

Use of multisite quantitative ultrasonography for noninvasive assessment of bone in horses

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Objective—To evaluate the usefulness of multisite quantitative ultrasonography for noninvasive assessment of bone in horses.

Sample Population—12 healthy horses and both forelimbs from 8 clinically normal horses.

Procedure—For in vivo measurements, various regions of interest (ROI) were examined on the third metacarpal bone, radius, and tibia. Precision error for speed of sound (SOS) measurements was obtained by measuring each ROI of 4 horses 10 times with probe repositioning. Additionally, 3 operators measured each aspect of the third metacarpal bone of 6 horses 5 times each. For ex vivo measurements, third metacarpal bones were examined at 9 ROI, and SOS measurements were performed before and after soft tissue removal. One ROI of a single forelimb was subjected to 96 ex vivo measurements with 3 different contact media.

Results—The lateral aspect of the third metacarpal bone had significantly higher SOS values than the dorsal and medial aspect of the third metacarpal bone. No difference was obtained between SOS values of the lateral and medial aspect of the radius. The tibia had significantly higher SOS values than the lateral aspect of the radius and the dorsal and medial aspect of the third metacarpal bone. Intraoperator coefficients of variation ranged from 0.62 to 3.15%, and interoperator coefficients of variation ranged from 0.78 to 2.70%. Values of SOS were highest when silicone oil was used as the contact medium.

Conclusions and Clinical Relevance—Speed of sound measurements obtained by quantitative ultrasonography in axial transmission mode can be used to precisely measure superficial cortical bone properties of third metacarpal bone, radius, and tibia in horses. (*Am J Vet Res* 2002;63:1464–1469)

Musculoskeletal diseases of sport and leisure horses are an important problem for the equine industry.¹ Several noninvasive methods for bone property evaluation in horses are presently available, but the early detection of bone diseases, the serial monitoring

of bone, and the evaluation of bone metabolism remain a difficult problem.^{2,3}

In horses, bone properties at well-defined skeletal sites can be detected by use of single photon absorptiometry,⁴ dual-energy x-ray absorptiometry,⁵ radiographic absorptiometry,⁶ quantitative computed tomography,⁷ or magnet resonance imaging.⁸ An additional tool for bone turnover assessment at precise skeletal sites is scintigraphy.⁹ These techniques are mostly radiation-based, expensive, or time-consuming. Another method for monitoring the metabolism of the whole skeleton includes the measurement of biochemical markers in serum, plasma, or urine.^{10,11}

An inexpensive alternative to mostly ionizing radiation-based techniques is **quantitative ultrasonography (QUS)**. Various QUS devices have been developed in human medicine to measure **speed of sound (SOS)** or broadband ultrasound attenuation.¹² The assessment of bone by use of ultrasonography in horses has been described for more than 20 years.¹³ However, the use of 2 percutaneously applied transducers restricts ultrasonographic evaluations of the distal limb.¹⁴ Additionally, the influence of soft tissue, bone shape, and limb temperature affects results and limits the usefulness of ultrasonography.^{15,16}

The recently described multisite QUS device^a is a new promising tool for noninvasive bone assessment in horses.^b The QUS device includes a simple hand-held probe, which is percutaneously applied to the site to be measured. The use of a set of transducers within the hand-held probe allows SOS measurements in axial transmission mode of various **regions of interest (ROI)**, even if accessible from only a single side. The velocity of an ultrasound wave propagating through a fixed distance of bone, parallel to the long axis of the bone, is evaluated, and the software is used to calculate the shortest propagation time of the sound wave through bone (Fig 1). Because of the special design of the probe, a soft-tissue layer of ≤ 9 mm of thickness is claimed not to influence SOS measurements.

The purpose of the study reported here was to evaluate the usefulness of QUS for noninvasive assessment of bone in horses. The precision of SOS measurements in axial transmission mode was determined at multiple skeletal sites in horses. Additionally, the possible influence of soft tissue and contact medium on SOS measurements was investigated.

Materials and Methods

Measurements of SOS in axial transmission mode were performed with a multisite QUS device.^a The manufacturer's largest hand-held probe^c was used, which allows SOS measurements of bone underneath soft tissue ≤ 9 -mm thick. The

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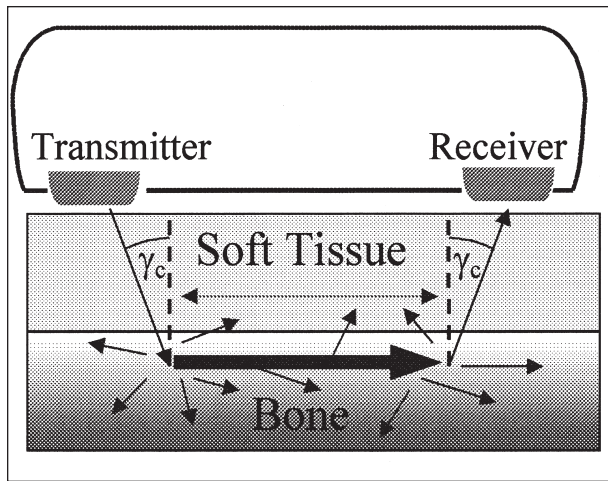


Figure 1—Schematic of the path of an ultrasound wave through soft tissue and bone. Acoustical waves are created by the transmitting transducers and are received by receiving transducers. Paths of the signal are determined on the basis of how the sound wave is refracted through a critical angle, how it propagates along the bone, and how it is scattered out of the bone through a critical angle. The traveling time between transmitters and receivers is recorded and speed of sound calculated. Transmitter = Transmitting transducers. Receiver = Receiving transducers. γ_c = Critical angle.

QUS device creates pulsed acoustic waves at a center frequency of 1.25 MHz (bandwidth, 0.7 to 1.8 MHz). Each probe is composed of 4 sets of transducers. Two transducers act as transmitters while 2 act as receivers. The ultrasound waves travel through soft tissue and thereafter along and under the bone surface, at a bone layer depth of 3 to 5 mm.¹⁷ This technique is based on Snell's law in terms of how the soundwave is refracted (through a critical angle when entering bone), how it travels through bone tissue, and how it is scattered out of the bone and is received by the receivers (Fig 1). Software is used to measure the shortest propagation time of the sound wave through bone and calculates the velocity of the ultrasound (ie, SOS).

At the beginning of each day that SOS measurements were obtained, system quality verification was performed to test the accuracy status of the measurement system by use of a phantom.⁴ The ROI were marked with an oily pen, and a coupling medium was applied to the horse's skin without prior shaving. All measurements were performed as static measurements without moving the probe. Each QUS scan cycle took approximately 20 seconds. The computer software checked 3 to 5 subsequent cycles for consistency and quality, and a significantly consistent result was then obtained. An operator who was blinded to information on the computer screen performed the QUS measurements.

Experiment 1—Use and precision of in vivo SOS measurements were determined on 12 clinically normal, randomly selected, slightly exercised warmblood geldings and females that were 3 to 18 years old. All measurements were performed on full weight-bearing, standing, unsedated horses. Silicone oil^c was used for acoustic coupling. For SOS measurements of the third metacarpal bone, the probe was placed midway between the proximal end of the second and fourth metacarpal bone, and the apex of the proximal medial and lateral sesamoid bones and 3 aspects (lateral, dorsal, medial) were measured (Fig 2a). For SOS measurements of the radius, a horizontal line, originating from the distal edge of the chestnut, was drawn dorso-laterally and 2 aspects (lateral, medial) were measured. For SOS measurements of the tibia, the probe was placed 10 cm proximal to the medial malleolus of this bone (Fig 2b).

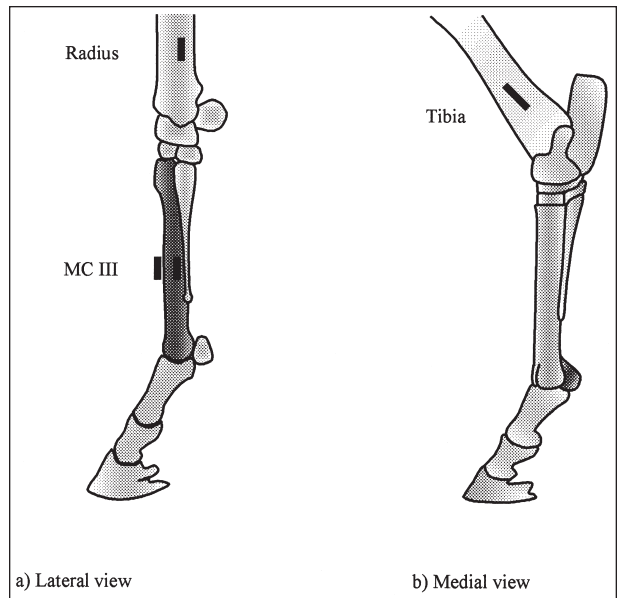


Figure 2—Schematic of probe positioning sites for axial transmission speed of sound (SOS) measurements on the radius, third metacarpal bone (MC III), and tibia in standing full weight-bearing horses. Panel a) Diagram of the lateral view of the forelimb. For SOS measurements of the MC III, a horizontal line was drawn midway between the proximal end of the fourth metacarpal bone and the apex of the proximal lateral sesamoid bone. The probe was placed in axial direction and perpendicular against MC III and measurements were performed at the lateral, dorsal and medial aspect. For SOS measurements of the radius, a horizontal line originating from the distal edge of the chestnut was drawn dorso-laterally and 2 aspects (lateral, medial) were measured. Panel b) Diagram of the medial view of the hind limb. For SOS measurements of the tibia, the probe was placed 10 cm proximal to the medial malleolus of the tibia in an axial direction and perpendicular against the bone.

Measurements were made 10 times on 4 horses. Each time, the probe was repositioned for each ROI of the third metacarpal bone, radius, and tibia to obtain intraoperator precision error for SOS measurements. To obtain the interoperator precision error for SOS measurements, 3 operators additionally measured all aspects of the third metacarpal bone 5 times each on 6 horses.

Experiment 2—The influence of soft tissue on ex vivo SOS measurements was determined by collecting both forelimbs of 8 musculoskeletally normal adult horses, which were euthanatized for reasons unrelated to our study. The limbs were dissected in the intercarpal joint within 30 minutes after death, and the skin was stabilized with sutures. Both third metacarpal bones of each horse were divided in 9 ROI (ie, 3 aspects [lateral, dorsal, medial] at 3 levels [proximal, middle, distal]). Ex vivo SOS measurements at each ROI with and without soft tissue were performed at room temperature (approx 19°C), within 8 hours after death. Silicone oil^c was used as contact medium.

Experiment 3—The effect of various coupling media on SOS measurements was studied in an ex vivo experiment. The skin over the middle level of the lateral aspect of the third metacarpal bone of a freshly dissected forelimb of a horse, euthanatized for reasons unrelated to our study, was cleaned, shaved, and dried. The ROI margins were marked before starting SOS measurements. Three series of 96 measurement cycles were performed with a probe^e by use of consecutively 3 contact media as follows: contact medium 1,^f contact medium 2,^g and contact medium 3.^e

Statistical analysis—An analysis of variance of SOS values was performed by use of a commercially available software system.^b Horses were considered as random in all models. In experiment 1, the fixed effects were the 3 aspects of the third metacarpal bone, both ROI of the radius, and the effect of the tibia, nested within left and right limbs. Least square means were computed and compared between aspects, sites, and limbs. In experiment 2, the fixed effects were the presence or absence of soft tissue, the 3 aspects and the 3 levels of the metacarpal bone, the 2 limbs, and their interactions. In experiment 3, the effect of the contact medium (3 types) was the only fixed effect included in the model. Least square means (\pm SE) were computed for all experiments. Significance level was set at $P < 0.05$. The intraobserver precision of 10 successive SOS measurements was computed for each ROI as SD divided by the mean value. The interobserver precision of third metacarpal bone was calculated for 6 horses as the SD divided by the pooled mean value of 5 repeated measurements performed by 3 operators.

Results

Experiment 1—Least square means (\pm SE) of SOS values obtained at each ROI were determined (Table 1). No difference in SOS values was obtained when comparing each side-matched ROI of the right and left limb. A significant difference was obtained between SOS values of each aspect of the left third metacarpal bone. In the right limb, significant differences between SOS values were obtained between all aspects of the third metacarpal bone, except between the dorsal and medial aspect of the third metacarpal bone. Values of SOS obtained in the lateral and medial aspect of the left radius were significantly different from those obtained in the lateral and dorsal aspect of the left third metacarpal bone. In the right limb, a significant difference was obtained between SOS values of the lateral and medial aspect of the radius and those obtained in the lateral aspect of the third metacarpal bone. The medial aspect of the radius had additional significantly different SOS values when compared with the dorsal aspect of third metacarpal bone. Values of SOS obtained in the tibia were significantly different from those obtained in side-matched medial and dorsal aspects of the third metacarpal bone and the lateral aspect of the radius. Additionally, SOS values obtained in the right tibia were significantly different from values obtained in the medial aspect of the radius.

The in vivo intraobserver coefficients of variation for successive SOS measurements ranged from 0.62 to 3.15%

Table 1—Least square mean (\pm SE) values of speed of sound measurements at various regions of interest of warmblood horses ($n = 12$)

ROI	Speed of sound (m/s)		Intraobserver CV (%) ^a
	Left limb	Right limb	
Lateral MC III	4281.67 \pm 49.64	4220.33 \pm 49.64	0.62–1.82
Dorsal MC III	3754.56 \pm 49.64	3791.33 \pm 49.64	0.94–1.30
Medial MC III	3973.11 \pm 49.64	3905.22 \pm 49.64	0.66–1.79
Lateral radius	3920.20 \pm 49.64	3932.50 \pm 49.64	0.97–1.91
Medial radius	4018.30 \pm 49.64	4017.80 \pm 49.64	0.77–1.90
Tibia	4175.90 \pm 49.64	4234.30 \pm 49.64	1.02–3.15

^aIntraobserver precision error from 10 successive SOS measurements was computed for each region of interest as SD divided by the mean value ($n = 4$ horses). ROI = Region of interest. CV = Coefficients of variation. MC III = Third metacarpal bone.

(Table 1). The interobserver coefficients of variation ranged between 0.90 and 2.70% for the lateral aspect of the third metacarpal bone, between 0.91 and 1.68% for the dorsal aspect of third metacarpal bone, and from 0.78 to 1.85% for the medial aspect of the third metacarpal bone.

Experiment 2—No significant difference was obtained between ex vivo SOS data of the third metacarpal bone with (least square means, 4142.57 \pm 43.73 m/s) and without (least squares means, 4095.26 \pm 43.73 m/s) soft tissue. The influence of soft tissue on ex vivo SOS measurements was therefore not significant.

Experiment 3—Mean SOS values (\pm SD) for contact medium 1, 2, and 3 were 4328.79 \pm 24.7, 4323.95 \pm 18.97, and 4337.54 \pm 22.35 m/s, respectively. Contact medium 3 had significantly higher SOS values than contact medium 1 and 2.

Discussion

Bone has an anisotropic structure and undergoes a continuous formation and resorption process. Several conventional radiation-based techniques allow to determine bone mineral density and may partially explain variations in bone strength.^{18,19} However, these tools provide only limited information on bone material and structure.²⁰

There is a known relationship between SOS and Young's modulus, which in turn is related to the biomechanical strength.²¹ Speed of sound can therefore be related to the properties of a material as follows: $SOS = E/\rho$, where E is Young's modulus and ρ is the density of the material. The use of QUS provides information about factors that influence strength, such as bone mineral density,²² bone size, mass distribution, cortical thickness, architecture, fiber orientation, porosity, elasticity, stiffness, and others.^{23,24} The evaluation of breaking strength might therefore be the most appropriate variable for QUS accuracy assessment.²⁰ The relationship between bone strength and QUS measurements has been validated in in vivo and in vitro studies on humans. However, the assessment of the breaking strength as assessed by biomechanical testing was not possible in our study. Information in the literature on humans indicates that QUS measurements at peripheral bone sites allow for a distinction between women with osteoporosis and healthy women.^{25,26} Additionally, the use of QUS is as sensitive as dual-energy x-ray absorptiometry in distinguishing between patients with and without fractures.^{20,25,27,28} In addition, QUS results provide a good predictor of fracture risk.^{29,30} Phantom measurements have been advocated to assess accuracy.²⁰ Such measurements are impractical without defined standards; however, they may at least detect imprecision of QUS devices.

Use of the recently introduced multisite QUS device provides SOS measurements in axial transmission mode at various skeletal sites, even if accessible from only a single side. In our study, axial transmission SOS measurements were performed in horses by measuring SOS values along a fixed distance of bone perpendicular to the load vector of the bone and parallel to the bone axis. This positioning of the probe is important because of the anisotropy of bone.

Values of SOS are thought to reflect a combination

of material (ie, elasticity) and structural characteristics (ie, cortical wall thickness) of the long bone cortex.^{31,32} Any reduction of the cortical thickness similar to or less than the ultrasound wavelength results in a decrease in SOS values, which is caused by the dispersion effect.¹⁷ Based on a previously published radiographic study, we can conclude that the cortical thickness of the ROI considered in our study was > 4 mm.³³ Values of SOS in cortical bone of warmblood horses varied in our study from 3800 to 4200 m/s, depending on the ROI considered. These results are in accordance with previous observations obtained by use of the same QUS device in Thoroughbreds.^{b,i} At a SOS value of 4200 m/s and at a frequency of 1.25 MHz, the sound wave has a wavelength of 3.3 mm.³⁴ Speed of sound propagates at a maximum depth of approximately 3.3 mm; therefore, SOS values should not be influenced by the dispersion effect in long bone measurements of adult warmblood horses and Thoroughbreds.

An association between SOS values, material properties (eg, elastic modulus, bone strength), and cortical density has been observed for the tibia in humans.^{35,j} Additionally, a weak to modest correlation between SOS and dual-energy x-ray absorptiometry measurements has been reported for humans³⁶ and horses.^k In humans, a significant relationship is reported between SOS values and bone mineral density and cortical thickness of phalanges.¹ These findings indicate that SOS values might depend on the geometry and density of the cortex.

Despite discordant results of bone mineral density measurements among various ROI,³⁷ a combination of SOS values from various skeletal sites improves the evaluation of the global bone status in humans¹⁷ and may improve diagnostic accuracy.³⁸ In 1 study, the combination of 3 ROI (radius, phalanx, and metacarpal bone) SOS values did result in a significant increase in the area under the receiver-operating curve, compared with measurements at a single site.¹⁷ Results of another study indicate a diagnostic improvement when combining 2 ROI (ie, calcaneus and distal radius).³⁹ Additionally, a significant correlation among SOS values of various skeletal sites was found for humans.³⁴ A further advantage of multiple skeletal site testing is to have various measurement sites if a particular site is not accessible. The same might be true for horses. Various measurement sites are described in our study, on the basis of easily detectable anatomic landmarks. Sites that were selected were on the radius, tibia, and third metacarpal bone. These ROI correspond to areas with bone property changes associated with physical activity, pharmaceutical drug treatments, bone diseases, or development.^{25,40-42} No limb differences were found between SOS values of the left and right limb of our slightly exercised horses of experiment 1. These findings correspond to SOS data obtained for the tibia in humans.⁴³

Precision is of highest importance to recognize subtle changes in bone properties. In our study, precision error for in vivo SOS measurements obtained at various ROI in horses was higher (coefficient of variation, $\leq 3.15\%$) than reported for humans (coefficient of variation, $\leq 0.3\%$ ¹⁷; coefficient of variation, $\leq 0.81\%$ ⁴²).

Interoperator coefficient of variation has been reported as $\leq 0.7\%$ for humans¹⁷ and was $\leq 2.7\%$ for horses of our study. The higher precision error in horses, when compared with humans, could be explained by differences in patient cooperation and differences in the accessibility of measurement sites among patients. The QUS technique requires complete immobilization of the patient for approximately 1 minute. Despite this disadvantage, in our study in vivo SOS measurements were routinely performed without prior sedation of horses. Additionally, difficulties in optimal limb positioning in horses might result in a decrease in precision. However, the precision of SOS measurements in horses is good when compared with other noninvasive measurement techniques used in equine medicine like ex vivo dual-energy x-ray absorptiometry⁴⁴ or biochemical bone marker evaluation.^{45,46}

In humans, use of the multisite QUS allows for differentiation between pre- or postmenopausal women and women with vertebral fractures.³⁶ In another study, it was possible to discriminate between patients with fractures and those without fractures (controls) by use of SOS measurements at the phalanx, radius, and metatarsus.¹⁷

In horses, soft-tissue swelling is often associated with bone disease and might influence SOS values. This was not evaluated in our study. However, results of our study indicate that ex vivo SOS measurements of third metacarpal bone were independent of soft tissue characteristics in clinically normal horses. The multisite QUS device,⁴ through a special design and nondisclosed mathematic algorithms, measures SOS and soft-tissue thickness as independent variables. However, soft tissue limits the use of the device to anatomic locations where a soft-tissue thickness is ≤ 9 mm.

The use of a coupling medium is necessary for the transmission of the ultrasound energy into tissues. Differences in the contact medium viscosity may influence SOS transmission. In our study, silicone oil, compared with other media, had a higher viscosity and provided better coupling without air bubble inclusion and therefore slightly faster sound transmission. Care should therefore be taken when comparing SOS measurements obtained with various contact media. Additionally, when using standard ultrasound coupling gel, it was often difficult or even sometimes impossible to get an appropriate coupling contact. Especially with horses that had thick hair, it was necessary to clip the hair before performing measurements with standard ultrasound gel. In contrast, silicone oil allowed measurements after short skin contact time. This fact may be the result of physical properties of the oil. Disadvantages of silicone oil, compared with standard ultrasound gel, are price and its oily consistency. We therefore recommend that examination gloves be used to protect the examiner's skin.

Skin and subcutaneous tissue temperature are variable in horses, especially in summer and winter months, and might influence SOS measurements. Results of a study indicate that temperature changes between 17 and 35°C did not influence in vivo SOS measurements at peripheral skeletal sites in humans obtained with the multisite QUS device.^m It is therefore

concluded that temperature changes in the mentioned range should not influence in vivo SOS measurements in horses. Limb positioning in horses might additionally influence SOS results; therefore, horses need to bear full weight on all 4 limbs and foot rotation must be avoided during ROI determination and SOS measurements.

In humans, the patient's final SOS result is expressed as Z- and T-scores. The Z-score is defined as the patient's deviation from the mean result for the age-matched controls divided by the SD of the age-matched controls. The T-score is similar but obtained using younger controls as a reference group. However, in horses, the monitoring of each individual over time and a comparison of serial SOS values is probably a better option. A further challenge is to correlate QUS measurements with bone mineral density measurements and the results of biochemical bone marker analysis.⁴⁷ The combination of various bone assessment techniques might increase the understanding of bone status in horses.

¹Omnisense, Sunlight Ultrasound Technologies, Rehovot, Israel.
²Lepage OM, Whitton C, Danieli A, et al. Multi-site quantitative ultrasound measurements in horses: preliminary results for metacarpal bone assessment. *Bone* 1998;23:F214.
³CM probe, Sunlight Ultrasound Technologies, Rehovot, Israel.
⁴Perspex phantom, Sunlight Ultrasound Technologies, Rehovot, Israel.
⁵Silicone DC-200, Fluka, Steinheim, Germany.
⁶Aquasonic Clear, Parker Laboratories Inc, Fairfield, NJ.
⁷Aquasonic 100, Parker Laboratories Inc, Fairfield, NJ.
⁸SAS/STAT Software, Changes and Enhancements through Release 6.11, SAS Institute Inc, Cary, NC.
⁹Carstansen B, Lepage OM, Langlois P. Quantitative ultrasound: a non-invasive method for bone assessment in thoroughbred horses (abstr). *J Equine Vet Sci* 1999;19:556.
¹⁰Bouxein ML, Coan B. Relationships among tibial ultrasound velocity and bone mineral density of the tibia and hip (abstr). *Osteoporos Int* 1996;6:S139.
¹¹Carstansen B, Duboeuf F, Dettelleux J, et al. Equine third metacarpal bone assessment by quantitative ultrasound and dual x-ray absorptiometry measurements (abstr). *Vet Surg* 2001;30:307-308.
¹²Fan B, He YQ, Hans D, et al. A comparison between ultrasound velocity at the phalanx with site-matched cortical thickness and bone density (abstr). *Osteoporos Int* 1997;7:S62.
¹³Yaniv I, Schwartz T, Niv Y. Temperature effect on speed of sound measurements at the third proximal phalanx (abstr), in *Proceedings*. 3rd Int Congr Osteoporosis Xian PR China 1999:P83.

References

1. Estberg L, Stover SM, Gardner IA, et al. High-speed exercise history and catastrophic racing fracture in thoroughbreds. *Am J Vet Res* 1996;57:1549-1555.
2. Jeffcott LB, Buckingham SH, McCarthy RN, et al. Non-invasive measurement of bone: a review of clinical and research applications in the horse. *Equine Vet J* 1988;(suppl 6):71-79.
3. Lepage OM, Carstansen B, Uebelhart D. Non-invasive assessment of equine bone: an update. *Vet J* 2001;161:10-22.
4. Jeffcott LB, McCartney RN, Speirs VC. Single photon absorptiometry for the measurement of bone mineral content in horses. *Vet Rec* 1986;118:499-505.
5. Grier SJ, Turner AS, Alvis MR. The use of dual-energy x-ray absorptiometry in animals. *Invest Radiol* 1996;31:50-62.
6. Meakim DW, Ott EA, Asquith RL, et al. Estimation of mineral content of the equine third metacarpal by radiographic photometry. *J Anim Sci* 1981;53:1019-1026.
7. Waite KL, Nielsen BD, Rosenstein DS. Computed tomography as a method of estimating bone mineral content in horses. *J Equine Vet Sci* 2000;20:49-52.

8. Choquet P, Sick H, Constantinesco A. MRI of the equine digit with a dedicated low-field magnet. *Vet Rec* 2000;20:616-617.
9. Ueltschi G. 1st results of scintigraphic skeletal examinations in horses using 99mTc phosphates as tracers. *Schweiz Arch Tierheilkd* 1975;117:383-392.
10. Lepage OM, Marcoux M, Tremblay A. Serum osteocalcin or bone Gla protein, a biochemical marker for bone metabolism in horses: differences in serum levels with age. *Can J Vet Res* 1990;54:223-226.
11. Price JS. Biochemical markers of bone metabolism in horses: potentials and limitations? *Vet J* 1998;156:163-165.
12. Njeh CF, Boivin CM, Langton CM. The role of ultrasound in the assessment of osteoporosis: a review. *Osteoporos Int* 1997;7:7-22.
13. Pratt GW. An in vivo method of ultrasonically evaluating bone strength, in *Proceedings*. 26th Annu Meet Am Assoc Equine Pract 1980;295-306.
14. Jeffcott LB, McCartney RN. Ultrasound as a tool for assessment of bone quality in the horse. *Vet Rec* 1985;116:337-342.
15. McCartney RN, Jeffcott LB. Combined 2.25 MHz ultrasound velocity and bone mineral density measurements in the equine metacarpus and their in vivo applications. *Med Biol Eng Comput* 1987;25:620-626.
16. Buckingham SH, McCarthy RN, Anderson GA, et al. Ultrasound speed in the metacarpal cortex—a survey of 347 thoroughbreds in training. *Equine Vet J* 1992;24:191-195.
17. Barkmann R, Kantorovich E, Singal C, et al. A new method for quantitative ultrasound measurements at multiple skeletal sites: first results of precision and fracture discrimination. *J Clin Densitom* 2000;3:1-7.
18. Hayes WC, Piazza SJ, Zysset PK. Biomechanics of fracture risk prediction of the hip and spine by quantitative computed tomography. *Radiol Clin North Am* 1991;29:1-18.
19. Mosekilde L, Bentzen SM, Ortoft G, et al. The predictive value of quantitative computed tomography for vertebral body compressive strength and ash density. *Bone* 1989;10:465-470.
20. Glüer CC. Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. The International Quantitative Ultrasound Consensus Group. *J Bone Miner Res* 1997;12:1280-1288.
21. van den Bergh JP, van Lenthe GH, Hermus AR, et al. Speed of sound reflects Young's modulus as assessed by microstructural finite element analysis. *Bone* 2000;26:519-524.
22. Prevrhal S, Fürst T, Fan B, et al. Quantitative ultrasound of the tibia depends on both cortical density and thickness. *Osteoporos Int* 2001;12:28-34.
23. Lees S, Ahern JM, Leonard M. Parameters influencing the sonic velocity in compact calcified tissue of various species. *J Acoust Soc Am* 1983;74:28-33.
24. Kohles SS, Vanderby R Jr, Ashman RB, et al. Ultrasonically determined elasticity and cortical density in canine femora after hip arthroplasty. *J Biomech* 1994;27:137-144.
25. Funck C, Wüster C, Alenfeld FE, et al. Ultrasound velocity of the tibia in normal German women and hip fracture patients. *Calcif Tissue Int* 1996;58:390-394.
26. Grampp S, Genant HK, Mathur A, et al. Comparisons of noninvasive bone mineral measurements in assessing age-related loss, fracture discrimination, and diagnostic classification. *J Bone Miner Res* 1997;12:697-711.
27. Bauer DC, Glüer CC, Genant HK, et al. Quantitative ultrasound and vertebral fracture in postmenopausal women. Fracture Intervention Trial Research Group. *J Bone Miner Res* 1995;10:353-358.
28. Frost ML, Blake GM, Fogelman I. Contact quantitative ultrasound: an evaluation of precision, fracture discrimination, age-related bone loss and applicability of the WHO criteria. *Osteoporos Int* 1999;10:441-449.
29. Bauer DC, Glüer CC, Cauley JA, et al. Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. *Arch Intern Med* 1997;157:629-634.
30. Hans D, Dargent-Molina P, Schott AM, et al. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet* 1996;348:511-514.
31. Sievanen H, Cheng S, Ollikainen S, et al. Ultrasound velocity and cortical bone characteristics in vivo. *Osteoporos Int* 2001;12:399-405.

32. Njeh CF, Hans D, Wu C, et al. An in vitro investigation of the dependence on sample thickness of the speed of sound along the specimen. *Med Eng Phys* 1999;21:651–659.
33. Hanson PD, Markel MD. Radiographic geometric variation of equine long bones. *Am J Vet Res* 1994;55:1220–1227.
34. Njeh CF, Saeed I, Grigorian M, et al. Assessment of bone status using speed of sound at multiple anatomical sites. *Ultrasound Med Biol* 2001;27:1337–1345.
35. Lee SC, Coan BS, Bouxsein ML. Tibial ultrasound velocity measured *in situ* predicts the material properties of tibial cortical bone. *Bone* 1997;21:119–125.
36. Knapp KM, Blake GM, Spector TD, et al. Multisite quantitative ultrasound: precision, age- and menopause-related changes, fracture discrimination, and T-score equivalence with dual-energy X-ray absorptiometry. *Osteoporos Int* 2001;12:456–464.
37. Njeh CF, Boivin CM. Variation in bone mineral density between different anatomical sites in a normal local population. *Appl Radiat Isot* 1998;49:685–686.
38. Lu Y, Genant HK, Shepherd J, et al. Classification of osteoporosis based on bone mineral densities. *J Bone Miner Res* 2001;16:901–910.
39. Hans D, Srivastav SK, Singal C, et al. Does combining the results from multiple bone sites measured by a new quantitative ultrasound device improve discrimination of hip fractures. *J Bone Miner Res* 1999;14:644–651.
40. Nunamaker DM, Butterweck DM, Provost MT. Some geometric properties of the third metacarpal bone: a comparison between the thoroughbred and standardbred racehorse. *J Biomech* 1989;22:129–134.
41. Pande KC, Bernard J, McCloskey EV, et al. Ultrasound velocity and dual-energy X-ray absorptiometry in normal and pagetic bone. *Bone* 2000;26:525–528.
42. Weiss M, Ben-Shlomo A, Hagag P, et al. Discrimination of proximal hip fracture by quantitative ultrasound measurement at the radius. *Osteoporos Int* 2000;11:411–416.
43. Foldes AJ, Rimon A, Keinan DD, et al. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. *Bone* 1995;17:363–367.
44. McClure SR, Glickman LT, Glickman NW, et al. Evaluation of dual energy x-ray absorptiometry for *in situ* measurement of bone mineral density of equine metacarpi. *Am J Vet Res* 2001;62:752–756.
45. Plebani M, Bernardi D, Meneghetti MF, et al. Biological variability in assessing the clinical value of biochemical markers of bone turnover. *Clin Chim Acta* 2000;299:77–86.
46. Seibel MJ, Lang M, Geilenkeuser WJ. Interlaboratory variation of biochemical markers of bone turnover. *Clin Chem* 2001;47:1443–1450.
47. Ingle BM, Hay SM, Bottjer HM, et al. Changes in bone mass and bone turnover following distal forearm fracture. *Osteoporos Int* 1999;10:399–407.