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ABSTRACT: An ultrasonometric and computed-tomographic study of bone healing was undertaken using a model of a transverse mid-shaft osteotomy of sheep tibiae fixed with a semi-flexible external fixator. Fourteen sheep were operated and divided into two groups of seven according to osteotomy type, either regular or by segmental resection. The animals were killed on the 90th postoperative day and the tibiae resected for the in vitro direct contact transverse and axial measurement of ultrasound propagation velocity (UV) followed by quantitative computer-aided tomography (callus density and volume) through the osteotomy site. The intact left tibiae were used for control, being examined in a symmetrical diaphyseal segment. Regular osteotomies healed with a smaller and more mature callus than resection osteotomies. Axial UV was consistently and significantly higher ($p \leq 0.01$) than transverse UV and both transverse and axial UV were significantly higher for the regular than for the segmental resection osteotomy. Transverse UV did not differ significantly between the intact and operated tibiae ($p = 0.20$ for regular osteotomy; $p = 0.02$ for resection osteotomy), but axial UV was significantly higher for the intact tibiae. Tomographic callus density was significantly higher for the regular than for the resection osteotomy and higher than both for the intact tibiae, presenting a strong positive correlation with UV. Callus volume presented an opposite behavior, with a negative correlation with UV. We conclude that UV is at least as precise as quantitative tomography for providing information about the healing state of both regular and resection osteotomy. © 2011 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 30:1076–1082, 2012

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Any diaphyseal fracture of a long bone is expected to heal in <3 months, depending on factors such as fracture type, patient’s health, and appropriate immobilization or fixation.1,2 Fractures treated conservatively or with external or internal fixation usually heal by means of a bulky callus that envelopes the fragments, while fractures treated with open reduction and rigid internal fixation tend to heal directly without callus formation. Whichever the healing mechanism, the quick changes in the consistency of the tissue formed between and around bone fragments are relatively easy to recognize on conventional radiographs or by computer-aided tomography (CT) for most fractures. Healing anomalies, such as delayed union and non-union, are common, but can go undiagnosed with usual imaging resources.3-4 Information provided by such techniques largely depends on subjective interpretation by an examiner and may be hindered by the presence of metal implants. Although versatile, conventional radiographs are not entirely reliable for the diagnosis of a healing anomaly.1,3 CT scans are the method of choice for doubtful cases, since they show sliced images of the cortex, exposing fracture lines of different width and direction, besides demonstrating bone fractions of different densities and quantifying mineral content and distribution among fragments. However, both involve the use of ionizing radiation and should be avoided for prolonged and repeated use, particularly for pregnant women and children.5,6

Ultrasonometry, particularly the measurement of ultrasound propagation velocity (UV), appears as a possibility to evaluate healing status, since experimental and clinical studies demonstrated that UV decreases through a fractured bone and progressively increases as healing takes place.5-9 However, not many studies exist on the behavior of UV through a fractured bone.9,10 Also, UV varies between bones in the same individual and in homonymous bones of different individuals, so that comparisons are often made with the homonymous contralateral bone at a point corresponding to the fracture site, based on the demonstration that the physical and biomechanical properties of the intact contralateral bone do not change during healing of the fractured bone.6,11-14

UV can be measured both transversely and axially, just by changing the position and interrelation of the emitting and recipient transducers. For the transverse mode, the ultrasound waves go through the bone–callus complex, while for the axial mode, the waves tend to run superficially, providing an evaluation of the subperiosteal region, depending on the wave length of the emission.6,7,15,16

Most of the knowledge about the behavior of ultrasound wave transmission through bone and fracture was derived from experimental investigations with animal bones. The macro and microscopical structure of animal bones, as well as fundamental phenomena...
involved in fracture healing, are the same in all mammals. Thus, such knowledge can be directly extrapolated to humans.

UV is probably reduced through a delayed union or nonunion, but ultrasound wave transmission through a healing anomaly has not been well studied, despite the potential clinical use of the method. Thus, our objective was to compare transverse and axial UV with quantitative CT in the evaluation of a healing anomaly resulting from a resection transverse osteotomy of the midshaft of sheep tibiae fixed with a semi-flexible external fixator by comparing it with the uneventful healing resulting from a simple transverse osteotomy in the same experimental design.

MATERIALS AND METHODS

The experiment was approved by the Ethics Committee on Experimental Use of Animals of our institution. Fourteen young adult sheep (10 months of age, average body weight = 37 kg) of the Santa Inez breed were divided into two groups of seven animals according to the type of procedure: a complete linear transverse osteotomy (Group 1) and a 5 mm-thick resection transverse osteotomy (Group 2). The right tibia was operated upon; the left tibia was left intact and used for control with comparisons always being made between sides for each animal.

Operative Procedure

The animals were fasted for 24 h before surgery to avoid intraoperative problems with vomiting and aspiration, since they were not intubated. Under general intravenous anesthesia (jugular vein; Ketamine, 3 mg/kg; Xylazine, 0.1 mg/kg; Acepromazine, 0.1 mg/kg; Tramadol, 2 mg/kg) and plain oxygen continuously administered within a nostril, a semiflexible external fixator was installed on the anteromedial aspect of the right tibia with four percutaneous self-tapping threaded pins (4 mm diam). Each pin was manually introduced down to the opposite cortex through a 1.5 cm × 1.5 cm cross incision and through a 3 mm hole perforated with a drill. The shaft was exposed through a 4 cm-long skin incision between the 2 central pins and the transverse linear or 5 mm-thick resection transverse osteotomy was made at the midpoint between the medial tibial condyle and the medial malleolus with an oscillatory saw (0.8 mm-thick blade). Saline was administered intravenously at the rate of 1 ml/min throughout the procedure, and an occasional extra dose of the above anesthetic mixture was administered to maintain the anesthetic level. The procedure took ~45 min, and both periosteum and skin were closed with separate stitches of absorbable suture (4/0 Vicryl, Ethicon). An occlusive dressing was applied to protect the incision and the entry points of the pins, and the animals were left free to walk immediately after recovering from anesthesia.

Postoperative Management

Prophylactic antibiotics (crystalline penicillin, 40,000 UI/kg, a single dose at the end of the surgery) and analgesics (Ketoprofen, 2 mg/kg TID, for 5 days) were injected intramuscularly. Control radiographs were taken postoperatively and at 15-day intervals until healing was ensured. All animals were killed on the 90th postoperative day with excess general anesthetic (Thiopental) injected intravenously, and both tibiae were resected and stripped of all soft tissues including the periosteum. Bones were stored in plastic bags at −20 °C until the day before ultrasonometric analysis (2 weeks on average), when slow defrosting was started to last 24 h, including the last 2 h period at room temperature.

Ultrasonometric Analysis

UV was measured with an ultrasound generator–receiver–amplifier source (Biotecnosis do Brasil Ltda, Model US01, Ribeirão Preto, São Paulo, Brazil) equipped with two unfocused ultrasound transducers (2 mm-thick PZT-5 disc, 12 mm diam, 1 MHz freq.), one for emission, the other for reception, able to generate high power (up to 300 V) narrow (1 μs) well defined ultrasonic pulses and linked to a computer loaded with software capable of calculating UV, based on time of flight of the first arrived signal (FAS), as recognized on a digital storage oscilloscope (Agilent Tech., Inc., model DSO3062A, Shangai, China) together with the emitted ultrasound signal. The equipment was calibrated after every five measurements with a 20 mm-thick Teflon disc, the UV through which is known to be constant (1,274 m/s at 35 °C, 0.3% variation). The diameters of the tibiae were measured on the frontal plane, at the osteotomy site for the operated tibiae, and at a corresponding point for the intact tibiae, as previously described, the values obtained being used to calculate UV. The UV measurement was conducted according to the direct contact transverse (DCT) and direct contact axial (DCA) modalities.

The transducers were mounted onto a U-shaped aluminum accessory facing each other and aligned with each other along the central axis for the DCT modality. For DCA, they were mounted parallel to each other, so as to be positioned one above (emitting) and the other one below (recipient) the osteotomy site (Fig. 1). In both cases, abundant ultrasonic coupling gel was used to ensure intimate contact with the bone. The time elapsed between the emitted ultrasound signal and the FAS was automatically introduced into the computer software; the variable distance, consisting of each individual diameter for DCT and the distance between transducers for DCA, was manually inserted. Five consecutive UV measurements were made for each modality, and an average value was calculated and used for the analyses. The highest and lowest values were discarded to ensure homogeneity. Both DCT-UV and DCA-UV were calculated as:

\[ V_u = \frac{d}{t} \]  

where \( V_u \) = velocity; \( d \) = distance (frontal diameter of the specimen for DCT and distance between the centers of the transducers for DCA); and \( t \) = time.

Quantitative CT Analyses

Heliocoidal CT scans were performed with conventional equipment (Siemens, Somaton Emotion, Forchheim, Germany). Both tibiae were positioned side by side ~5 cm from each other, with the tibial crest perpendicular to the table plane. Twenty-six 1 mm-thick tomographic sections were obtained from the interest area, involving the osteotomy site of the operated tibiae and a corresponding segment of the intact tibiae. A soft tissue protocol (130 kV, 125 mA) was used to demonstrate the still young and incompletely calcified bone callus above and below the osteotomy (Fig. 2). The tomographic images were initially processed and stored and later transferred to another computer fed with the Image J software (NIH), for the analysis of callus volume and density (Hounsfield units, HU) based on automatic segmentation of...
the region of interest with a previously defined 100 HU threshold. Density was calculated by the quotient between the intensity (HU) of each individual pixel and the total number of pixels:

\[
D(Im) = \frac{\sum_{i=1}^{n} d(i)}{n}
\]  

(2)

where \(d(i)\) is the density (HU) of each pixel, and \(n\) is the number of pixels in the region of interest.

Callus volume was calculated based on the number of pixels in the region of interest using Cavalieri’s principle, according to which the volume of each individual tomographic slice is the product of the slice area by its thickness, and the total callus volume is the sum of the volumes of all slices.\(^{17}\) A percent volume index was calculated by dividing the volume obtained for each operated tibia by the volume of a corresponding area of the opposite intact tibia, and then multiplying the product by 100.

**Statistical Analysis**

Data referring to UV and callus density and volume were compared between operated and intact tibiae using a mixed effects linear model,\(^{18}\) adjusted by the Proc Mixed procedure of the SAS v.9 software at a 1% level of significance.\(^{19}\) An analysis of residues was conducted to check assumptions; a logarithmic transformation was adequate to satisfy this demand in some cases. A raw comparison between average values was also performed for each situation and the Spearman correlation coefficient was used to quantify correlation between density and transverse and axial UV.

**RESULTS**

Anesthetic and operative procedures were well tolerated by the animals, and all presented with uneventful wound healing and resumed weight bearing on the operated leg and normal gait by the 7th postoperative day. The results obtained for the tibiae are summarized in Table 1. The average frontal diameter of the intact tibiae was 13.4 mm (range: 12–14 mm); for the operated tibiae, it was 19.8 mm (range: 16–22 mm) in Group 1 and 23.9 mm (range: 21.3–31.2 mm) in Group 2. These differences between groups were significant (\(p \leq 0.01\)).

The average DCT-UV was 2,132 m/s (range: 1,974–2,282 m/s) for the intact tibiae. For the tibiae in Group 1 (assumed normal healing), it was 2,229 m/s (range: 2,062–2,389 m/s), while in Group 2 (delayed healing) it was 1,934 m/s (range: 1,545–2,338 m/s), with a significant difference between Groups 1 and 2 (\(p \leq 0.01\)), but not between the intact tibiae and Groups 1 (\(p = 0.20\)) and 2 (\(p = 0.02\)). The average DCA-UV was 3,553 m/s (range: 3,230–3,750 m/s) for the intact tibiae. For the tibiae in Group 1 it was 3,181 m/s (range: 2,755–3,534 m/s), dropping to 2,377 m/s (range: 1,718–2,846 m/s) in Group 2, with significant differences for any comparison (\(p \leq 0.01\)).
Bone density was 1,756 HU (range: 1,712–1,875 HU) for the intact tibiae, decreasing to 1,383 HU (range: 1,193–1,568 HU) for Group 1 and to 1,027 HU (range: 795–1,341) for Group 2, with significant differences for any comparison \((p < 0.01)\). Callus volume was 3.6 cm\(^3\) (range: 2.4–4.6 cm\(^3\)) for the intact tibiae, increasing to 9.0 cm\(^3\) (range: 6.0–11.9 cm\(^3\)) in Group 1 and to 12.2 cm\(^3\) (range: 9.2–18.3 cm\(^3\)) in Group 2, with a significant difference for any comparison \((p < 0.01)\). A strong positive linear (rho: 0.8) and significant \((p < 0.01)\) correlation existed between DCA-UV and density (Fig. 3). On the contrary, a weak positive linear (rho: 0.32) nonsignificant \((p = 0.1)\) correlation existed between the DCT-UV and density (Fig. 4). Similarly, there was a strong negative linear (rho: −0.67) and significant \((p < 0.01)\) correlation between the DCA-UV and callus volume (Fig. 5). On the contrary, there was a weak negative linear (rho: −0.12) nonsignificant \((p = 0.55)\) correlation between the DCT-UV and callus volume (Fig. 6).

**DISCUSSION**

Most diaphyseal fractures of long bones proceed to uneventful healing whatever the treatment method used. Conservatively treated fractures tend to heal with a bulky periosteal bone callus bridging the main fragments and enveloping smaller intermediate fragments, also called irritative callus. Irritative callus is stimulated by slight repetitive motions between fragments, usually induced by unstable fixation devices, such as a plaster cast and external or internal fixator. Fractures treated with rigid internal fixation, particularly with plates and screws or screws only, heal directly from one fragment to the other, without callus formation. The irritative callus takes longer to ossify than direct healing and may not result in complete healing in the presence of an excessive motion between fragments.

Some diaphyseal fractures evolve to delayed union, which still bears some healing potential, and nonunion, which will probably never heal, instead resulting in pseudarthrosis. The stages of delayed and...
nonunion can present diagnostic problems, since the situation may be obscured by the geometry of the fracture, superimposition of bone fragments, and presence of an incomplete callus or metal implants. Conversely, the radiographic diagnosis of a healing anomaly largely depends on the surgeon’s ability to recognize the situation, which involves a great deal of subjectivity in image interpretation. From this standpoint, a diagnostic method applicable to the earliest phases of healing and, preferably, does not involve ionizing radiation, would be of great help in clinical practice, particularly when repetitive and prolonged use of diagnostic resources becomes necessary. Diagnostic quantitative ultrasound fulfills these requirements, since it is almost entirely devoid of harmful effects on biological tissues within certain parameters. Already used for diagnosis and quantification of osteoporosis, it is quite sensitive, practical, and economic. Fracture healing can be adequately quantified by measuring UV and attenuation, with UV increasing and attenuation decreasing as healing takes place, even in the earliest phases. 8,9,11

We undertook the present in vivo investigation using sheep tibiae as a model to compare a simple transverse midshaft osteotomy with a segmental resection osteotomy, with the aim of producing normal and delayed healing, respectively. The model of a simple transverse osteotomy has been successfully tested to evolve to a complete healing and remodeling situation within a normal period. 6,15 Segmental resection osteotomy mimics the bone-aching fracture and tends to take longer to heal than simple osteotomy. Both types of osteotomy were fixed with a semi-flexible external fixator, which permits some axial motion between fragments so as to stimulate irritative callus formation, but prevents sideways displacements, which impose a shear stress upon the newly formed callus, with a potential to prevent healing.

UV was the preferred parameter, since it is considered a fundamental acoustic property of the tissues and is easier to calculate than broadband ultrasound attenuation (BUA), the other frequently used parameter, particularly for osteoporosis evaluation. 24 In a previous investigation, UV and BUA were compared to evaluate an experimental fracture healing and presented a strong negative correlation, with BUA decreasing while UV increased. But BUA was less consistent, presenting momentary variations that were difficult to interpret. 6

Besides UV, callus volume and density were measured with quantitative CT to test the hypothesis that UV is directly proportional to density and indirectly proportional to callus volume.

Ultrasound waves propagate better in water than in any other medium, including the coupling gel used for the direct contact modality. Underwater UV is consistently higher than direct contact UV, but both present the same behavior, that is, increasing as healing takes place, with nonsignificant differences between them. Also, axial UV is consistently and significantly higher than the transverse modality, but we decided to test both modalities, since both present potential for clinical application. The transverse modality was tested in the coronal plane only, since no significant difference

![Figure 5](image_url)

Figure 5. The correlation between direct contact axial ultrasound velocity (m/s) and callus volume (cm³). The intact tibiae are clustered around 3,500 m/s UV and below 5 cm³ volume, while Group 1 and Group 2 tibiae are scattered at progressive lower UV but higher callus volume level, presenting a descending distribution, with an intermediate negative Spearman’s correlation coefficient (rho: −0.67; p ≤ 0.01).

![Figure 6](image_url)

Figure 6. The correlation between direct contact axial ultrasound velocity (m/s) and callus volume (cm³). The distribution has a low negative Spearman’s correlation coefficient (rho: −0.12), with the intact tibiae clustered between 2,000 and 2,200 m/s UV and below 5 cm³ volume, and Group 1 and Group 2 tibiae presenting a scattered distribution, the first at a similar UV but higher volume level, and the second at lower UV but higher volume level (p = 0.55).
between coronal and sagittal planes was previously observed.

We showed that the average transverse UV was significantly higher (~13%) for the simple osteotomy than for the resection osteotomy. On the other hand, the average transverse UV was slightly higher (~4.5%) for the simple osteotomy and slightly lower (~9%) for the resection osteotomy than for the intact tibiae, though not significantly so. A significant difference (~13%) was observed between the two osteotomies. The average axial UV presented a different behavior, with values for the intact tibiae being consistently and significantly higher than for the simple (~10%) and resection (~33%) osteotomies, with the average axial UV being also consistently and significantly higher (~25%) for the simple versus the resection osteotomies. We believe that such behavior is consistent with the way ultrasound waves travel through bone. Axial ultrasound wave transmission depends on the relation between cortical bone thickness and ultrasound wavelength, with waves of narrower wavelength than the cortical thickness propagating along the subperiosteal region of the entry surface, while waves of wider wavelength propagate through the entire cortex, the bone acting as a waveguide. In such a situation, the received signal waveform is a superimposition of multiple guided wave modes, which propagate throughout the cortex and are sensitive to the bone’s mechanical and geometrical properties, thus generating complicated wave guidance phenomena difficult to interpret only by UV measurement. The same mechanism applies to transverse transmission through the cylindrical bone surface, but a contingent of waves goes directly through the marrow, resulting in a lower velocity.

UV is consistently higher for superficial than for the cortical thickness transmission. Despite superficial conduction, axial transmission is also favored by the osteonal structure of cortical bone, responsible for its anisotropy, whereby its physical properties vary according to trabecular alignment, connectivity, and direction of application of mechanical energy, like ultrasound. For axial transmission, the ultrasound waves go in the direction of the normal lengthwise distribution of the collagen fibers instead of crossing them as in the transverse modality, thus resulting in higher UV for the axial transmission.

Tomographic bone density at the osteotomy site was consistently and significantly higher for the intact tibiae than for the simple (21%) and resection (41%) osteotomies, while the callus volume showed the opposite behavior, with the simple (146%) and resection (234%) osteotomies presenting significantly greater volume than the intact tibiae at a corresponding length of the midshaft. The larger calluses characterized the anomalous healing process with the resection, which was probably still ongoing, perhaps on the verge of becoming a nonunion if the external fixator were removed. The overall positive correlation between density and axial and transverse UV was perhaps due to higher densities indicating a more mature, ossified phase of healing, with a higher capacity to transmit ultrasound waves. Correlation was stronger for axial than for transverse propagation, confirming the transmission mechanism along the osteonal structure, suggesting that axial examination would be more reliable. Conversely, a strong negative correlation existed between callus volume and axial UV, indicating that the voluminous callus is an immature structure, not well prepared to transmit ultrasound waves. There was no clear correlation between transverse UV and volume, probably indicating the greater anisotropy of the larger callus.

We conclude that the transverse linear osteotomy tends to heal faster and with a smaller and more mature callus than the 5 mm-thick resection osteotomy, healing of which involves a larger, less mature callus within the same time span, thus resulting into higher and lower UV, respectively. Both transverse and axial UV correlated positively with callus density and negatively with callus volume, as measured by quantitative CT, thus showing that UV permits diagnosis at least as precisely as CT.

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