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Influence of Bone Thickness on Densitometric and Ultrasonic Parameters, an *in vivo* Study

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Abstract: DXA and QUS assessments *in vivo* have been shown to be predictive of osteoporosis and future fractures. In clinical measurements, bone thickness can affect bone mineral density and ultrasound parameters. Previous *in vitro* studies have demonstrated contradictory reports about relationship between bone mineral density and so ultrasound parameters with bone thickness, separately. In this study, DXA, phalangeal QUS and calcaneus QUS 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 thickness on the measured parameters, two regions of femur and tibia bones (N = 44) were processed: up (1/3 of length) and down (2/3 of length) for BMC, areal BMD, volumetric BMD, AD-SOS, UBPI, BTT, SOS, BUA and SI measurements and bone thickness-corrected SOS and bone thickness corrected BUA. The paired student's t-test analysis of densitometric and ultrasonic characteristics extracted by DXA, Phalangeal and calcaneus quantitative ultrasound showed significant differences ($p<0.05$) between densitometric and ultrasonic parameters of two groups of up and down of the femur and two groups of up and down of the tibia, with the exception of SOS and SI ($p>0.05$). It shows that BMC, BMD_a, AD-SOS, UBPI, BTT and BUA correlate well with the bone thickness of the tibia and the femur. Among the femur parameters, the highest correlation ($r = 0.755$) was obtained for BMC parameter. But in the tibia, measurements at AD-SOS, UBPI and BUA inversely correlated with bone thickness, that could be arise from the tibia bone structure. This bone has collagen and non mineral structures more than bone mineral density. Correlation analyses of the bone thickness with the thickness-corrected DXA and ultrasound parameters revealed that corrected BMD (BMD_v) is independent from thickness, but corrected parameters excluding SOSc and BUA_c showed significant correlation coefficient than uncorrected. Linear regression analyses were used to examine the relationship between DXA and ultrasound parameters with bone thickness and the regression functions for each parameters (with correlated significant) is given. We concluded that BMD_v, SOS and SI are independent from bone thickness (with range of 5-9 mm). Thus, the ability of these parameters to discriminate low density or osteoporotic bone from normal bone may be limited if differences in bone thickness are not accounted for. This result may be at least in part due to large precision error measurement of the bone thickness, *in vivo* study.

Key words: Dual-energy X-ray Absorptiometry (DXA), ultrasound, correlation, bone thickness

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. Recently, Quantitative Ultrasound (QUS) instruments have obtained Food and Drug Administration (FDA) approval for use in clinical diagnostics (Prins *et al*., 1998; Compston *et al*., 1995).

BMD only gives the amount of bone mineral present, without giving any information regarding structure or how mass is distributed and therefore limiting its ability. The acoustic properties of a bone are measured by quantitative ultrasound of the hand phalanges and calcaneus bone. These parameters are: the slope of the frequency dependent attenuation known as Broadband Ultrasonic Attenuation (BUA), Speed of Sound (SOS), Amplitude Dependent Speed of Sound (AD-SOS) and Bone Transmission Time (BTT) (Johansen *et al*., 1999; Litniewski *et al*., 2000; Folds *et al*., 1995; Toyras *et al*., 2002; Oliveri *et al*., 2002). However, the precision of these ultrasonic methods may be adversely affected by the bone thickness, surrounding soft tissue, as well as bone

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shape irregularity that interferes with correct probe positioning (Folds *et al*., 1995; Duquette *et al*., 1997; Serpe and Rho, 1996; Wu *et al*., 1995). *In vitro* studies have demonstrated that QUS parameters are sensitive to structure (Pande *et al*., 2000; Gluer *et al*., 1993, 1994; Hans *et al*., 1995), but this has been demonstrated conclusively *in vivo*.

Although the dependence of BMD (g cm^{-2}) on bone width is recognized *in vitro* (Toyras *et al*., 1999; Mokhtari-Dizaji *et al*., 2006), *in vivo* studies on bone have inconsistently demonstrated the relation between BMD and bone width. In QUS instruments, SOS and AD-SOS is determined without knowledge of the bone width, which makes the result width dependence (Toyras *et al*., 1999; Duquette *et al*., 1997). The velocity in tibia was shown to correlate not only with bone mieral density, but also to be affected by cortical thickness (Prevrhal *et al*., 2001). Tatarinov *et al*. (2005) show a non-linear increase of velocity vs. wall thickness of bone phantom. In a recent study, no significant effect of heel width on BUA was found (Kotzki *et al*., 1994), but in other study, BUA was shown to increase linearly with bone width (Serpe and Rho, 1996). Although theoretical models have been used to ultrasound propagation and attenuation in bone, understanding of interactions between ultrasound and bone is still limited, however, unlike previous investigations have found contrariety results. Also, in clinical measurements, there are contradictory reports about effect of bone width on BMD and ultrasound parameters (Toyras *et al*., 1999; Johansen and Stone, 1997; Gomez *et al*., 1997).

It seems by normalizing the DXA and QUS results with the bone width, we could systematically improve their ability to predict bone strength. Therefore, the propose of this study was to examine the dependence of BMC, BMD, AD-SOS, UBPI, BTT, SOS, BUA and stiffness index on bone width. 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. Then the dependence of densitometric and ultrasonic parameters SOS, BUA and stiffness index on bone width of the tibia and the femur of rabbit was characterized *in vivo*.

MATERIALS AND METHODS

Study design: A total of 44 two-month-old New Zealand white rabbits weighting 1851±271 g were anaesthetized by intra peritoneum Ketamin Hydrochloride (10%) and Xylazin Hydrochloride (2%) at a 4 to 3 ratio respectively, with dosage 0.7 mL per kg of rabbit's weight. For evaluation how the difference in structure affects, the BMD and ultrasound parameters of rabbit's tibia and femur bones in two regions: up and down were measured and compared. We have assumed that the density of the femur and the tibia are uniform throughout up and down.

The femur and tibia regions were shaved. The rabbit femora (n = 22) and tibiae (n = 22) lengths were measured with digital caliper (Kanon Co., 0-200 mm /±0.03 mm). To investigate the effect of bone thickness on the measured parameters, two regions of femur and tibia bones were processed: up (1/3 of length) and down (2/3 of length) for BMC, BMD, AD-SOS, UBPI, BTT, SOS, BUA and stiffness index measurements and bone-thickness-corrected BMD, SOS and BUA. We have measured bone thickness with A-mode ultrasound machine (Echoscan US-2500 NIDEK, 10MHz). The tall of bones were measured with digital calipers (Kanon, Japan, 0-200 mm, ±0.03 mm). This study was performed from Mar 2004 to Mar 2005 at Tarbiat Modares University and Metabolism and Endocrine Research Center. All the animals were under general anesthesia throughout measurements. All *in vivo* measurements were performed on the same day.

BMD measurements: The Bone Mineral Content (BMC) and Bone Mineral Density (BMD) of two regions of femur and tibia were measured using DXA (Lunar Expert, Lunar Corp., Madison, WI, ±3%) with a scansion speed of 1mm/s and a resolution 0.5×0.5 mm. The measurement protocol for the femoral with 167 scan lines, scan thickness of 180mm and 15×12 (pixel) window size of 15×12 (pixel) were used. For measurement of bone mineral parameters, animals' legs were placed in sandy phantom. To evaluate relationship between the BMC, BMD (g cm^{-2}) and thickness, we measured bone length (pixel) and placed Region of Interest (ROI) in 1/3 and 2/3 of bone length, then BMC and BMD in two regions of bones were estimated by using the compare function of software (DPX-MD V.4.6 d). To determine apparent volumetric mineral density, BMD_v, (g cm^{-3}), then 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 two regions of the tibia and the femur bones.

Bone ultrasound measurements: Ultrasound measurements were performed using a phalangeal QUS device and calcaneus QUS device:

Phalangeal QUS: The phalangeal QUS system (Digital Bone Measurement [DBM] Sonic Bone Profiler 1200, IGEA Srl, Carpi, Italy) uses fixed-point transmission

techniques to measure Amplitude-Dependent Speed of Sound (AD-SOS) through the proximal phalanges of the fingers. The instrument is equipped with an electronic high precision caliper (±0.02 mm) that measures the distance between two ultrasound probes (diameters of 16 mm): one probe generates the ultrasound (1.25 MHZ) while the other receives the ultrasound energy (Max 1.8 W cm^{-2}). The caliper closes tangentially on the femur (up and down regions) and tibia (up and down regions) axis and measures the velocity of propagation through the bone and the bone thickness. The system automatically calculates the ultrasound transmission velocity when the signal received reaches the predetermined amplitude of 2 mV amplitude-dependent-speed of sound. The caliper of the device was positioned on the tibia (up and down) and the femur (up and down) bones in the same ROI with DXA. Coupling was achieved with standard gel and the caliper was rotated until the number of peaks and the amplitude of the peaks recorded on the screen were not influenced by ultrasound scattering. The device also routinely measures the soft tissue of the surrounding femur as a soft tissue reference to correct the influence soft tissue. Ultrasound Bone Profile Index (UBPI) was developed on the basis of the analysis of the US received signal. It is a combination of specific features of the US signal, intrinsically associated with particular physical properties of bone. These features, combined in a statistically supported mode, provide discriminant information on the biomechanical competence of bone and hence on bone fragility, related to fracture risk. The received US signal is evaluated in terms of the following physical quantities: Amplitude of the Fast Wave (FWA) expressed in mV; Signal Dynamic (S.Dy) expressed in mV μs^{-2} and Time Frame (TF) expressed in μ sec (Fig. 1). The combination of these three quantities is the fracture predictive value and is the expression of a probability of an actual fracture being present in a subject at the moment of measurement. It is expressed on a scale from 0 to 1 (Eq. 1).

$$UBPI = - (-0.0018SDy-0.0560FWA-1.1467TF+3.0300) \quad (1)$$

Bone Transmission Time (BTT) is the time interval between the instant corresponding to the top of the first peak, the instant corresponding to the cut-off on soft tissue velocity. It has been shown mathematically that BTT does not depend on soft tissue thickness but on bone tissue thickness and that it is representative of changes in bone tissue independently from the thickness of soft tissue (Fig. 1).

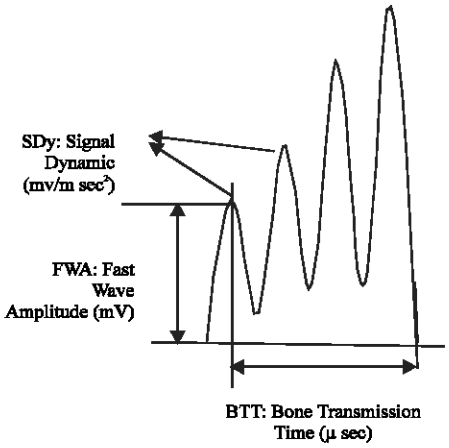

Fig. 1: The graphic trace is analyzed and a extracted series of parameters (UBPI and BTT)

In this study, instrument was calibrated and measurements were done by two operators. The results of ultrasound parameters were obtained by calculating the mean of two sets of four measurements in ever region of femur and tibia bones in order to reduce repositioning errors, then, we have reported AD-SOS, BTT and UBPI in femur and tibia bones with thickness change. The ultrasonic measurements were repeated three times for each region.

Calcaneus QUS: Following a standard procedure that is typically used in clinical settings, we assessed Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA) in the femur and the tibia bones at up and down by means of Lunar Achilles + ultrasound instrument (Lunar, Co., Madison, WI, with center frequency of 500 kHz). This ultrasound densitometry is usually made at the calcaneus, by the system incorporating two transducers, one acting as a transmitter, the other as a receiver. The basic principle of bone measurements is the same. The speed at which ultrasound propagates in bone or the extent of their attenuation through bone are determined by bone density and some physical properties that are intimately correlated with bone strength (Stiffness Index: SI). BUA is derived as the slope of the regression line in a plot showing attenuation vs. frequency (the frequencies used range from 0.2 to 0.6 MHZ).

It has been demonstrated that SOS is related to the elasticity and the density of bone whereas BUA is related to the density and structure (Sone *et al*., 1998).

The leg's rabbit was placed between the ultrasound transmitter and receiver to obtain parallel sides of the legs perpendicular to ultrasound beam. After localization the

beam path with the help of a plastic collimator, the diameter of which was the same as that of the ultrasound beam (1 cm). SOS (±4 m sec^{-1}), BUA (±2 db MHZ^{-1}) and stiffness index were measured through the tibia and the femur bones in two regions (up and down) using Achilles V 1.0.47 software. All ultrasound measurements were repeated twice and the mean SOS. BUA and combination of SOS and BUA (stiffness index) values were calculated to reduce experimental variation. In Eq. 2, stiffness index are introduced:

$$\text{Stiffness Index} = (0.67\ \text{BUA} + 0.28\ \text{SOS}) - 420 \quad (2)$$

We have normalized SOS and BUA with the bone thickness (SOS_c and BUA_c).

Statistical analysis: Statistical analysis was performed with SPSS V. 11 software (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 homogeneity variances, Paired student's t-test was done with a significance level of less than 0.05. Analyses of the Pearson correlations between densitometry and ultrasound parameters with bone thickness were carried out in the characterized regions of the tibia and the femur bones and Pearson correlation coefficients (r) were estimated. Finally, simple linear regression was used to determine the associations between densitometric and ultrasonic parameters with bone thickness.

RESULTS

When assessing reproducibility for DXA and ultrasound measurements *in vivo*, the maximum of coefficients of variation (CV_m%) were 8, 3.6, 1.2, 8.9, 5.5, 0.6, 4.3 and 6.1% for BMC, BMD_a, AD-SOS, UBPI, BTT, SOS, BUA and stiffness index, respectively.

The mean and standard deviation values (CV%) for BMC (g), BMD_a (g cm^{-2}), BMD_v (g cm^{-3}), AD-SOS (m sec^{-1}), UBPI, BTT (µ sec), SOS (m sec^{-1}), BUA (db MHZ^{-1}), SOS_c (sec^{-1}), BUA_c (db/MHz.cm) and Stiffness Index (SI) in two regions (up and down) of the femur and the tibia are reported in Table 1. In this Table 1, we have reported the results of thickness measurements of bones that were made with A-mode ultrasound. All variables were normally distributed.

The densitometric parameters were, in two regions of the tibia and two regions of the femur, separately, significantly different ($p<0.05$), with paired student's t-test analysis.

The paired student's t-test analysis of ultrasonic characteristics extracted by phalangeal and calcaneus Quantitative Ultrasound showed significant differences ($p<0.05$) between ultrasonic parameters of two groups of up and down of the femur and two groups of up and down of the tibia, with the exception of SOS and SI ($p>0.05$).

Significant differences were observed for bone thicknesses between two groups (up and down) in the tibia and the femur bones, separately.

Table 1: Characteristics of bone [mean±SD (CV%)], BMC (g), BMD_a (g cm^{-2}), BMD_v (g cm^{-3}), AD-SOS (m sec^{-1}), UBPI, BTT (µs), SOS (m sec^{-1}), BUA (db MHZ^{-1}), SOS_c (sec^{-1}), BUA_c (db $MHz.m^{-1}$) and Stiffness Index (SI) in two regions (up and down) of femur and tibia bones

	Femur (l* = 89.0±7.6 mm) N = 22		Tibia (l* = 98.0±3.7 mm) N = 22	
Parameters	Up (CV%)	Down (CV%)	Up (CV%)	Down (CV%)
DXA				
BMC (g)	0.231±0.058 (5.3)	0.138±0.052 (8.0)	0.171±0.032 (4.0)	0.122±0.034 (5.9)
BMDa (g cm^{-2})	0.315±0.042 (2.8)	0.247±0.033 (2.8)	0.274±0.041 (3.2)	0.210±0.035 (3.6)
BMDv (g cm^{-3})	0.413±0.052 (2.7)	0.396±0.051 (2.7)	0.317±0.050 (3.4)	0.452±0.083 (3.9)
Phalangeal QUS				
AD-SOS (m sec^{-1})	2194±103 (1.0)	2032±71 (0.7)	1843±66 (0.8)	2142±125 (1.2)
UBPI	0.66±0.21 (6.8)	0.49±0.13 (5.6)	0.31±0.13 (8.9)	0.54±0.19 (7.5)
BTT (µ sec^{-1})	1.22±0.13 (2.3)	0.93±0.16 (3.7)	0.89±0.19 (4.5)	0.58±0.15 (5.5)
Calcaneus QUS				
SOS (m sec^{-1})	1517±38 (0.5)	1522±20 (0.3)	1525±27 (0.4)	1520±39 (0.6)
BUA (db MHZ^{-1})	59±10 (3.6)	42±6 (3.0)	55±11 (4.3)	44±4 (1.9)
SOSc (sec^{-1})	199575±12131 (1.3)	244328±17377 (1.5)	172002±20027 (2.5)	326699±25371(1.7)
BUAc (db/MHz.cm)	78±15 (4.1)	71±8 (2.4)	62±15 (5.2)	95±9 (2.0)
Stiffness Index (SI)	44±11 (5.3)	52±9 (3.8)	44±11 (5.3)	49±14 (6.1)
A-mode, Ultrasound Thickness (cm)	0.763±0.044 (1.2)	0.626±0.048 (1.6)	0.898±0.102 (2.7)	0.468±0.034 (1.5)

*Bone length (mm)

Table 2: Pearson's correlation coefficients (r) between DXA and ultrasound parameters with bone width

r	BMC	BMD_a	BMD_v	AD-SOS	UBPI	BTT	SOS	SOS_c	BUA	BUA_c	SI
Femur-thickness	0.76**	0.70**	0.05	0.60**	0.31*	0.66**	0.08	-0.98*	0.62**	-0.73**	-0.28
Tibia-thickness	0.62**	0.65**	-0.75**	-0.78**	-0.57**	0.65**	0.07	-0.98**	0.50**	-0.85**	-0.17

**Correlation is significant the 0.01 level (2 tailed), *Correlation is significant the 0.05 level (2 tailed)

Analyses of the correlations between DXA and ultrasound measurements with bone thickness were carried out in the femur and the tibia bones. Table 2 presents correlation coefficients between bone thickness and all measured variables. It shows that BMC, BMD_a, AD-SOS, UBPI, BTT and BUA correlate well with the bone thickness of the tibia and the femur. Among the femur parameters, the highest correlation (r = 0.755) was obtained for BMC parameter.

But measurements at AD-SOS, UBPI and BUA inversely correlated with bone thickness that may be arising from the tibia bone structure. This bone has collagen and non mineral structures more than bone mineral density. However, both the femur and the tibia BMD_v, SOS and stiffness index didn't correlate with bone thickness. Correlation analyses of the bone thickness with the thickness-corrected DXA and ultrasound parameters revealed that the corrected parameters excluding SOS_c showed significant correlation coefficient than uncorrected SOS, but corrected BUA showed high correlation coefficient than uncorrected BUA. Corrected BMD (BMD_v) is independent from thickness at the femur but this parameter is dependent to thickness at the tibia. Linear regression models were used to examine the relationship between DXA and ultrasound parameters with bone thickness. The regression function for each parameters (with correlated significant) is given in Table 3.

There were no correlation between BMD_v at the femur, SOS, SI at the femur and the tibia with the bone thickness then linear regression functions are not calculated.

Table 3: Summary of the linear regression functions between DXA and Ultrasound parameters with bone width (cm) at the femur and the tibia with signification of less than 0.05

Method	Linear regression functions	r
Femur		
DXA	0.427 (T*) -0.015 = BMC (g)	0.701
	0.659 (T) -0.273 = BMD_a (g cm^{-2})	0.755
Phalangeal QUS	872 (T) +1507 = AD-SOS (m sec^{-1})	0.602
	0.72 (T) +0.07 = UBPI	0.313
	1.67 (T) +0.08 = BTT (μ sec^{-1})	0.663
Calcaneus QUS	-32173 (T) +445331 = SOS_c (sec^{-1})	0.983
	84 (T) -6 = BUA (db MHZ^{-1})	0.624
	11 (T) +67 = BUAc (db/MHZ cm)	0.730
Tibia		
DXA	0.121 (T) -0.065 = BMC (g)	0.622
	0.153 (T) -0.140 = BMD_a (g cm^{-2})	0.652
	-0.338 (T) +0.610 = BMD_v (g cm^{-3})	0.748
Phalangeal QUS	-660 (T) +2434 = AD-SOS (m sec^{-1})	0.777
	0.53 (T) +0.78 = UBPI	0.562
	0.71 (T) +0.26 = BTT (μ sec^{-1})	0.647
Calcaneus QUS	-34774 (T) +486731 = SOS_c (sec^{-1})	0.982
	22 (T) -35 = BUA (db MHZ^{-1})	0.504
	-77 (T) +131 = BUAc (db Mhz^{-1} cm)	0.851

*Thickness

DISCUSSION

DXA and QUS assessments *in vivo* have been shown to be predictive of osteoporosis and future fractures (Toyras *et al*., 2002; Han *et al*., 1997, 1998; Lee *et al*., 1997; Rho *et al*., 1997). In clinical measurements, bone thickness can affect bone mineral density and ultrasound parameters. Thus, the ability of these parameters to discriminate low density or osteoporotic bone from normal bone may be limited if differences in bone thickness are not accounted for. Previous *in vitro* studies have demonstrated contradictory reports about relationship between bone mineral density and so ultrasound parameters with bone thickness, separately (Toyras *et al*., 1999, 2002; Serpe and Rho, 1996). In this study, DXA, phalangeal QUS and calcaneus QUS measurements were conducted on rabbit's femur and tibia bone *in vivo*, respectively. The results revealed a significant effect of rabbit's femur and tibia bones thickness on the densitometry and the ultrasound parameters measured *in vivo* using DXA, phalangeal and calcaneus QUS instruments. We have normalized the DXA results with the mean then the effect bone thickness of the femur and the tibia on BMC, BMD_a and BMD_v are shown in Fig. 2a and b. BMD_v was independent of the femur thickness (Fig. 2a) but dependent of the tibia thickness (Fig. 2b). We have selected rabbit's bone, because rabbit's bone mineral is low relative to collagen tissue, specially, in the tibia bone, therefore, BMD_v is dependent of the tibia thickness. BMD_a and BMC values showed highly linear positive correlations with the bone thickness.

In Fig. 3, the AD-SOS, SOS and BUA normalized with the mean-thickness relation was shown for the femur (a) and the tibia (b). AD-SOS and BUA showed linear positive correlations with the femur thickness that is similar to previously reported values in animal bone. Correlations of AD-SOS and BUA with the tibia thickness were linear negative. This negative behavior can be caused high collagen tissue in the rabbit's tibia bone. Based on theoretical analyses, SOS is typically determined without knowledge of the true bone thickness and depends also on the bone structure that our results confirmed this thickness-independent.

In this study, we have supposed that bone structure in up and down regions were similar with various thicknesses.

The effect of bone size on BMD and BUA and SOS parameters has been studied in animal bone *in vitro* (Duquette *et al*., 1997; Kotzki *et al*., 1994; Serpe and Rho, 1996; Longton *et al*., 1984; Wu *et al*., 1995; Blake *et al*., 1994) and *in vivo* (Fricke *et al*., 2005). In this study, the relationship of BMC, BMD_a, AD-SOS, UBPI, BTT, SOS,

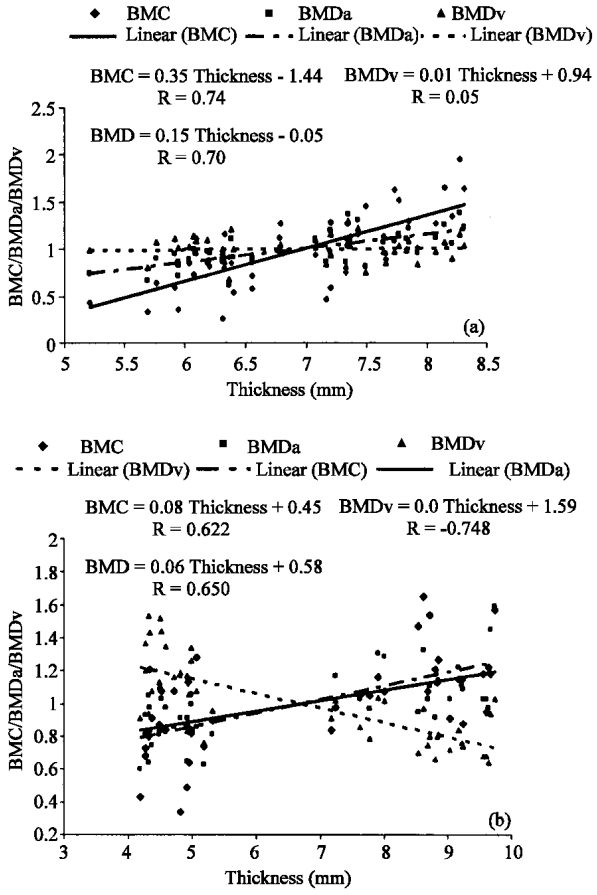

Fig. 2: Effect of bone thickness (mm) on BMC, BMD_a and BMD_v normalized with the mean, *in vivo* measurement, femur (a) and tibia (b)

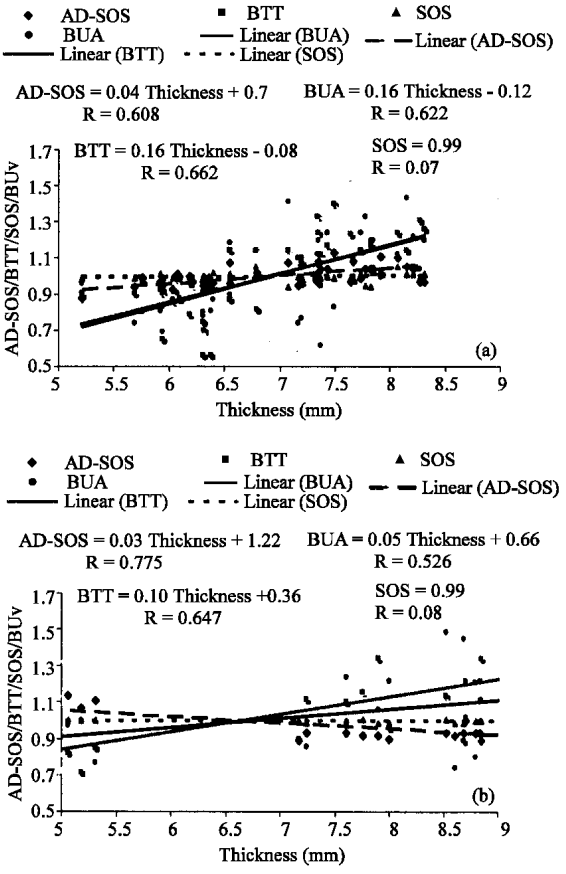

Fig. 3: Effect of bone thickness (mm) on AD-SOS, BTT, SOS and BUA normalized with the mean, *in vivo* measurements, the femur (a) and the tibia (b)

BUA, SI and thickness-corrected BMD, SOS and BUA with bone thickness were evaluated *in vivo* measurements. Although the dependence of DXA-measured areal bone mineral density (BMD_a on bone thickness is recognized, but the effect size on volumetric bone mineral density (BMD_v) was different for cortical thickness. In recently study, there were no significant differences in cortical BMD_v at the radius or tibia diaphysis between the groups (gymnasts and control with various cortical areas (Ward *et al*., 2005). Backstrom (2005) studied bone cortical and trabecular density and bone site, volumetric trabecular and cortical densities were not associated to thickness. In other study was reported that BMD_v is a measurement that minimize the effect of bone size on BMD_a (Shane *et al*., 1996). Fricke shows that bone size have a stronger influence on SOS than BMD_v and the influence of bone width on SOS also depends on the location of measurement (Fricke *et al*., 2005).

Based on theoretical and experimental analyses, ultrasound behavior in bone depends also on the structural features of the bone and thus may extra information regarding bone quality not obtainable using DXA, therefore we have 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.

The effect of bone thickness on AD-SOS, UBPI and BTT parameters has not been studied as *in vivo*. In this study, we have shown that there was linear positive correlation between bone thickness and these parameters that is according to theoretical models. The *in vitro* studies have revealed a correlation between bone thickness and BUA as linear (Serpe and Rho, 1996; Gomez *et al*., 1997), nonlinearity at high value (Toyras *et al*., 1999; Duquette *et al*., 1997; Wu *et al*., 1995) and the *in vivo* study have showed that BUA increases in a highly linear manner, but clearly reaches a equilibrium value with higher thickness (Toyras *et al*., 1999). In

contrast, Kotzki *et al.* (1994) and Blake *et al.* (1994) concluded our results are consistent BUA increases as linearity with bone thickness. In this study, the femur with 3.7 mm difference in bone thickness may show difference of 0.93 g, 0.68 g cm^{-2}, 172 m sec^{-1}, 17, 0.29 μ sec^{-1} and 17 db $Mhz\mu^{1}$ for BMC, BMD_a, AD-SOS, UBPI, BTT and BUA, respectively. The tibia with 4.3 mm difference in bone thickness may show difference of 0.49 g, 64 g cm^{-2}, 199 m sec^{-1}, 13, 0.31 μ sec^{-1} and 11 db MHZ^{-1} for BMC, BMD_a, AD-SOS, UBPI, BTT and BUA, respectively.

The linear SOS-bone thickness relation from the *in vitro* study was reported (Toyras *et al.*, 1999; Kotzki *et al.*, 1994) whereas SOS was based on the time of flight principle, in Calcaneus QUS. In this *in vivo* study, a significant correlation was not found between SOS and bone thickness. According to previous study (Toyras *et al.*, 1999), correlation analyses of the thickness corrected ultrasound (SOS_c and BUA_c) parameters revealed that these parameters have higher correlation coefficients than uncorrected parameters with bone thickness.

In summer, our DXA, phalangeal and calcaneus QUS showed a linear behavior on BMC, BMD_a, AD-SOS, UBPI, BTT and BUA as a function of the bone thickness, *in vivo*. No correlation between bone thickness with SOS and SI was detected. In the tibia bone, we have collagen tissue more than bone mineral then the behavior of the tibia was slightly different with the femur.

We concluded that SOS and SI are independent bone thickness (with range of 5-9 mm). However, recently studies found that division of densitometry and ultrasound parameters of dependent-thickness by bone thickness did not improve diagnostic sensitivity due to the dispersion in bone thickness measurements. However, further studies are needed to evaluate correlation between human bone thickness with densitometric and ultrasonic parameters since the measurement of bone thickness has been found to have significantly large error in diagnosis. This result may be at least in part due to large precision error measurement of the bone thickness, *in vivo* study.

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