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Original Research Article

Association of Body Composition, Lipid Parameters and Lifestyle Factors with Bone Health Status in Males and Females of North India: A Case-Control Study

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Abstract

A number of epidemiological and animal studies have indicated the fine balance among bone and fat metabolism as the connecting link between osteoporosis and obesity. Excess abdominal fat is associated with an abnormal lipid profile causing greater predisposition towards metabolic diseases like osteoporosis. Quantitative ultrasonometry (QUS) T-score being the surrogate marker of osteoporosis was studied in this case-control study (254 cases, 250 controls) comprising of both males and females to investigate its potential association with anthropometric predictors of adiposity and lipid parameters. According to the WHO criteria, patients with a T-score <-1.0 SD were marked as cases and T-score ≥-1.0 SD as controls. Information on demographic and lifestyle factors, anthropometric measurements and lipid profile was recorded for all the participants. Results depicted that QUS T-score was inversely correlated with the predictors of obesity including BMI, WHtR and BAI in the pooled group, BMI, WHtR and TC in females, and directly correlated with TGL in males. After attenuating for the confounding factors, while obesity indices remained significant in pooled and females, the influence of lipid parameters among males was nullified. However, principal component analysis in all groups pointed obesity to be the major determinant, followed by lipid parameters, accounting for 90% of the variance. Additionally, calcium and fruit intake, post-menopausal and socio-economic status, and smoking had a significant role to play. To conclude, the present data indicates that individuals with greater abdominal obesity might pose a higher risk of developing bone metabolic diseases like osteopenia and osteoporosis, necessitating the need for evaluating the bone status in obese individuals, whilst, the role of lipid parameters still remains conflicting. This might open new avenues in understanding the mechanism underlying bone metabolism.

Keywords: Body Composition, Lipid Parameters, Quantitative ultrasonometry.

1. Introduction

Osteoporosis, the silent epidemic is characterized by low bone mass and micro architectural deterioration of the bone tissue resulting in an increased risk of spontaneous fractures [1][2]. The strength of bone tissue is mainly determined by two features- bone density and quality. While bone density is expressed in grams of mineral content per unit volume, bone quality is assessed with regard to bone microstructure, elasticity, turnover and mineralization [3][4]. Till date, there is no accurate method for evaluating bone strength. However, estimation of bone mineral density (BMD) using dual energy x-ray absorptiometry (DXA), quantitative ultrasonometry (QUS) and quantitative computed tomography (QCT) is widely being performed. Although, DXA determines the occurrence of fragility fractures and is considered the gold standard technique, it does not provide information regarding bone quality. For this purpose, QUS being an easily available, cost-effective, portable and radiation-free method is commonly used as a surrogate for BMD in addition to determining the bone quality to aid in prediction of fracture risk [5].

Bone quality is compromised in obesity giving rise to an increased risk of fracture [6]. This is evident from the well-established relationship between bone and fat tissue where maintenance of bone mass is contributed by a fine balance between the two mechanistically coupled arms of bone remodeling i.e. osteocytes (bone cells) and adipocytes (fat cells). This dysregulation leads to the bone debilitating disease, osteoporosis [7]. Thus, multipotent mesenchymal stem cells (MSCs) being the common progenitor of these cells might be responsible for causing disharmony and leading to bone metabolic diseases [8][9]. *In vitro* and *in vivo* studies suggest that oxidized lipids and hyperlipidemia in

both bone marrow and around blood vessels inhibit osteoblast differentiation along with promotion of bone resorption [10]-[14]. This finding has been further corroborated by the effect of widely used treatment therapies such as statins (HMG co-A reductase inhibitors), hormone replacement therapy (HRT) and aminobisphosphonates which have been shown to exert beneficial effects on the bone by lowering lipid levels [15]-[18]. Considering the reciprocal relationship, these studies might directly or indirectly support the fact that excess abdominal fat results in insulin resistance, hyperglycemia, hypertension and an abnormal lipid profile, eventually leading to metabolic diseases like type 2 diabetes mellitus (T2D) and cardiovascular disease (CAD), which are both the risk factors for osteoporosis [19]. Additionally, evidence from epidemiological data has been supportive for the above observations by suggesting a significant correlation between obesity, lipid parameters and susceptibility for osteoporosis [20][21]. Contradicting the traditionally accepted belief that higher body weight or BMI are associated with greater bone mass attributed to more mechanical loading of the bone [22][23], the negative influence of fat on the bone tissue has been well reported[24]-[26]. Taking into account obesity-related comorbidities, conflicting results have emerged with some studies showing a positive or negative correlation with lipid parameters, while others reporting no relationship at all [27]-[29].

Considering this background data and the emerging concern for the increased percentage of elderly people in the rapidly growing Indian population of 1.2 billion [30] makes it an urgent need to carry out screening studies for the presumptive risk of fracture. This requirement is further accentuated by the fact that the process of aging is not only accompanied by abdominal adiposity, but also results in an increase in bone marrow adipocytes leading to porous bones [31]. Thus, the alarming prevalence of osteoporosis to be 24.6% (males) and 42.5% (females) [32] and that of obesity being 46.1% (males) and 22.26% (females) [33][34] in the North Indian region along with the changing lifestyle pattern including a calorie-rich diet and sedentary work profile has warranted the need to ascertain the likelihood of this relationship. Introspecting into this association requires further attention owing to the lack of adequate data from this region. Hence, the present case-control study was initiated with the objective to investigate the relationship of bone health status with body composition, lipid parameters and lifestyle factors to elucidate the connecting etiology between osteoporosis and obesity in the males and females of North India.

2. Materials and methods

2.1 Subjects

The study group consists of age, gender and ethnicity matched 254 cases (62.20% osteopenic, 37.80% osteoporotic) and 250 healthy controls comprising of both males and females aged 20-81 years from North India. The samples were collected from hospital-based bone check-up camps using QUS. All the participants underwent clinical examination and a detailed questionnaire was scored regarding socioeconomic status (SES), dietary history, alcohol and caffeine consumption, calcium and vitamin intake, medical history, reproductive history, physical activity, sunlight and pesticide exposure. For calculating SES, the scale based on education, occupation and income level of the individual was considered separately for the urban and rural population [35]-[37]. Dietary information was collected using the food frequency questionnaire (FFE) and interview technique for retrospectively inferring the frequency of the consumed food products. Postmenopausal status was noted where menopause was defined as the absence of menstruation for at least 12 months caused by permanent cessation of ovarian function. Patients suffering from diabetes mellitus, coronary heart disease, chronic liver and kidney problems, thyroidism and bone disorders were excluded from the study. A written informed consent of all the individuals was obtained prior to sample collection according to the guidelines given by the Declaration of Helsinki (1964). The research work has been cleared by the ethical committee of Guru Nanak Dev University, Amritsar, Punjab.

2.2 Measurements

All the QUS scans for measuring bone density at the distal radius and calcaneus were carried out using manufacturer's instructions by a trained medical technician, with calibrations made periodically. According to the World Health Organization (WHO) classification, a T-score ≤-2.5 SD was considered as osteoporosis, -1.0 to -2.5 SD as osteopenia and ≥-1.0 SD as normal (WHO, 2003). Additionally, anthropometric measurements for body weight, height, waist circumference (WC) and hip circumference (HC) were taken to the nearest 0.1 unit using the standard protocols. BMI was calculated by dividing weight by height square (kg/m^2) and body adiposity index (BAI) as hip circumference in cm divided by height in meters to the 1.5 power minus 18.

Blood samples were drawn and plasma was separated within 24 hrs and stored at -20ºC. Biochemical analysis [triglyceride (TGL), total cholesterol (TC) and high density lipoprotein- cholesterol (HDL-C)] was performed using ERBA kits (Trans Asia Bio-medicals Ltd., India) according to the instructions provided by the supplier. Low-density lipoprotein- cholesterol (LDL-C) and very low-density lipoprotein- cholesterol (VLDL) were calculated using the Friedewald-Fredrickson method [38].

Normal range for the lipid parameters includes: TGL (≤150 mg/dl), TC (≤200 mg/dl), HDL-C (≥40 mg/dl) [39]. Cut off values for the normal anthropometric measurements were as follows: BMI (≤23 Kg/m^2) [40], WC (M: ≤85 cm, F: ≤80 cm), WHR (M: ≤0.89, F: ≤0.81), WHtR (≤0.5) [41] and BAI (M: ≤27, F: ≤32) [106].

2.3 Statistical Analysis

The study was divided into three groups: pooled (both males and females), males and females. This stratification of data was done taking into consideration that gender is among the important predictors of bone mass. Mean ± SD was calculated for the basic characteristics of all groups and analyzed using an independent samples t-test. Pearson's bivariate correlation model was applied to assess the strength of relationship of QUS T-score with anthropometric parameters and lipid profile. Univariate logistic regression analysis was performed to evaluate the potential determinants among the studied variables. Significant ones were further analyzed using the multivariate model. Principal component analysis (PCA) was performed to extract the variables with the highest loadings to explain the maximum variance and contribution. Odd's ratio was calculated using 2x2 contingency tables and their confidence intervals taken at 95%. P-values < 0.05 were considered statistically significant unless specified. All statistical analysis was performed using the software SPSS for windows version 20 (SPSS, Inc, Chicago, USA).

3. Results

A summary of the basic characteristics of the pooled study group, males and females is shown in Table 1. The pooled mean age of the cases and controls included in the study was 45.54 and 43.60 years, respectively. Significant differences were observed among the pooled group of cases and controls in terms of BMI (p=0.001), WHtR (p=0.000), BAI (p=0.000), TGL (p=0.003) and VLDL (p=0.005). Similar trend was also observed when female cases were compared with controls with WC (p= 0.006) and WHR (p=0.000) also contributing to the above factors. However, in case of males, only TGL (p=0.002) and VLDL (p=0.001) showed significant difference. It was observed that obesity profile comprising of BMI, WC, WHR, WHtR and BAI was higher among the cases than controls in the females and pooled group. TGL and VLDL were more among the controls. However, no significant differences were observed among other lipid parameters. The average SD values of QUS T-score were lower in females (cases: -2.28±0.97, controls: -0.19±1.17) as compared to males (cases: -2.00±0.90, controls: -0.12±0.99). The frequency of milk intake was also analyzed, and found to be higher among the controls.

Considering the binary data, it was observed that 11.62% of the males were smokers while, 29.56% consumed alcohol on a daily basis. 65.33% males and 57.09% females were literate at least to the matric level. 38.87% males were vegetarian (68.52% females), 57.81% were non-vegetarian (29.32% females) and 3.32% conditional vegetarians (2.16% females). 85.37% males and 84.17% females consumed fruits on a daily basis (>3 times/week), with majority of them taking those rich in vitamin A such as apple, banana, mango, tomato and carrot. Out of the total persons evaluated, mere 1.8% and 3.1% were taking vitamin A supplements and cod liver oil, hence making it extremely difficult to analyze the potential role of this micronutrient in affecting the bone status. However, 42.56% of total people routinely consumed desi ghee/butter, another important source of vitamin A. Potential role of other micronutrients like calcium and vitamin D was also evaluated. 74.08% participants were taking milk in their diet, 25.86% using calcium as supplements and 27.21% consuming both. 13.77% were using vitamin D supplementation, 75% being exposed to sunlight for at least 15 minutes daily and 14.02% benefitting from both.

Pearson's correlation between the QUS T-score and various studied variables is presented in Table 2. Age and age of onset of osteopenia were inversely correlated with the T-score in the pooled and female group. Significant positive correlative effect was observed with milk frequency. Different measures of adiposity including BMI (pooled [P]: r=-0.13, p=0.004; females [F]: r=-0.15, p=0.019), WC (P: r=-0.11, p=0.016; F: r=-0.20, p=0.001), WHR (P: r=-0.11, p=0.010; F: r=-0.26, p=0.000) and WHtR (P: r=-0.21, p=0.000; F: r=-0.23, p=0.000) showed an inverse relationship in pooled and female group. BAI showed similar effects only in pooled group (r=-0.17, p=0.000). Among the lipid profile, TGL (r=0.15, p=0.015) and VLDL levels (r=0.16, p=0.013) were positively correlated with T-score in males but TC (r=-0.13, p=0.038) showed a negative correlation in females. Correlations of modest magnitude (0.09-0.40) were observed for all the variables of the three groups. The strongest inverse associations after age and age of onset of osteopenia had been observed with WHtR followed by BAI among pooled group and WHR followed by WHtR among females. Males only showed correlation with TGL and VLDL. However, absence of a significant association was seen with other parameters including tea intake, sunlight exposure, age at menarche, age at menopause, age since menopause, weight, HC, HDL-C, LDL, HDL-C/LDL.

Univariate regression analysis was performed to establish the individualistic effects of all the variables on the QUS T-score values, considering it as the dependent variable (Table 3). Out of all the variables, WC, BMI, BAI, WHR, WHtR, TGL, VLDL, milk frequency, SES, physical activity, fruits (especially tomato, carrot, apple, banana and mango), supplemental calcium, smoking and post-menopausal status were observed to have a statistically significant effect on T-score among any of the three groups. Further, multivariate regression model was applied only on these variables to estimate the independent risk factors (Table 4). While, WHtR [P: p=0.004, OR=10.90 (2.13-55.92); F: p=0.008, OR=7.56 (1.71-33.39)] and postmenopausal status [P: p=0.016, OR=2.12 (1.15-3.92); F: p=0.005, OR=2.48 (1.32-4.63)] emerged as the major risk factors between the pooled category and females, smoking posed a 2-fold risk among males [p=0.041, OR=2.32 (1.03-5.19)]. However, BMI

[p=0.001, OR=1.15 (1.06-1.25)] only gave a marginal risk in the pooled group. Consumption of fruits [P: p=0.049, OR=0.15 (0.02-0.99)] like tomato [P: p=0.039, OR=0.34 (0.12-0.93)] and carrot [P: p=0.047, OR=0.28 (0.08-0.98); F: p=0.035, OR=0.28 (0.08-0.91)] had a protective effect on the bone structure and quality evaluated by the QUS T-score. Along with these, intake of supplemental calcium showed a protective role in females [p=0.007, OR=0.41 (0.21-0.79)]. While, SES exerted such an effect only in males [p=0.026, OR=0.47 (0.20-0.90)].

PCA was performed on 10 phenotypic traits in each group to make a predictive model for finding clusters in the data set. It resulted in data reduction and yielded 5 principal components (PCs), which jointly explained upto 90% of the total variation (Table 5). Among all the three groups of pooled, males and females, PC1 had the highest loadings for BAI, WHtR and BMI, and additionally WHR in males suggesting obesity to account for 26-31% of the whole effect. PC2, 3 and 4 mainly constituted an index of lipid profile in the order TGL> TC> HDL-C, respectively for pooled and males, but TC> TGL> HDL-C, respectively for females. PC4 had an additional loading of WHR in case of females. PC5 comprising of WHR was prevalent only in the pooled group. The communalities below 0.4 were excluded from the analysis as they are considered to be low estimators of prediction. Communality estimates >0.9 considered as good predictors were observed for TGL, VLDL, TC and LDL among all groups, along with BAI, WHtR and WHR among pooled, only WHtR among males, BAI and BMI for females. Therefore, these results indicate that obesity and lipid profile might be significant risk factors underlying complex diseases like osteoporosis.

Table 1: Demographic, anthropometric and clinical characteristics of the study population

Variables	Pooled (n=504)					Male (n=250)					Female (n=254)				
	Cases (n=254)		Control (n=250)		P-value	Cases (n=107)		Controls (n=143)		P-value	Cases (n=147)		Controls (n=107)		P-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Age (y)	45.54	10.08	43.60	12.00	0.051	44.31	10.21	45.29	12.86	0.514	46.43	9.92	41.35	10.38	0.000*
Tea	2.53	1.72	2.49	1.70	0.601	2.59	2.00	2.42	1.54	0.449	2.48	1.91	2.49	1.91	0.989
Milk frequency (l)	0.13	0.36	0.22	0.49	0.012*	0.14	0.39	0.21	0.44	0.158	0.12	0.34	0.24	0.55	0.043*
Sunlight exposure (h)	1.34	1.93	1.64	2.03	0.087	1.54	2.17	1.90	2.36	0.224	1.19	1.71	1.31	1.44	0.555
QUS T-score (SD)	-2.16	0.95	-0.14	1.07	0.000*	-2.00	0.90	-0.12	0.99	0.000*	-2.28	0.97	-0.19	1.17	0.000*
Age at menarche (y)	14.68	1.69	14.64	1.40	0.877	-		-		-	14.68	1.69	14.64	1.40	0.877
Age at menopause (y)	45.74	4.99	45.43	5.53	0.747	-		-		-	45.74	5.00	45.43	5.53	0.747
Age since menopause (y)	10.59	10.94	10.26	12.34	0.879	-		-		-	10.59	10.94	10.26	12.34	0.879
Weight (Kg)	70.74	13.76	71.84	13.53	0.366	75.67	14.85	76.79	12.90	0.526	67.15	11.71	65.22	11.40	0.192
BMI (Kg/m^2)	27.58	4.70	26.25	4.08	0.001*	26.86	4.60	26.24	3.99	0.260	28.11	4.71	26.25	4.17	0.001*
WC (cm)	95.57	10.73	94.10	11.34	0.133	94.84	12.26	95.47	10.15	0.660	96.10	9.46	92.24	12.57	0.006*
HC (cm)	98.76	9.71	98.24	9.80	0.551	96.12	9.39	96.69	8.93	0.565	100.69	9.52	100.17	10.60	0.683
WHR	0.97	0.07	0.96	0.08	0.140	0.99	0.07	0.99	0.07	0.890	0.96	0.06	0.92	0.08	0.000*
WHtR	0.60	0.07	0.57	0.07	0.000*	0.57	0.07	0.56	0.06	0.373	0.62	0.07	0.59	0.08	0.000*
BAI	31.01	6.92	28.53	6.18	0.000*	26.16	5.12	25.36	4.27	0.180	34.57	5.84	32.76	5.80	0.015*
TC (mg/dl)	160.15	21.21	159.72	51.57	0.924	158.58	51.70	162.38	59.77	0.600	161.29	51.01	156.10	37.63	0.377
TGL (mg/dl)	133.35	77.39	158.25	107.23	0.003*	135.64	78.42	175.04	119.76	0.002*	131.69	76.86	135.40	82.53	0.714
HDL-C (mg/dl)	37.68	16.13	38.32	18.64	0.680	35.74	17.75	36.20	18.45	0.843	39.08	14.76	41.21	18.58	0.311
LDL (mg/dl)	96.68	48.72	94.18	50.06	0.571	97.16	46.30	96.39	57.77	0.911	96.34	50.53	91.16	37.14	0.349
VLDL (mg/dl)	26.87	16.04	31.62	21.27	0.005*	26.85	15.80	34.96	23.68	0.001*	26.88	16.26	27.08	16.51	0.925

Data is shown as mean ± SD. BMI= body mass index; WC= waist circumference; HC= hip circumference; WHR= waist hip ratio; WHtR= waist height ratio; BAI= body adiposity index; TC= total cholesterol; TGL= triglyceride; HDL-C= high density lipoprotein-cholesterol; LDL-C= low density lipoprotein-cholesterol; VLDL= very low density lipoprotein. *P< 0.05, determined by t-test is statistically significant.

Table 2: Bivariate correlation matrix of various risk factors with QUS T-score

Variables	Pooled (n=504)		Male (n=250)		Female (n=254)	
	r	P-value	r	P-value	r	P-value
Age	-0.22**	0.000*	-0.06	0.371	-0.40**	0.000*
Milk frequency	0.09*	0.037*	0.03	0.629	0.15*	0.021*
Age of onset	-0.24**	0.007*	-0.19	0.142	-0.28*	0.025*
BMI	-0.13**	0.004*	-0.08	0.224	-0.15*	0.019*
WC	-0.11*	0.016*	-0.02	0.771	-0.20**	0.001*
WHR	-0.11*	0.010*	-0.08	0.235	-0.26**	0.000*
WHtR	-0.21**	0.000*	-0.10	0.107	-0.23**	0.000*
BAI	-0.17**	0.000*	-0.08	0.223	-0.11	0.095
TC	-0.04	0.408	0.04	0.509	-0.13*	0.038*
TGL	0.07	0.122	0.15*	0.015*	-0.08	0.231
VLDL	0.07	0.149	0.16*	0.013*	-0.08	0.199

r= Pearson correlation coefficient. **Correlation significant at 0.01 level (2-tailed). *Correlation significant at 0.05 level (2-tailed).

Table 3: Univariate Logistic Regression analysis of various risk factors with QUS T-score

Variables		Pooled (n=504)				Male (n=250)				Female (n=254)			
		P-value	OR	(95% CI)		P-value	OR	(95% CI)		P-value	OR	(95% CI)	
				Upper limit	Lower limit			Upper limit	Lower limit			Upper limit	Lower limit
Continuous	**Milk frequency**	0.013	0.580	0.377	0.893	NS	NS	NS	NS	0.036	0.531	0.294	0.959
	WC	NS	NS	NS	NS	NS	NS	NS	NS	0.007	1.034	1.009	1.059
	BMI	0.001	1.072	1.029	1.117	NS	NS	NS	NS	0.002	1.098	1.036	1.164
	BAI	0.000	1.060	1.031	1.090	NS	NS	NS	NS	0.017	1.056	1.010	1.104
	TGL	0.004	0.997	0.995	0.999	0.005	0.996	0.993	0.999	NS	NS	NS	NS
	VLDL	0.006	0.986	0.977	0.996	0.004	0.979	0.966	0.993	NS	NS	NS	NS
	SES	0.005	0.450	0.259	0.783	0.019	0.410	0.195	0.861	0.073	0.459	0.195	0.861
	Physical activity	NS	NS	NS	NS	NS	NS	NS	NS	0.041	0.575	0.338	0.978
Binary	**Fruits**	0.024	0.473	0.247	0.907	NS	NS	NS	NS	NS	NS	NS	NS
	Tomato	0.021	0.563	0.345	0.917	NS	NS	NS	NS	0.004	0.275	0.116	0.655
	Carrot	0.018	0.513	0.295	0.893	NS	NS	NS	NS	0.003	0.218	0.081	0.587
	Apple	0.025	0.529	0.303	0.921	NS	NS	NS	NS	0.038	0.443	0.205	0.958
	Banana	0.006	0.468	0.273	0.802	NS	NS	NS	NS	NS	NS	NS	NS
	Mango	NS	NS	NS	NS	NS	NS	NS	NS	0.018	0.478	0.259	0.883
	Supplemental calcium	NS	NS	NS	NS	NS	NS	NS	NS	0.012	0.497	0.289	0.856
	Smoking	NS	NS	NS	NS	0.029	2.427	1.094	5.384	-	-	-	-
	Postmenopausal status	0.001	2.464	1.471	4.128	-	-	-	-	0.001	2.464	1.471	4.128
	WHR	0.004	4.313	1.593	11.680	NS	NS	NS	NS	0.031	10.150	1.230	83.780
	WHtR	0.002	2.487	1.383	4.471	NS	NS	NS	NS	0.004	5.344	1.707	6.735

OR= Odds ratio; CI= confidence interval; NS= non-significant. Given P< 0.05 is statistically significant.

Table 4: Multivariate Logistic Regression analysis of various risk factors with QUS T-score

Variables		Pooled (n=504)				Male (n=250)				Female (n=254)			
		P-value	OR	(95% CI)		P-value	OR	(95% CI)		P-value	OR	(95% CI)	
				Upper limit	Lower limit			Upper limit	Lower limit			Upper limit	Lower limit
Continuous	**Milk frequency**	NS	NS	NS	NS	-	-	-	-	NS	NS	NS	NS
	WC	-	-	-	-	-	-	-	-	NS	NS	NS	NS
	BMI	0.001	1.15	1.06	1.25	NS	NS	NS	NS	NS	NS	NS	NS
	BAI	0.000	0.88	0.83	0.94	NS	NS	NS	NS	NS	NS	NS	NS
	TGL	NS	NS	NS	NS	-	-	-	-	-	-	-	-
	VLDL	NS	NS	NS	NS	-	-	-	-	-	-	-	-
	SES	NS	NS	NS	NS	0.026	0.47	0.20	0.90	NS	NS	NS	NS
	Physical activity	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Binary	**Fruits**	0.049	0.15	0.02	0.99	NS	NS	NS	NS	NS	NS	NS	NS
	Tomato	0.039	0.34	0.12	0.93	NS	NS	NS	NS	NS	NS	NS	NS
	Carrot	0.047	0.28	0.08	0.98	NS	NS	NS	NS	0.035	0.28	0.08	0.91
	Apple	NS	NS	NS	NS	-	-	-	-	NS	NS	NS	NS
	Banana	NS	NS	NS	NS	-	-	-	-	-	-	-	-
	Mango	-	-	-	-	-	-	-	-	NS	NS	NS	NS
	Supplemental calcium	NS	NS	NS	NS	NS	NS	NS	NS	0.007	0.41	0.21	0.79
	Smoking	NS	NS	NS	NS	0.041	2.32	1.03	5.19	-	-	-	-
	Postmenopausal status	0.016	2.12	1.15	3.92	-	-	-	-	0.005	2.48	1.32	4.63
	WHR	NS	NS	NS	NS	-	-	-	-	NS	NS	NS	NS
	WHtR	0.004	10.90	2.13	55.92	NS	NS	NS	NS	0.008	7.56	1.71	33.39

OR= Odds ratio; CI= confidence interval; NS= non-significant. Given P< 0.05 is statistically significant.

Table 5: Principal Component Analysis of various risk factors with QUS T-score

	Variables	PC1	PC 2	PC 3	PC 4	PC 5
Pooled	BAI	0.916	-	-	-	-
	WHtR	0.912	-	-	-	-
	BMI	0.847	-	-	-	-
	TGL	-	0.979	-	-	-
	VLDL	-	0.976		-	-
	LDL	-	-	0.971	-	-
	TC	-	-	0.968	-	-
	HDL-C	-	-	-	0.859	-
	HDL-C/LDL	-	-	-	0.838	-
	WHR	-	-	-	-	0.988
	Eigen values	2.626	2.097	1.659	1.492	1.089
	% of variance	26.256	20.973	16.588	14.923	10.894
	Cumulative %	26.256	47.229	63.817	78.740	89.635
Males	WHtR	0.952	-	-	-	-
	BMI	0.804	-	-	-	-
	BAI	0.713	-	-	-	-
	WHR	0.651	-	-	-	-
	TGL	-	0.979	-	-	-
	VLDL	-	0.978	-	-	-
	LDL	-	-	0.975	-	-
	TC	-	-	0.957	-	-
	HDL-C	-	-	-	0.885	-
	HDL-C/LDL	-	-	-	0.882	-
	Eigen values	3.066	1.915	1.777	1.494	
	% of variance	30.658	19.154	17.773	14.937	
	Cumulative %	30.658	49.812	67.585	82.522	
Females	BAI	0.954	-	-	-	-
	BMI	0.901	-	-	-	-
	WHtR	0.877	-	-	-	-
	LDL	-	0.956	-	-	-
	TC	-	0.905	-	-	-
	HDL-C/LDL	-	-0.618	-	-	-
	TGL	-	-	0.968	-	-
	VLDL	-	-	0.966	-	-
	WHR	-	-	-	0.798	-
	HDL-C	-	-	-	0.729	-
	Eigen values	2.635	2.343	1.819	1.355	-
	% of variance	26.355	23.431	18.192	13.546	-
	Cumulative %	26.355	49.786	67.978	81.524	-

PC= principal component. Rotated component values ≥0.4 are given.

4. Discussion

The emerging concept of the obesity of bone has further reiterated the strong association between the fat and bone tissue [31]. The reciprocal relationship between adipocytes and osteocytes very appropriately reassert that obesity might pose risk for skeletal diseases like osteopenia and osteoporosis [7]. In addition, the altered adipokine and hormonal milieu prevalent during obesity has a distinct effect on bone metabolism, thus hampering bone density, geometry and microarchitecture [42]. This has previously been shown by epidemiological data confirming the negative association between osteoporosis and obesity [43][44]. Thus, validating this concept, the present study is first of its kind from the region of North India showing obesity profile to be the major risk factor in the etiology for altered bone quality.

It has become quite evident from both clinical trials and animal studies that bone quality and fracture risk are altered in obesity [45][46]. A study by Wannenes *et al*[47] carried out on males and females stratified according to BMI, which is the most widely used and simple measure of body size showed the negative influence of obesity on bone metabolism. In addition, they carried out *in vivo* characterization of osteoblasts using sera of obese patients and found reduced osteoblastic differentiation mediated through a Wnt/β-catenin-dependent pathway. This finding can further be claimed through a study which demonstrates a U-shaped pattern of BMI, where upto 35 kg/m^2 might give protection to the bone but greater than this confers risk for fractures [48]. Greco *et al*[49] however reported 30 kg/m^2 as the threshold. In the present population as the BMI range falls into the obese category due to high calorie intake in the North region, this might be the probable reason for the observed inverse relationship.

Although, BMI is considered a good predictor, but it does not take into account the variation in body fat distribution. This problem was confronted using WHR, a measure of visceral or abdominal fat mass [50][51]. WHR was significantly higher among cases and showed a negative correlation in females, but it did not emerge as a risk factor in either of the groups after applying multivariate logistic regression. However, reducing the data into its basic components using PCA analysis revealed it to be the principal factor among males, while it appeared later in the list for females. This implies that it might be considered as a risk factor for both genders but a clear-cut answer can only be provided upon validation in a larger set of samples. These observations are supported by data showing its role in both the genders [52].

Although, BMI and WHR provide a good indication of a person's body fat, evidence suggests that WHtR is a better predictor for disease risk and mortality [53][54]. It is evident from the present study where it shows strong correlation as well as markedly high risk among the pooled group and females as compared to males. This risk can be attributed to the broader WC and short stature of the females of North India. According to the biological theory of sexual dimorphism, males are taller in places with lowest female to male ratio [55]. The 2011 census marks the North Indian states of Punjab, Haryana, Delhi and Uttar Pradesh to have the lowest sex ratio in the country [56], thus justifying the gender differences.

To further substantiate the role of visceral obesity, BAI was included in the analysis. It is a newer surrogate measure of body fat considered good for both genders and varied ethnicities [57]. Correlation analysis revealed a negative relationship between QUS T-score and BAI, however, significant only in pooled group. Upon multivariate logistic regression it was found to have a marginal odd's ratio making it difficult to ascertain its associated risk. However, upon PCA it was observed to be among the primary factors with a very high communality among all three groups. These results would substantiate and give a better estimation only once a larger sample size is considered, as large cohort studies of Chinese and Korean subjects have shown greater percentage of body fat associated with reduced bone density resulting in a higher risk of osteoporosis [58][24]. The association of these parameters reveals that obesity and bone health share a complex relationship as both are affected by physical inactivity and aging, and result in physical disability [59], implying the importance of reducing fat mass for achieving greater bone health.

Traditionally, osteoporosis has been regarded as a disease of females with a high prevalence among post-menopausal females [60]. This is consistent with the present results showing a 2.5-fold risk associated with the post-menopausal status, which might be attributed due to the decrease in estrogen after menopause resulting in sudden bone loss. This reduction is generally more stable in males [61], underestimating their screening process [62]. But growing evidence shows males to be substantially incurring bone loss with aging [62]-[64]. Asian males have been shown to have comparable risk of fracture as females [65]-[67]. Male osteopenia/osteoporosis has also been related to visceral adiposity [68][69]. Gender differences were observed in the present study that might be attributed to differences in bone size, geometry and strength as well as hormonal variations [65]-[71]. Although, an inverse correlation was observed between QUS T-score and obesity parameters but it was not statistically significant to confer risk in case of males. But this again cannot give a clear-cut answer, as obesity profile was PC1 even among males. Hence, a larger sample size would be needed to further validate these results.

Excessive abdominal fat, characteristic of obesity has been found to result in an abnormal lipid profile among patients. This leads to the metabolic menace of T2D and CAD, which eventually gives rise to complications like osteoporosis [19]. Through the present results, the major risk factor among the lipid parameters can be attributed to TGL in pooled and males and to TC among females. While the borderline odds ratio of the TGLs correspond to neither risk nor protection, but TCs seem to be giving risk to the females. This is consistent with a study conducted among European male and female patients with vascular disease who found that those having higher levels of TC had lower BMD resulting in a greater risk for fractures, with no influence from other lipid parameters [72]. Another European community-based study of healthy individuals demonstrated positive relationship for TGL and negative for HDL-C [27]. However, HDL-C in this study population did not account for any significant effect but as it is the fourth PC so in a larger cohort its role might become visible. These differences arise as a result of the genetic heterogeneity associated with different ethnic groups. Collectively, these suggest the possible collinearity between an abnormal lipid profile and skeletal health. But due to scarce data and debatable results in Indian population, inconsistency still prevails.

In addition to the hormonal milieu in obesity, other factors such as genetics, diet, physical activity, environment and altered physical activity are known to influence bone quality and risk of fracture [6]. According to a WHO survey, 60% of mortality rate is due to unhealthy diet and physical inactivity where 79% of this share is from developing countries [73]. Inadequate calcium intake [74] and vitamin D deficiency [75] is highly prevalent among Indians, which can be a leading cause for fractures [76]-[77]. However, in North Indians, supplemental calcium gave protection to females but no significant contribution of vitamin D was observed either in supplemental form or when absorbed through sunlight. This can ascribe to the fact that 60% of the study population was urban which due to modern lifestyle had minimum exposure to sunlight. This is in harmony with previous reports

exhibiting a lack of association between BMD and vitamin D intake [78][79]. Milk did not exert any protective effect on the bone, which can be due to inadequate fortification with calcium, and the presence of pesticide residues detected in the milk samples [80][81]. In fact, tomato and carrot that are vitamin A-rich foods included in the daily North Indian diet showed a protective effect. This contradicted the long-established risk of fracture associated with vitamin A intake [82]-[85].

Appraisal of the SES comprising of education, occupation and annual-income led to the conclusion that good education and higher income in males was associated with better bone quality. The possible explanation could be that only the ones with a reasonably good education level and income can understand the need and are able to afford the costly regular orthopedic check-ups and calcium and vitamin D supplements required for maintaining skeletal health after a certain age. In accordance with epidemiologic data, smoking had a negative impact on the bone. Such an adverse effect is attributable to its effect on bone cells and role in skeletal remodeling [86], which further decrease calcium absorption [87]. This might also be the reason that calcium intake did not exert any protection in case of males in the present study. Smoking lowers the testosterone activity as well. Inclusion of rural population, along with statistics showing pesticide residues in ~80% of blood samples collected from Punjab prompted the study of pesticide exposure as these are known to cause compromised bone formation [88]. No associative effect was observed, but further quantification studies are needed for an elaborative analysis.

Irrespective of being a tropical country, widespread vitamin D deficiency is prevalent particularly in the urban area accounting for ~80% among both males and females [89]-[93]. This has lowered down the age of occurrence of hip fractures by about a decade in comparison to the Western nations [94]. A recent survey by the International Osteoporosis Foundation (IOF) Asian Audit has estimated that 50 million Indians have a low bone mass (T-score$\leq$ -1) [95]. Single Nucleotide Polymorphisms (SNPs) in the main and most studied SNPs of the genes like Vitamin D Receptor (VDR) and Estrogen receptor alpha (ERα) which are associated with osteoporosis have also been observed to confer risk among urban Asian Indians [96]. Thus, clinical parameters, nutritional deficiency, genetic differences and smaller skeletal size [97] are the result of lower bone health among Indians. Thus further accompanied by a high calorie diet and sedentary lifestyle, it poses a risk for developing obesity. These figures necessitate the demand for conducting studies for delineating the risk factors and creating awareness among the general population through easily accessible bone assessment camps.

The occurrence of fractures is more prevalent at certain anatomical sites like the phalanges where trabecular bone is present [98]. These are the areas of active bone turnover [99]. So, micro architectural changes at these sites influence the susceptibility to fracture [100]. Hence, axial sites like calcaneus and distal radius were chosen to be measured using QUS because they are easily accessible, contain ~90% trabecular bone, have a high metabolic turnover rate and are similar to the lumbar spine and femoral neck which are the major sites for diagnosing osteoporosis [101]. Frost *et al*[102] has found both calcaneal QUS and the DXA measurements to have similar sensitivity towards the risk factors for osteoporosis. It thus serves as a highly prognostic tool with respect to early detection of bone fractures and for monitoring the response of various therapies. Thus, it provides an edge over DXA, which does not distinguish between trabecular and cortical bone [101]. Additionally, as India is still at the verge of socio-economic development, very few health-care centers in the country have access to the costly densitometry instrumentation as compared to the large population of all age groups who are in need of early assessment of their bone status. Moreover, the risk factors for BMD are well studied, but scarce data is available regarding the factors influencing QUS T-score, implying the necessity for conducting this study. The estimated risk of adiposity indices like WHtR and BAI with respect to bone health in Indian population has earlier not been studied. It thus widens the outlook of the body measures with this regard. Analysis of certain risk factors like vitamin A intake and pesticide exposure, which present novelty, adds credibility to the study. Moreover, to the best of knowledge, this is the first case-control study indulging both males and females from North India to make an attempt towards investigating the association of various risk factors with the predictor of bone strength using an easily available tool.

Even though substantial evidence regarding the gender variations was not completely elucidated, but further scaled-up studies in larger cohorts considering the extreme pathologies of obesity and osteoporosis would give a clear picture. The study is also accompanied by certain limitations of restricted sites of evaluation, which hinders the extrapolation of the data to the whole skeleton. Also, densitometry data assessing BMD is lacking which if considered alongside would further substantiate the results. Furthermore, studies show that despite of a normal BMD T-score, osteoporotic fractures have been shown to occur [103][104]. This holds true especially for obese people who are more susceptible to fractures for a given BMD as compared to their normal-weight counterparts [46]. So, it is desirable to incorporate the non-BMD clinical risk factors using the Fracture Risk Assessment Tool (FRAX), which would give a 10-year probability of osteoporotic fracture [105] and bone resorption assessment from urine along with the evaluation of other bone markers for an accurate detection of the bone status. Despite of the various pitfalls, the present study highlights the possible role of obesity parameters like BMI and WHtR, and lifestyle factors including SES, smoking

and postmenopausal status in affecting the bone health status among the population of North India. It thus opens new avenues for developing therapeutic interventions in a population-specific manner that would help to combat the skeletal menace among the population of North India.

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