Assessment of bone mineral density; and comparative
densitometric, morphometric and breaking strength analysis
of the equine third metacarpal bone and proximal phalanx

PhD theses

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Introduction

Bone fractures, which occur as a result of greater traumas or structural damages due to extensive use, lead to significant economic losses in equestrian sport and horse racing. Nevertheless, there is no adequate method at present to accurately assess fracture risk in horses.

Dual-energy X-ray absorptiometry (DXA), which has come to the center of researchers’ and practitioners’ attention in medical science in the past two decades, may play a pivotal role in fracture risk evaluation. This technique, which has already become standard practice in human medicine, is not yet applied widely in veterinary science and equine medical practice. Its use has been mainly limited to ex vivo research; the development of technology which can be used for in vivo examinations is still underway.

Biomechanics is a science that examines and models the mechanic operation and features of the body’s supporting structures. It studies, for example, the breaking, bending, stretching, and rotating features of bones.

Due to the large number of factors and parameters that contribute to the results of biomechanical analyses, as well as the limitations of measurement techniques, the examination of the bone’s biomechanical qualities is a very difficult task. Although researchers try to reproduce as much as possible the parameters that are present under in vivo loading circumstances, there are a number of difficulties to tackle with in the course of these tests, such as the heterogeneity of the bone’s physical features and the complications that arise from the asymmetry of bones.

Studies are very often based on the simplification of bone features, and take into consideration only one aspect of the bone during the examinations. However, under in vivo circumstances the different features and parameters are all present in their complexity, which means that if researchers want to simulate an in vivo environment, they have to take a large number of parameters into consideration. Many research groups deal with the examination and analysis of
bones in horses, and their results – mainly because of the reasons stated above – are varied. Nevertheless, there has been significant progress in the description and understanding of in vivo circumstances.

The third metacarpal bone (McIII) is structured to enhance and modulate sagittal bending, rather than to resist it. The material distribution within the bone is intended to provide more predictable behaviour under load. Moreover, the natural curvature of the bone is assumed to represent a balance between the maximization of load and capacity, and the predictability of bone behaviour under different loading conditions.

According to some research in the UK, fractures of the lateral condyle are the most common fractures with a fatal outcome, probably because of the continuous concentrated stress in the condylar groove, as the bone is subject to continual compressive strength in the proximo-distal axis. This locally stimulates the process of bone remodelling, which in turn decreases bone mineral density in the condylar region and can result in higher fracture risk.

Stress fractures are considered as one of the most significant causes of economic losses in the race horse industry. According to comprehensive retrospective studies, a large number of musculoskeletal injuries are fatal in racehorses. Fractures of the proximal phalanx (P1) are one of the most common incidences during training, and they are also one of the most common types of fractures requiring osteosynthesis at equine clinics. The most severe longitudinal and comminuted fractures are also observed in the first phalanx. Most fractures appear ‘spontaneous,’ despite the fact that they are the summation of complex processes with numerous factors involved.

Due to all the various reasons outlined above, the assessment of fracture risk proves to be an important issue in equine medical practice. The major objective of the present study is to provide further data from the examination of the third metacarpal bone and the proximal phalanx in horses, in order to contribute to the development of more accurate fracture risk assessment methods.
Objectives of the study

1. Our major objective was to develop an examination routine for the *ex vivo* densitometry of equine third metacarpals and first phalanges. We aimed to detect correlations between bone mineral density (BMD) and bone mineral content (BMC) values and measurement directions.

2. We wanted to test the accuracy of our measurements, as well as find correlations between BMD and BMC values, and the animal's breed, age, sex and type of use. We also planned to describe the differences in values according to the side of the limb, if there were to be any.

3. We also aspired to find out whether it is the BMD values of third metacarpi and proximal phalanges or rather their morphometric parameters that more strongly correlate with breaking features. CT scans provided a method for measuring the morphometric parameters of the bones. We measured the breaking features of the third metacarpi with three-point bending tests, while we examined the same features of the proximal phalanges with vertical compression tests.

Materials and methods

1. Densitometric analysis of equine third metacarpal bones and proximal phalanges, designing and testing the method, other correlations

All the animals sampled in the present study were euthanised at the Clinic for Large Animals, Faculty of Veterinary Science, Szent István University, Úllő, Hungary, for reasons unrelated to this research. Following the removal of the soft tissues covering the third metacarpal bones and the proximal phalanges, we stored the bones in 70% alcohol until further study. We identified the bones by age, sex, breed, and type of use, as well as the side of the limb from which the sample was taken.
The bones were subjected to post mortem densitometric analysis at the ODM Laboratory of the First Department of Medicine, Semmelweis University of Medicine, with the help of a Norland XR-26 densitometer (Norland XR-26, manufacturer: Norland Corporation, Fort Atkinson, WI, USA).

In the first part of the study, we examined 11 third metacarpi of 9 horses from dorsopalmar (DP), palmarodorsal (PD), lateromedial (LM), and mediolateral (ML) directions, using the ‘spine’ mode. During measurement the bones were placed on a plexi plate of 20mm in width.

We set the whole bone as our region of interest (ROI), and we evaluated both BMD and BMC, similarly to a previous study in other species. Average and spread values were calculated from repeated measurements, after which we compared the results obtained from different directions with paired t-tests.

In the second part of the study, we measured 34 third metacarpi and 34 proximal phalanges of 17 horses three times from the dorsopalmar (DP) direction.

The main objective of these measurements was to establish the accuracy of our own measurements by calculating the coefficient of variation between measurements in equine third metacarpal bones and proximal phalanges, in order to verify that we can precisely determine the BMD and BMC values without repeated measurements. We evaluated the BMD and BMC values, looking for correlations according to the side of the limb, as well as the animal’s age, sex, and breed. We calculated the coefficients of variation (CV%), then we applied paired t-tests for the statistical comparison of the BMD values of the third metacarpi and the proximal phalanges. To test the relationship between BMD and BMC values, and the type of bone (McIII or P1), age, sex, and breed, we applied general linear model and the Tukey-test. We analysed our statistical data with the help of the R 2.6.0 program (manufacturer: R Development Core Team 2007).
2. Comparative densitometric, CT and breaking test analysis of equine third metacarpal bones

Sampling
The bone specimens used in the present study were all taken from horses euthanised at the Clinic for Large Animals, Faculty of Veterinary Science, Szent István University, Úllő, Hungary, for reasons unrelated to this research. We used the specimens from 26 front limbs of 13 horses. After dissection and the manual removal of all soft tissue, we stored the bones in 70% ethyl alcohol until the measurements.

BMD measurements
The post mortem examinations were done by using a Norland XR-26 densitometer (manufacturer: Norland Corporation, Fort Atkinson, WI, USA) at the OMD Laboratory of the First Department of Medicine, Semmelweis University of Medicine, Budapest, Hungary. During the measurements the bones were placed on a plexi glass of 20mm in width. The bones were measured three times from the dorsopalmar (DP) direction, and the three values were averaged. The regions of interest (ROIs) were the whole bone, the medial cortex region (1), the lateral cortex (2), the transverse area of the longitudinal center (3), and the perpendicular medial cortex (4). The ROIs were selected in the loaded area of the assessed bones because our research aim in this part was to identify the correlation between BMD and failure strength.

CT measurements
The bones were scanned with a Siemens Somaton Emotion 6 Multislice CT (130 kV, 20 mAs, slides: 2mm; manufacturer: Siemens AG, Erlangen, Germany) at the Institute of Diagnostic Imaging and Radiation Oncology, Kaposvár University, Kaposvár, Hungary. The cortical width was measured three times and averaged at each ROI, after which the cross-sectional area was
calculated with the Siemens SIENET software (manufacturer: Siemens AG, Erlangen, Germany).

**Loading tests**

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

**Statistical analysis**

The statistical analysis of the data from the measurements was performed with the commercially available Minitab 16 software (manufacturer: Minitab Inc., PA, USA). Descriptive statistical analyses were performed to calculate the mean, the standard deviation, the median and the range of each individual variable. The distribution of data was tested with the Shapiro-Wilk method. Pearson’s linear regression analyses were performed to reveal any correlations between bone length, the BMD parameters of the selected ROIs, and such mechanical parameters as bending strength, elastic moduli and breaking force. Pearson’s linear regression analyses were also performed to reveal any correlations between morphometric CT measurements (lateral, medial, dorsal, palmar width and area) and the above mentioned mechanical parameters. 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.
3. Comparative densitometric, CT and breaking test analysis of equine proximal phalanges

**Sampling**
The bone specimens used in the present study were all taken from horses euthanised at the Clinic for Large Animals, Faculty of Veterinary Science, Szent István University, Üllő, Hungary, for reasons unrelated to this research. We used the specimens from the front limbs of 7 horses. After dissection and the manual removal of all soft tissue, we stored the bones in ethyl alcohol at room temperature until the measurements.

**BMD measurements**
We carried out the post mortem examinations of the bones with a Norland XR-26 densitometer (manufacturer: Fort Atkinson, WI, USA) at the OMD Laboratory of the First Department of Medicine, Semmelweis University of Medicine, Budapest, Hungary. Imitation of soft tissue around the bone was required for the software algorithm used to measure the BMD. Therefore, a 20mm plexiglass was used as a substitution for soft tissue. We measured the bones once from the dorsopalmar direction. First the BMD of the whole bone was measured, then three 1x1 cm ROIs were selected and measured. The ROIs were the entire bone; the medial cortex; the lateral cortex at the level of the horizontal axis of the mid-third of the bones; and the trabecular region of the sagittal plane of the proximal third, 3mm under the level of the deepest point of the metacarpophalangeal joint surface of the proximal phalanges.

**CT measurements**
The P1 bone specimens were also scanned with a Siemens Somaton Emotion 6 Multislice CT (settings: 130 kV, 20 mAs; slides: 2mm; manufacturer: Siemens AG, Erlangen, Germany) at Kaposvár University, Kaposvár, Hungary. The morphometric parameters of the proximal phalanges were measured at the mid-diaphyseal plane using the Siemens SIENET software. Cortex width was
measured from the dorsal, lateral, medial and palmar sides, then we measured total bone width from both the dorsopalmar and the lateromedial direction. Cortical area was calculated from these data using an equation described by Sherman et al. (1995). Subsequent CT images were taken after the loading tests to identify the fracture sites.

**Loading tests**
The biomechanical properties of the P1 specimens were assessed at the Laboratory of Biomechanical Research, Budapest University of Technology and Economics, Hungary. For the loading pressure tests a universal bone crusher (ZD-20 universal testing machine) was used. Due to the lack of previous *ex vivo* data regarding the breaking force measurements of the first phalanx, the machine was arbitrarily set for human lumbar spinal preset due to the similar cuboid shape. In order to position the bone properly during measurements, the proximal and distal articular rims of the P1 bones were removed at the level of the deepest point of the metacarpophalangeal (proximal) and proximal interphalangeal joint (distal) articular surfaces of the proximal phalanges by a bandsaw. The bones were subjected to loading pressure from a proximodistal direction, as this kind of pressure is similar to that experienced in *in vivo* circumstances. Bone failure strength was calculated from loading pressure values.

**Statistical analysis**
The statistical analysis of the data was performed with the commercially available Minitab 16 program. Mean, standard deviation, median and range values were calculated for bone mineral density, cortical area, cortex width, bone width, loading pressure, and breaking force. The distribution of the data was assessed with the Shapiro-Wilk test. Pearson’s linear correlation was used to reveal any association between bone mineral density values (whole bone BMD, trabecular BMD, lateral cortical BMD, medial cortical BMD), cortical area,
cortical width (lateral, medial, dorsal, and palmar regions), bone width (sagittal and transversal) and bone breaking strength and loading pressure. Statistical significance was set at P<0.05 and an adequate linear correlation was assumed if the regression coefficient was greater than 0.6 (r>0.6).

Description and evaluation of results

1. Densitometric analysis of equine third metacarpal bones and proximal phalanges, designing and testing the method, other correlations

In the first part of our study we proved that there are no significant differences between the BMD and BMC values of the metacarpal bone measured from the dorsopalmar (DP) and palmarodorsal (PD), as well as from the lateromedial (LM) and mediolateral (ML) directions. This means that there is no significant difference between the BMD and BMC values measured from opposite directions (P>0.05). The variations in values according to different measurement directions can be explained with the geometry of the third metacarpal bone. In future studies we advise to choose the same positioning to detect the possible biological differences between bones. In the future the standardization of the measurement technique will be important to maintain and guarantee the precision of measurements.

In the second part of the experiments, we measured 34 third metacarpi of 17 horses three times from the dorsopalmar (DP) direction. In this part of the study we established that there are no significant differences between serial measurements of the same bone. Therefore, we concluded that one measurement is sufficient for the evaluation of the third metacarpal bones (McIII) and proximal phalanges when the DXA method is used. The CV% values of the dorsopalmar metacarpal bone mineral density values (g/cm²) are between 0.09 and 0.55 and those of the bone mineral content values (g) are between 0.05 and 0.89. CV% values of the first phalanx
dorsopalmar bone mineral density values (g/cm$^2$) are between 0.10 and 0.76 and those of the bone mineral content values (g) are between 0.07 and 0.76. The comparison of BMD and BMC of the same bones from the right or the left side limb did not result in statistically significant differences either ($P>0.05$). We could not detect the presence of ‘sidedness’ known from human studies, although our pool included only one English thoroughbred specimen. As the BMD and BMC values of the right and the left side did not differ significantly, in the rest of our study we calculated only the data obtained from the right side.

In the study we showed that the BMD values of equine third metacarpal bones are higher than those of proximal phalanges. We were also the first to use the DXA method for the evaluation of the equine proximal phalanx.

We found that there is no correlation between BMD and BMC values of equine McIII and P1 bones concerning age ($P=0.053$ for BMD and $P=0.192$ for BMC) or breed ($P=0.227$ for BMD and $P=0.071$ for BMC). On the other hand, we showed that the gender of the horse has a significant effect on BMD and BMC ($P=0.005$ for BMD and $P=0.015$ for BMC). Both BMD and BMC were significantly higher in geldings compared to mares ($P=0.002$ for BMD and $P=0.004$ for BMC) or to stallions ($P=0.003$ for BMD and $P=0.012$ for BMC), but the BMD and BMC values of mares and stallions do not differ significantly ($P=0.923$ for BMD and $P=0.999$ for BMC). Our results indicate that there is a need for further and more extensive BMD research which includes greater samples.

2. Comparative densitometric, CT and breaking test analysis of equine third metacarpal bones

The highest mineral density among our measurements was observed in the transverse ROI (mean±SD=$2.4225±0.2637$ g/cm$^2$), which encompasses all four bone quadrants in a cross sectional slice at the longitudinal centre of the bone, and includes the palmar region, which has been described in human studies to
have the highest density due to its subjection to compressive stress under physiological load conditions.

Also in line with human research, the mineral density measurements of the medial cortex were the second highest in our study (2.3767±0.2581 g/cm²). The mineral density of the whole bone was the lowest of all measured regions (2.1630±0.2181 g/cm²), which can be explained by the inclusion of the lower-density epiphyseal regions.

The most significant correlations identified in the study involved breaking force and bone mineral density. The whole bone and medial cortex BMD measurements were found to be the strongest indicators of bone strength ($P<0.001$, $r=0.72$ and $P<0.001$, $r=0.68$, respectively). One possible explanation for the strong correlation between the whole bone BMD and 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 correlation regarding the medial cortex may be partially due to the fact that it is the region with the highest cortical width among the studied specimens, which makes it one of the main contributors to overall bone density and stability.

Breaking force was also found to correlate with the BMD of the medial cortex ROI ($P<0.001$, $r=0.68$), the transverse ROI ($P<0.001$, $r=0.61$), the lateral cortex ROI ($P<0.001$, $r=0.59$), and the medial perpendicular ROI ($P < 0.001$, $r = 0.6$).

The elastic modulus was also found to correlate with the bone’s diameter ($P<0.001$, $r=0.67$), and we also found that it weakly correlates with dorsal cortex width ($P=0.004$, $r=0.55$), following a general regression pattern similar to that of breaking strength.

The dimensions of the metacarpal bones analyzed in this study were not found to significantly correlate with breaking force. There was only a weak positive correlation between breaking force and cortical area ($P=0.006$, $r=0.52$). Bone diameter also presented only a slight positive correlation ($P=0.003$, $r=0.5$). The widths of each cortical quadrant could not be statistically correlated to any of the measured mechanical properties. Bending strength did not correlate
significantly either with the various widths of the cortical quadrants or with bone length.

Our study proved that bending strength, defined as the bone’s ability to resist deformation under load, was found to correlate with the bone’s diameter ($P<0.001$, $r=0.7$). Similar correlations were also suggested in other studies. The widths of each cortical quadrant could not be statistically correlated to any of the measured mechanical properties. This conflicts with some studies that have indicated differences in strength and stiffness between the cortical quadrants, suggesting that the lateral quadrant has the highest strength. These studies often analyze samples taken from different cortical regions, rather than a full cross-section or the entire bone.

During the dimensional analysis of the cross-sections in this study, fused metacarpals were not considered in the cortical area, and were not specifically tracked as a variable or possible indicator of bone strength. Prior research suggests that these fused metacarpals contribute to bending strength and bone rigidity. It remains to be seen how the difference in area would influence the correlation between area and breaking force or bending strength.

The loading test method used in this study involved the artificial application of force in the palmarodorsal direction at the longitudinal centre of the bone. However, it should be considered that the most frequent fracture site in racehorses is the lateral condyle. It is therefore a potential region of interest that can be considered in future research.
3. Comparative densitometric, CT and breaking test analysis of equine proximal phalanges

14 proximal phalanges from 7 horses were used in this part of the study. One phalanx was excluded from the statistical evaluation because following loading test examinations the fracture site could not be located on the CT images. In the course of the ODM examinations of the equine proximal phalanges, the lowest BMD was observed in the trabecular ROI. In horses both the anatomy and physiology of the trabecular region differ from those of the cortical region. The trabecular region is composed of small trabeculae with a lower mineral content, and unlike the cortical region, the trabecular region is not only under the regulation of vitamin D, calcitonin or parathyroid hormone, but local forces can also induce marked remodelling, which enables this region to directly respond to mechanical forces. Similarly, loss of trabecular bone induced by immobilization has been well documented in people, and is characterized by a complete loss or thinning of trabeculae.

Fracture lines were identified in the sagittal plane in 13 of 14 (92.86%) specimens. In 5 of 13 (38.46%) cases the fracture lines were located in the proximal and mid-third, in 6 of 13 (46.15%) cases the fracture lines were located in the mid- and distal third, and in 2 of 13 (15.38%) cases fracture lines were located in the mid-third in the sagittal plane.

Our in vitro study showed significant positive linear correlation between trabecular BMD and breaking force (P=0.023, r=0.62). Other parameters did not significantly correlate either with breaking force or compression force. This result coincides with the clinical evidence that the fracture of the trabecular region is the most common in the proximal phalanx. Our post-fracture CT images revealed that most fracture lines were located in the sagittal plane at the proximal or distal trabecular regions, similarly to an earlier retrospective study. Thus, it would be important to focus on this region in further studies, as the
trabecular region appears to be the key component in sagittal proximal phalanx fractures. Despite the coinciding results, it must be emphasized that sagittal trabecular fractures are currently attributed to the mechanical effect of the sagittal ridge of the third metacarpal bone, which was not part of our in vitro model. In conclusion, our study suggests that the trabecular region is the only ROI which may predict the strength failure of the proximal phalanx. Thus, the identification of the fracture lines and the measurement of the BMD values of this particular area should also be investigated in future studies. In vivo experiments are also warranted to reveal whether or not the trabecular BMD data are useful indicators for the risk evaluation of fractures in equine proximal phalanges.
New scientific results

1. In the process of our *ex vivo* equine densitometric analysis, we managed to work out a convenient, simple and useful method for the substitution of soft tissue. Also, we were the first to apply the DXA method for the densitometry of equine proximal phalanges.

2. We proved that there are no statistical differences between the BMD and BMC values of the third metacarpal bone measured from opposite (DP-PD and LM-ML) directions ($P>0.05$). We also established that there are no significant differences between subsequent measurements of the same bone. Thus, we arrived at the conclusion that one measurement is enough for the evaluation of the third metacarpal bone (McIII) and the proximal phalanx (P1) in DXA analysis.

3. We concluded that the mineral density of the third metacarpal bone is higher than that of the proximal phalanx. According to our research results, it is only the sex of the animal that has a significant influence on BMD and BMC values ($P>0.005$).

4. In the comparative analysis of the third metacarpal bone, whole bone BMD and medial cortex BMD proved to be the most significant indicators of bone strength ($P<0.001$, $r=0.72$; and $P<0.001$, $r=0.68$, respectively). We also showed that in the case of the third metacarpal bone, bending strength correlates with bone diameter ($P<0.001$, $r=0.7$). The elastic modulus also correlates with McIII bone diameter ($P<0.001$, $r=0.67$), and we also found that it weakly correlates with dorsal cortex width ($P=0.004$, $r=0.55$), following a general regression pattern similar to that of breaking strength.

5. In the case of the proximal phalanx, the lowest BMD value was measured in the trabecular ROI. There is a significant positive linear correlation between the BMD value of the trabecular region and breaking force.
Publications on the results of the present research

*Peer reviewed publications published in academic journals with impact factors*


*Conference presentations*


Tóth P., Bodó G.: A csontsűrűség vizsgálata kettős energiájú röntgenfoton abszorpciometria (DEXA) módszerrel a ló metacarpalis csontjain 2008. január MTA Állatorvos-Tudományi Bizottsága, Akadémiai beszámoló (oral presentation, abstract)


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