M. Mokhtari-Dizaji PhD[1].
M.R. Dadras MSc[2].
B. Larijani MD[3].

Bone Mineral Density Value Dependence on Bone Width

Dual x-ray absorptiometry (DXA) is the most widely used measurement for the assessment of bone mass in osteoporosis. In clinical measurement, bone width can affect bone mineral parameters. The purpose of this study was to examine the dependence of bone mineral parameters on bone width. In this study, DXA measurements were conducted on rabbit bone *in vivo* using clinical instruments. We have selected rabbit's bones that have low BMD and more collagen tissue to predict structure not only measures BMD, but is also sensitive to the structure of the bone. To investigate the effect of bone width on the measured parameters, three regions of femur and tibia bones (N=132) were processed: upper (1/3 of length), middle (1/2 of length) and lower (2/3 of length) for BMC, areal BMD and volumetric BMD. The ANOVA analysis of bone mineral extracted by DXA showed significant differences ($P<0.05$) between BMC, BMD_a and BMD_v of six groups of upper, middle and lower parts of the femur and the tibia. It shows that BMC and BMD correlate well with the bone width, but BMD_v inversely correlates with bone width. Linear and nonlinear regression analyses were used to examine the relationship between DXA characteristics with bone width and the regression function for each parameter is given. We concluded that BMC, areal BMD, and volumetric BMD in rabbit's bone with collagen fibers more than bone mineral are dependent on bone width. This result may be at least in part due to large precision error measurement of the bone width, in vivo.

Keywords: dual-energy x-ray absorptiometry (DXA), correlation, nonlinear regression, bone width

1. Associate professor, Department of Medical Physics, Tarbiat Modarres University, Tehran, Iran.
2. Department of Medical Physics, Tarbiat Modarres University, Tehran, Iran.
3. Professor of Endocrinology and Metabolism, Head of Endocrinology and Metabolism Research Center, Tehran University of Medical sciences, Tehran, Iran.

Corresponding Author:
Manijhe Mokhtari Dizaji
Address: Medical Physics department, Tarbiat Modarres University, Tehran, Iran.
PO Box: 14115/331
Tel: 009821-88011001
Fax: 009821-88013030
E-mail: mokhtarm@modares.ac.ir

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Introduction

During the last decade, dual energy x-ray absorptiometry (DXA) has been established as the most widely used and accepted method of *in vivo* bone mineral analysis, and is now the technique of choice in the diagnosis of osteoporosis.[1] Bone mineral density (BMD) can be measured using densitometric techniques such as dual energy x-ray absorptiometry. DXA can be diagnostic of osteoporosis, but is relatively expensive, and currently of limited availability in some countries.[2] BMD only gives the amount of bone mineral present, without giving any information regarding structure or how bone width is affected on bone mineral, hence limiting its value. Thus, the ability of these parameters to discriminate low-density or osteoporotic bone from normal bone may be limited if differences in bone width are not accounted for. The precision of this method may be adversely affected by the bone width, surrounding soft tissue, as well as bone shape irregularity. Although the dependence of BMD (gr/cm^2) on bone width is recognized *in vitro,* [3-8] *in vivo* studies on bone have inconsistently demonstrated the relation between BMD and bone width.

Bone mass is expressed as bone mineral density (BMD, gr/cm^2). The relationship between bone size and bone mineral density is. There are also contradictory reports about the effect of bone width on BMD in clinical measurements. [6-10] Although the dependence of DXA-measured areal bone mineral density (BMD_a) on bone thickness is recognized, the effect of size on volumetric bone mineral density (BMD_v) was different for cortical thickness.

In recent studies, there have been no significant differences in cortical

BMD_v at the radius or tibia diaphysis between the groups (gymnasts and controls) at various cortical areas. [11] Backstrom studied bone cortical and trabecular density, and bone site, and showed that volumetric trabecular and cortical densities were not associated with thickness.[12] In another study, it was reported that BMD_v is a measurement that minimizes the effect of bone size on BMD_a. [13]

In this study, we have selected rabbit's bones that have lower BMD and more collagen on assumption that BMD is affected by the structure of the bone. Then, the dependence of BMC and BMD on the bone width of the tibia and the femur of rabbit was characterized *in vivo*. To evaluate how the difference in structure affects, the BMC and the BMD of rabbit's tibia and femur bones in three regions: upper, middle and lower parts were measured and compared, separately. We have assumed that the density of the femur and the tibia are uniform throughout upper, middle and lower parts of the bones. Finally, the correlation between densitometry characteristics with bone thickness was evaluated and regression functions between these variables and bone width were estimated.

Materials and Methods

A total of 66 two-month-old New Zealand white rabbits weighting 1942±267 g were anesthetized by intraperitoneal ketamine hydrochloride (10%) and xylazine hydrochloride (2%) at a 4 to 3 ratio, respectively, at the dosage of 0.7 ml per kg of rabbit's weight. The femural and tibial regions were shaved. The rabbits' femora (n=22) and tibiae (n=22) lengths were measured with digital caliper (Kanon Co., 0-200mm±0.03mm). To investigate the effect of bone width on the measured parameters, three regions of the femur and three regions of the tibia bones were processed: upper (1/3 of length), middle (1/2 of length) and lower (2/3 of length) for BMC and areal BMD (BMD_a) measurements and bone-width-corrected BMD (volumetric BMD: BMD_v). We measured bone width with an A-mode ultrasound machine (Echoscan US-2500 NIDEK, 10MHz). All animals were under general anesthesia throughout measurements. All *in vivo* measurements were performed on the same day.

The bone mineral content (BMC) and bone mineral density (BMD) of the six regions of femur and tibia were measured using DXA (Lunar Corp., DPX series, Lunar MD 7164, USA, ±3%) with a scansion speed of 1mm/s and a resolution of 0.5×0.5mm. The measurement protocol for the femoral with 167 scan lines, scan width of 180mm and 15×12 (pixel) window size of 15×12 (pixel) were used. For measurement of bone mineral parameters, the animals' legs were placed in sandy phantom (Figure 1). To evaluate the relationship between the BMC, BMD (g/cm^2) and thickness, we measured bone length (pixel) and placed the region of interest (ROI) at 1/3, 1/2 and 2/3 of bone length. Then, BMC and BMD in three regions of bones were estimated by using the "compare" function of software (DPX-MD V. 4.6d). To determine apparent volumetric bone mineral density, BMD_v, (g/cm^3), the measured BMD was divided by the bone thickness. The BMC (g), areal BMD (BMD_a) and volumetric BMD (BMD_v) values were reported for each of the six regions of the tibia and femur bones. In this study, we have assumed that the density of the femur and the tibia are uniform throughout the upper, middle and lower regions.

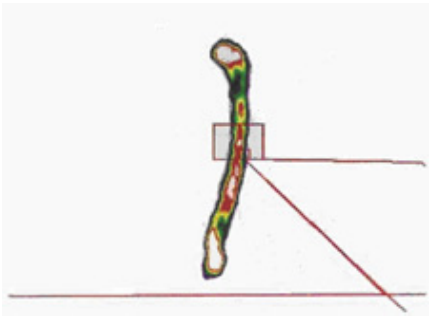

Fig 1. Bone mineral density image of the tibia of rabbit.

Statistical analysis was performed with SPSS 11 (SPSS/PC Inc. Chicago, IL). Summary statistics for all normally distributed variables are presented as mean and standard deviation. After having verified normal distribution and homogenicity variances, ANOVA was done with a significance level of P-value<0.05. Analyses of the Pearson correlations between densitometry parameters with bone width were carried out in the characterized regions of the tibia and the femur bones, and Pearson correlation coefficients (r) were calculated. Finally, linear and nonlinear regressions were used to determine the associations between densitometry parameters with bone width.

Table 1. Mean, standard deviation and 95% of confidence interval (CI) values of bone width (mm), BMC (g), BMD_a (g/cm^2) and BMD_v (g/cm^3) in three regions (upper, middle, and lower) of the femur and the tibia bones.

	Femur (n=66)			Tibia (n=66)		
Region	1/3 L*	1/2 L	2/3 L	1/3 L	1/2 L	2/3 L
Bone width	7.63±.44	6.26±.48	5.90±.46	8.71±.79	4.68±.34	5.65±.44
(mm)	(7.43-7.82)	(6.05-6.47)	(5.69-6.10)	(8.36-9.06)	(4.53-4.83)	(5.46-5.85)
BMC (g)	.229±.059	.138±.052	.202±.073	.171±.032	.122±.034	.178±.053
	(.202-.255)	(.115-.161)	(.168-.235)	(.156-.185)	(.107-.137)	(.154-.202)
BMD_a (g/cm^2)	.315±.042	.247±.033	.295±.053	.274±.041	.210±.035	.286±.054
	(.296-.333)	(.233-.262)	(.272-.319)	(.256-.292)	(.195-.226)	(.262-.310)
BMD_v (g/cm^3)	.413±.052	.396±.051	.502±.089	.317±.050	.452±.083	.507±.103
	(.390-.436)	(.373-.419)	(.462-.541)	(.295-.339)	(.415-.488)	(.462-.553)

* *bone length*

Results

When assessing reproducibility for DXA measurement *in vivo*, the maximum of coefficients of variation (CV_m %) were 8.0%, 4.0% and 4.3% for BMC, BMD_a and BMD_v, respectively. The mean and standard deviation values for BMC (g), BMD_a (g/cm^2) and BMD_v (g/cm^3), in the six regions (upper, middle, and lower) of the femur and the tibia are reported in Table 1. In this Table, we have reported the results of thickness measurements of bones that were made with A-mode ultrasound. In this study, the bone length of femur and tibia are 89.0±7.6 mm and 98.0±3.7 mm, respectively. All variables were normally distributed.

The ANOVA test of bone mineral characteristics extracted by DXA showed significant differences ($P<0.05$) between BMC, BMD_a and BMD_v of the six measured regions of the femur and the tibia. Significant differences were observed for bone width between groups in the femur and the tibia bones, separately. Analyses of the correlation between DXA measurements with bone width were carried out in the bones. It shows that BMC and BMD_a correlate well with bone width; however, BMD_v inversely correlates with bone width. Rabbit's bone has collagen and nonmineral structures more than bone mineral density therefore; our BMD_v results are contradictory to others. Figure 2 presents correlation curves between bone width with BMC (a), BMD_a (b) and BMD_v (c). These figures show that BMC, BMD_a and BMD_v correlate well with bone width, 0.520, 0.599 and -0.588, respectively. In this analysis, correlation is significant at the 0.01 level (2-tailed).

Linear and nonlinear regression models were used to examine the relationship between DXA bone mineral characteristics with bone width. The estimated functions are linear ($Y=b_0+b_1X$), logarithmic ($Y=b_0+b_1\ln X$), inverse ($Y=b_0+b_1/X$), quadratic ($Y=b_0+b_1X+b_2X^2$), cubic ($Y=b_0+b_1X+b_2X^2+b_3X^3$), compound ($Y=b_0\times b_1^2$), power ($Y=b_0\times X^{b1}$), S ($Y=e^{b0+b1/X}$), growth ($Y=e^{b0+b1X}$), exponential ($Y=b_0\times e^{b1X}$) and logistic regressions ($Y=\{1/u+(b_0\times b_1^X)\}^{-1}$. The regression function for each parameter is given in Table 2. The evaluation of regression functions show that cubic regression functions in BMC (g), BMD_a (g/cm^2) and BMD_v (g/cm^3) have the highest estimated curves.

Discussion

The mechanical properties of a tissue are determined by its material and structural properties. Bone mineral density as determined by DXA is a material property. Material properties are independent of geometry and architecture, whereas structural properties are determined by these factors. Bone size is influenced by body height, body mass index (BMI), appendicular skeletal muscle mass and long-term intense physical activity.[14] In clinical measurements, bone width can affect densitometric parameters. Previous in vitro studies have demonstrated contradictory reports about the relationship between bone mineral density, and therefore ultrasound parameters, with bone width, separately. [6,7,9] Because DXA iprojectional technique, the measured BMD is areal rather than the true volumetric density. It has been shown that, for a constant volumetric bone density, a large vertebra would yield higher areal BMD than a small one. [15] Pande has shown that the increase in the a real BMD appears to be almost totally explained by

Table 2. Summary of the linear and nonlinear regression functions between bone mineral characteristics.

Parameters	Regression function	B_0	B_1	B_2	B_3	R
BMC(g)	Linear	.0302	.0197			.520
	Logarithmic	-.0843	.1317			.532
	Inverse	.2921	-.8181			.533
	Quadratic	-.1669	.0810	-.0045		.551
	Cubic	-.1163	.0542		.0002	.557
	Compound	.0624	1.1406			.529
	Power	.0294	.8725			.539
	S	-1.0372	-5.3934			.537
	Growth	-2.7736	.1315			.529
	Exponential	.0624	.1315			.529
	Logestic	15.6981	.8561			.529
BMD_a (g/cm^2)	Linear	.1272	.0197			.593
	Logarithmic	.0104	.1328			.612
	Inverse	.3917	-.8355			.621
	Quadratic	-.0943	.0886	-.0051		.637
	Cubic	-.0269	.0559		.0003	.640
	Compound	.1494	1.0822			.604
	Power	.0932	.5346			.627
	S	-.8366	-3.3761			.639
	Growth	-1.9012	.0790			.604
	Exponential	.1494	.0790			.604
	Logestic	5.9162	.8996			.602
BMD_v(g/cm^3)	Linear	.5868	-.0282			-.587
	Logarithmic	.7372	-.1814			-.579
	Inverse	.2229	1.1003			-.566
	Quadratic	.4921	.0012	-.0022		-.592
	Cubic	.4848		-.0013	-.0001	-.592
	Compound	.6376	.9293			-.590
	Power	.9320	-.4654			-.574
	S	-1.3844	2.7886			-.554
	Growth	-.4501	-.0733			-.590
	Exponential	.6376	-.0733			-.590
	Logestic	.6797	1.1289			-.591

the increased size of the pagetic tibia.[16] The major limitation of the present study is that we have supposed that bone structure in upper, middle, and lower regions were similar with various thicknesses.

In this study, DXA measurements were conducted on rabbits' femura and tibiae *in vivo*, respectively. The results revealed a significant effect of the rabbits' femur and tibia bone widths on the densitometric parameters measured in vivo using DXA instrument. The effect of the femur and the tibia's bone widths on BMC, BMD_a and BMD_v are shown in Table 1. BMD_v was dependent on bone width. We selected rabbit's bone, because its bone mineral is low relative to collagen fibers resulting in greater flexibility, especially in the tibia (similar to Paget's disease). Therefore, BMD_v is dependent on bone width. BMD_a and BMC values showed linear positive correlations with the bone width.

In this study, the relationship of BMC, BMD_a and BMD_v with bone width were evaluated using *in vivo* measurements. Based on theoretical and experimental analyses, volumetric bone mineral density behavior in bone depends also on the structural features and mineral density of the bone. We have therefore studied rabbit's bone which bring have low bone mineral density, and high collagen, these bones show the structural features of the bone better than human bone. In this study, we showed that there was a linear positive correlation between bone width and these parameters that is in concordance wit theoretical models. The effect of bone size on BMD has been studied in animal bone *in vitro*. [9, 11, 17-20]

Although the dependence of DXA-measured areal bone mineral density (BMD_a) on bone width is recognized [7], but the effect size on volumetric bone mineral density (BMD_v) was different for bone thickness. In recent studies, there were no significant differences in cortical BMD_v at the radius or tibia diaphyses between the groups (gymnasts and control) with various cortical areas. [11] Backstrom studied bone cortical and trabecular density, and found out that bone site, volumetric trabecular and cortical densities were not associated to thickness.[21] Another study has reported that BMD_v is a measurement that minimizes the effect of bone size on BMD_a. [22] Pande showed that there was no significant difference in volumetric BMD between the normal and pagetic tibia.[16]

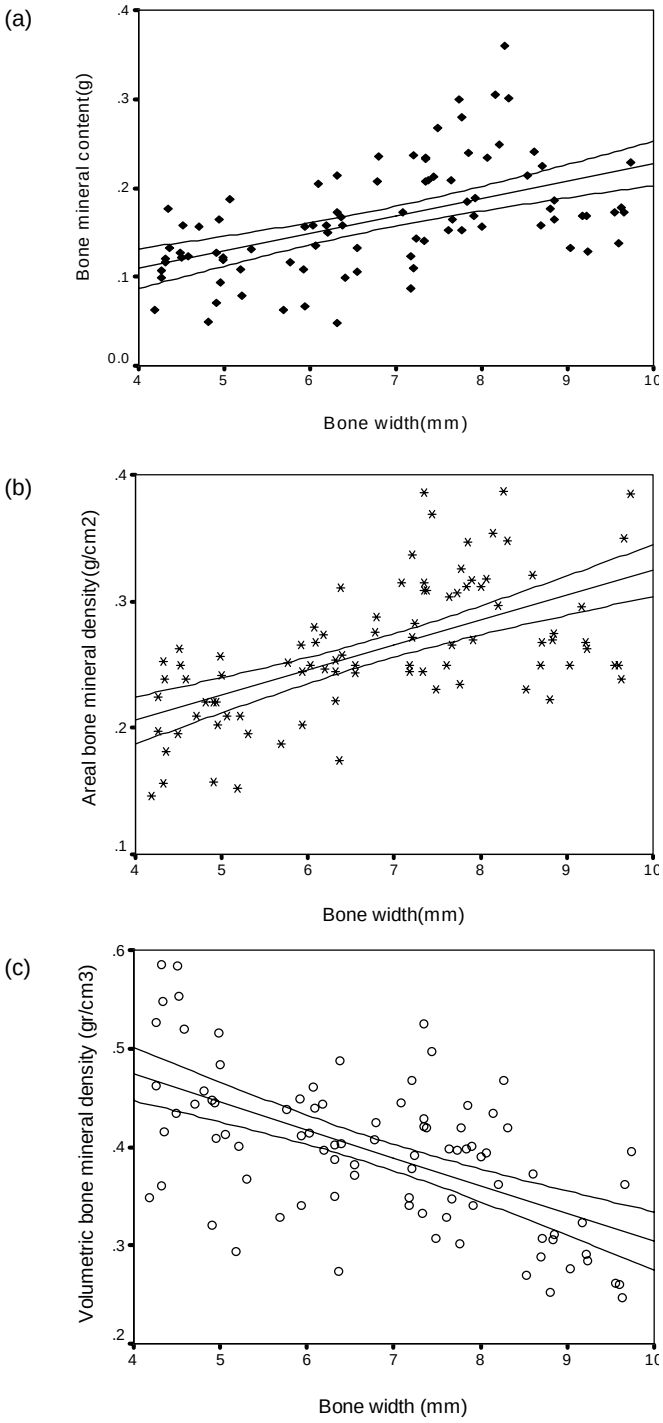

Fig 2. Pearson correlation plots between (a) BMC: bone mineral content (g)-Bone width (mm) and (b) BMD_a: areal bone mineral density (g/cm^2) and (c) BMD_v: volumetric bone mineral density (g/cm^3)-bone width (mm). ** Correlation is significant at the 0.01 level (2-tailed).

In summary, our DXA measurement showed a linear behavior on BMC and BMD as a function of the bone width, *in vivo*. Tibia has more collagen tissue than bone mineral, thus its behavior is slightly different with that of femur.

We conclude that BMC, areal BMD and volumetric BMD in rabbit's bone with collagen fibers more than bone mineral are dependent on bone width (4-9mm). However, recent studies have found that division of densitometric parameters of dependent-thickness by bone width did not improve diagnostic sensitivity due to the dispersion in bone width measurement. Nevertheless, further studies are needed to evaluate the correlation between human bone widths with densitometric parameters, since the measurement of bone width has been found to cause significantly large errors in diagnosis. This result may be at least in part due to the large precision error measurement of the bone thickness, *in vivo*.

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