Evaluation of dual energy x-ray absorptiometry for in situ measurement of bone mineral density of equine metacarpi

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Objective—To determine the accuracy and precision of dual energy x-ray absorptiometry (DEXA) for measuring bone mineral density in horses in situ.

Sample Population—12 randomly selected forelimbs from 12 horses.

Procedure—Metacarpi were scanned in 2 planes and DEXA measurements obtained for 6 regions of interest (ROI). Each ROI was isolated and bone density measured by Archimedes’ principle. Linear regression analysis was used to determine the correlation between the 2 measurements at each ROI. An additional metacarpus was measured 10 times to determine the coefficient of variation for both techniques.

Results—Dual energy x-ray absorptiometry and bone density were significantly associated at multiple ROI. The addition of age, weight, and soft tissue or bone thickness improved these associations. Repeated measurements had a low coefficient of variation.

Conclusions and Clinical Relevance—Dual energy x-ray absorptiometry can be used to accurately and precisely measure the bone density in the equine metacarpus. Dual energy x-ray absorptiometry appears suitable for serial in vivo measurement of bone density of the equine metacarpus. Dual energy x-ray absorptiometry may be used for studies to evaluate the effects of diet or drugs on bone density or density changes from bone remodeling that develop prior to stress fractures. (Am J Vet Res 2001; 62:752-756)

Bone mineral density (BMD) is the concentration of mineral per unit volume of bone. The BMD correlates with bone material properties and histologic features.

Bone mineral content (BMC), another measure of bone density, is the concentration of mineral per unit volume of bone. The BMC correlates with bone material properties and histologic features.

Bone density correlates with BMD, but is not a direct measure of bone density. Radiographic techniques such as DEXA for determination of BMD in humans and animal bone mineral density in vivo.

Current techniques to serially and noninvasively measure bone mineral density in vivo in horses are limited. Percutaneous ultrasonography has been used in horses to measure bone elasticity. Ultrasound transmission is directly related to bone density and inversely related to elasticity. This technique has multiple limitations such as being affected by limb temperature and soft tissue swelling and can only be used in the frontal plane of the distal portion of the limb. Therefore, changes in the dorsal and palmar and plantar cortices of the third metacarpal bone cannot be measured. Ultrasonographic determination of bone density correlates with BMD, but is not a direct measurement such as dual energy x-ray absorptiometry (DEXA) or single photon absorptiometry.

Single photon absorptiometry uses a monenergetic radionucleotide source. A scintillation detector measures the attenuation of the beam by bone in relation to soft tissue. There is a direct relationship between photons absorbed and bone mineral content. Bone mineral content can be divided by the area of bone measured to calculate the BMD. Thus, ultrasonography can accurately measure the cross sectional area of the bone and, when combined with mineral mass measurements of single photon absorptiometry, will yield BMD. Although this combination of techniques provides accurate information, it requires 2 procedures. In addition to the limitations of ultrasound transmission mentioned, single photon absorptiometry requires a long scan time of up to 1 min/site, and decay of the radioactivity source affects long-term accuracy. Radiographic techniques such as DEXA have replaced single photon absorptiometry for determination of BMD in humans.

Dual-energy x-ray absorptiometry is considered the state of the art technique for measuring BMD in vivo. X-rays of 2 energy levels are impeded by bone, fat, and muscle differently. Proprietary algorithms in the system software are used to calculate the type and quantity of each tissue. Dual-energy x-ray absorptiometry equipment is available for use in humans and laboratory animals, with software containing the appropriate algorithms to correct for the size of the specimen. Use of DEXA for studies in horses has been limited to in vitro analysis of excised bones. For example, a study in horses was performed on long bones devoid of soft tissue placed in a water bath. Soft-tissue structures present in vivo such as the dense collagen in the flexor tendons may contribute, however, to errors in BMD measurement, because the algorithms in the software have not been designed for...
equine limbs. Therefore, DEXA evaluation of bone in situ is necessary.

A mechanism to accurately, noninvasively, and repeatedly measure BMD would allow investigators to perform studies of the pathophysiologic mechanisms of orthopedic problems such as developmental orthopedic disease and potentially predict animals at risk of developing orthopedic injuries. Furthermore, the effects of diet, pharmaceuticals, exercise, and other factors that affect BMD could be evaluated. The purpose of the study reported here was to evaluate DEXA for measuring in situ BMD in horses.

**Materials and Methods**

Twelve forelimbs from the distal portion of the radius through the hoof with soft tissue intact were removed from horses that had been euthanatized for reasons not associated with our study. To test this technique over a wide range of BMD, limbs expected to have dissimilar BMD on the basis of clinical criteria including age, weight, activity level, and chronic lameness were used. All limbs were frozen at -20°C in saline (0.9% NaCl) solution-soaked towels and thawed to room temperature (approx 25°C) prior to testing. For DEXA measurement, limbs were placed in the densitometer and scanned twice, once in a dorsopalmar (DP) plane and once in a lateromedial (LM) plane. Limbs were scanned submerged in 12 cm of water with clay proximally and distally to hold the limb in position. Scans were obtained with the high-resolution medium spine mode setting (3.0 mA, 30 kVp/70 kVp, 0.6-mm point resolution, 1.2 mm line spacing, 1.68 overall resolution). One limb was scanned 10 times with another limb being scanned between each of the 10 measurements. The DEXA measurement of BMD (D-BMD, g/cm²) was obtained for each region of interest (ROI). Location of each ROI was determined by measuring the length of the metacarpus and placing it at 20, 40, and 80% the length of the metacarpus measured from the proximal end (Fig 1). Each ROI was 5 mm in length in a proximal to distal direction. With the exception of the whole bone measurement, width was maintained at 5 mm where possible. In the LM plane, the width of the dorsal and caudal cortex did not allow for a full 5 mm width in all bones. The ROI for the 40% whole measurement in the DP and LM sites included the second and fourth metacarpal bones. One technician who routinely performs DEXA analysis in multiple species obtained all DEXA data.

Bones were stripped of soft tissues and cut with a bandsaw to isolate each ROI. Bone and soft tissue thickness were measured in the appropriate planes at each ROI during the dissection (Fig 2). For the 40% whole measurements and specimens, the second and fourth metacarpal bones were left attached to the third metacarpal bone. Using the apparent
BMD by Archimedes’ principle (A-BMD, g/cm³), the bone specimens were hydrated for 36 hours at ambient atmospheric pressure (approx 760 mm Hg) and room temperature (approx 25 C) in distilled deionized water. Bone specimens were weighed out of water and weighed submerged in distilled deionized water with a commercial density determination system. Density was calculated, using the formula: Density = (A−B) / P, where A is the weight of the hydrated bone out of water, B is the weight of the hydrated bone submerged in water, P is the density of distilled water at a given temperature, and A−B is the difference in weight, which is equivalent to the volume. Bone specimens were dehydrated at 110 C for 16 hours and defatted in methanol as follows: chloroform (1:2) solution in a shaker for 24 hours, rehydrated in distilled deionized water for 36 hours. Density was again determined as described. The group of specimens from the limb that was measured 10 times by DEXA was measured 10 times by Archimedes’ principle, before and after defatting in a random pattern. A laboratory technician who routinely performs these measurements in multiple species obtained the A-BMD data.

Statistical analysis—A database was established, using commercially available software. Statistical analysis was performed, using computer software. A P value of ≤ 0.05 was considered significant. A paired t-test was used to compare nondefatted and defatted A-BMD and LM and DP D-BMD measurements at the 40% whole ROI.

For each ROI, linear regression analysis was used to determine the association between D-BMD and A-BMD including the R, R², slope, and P value. Multiple linear regression models were used to determine whether age and weight of the horse, surrounding soft tissue thickness, and bone thickness were correlated with the D-BMD and A-BMD.

Sample size and power—The correlation between traditional radiographic absorptiometry and BMD in a previous study was found to range between 0.887 and 0.993. Assuming a correlation between DEXA and actual BMD in our proposed study of at least 0.8, a sample size of 10 bones should yield a power of 95% to detect a significant correlation at an alpha level of 0.05 (two-tailed).

Results

Horses had a median age of 6.5 years (range 2 days to 25 years) and weighed a median of 427 kg (range, 46 to 523 kg). There were 3 sexually intact males, 6 castrated males, and 3 females. Five breeds were included (4 Arabian, 4 Quarter Horse, 2 Thoroughbred, 1 American Paint Horse, 1 cross-bred). A bone specimen from a 10-year-old 523-kg Quarter Horse gelding was selected at random to be scanned repetitively.

The D-BMD ranged from 0.293 to 4.804 g/cm³ and A-BMD from 1.054 to 1.9962 g/cm³. The D-BMD could not be obtained for 2 sites, 20% DP and 40% DP, in the foal that was 2 days old. The 40% whole DP and whole lateral D-BMD sites were compared with the A-BMD at the same sites. The A-BMD of nondefatted bone specimens was significantly (P = 0.03) higher than that of defatted bone specimens at the 80% DP ROI, and a similar but not significant (P = 0.15) finding was observed at the 20% DP ROI. The D-BMD at the 40% whole lateral ROI was significantly higher (P = 0.006) than the 40% whole DP ROI measurement. The A-BMD of defatted bone specimens was compared with D-BMD. The association of the D-BMD and A-BMD data was increased by adjustment for age, weight, and soft-tissue thickness and bone thickness were not included in the same model. The best regression models were found with measurements taken at the 40% locations. The coefficients of variation for A-BMD were lower than for the D-BMD measurements; however, all measurements were highly repeatable (Table 2).

Discussion

Archimedes’ principle and variations of it have been used to determine apparent density of bone in

| Dependent variable | Independent variable | Unadjusted Adjusted for age and weight Adjusted for soft tissue, age, and weight Adjusted for bone thickness, age, and weight |
|--------------------|----------------------|------------------------------------------|------------------------------------------|------------------------------------------|
|                    |                      | R² | P   | R² | P   | R² | P   | R² | P   |
| A-BMD 20% DP       | D-BMD 20% DP         | 0.16 | 0.19 | 0.17 | 0.51 | 0.21 | 0.40 | 0.35 | 0.49 |
| A-BMD 40% Cd       | D-BMD 40% Cd         | 0.26 | 0.08 | 0.71 | 0.33 | 0.90 | 0.005 | 0.72 | 0.41 |
| A-BMD 40% Dor      | D-BMD 40% Dor        | 0.42 | 0.02 | 0.65 | 0.17 | 0.75 | 0.06 | 0.66 | 0.24 |
| A-BMD 40% Whole    | D-BMD 40% DP Whole   | 0.74 | 0.0003 | 0.80 | 0.05 | 0.81 | 0.09 | 0.94 | 0.0002 |
| A-BMD 40% Lat      | D-BMD 40% Lat        | 0.71 | 0.0005 | 0.86 | 0.01 | 0.93 | 0.001 | 0.87 | 0.03 |
| A-BMD 80% DP       | D-BMD 80% DP         | 0.11 | 0.29 | 0.32 | 0.96 | 0.43 | 0.85 | 0.39 | 0.75 |

Table 1—Relationship between bone mineral density (BMD) as determined by Archimedes’ principle (A-BMD; dependent variable) and BMD as determined by dual energy x-ray absorptiometry (D-BMD; independent variable) in equine metacarpi

<table>
<thead>
<tr>
<th>Regions of interest</th>
<th>Mean</th>
<th>SD</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-BMD 20% DP</td>
<td>2.45</td>
<td>0.07</td>
<td>2.86</td>
</tr>
<tr>
<td>D-BMD 40% DP</td>
<td>2.93</td>
<td>0.14</td>
<td>4.78</td>
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<tr>
<td>D-BMD 40% DP Whole</td>
<td>3.62</td>
<td>0.08</td>
<td>2.21</td>
</tr>
<tr>
<td>D-BMD 40% Lat Whole</td>
<td>3.71</td>
<td>0.14</td>
<td>3.77</td>
</tr>
<tr>
<td>D-BMD 80% DP</td>
<td>2.08</td>
<td>0.03</td>
<td>1.44</td>
</tr>
<tr>
<td>D-BMD 40% Dor</td>
<td>4.63</td>
<td>0.12</td>
<td>2.59</td>
</tr>
<tr>
<td>D-BMD 40% Cd</td>
<td>4.56</td>
<td>0.17</td>
<td>3.73</td>
</tr>
<tr>
<td>A-BMD 20% DP defat</td>
<td>1.59</td>
<td>0.04</td>
<td>2.60</td>
</tr>
<tr>
<td>A-BMD 40% Dor defat</td>
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<tr>
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<td>A-BMD 80% DP defat</td>
<td>1.15</td>
<td>0.06</td>
<td>5.54</td>
</tr>
</tbody>
</table>

*Coefficient of variation (%) = (SD/mean) × 100. defat = Defatted specimens. See Table 1 for key.
previous studies.21,22 Density measurements of cortical bone are likely similar in defatted and nondefatted bone. However, density of trabecular bone is artificially increased by fat within trabecular spaces when using Archimedes’ principle.19 This is supported by our findings that indicate that metaphyseal segments have higher BMD in nondefatted segments than defatted segments. The removal of fat leaves only bone such that there is a linear relationship between density, mineral, and organic components.7 Therefore, defatted A-BMD measurements were used for comparison with D-BMD. One limitation observed with Archimedes’ principle is that the CV for defatted segments was higher at the 20 and 80% ROI, possibly as a result of small air bubbles trapped within trabecular bone during the weighing process.

Results of our study indicate that currently available software can be used for DEXA determination of BMD in horses. We chose to use the high-resolution medium spine mode for our study for multiple reasons. During preliminary studies, the spine mode results were more consistent than the femur mode. The high-resolution slow spine mode that used a 0.75 mA x-ray beam did not provide consistent measurements. The high-resolution medium spine mode provided the most repeatable results and was also recommended by the manufacturer. The D-BMD measurements were not obtained at 2 sites in the 2-day-old foal. The lowest A-BMD measurements were obtained at both of these sites, suggesting it was below the sensitivity of the DEXA software selected.

Because the software algorithms account for a minimal amount of soft-tissue density surrounding the bone,20,21 which is lacking in the lower limb of horses, actual soft tissue density was approximated by submerging the limbs in 12 cm of water. Other studies have evaluated lard, paraffin, polycarbonate, and rice to provide soft-tissue radiodensity.20 In preliminary studies, D-BMD measures made with rice bags were not as consistent as with limbs submerged in 12 cm of water.

The D-BMD can be affected by the direction of the scan. Mediolateral scans yielded a higher BMD at the same ROI than DP in a previous study,21 and similar results were obtained in our study at the 40% whole location where measurements were made in both planes. Therefore, for studies measuring changes in BMD over time, serial measurements must be made in the same plane to be comparable. Because BMD will vary between diverse locations of the bone, serial studies must use similar ROI throughout the study, preferably at multiple locations that will identify changes of BMD in cortical and trabecular bone. In our study, the D-BMD and A-BMD correlated best at the measurements taken at the 40% locations in the dense cortical bone. However, with serial evaluations, multiple ROI should be included, because various remodeling processes affect different areas of bone.20,21 Alternatively, with DEXA, an entire bone can be included. However, small changes in specific locations may not be identified when evaluated in this fashion.

The D-BMD CV was similar to previous studies in other species where it has been reported to range from 1.8 to 1.9%21 in human hips to 2.7% in the rat femur.22 When using a technique for serial studies, a low CV is critical. The minimum detectable significant difference between 2 measurements in a single subject is 2.8% if the CV is 1% and 14% with a CV of 5%.23 The CV for DEXA in our study ranged from 1.58 to 4.5%.

The magnitude of the CV usually depends on multiple factors including positioning, precise delineation of the ROI, intraobserver variability, and the intrinsic precision of the equipment. The ROI were identified by measuring pixels in a proximal to distal direction. However, the exact location and ROI size was operator dependent. Further, small ROI as used in our study have a lower CV, compared with using larger ROI.24 The CV determined by measurement of a phantom for the DEXA system used was 0.32% for the year that this project was performed. The CV for A-BMD was lower than for the D-BMD. This was to be expected, because there is no positioning effect and minimal operator dependence when using Archimedes’ principle.

The D-BMD and A-BMD measurements were similar to data previously reported for these techniques and varied as expected by age and weight.19,21,22 Over the range of BMD in our study, there was a high correlation between A-BMD and D-BMD. One limitation observed in our study was that the D-BMD of a young foal could not be determined at 2 ROI.

In summary, our findings indicate that DEXA can be used to accurately measure BMD in situ in the metacarpus of horses, and the addition of age, weight, and soft-tissue thickness or bone width to a regression model substantially improves the correlation between measured and apparent BMD. Serial in vivo determinations of BMD in horses are feasible when techniques are standardized to maintain precision.

References


