Effects of extracorporeal shock wave therapy and radial pressure wave therapy on elasticity and microstructure of equine cortical bone

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Objective—To measure changes in the modulus of elasticity (E) and describe histologic findings after extracorporeal shock wave therapy and radial pressure wave therapy on equine cortical bone specimens.

Sample Population—16 bone specimens from the proximodorsal cortex of an equine third metacarpal or metatarsal bone.

Procedure—Baseline E was determined by the density (ρ) and unidirectional ultrasound transmission velocity (C) of each specimen according to the equation $E = \rho C^2$. Eight specimens were treated with 500 pulses of 0.15 mJ/mm^2 of extracorporeal shock wave therapy, and 8 specimens were treated with 500 pulses of 0.16 mJ/mm^2 of radial pressure wave therapy. After treatment, C was determined again. Four treatment sessions resulted in 2,000 pulses and 5 C measurements. The ρ of each sample was measured again. Mean post-treatment E was calculated for each group. Nondecalcified sections of all specimens were stained with toluidine blue or basic fuchsin for histologic evaluation.

Results—Overall treatment group effect was not significant for C or E. Final E was not different from baseline values for extracorporeal shock wave therapy and radial pressure wave therapy. No histologic changes could be attributed to either treatment modality.

Conclusions and Clinical Relevance—Extracorporeal shock wave therapy and radial pressure wave therapy did not affect the material properties of equine bone at the energy and pulse values used in this study. (*Am J Vet Res* 2004;65:207–212)

Extracorporeal shock wave therapy (ESWT) and **radial shock wave therapy (RSWT)** have become popular treatment modalities for equine musculoskeletal problems. Little is known about their mechanism of action and, importantly, about their effect on cortical breaking strength of equine bone.

Shock waves are transient pressure waves propagating in 3-dimensional space.[1] Extracorporeal shock waves are pressure waves generated outside the body that can be focused at a specific site within the body. Shock waves are characterized by high positive pressures up to 100 bar, and negative pressures of 5 to 10 bar.[1] They have a rapid rise time of 30 to 120 nanoseconds and a short, 5-microsecond, pulse duration.[1] More recently, RSWT has been developed.[2,3] Radial shock wave therapy uses a projectile mechanism to stimulate a pressure wave. Pressure waves generated by this mechanism are transmitted radially, decreasing in energy proportional to the square of the distance from the surface. These waveforms are different from the focused shock waves of ESWT.[a]

Extracorporeal shock wave therapy can stimulate bone healing and bone remodeling.[4] The exact mechanism of this effect is unknown. Multiple factors have been hypothesized to play a role, including biochemical and physical effects of the energy release, which occur when a shock wave traveling through fluid and soft tissue hits an interface with a different acoustic impedance. In vitro stimulation of growth factors and cellular division have been documented.[1,5,6] Shock waves can increase cellular permeability and stimulate cytokine production by cells.[5,6] Extracorporeal shock wave therapy also has a positive effect on the concentration of transforming growth factor-β 1, which has a chemotactic and mitogenic effect on osteoblastic cells.[5] At this time, however, the biological and cellular mechanisms induced by shock waves that simulate bone healing in vivo are largely unknown.

Physical effects include compression, tension, shear, and cavitation.[7] Early studies on bones revealed that ESWT induces microfractures and gross cortical fractures dependent upon the energy levels and number of pulses used.[4,8] In a study of formalinized rabbit bones, defects and fractures were induced with ESWT in a dose-responsive manner.[4] In many specimens, microfractures were seen and thought to be the mechanism that stimulated bone healing.[4] In a preliminary investigation with ESWT on a limited number of horses, no microfractures were seen with 1,000 pulses at an energy flux density of 1.8 mJ/mm^2.[9] However, that study did not include RSWT and was stopped after 1,000 pulses. No microfractures could be induced in 1 study on equine metacarpal bones with therapeutic levels of RSWT.[10]

A safety issue has arisen because ESWT and RSWT are frequently used to treat equine athletes. It is not known whether these therapeutic modalities can alter the material properties of equine cortical bone. An

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effect on a material property such as the **modulus of elasticity (E)** could influence the risk of structural failure. The E is an important predictor of the breaking strength of bone and any change in the modulus would indicate a change in the structure of the bone.[11-14] The E of a material can be derived from the product of the **density (ρ)** and the **ultrasound transmission velocity (C)** squared, as $E = \rho C^2$.[14] If the ρ remains unchanged under the influence of either treatment modality, any changes in ultrasound transmission speed are the result of a change in E. The E determined by C testing correlates strongly with values derived from mechanical testing.[11-15] Use of ultrasound to determine E provides a nondestructive mechanism of evaluation; therefore, serial testing can be performed.

Simply evaluating bone for microcracks lacks sensitivity. The absence of microcracks does not mean the mechanical properties are normal.[16] No substantial accumulation of microscopic damage was detected until canine femurs had lost 15% of their E.[17] The accumulation of microcracks is threshold dependent, and they accumulate in a nonlinear fashion with loading cycles. Therefore, it is difficult or impossible to predict the extent of degradation of mechanical properties from measurements of the number or length of microcracks alone.[17] Furthermore, the presence of some microcracks is normal.[18] Therefore, evaluation of both E and microstructure is necessary.

Presently, there are only pilot studies available on any physical effect of ESWT and RSWT on the material properties and the microstructure of equine cortical bone.[9,10] Using a nondestructive materials testing procedure, such as measurement of C, is a sensitive way of assessing changes in breaking strength of bone. As well, a dose-response relationship can be established through repeated measures of E after treatment, and histologic evaluation can be performed on these same specimens. Therefore, the purpose of the study reported here was to investigate the physical effects and cumulative dose response of ESWT and RSWT on equine cortical bone by measuring their effects on bone E and microstructure as an indicator of the safe use of these treatment modalities. Our hypothesis was that ESWT and RSWT have no effect on E and microstructure of equine cortical bone specimens.

Materials and Methods

Bone specimens were harvested from the dorsal cortex of the third metacarpal and metatarsal (cannon) bones of 5 adult horses euthanatized for reasons not associated with this project. There were 3 Quarter Horses (8, 10, and 14 years of age), 1 Warmblood (3 years of age), and 1 Thoroughbred (5 years of age). Sixteen rectangular bone specimens of approximately 1 cm^2 in cross section and 3 cm long were cut from the dorsal cortex of 16 metacarpal and metatarsal bones with a water-cooled band saw. The proximal aspect of the specimens started 10 cm from the proximal aspect of the bone, and the long axis of the specimens was oriented with the long axis of the bone. The location of the proximal periosteal surface was marked to maintain orientation. A precision wet grinder[b] was used to polish the periosteal and endosteal surfaces until they were parallel as assessed visually and by 3 thickness measurements.[c] The specimens were labeled and wrapped in saline (0.9% NaCl) solution-soaked sponges and stored at –20°C until testing.

Specimens were removed from the freezer and allowed to thaw in saline solution at room temperature (22°C) for 24 hours. The ρ (grams per cubic centimeter) of each specimen was measured by use of Archimedes' principle as described for nondefatted specimens.[19] One additional specimen was measured 10 times to determine the coefficient of variation.

The 16 specimens were randomly allocated into either the ESWT or RSWT treatment group. The width of the specimen in periosteal-to-endosteal orientation was obtained with an electronic caliper[d] to the one-hundredth of a millimeter with measurements taken twice at points 10, 15, and 20 mm distal to the proximal border (**Fig 1**).

Ultrasound testing—The baseline ultrasound **travel time (t0** [microseconds]) was established by measuring the ultrasound travel time through saline solution. Two similar transducers, each with center frequency of 2.25 MHz, diameter of 1 inch (25.4 mm), and focused at 4 inches (101.6 mm), were used at a repetition rate of 1 KHz. In this method, 1 transducer is the transmitter and the other is the receiver.[e] The transducers were positioned so they were parallel and facing each other. The transducers were fixed at 8 inches

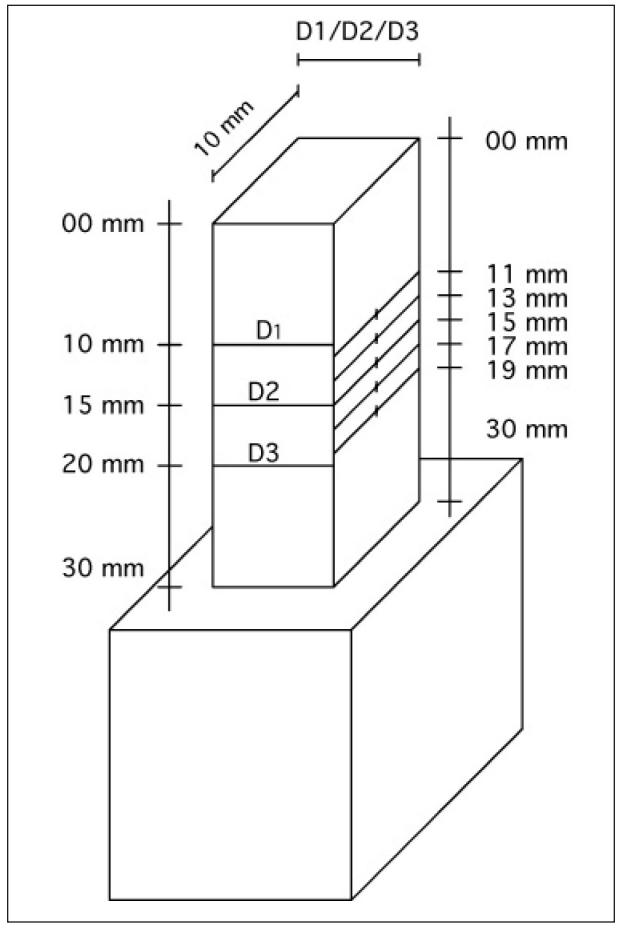

Figure 1—Schematic diagram of an equine bone specimen (upper rectangular structure) positioned on a pedestal (lower rectangular structure) for thickness measurements. Six thickness measurements were made: 3 from the periosteal to the endosteal side of the specimen at 10, 15, and 20 mm distal to the proximal border (levels D1, D2, and D3) and 3 from the endosteal to the periosteal side at 10, 15, and 20 mm distal to the proximal side (level D1/D2/D3). Ultrasound wave transmission time was measured 5 times, approximately 11, 13, 15, 17, and 19 mm distal to the proximal border of the specimen, coinciding with the area of treatment via extracorporeal shock wave therapy or radial pressure wave therapy.

apart (twice the focal distance) to maintain the specimens in focus for both the transmitter and receiver. The focus of the ultrasound beam is approximately 2.7 mm wide for a 2.25 MHz frequency transducer. Time for the ultrasound signal to reach from transmitter to receiver through saline solution was recorded 3 times and the mean of the 3 values was used for t0. A bone specimen was placed upright on a pedestal in the saline solution bath at the center of the distance between the 2 transducers, ensuring that the periosteal and endosteal surfaces were perpendicular to the ultrasound beam and faced the transmitter and receiver, respectively **(Fig 2)**. After placing the specimen, the time for the ultrasound signal to reach from transmitter to receiver (ultrasound wave transmission time) through saline solution and bone was recorded 5 times (t1 to t5) at 5 locations (11, 13, 15, 17, and 19 mm distal to the proximal border of the specimen [Fig 1]) by use of direct cursor measurements of echo signals displayed on a digital oscilloscope.[f] Each signal was a mean of 32 repetitions. To reduce noise in the signal, it was digitized at a sampling rate of 200 MHz; 2,000 digital points were displayed. High sampling rate allowed precise measurement of signal peaks and corresponding time for echo signals. A computer-controlled, high-precision (10,000 steps/2-mm motion), 3-axis motion stage was used to move the transducer for measurements through the different locations on the specimens. A standard substitution method was used for measurements of velocity in bone specimens. The ultrasound velocity in the bone specimens was determined by use of the formula:

$$Vs = 1 / (1/Vw - \Delta T/d)$$

where Vs is the velocity in the specimen, Vw is the velocity in saline solution, ΔT is the difference between arrival times without and with the specimen, and d is the specimen thickness at the measurement site.

The temperature of the saline solution in the bath was constantly measured with a thermocouple so any changes in temperature could be included in the saline solution velocity calculation. The specimens were kept soaked in saline solution throughout the testing procedure. After the 5 baseline ultrasound wave transmission time measurements were taken, the specimen was transferred to a treatment bath. The specimen was placed on a wooden treatment block, endosteal side down, and covered with a piece of fresh horse skin. It was ensured that the specimen was fully immersed in saline solution and that no air was present between skin and bone to mimic the normal anatomy of the dorsal portion of the cannon bone. The ESWT specimens were treated with 500 pulses with an energy density of 0.15 mJ/mm^2 by use of the 5-mm probe.[g] The RSWT specimens were treated with 500 pulses with an energy density of 0.16 mJ/mm^2 by use of the wide head probe.[h] After treatment, the ultrasound wave transmission time was measured again as described and the sequence of treatment and measurement was executed 3 more times until 2,000 pulses of treatment and 5 velocity measurements were completed on each specimen. One separate control specimen underwent ultrasound transmission measurement 10 times, interspersed with the test specimens, to establish the coefficient of variation.

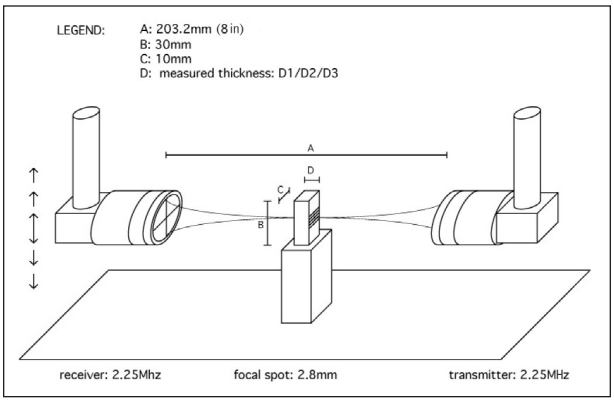

Figure 2—Schematic diagram of an equine bone specimen positioned for measurement of ultrasound wave travel time. The ultrasound transducer on the right served as a transmitter and the transducer on the left served as a receiver. The transducers were positioned so that the focus of the ultrasound beam coincided with the center of the specimen in the lateromedial plane. Arrows indicate how the transducer was moved up and down for the 5 measurements of transmission time. *See* Figure 1 for key.

After testing, the ρ of every specimen was measured again as described. The bone specimens were wrapped in saline solution-soaked sponges and refrozen at –20°C. The specimens were thawed in cold 70% ethanol and permitted ample time for fixation. Sections approximately 150 to 200 µm thick (full length, longitudinal) were cut in an endosteal-periosteal orientation with a macro cutting device,[i] resulting in 2 sections from each specimen. Both sections were bulk stained; 1 section was stained with basic fuchsin and the other with toluidine blue. Sections were embedded in polymethylmethacrylate, ground to a thickness of 100 to 125 µm with a precision wet grinder,[b] and polished to remove stain precipitant and any microcracks that resulted from processing. Sections were dehydrated through increasing percentages of ethanol, cleared in xylene, and coverslipped with a synthetic mounting medium to obtain a permanent mount, which prevents cracks caused by drying of the bone matrix. Slides were microscopically screened systematically with 4X and 20X objective lenses primarily for microcracks, as well as stain intensity and alignment of blood vessels.

Calculations—For each specimen, the first, third, and fifth velocity measurements corresponded with width measurements 1 through 3, respectively. The **bone width (D)** used to calculate velocity at the second and fourth ultrasound measurement was the mean of width measurements 1 and 3, and 3 and 5, respectively. A temperature-corrected value of C in saline solution (Cw; millimeters per microsecond) was calculated for every measurement series according to the equation:

$$Cw = 1{,}402.9 + (4.835 \times T) - (0.047016 \times T^2) + (0.00012725 \times T^3)\ mm/\mu s$$

where T is the saline solution bath temperature (°C) at the time of the measurement.[20] The time required for the ultrasound wave to transmit through the bone was determined by subtracting the time needed to transmit through saline solution alone (Δt) from the time for the ultrasound wave to transmit through bone and saline solution. This was calculated for each of the 5 sites for a given specimen. A transmission C (Cs; millimeters per microsecond) was calculated at 5 sites on each specimen each time by use of the equation:

$$Cs = Cw/(1-[Cw \times \Delta t/mean\ D])^{20}$$

Mean ultrasound transmission velocity per measurement series was calculated from the 5 for every specimen. The final E for each step for each specimen was calculated from the equation:

$$E = mean\ \rho \bullet Cs^2$$

Statistical analyses—Data were entered into a spreadsheet[j] and statistical analyses were completed with a commercially available software package.[k] Coefficients of variation were calculated for velocity, E, and ρ from the control specimen data. A pairwise *t* test was used to compare pre- and post-treatment ρ in a 2-group (ESWT and RSWT)

repeated measures over time design, with 8 units/group. Each experimental unit was measured at baseline and after each of 4 shock wave treatments, yielding 5 measurements/unit. Data were analyzed with **multivariate analysis of covariance (MANCOVA)**, which accommodates the correlation induced by repeated measurements on the same subjects as well as adjusting for baseline to determine whether there was a difference between treatments.[21] The Dunnet test was used to determine whether the C or E changed from baseline over time.

Results

The coefficient of variation was 0.07% for ρ, 1.4% for C, and 2.7% for E. Pre- and post-treatment ρ were not significantly different ($P = 0.83$), so mean of the 2 ρ measurements was used in the calculation of E. The MANCOVA for C revealed that the time by treatment group interaction was not significant ($P = 0.43$), and the overall treatment group effect was not significant ($P = 0.77$). Similarly, the time by group interaction for E was not significant ($P = 0.48$), and the overall treatment group effect was not significant ($P = 0.79$). Neither the C for ESWT ($P = 0.96$) and RSWT ($P = 0.78$) nor E for ESWT ($P = 0.96$) and RSWT ($P = 0.82$) changed from baseline (**Table 1**).

The bone specimens had differences in blood vessel orientation, diameter, and the number of cutting cones and microcracks characteristic of horses of different ages and stages of remodeling. Specimens from both treatments were histologically similar with no microstructural changes attributed to either treatment modality. Microcracks at 4X and 20X were found in specimens of both treatment modalities and considered to be normal remodeling. Microcracks could be seen better with basic fuchsin than toluidine blue stain. Because of the acute nature of this in vitro study, the presence of stained material within the microcrack indicated that the microcrack was present prior to the study.

Table 1— Ultrasound transmission velocity (vol [millimeters per microsecond]) and modulus of elasticity (E) for equine cortical bone specimens at baseline (0 pulses) and after 500 to 2,000 pulses of extracorporeal shock wave therapy (ESWT) or radial pressure wave therapy (RPWT)

Variable	0 pulses	500 pulses	1,000 pulses	1,500 pulses	2,000 pulses
ESWT-vol	3.362	3.388	3.385	3.382	3.385
RPWT-vol	3.415	3.384	3.405	3.392	3.374
ESWT-E	22.886	23.277	23.244	23.196	23.235
RPWT-E	23.682	23.235	23.501	23.327	23.091

Discussion

This study revealed that there was no effect on E associated with either ESWT or RSWT. The treatments did not induce detectable microstructural changes with the number of pulses used in the study.

Determination of E via means of ultrasound transmission velocity measurement is a reliable, nondestructive testing mechanism.[11-15] This mechanism allows for repeated measurements on a single sample and is a more sensitive test than mechanical testing. Furthermore, a nondestructive test allows for a dose-response study on a single sample. Transmission ultrasound speed varies inversely with the temperature.[15] The saline solution bath temperature was therefore constantly measured, and a correction factor was inserted into the calculation of the Cw in saline solution.

An identical pair of transducers with a frequency of 2.25 MHz was used. The focal zone of a 2.25-MHz transducer at 101.6 mm (4 in) is 2.7 mm, which could be centered on the specimen with minimal scatter. The use of the equation $E = \rho C^2$ requires that the wavelength (wavelength = velocity/frequency) of the transmitted ultrasound wave must be larger than the characteristic dimension of the structure; for a frequency of 2.25 MHz, the wavelength in cortical bone is approximately 1 mm, which satisfies that condition.[12] Ultrasonic scatter can increase the variation of measurements. Scatter is minimized if the sound wave travels perpendicular through the surfaces of the specimen. For that reason, a water-cooled polishing wheel was used to make the periosteal and endosteal surfaces parallel. Three thickness measurements with an electronic micrometer were used to determine adequate quality of the specimen for inclusion in the experimental group. Transmission C is also influenced by water content of the bone.[22] The specimens were therefore kept immersed or moist at all times.

Hematomas, diffuse hemorrhages, fragmentation of bony trabeculae in the marrow, and the transport of marrow content under periosteum were detected in experiments on rabbit femurs in vivo, sheep calcaneus in vivo, and formalin-fixed rabbit femurs.[4] Microcracks have been suggested as a possible mechanism of the stimulation of bone formation and this may be true in some laboratory species; however, ESWT and RSWT did not create microcracks in the equine bone in our study. It is possible that higher energy flux ρ and number of pulses may induce microcracks in healthy equine cortical bone. However, the doses used in this protocol are the highest used in a single treatment in clinical settings. No formal dosage recommendations are published yet. An equal dosage of energy was delivered by the RSWT machine following the manufacturer's information in regards to energy flux ρ.

Our histologic evaluation revealed no microstructural effects of the treatment, which supported the ultrasound findings. The technique used in this study was different than en bloc basic fuchsin staining that is used by many investigators to detect bone microcracks.[23] The basic fuchsin stain fills microcracks and makes it easy to identify their presence, whereas toluidine blue metachromatically stains the glycosaminoglycans of proteoglycans that fill the microcracks during the initial stage of repair.[24,25] For this reason, toluidine blue stain was compared with basic fuchsin stain in this protocol. We did, indeed, see basic fuchsin blebs at the ends of the canaliculi at the microcracks. From our experience, basic fuchsin stain was much better for detecting the preexisting microcracks than toluidine blue. Had multiple microcracks been seen that did not stain with toluidine blue but did stain with basic fuchsin, the technique could have been a concern, but because they were not seen, the histologic technique did not compromise the study. However, no control specimens from the same horses were evaluated.

Compact cortical bone is an anisotropic material,

and therefore the velocity of an ultrasound wave through the bone will be affected by the orientation and the origin of the specimen.[15] Care was therefore taken to test all specimens in the same orientation. The anisotropy of the equine metacarpus also necessitated the specimens to come from the same area of the dorsal metacarpus, as we did in our harvesting procedure. There is a greater variation in E and ultimate breaking strength between bone locations within a bone than between bones.[26] Burr et al[17] found that there was 25% more damage in tensile than compressive cortices in canine femurs under cyclic 4-point bending. Bone cracks are more easily started in tensile cortices, but their growth is limited; cracks are started less easily in compressive cortices, but their growth is less constrained. Because E degradation is associated with crack accumulation rather than growth, the E of tensile cortices should decline more quickly than that of compressive cortices, accounting for the shorter tensile fatigue life of cortical bone.[17] This was another reason to use only specimens from the dorsal cortex. Accumulation of microdamage is also anisotropic with damage initiated differently in different directions; therefore, all specimens were treated dorsal to palmar.[17] Microcracks will propagate in the direction of the osteons in cortical bone rather than advance perpendicular to them. The histologic sections were therefore performed in a longitudinal plane. It is possible that additional evaluation in the transverse plane would have identified additional microcracks.

Other imaging means of assessing microcracks such as oblique illumination 3-dimensional imaging may be more sensitive,[10] but even with these techniques, true microcracks are difficult to detect and differentiate from cracks from the sectioning and grinding or polishing procedure. Nondestructive assessment of bone microstructure such as with microcomputed tomography would allow pre- and post-treatment analysis and could be a quantifiable and reliable measurement option.

Our specimens originated from horses of different ages and occupations. In this study, we saw a wide variation in blood vessel orientation, diameter, and the number of cutting cones and microcracks between specimens. This is consistent with horses of different ages and stages of remodeling. This study did not include histologic examination of untreated control specimens from the same horse nor were specimens assigned as paired comparisons between treatments; therefore, we cannot further interpret histologic findings.

The influence of weight bearing on treatment in vivo is unknown. Furthermore, it is possible that the effect of preexisting cortical microcracks in horses with dorsal metacarpal disease could affect the acoustical parameters of shock waves and therefore the treatment effects. Gerdesmeyer et al[l] showed the strongly attenuating effects of intact cortical bone by use of a focused shock wave system and advocated exact positioning systems to hit the fracture gap to stimulate fracture healing in nonunion fractures.

No significant change in the E or dose-response relationship could be detected. Therefore, at this dose neither ESWT nor RSWT induced changes in E in equine cortical bone specimens. Histologically, we found no changes that could be attributed with certainty to either ESWT or RSWT. If we combine these findings with the strong correlation of E and breaking strength,[11-14] we can conclude that there is no immediate substantial effect of ESWT and RSWT on material properties of equine cortical bone specimens that would predispose these bone specimens to fracture.

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[a]Cleveland RO, Chitnis PU. Comparison of the acoustic and cavitation fields produced by SWT devices with different generating principles (abstr), in *Proceedings*. 5th Surg Int Soc Musculoskelet Shockwave Ther 2002;2.

[b]Wet grinder, Buehler Econet, Lake Bluff, Ill.

[c]Electronic micrometer, Exakt, Norderstedt, Germany.

[d]Vernier caliper, Absolute Digimatic model CD-6 CS, Mitutoyo Corp, Aurora, Ill.

[e]Pulser/Receiver, Parametrics model 5058, Waltham, Mass.

[f]Digital oscilloscope, Lecroy Corp, Model 9450, Chestnut Ridge, NY.

[g]Equitron, kindly provided by High Medical Technologies, Lengwil, Switzerland.

[h]Swiss Dolorclast Vet, kindly provided by Electro Medical Systems, Dallas, Tex.

[i]Exakt Macro cutting device, Norderstedt, Germany.

[j]Excel, Office 2000, Microsoft Corp, Redmond, Wash.

[k]JMP Statistical Software, SAS Institute Inc, Cary, NC.

[l]Gerdesmeyer L, Hausch F, Ueberle F. The influence of cortical bone on acoustical parameters of shock wave focus (abstr), in *Proceedings*. 4th Cong Int Soc Musculoskelet Shockwave Ther 2002;24.

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