Ultrasonometric evaluation of the healing of a transverse diaphyseal osteotomy in different periods. Experimental study in sheep tibiae.

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SUMMARY

An experimental study on the ultrasonometric evaluation of the bone healing process of the tibia of sheep submitted to a transverse diaphyseal osteotomy for different periods was carried out using 15 sheep weighing 37 kg on average and divided into three groups of five according to the postoperative period of observation of 30, 45 and 60 days. The osteotomies were performed on the right tibiae while the left tibiae were left for control. Radiographic control was done at 2-week intervals and the animals were killed at the end of the period of observation for removal of the tibiae and ultrasonometric evaluation. Both diameter and ultrasound transverse propagation velocity were measured in two directions (perpendicular and parallel to the anterior tuberosity) at the osteotomy site and compared. The ultrasound propagation velocity increased with time (±5%), with differences between the experimental and control groups being significant, but not within the experimental groups. The diameters decreased in both directions, with differences between the experimental groups being significant, with a strong negative correlation with velocity. It was concluded that ultrasonometric evaluation of the bone healing process is feasible and the results are precise and reliable.

Keywords: Sheep; Tibia; Osteotomy; Bone callus; Ultrasonometry.

INTRODUCTION

Healing process of osseous fractures is traditionally investigated through radiographic evaluation, which identifies the visually detectable amount of a callus formation[1]. Radiographic examinations show osseous healing evidences only when the calcification process on periosteal callus starts, and it is not fully precise[2]. Modern techniques, such as densitometry and computed tomography, are now being used, not only as osteoporosis diagnostic methods, but also for evaluating bone healing. Despite the evolution of those techniques today, they involve the use of ionizing radiation, imposing deleterious cumulative effects on the body. Perhaps this is the reason why the search for other methods similarly sensitive, but at a lower cost, more feasible, and free of deleterious effects is justified, with ultrasound being one of the most powerful resources.

Ultrasound (US) applications began in the 1980's with the purpose of diagnosing and measuring osteoporosis, as well as for predicting an osseous fracture risk[3,4]. In the United States, the Food and Drug Administration (FDA) has authorized the clinical use of some osseous ultrasonometry (OUS) equipment for osteoporosis diagnostics, particularly those employing the calcaneus as a measurement site and sound waves transmission systems with coupling by means of gel or water[4]. Many authors showed that the OUS provides an indirect measurement for anisotropy (physical characteristics variation on a given material according to the direction in which it is investigated) and for osseous tissue quality. Turner and Eich[5], using an *in vitro* model of bovine trabecular bone, demonstrated the ability of OUS to predict the mechanical resistance of the osseous tissue. Gluer et al.[6], by evaluating cubes of trabecular bone removed from a bovine femur, noticed that the attenuation coefficient and the ultrasound propagation velocity (USPV) in the bone were linearly changed according to trabecular alignment and osseous tissue connectivity.

Njeh et al.[7] evaluated the OUS as a method to predict the mechanical resistance of human osseous tissue. Twenty-three femoral heads, removed from patients with osteoarthrosis have

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Received in: 06/16/04. Approved in: 09/08/04

been submitted to OUS and osseous density examinations. The Young module (mechanical resistance and stiffness) was subsequently determined through compression tests. The authors observed a positive correlation among OUS and osseous density, and the mechanical resistance of the assessed bone.

In an *in vivo* study, where human tibiae have been assessed, a positive correlation was demonstrated between the USPV and cortical wall density and width. For each individual, three speed measurements were performed at the same evaluation site (average point of the line between the medial malleolus apex and the medial condyle apex), using a frequency of approximately 1 MHz. In the cortical bone, VS is dependent on elasticity, density and homogeneity(3).

Regarding the evaluation of fracture unions, Siegel et al.(8) had already demonstrated in an experimental study in rabbits that the ultrasound propagation velocity tends to be close to that of the intact bone as healing process proceeds. Gerlanc et al.(9), in a study conducted in human fractured tibiae, in which the normal osseous healing has been analyzed, showed that USPV initially decreases, followed by a gradual increase, achieving up to 95% of the normal value (contralateral bone), after 12 months following fracture. The authors concluded that the USPV measurement can be clinically used as a tool to monitor osseous healing. In both researches, the technique used was the US transmission over cortical surface, in which transducers (emission and reception) are perpendicularly positioned on the tibia (*in vivo*), parallel-oriented from each other and separated by a pre-determined distance(8,9).

In a more recent study, in which a device available in the market* has been used, usually employed for the analysis of intact human tibiae for diagnosing osteoporosis, the authors also demonstrated a progressive increase of USPV as osseous healing progresses(10).

USPV varies from patient to patient, and, in a single patient, from a bone to other. A single value proposed for speed cannot be used as an indicative of the full osseous union. The intact contralateral bone can be used as control, indicating the value of the end-point osseous healing, because some authors demonstrated that significant changes do not occur on a contralateral, non-fractured bone density, from the moment of the fracture until the complete healing of the fractured bone(10,11,12).

Whereas techniques using ionizing radiation for osseous evaluation can impose tissue changes, potentially influencing fetus development and a child's normal growth process, the use of osseous ultrasonometry (OUS) in pregnant women and children might be advantageous(13,14), since the existence of a positive correlation among children's ages, sound velocity and osseous cortical density(14) has already been demonstrated.

The purpose of this study was to develop an evaluation method for the osseous healing through the use of a quantitative osseous ultrasound (QOUS), by the analysis of the USPV, employing mediophyseal transverse osteotomy in sheep tibiae attached with an external fixing device for different periods as a model.

MATERIALS AND METHODS

Fifteen young adult Santa Inês sheep (average age of 1 year old) have been used, with average body mass of 37 kg, coming from the Central Biotery belonging to USP Campus Hall in Ribeirão Preto. The animals were divided into three groups of five animals each (Groups 1, 2, and 3, respectively), according to the period of post-operative follow-up, until they were killed, of 30, 45, and 60 days, respectively. Non-treated tibiae of the 15 sheep have been used as a Control group.

Anesthesia and surgical technique

The animals were submitted to a complete fasting regimen for a preoperative period of 24 hours. Pre-anesthesia was performed using 1% atropine sulfate 0.05mg/kg, subcutaneously (SC), and xylazine dosed 0.22 mg/kg, via intramuscular (IM). Anesthesia was performed with thiopental sodium dosed 12.5 mg/kg intravenously (IV). Support fluidotherapy was performed with 0.9% sodium chloride solution.

It was determined that surgical procedures should be performed on the anteromedial surface of the right tibia. The first surgical step was setting up an external fixation device, which was performed with the help of a gauge instrument in order to precisely locate the insertion points of Schanz's autoperforating threads, 4 cm distant from each other. When the points were marked, cross-sectioned incisions of 1.5 x 1.5 cm were performed on the skin and the threads could be passed with the help of a guide, so as to transfix the two diametrally opposed cortical. Four threads were introduced in each tibia, the most parallel from each other as possible, which was facilitated by the use of the gauge. Then, the connection shaft of the fixation device was setup, stuck to the threads by means of clamps equipped with clockwise locknuts, screwed before osteotomy; the distance from the fixation device shaft to the limb surface was determined as 2 cm, intending to achieve better system stability (Figure 1A). The tibia was accessed through a longitudinal skin incision of about 3 cm long, between the two central threads of the fixation device. The periosteum was longitudinally incised and carefully shifted with a knife to an extension enough to allow the osteotomy to be performed. By using a vibrating saw with a 1mm-thick blade, a mediophyseal transversal osteotomy was performed (Figure 1B). After this procedure, the musculature was sutured with a polyglactin 910 absorbable suture (Vycril 2/0, EthiconÒ) and the skin was sutured with monofilament nylon (Superlon 2/0, CirumédicaÒ). An occlusive dressing was placed over operative wounds.

Post-operative period

Prophylactic therapy with antibiotics was provided with a single dose of a penicillin association, dosed as 40,000 UI/Kg via IM. Additionally, animals received analgesics and antiinflammatory therapy with ketoprofen dosed 2 mg/kg via IM, for five days. Radiographic controls of the operated tibiae were obtained during the immediate post-operative period and each 15 days subsequently, up to the date in which the animals were killed for tibia resection, aiming to control healing progress (Figures 2 – ABCD).

* SoundScan 2000® - Myriad Ultrasound Systems Ltd., Israel.

After the maintenance of the external fixation device during the time determined for each experimental group, the animals were killed with an intravenous overdose of 2.5% thiopental sodium. Tibiae were dried, totally cleared of soft parts and stored at a freezer under -20 º C until the day before ultrasound analysis was conducted.

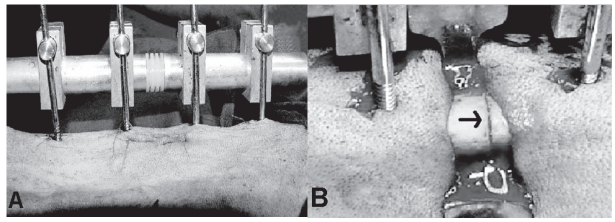

***Figure 1** - Details of the surgical procedure. External fixation device setup (A); mediodiaphyseal transverse osteotomy (B).*

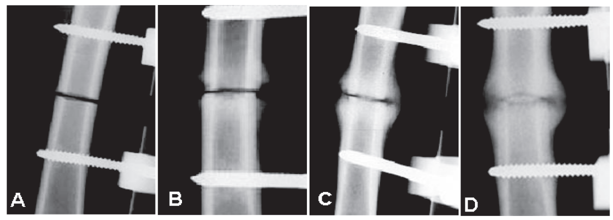

***Figure 2** - Radiographic controls obtained at the immediate postoperative period (A), and after 30 (B), 45 (C), and 60 days (D), showing union progression.*

Ultrasound analysis

Sound speed measurements were performed in an acrylic acoustic tank, 35 cm long, 10 cm high and 9 cm wide, appropriate dimensions to fully fit the tibia inside it. A square window of 9 cm was built in each side walls of the tank in order to attach the ultrasonic transducers made with PZT-5 inserts (piezoelectric material), disc-form, with a diameter of 12 mm and 1MHz frequency. Transducers were attached, then, one at each side of the acoustic tank, centers aligned, one acting as emitter and the other as receptor.

In addition to the acoustic tank equipped with ultrasonic transducers[(1)], time capture system consisted on ultrasound pulse generator-receptor-amplifier equipment[*(2)], one oscilloscope[*(3)], for visualization of the received signs, and a microcomputer[(4)], for processing and storing signs (Figure 3). The sign received by the receptor transducer was, then, amplified by a specific circuit with a build-in switch enabling or not this option.

Before bone analysis, the equipment was calibrated using a Teflon cylinder with a known and constant ultrasound propagation velocity. The cylinder was positioned between the transducers so that the ultrasonic wave could rest on the plane surface of the piece. Initially, the Teflon cylinder was heated in thermal bath with water at 35º C for 30 minutes. The water inside the acrylic stack was also kept at 35º C (± 2ºC). Subsequently, the ultrasound propagation velocity was measured only in water and, then, with the Teflon cylinder positioned inside it. The Teflon cylinder was removed and returned to thermal bath, assuring that temperature remained constant. Ultrasound propagation velocity in water and in the Teflon cylinder was, in average, of 1,273.8 m/s and 1,518 m/s, with a percentage variation of 0.2% and 0.3%, respectively.

The region of interest in the tibiae was already determined as being the medium portion between medial condyle and medial malleolus apexes, both for osteotomy and central diameter measurement purposes, with a caliper, and for USPV analysis, in the acoustic tank. Measurements of tibiae diameters and USPV were performed separately on two planes relatively to the sagittal plane of the anterior tuberosity of the tibia: 1) according to a parallel plane, corresponding to the anteroposterior radiographic incidence (rates at 180º), and 2) according to a perpendicular plane, corresponding to the lateral radiographic incidence (rates at 90º); the plane was shifted for USPV measurement by simply changing the tibia position inside the acoustic tank. Measurement results in those two planes were analyzed separately.

On the day before analysis performance, tibiae were transferred from the storage freezer (-20ºC) to other freezer at -12ºC for 12 hours. After that, they were transferred to a refrigerator at +4ºC for 12 additional hours. Before analysis performance, tibiae remained for two additional hours at controlled room temperature (25ºC). Five sequential measurements were performed in each specimen for each ultrasound incidence plane (rates 90º and 180º), removing and repositioning the specimen at the same pre-determined position, which allowed for measurements dispersion analysis. An average value for those five measurements was obtained, which was subsequently used for statistical calculations.

In the statistical analysis of results, the variance analysis was used for averages equality test for the four groups (one control group and three experimental groups) in the 180º measurements, variables speed and diameter (the latter by its fourth root), with significance level of 5% ($p \leq 0.05$). The Student's T-Test was used for comparing the averages of the four groups (one control group and three experimental groups) of the tibiae at 90º (average pairs with unequal variances), variants speed and diameter, with significance level of 1% ($p \leq 0.01$) for each comparison, thus establishing the resulting probability for the comparisons set of 5.8%. Correlations between speed and diameter were calculated and significances were tested for the four groups as a whole.

RESULTS

Rates at 180°

The average diameter measured at the control group was 13.31 mm (range: 12.1 mm – 14.35 mm). For experimental groups, the average was 26.93 mm (range: 21.8 mm – 33.5 mm) on Group 1 (30 days), 22.06 mm (range: 18.5 mm – 24.0 mm) on Group 2 (45 days), and 20.9 mm (range: 17.7 mm – 23.1 mm) on Group 3 (60 days), with a noticeable progressive decrease, with differences between the control and experimental groups being significant ($p \leq 0.0005$ for each comparison). Differences were also significant on the comparison between Groups

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*** TDS 210 Digital, Tektronix®.

1 and 2 (p=0.018) and between Groups 1 and 3 (p=0.003), but not between Groups 2 and 3 (p=0.876) (Table 1).

Average USPV on control group was 2,838.89 m/s (range: 2,736.5 m/s – 2,908.1 m/s). On Group 1, was 2,290.27 m/s (range: 2,154.3 m/s – 2,377.2 m/s), on Group 2, 2,399.48 m/s (range: 2,331.4 m/s – 2,477.0 m/s) and on Group 3, 2,382.62 m/s (range: 2,258.9 m/s – 2,500.4 m/s), with differences between control group and the others being significant (p≤0.0005 for each comparison). Despite a noticeable increase trend (±5%) as healing progresses, coming closer to values measured on control group, there was no significant difference on USPV comparisons between Groups 1 and 2 (p=0.116), 1 and 3 (p=0.225), and 2 and 3 (p=0.984) (Table 2).

When significances for the four groups as a whole were tested, the Pearson correlation rate (-0.8998) showed a significant negative correlation (p=0) between the diameter and USPV measured on the tibia at 180º orientation (Figure 4).

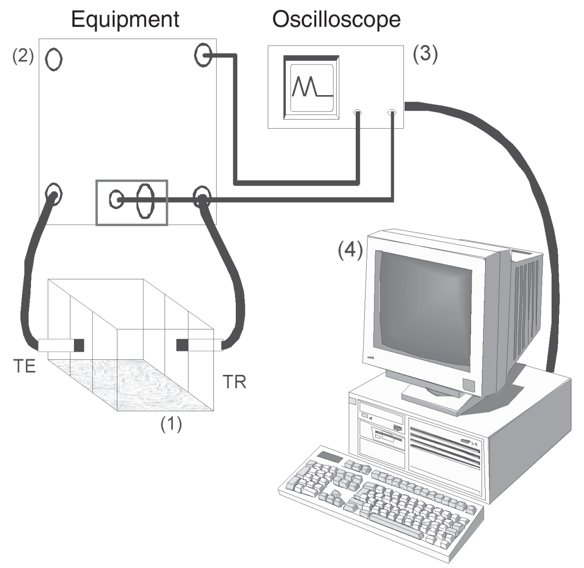

Figure 3 – *Schematic illustration of the ultrasonic measurement system: acoustic tank with attached transducers (1), ultrasound-generator equipment (2), oscilloscope (3) and microcomputer (4).*

Group	Average	SD	Frequency (n)
control	13,31	0,66	15
3	20,9	2,09	5
2	22,06	2,58	5
1	26,93	4,40	5

Table 1 – *Average diameter (mm) values and their respective standard deviations measured on tibiae at 180°.*

Group	Average	SD	Frequency (n)
control	2.838,9	50,67	15
3	2.382,6	102,70	5
2	2.399,5	53,11	5
1	2.290,3	86,35	5

Table 2 - *Average ultrasound propagation velocity values (m/s) measured on tibiae at 180°.*

Rates at 90º

The average diameter on the control group was 15.52 mm (range: 14.25 mm – 17 mm). For treated tibiae, average was 29.28 mm (range: 25.2 mm – 32.65 mm) on Group 1, 23.88 mm (range: 22.10 mm – 25.6 mm) on Group 2, and 22.95 mm (range: 20.75 mm – 24.6 mm) on Group 3, characterizing a progressive decrease, as observed for rates at 180º. Differences between control group and experimental groups were significant (p≤0.0005 for each comparison), also being significant between Groups 1 and 2 (p=0.006) and between Groups 1 and 3 (p=0.003), but not between Groups 2 and 3 (p=0.203) (Table 3).

USPV on control group was 2,891.04 m/s (range: 2,845.7 m/s – 2,916.0 m/s). On Group 1, it was 2,375.76 m/s (range: 2,249.2 m/s – 2,443.5 m/s), on Group 2, 2,471.81 m/s (range: 2,420.4 m/s – 2,536.7 m/s) and on Group 3, 2,466.03 m/s (range: 2,330.4 m/s – 2,538.2 m/s), with differences between control group and experimental groups being significant (p≤0.0005 for each comparison), but not between Groups 1 and 2 (p=0.032), 1 and 3 (p=0.06), and 2 and 3 (p=0.901), despite a noticeable increase trend (±4%) and a trend to get closer to the speed measured on control group and with the progression of healing (Table 4).

When the significances for the four groups as a whole were tested, the Pearson correlation rate (-0.9192) showed a significant negative correlation (p=0) between diameter and USPV measured on the tibia at 90º orientation (Figure 5).

DISCUSSION

Ultrasound does not measure osseous density en masse by area unit as densitometry (g/cm2) does, nor en masse by volume unit as computed tomography (g/cm3) does. In fact, the measure is the ultrasound propagation velocity (USPV) across or along a bone, whether it is a calcaneus or a tibia, and the broadband ultrasound attenuation, or BUA, which measures the percentage of sound waves absorbed by body when crossing or traversing it. Such ultrasonic parameters vary according to density, structure, and elasticity variation, and to other bone biomechanical changes[15].

Fracture union process in long bones involves fast changes on fractured bone consistence, because the fracture callus changes from a virtually liquid phase (fracture hematoma) to a half-solid phase (fibrous callus) and, then, to a solid phase (calcified callus), the three of them having variable duration; the full phenomenon occurs within an average term of one or two months. The theory of osseous ultrasonography by transmission is based on the observation that the sound wave, when crossing a solid body, can suffer changes in its speed and amplitude, depending on material's physical properties. Similarly, ultrasound passing through a bone in union process would certainly receive interference depending on the bone callus consistency, producing changes on the ultrasonic parameters above mentioned, which magnitude has not been precisely determined, but which would exhibit a great potential for clinical applications for complementing information provided by radiographic tests.

Osseous ultrasonometry by transmission is performed in an acoustic tank, with two transducers (emitting and receiving) built in the same axis, and in which the tissue to be studied is sunk in water between them. The 'transmission by direct contact' technique can also be used with an attachment gel placed between them in order to facilitate transducer wave passing through tissue[7]. Temperature is an important variant to consider when sound speed measurements are performed. In a study performed on human cadavers' calcaneus, using the transmission technique, by the moment the piece was sunk in water during the analysis, the authors found a negative correlation between sample temperature and water temperature with the USPV[16]. For this reason, the acoustic tank temperature was strictly controlled during this investigation.

For calculating sound speed in water and in other media, the principle of energy course time of the received sign is employed. In this situation, the course time in the reference media is measured, and, then, the same measure is taken from the sample between transducers. Sound speed calculation at a given object is performed according to the equation[17,18,19,20]:

where:

$$V_s = \frac{1}{\frac{1}{V_r} - \frac{(\tau_r - \tau_s)}{d}}$$

t_s :sign course time with sample;

t_r: sign course time in reference medium (water);

V_s: sound speed in sample;

V_r: sound speed in reference medium (water); and

d: sample width.

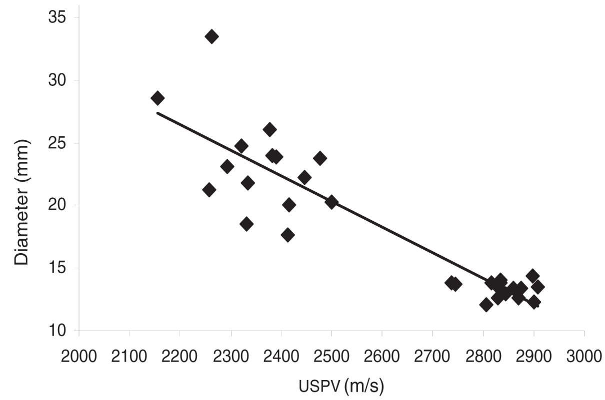

Figure 4 – Chart showing the correlation between the diameter and USPV measured on tibiae at 180° (linear adjustment).

Group	Average	SD	Frequency (n)
control	15,5	0,85	15
3	23,0	1,79	5
2	23,9	1,55	5
1	29,3	3,02	5

Table 3 - Average diameter values (mm) and their respective standard deviations measured on tibiae at 90°.

Group	Average	SD	Frequency (n)
control	2.891,0	25,85	15
3	2.466,0	83,04	5
2	2.471,8	56,13	5
1	2.375,8	80,94	5

Table 4 – Average ultrasound propagation velocity values (m/s) measured on tibiae at 90°.

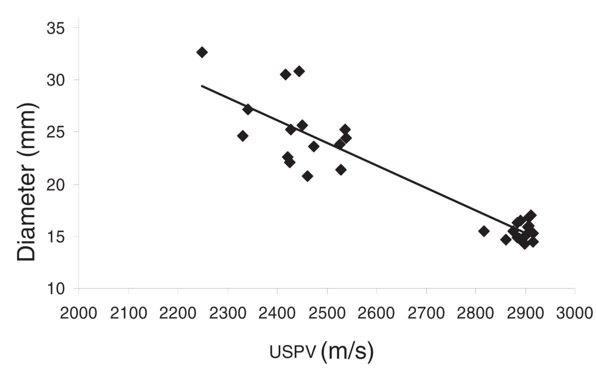

Figure 5 – Chart showing the correlation between diameter and USPS measured on tibiae at 90° (linear adjustment).

The reproducibility of measurements taken with the equipment is verified by periodically taking measurements on a reference material (Teflon), which allows the correction of values and assures better results for speed and attenuation parameters[15].

Quantitative evaluation of the osseous tissue quality through ultrasound conduction speed measurement has been the object of a high number of investigations, specially those targeting osteoporosis measurements. Regarding the use of the same device for osseous healing evaluation, however, available literature is very restrict, although there are some evidences showing that it could be used for that purpose. This was, thus, the main reason for conducting the present study. Diagnostic ultrasound presents the advantage of being a sensitive method, free of ionizing radiation, of low cost (compared to other methods) and more practical. Despite its advantages, ultrasound should not be employed as a replacement to traditional methods for osseous tissue evaluation, but as an aid method, reducing the need of using ionizing radiation, especially in pregnant women and children.

The experimental model selected was transversal diaphyseal osteotomy in sheep tibiae, because this is a large bone with easy surgical access and free of adjacent bones (absence of the fibula, typical of those animals), which provides the formation of a more homogeneous bone callus due to the quality on load distribution on osteotomy focus. Osteotomy procedure was performed on the right tibia in all cases, but both tibiae were used in the ultrasonometric analysis, because of the need to compare operated side to the healthy one. The comparison with the contralateral healthy limb co-validates the methodology because it presents safer and accurate results[8,9,10,11,12]. The shortcoming regarding the use of sheep is the poor availability of those animals, which must be found in slaughterhouses at a relatively high cost by animal, which makes the use of such animals to be expensive and difficult, also due to lodging limitations.

The medium portion between the medial proximal condyle apex and the medial malleolum apex of the tibia was transversal diaphyseal osteotomy purposes due to many reasons: easier surgical access, through a small skin incision; more favorable region for obtaining a large periosteal bone callus; more appropriate site for performing further analysis on ultrasound propagation velocity[3,13,14]. Moreover, the standardization of the region of interest provided a higher reliability of results evaluation.

Post-operative radiographic follow-up of the osteotomy union was carried on each two weeks. In addition to the union itself, such periodicity allows for the evaluation of osseous proximal and distal segments alignment and of the implant position, providing enough time to detect and enable correction for eventual deviations.

Regarding the quantitative analysis of fractures or osteotomies union by using USPV evaluation, there is no equipment specifically developed and commercially available for that purpose. Some authors have conducted studies on that purpose, but they employed obsolete equipment, without digital technology and microcomputer-based signs processing, obtaining results capable of errors on measurements and calculations and with no methodological co-validation, in view of current knowledge and technologies[8,9].

In a more recent study, the authors used a commercially available equipment, developed for osteoporosis analysis in human tibiae (SoundScan 2000® - Myriad Ultrasound Systems Ltd, Israel), for evaluating fractures, detecting an ultrasound conduction velocity increase as healing progresses[16]. The same equipment was used in other board study, where the USPV was measured in bodies of evidence of various materials having known length failures simulating simple transverse diaphyseal fractures in order to evaluate the variations imposed by the failure. The authors concluded that the equipment could be used on the evaluation of tibia fractures *in vivo*[21]. Nevertheless, this equipment is restricted to use on human tibiae, and was built for evaluating intact cortical surface, which means that transducers are fixed at an angle, both for emission and for reception of the ultrasonic pulses, which must travel over a surface without exaggerated deformations, being inappropriate for irregular surfaces analysis, as it is the case of a periosteal bone callus.

The device used on the present experiment was initially developed for osteoporosis evaluation and co-validated by previous studies, but, for being relatively simple and versatile, it was easily adjusted for the intended evaluation[15]. There are a lot of advantages in using it, because it is a digital technology, it is connected to a computer enabling the storage and comparative analysis of gathered data, and it has mobile transducers, which can be adjusted to many kinds of surfaces and uses both water and gel for attachment, depending on the analysis requirements.

The ultrasound technique used by previously mentioned authors was that of the transmission over cortical surface, being the ultrasonic sign introduced at a given point in one of the bone sides and captured in an other point on the same side[8,9,10]. In the present study, the transducers were positioned at the opposite sides of the bone being studied, with centers aligned, so that emitted ultrasonic sign in one of the sides of the bone was captured from the other, at a transversal orientation to bone's axis, crossing not only cortical, but also the medulla and all bone callus, allowing for the evaluation of each bone as a whole, and not only tibia's cortical. Due to the diameter differences in the region of interest, the conduction of ultrasonic pulses was evaluated with bones positioned in two ways (rates at 180º and at 90º). Furthermore, ultrasound water transmission was preferred, with tibiae fully sunk in the acoustic tank filled with water, because this is the best wave propagation medium, totally hitting the bone, crossing it and exiting it from the opposite side they have entered, reaching the captivating transducer. Direct irradiation with attachment gel, would certainly not have the same efficiency, due to irregularities on bone callus surface. Considering the ultrasonic waves propagation route in water, forming a cone, surely a percentage of the waves did not reach the bone, being lost within the tank or being eventually reflected on its walls until they are dispersed. It is not possible to precisely quantify the percentage of ultrasonic waves' loss, but, anyway, this phenomenon was equal in all samples.

The parameter selected for analysis was ultrasound propagation velocity through the bone, because it is considered as a crucial feature of the acoustic propagation on tissues, more characteristic than attenuation (BUA) or spreading[22]. On the other hand, USPV varies according to the reference medium (water) temperature and to the temperature of the sample to be analyzed, this is because water, Teflon, and all samples' temperatures were also standardized during the analysis performance[16]. USPV itself is calculated by means of an equation, which may vary according to the source referred, with the one that best fitted the kind of analysis employed being selected for this study[17,18,19,20].

At least three ultrasound conduction velocity measurements are required for each region of interest, which provides a higher reliability of the results[3]. In this study, five measurements were performed in each of the two evaluation positions, which have probably increased results' level of reliability.

The results obtained in this study clearly showed that ultrasound propagation velocity has progressively increased, along with the progression of osteotomy healing, in a noticeable trend to be closer to that observed on normal tibiae. Yet, differences among experimental groups with the course of time, at the two evaluation positions, were not considered as statistically significant, which may be a consequence of the relatively small number of animals in each group, and, maybe, of the short healing time interval determined for the study (30, 45, and 60 days); as for experimental groups compared to the control group, differences were always significant. It is worthy to highlight that the analysis on this study was performed in an *in vitro* fashion, thus temporal comparisons between the treated tibiae and the healthy tibiae during all healing process *in vivo* were not performed, which means that all comparisons were performed only by the end of the period established for each group. On Group 3, for example, the average value for ultrasound propagation velocity was negatively influenced by the result observed in one of the animals, from which resulted a non-significant difference compared to Groups 1 and 2. It is possible that differences between groups would be stronger and even more significant if ultrasound propagation velocity was evaluated from the beginning of the healing process.

In regard of the bone diameter on the region of interest, which progressively reduced from the 30th day on, as a response to the remodeling process, differences were significant in both positions, both between the control group and the experimental groups, and between an experimental group and the others. Along with this, a strong negative correlation was found between ultrasound propagation velocity and the bone callus diameter on the two evaluation positions, meaning that the narrower the diameter, the higher the speed. This finding demonstrates that the USPV depends on bone callus constitution.

An issue to be addressed in the future – when and if the method of ultrasonic evaluation of bone healing is proven to be feasible in clinical situations – should be the way of performing the test. The method used in this study – the underwater ultrasound – will probably not be practical, and will not provide precise data as well, considering tissues mass around the bone. Probably, the method should be improved for transcutaneous applications with an attachment gel.

CONCLUSION

The authors conclude that the USPV measurement is a method of relatively easy application, which could be useful for evaluating the status of the healing process of a diaphyseal fracture, with a potential to clinical applications *in vivo*.

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