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INFLUENCE OF CORTICAL BONE THICKNESS ON THE ULTRASOUND VELOCITY

Luiz Garcia Mandarano-Filho, Márcio Takey Bezuti, Nilton Mazzer, Cláudio Henrique Barbieri

ABSTRACT

Objective: An experimental in vitro study was carried out to evaluate the influence of cortical bone thickness on ultrasound propagation velocity. Methods: Sixty bone plates were used, made from bovine femurs, with thickness ranging from 1 to 6 mm (10 of each). The ultrasound velocity measurements were performed using a device specially designed for this purpose, in an underwater acoustic tank and with direct contact using contact gel. The transducers were positioned in two ways: on opposite sides, with the bone between them, for the transverse measurement; and parallel to each other, on the same side of the bone plates, for the axial measurements. Results: In the axial transmission mode, the ultrasound velocity speed increased with cortical bone thickness, regardless of the distance between the transducers, up to a thickness of 5 mm, then remained constant thereafter. There were no changes in velocity when the transverse measures were made. Conclusion: Ultrasound velocity increased with cortical bone thickness in the axial transmission mode, until the thickness surpasses the wavelength, after which point it remained constant. ***Level of Evidence: Experimental Study.***

Keywords: Ultrasonics. Acoustics. Bone and bones.

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INTRODUCTION

The use of ultrasound as a medical diagnostics method has generated considerable interest due to the low cost, portability, ease of handling, possibility of managing to generate images in real time, providing information on the physical properties of tissues, non-invasiveness, and above all, having the fact that it does not produce ionizing radiation as a characteristic.[1]

In the last two decades the use of ultrasound for evaluation of bone quality through the calculation of its propagation velocity was the subject of countless investigations, emerging as an accurate and reproducible method, which can be used as an auxiliary technique with bone densitometry in the assessment of osteoporosis and of the clinical follow-up of patients. Studies on the normal bone consolidation process, its disorders and the influence of a wide variety of types of implants when fractures are treated surgically have been started recently, using the same methodology. The results show that the technique is practicable, yet many aspects still need to be studied for quantitative bone ultrasonometry to be validated as an auxiliary method in connection with radiography and computed tomography in the evaluation and follow-up of fractures.

The objective of this study was to evaluate the influence of cortical bone thickness on ultrasound propagation velocity, employing bovine femoral bone plates as an experimental model and performing the quantitative ultrasonometry by the underwater and direct contact technique.

MATERIAL AND METHOD

Bovine femur was the bone chosen to carry out the study because of the availability of fresh and frozen pieces at cold-storage plants; due to the fact that they came from animals with known weight, age and sex; as they had been sacrificed for consumption, which would avoid the use of animals exclusively for the survey, and as this bone presents thick cortex as a characteristic, making the idea of the study practicable. We used 60 femurs (39 left and 21 right), from Nelore cattle, all males, aged approximately three years and with 500 kg of weight.

The femurs were then submitted to removal of all the soft parts, still frozen, and only their diaphysis was used to make the bone plates. Each diaphysis enabled the creation of just one plate, since the anterior surface of the bone was always used as a means of standardizing the samples. The plates were made in a length of 130mm, with a width of 30mm and different thicknesses, ranging

All the authors declare that there is no potential conflict of interest referring to this article.

Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto – SP, Brazil.

Study conducted at the Department of Biomedicine, Medicine and Rehabilitation of the Musculoskeletal System. of Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo - Ribeirão Preto, SP, Brazil.
Mailing address: DBMRAL - Hospital das Clínicas de Ribeirão Preto da Universidade de São Paulo (11 andar). Av. Bandeirantes, 3900 Campus USP. Ribeirão Preto - São Paulo. Brazil. CEP: 14048-900. *lgmandarano@yahoo.com.br*

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from 1mm to 6mm. The minimum thickness of 1mm was owing to the fact that thinner plates were hard to make and became excessively fragile; when over 6mm, it was no longer possible to achieve uniformity of thickness throughout the length of the sample. Once they were ready, the bone plates were packed in properly identified individual plastic bags, sealed and frozen at an average temperature of -20°C.

The sample size was calculated using R software in version 2.6.2, assuming that six different thicknesses would be compared in relation to sound velocity. Thus we opted to work with 10 samples of each thickness and the ultrasonometric measurements were taken under the following conditions: AUW (axial underwater) with a distance of 3, 5 and 7cm between the transducers; TUW (transverse underwater); ADC (axial direct contact) with a distance of 3, 5 and 7cm between the transducers and TDC (transverse direct contact).

The underwater sound velocity measurements were performed in an acrylic acoustic tank, with a length of 36cm, height of 10cm and width of 7cm, dimensions considered appropriate to adapt from small bone segments up to some whole bones. A circular window was made in the geometric center of each side wall of the tank, for coupling of the ultrasonic transducers precisely aligned with one another by their axial axis.

Two disc-shaped transducers (one emitter and one receptor) made from PZT-5 wafers, a ceramic with piezoelectric properties, with a diameter of 25mm, were used for the performance of the procedures. The transducers were connected to ultrasound pulse generator-receptor-amplifier (Biotecnosis do Brasil®) equipment, connected to an oscilloscope (Digital Storage Oscilloscope 3062A, Agilent Technologies®) for visualization of the signal received. This equipment, in turn, is connected to a microcomputer fed with a program for signal processing and for ultrasound velocity calculation. The ultrasonic equipment used functions with a circuit that generates narrow pulses with a frequency of 1 MHz. The input voltage in the source transformer is adjustable, but it was set at 100 V, thus fixing the voltage applied in the emitter transducer, with sufficient power for the pulse to cross the bone sample without being totally attenuated.

The signal received by the receptor transducer is amplified by a specific circuit, featuring a selector switch that allows users to amplify the signal or not, with 3X amplification having been established for better visualization of the waves. The oscilloscope visualizes wave reception and the microcomputer processes the signs received and stores the information.

In velocity calculation it is important to identify the point of arrival of the first wave (FAS, or first arrived signal), which will define the travel time on the path. (Figure 1) Several frames of references can be used to confirm signal arrival. In this case it was defined as wave deflection greater than 5% from the baseline, and that is calculated automatically by the computer program.

The equipment was calibrated using a polytetrafluorethylene cylinder, with known and constant ultrasound propagation velocity. The cylinder was positioned between the transducers so that the ultrasonic wave could be incident on the flat surface of the piece. The room temperature was kept at 23°C. The ultrasound propagation velocity was only measured in the water, and afterwards, with the polytetrafluorethylene cylinder positioned inside it. In the case of the direct contact technique, contact gel was used between the transducers and the polytetrafluorethylene piece. This procedure was repeated before the evaluation of each bone plate, to ensure the reproducibility of the measurements. The ultrasound propagation velocity in the water and in the Teflon cylinder averaged 1,470 m/s and 1,156 m/s, respectively.

Before the performance of the ultrasonic measurements, the bone plates were transferred from the storage freezer to a domestic freezer, remaining at -12° C for 12 hours. After that, they were transferred to a refrigerator for a further 12 hours at an average temperature of +4° C. Before the measurements, the assembled pieces remained at a controlled room temperature of 23° C, the same as the water in the acoustic tank.

The measurements by direct contact between the transducers and the bone plates were performed with the help of contact gel. Two types of assemblies were created, one for measuring the ultrasound velocity through axial transmission, and the other for transverse transmission.

For the measurement by axial direct contact (ADC) we used rubber mounts that allowed the exposure of the entire surface of the bone plate and the distance between the transducers could be freely altered. (Figure 2) For the measurement by transverse direct contact (TDC) a rubber mount was also used just to support the assembly. (Figure 3)

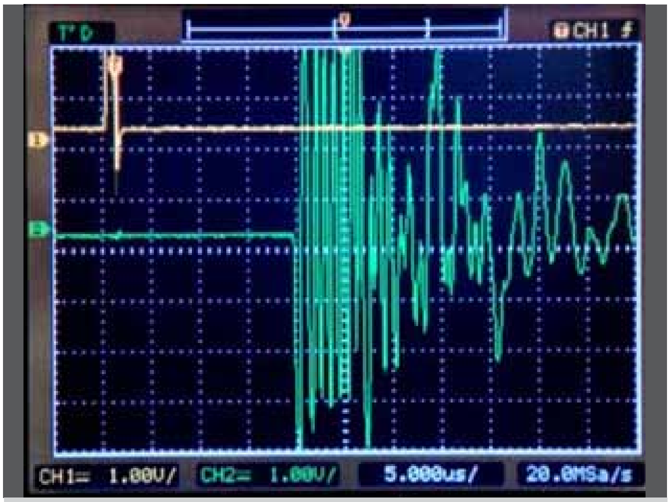

Figure 1. *Image of the wave emitted and received visualized in the oscilloscope.*

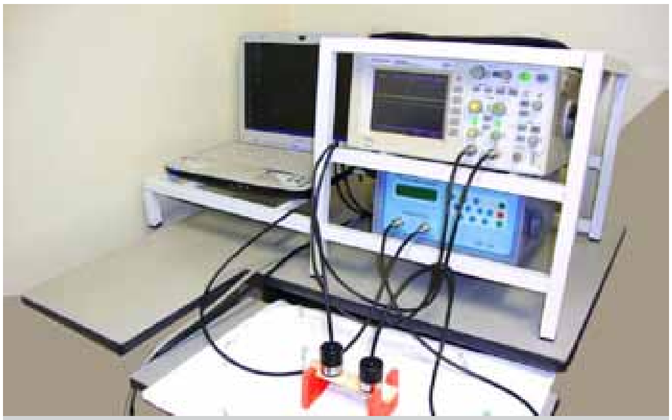

Figure 2. *Measurement of ultrasound velocity via the axial direct contact technique.*

Figure 3. *Measurement of ultrasound velocity via the transverse direct contact technique.*

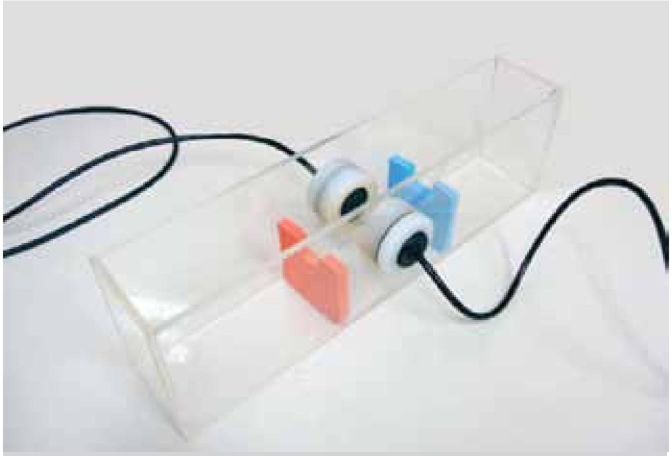

Figure 4. *Measurement of ultrasound velocity via the transverse underwater technique.*

For the measurements by ADC, each group of bone plates with a particular thickness was tested placing the transducers (using the center of the contact surface of the transducer as a reference) at a distance of 3cm, 5cm and 7cm from one another, gauged by means of prebuilt rubber molds, with measurements corresponding to each distance, coupled to the transducers. In this manner we were able to evaluate the influence of the cortical thickness and of the distance between the transducers on the US propagation velocity. In the case of the measurements by TDC, the only variable to be analyzed was the cortical thickness.

The underwater measurements were performed with the help of the acoustic tank. Two types of assembly were made, one to gauge the ultrasound velocity by axial transmission, and the other, for transverse transmission. The same rubber mounts used in the ADC technique were applied for the axial underwater (AUW measurement), allowing the exposure of the entire surface of the bone plate with free alteration of the distance between the transducers. In this situation, the windows of the side walls of the tank were sealed and the transducers were positioned on the surface of the bone plate through the upper opening. For the transverse underwater (TUW) measurement, the transducers were coupled on the side windows and the distance between them was kept constant to allow the accurate calculation of the US velocity. (Figure 4)

Six groups of bone plates were prepared for the performance of the study, each one with 10-plate samples of the same thickness for each group. Each group was submitted to analysis of the sound propagation velocity by the two techniques (direct contact and underwater), in two different ways, by axial transmission and by transverse transmission. (Table 1)

In each specific case we performed three sequential measurements of the SV and extracted the mean of the values obtained for each bone plate. After this, we calculated the mean value corresponding to each group, which was employed in the statistical calculations.

The results were compared to evaluate the influence of cortical thickness on axial and transverse US propagation velocity, to compare the bone ultrasonometry methods (direct contact and underwater) and to evaluate the influence of the distance between the transducers on the *SV* in axial transmission.

Table 1. *Study design. ADC: Axial direct contact, TDC: Transverse direct contact, AUW: axial underwater, TUW: transverse underwater.*

Thickness of the bone plates	N (number of samples)	Bone ultrasonometry
1	10	ADC, TDC, AUW and TUW
2	10	ADC, TDC, AUW and TUW
3	10	ADC, TDC, AUW and TUW
4	10	ADC, TDC, AUW and TUW
5	10	ADC, TDC, AUW and TUW
6	10	ADC, TDC, AUW and TUW

The linear regression model with mixed effects (random and fixed effects) was used to achieve the objectives. Linear mixed-effects models are used in data analysis in which the responses are grouped (measurements repeated for the same individual) and the supposition of independence between observations in the same group is not adequate.[2] These models are based on the assumption that their residues have normal distribution with mean 0 and variance σ^2. In situations in which such an assumption was not observed, transformations in the response variable were used. The Bonferroni simultaneous confidence interval was used with consequent correction of p-value, in order to guarantee that the simultaneous comparisons between the means maintain 95% of confidence.[3] This procedure was executed through the SAS® 9.0 software (SAS Institute Inc., SAS/STAT® User's Guide, Version 9.0, Cary, North Carolina, USA), using PROC MIXED.

RESULTS

The USPV increased consistently with the increase in the thickness of the bone plates, in the axial direct contact (ADC) and underwater (AUW) measurements, but presenting uniformity in the transverse direct contact (CDT) or underwater (TUW) measurements, practically without variation accompanying the thickness of the plates. On the other hand, the distance between the transducers (3, 5 and 7cm) in the axial director contact (ADC) or underwater (AUW) measurements did not produce significant differences in the velocities beyond those observed for the thickness of the plates.

In the ADC measurements, the mean value of the USPV increased from 3491.40 m/s in the thickness of 1mm to 4201.20 m/s in the thickness of 6mm, for the distance of 3cm between the transducers. For the distance of 5cm, the mean USPV increased from 3497.50 m/s in the thickness of 1mm to 4200.30 m/s in the thickness of 6mm. For the distance of 7cm, the mean USPV increased from 3497.90 in the thickness of 1mm to 4200.60 in the thickness of 6mm. (Table 2) The differences between the measurements were significant for all the comparisons ($p<0.0001$), with the exception of those between the thicknesses of 5 and 6mm ($p=1$), in the three different distances between the transducers. For each individual thickness from 1 to 6 mm, there were no significant differences observed for any comparison between the measurements in keeping with the distance between the transducers, of 3, 5 and 7cm, evidencing that this parameter is not important, within the limits investigated.

In the AUW measurements, the mean USPV increased from 3498.90 m/s in the thickness of 1mm to 4200.20 m/s in the thickness of 6mm, for the distance of 3cm between the transducers. For the distance of 5cm, the mean USPV increased from 3493.10 m/s in the thickness of 1mm to 4201.10 m/s in the thickness of 6 mm, and for the distance of 7cm, the mean USPV increased from 3491.70 m/s in the thickness of 1mm to 4200.10 m/s in the thickness of 6mm. (Table 3) The differences between the measurements were significant for all the comparisons ($p<0.0001$), with the exception of that between the thicknesses of 5 and 6 mm ($p=1$), in the three different distances between the transducers. For each individual thickness from 1 to 6mm, there were no significant differences observed for any comparison between the measurements in keeping with the distance between the transducers, of 3, 5 and 7cm, once again demonstrating that this parameter is not important, within the limits investigated.

Comparisons were also made for the same thickness (from 1 to 6mm) of the bone plate and the same distance (3, 5 and 7cm) between the transducers, analyzing the ADC and AUW techniques, with no significant differences having been demonstrated for any comparison, indicating that the two techniques are equivalent.

Grouping the aforesaid data, knowing that there is no significant difference between the velocities with the two techniques (direct contact and underwater), we can set out the results of the axial measurements in a graph that allows us to visualize the pattern of growing ultrasound propagation velocity with the increase in plate thickness, until stabilization from five millimeters of thickness. (Figure 5) In the TDC and TUW measurements, the mean USPV varied very slightly between the thicknesses of 1 to 6mm, remaining between the minimum of 3438.40 m/s and the maximum of 3441.50 m/s for the first, and between the minimum of 3436.90 m/s and the maximum of 3442.90 m/s for the second. (Table 4) There was no significant difference between the measurements of TDC ($p=1$) and, also, of TUW ($p=1$), when comparing the different thicknesses.

Comparisons were also made for the same thickness (from 1 to 6 mm) of the bone plate analyzing the TDC and TUW techniques, without any significant differences having been demonstrated for any comparison, which also indicates that the two techniques are equivalent. (Figure 6)

Table 2. *Description of the velocities obtained with the direct contact technique with axial transmission, for the distances of 3, 5 and 7cm between the transducers.*

		1mm		2mm	3mm		4mm		5mm		6mm		
		Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD
	3	3491.4	2.55	3650.1	1.52	33802.8	2.3	3998.2	2.3	4200.9	1.79	4201.2	2.15
ADC	5	3497.5	3.81	3650.1	3	3800.4	3.57	4001	4.67	4199.3	1.7	4200.3	2.45
	7	3497.9	5.36	3651	2.67	3799.5	4.03	3998.1	2.92	4200.3	2.63	4200.6	1.35

ADC: Axial direct contact; mm: millimeters; SD.: standard deviation.

Table 3. *Description of the velocities obtained with the underwater technique with axial transmission, for the distances of 3, 5 and 7cm between the transducers.*

		1mm		2mm	3mm		4mm		5mm		6mm		
		Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD
	3	3498.9	7.14	3651	5.37	3800	5.52	3998.1	5.84	4199.6	6.75	4200.2	6.16
AUW	5	3493.1	4.63	3649.9	5.28	3798.7	3.4.27	3999.9	5.95	4201	5.06	4201.1	4.75
	7	3491.7	4.76	3649	3.89	3804.2	4.18	3998.8	4.94	4202.1	2.69	4200.1.6	2.85

AUW: Axial underwater; mm: millimeters; SD.: standard deviation.

Table 4. *Description of the velocities obtained with the direct contact and underwater techniques for transverse transmission, with the 6 plate thicknesses.*

	1mm		2mm	3mm		4mm		5mm		6mm		
	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD	Mean (m/s)	SD
TDC	3438	9.12	3442	12.2	3439	10.2	3441	11.8	3436	11.5	3441	9.96
TUW	3440	11.9	3440	8.75	3437	13.6	3443	11.5	3441	11.4	3437	8.44

TDC: transverse direct contact; TUW: transverse underwater; SD.: standard deviation.

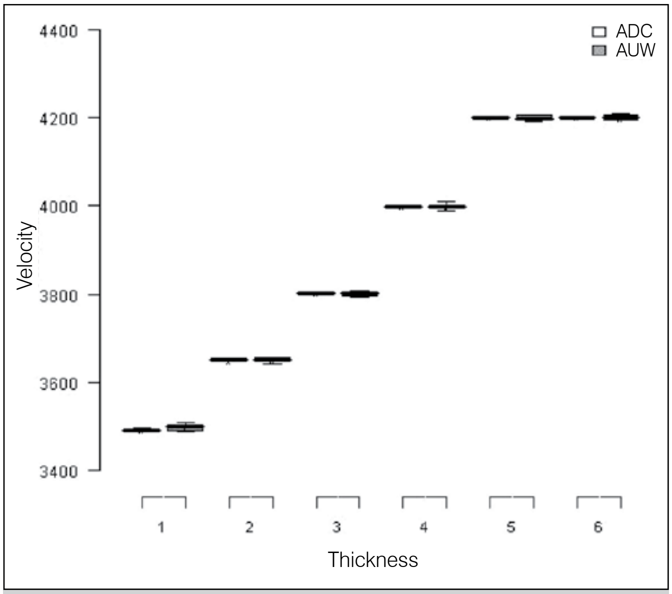

Figure 5. *Box plot of comparison of the USPV in the different thicknesses and with the direct contact and underwater techniques, for axial transmission with a distance of 3, 5 and 7cm between the transducers.*

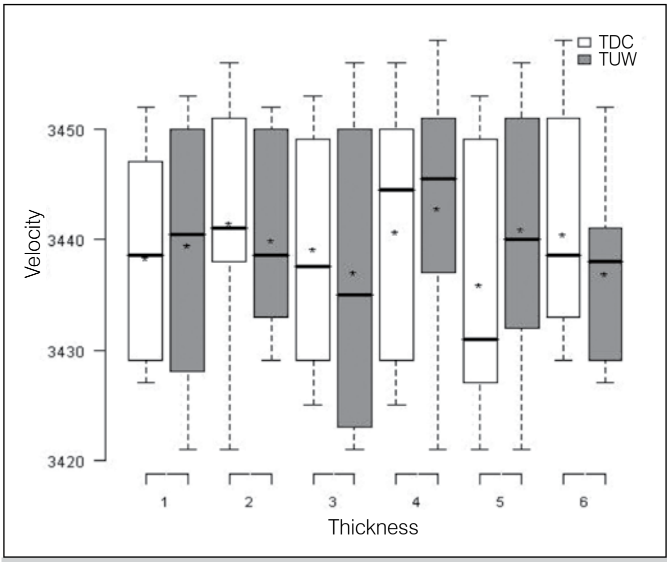

Figure 6. *Comparison of TDC and TUW for the thicknesses of 1mm to 6mm of the bone plates.*

DISCUSSION

The quantitative evaluation of the quality of bone tissue by means of the measurement of the ultrasound conduction speed has been the subject of countless investigations, mainly geared towards the measurement of osteoporosis and of bone healing. With regard to the measure of osteoporosis, the current literature is richer and provides solid subsidies for clinical applicability. On the other hand, this is not true when the focal point is the bone healing process. There are in vitro and in vivo studies, with strong evidence that the method can be applied clinically, yet there is still a lot to be understood until it can be standardized and the results considered reliable.

The fracture consolidation process in humans and animals is usually evaluated through radiographs or computerized tomography, methods that involve the use of ionizing radiation with known deleterious effects on the tissues.[4] This, added to the fact that the bone callus is only visible upon examination if sufficiently calcified, that bone consolidation does not always involves callus formation, as in cases of rigidly fixed diaphyseal fractures submitted to osteosynthesis by the absolute stability method, and that the fracture line can often not be visualized as there are overlapping metal implants, justifies the search for an alternative resource in this field. In addition, there is the fact that it may be necessary to obtain many and repeated radiographs over the course of the treatment, exposing the patient to an overdose of radiation, with significant potential for secondary lesions, particularly in children and pregnant women.

The availability of a resource that involves the employment of a non-ionizing physical agent and that can be used in the initial phases of consolidation would be very useful, especially when many successive evaluations are necessary. Magnetic resonance (MR) has all the above characteristics, but is costly, not always available and the images obtained suffer the influence of metal implants, hindering an adequate interpretation. The use of conventional ultrasound is a possibility, since the method is inexpensive when compared to the other techniques, widely available and easy to handle. However, the images obtained are frequently hard to interpret, with limited reproducibility and dependent on the examiner's experience. The equipment that quantitatively evaluates transosseous ultrasound conduction, such as that used in the diagnosis of osteoporosis and osteopenia, has all the ideal characteristics to be an auxiliary method in the study of the fracture consolidation process, since this equipment has the abovementioned advantages of ultrasound, as well as objective results.

The use of ultrasonometry for bone evaluation was initially described by Siegel et al.[5] in 1958, using rabbit tibias. Its use for the evaluation of bone density through BUA (Broadband Ultrasound Attenuation) was described by Langton et al.[6], and is capable of predicting the quality and the quantity of bone mass, gaining space in recent years with several commercially available models. The use of ultrasonometry for monitoring the fracture consolidation process is more recent, and few studies demonstrate its clinical applicability;[7,8] however, without adequate standardization, due mainly to methodological issues.

In our area, Barbieri et al.[9] conducted an in vitro study on the use of transverse underwater ultrasonometry to evaluate the consolidation of transverse diaphyseal osteotomies of sheep tibia in different periods, demonstrating that the velocity of ultrasound propagation through the bone increases as the consolidation process progresses. This investigation was carried out with a tibial osteotomy external fixation model, which favors bone callus consolidation, a process entirely different from the direct consolidation obtained with the rigid fixation plates using axial compression. This latter type of fixation has become increasingly frequent in the clinical practice, equally entailing an increase in complications such as the delay of consolidation, which can have its diagnosis hindered by the overlapping of the implant as presented previously. Accordingly, Bezuti[10] proposed the in vitro study of the interaction between bone and metal fracture fixation plate, by the measurement of the ultrasound propagation velocity in different planes, showing that the method is efficient in detecting mid-diaphyseal transverse osteotomy furrow of sheep tibia, with no significant influence of the implant on dependency of the plane of incidence of the ultrasonic waves.

Bone ultrasonometry can be performed with the transducers positioned in two different ways; on opposite sides, with the bone between them, for the transverse measurement; or parallel to each other, on the same cortical surface, for the axial measurement. The technique used can also differ between that by direct contact, in which the transducers are placed directly on the bone surface with the help of a contact gel, or the underwater technique, in which the bone is completely submerged in a tank of water. The aquatic medium presents better conditions for the propagation of sound and the study of its velocity, yet the direct contact technique is interesting as the in vivo underwater analysis is often unfeasible.

Two were questions that motivated this study. The first is regarding the type of influence that the thickness of the cortical bone would have on ultrasound propagation velocity, since we know that the thickness can be different according to sex, age, race, level of physical activity and presence of metabolic diseases, with possible impacts on the ultrasonic evaluation. The second concerns the degree of equivalence existing between ultrasonometric techniques, in this case the underwater technique, and the direct contact technique. An in vitro study was idealized for this purpose, using homogeneous bone plates, with all the possible variables well controlled. This kind of comparison is unviable in whole bones, which present an unfavorable relief and significant variability between one another.

The experimental model chosen was that of using bone plates made from the anterior diaphyseal cortex of bovine femur. Bovine femur was chosen for the ease in acquiring samples of fresh and frozen pieces at cold-storage plants, from animals with known weight, age and sex; as it was not necessary to sacrificed animals for the survey, and as this bone presents thick cortex as a characteristic, making the idea of the study viable. The anterior surface of the bone was standardized to make the plates. Due to the surface anatomy of this bone and its average diameter, the largest plate that can be manufactured and always replicated, was 130mm long by 30mm wide, dimensions sufficient for adequate adaptation to the ultrasonic transducers, in a diameter of 25mm. As regards thickness, it was not always possible to obtain plates thicker than 6mm and that were regular all along their length. Likewise, plates with a thickness of less than 1mm were hard to produce and became very fragile. Therefore, the plates from the study ranged from 1mm to 6mm in thickness. To evaluate larger thicknesses, the only possible option would be the use of an animal of greater size or the use of synthetic bone models.

The equipment used for the measurements was built by a specialized company, for the specific purposes of bone ultrasonometry, and could be adapted both for the underwater and for the direct contact measurements, having already been used in previous investigations. It is a prototype that should be implemented for commercial availability and is endowed with digital technology. The computer program developed allows users to measure the ultrasound velocity in both situations (underwater and by direct contact) with high reliability.

As specified by Hill[11] the main parameter chosen for analysis was ultrasound propagation velocity through the bone, as it is considered the essential property of acoustic propagation in tissues. Pocock et al.[12] showed that ultrasound propagation velocity varies with the temperature of the medium of reference (water) and of the actual sample to be analyzed, which is the reason why the temperature of the water and of all the samples was also standardized during the execution of the analyses. The actual velocity is calculated by means of an equation that can vary according to the source consulted, and in this case, we used that proposed by Evans and Tavakoli.[13]

Sievänen et al.[14] commented on the need for at least three ultrasound propagation velocity measurements for each region of interest, which would enhance the reliability of the results obtained. This guideline was followed in the present study, and after the measurements we calculated the mean to arrive at the final value.

The ultrasound propagation velocity results obtained showed that the distance between the emitter and receptor transducers in axial transmission did not influence the measurements, regardless of the technique used, whether by direct contact or underwater. Three distances were used between the transducers in this study: 3cm, 5cm and 7cm. Longer distances were not practicable due to the power of the signal generating equipment and to the high impedance of the bone.

There was no difference in velocity, either, when comparing the different thicknesses of the bone plates with transverse transmission, regardless of the technique used, whether via direct contact or underwater. This data is theoretically expected, since the thicker the plates, the greater the distances and the longer the time needed to cover them.

Another important fact is that the values obtained for ultrasound velocity in bovine cortical bone was similar to the values reported previously by Evans and Tavakoli,[13] which validates the techniques used in this study and the device developed for this purpose.

It is possible to notice that all the measurements performed, with axial or transverse transmission, failed to present differences in keeping with the technique used. Although not the initial objective of the study, we ended up demonstrating that the direct contact technique is comparable with the underground technique. This comparison was only possible due to the object studied, in this case relatively regular bone plates without significant differences between each other. This would not be possible if whole bones had been used, as these differ from one another, in spite of being from the same species and from the same limb. Moreover, superficial anatomical irregularities, even though small, mean that the coupling of the transducers is not complete and this can contribute to the difference in results when compared with the underwater technique. Until now, this technique was considered more reproducible since water is an excellent conductor of sound waves. However, the direct contact technique appears to be more adaptable to clinical situations, as it is possible to collimate the area of interest to be analyzed with greater ease than in an acoustic tank.

As already demonstrated previously by Njeh et al.[15] sound velocity in axial transmission depends on the thickness of the cortical bone; the thicker the bone, the higher the velocity. This fact is true up to a certain limit, and the authors postulate that velocity increases up to the point where the wavelength is even smaller than the thickness of the cortex. The same fact was observed in this study, in which the ultrasound propagation speed increased progressively from about 3,500m/s in the bone plates with a thickness of 1mm (in both techniques, by direct contact and underwater), passing through 3,650m/s in the 2mm plates, 3,800m/s with 3mm, 4,000m/s with 4mm, to arrive at 4,200m/s with 5mm. The velocity remained constant at 4,200m/s in the thickness of 6mm. If we take into account the fact that the frequency of the device is 1 MHz and that the wavelength is calculated by dividing velocity by frequency, we obtain approximately 4.3mm as the wavelength value, since it was the maximum velocity reached in the samples. Therefore, the velocity is not expected to change any more, even if the sample thickness increases, since the thickness has already exceeded the wavelength starting from 5mm.

For the plates of lesser thickness (1 to 4mm), the ultrasonic wavelength was always equal to or greater than the thickness. In these situations, wave conduction occurs throughout the bone thickness and not only on its surface, so that it reflects the physical properties of the bone with greater precision. When the wavelength is smaller than the thickness, its conduction changes, becoming superficial and more rapid, but not reflecting the integral properties of the bone.[16,17]

Knowing the ratio between cortical bone thickness and ultrasound propagation velocity is extremely important for the continuity of studies and the standardization of bone ultrasonometry. Age and osteometabolic diseases alter the thickness of the bone cortex and influence the results obtained, besides the actual mean variability of cortical thickness of the bones of the human body. We know that the mean cortical thickness, measured by radiographic examination, is 1.7mm for the proximal phalanx of the fingers of the hand, 2 to 3mm for the metacarpals, 3 to 3.6mm for the proximal region of the radius, 5 to 8 mm for the tibial diaphysis and 2.3 to 7.4mm for the femoral diaphysis.[18] Accordingly, with the methodology employed in this study, the maximum wavelength was 4.2mm, in the thicknesses of 5 and 6mm. Nevertheless, there are many situations in which ultrasound velocity can be directly affected by the thickness of the bone cortex above the studied limits, which opens up a perspective for new investigations.

The continuity of research in quantitative bone ultrasonometry should focus on applicability in real clinical situations, as well as the influence of implants, that of the geometry of the different patterns of fractures and of their consolidation, and of the physical conditions of bones. Considering the envelope of soft parts around the bones and the difficulties involved in using the underwater technique, the technique of direct contact with the help of contact gel appears, up to this point, to be the most likely path for bone ultrasonometry to occupy its space as a safe, low-cost auxiliary method free of ionizing radiation in the evaluation of the bone healing process, irrespective of the therapeutic approached used, whether conservative or surgical.

CONCLUSION

The thickness of the cortical bone influences ultrasound propagation velocity, when the axial transmission technique is employed. The greater the thickness, the higher the speed, up to the point where the thickness exceeds the wavelength. From this point on, the velocity remains constant. Both bone ultrasonometry techniques, via direct contact and underwater, proved practicable and had comparable results.

REFERENCES

1. Njeh CF, Saeed I, Grigorian M, Kendler DL, Fan B, Shepherd J, et al. Assessment of bone status using speed of sound at multiple anatomical sites. Ultrasound Med Biol. 2001;27(10):1337-45.
2. Schall R. Estimation in generalized linear models with random effects. Biometrika. 1991;78(4):719-27.
3. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ. 1995;310(6973):170.
4. Markel MD, Chao EY. Noninvasive monitoring techniques for quantitative description of callus mineral content and mechanical properties. Clin Orthop Relat Res. 1993;(293):37-45.
5. Siegel IM, Anast GT, Fields T. The determination of fracture healing by measurement of sound velocity across the fracture site. Surg Gynecol Obstet. 1958;107(3):327-32.
6. Langton CM, Palmer SB, Porter RW. The measurement of broadband ultrasonic attenuation in cancellous bone. Eng Med. 1984;13(2):89-91.
7. Saulgozis J, Pontaga I, Lowet G, Van der Perre G. The effect of fracture and fracture fixation on ultrasonic velocity and attenuation. Physiol Meas. 1996;17(3):201-11.
8. Protopappas VC, Baga DA, Fotiadis DI, Likas AC, Papachristos AA, Malizos KN. An ultrasound wearable system for the monitoring and acceleration of fracture healing in long bones. IEEE Trans Biomed Eng. 2005;52(9):1597-608.
9. Barbieri G, Barbieri CH, de Matos PS, Pelá CA, Mazzer N. Ultrasonometric evaluation of bone healing: Experimental study using a model of diaphyseal transverse osteotomy of sheep tibiae. Ultrasound Med Biol. 2006;32(6):875-82.
10. Bezuti MT. Avaliação ultrassonométrica in vitro da osteotomia transversal da tíbia de ovelhas fixadas com placa de compressão [dwissertação]. Ribeirão Preto: Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo; 2010.
11. Hill CR. Physical principles of medical ultrasonics. Chischester: Ellis Horwood; 1986.
12. Pocock NA, Babichev A, Culton N, Graney K, Rooney J, Bell D, et al. Temperature dependency of quantitative ultrasound. Osteoporos Int. 2000;11(4):316-20.
13. Evans JA, Tavakoli MB. Ultrasonic attenuation and velocity in bone. Phys Med Biol. 1990;35(10):1387-96.
14. Sievänen H, Cheng S, Ollikainen S, Uusi-Rasi K. Ultrasound velocity and cortical bone characteristics in vivo. Osteoporos Int. 2001;12(5):399-405.
15. Njeh CF, Hans D, Wu C, Kantorovich E, Sister M, Fuerst T, Genant HK. An in vitro investigation of the dependence on sample thickness of the speed of sound along the specimen. Med Eng Phys. 1999;21(9):651-9.
16. Dodd SP, Cunningham JL, Miles AW, Gheduzzi S, Humphrey VF. Ultrasonic propagation in cortical bone mimics. Phys Med Biol. 2006;51(18):4635-47.
17. Protopappas VC, Fotiadis DI, Malizos KN. Guided ultrasound wave propagation in intact and healing long bones. Ultrasound Med Biol. 2006;32(5):693-708.
18. Havelka S, Bartůnková V, Streda A. Bone indexes and cortical thickness in assessing osteoporosis in rheumatic patients. Scand J Rheumatol. 1973;2(2):57-60.