	0.90 (0.10)	8.04	0.02*	0.74-0.99
25(OH)D (nmol/L)	56.8 ± 23.0	61.7 ± 21.2	59.4 ± 22.4	1.718†	0.181	≥ 50
PTH (pmol/L)	5.40 (3.75)	5.30 (3.35)	5.30 (3.05)	1.23	0.54	1.48-7.63
ALP (U/L)	73.0 (25.0)	80.0 (25.0)	84.0 (22.5)	11.85	<0.001*	38-124
CTX (ng/L)	350 (230)	460 (280)	550 (270)	45.01	<0.001*	100-800
P1NP (μg/L)	47.23 (26.7)	61.6 (34.6)	64.85 (28.03)	27.89	<0.001*	15 -115
FBS (mmol/L)	5.58 (1.76)	5.23 (1.24)	4.98 (1.11)	4.398	0.013*	3.0-6.1
TC (mmol/L)	5.3 (1.55)	5.6 (1.7)	5.8 (1.55)	5.15	0.08	<5.2
TG (mmol/L)	1.4 (0.90)	1.2 (0.70)	1.3 (0.7)	6.45	0.04*	<1.7
HDL (mmol/L)	1.3 (0.50)	1.5 (0.5)	1.6 (0.5)	25.67	<0.001*	>1.0
LDL (mmol/L)	3.3 (1.3)	3.3 (1.5)	3.4 (1.55)	1.57	0.46	<2.6
Non-HDL (mmol/L)	3.8 (1.3)	3.8 (1.6)	3.8 (1.55)	0.36	0.83	<3.4

Mean ± Standard Deviation: Mean ± SD; Median (interquartile range): Median (IQR)
Chi-Square statistical test (χ^2); Anova statistical test (F)†; Kruskal Wallis Test statistical test (H); statistical significance at p <0.05*

Table 2: Sociodemographic factors, clinical characteristics and biochemical parameters according to MetS status

Sociodemographic factors	Absent n=199 n (%)	Present n=132 n (%)	χ^2	p value
Age (years old)				
< 61	102 (51.3)	66 (50.0)	0.050	0.823
≥ 61	97 (48.7)	66 (50.0)		
Gender				
Male	69 (34.7)	43 (32.6)	0.156	0.693
Female	130 (65.3)	89 (67.4)		
Menopausal status (n=219)				
Pre-menopausal	17 (13.1)	9 (10.1)	0.444	0.505
Post-menopausal	113 (86.9)	80 (89.9)		
Race				
Malay	34 (17.1)	28 (21.2)	10.05	0.007*
Chinese	116 (58.3)	54 (40.9)		
Indian	49 (24.6)	50 (37.9)		
BMD status				
Normal	74 (37.2)	63 (47.7)	3.803	0.149
Osteopenia	87 (43.7)	50 (37.9)		
Osteoporosis	38 (19.1)	19 (14.4)		

	Median (IQR)	**Median (IQR)**	**Z**	**p value**
BMI (kg/m^2)	23.73 (4.76)	26.74 (5.91)	-5.42	<0.001*
WC (cm)	85 (14.0)	92.00 (10.88)	-5.61	<0.001*
SBP (mmHg)	126 (17.0)	137.0 (15.0)	42.97	<0.001*
DBP (mmHg)	78.0 (13.0)	87.0 (13.0)	51.8	<0.001*

DXA measurements	**Absent Mean ± SD Median (IQR)**	**Present Mean ± SD Median (IQR)**	**t-test**	**p value**
LS BMD (g/cm^2)	0.93 ± 0.18	0.97± 0.17	-2.30	0.022*
Left FN BMD (g/cm^2)	0.68 ±0.14	0.72 ±0.15	-2.46	0.014*
Left TH BMD (g/cm^2)	0.81 ± 0.15	0.86 ± 0.16	-2.84	0.005*
Total body BMD (g/cm^2)	1.01 ±0.13	1.034 ± 0.14	-1.338	0.182
TBS (L1 – L4)	1.35 ± 0.10	1.34 ± 0.10	1.075	0.283
Total fat (%)	32.66 ± 7.78	35.24 ±7.85	-2.94	0.003*

Biochemical variables	**Absent Mean ± SD Median (IQR)**	**Present Mean ± SD Median (IQR)**	**Z/t-test‡**	**p-value**	**Ref. range**
Adjusted Cal (mmol/L)	2.32 ± 0.07	2.32 ± 0.06	-0.25‡	0.81	2.12-2.52
PO4 (mmol/L)	1.17 ± 0.15	1.18 ± 0.13	-0.77‡	0.44	0.80-1.60
Mg (mmol/L)	0.90 (0.09)	0.87 (0.12)	-3.79	<0.001*	0.74-0.99
25 (OH)D (nmol/L)	61.31±22.0	56.22±22.33	2.05‡	0.04*	≥ 50
PTH (pmol/L)	5.40 (3.20)	5.30 (3.63)	-0.75	0.45	1.48-7.63
ALP (U/L)	77.00 (25.00)	80.50 (27.5)	-1.44	0.15	38-124
CTX (ng/L)	460 (280)	390 (270)	-1.84	0.07	100-800
P1NP (μg/L)	58.57 (32.58)	54.11 (28.47)	-1.12	0.26	15 -115
FBS (mmol/L)	4.90 (0.60)	5.20 (1.58)	-4.47	<0.001*	3.0-6.1
TC (mmol/L)	5.6 (1.6)	5.3 (1.6)	-1.82	0.07	<5.2
TG (mmol/L)	1.10 (0.6)	1.6 (0.90)	-6.55	<0.001*	<1.7
LDL (mmol/L)	3.4 (1.4)	3.2 (1.50)	-1.97	0.05	<2.6
HDL (mmol/L)	1.60 (0.6)	1.40 (0.40)	-4.44	<0.001*	>1.0
Non-HDL (mmol/L)	3.8 (1.3)	3.8 (1.58)	-1.06	0.29	<3.4

Mean ± Standard Deviation: Mean ± SD; Median (interquartile range): Median (IQR); Chi-Square statistical test (χ^2); Mann-Whitney statistical test (Z); Independent-samples t-test (t)‡; statistical significance at p <0.05*

with high HDL levels were 4.7 times more likely to be osteoporotic after adjustment. However, the confidence interval (CI) was large rendering the model unstable.

Table 4 shows the significant CV risk factors related to BMD at all skeletal sites and TBS. WC was positively associated with LS-BMD, L-TH-BMD, L-FN-BMD and TB-BMD in all subjects and males after adjustment. WC was positively associated with LS-BMD, L-TH-BMD and L-FN-BMD in females after adjustment. However, for TB-BMD in females, the association remained significant after adjusting for age, race and fat percentage but became attenuated after further adjustment for menopausal status. The strength of the association of WC with BMD was strongest for males (highest β value), specifically for L-TH-BMD (β = 0.530). In contrast, WC was negatively associated with TBS for all subjects and females after adjusting for demographic factors but this association became attenuated when adjusted for fat percentage. FBG was positively associated with LS-BMD, L-TH-BMD; and L-FN-BMD in all subjects after adjustment. In males, only LS-BMD and L-TH-BMD whereas in females, only L-TH-BMD and L-FN-BMD were positively associated with FBG, respectively after adjustment. TG

was positively associated with LS-BMD and L-TH-BMD in all subjects after adjustment. In males, only L-TH-BMD; whereas in females, LS-BMD, L-TH-BMD and TB-BMD were positively associated with TG, respectively after adjustment. HDL was negatively associated with LS-BMD, L-TH-BMD and L-FN in all subjects and males after adjustment. MetS was positively associated with LS-BMD, L-TH-BMD and L-FN in all subjects and males after adjustment.

In Table 5, Mg remained significantly protective for MetS in all subjects and females after adjustment. PTH was protective for Mets in all subjects but not significant in either males or females. All subjects and males with high LS-BMD and L-TH-BMD levels were more likely to have MetS after adjustment. However, the CI was considered very large, especially in males due to the small sample size rendering the model unstable.

DISCUSSION

The increasing prevalence of traditional CV risk factors such as dyslipidaemia, obesity, hypertension and diabetes[15] is reflected in this study population by the majority having raised WC, abnormal BP and deranged lipid profile. The relatively high prevalence of MetS of 39.9% is consistent with the markedly growing rates of MetS in Malaysia reported as approximately 25-40% of adults, depending on the criteria used.[18] In a similar study looking at an urban population, the prevalence of OPe and OP was 38.0% and 12.3% respectively,[19] slightly lower compared to this study's results of 41.4% and 17.2%, respectively.

From normal to OPe to OP groups, the significant decreasing trend of BMI and WC is in keeping with low body weight as a risk factor for OP, while the decreasing trend of LS-BMD, L-FN-BMD, L-TH-BMD, TB-BMD, TBS across the groups is consistent with the definition of OPe/OP.[16] In addition, as expected, CTX (bone resorption marker), ALP and P1NP (bone formation markers) showed a significant increasing trend. This is consistent with the notion of increasing bone turnover results in loss of bone leading to decreasing BMD and OP ultimately.

Significantly higher median values of BMI, WC, SBP, DBP, FBG, TG, fat percentage and lower HDL in the MetS subjects compared to those without MetS are characteristic features of MetS. BMD values at all skeletal sites were significantly higher in subjects with MetS

Table 3: CV Risk factors related to Abnormal BMD status (OPe + OP)

CVD Risk Factors	Abnormal BMD																			
	ALL						MALE						FEMALE							
	Model 1			Model 2			Model 1a			Model 2a			Model 1a			Model 2a			Model 3		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
WC	**0.944**	**0.919-0.970**	**<0.001***	**0.942**	**0.913-0.971**	**<0.001***	**0.920**	**0.868-0.976**	**0.006***	**0.897**	**0.833-0.965**	**0.004***	**0.944**	**0.914-0.975**	**<0.001***	**0.944**	**0.910-0.980**	**0.002***	**0.947**	**0.912-0.982**	***.004***
HDL	**2.743**	**1.293-5.818**	**0.009***	0.966	0.918-1.016	0.183	**4.804**	**1.128-20.462**	**0.034***	**4.705**	**1.082-20.446**	**0.039***	2.180	0.896-5.300	0.086	1.912	0.763-4.795	0.167	1.903	0.754-4.804	0.173

Binary logistic regression analysis - Model 1: adjusted for age, race, gender; Model 2: adjusted for Model 1 + total fat (%); Model 1a: adjusted for age, race; Model 2a: adjusted for Model 1a + total fat (%); Model 3: adjusted for Model 2a + menopausal status; OR: odds ratio, CI: confidence interval; ***statistical significance at p <0.05***

(Table 3). A longitudinal study in postmenopausal Korean women assessing MetS and its association with changes in BMD indicated that the higher mechanical loading in MetS subjects essentially explains the favourable effects of MetS on bone.[20] Our study confirms this finding of a favourable effect of MetS on BMD.

For a given WC, body fat distribution varies considerably according to age, gender and menopausal status.[21] This study showed that increased WC was protective for abnormal BMD status (OPe and OP) in both males and females after adjusting for confounders (Table 4). Significant CV risk factors related to BMD at all skeletal sites and TBS included WC, FBG, TG, HDL and MetS (Table 5). Although the strongest association between abnormal BMD status and WC was seen in females (highest OR), the strength of the association of WC with BMD *per se* was strongest for males (highest β value). This disparity may be due to gender differences in abdominal adipose tissue distribution.[21] Another interesting finding was WC related to TB-BMD in females remained significant after adjusting for age, race and fat percentage but became attenuated after further adjustment for menopausal status suggesting that increase in TB-BMD with increase in WC is driven by menopausal status. Kim *et al*.,[20] showed that a higher WC was significantly associated with reduced bone loss at all skeletal sites but this association was attenuated by the inclusion of weight and height signifying that the beneficial effects of central adiposity on bone mass is driven predominantly by a larger load on the trunk and lower limbs. In our study, the β value for WC with its BMD association became smaller for females but larger for males after adjustments. However, since the statistical significance remained even after these additional adjustments at LS-BMD, L-TH-BMD and L-FN-BMD for both genders and at TB-BMD for males only, other protective mechanisms linked to central obesity including higher 17 β-oestradiol, insulin or leptin levels or lower adiponectin levels may partially explain the results.[20]

Conversely, adipose tissue can also produce pro-inflammatory cytokines resulting in low-grade inflammation that may lead to bone loss.[20] Kim *et al*.,[20] further adjusted for serum high sensitivity C-reactive protein level to investigate this possibility. Nevertheless, the detrimental effects of central obesity were not observed. This suggests that the protective influences of hormonal and adipokine changes as well as body size are strong enough to counteract the negative effects of inflammation on bone mass.[20] This could be a possible explanation in our study although we did not adjust for inflammatory mediators. The Henan Rural Cohort Study concluded that in 8475 Chinese rural adults, higher adiposity indices were significantly associated with increased estimated BMD and a reduced risk of OP. Additionally, it was noted that the elderly were protected against poor bone health and a possible role of moderate adiposity in OP prevention,[22] similar to our study.

In contrast, WC was significantly and negatively associated with TBS for all subjects and females after adjusting for demographic factors, but this association became attenuated when adjusted for fat percentage. TBS is associated with bone microarchitecture and offers skeletal information that is not obtained from BMD measurement. Increased and decreased TBS values correlate with stronger and weaker skeletal microstructure, respectively.[17] Hence, our study findings suggest a higher WC was associated with a weaker skeletal microstructure in females driven by fat percentage.

In this study, FBG was positively related to BMD in males and females but significance was skeletal-site specific. Insulin resistance is a fundamental feature of MetS, and previous studies have revealed that hyperinsulinaemia is associated with increased BMD.[23] Increased circulating insulin levels in MetS may be a contributing factor in the positive association between FBG and BMD considering that insulin is said to stimulate osteoblast differentiation. Hyperinsulinaemia may be a marker for raised sex hormones (oestrogen in females and androgen in males), which are in turn responsible for higher BMD levels.[24] Preptin and amylin that are co-secreted with insulin from pancreatic β-cells may also promote bone formation.[25]

Previous experimental data suggest that TG, an apolar lipid, forms a layer between mineral crystals and collagen fibers.[26] Therefore, TG may facilitate the interaction between bone minerals and the protein matrix enhancing bone qualitative properties and thereby increasing bone stability.[27] This could explain the positive correlation between TG and BMD in this study. High TG level is also associated with osteoclastic potential in animal models for example resorption pits, whereas TG-rich lipoprotein is reported to regulate secretion of osteocalcin or osteoblast activity.[20] Further research should elucidate the predominant pathogenesis in gender-specific

Table 4: CV Risk Factors related to BMD at all skeletal sites (LS, L-TH, L-FN, TB) and TBS

CVD Risk Factors	LS BMD																				
	ALL						MALE						FEMALE								
	Model 1			Model 2			Model 1a			Model 2a			Model 1a			Model 2a			Model 3		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WC	**0.276**	**0.003-0.006**	**<0.001***	**0.245**	**0.002-0.006**	**<0.001***	**0.401**	**0.004-0.012**	**<0.001***	**0.471**	**0.005-0.014**	**<0.001***	**0.288**	**0.002-0.006**	**<0.001***	**0.235**	**0.001-0.005**	**0.002***	**0.216**	**0.001-0.005**	**0.004***
FBS	**0.138**	**0.005-0.028**	**0.007***	**0.110**	**0.001-0.025**	**0.030***	**0.257**	**0.007-0.045**	**0.007***	**0.239**	**0.005-0.043**	**0.013***	0.064	-0.008-0.023	0.319	0.030	-0.012 - 0.019	0.632	0.030	-0.011-0.019	0.639
TG	**0.138**	**0.010-0.066**	**0.008***	**0.124**	**0.007-0.061**	**0.015***	0.136	-0.014-0.084	0.157	0.132	-0.005-0.083	0.167	**0.173**	**0.013-0.079**	**0.007***	**0.151**	**0.007-0.073**	**0.016***	**0.147**	**0.007-0.072**	**0.018***
HDL	**-0.175**	**-0.125-0.031**	**0.001***	**-0.119**	**-0.103-0.003**	**0.038***	**-0.222**	**-0.253-0.022**	**0.020***	**-0.203**	**-0.243-0.009**	**0.035***	**-0.154**	**-0.108--0.010**	**0.018***	-0.074	-0.08-0.024	0.289	-0.065	-0.077-0.027	0.351
MetS	**0.136**	**0.013-0.084**	**0.007***	**0.103**	**0.001-0.073**	**0.042***	**0.225**	**0.014-0.156**	**0.019***	**0.209**	**0.008-0.152**	**0.030***	0.112	-0.005-0.075	0.085	0.067	-0.019-0.061	0.301	0.066	-0.019-0.060	0.304
CVD Risk Factors	L-TH BMD																				
	ALL						MALE						FEMALE								
	Model 1			Model 2			Model 1a			Model 2a			Model 1a			Model 2a			Model 3		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WC	**0.297**	**0.003-0.006**	**<0.001***	**0.247**	**0.002-0.005**	**<0.001***	**0.485**	**0.006-0.011**	**<0.001***	**0.530**	**0.006-0.013**	**<0.001***	**0.275**	**0.002-0.005**	**<0.001***	**0.188**	**0.001-0.004**	**0.007***	**0.181**	**0.001-0.004**	**0.010***
FBS	**0.160**	**0.007-0.027**	**0.001***	**0.127**	**0.004-0.023**	**0.060***	**0.160**	**0.007-0.027**	**0.001***	**0.127**	**0.004-0.023**	**0.060***	**0.177**	**0.007-0.032**	**0.003***	**0.141**	**0.003-0.028**	**0.016***	**0.140**	**0.003-0.028**	**0.016***
TG	**0.136**	**0.011-0.056**	**0.004***	**0.119**	**0.007-0.051**	**0.010***	**0.136**	**0.011-0.056**	**0.004***	**0.119**	**0.007-0.051**	**0.010***	**0.187**	**0.017-0.072**	**0.002***	**0.161**	**0.011-0.065**	**0.005***	**0.159**	**0.011-0.065**	**0.006***
HDL	**-0.206**	**-0.121-0.044**	**<0.001***	**-0.139**	**-0.096-0.015**	**0.007***	**-0.206**	**-0.121-0.044**	**<0.001***	**-0.139**	**-0.096-0.015**	**0.007***	**-0.185**	**-0.104-0.023**	**0.002***	-0.094	-0.075-0.011	0.144	-0.090	-0.074-0.012	0.162
MetS	**0.161**	**0.023-0.081**	**0.001***	**0.122**	**0.010-0.068**	**0.008***	**0.161**	**0.023-0.081**	**0.001***	**0.122**	**0.010-0.068**	**0.008***	**0.131**	**0.003-0.070**	**0.031***	0.079	-0.011-0.055	0.184	0.079	-0.011-0.055	0.186

CVD Risk Factors	L-FN BMD																				
	ALL						MALE						FEMALE								
	Model 1			Model 2			Model 1a			Model 2a			Model 1a			Model 2a			Model 3		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WC	**0.294**	**0.003-0.005**	**<0.001***	**0.279**	**0.002-0.005**	**<0.001***	**0.294**	**0.003-0.005**	***<0.001***	**0.279**	**0.002-0.005**	**<0.001***	**0.284**	**0.002-0.005**	**<0.001***	**0.212**	**0.001-0.004**	**0.002***	**0.206**	**0.001-0.004**	**0.003***
FBS	**0.125**	**0.003-0.022**	**0.008***	**0.099**	**0.001-0.019**	**0.037***	0.176	0.000-0.032	0.051	0.140	-0.003-0.028	0.119	**0.159**	**0.004-0.028**	**0.008***	**0.124**	**0.001-0.024**	**0.034***	**0.124**	**0.001-0.024**	**0.035***
HDL	**-0.170**	**-0.100-0.026**	**0.001***	**-0.116**	**-0.082-0.004**	**0.029***	**-0.282**	**-0.248-0.061**	**0.001***	**-0.251**	**-0.231-0.043**	**0.005***	**-0.136**	**-0.082-0.005**	**0.026***	-0.042	-0.054-0.027	0.511	-0.038	-0.053-0.028	0.552
MetS	**0.138**	**0.014-0.070**	**0.003***	**0.108**	**0.005-0.060**	**0.022***	**0.275**	**0.034-0.150**	**0.002***	**0.250**	**0.026-0.141**	**0.005***	**0.124**	**0.001-0.064**	**0.040***	0.076	-0.011-0.051	0.207	0.075	-0.011-0.051	0.209
CVD Risk Factors	**TB BMD**																				
	ALL						**MALE**						**FEMALE**								
	Model 1			**Model 2**			**Model 1a**			**Model 2a**			**Model 1a**			**Model 2a**			**Model 3**		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WC	**0.157**	**0.001-0.003**	**0.002***	**0.196**	**0.001-0.004**	**0.001***	**0.253**	**0.001-0.005**	**0.010***	**0.493**	**0.003-0.009**	**<0.001***	**0.170**	**0.001-0.003**	**0.007***	**0.158**	**0.000-0.004**	**0.039***	0.139	0.000-0.003	0.072
TG	**0.123**	**0.005-0.046**	**0.015***	0.042	-0.002-0.003	0.540	0.107	-0.013-0.047	0.263	0.111	-0.012-0.047	0.244	**0.166**	**0.010-0.065**	**0.009***	**0.156**	**0.007-0.063**	**0.014***	**0.152**	**0.007-0.062**	**0.016***
CVD Risk Factors	**TBS**																				
	ALL						**MALE**						**FEMALE**								
	Model 1			**Model 2**			**Model 1a**			**Model 2a**			**Model 1a**			**Model 2a**			**Model 3**		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
WC	**-0.125**	**-0.002-0000**	**0.012***	-0.028	0.001-0.001	0.633	-0.023	-0.002-0.002	0.813	0.218	0.000 – 0.004	0.052	**-0.146**	**-0.002-0.000**	**0.016***	-0.073	-0.002-0.001	0.315	-0.096	-0.002-0.000	0.191

Model 1: adjusted for age, race, gender; Model 2: adjusted for Model 1 + total fat (%); Model 1a: adjusted for age, race; Model 2a: adjusted for Model 1a + total fat (%); Model 3: adjusted for Model 2a + menopausal status; β: standardised regression coefficient, CI: confidence interval; **statistical significance at p <0.05***

Table 5: Bone Parameters related to MetS

Bone Parameters	MetS																				
	ALL						MALE						FEMALE								
	Model 1			Model 2			Model 1a			Model 2a			Model 1a			Model 2a			Model 3		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Mg	**0.016**	**0.001-0.294**	**0.005***	**0.025**	**0.001-0.474**	**0.014***	0.024	0.000-2.842	0.126	0.036	0.000-4.817	0.183	**0.005**	**0.000-0.275**	**0.009***	**0.008**	**0.000-0.441**	**0.018***	**0.008**	**0.000-0.438**	**0.018***
PTH	**0.912**	**0.836-0.995**	**0.038***	**0.894**	**0.818-0.977**	**0.014***	0.886	0.762-1.029	0.112	0.859	0.737-1.002	0.053	0.927	0.829-1.037	0.183	0.915	0.815-1.026	0.129	0.914	0.814-1.027	0.130
LS BMD	**5.794**	**1.387-24.208**	**0.016***	**4.471**	**1.039-19.238**	**0.044***	**13.503**	**1.289-141.467**	**0.030***	**12.771**	**1.197-136.220**	**0.035***	4.356	0.625-30.337	0.137	2.852	0.385-21.118	0.305	2.951	0.391-22.245	0.294
L-FN BMD	**8.236**	**1.281-52.939**	**0.026***	6.272	0.952-41.311	0.056	15.427	0.892-266.830	0.060	15.218	0.861-268.970	0.063	7.029	0.541-90.394	0.135	4.240	0.307-58.520	0.281	4.274	0.309-59.170	0.279
L-TH BMD	**11.709**	**1.874-73.173**	**0.009***	**8.481**	**1.315-54.694**	**0.025***	**46.190**	**2.417-863.544**	**0.010***	**40.706**	**2.130-778.053**	**0.014***	6.586	0.556-78.030	0.135	4.148	0.327-52.597	0.272	4.176	0.328-53.140	0.271

Binary logistic regression analysis - Model 1: adjusted for age, race, gender; Model 2: adjusted for Model 1 + total fat (%); Model 1a: adjusted for age, race; Model 2a: adjusted for Model 1a + total fat (%); Model 3: adjusted for Model 2a + menopausal status;
OR: odds ratio, CI: confidence interval; **statistical significance at p <0.05**

BMD regulation considering that this study's results indicate that the association between BMD and TG is gender-dependent.

An association between BMD and HDL has been reported in many studies; however, results are inconclusive. There are several proposed reasons for the negative correlation between HDL and BMD. An unbalanced diet and limiting calcium intake to correct lipid profile could be an OP risk factor. Increased calcium appears to correlate with MetS, insulin resistance, and a deranged lipid profile. Several mechanisms have been hypothesised in the association between calcium and lipids. Calcium decreases cholesterol catabolism, and stimulates lipids synthesis. Calcium supplement decreases the activity of the 7α-hydroxylase enzyme involved in cholesterol catabolism and stimulates Sterol Regulatory Element-Binding Protein-1c expression that is a transcription factor involved in de-novo lipid synthesis.[28] Oxysterols (oxygenated derivatives of cholesterol) are recognised in inhibiting adipogenic differentiation while stimulating osteogenic differentiation of mesenchymal stem cells. Although a raised HDL is cardioprotective, it can be detrimental to BMD as HDL removes oxysterols in the periphery hence decreasing osteogenic differentiation of mesenchymal stem cells.[29] In this study, HDL association with BMD was driven by fat percentage in females as it became attenuated after adjusting for it but not in males.

The diverse rates of bone loss at different skeletal sites are due to the variations in the composition of each bone. Furthermore, heterogeneity in bone microstructure may be an additional contributing factor.[30] Hence, the relative contribution of these risk factors would vary with skeletal sites.

The only significant bone parameter related to MetS was Mg, which was protective for MetS in all subjects and females after adjustment (Table 6). Studies have demonstrated the effect of Mg on components of MetS (glucose, HDL, TG and BP). Mg deficiency is involved in the pathogenesis of dyslipidaemia through increasing the activity of lecithin cholesterol acyltransferase and HMG-CoA reductase, and decreasing lipoprotein lipase activity. Furthermore, Mg is involved in enzymatic reactions of glycogen breakdown and ATP synthesis, decreases in both tyrosine kinase activity at insulin receptors and insulin secretion, as well as in increases in inflammatory markers. Thus, through the reduction of insulin sensitivity and insulin secretion, and the triggering of the acute phase reaction, hypomagnesaemia is associated with hyperglycaemia. It has also been reported that Mg, by competition for calcium receptors, inhibits calcium flux into vascular smooth muscle sarcoplasmic reticulum, attenuates Na-K ATPase, and improves myocardial contractility and endothelium-dependent vasodilation. Thus, it is not surprising that Mg deficiency contributes to hypertension.[31]

PTH was protective for Mets only in all subjects after adjustment but neither in males nor females (Table 6). The risk of developing hypertension, obesity and diabetes that are components of MetS may be increased by secondary hyperparathyroidism. Reis *et al*.,[32] suggested an increased risk of MetS with raised PTH levels in older males but not in females and showed that PTH values ≥6.7pmol/L gave an OR >1 whereas for PTH <6.7pmol/L, the OR <1. The reason for the gender difference in the association between MetS and PTH is unknown. In contrast, our study's results showed that PTH was protective for MetS in all subjects but not significant in males or females. This could be because PTH was not raised in this study's subjects (median value of 5.60pmol/L).

In the Camargo Cohort Study, WC was highly correlated with BMD for females with MetS at the three sites. Females with MetS showed higher BMD mainly driven by their higher body weight. No significant difference in BMD was seen in males, indicating that the effect of MetS on bone is gender-dependent.[33] In contrast, in this study, significant associations were only in males with MetS for LS-BMD and L-TH-BMD in the adjusted model, despite a large CI (Table 6). Previous research has suggested that males with MetS characteristically have lower BMD.[21,34] Moreover, due to dissimilar body fat distribution between genders, it has been hypothesised that oestrogen and mechanical loading are the main mechanisms in females, while chronic inflammation and oxidative stress predominate in males. Not all studies, however, are consistent regarding this issue, similar to our study. One of the adjustment factors in the previous studies for BMD was BMI or body weight, which clinically distorts the MetS profile as MetS encompasses raised WC, body weight or BMI.[35] Therefore, adjusting for fat percentage was chosen in this study. Furthermore, body composition measured by DXA is a more accurate measure of body adiposity compared to the commonly used body weight or BMI.[36]

Although this study included the gold standard DXA-derived assessment of BMD measured at multiple sites, TBS and adjustment for percentage fat and menopausal status, it has several limitations. Being a cross-sectional study, the causal relationship between CV risk factors and bone health cannot be clearly established. Furthermore, not all potential confounding factors were considered in the analyses such as energy or nutrients intake, physical activity, previous fracture history and/or Fracture Risk Assessment Tool (FRAX®) and factors mediating the relationship between MetS and BMD, such as insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), pro-inflammatory cytokines, adipokines and sex hormones. Smoking and alcohol intake, however, were not included on purpose as the number of subjects who smoked or consumed alcohol was very small, hence it would have rendered the adjustment model unstable. Since sample size was relatively small, race-specific associations could not be done and some gender-specific adjustment models were unstable.

In conclusion, there are skeletal site and gender-specific differences in the association between CV risk factors with abnormal BMD status and BMD *per se*. A higher BMD was demonstrated among MetS. Mg is a significant protective factor for MetS. MetS is a combination of CV risk factors that include obesity, a factor associated with increased BMD, and inflammation, a factor that lowers BMD. In this study, the findings suggest that adiposity may have a protective effect on BMD.

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