A COMPARATIVE STUDY OF ULTRASONIC VELOCITY AND ATTENUATION IN THE EVALUATION OF BONE HEALING

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ABSTRACT

Objectives: The objective of this study was to compare the in vitro ultrasonic velocity and attenuation in bone healing evaluation. Methods: Seventeen sheep weighting 37 kg in average were used, being divided into two groups of five animals each and one group of seven animals, according to the postoperative follow-up time (30, 60 and 90 days, respectively). Osteotomies were performed on the right tibiae and the intact left tibiae of the 17 animals were used as control. The healing process was monitored with conventional conventional radiographs taken at two-week intervals. The animals were sacrificed at the end of the corresponding follow-up period and both right and left tibiae were removed for in vitro underwater and contact ultrasound evaluations. The transverse and longitudinal ultrasound propagation velocity (USPV) and the broadband ultrasound attenuation (BUA) were measured and correlated. Results: USPV increased with the progression of the healing process, while BUA decreased, with significant differences between the experimental and control groups and between the experimental groups, for most of the comparisons. Conclusion: It was concluded that the method using ultrasound as employed in this investigation is feasible and reliable for evaluating cortical bone healing.

Keywords: *Sheep. Tibia. Fracture Healing. Bone callus. Ultrasonic.*

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INTRODUCTION

Conventional diagnostic tests by imaging (X-Ray, computed tomography, densitometry) involve using ionizing radiation, which may cause tissue changes, potentially influencing fetus development and child growth. For this reason, alternative methods that do not depend on those radiations and are free of deleterious effects, such as magnetic nuclear resonance and ultrasound are preferable, particularly in pregnant women and children.[1,2]

Ultrasound in usually preferred over magnetic resonance for being cheaper and are easily available, but ultrasound is still experiencing developments. Studies on image capturing and ultrasound parameters for human body and animal tissues, including bone, were first conducted in the 1950's, where the potential to characterize and distinguish each tissue was demonstrated. In the 1980's, quantitative ultrasound started to be used with the objective of diagnosing and measuring osteoporosis, and also to predict risks of bone fractures, but there are devices properly tested and authorized available for that end, especially those using calcaneus as a measurement site and sound wave transmission systems with gel or water coupling.[3-5]

With ultrasound, bone mass density is not measured by area unit, as happens with densitometry (g/cm^2), nor in mass by volume unit as in computed tomography (g/cm^3), but the ultrasound wave propagation speed through a body of evidence (ultrasound propagation velocity, or USPV) and the energy lost by the wave when doing it, a phenomenon known as broadband ultrasound attenuation, or BUA. Those ultrasound parameters vary according to density, structure, elasticity and other physical and mechanical characteristics of the bone, which differ according to the region of the assessed bone, characteristic of every anisotropic material.[6]

USPV is expressed as meters per second (m/s) and is regarded as a critical characteristic of the acoustic propagation of tissues, more reliable than attenuation or spreading characteristics, which are influenced by some velocity variations.[7] BUA measures the percentage of sound waves absorbed by the bone when crossing or passing through it, and is expressed as decibels by megahertz (dB/MHz).[8]

Ultrasound waves are better propagated in water than in any other medium, so that underwater ultrasound is usually preferred over the one applying contact transducers with the aid of coupling gel, because both USPV and BUA measurements are more accurate.[8] On the other hand, contact measurements are easier to apply in clinical environment, because it does not require the use of large water containers to dip the limb being studied**.**

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Furthermore, ultrasound can be transversal or longitudinal. In the transversal mode, emitting and receiving transducers are placed at the sides of a body of evidence, and the ultrasound waves transversally pass through them, this being a way to get underwater and contact measurements.[9] In the longitudinal mode, emitting and receiving transducers are perpendicularly placed on the body of evidence, aligned with its long axis, separated by a pre-determined distance and in parallel to each other where ultrasound waves pass through one of its surfaces, with the area of interest lying between transducers; this is an optional way to get contact measurements.[10,11]

By means of experimental and clinical studies, it has been demonstrated that, in a fractured bone, USPV gradually approximates the intact bone, with the progression of union process, meaning that USPV increases as the bone callus is established, acquiring physical characteristics that are close to those of an intact bone.[9-11] As for BUA, very little discussed in ultrasound studies on bones, is gradually reduced with fracture union, meaning that as a bone callus is established, the energy spent by ultrasound waves to cross it is reduced.[12] USPV values vary from person to person, thus, a fixed value cannot be used as an indication of total bone healing. Thus, in a clinical environment, the contralateral bone must be used for comparison; the USPV obtained for it indicating the value corresponding to union endpoint, because some authors show that no significant changes occur on the density of a non-fractured contralateral bone, since the occurrence of fracture to total bone healing.[12-14]

The objective of this study was to compare the key ultrasound parameters on bones - USPV and BUA - in an in vitro evaluation of bone healing using mid-physeal transverse osteotomy of sheep tibiae fixated with a flexible external fixator for different periods as a model.

MATERIAL AND METHOD

The experiment was approved by the Committee of Ethics on Animal Experimentation at the institution which the involved researchers belong to. Seventeen (17) young adult Santa Inês sheep were used. The animals were 10 months old and had a mean body mass of 37 kg (range: 35.3 kg - 38.6 kg).

This was a self-control type experiment, in which intact left tibiae composed the control group (henceforth named intact) for right operated tibiae (henceforth referred to as operated). There were 17 control tibiae, which were submitted to the same ultrasound analyses as the operated ones. The 17 animals were divided into groups, according to the postoperative follow-up period up to sacrifice, of 30, 60 (5 animals each) and 90 days (7 animals), which will henceforth be identified according to their respective periods, namely: Group 30, Group 60, and Group 90.

ANESTHETIC AND SURGICAL TECHNIQUE

The animals were submitted to total fastening for a preoperative period of 24 hours. Pre-anesthesia was provided with a combination of xylazine (0.1 mg/kg), acepromazine (0.1 mg/kg) and tramadol (2 mg/kg), and the anesthesia, with a combination of ketamine (1 g/l), guaiacol glyceryl ether (GGE, 50 g/l) and xylazine (100 mg/l), and, for support fluidics, 0.9% saline solution. The solution infusion speed for maintaining anesthesia was established as 3 ml/Kg/h.

In order to get sample uniformity, surgical procedures were determined to be performed on the right tibiae, duly prepared with broad trichotomy, antisepsis with 20% iodine alcohol solution and insulation with sterile surgical drapes. Both the external fixator placement and osteotomy were made through the anteromedial tibial surface. The first step of the procedure was marking on the skin the mean point between tibial medial condyle and medial malleolum, as a reference for tibial osteotomy. Then, a semi-flexible external fixator was placed with four self-perforating 4-mm wide Schanz wires positioned at a distance of 4 cm from each other. That distance was marked with the aid of a metal template allowing for marking the entry points of the wires and assuring a parallel positioning. Once the entry points were marked, 1.5cm-long skin incisions were made on them, and the wires were passed with the aid of a drill guide in order to transfix both diametrally opposed corticals. Following, the connection nail of the fixator was inserted, held to wires by means of staples equipped with washers, which were fastened prior to osteotomy, located between two central wires. A distance of 2 cm was established between the nail and limb's surface for a better system stability. The tibia was accessed by means of a longitudinal 3-cm skin incision between both central wires and on the reference point marked on the skin. Muscles were retracted and the periosteum was incised at skin level and carefully dissected, providing access to the bone, where a transversal osteotomy was made with a vibrating saw with 1mm-thick blade. After this procedure, muscles were sutured with absorbable polyglactin 910* thread, and the skin with monofilament nylon**. An occlusive dressing was places on the wounds and there kept by means of a slightly compressive band.

Prophylactic antibiotic therapy was provided with a combination of penicillin (40.000 UI/kg) and analgesic and anti-inflammatory therapy with ketoprofen (2 mg/kg), both intramuscular, for five days. Dressings were refreshed at two-day intervals until operative wound healing. The animals were submitted to X-ray tests to assess bone callus progression at the early postoperative period, and at each 15 days to sacrifice date for tibial resection.

After remaining with the external fixator for the time set for each experimental group, the animals were anesthetized with an intravenous injection of a high dose of 2.5% sodium thiopental followed by an intravenous injection of some amount of potassium chloride solution enough to cause a heart attack. Soon after euthanasia, both tibiae were disconnected and dissected, removing all soft parts, leaving just the intact bone. Then, the pieces were packed into individual plastic bags and removing the internal air as much as possible, and frozen at a temperature of -20°C according to thermostat readings, until ultrasound analyses were conducted.

ULTRASOUND TECHNIQUES

The tibiae were submitted to underwater transversal ultrasound (insertion technique) and to contact ultrasound - both transversal and longitudinal. The first was made with the tibia sunk into an acoustic container; the latter were made with transducers directly touching the bone, interposed by a coupling gel. Transducers were made with 13mm-wide disk-like PZT-5 pastilles (piezoelectric material), and the frequency was set to 1 MHz.

The system for measuring ultrasound wave transmission time consisted of an acoustic container (insertion technique) or of an aluminum U-shape tool (contact technique), where emitting and

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* Vicryl 2/0, Ethicon®

** Superlon 2/0, Cirumédica®

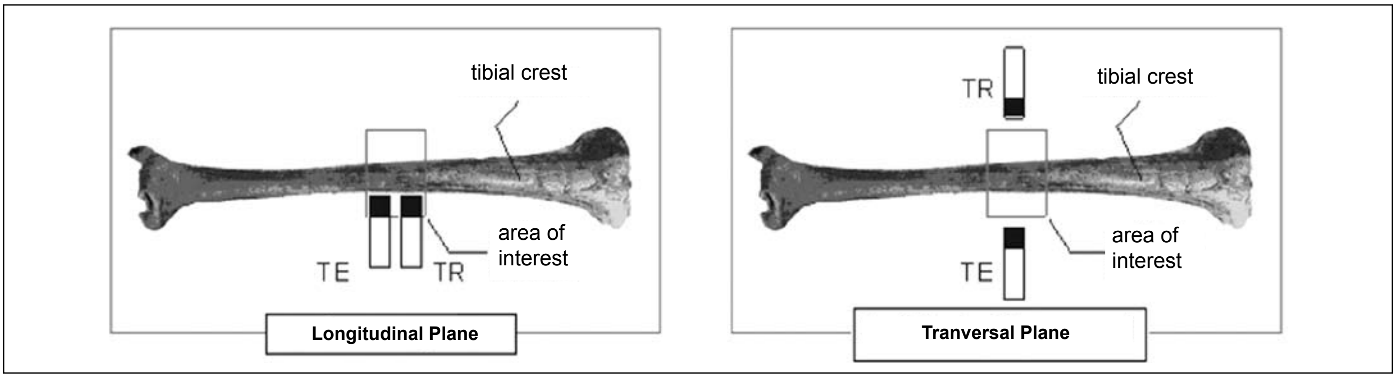

Figure 1 – *Longitudinal plane (used for contact; USPV) and Transversal Plane (used for contact and insertion; USPV and BUA)*

receiving transducers were set, between which tibiae were placed. The system also counted on a ultrasound pulse generator-receptor-amplifier equipment connected to an oscilloscope to view a received signal, which, in turn, is connected to a micro computer for signal processing.[9] The employed ultrasound device works with a circuit generating short pulses (1 μs) and up to 200 V of amplitude to excite the emitting transducer with enough power for the pulse to cross a bone sample without being completely attenuated. The entrance voltage on the source transformer is adjustable, allowing different tensions on the US emitting transducer. Both the hi- and low-tension sources are used to feed a circuit generating well-defined rectangular short-duration pulses. The signal received by the receiving transducer is amplified by a specific circuit, with a built-in switch enabling signal amplification or not. The oscilloscope provides the view and the computer processes any signals received and store all data.

For taking ultrasound measurements, tibiae were removed from the freezer and gradually unfrozen, remaining at a temperature of -12°C for 12 hours, and for additional 12 hours at a mean temperature of +4°C (refrigerator). Prior to analyses, the tibiae remained for additional two hours at a controlled room temperature (room with air conditioner at 25°C measured with a thermometer). The equipment was calibrated prior to each measurement, which were made with the transducers focused to the area of interest, which was the medial portion between the medial condyle and the tibial medial malleollum, where osteotomy had been done, in all groups already covered by bone callus and not identifiable. The positioning of the tibiae into the acoustic container was adjusted so that the ultrasound pulse would lie exactly on the area of interest. Measurements were made at two planes: 1) transversal (transversal velocity, or TV), in which the transducers were positioned on both sides of the osteotomy and perpendicular to the long axis of the tibia and at sagittal plane; 2) longitudinal (longitudinal velocity, or LV) on the anterolateral surface of the tibia in which the emitting transducer was positioned above, while the receptor below the osteotomy, both at equal distance from it. (Figure 1) Six sequential measurements were made in each specimen for each ultrasound plane, allowing for evaluating measurement dispersion. A mean value was calculated for those six measurements and used for statistical calculations.

The results concerning longitudinal velocity, measured by the contact technique (LV), and transversal velocity measured by contact techniques (TVC) with gel and by insertion technique in the acoustic container (TVI) on operated and intact tibiae were compared among the groups. BUA was measured only with the transversal velocity techniques.

For statistical analysis, a linear model of mixed effects was proposed.[15] The adjustment of the model was made by means of the procedure PROC MIXED on SAS software (V. 9), with the significance level established at 1% ($p \leq 0.01$).[16] After the model was built, an analysis of the residues was performed to check hypothesis for the model, being logarithmic transformation regarded as appropriate to meet this requirements in some cases. In addition, a gross comparison of the mean values was suggested for each situation. With the purpose of checking for the existence of a linear correlation between those variables, a linear regression model was employed. The magnitude of the adjusted line bending coefficient indicates how much the respective variables are correlated.

RESULTS

Longitudinal velocity (LV)

For intact tibiae, mean LV was 3.818.11 m/s (range: 2,914.95 m/s – 4,166.32 m/s), 3,852,61 m/s (range: 3,138.72 m/s – 4,144.62 m/s), and 4,369.33 m/s (range: 3,960.05 m/s – 4,861.1 m/s), and, for operated tibiae, 2,727.46 m/s (range: 2,423.47 m/s – 3,217.15 m/s), 2,936.65 m/s (range: 2,329.55 m/s – 3,584.52 m/s), and 4,001.26 m/s (range: 3,642.67 m/s – 4,320.32 m/s), on Groups 30, 60 e 90, respectively. Therefore, LV gradually increased with osteotomies healing, getting closer to the values measured on intact tibiae. The differences were significant for comparisons between intact and operated tibiae at each follow-up period ($p \leq 0.01$), for all comparisons. Differences were significant both for operated and intact tibiae in the comparison between Groups 30 and 90, and between 60 and 90 ($p \leq 0.01$), except for 30 and 60 ($p = 0.19$ and $p = 0.82$, respectively, for operated and intact tibiae. (Table 1)

Table 1 – *Results achieved for variable LV (m/s)*

		Group I		Group II		
		Mean	SD	Mean	SD	P value
Groups	I30 vs O30	3818,11	516,11	2727,46	342,85	<0,01
	I60 vs O60	3852,61	432,54	2936,65	467,54	<0,01
	I90 vs O90	4369,33	297,87	4001,26	202,14	<0,01
Intact	30 vs 60	3818,11	516,11	3852,61	432,54	0,82
	30 vs 90	3818,11	516,11	4369,33	297,87	<0,01
	60 vs 90	3852,61	432,54	4369,33	297,87	0,01
Operated	30 vs 60	2727,46	342,85	2936,65	467,54	0,19
	30 vs 90	2727,46	342,85	4001,26	202,14	<0,01
	60 vs 90	2936,65	467,54	4001,26	202,14	<0,01

Transversal velocity by the contact method (TVC)

For intact tibiae, mean TVC was 2,505.18 m/s (range: 2,399.33 m/s – 2,586.82 m/s), 2,490.15 m/s (range: 2,476.2 m/s – 2,510.75 m/s) and 2,992.00 m/s (range: 2,841.97 m/s – 3,159.02 m/s), and for operated tibiae, 2,147.61 m/s (range: 2,070.47 m/s – 2,255.3 m/s), 2,328.82 m/s (range: 2,254.78 m/s – 2,402.83 m/s) and 2,839.97 m/s (range: 2,657.35 m/s – 3,033.78 m/s), on Groups 30, 60 and 90, respectively, showing that TVC on operated tibiae gradually approximated the values measured on intact tibiae. Differences were significant in the comparisons between intact and operated tibiae in each follow-up period ($p \leq 0.01$), for all comparisons, and for operated tibiae in all comparisons between Groups 30, 60 and 90 ($p \leq 0.01$). For intact tibiae, differences were significant in the comparison between Groups 30 and 90 and between 60 and 90 ($p \leq 0.01$), but not between 30 and 60 (p=0.65). (Table 2)

Table 2 – *Results achieved for variable TVC (m/s)*

		Group I		Group II		
		Mean	SD	Mean	SD	P value
Gel Groups	I30 vs O30	2505,18	78,16	2147,61	75,9	<0,01
	I60 vs O60	2490,15	12,75	2328,82	52,51	<0,01
	I90 vs O90	2992	96,08	2839,97	147,09	<0,01
Intact	30 vs 60	2505,18	78,16	2490,15	12,75	0,65
	30 vs 90	2505,18	78,16	2992	96,08	<0,01
	60 vs 90	2490,15	12,75	2992	96,08	<0,01
Operated	30 vs 60	2147,61	75,9	2328,82	52,51	<0,01
	30 vs 90	2147,61	75,9	2839,97	147,09	<0,01
	60 vs 90	2328,82	52,51	2839,97	147,09	<0,01

Transversal velocity by the insertion method (TVI)

For intact tibiae, mean TVI was 2,987.70 m/s (range: 2,929.37 m/s – 3,015.23 m/s), 2,921.56 m/s (range: 2,859.37 m/s – 3,037.17 m/s) and 2,946.49 m/s (range: 2,883.75 m/s – 2,988.63 m/s), and for operated tibiae, 2,281.46 m/s (range: 2,195.78 m/s – 2,400.7 m/s), 2,484.95 m/s (range: 2,369.78 m/s – 2,599.3 m/s) and 2,693.83 m/s (range: 2,529.45 m/s – 2,808.9 m/s), on Groups 30, 60 and 90, respectively, also showing that TVI on operated tibiae gradually approximated the values measured for intact tibiae. Differences were significant in the comparisons between intact and operated tibiae in all follow-up periods ($p \leq 0.01$), for all comparisons, and for operated tibiae in all comparisons between Groups 30, 60 and 90 ($p \leq 0.01$). For intact tibiae, no significant difference was found in any comparison (30 and 60, p=0.05; 30 and 90, p=0.18; and 60 and 90, p=0.42). (Table 3)

Table 3 – *Results achieved for variable TVI (m/s)*

		Group I		Group II		
		Mean	SD	Mean	SD	P value
Water Groups	I30 vs O30	2987,7	34,21	2281,46	77,59	<0,01
	I60 vs O60	2921,56	70,84	2484,95	95,71	<0,01
	I90 vs O90	2946,49	36,31	2693,83	104,57	<0,01
Intact	30 vs 60	2987,7	34,21	2921,56	70,84	0,05
	30 vs 90	2987,7	34,21	2946,49	36,31	0,18
	60 vs 90	2921,56	70,84	2946,49	36,31	0,42
Operated	30 vs 60	2281,46	77,59	2484,95	95,71	<0,01
	30 vs 90	2281,46	77,59	2693,83	104,57	<0,01
	60 vs 90	2484,95	95,71	2693,83	104,57	<0,01

Broadband ultrasound attenuation by the contact method (BUAC)

For intact tibiae, mean BUA was 44.22 dB/MHz (range: 33.53 – 53.84 dB/MHz), 45.50 dB/MHz (range: 36.57 – 52.14 dB/MHz) and 65.31 dB/MHz (range: 56.43 – 73.05 dB/MHz), and, for operated tibiae, 75.05 dB/MHz (range: 63.25 – 98.16 dB/MHz), 49.85 dB/MHz (range: 28.53 – 62.89 dB/MHz) and 75.54 dB/MHz (range: 66.15 – 85.03 dB/MHz), on Groups 30, 60 and 90, respectively, evidencing that attenuation is reduced as osteotomy heals, and its values gradually approximate those of the intact tibiae. Differences were significant in the comparisons between intact and operated tibiae on Groups 30 and 90 ($p \leq 0.01$), except for Group 60 (p=0.05). For operated tibiae, differences were significant in the comparison between Groups 30 and 60, and between 60 and 90 ($p \leq 0.01$), except for 30 and 90 (p=0.92). For intact tibiae, differences were significant in the comparison between Groups 30 and 90 and between 60 and 90 ($p \leq 0.01$), except for 30 and 60 (p=0.80). (Table 4)

Table 4 – *Results achieved for variable BUAC (dB/MHz)*

		Group I		Group II		
		Mean	SD	Mean	SD	P value
Gel Groups	I30 vs O30	44,22	9,51	75,05	14,24	<0,01
	I60 vs O60	45,5	6,13	49,85	13,06	0,05
	I90 vs O90	65,31	6,25	75,54	6,45	<0,01
Intact	30 vs 60	44,22	9,51	45,5	6,13	0,8
	30 vs 90	44,22	9,51	65,31	6,25	<0,01
	60 vs 90	45,5	6,13	65,31	6,25	<0,01
Operated	30 vs 60	75,05	14,24	49,85	13,06	<0,01
	30 vs 90	75,05	14,24	75,54	6,45	0,92
	60 vs 90	49,85	13,06	75,54	6,45	<0,01

Broadband ultrasound attenuation by the insertion method (BUAI)

For intact tibiae, mean BUAI was 55.,65 dB/MHz (range: 41.96 – 64.31 dB/MHz), 56.15 dB/MHz (range: 51.01 – 65.17 dB/MHz) and 57.05 dB/MHz (range: 49.48 – 64.72 dB/MHz), and, for operated tibiae, 95.20 dB/MHz (range: 86.75 – 104.21 dB/MHz), 75,60 dB/MHz (range: 66.37 – 87.53 dB/MHz) and 69.57 dB/MHz (range: 45.88 – 87.51 dB/MHz), on Groups 30, 60 and 90, respectively, also evidencing that attenuation is reduced as osteotomy heals, and its values gradually approximate those of intact tibiae. Differences were significant in the comparisons between intact and operated tibiae in all follow-up periods ($p \leq 0.01$), for all comparisons. For operated tibiae, differences were significant in the comparison between Groups 30 and 60 and between 30 and 90 ($p \leq 0.01$), except for 60 and 90 (p=0.19), whereas for intact tibiae, differences were not significant in any comparison (30 and 60, p=0.92; 30 and 90, p=0.76; 60 and 90, p=0.85). (Table 5)

Linear correlation (linear regression) TVC *versus* BUAC and TVI *versus* BUAI

The linear correlation (linear regression) was calculated between TVC and BUAC, and between TVI and BUAI, with significances tested as a whole. We found a strong negative and significant correlation, i.e., the broadband attenuation is inversely reduced as ultrasound propagation velocity increases with the progression of osteotomy healing. (Figures 2 and 3)

Table 5 – *Results achieved for variable BUAI (dB/MHz)*

		Group I		Group II		
		Mean	SD	Mean	SD	P value
Water Groups	I30 vs O30	55,65	8,51	95,2	6,96	<0,01
	I60 vs O60	56,15	5,55	75,6	9,19	<0,01
	I90 vs O90	57,05	5,2	69,57	13,32	<0,01
Intact	30 vs 60	55,65	8,51	56,15	5,55	0,92
	30 vs 90	55,65	8,51	57,05	5,2	0,76
	60 vs 90	56,15	5,55	57,05	5,2	0,85
Operated	30 vs 60	95,2	6,96	75,6	9,19	<0,01
	30 vs 90	95,2	6,96	69,57	13,32	<0,01
	60 vs 90	75,6	9,19	69,57	13,32	0,19

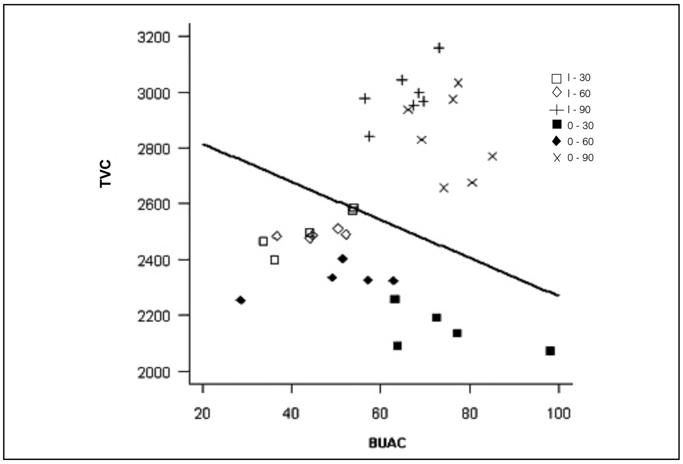

Figure 2 – *Linear correlation (linear regression) between TVC (m/s) and BUAC (dB/MHz). Bending coefficient: -6.82 (p≤0.01). Intact (I), Operated (O).*

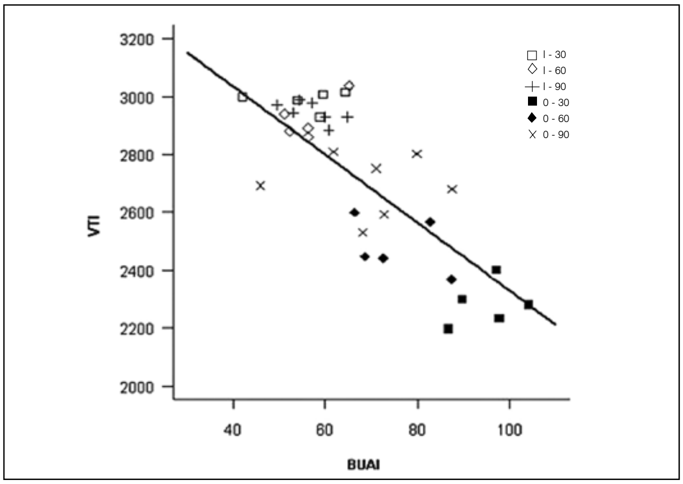

Figure 3 – *Linear correlation (linear regression) between TVI (m/s) and BUAC (dB/MHz). Bending coefficient: -11.73 (p≤0.01). Intact (I), Operated (O)*

DISCUSSION

Fracture healing process in human beings and in animals is usually evaluated by means of X-ray techniques, either conventional, or by bone densitometry, or computed tomography, which are obtained by ionizing radiation, well known to cause deleterious effects on tissues.[3,17,18] Additionally to this, first, is the fact that bone callus is only visible on radiographic tests when fully calcified, with no image being seen before that; secondly, bone union not always involve bone callus formation, so being an almost invisible process, as occurs in shaft fractures tightly fixated and in epiphyseal and metaphyseal fractures, of endosteal union. Therefore, a resource able to be used in the early phases of union, before a bone callus is formed, and preferably one that does not involve the use of ionizing radiation would be very useful, particularly when several successive evaluations are required. This would constitute a niche for using quantitative ultrasound for this purpose, because, within certain parameters, ultrasound is a physical agent totally free of deleterious effects on biological tissues.

Ultrasound has been used for diagnosing osteoporosis by means of specific devices, which could be adapted for evaluating fractures union, with the advantage of being sensitive and cheap, but it would not be designed to replace conventional methods, working only as a complementary test, contributing to a reduced need of using ionizing radiation, particularly in pregnant women and children. However, there are few investigation reported by peer-reviewed literature on the use of ultrasound to evaluate fractures progression, for which reason this study was conducted, focusing the evaluation of the correlation between USPV and BUA. In a previously published study, conducted on a model of tibial mid-diaphyseal transversal osteotomy on sheep, the authors of the present investigation reported the results of measurements only for USPV, within a period of 60 days at most, having demonstrated that this parameter gradually approximates the values measured on intact bones as osteotomy healing process evolves. Now, using an identical model, the follow-up time was extended to 90 days here, a time when bone callus remodeling is already at a late stage, and associated the analysis of broadband ultrasound attenuation (BUA), which is also a parameter of potential clinical use.

One of the intrinsic problems with developing such a study is the equipment employed. In the past, equipments counting on no digital technology and computer processing of signals were used, with which the results are susceptible to measurement and calculation errors.[10,11] More recently, clinical studies have been conducted on human beings using a commercially available equipment developed for studying osteoporosis (SoundScan 2000® - Myriad Ultrasound Systems Ltd., Rehovot, Israel), with digital technology, showing a progressive growth of USPV as tibial fractures healing process progresses.[12,19]

The device used in this experiment was initially developed to evaluate osteoporosis and covalidated by previous studies by De Matos[6], but, for being relatively simple and versatile, it was easily adjusted for assessing fractures healing by Barbieri et al.[9] Its use brings countless advantages, because of its digital technology, for being connected to a computer allowing data storage and a comparative analysis of those, and for counting on versatile transducers, able to be adapted to several kinds of surfaces, allowing the use of both water and gel for coupling, depending on the requirements of the analysis.

The ultrasound technique employed by Gerlanc et al.[11], Saulgozis et al.[12] and Siegel, Anast and Fields[10], was transmission on cortical surface (using a coupling gel), the ultrasound signal being introduced at a given point on one of bone surfaces and being captured at another point of the same surface. Barbieri et al.[9] used the technique where transducers were placed at opposite sides of the bone with centers aligned, so that the ultrasound signal emitted from one of the sides was captured on the other, at a transversal direction to bone axis, crossing not only the cortical, but also marrow and the whole bone callus, and allowing each bone to be assessed as a whole, and not only tibia cortical. The authors also report that due to diameter differences on the area of interest, ultrasound pulses transmission was assessed with the bone positioned in two different ways. Also, the underwater ultrasound transmission was preferred, with tibiae fully sunk into a container filled with water, because this is how waves are best propagated, hitting all the bone, crossing it and exiting through the opposite side to the entrance, reaching the transducer. In the present study, we used the same methodology as Barbieri et al.[9], but direct transmissions were also measured with a coupling gel, because, for future *in vivo* applicability, the underwater transmission technique becomes unfeasible. For that, additionally of cortical surface transmission, this study employed the transversal technique, which is applied just like the underwater technique, but, in this case, using a coupling gel.

As specified by Hill[7], the key parameter selected for analysis was ultrasound propagation velocity through the bone, for being regarded the essential property of acoustic propagation on tissues, but, differently from the studies mentioned above, we decided to use BUA as well.

The results achieved in this study clearly showed that ultrasound propagation velocity has progressively increased, together with osteotomy healing progression, with a clear trend to approximate that observed on intact tibiae. Furthermore, the differences between operated groups with time were significant from a statistical point of view for TVI. For LV and TVC some differences between operated groups were significant. Almost all differences between operated and intact groups were significant.

Concerning ultrasound attenuation (BUA), results show a progressive reduction with healing progression, with a trend to approximation to the values obtained on intact tibiae, in what concerns to BUAI. Furthermore, significant differences were found between operated and intact groups, and, similarly, some differences between operated groups were significant. For BUAC, the results were confusing and not significant; however, they have showed a progressive reduction (with variations) with the progression of the healing process, with a subtle trend to approximate the values obtained on intact tibiae.

In addition, TV *versus* BUA (insertion and contact) was correlated. Strong negative correlations were found with significant results for both correlations. These results show that the higher the ultrasound propagation velocity on bone callus the least ultrasound is attenuated.

These findings suggest that ultrasound velocity and ultrasound attenuation variables are dependent on bone callus constitution, which is initially predominantly fibrous (fibrocartilage), and, thus, shows a poorer ability to transmit mechanical waves, such as those of the ultrasound, more strongly attenuating them, and due to the fibrocartilaginous nature of the callus. With time, fibrocartilage is replaced by spongy bone and, finally, by cortical bone, making ultrasound transmission and attenuation to tend towards those measured on intact tibiae, reminding that only Saulgozis et al.[12] have also worked with attenuation (BUA).[9-12]

No longitudinal BUA measurements were made, because the signal achieved at that position was inaccurate for such calculations, what does not happen with velocity calculations, which are associated to ultrasound signal arrival time.

Ongoing studies, especially concerned to clinical environment (*in vivo*) are warranted to prove the strong applicability of ultrasound as a support, safe, cheap method, free of ionizing radiations in the evaluation of bone healing process.

CONCLUSION

The method for evaluating bone healing by quantitative measurements of ultrasound propagation and attenuation velocity in *in vitro* environments as described in this study has shown to be feasible and easy to apply, allowing to detect small differences even between short follow-up periods, with significant differences between a large portion of the comparisons. The method can potentially be developed also in clinical *in vivo* environments.

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