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Bone mineral density and computer tomographic measurements in correlation with failure strength of equine metacarpal bones

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Abstract

Information regarding bone mineral density and fracture characteristics of the equine metacarpus are lacking. The aim of this study was to characterize the relationship between mechanical properties of the equine metacarpal bone and its biomechanical and morphometric properties. Third metacarpal bones were extracted from horses euthanized unrelated to musculoskeletal conditions. In total, bone specimens from 26 front limbs of 13 horses (7.8 ± 5.8 years old) including Lipizzaner (n = 5), Hungarian Warmblood (n = 2), Holsteiner (n = 2), Thoroughbred (n = 1), Hungarian Sporthorse (n = 1), Friesian (n = 1), and Shagya Arabian (n = 1) were collected. The horses included 7 mares, 4 stallions and 2 geldings. Assessment of the bone mineral density of the whole bone across four specific regions of interest was performed using dual-energy X-ray absorptiometry. The bones were scanned using a computer tomographic scanner to measure cross-sectional morphometric properties such as bone mineral density and cross-sectional dimensions including cortical area and cortical width. Mechanical properties (breaking force, bending strength, elastic modulus) were determined by a 3-point bending test. Significant positive linear correlations were found between the breaking force and bone mineral density of the entire third metacarpal bones ($P < 0.001$, $r = 0.72$), the medial cortex region of interest ($P < 0.001$, $r = 0.68$) and the transverse region of interest ($P < 0.001$, $r = 0.61$). The correlation between the breaking force and bone mineral density of the equine third metacarpal bone found in this study warrants *in vivo* investigations.

Third metacarpal bone, densitometry, biomechanical properties, CT, horse

Although bone mineral densitometry is an essential diagnostic method to determine increased fracture risk in human patients, application in the veterinary medicine is negligible. In horses, the current works are in experimental stages (Shryver 1978; Hanson and Markel 1995; McClure et al. 2001). Evaluation of equine skeletal structures with dual energy x-ray absorptiometry (DXA) was first described by Lawrence and Ott (1985). Since then, DXA has mainly been applied for the measurements of bone mineral density of the metacarpal bones (van Harreveld et al. 2002; Walker et al. 2004). *In vitro* studies evaluated the bone density of different equine bones (Hanson and Markel 1995; Firth et al. 1999; Tóth et al. 2010). The DXA method could be valuable to assess fracture risk of the metacarpus in horses. However, data describing correlation between the failure strength and the different bone mineral density (BMD) values are missing.

Therefore, the aim of this *in vitro* study was to describe the relationship between mechanical properties of the equine metacarpal bone, such as breaking force, elastic moduli and bending strength, and its biomechanical and morphometric structural properties such as BMD and computer tomographic (CT) cross-sectional dimensions. We hypothesized that there is a linear correlation between BMD, CT morphometric indices and the bending strength, elastic modulus and breaking force of the equine third metacarpal bone.

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Materials and Methods

Specimen selection

Bone specimens used in this study were collected from horses euthanized for non-musculoskeletal causes at the Clinic for Large Animals, Üllő, Hungary. Bone specimens were collected from 26 front limbs of 13 horses (7.8 ± 5.8 years old). Horses included in the study consisted of Lipizzaner (n = 5), Hungarian Warmblood (n = 2), Holsteiner (n = 2), Thoroughbred (n = 1), Hungarian Sporthorse (n = 1), Friesian (n = 1), Shagya Arabian (n = 1). They were 7 mares (4 of which were Lipizzaner), 4 stallions and 2 geldings.

Bone dissection and preparation

After dissection and manual removal of all soft tissue, the bones were stored in 70% ethyl-alcohol at room temperature until measurements, as previously suggested by Beaupied et al. (2006).

Bone mineral densitometry

Post mortem examinations were done by using a Norland XR-26 densitometer (Norland Corporation, Fort Atkinson, WI, USA) at the First Department of Medicine, Semmelweis University of Medicine, Budapest, Hungary. During the measurements the bones were placed on a 20 mm wide plexiglass plate to imitate soft tissue density as described in detail elsewhere (Tóth et al. 2010). The bones were measured × 3 from the dorsopalmar (DP) direction and averaged. The regions of interest (ROI) were the entire bone, the medial cortex region, the lateral cortex, the transverse area of the longitudinal centre and the perpendicular medial cortex as shown in (Plate III, Fig. 1). Positions of the ROIs were chosen in the loaded area of the assessed metacarpal bones because the question was the correlation between the BMD and failure strength.

CT scan

The bones were scanned with a Siemens Somaton Emotion 6 Multislice CT (130 kV, 20 mAs, slides: 2 mm) (Siemens AG, Erlangen, Germany) at the Institute of Diagnostic Imaging and Radiation Oncology, Kaposvár University, Hungary. The cortical width was measured × 3 and averaged at each bone quadrant at the longitudinal centre and the cross-sectional area was calculated using Siemens SIENET software (Siemens AG, Erlangen, Germany).

Load testing

The bones were tested with an INSTRON 8872 servohydraulic Universal Testing Machine /UTM/ (Instron, Norwood, MA, USA) at the Laboratory of Biomechanics, University of Technology and Economics, Budapest, Hungary. The specimens were supported by proximal and distal metal rods placed 180 mm apart. A third rod fixated to the actuator was used to transmit the load to the palmar mid-diaphyseal cortex, 90 mm from the proximal and distal rod in a palmaro-dorsal direction at a speed of 25 mm/sec until breaking.

Data analysis

Statistical analysis of the data was performed using commercially available software (Minitab 16: Minitab Inc., PA, USA). Descriptive statistical analyses were performed to calculate the mean, standard deviation, median and range of each individual variable. Distribution of the data was tested with the Shapiro-Wilk method. Pearson's linear regression analyses were performed to reveal possible correlations between the bone length, BMD indicators of the selected ROIs and mechanical properties (bending strength, elastic moduli and breaking force). Pearson's linear regression analyses were also performed to reveal possible correlations between morphometric CT measurements (lateral, medial, dorsal, palmar width and area) and the above mentioned mechanical properties. A $P < 0.05$ was considered significant in all tests and a correlation coefficient (r) value greater than 0.6 was considered to be sufficient to assume a linear relationship between the two variables in a given model.

Results

Descriptive statistical values for each variable across the equine third metacarpal bone specimens are summarised in Table 1.

The breaking force was found to correlate with the BMD of the entire bone ($P < 0.001$, r = 0.72; Fig. 2), the medial cortex ROI ($P < 0.001$, r = 0.68) (Fig. 3), the transverse ROI ($P < 0.001$, r = 0.61), the lateral cortex ROI ($P < 0.001$, r = 0.59), and the medial perpendicular ROI ($P < 0.001$, r = 0.6). Breaking force was not found to correlate well with the bone dimensions such as length. Cortical width measured by CT did not correlate with breaking force.

There were no significant correlations between bending strength and any of the bone mineral density measurements. Furthermore, bending strength did not significantly correlate with cortical widths in each quadrant or bone length.

Table 1. Descriptive statistical values for each variable across the third metacarpal bone specimens in horses.

Variable	Mean ± SD	Median	Range
Bone length (cm)	26.2 ± 0.9	26.1	24.9-28.2
Whole BMD (g/cm²)	2.16 ± 0.22	2.19	1.65-2.59
Medial cortex BMD (1) (g/cm²)	2.38 ± 0.26	2.36	1.95-2.87
Lateral cortex BMD (2) (g/cm²)	2.22 ± 0.16	2.24	1.83-2.52
Transverse BMD (3) (g/cm²)	2.42 ± 0.26	2.45	1.81-2.98
Medial perpendicular cortex BMD (4) (g/cm²)	2.29 ± 0.21	2.32	1.88-2.68
Bending strength (MPa)	234.4 ± 56.6	214.8	129.6-341.3
Elastic modulus 1 (MPa)	7380 ± 2728	6570	3626-12864
Elastic modulus 2 (MPa)	2978 ± 1855	2397	542-6573
Breaking force (kN)	19.8 ± 4.5	20.5	13-28
Lateral cortex width (cm)	0.99 ± 0.13	1.00	0.69-1.21
Medial cortex width (cm)	1.38 ± 0.25	1.34	0.99-1.99
Dorsal cortex width (cm)	0.96 ± 0.12	0.94	0.74-1.19
Palmar cortex width (cm)	0.66 ± 0.15	0.66	0.39-1.01
Area (mm²)	755.4 ± 117	752.2	604.8-1063

BMD – bone mineral density, n = 26

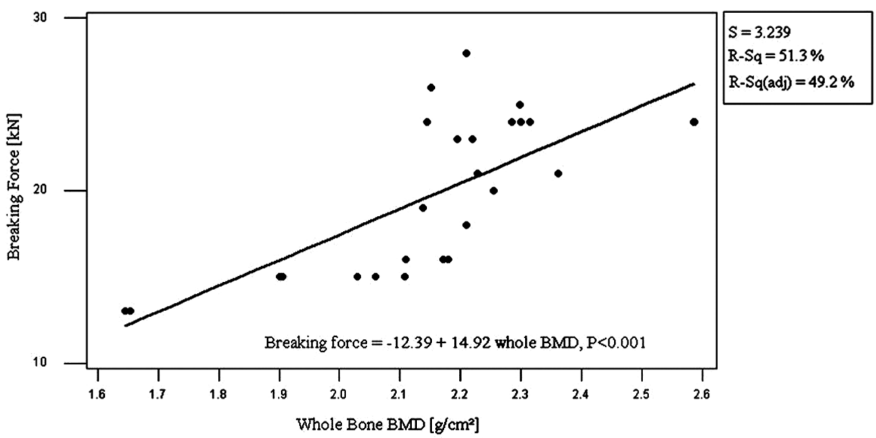

Fig. 2. Correlation between the whole bone mineral density and the breaking force of third metacarpal bone of horses

McIII – third metacarpal bone, BMD – bone mineral density, S – skewness, R-Sq – coefficient of determination, R-Sq(adj) - adjusted coefficient of determination

No significant correlations were found between the elastic modulus and BMD measurements. Furthermore, the elastic moduli were not found to adequately correlate with the dorsal cortex width and with the width of the remaining quadrants.

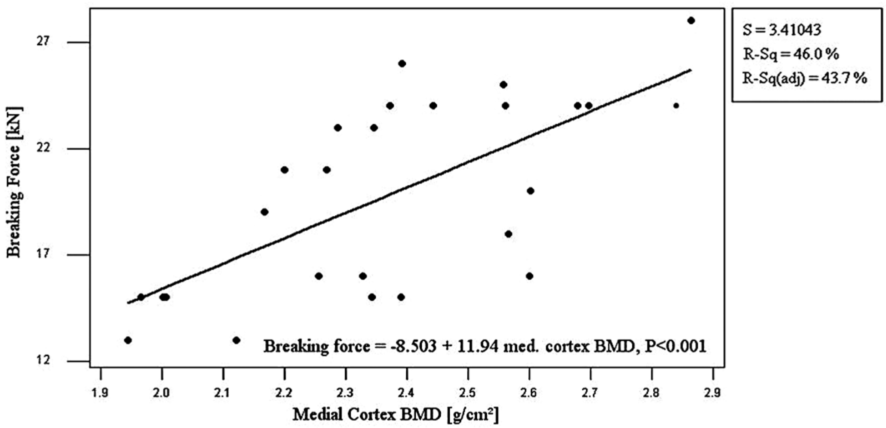

Fig. 3. Correlation between the medial cortex bone mineral density and the breaking force of third metacarpal bone of horses

McIII – third metacarpal bone, BMD – bone mineral density, S – skewness, R-Sq – coefficient of determination, R-Sq(adj) - adjusted coefficient of determination

Discussion

The highest BMD was found in the transverse ROI, which encompasses all four bone quadrants in a cross sectional slice at the longitudinal centre of the bone. This includes the palmar region which has been described in both equine (Shryver 1978) and human (Lai et al. 2005) studies to exhibit the highest density due to its subjection to compressive stress under physiological load conditions (Les et al. 1997). The BMD results of the medial cortex prove to be the second highest value in our study. The mineral density of the whole bone was the lowest among all measured regions, which might be explained by the inclusion of the lower-density epiphyseal regions. Selection of these specific ROIs was chosen within the loaded area of the metacarpal bones according to the findings of an *in vivo* study, which described the peak strain on the dorsal and dorsomedial aspect of the midshaft of the cannon bone (Davies 2005). However, this peak strain of the metacarpal bone has only been measured in Thoroughbred racehorses running on an artificial surface (treadmill) (Davies 2005, 2009). Thus the distribution of peak strains may be different on different track surfaces (dirt, turf, poly-track). Furthermore, it must be noted that fractures in racehorses occur commonly in the lateral condylar regions (Riggs et al. 1999).

We have found the whole bone and the medial cortex BMD measurements to be the strongest indicators of bone strength. One possible explanation for the strong correlation between the whole bone BMD and the breaking force is that, despite the lack of regional granularity, it measures the density of the overall bone, accounting for heterogeneity across its length. The medial cortex correlation may partially be owed to it being the region with the highest cortical width among the studied specimens, making it one of the main contributors to overall bone density and stability. A previous study of the third metacarpal bone has shown no significant variation between the bone mineral content or density values measured by DXA from different directions, indicating that *in vivo* studies do not need to focus on a specific angle, and that any whole bone assessment through DXA can be a sufficient indicator of the BMD in horses (Tóth et al. 2010). Studies comparing cut bone preparations to whole bone found that the mean bone density was significantly higher when analysing the entire bone, and that there were no significant differences between the whole bone measurements between dissected and intact metacarpal bones (Carter et al. 1992).

The load testing method used in this study involved the artificial application of force in the palmaro-dorsal direction at the longitudinal centre of the bone. This approach was chosen due to practical reasons, as we aimed to focus on the midshaft of the third metacarpal bone during the loading test. In this position the proximal and the distal rod of the testing machine lie under the cannon bone and not under the splintbones. In this positioning, the confounding effects of the splintbones are minimal on the breaking strength values of the cannon bones. It should be reiterated that the most prevalent fracture site in racehorses is the lateral condyle (Riggs et al. 1999); however, the loading test of that single region or the proximodistal compression described by Davies (2009) was not feasible in our settings.

In conclusion, the most determinant factors were those representing the entire bone rather than specific regions. Bone strength is determined by a complex set of structural variables that need to be collectively considered. Therefore, despite differences among cortical regions, the strong relationship between whole bone BMD and breaking force suggests that further studies should focus on the entire bone rather than specific regions. Complete understanding of fracture risk requires combining these factors into a single cohesive model. Considering the fact that portable DXA devices are a feasible, accurate and precise tool in standing horses (Donabedian et al. 2005), correlation between breaking force and whole bone mineral density identified in this study warrants *in vivo* investigations.

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