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Comparison of the strength parameters of the bilateral third metacarpal bones in horses by pQCT

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Summary

The third metacarpal (MC3) bone, along with the proximal phalanx, is one of the bones that are most prone to injury in sport horses. To date, no detailed analysis has been conducted that would compare the strength parameters of bilateral MC3 bones taking into account differences depending on the site of measurement. The aim of the study was to compare strength parameters between the left and right MC3 bones in horses at 10%, 20%, 30%, 40%, 50%, 60%, 70% and 80% of the bone length using peripheral quantitative computed tomography (pQCT). The research material comprised isolated bilateral MC3 bones from 21 horses (age range: 3-27 years). The structure of these bones was measured using high-resolution pQCT. The following bone parameters were determined: polar strength strain index, strength strain index X and strength strain index Y. The computed tomographic analysis of the MC3 bones was carried out at sections from 10% to 80% (every 10%) of the bone length. The statistical analysis showed that in most cases the strength parameters calculated using pQCT were significantly higher for the right MC3 bones at 10%, 20% and 50% of the bone length, i.e. at the proximal metaphysis and at the proximal diaphysis. However, strength parameters measured at 60% and 80% of the diaphyseal length, i.e. at the distal diaphysis, were significantly higher for the left MC3 bones. Further studies of the MC3 bones parameters should focus on the vicinity of the proximal metaphysis and at the proximal diaphysis.

Keywords: horse, third metacarpal bone, bilateral bones, computed tomography

Humans present one-sided dominance in both upper and lower limbs (27). This manifests as a clear dominance of one limb over the other in terms of precision, motor coordination and use (4). People are able to subjectively determine their dominant upper and lower limb. In animals, however, limb lateralisation can only be assessed using objective methods. Lateralisation, i.e. brain laterality, is a broader notion referring not only to the dominance of the right or left limb but also to the dominance of other organs, especially sensory ones, on a particular side of the body (4, 30, 33). Thus, there are two types of laterality. One is motor laterality (limb preference) and the other is sensory laterality, i.e. the preferential use of sensory organs on one side of the body (31). In view of the relationship between people and horses in sports, leisure and other types of activities, studies on laterality in horses are particularly important. Laterality may have an impact on the functioning and sport performance of horses and should therefore be taken into account in their training, so that it is as effective and safe as possible for both people and horses (15, 35). While there have been studies on motor laterality in horses, their results are inconclusive. A study on 2095 Thoroughbred race horses showed that there were both left-limb biased and right-limb biased horses among the animals studied, with a higher number of left-lateralised horses. It was also observed that motor bias is stronger in horses older than two years

(22). However, the results of a study by Williams and Norris on a population of Thoroughbreds and Arabians showed that 90% of the horses were right-biased (37). Similar conclusions were reached by Lucidi et al., who found that most of the two-year-old horses studied were right-limb biased (20). Other researchers have found that there is no correlation between motor laterality and sensory laterality in horses (1, 22). In turn, a study on Shetland ponies found that there was no correlation between age and sex and the preferential use of sensory organs on a particular side of the body (31). At the same time, the study found preference for the use of sensory organs on the left side of the body in the horses studied, especially those used for riding (31). Motor laterality in horses is mainly assessed based on the movement of thoracic limbs, especially in the gallop (2).

The results of studies on motor laterality in horses are inconclusive and often conflicting, which may suggest that there is no objective method of assessing limb laterality in this species (20). At the same time, researchers agree that the issue of motor laterality is particularly important in these animals, as it has implications for the athletic performance of horses, understanding their predispositions as well as for horse welfare and human safety (24, 31, 38).

While it is normal for there to be moderate musculoskeletal asymmetry in mammals (18), such asymmetry is a particularly common phenomenon in horses, which is most likely linked to motor laterality (18, 32). The relatively common musculoskeletal asymmetries in horses pose a significant problem, as they have an impact on the animals' locomotor performance and may result in lameness (5, 21). A study on 43 Thoroughbreds showed that in 78% of the horses, the right third metacarpal (MC3) bone was significantly longer than the left MC3 bone (36). Another study, conducted on a population of Warmblood horses, found a correlation between motor laterality and significant hoof unevenness in 24% of the animals (16).

Human studies carried out to determine the level of bone mineralization focus either on the bones on the dominant side or those on the opposite side, without analysing differences between the right and left bones. Such an approach is determined by economic factors (17).

Therefore, it is not clear whether and to what extent motor laterality has an impact on the strength parameters of the bones of the autopodium, which is the most injury-prone limb section in horses. Thus, the aim of this study was to compare the strength parameters of bilateral MC3 bones in horses using peripheral quantitative computed tomography (pQCT). The analysis aimed to determine whether there is asymmetry in terms of the microstructure and strength between the right and left MC3 bone in a population of non-ridden and non-athletic horses. The study also aimed to determine whether it is possible to assess motor laterality in horses based on geometrical differences between the right and left MC3 bone. The mineralization level is not the only factor affecting bone durability as the bone trabeculae architecture is also very important. Quantitative Computed Tomography (CT) allowed for us to study the differences of geometry of the examined bones, providing important information regarding the bilateral MC3 bone microarchitecture.

Material and methods

The research material comprised bilateral MC3 bones from Polish Coldblood horses. The animals (n = 21) came from private farms and were slaughtered for reasons unrelated to any musculoskeletal disease. The mean body weight of the animals was 520 ± 75 kg. The horses were between 3 and 27 years old. The study group comprised Polish Coldblood horses, which had never been used as saddle animals. The study population comprised Polish Coldblood horses. The horses' history was known to us. All the horses worked as carriage-pulling animals. However, there was no possibility to determine the intensity of the work. What is known is that all the horses studied worked pulling carriages for tourists in urban and suburban recreational areas. These were horses performing intense work in the spring-summer season; in the autumn-winter season the work was less intense. Although the precise intensity of work for each individual horse was unknown, they were all carriage horses in the same region and likely had similar forces acting on their MC3. For the purposes of the measurements reported here, comparisons between left and right limbs within the same horse helps to mitigate the potential bias introduced by variation in work between horses. Moreover, all these horses came from one region (around Wrocław, Poland), so it can be concluded that the environmental factor is uniform. The horses worked on flat, not mountainous terrain, which resulted in similar forces affecting the bones of all analyzed animals. The animals were slaughtered in early autumn, i.e. after the tourist season.

The bones were stripped of soft tissue, leaving the periosteum, and then tightly packed into plastic bags, labelled and stored at –22°C for a maximum period of 5 months. The length of the MC3 bones analysed was measured using a digital calliper from the proximal end to the distal articular surface, excluding the medial crest (up to the base of the crest on the medial side). During the CT analysis, the crest of the MC3 bone was positioned vertically and the dorsal surface was directed upwards (Fig. 1). In Figure 1 shows the places where CT measurements are made, respectively, on the left side – at the level of 10% of the MCIII bone length, while in the middle and on the right side – at the level of 40% of the bone length from its proximal end. In the bottom row we can see the bone cross-sections at the level of 10% (on the left side) and at the level of 40% (on the right), respectively.

The analysis of the densitometric and geometric parameters of the bones was carried out using a single-row Stratec XCT 2000L peripheral CT scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany). The measurements were obtained over a period of 5 months. During that time, the scanner underwent quality checks in accordance with the manufacturer's instructions. The measurement error calcu-

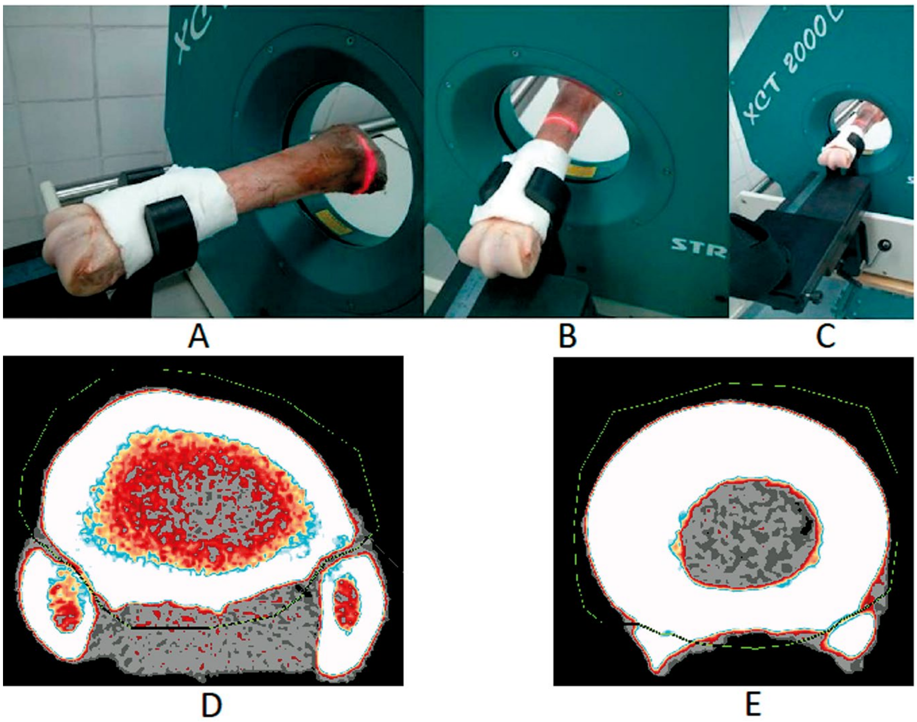

Fig. 1. Position of the MC3 bone during CT analysis; the places where CT measurements are made and the bone cross-sections at the level of 10% and at the level of 40%
Explanations: A – the place where CT measurements are made at the level of 10% of the MCIII bone length; B, C – the places where CT measurements are made at the level of 40% of the MCIII bone length from its proximal end; D – the bone cross-sections at the level of 10%; E – the bone cross-sections at the level of 40%

lated using a phantom model for the period concerned was 0.22% for the density of the entire bone section, 0.29% for the trabecular density, and 0.24% for the compact bone density. The following parameters were determined: the strength strain index X (SSI_X) mm³ – a parameter of the fracture resistance of bones in the transverse plane (forward-backward), strength strain index Y (SSI_Y) mm³ – a parameter of the fracture resistance of bones in the transverse (side-side) plane and the polar strength strain index (SSI_P) mm³ – a parameter of the expected fracture resistance of bones to torsional fractures.

The computed tomographic analysis of bilateral MC3 bones was carried out at 10%, 20%, 30%, 40%, 50%, 60%, 70% and 80% of the bone length. The measurements were started from proximal end of the MCIII (the bases of the metacarpal bone). The measurements were performed using a 0.5 mm³ voxel and a scanning speed of 30 mm/s. The algorithm threshold used to differentiate the cortical bone from the trabecular bone was set at 480 mg/cm³ (13). Threshold 480 includes the cortical and subcortical bone, which is an essential component of bone strength. (Threshold 480 for SSI calculations is recommended by the manufacturer, primarily for measurements in animals: Stratec XCT 2000L manual. Pforzheim. Stratec Medizintechnik; 2011) (13).

Statistical analysis. The parameter values obtained were analysed statistically. The normality of the distribution of the variables was tested using the Shapiro-Wilk test. As the parameters were not distributed normally, the non-parametric paired Wilcoxon signed-rank was used to compare data between left and right MC3. All calculations were performed using Statistica 10 PL software (StatSoft, Inc. Tulsa, USA). The significance level was set at $p < 0.05$.

Results and discussion

Differences in the values of the parameters measured along the long axis for bilateral MC3 bones are shown in the table and box plots. The whiskers of a box represent the minimum and maximum values of a given parameter (Fig. 2).

Table 1 shows a comparison of parameters between the left and right MC3 bone. Statistically significant differences at particular lengths of the bones ($p < 0.05$) are shown as *. The statistical analysis showed that, in most cases, the strength parameters calculated using pQCT were significantly higher for the right MC3 bone. The parameters were significantly higher in the right bones at 10%, 20% and 50% of the bone length, i.e. at the proximal metaphysis and at the proximal diaphysis. However, strength parameters measured at 60% of the diaphyseal length, i.e. at the distal diaphysis, were significantly higher for the left MC3 bone.

The subjective assessment of limb laterality is not always consistent with its objective presence (4). Considering the occurrence of this phenomenon in people, it is most valid to assess limb laterality in animals based on an objective assessment of the strength parameters for the autopodial bones of the limb. While strength parameters are most relevant with regard to the prediction of bone tissue resistance to mechanical forces, metric asymmetry between left and right bones should also be taken into account. Such asymmetry can be observed in different animal species but it may be of particular significance in horses, as they are intensively used to perform various physical activities. This parameter has implications for e.g. the initial risk assessment for some musculoskeletal disorders in horses. One such disorder is the navicular syndrome, which manifests as a group of pathological changes occurring bilaterally, i.e. in both thoracic limbs. While those pathological changes affect both the right and the left limb, one limb is affected earlier (8, 19, 26). A similar situation might occur in the case of other bones which, when overstrained, develop microcracks or even fatigue damage. One of such bones is the MC3 bone, which is the main pillar and component of the autopodium. There have been numerous studies on injuries to this bone in athletic or race horses. However, those studies do not always acknowledge the fact that a training regime and training methods may result in a better development

Tab. 1. Comparison of parameters between the left (L) and right (R) MC3 bone. Statistically significant differences at particular lengths of the bones ($p < 0.05$) are shown as *

Prox–dist	Differences between the right and left MCIII bone	Descriptive statistics for MC III	
	paired Wilcoxon test, p-value	Rights (IQR)	Left (IQR)
10% prox SSI polar	0.027*	6904.5* (1769.3)	6616.4 (2100.4)
10% prox SSI X	0.099	3449.6 (1054.1)	3386.1 (1209.3)
10% prox SSI Y	0.0496*	4811.7* (833.9)	4594.2 (1123.7)
20% prox SSI polar	0.13	6649.4 (1893.3)	6494.1 (2332.6)
20% prox SSI X	0.036*	3033.3* (1112.3)	2972.0 (986.1)
20% prox-SSI Y	0.17	4646.5 (1034.5)	4600.8 (1203.2)
30% prox SSI polar	0.69	6371.9 (1796.5)	6461.7 (1941.0)
30% prox SSI X	0.69	3031.8 (795.8)	3036.4 (824.6)
30% prox-SSI Y	0.18	4547.1 (1356.4)	4481.2 (1157.8)
40% prox SSI polar	0.64	6927.2 (2066.0)	6638.8 (1991.9)
40% prox SSI X	0.97	3032.3 (901.9)	3083.6 (902.2)
40% prox-SSI Y	0.068	4634.9 (1377.8)	4488.3 (1180.7)
50% dist SSI polar	0.092	6838.5 (2297.9)	6699.1 (2169.9)
50% dist SSI X	0.22	3160.5 (1090.6)	3200.8 (1137.9)
50% dist SSI Y	0.019*	4613.4* (1596.4)	4572.0 (1588.8)
60% dist SSI polar	0.16	6875.8 (1953.7)	6856.2 (2653.5)
60% dist SSI X	0.90	3111.4 (1048.4)	3154.4 (1296.7)
60% dist SSI Y	0.025*	4631.1 (1240.3)	4705.4* (1713.3)
70% dist SSI polar	0.11	6902.9 (1769.4)	6914.6 (2379.0)
70% dist SSI X	0.99	3074.9 (979.6)	3068.0 (1205.5)
70% dist SSI Y	0.11	4873.0 (1333.8)	5010.4 (1705.2)
80% dist SSI polar	0.030*	6635.2* (2335.6)	6474.2 (2748.3)
80% dist SSI X	0.030*	2658.1 (1039.2)	2673.5* (953.2)
80% dist SSI Y	0.13	5174.9 (1648.8)	5192.2 (2121.5)

Explanations: SSI – strength strain index (mm^3); prox – proximal; dist – distal; IQR – interquartile range

of one side of the body in such horses. In the case of horses used for show jumping or eventing, the objectivity of research may also be influenced by such factors as the individual habits of riders. Consequently, there can be differences as regards bone tissue remodeling and adaptation processes between the right and left limb resulting from training, where one side of the body is subjected to higher loading than the other. Therefore, to avoid this problem in our study, the choice of the horses was not random. The animals studied were farm horses, which had not been used in sports or racing. Moreover, the type of draft work they performed was not associated with the loading of only one side of their body, as is the case in e.g. race horses (9, 11, 12, 14). This allowed us to view the problem of asymmetry from the biological perspective, without interference from such factors as differences in training. Each horse was from a different farm and did not have the same number of hours in harness. These horses was not sent to a slaughterhouse of the same age. Therefore, we are aware that, despite our efforts, there are some limitations which, for independent reasons, cannot be avoided in a scientific experiment. As the authors, we found that despite the unknown in details of the history of the studied horses, it is worth showing the features of their MC III bones. The horses were of the same type and worked very similarly, although of course with different intensity. The same type of work was also associated with the specificity of the tourist season, because these horses worked the hardest during the summer months. Meanwhile, the late autumn, winter and early spring was for them a time of light work, or even a time of rest in the paddocks. Due to these factors, their MCA III bones can be considered as a homogeneous material for analysis. The great advantage of the study is the fact, that it was not done on a few, but on twenty one pairs (left and right), which gives credibility to the conclusions drawn. As for anatomical research, this number is large and to some extent reduces the hypothetical errors that could arise from the above-presented limitations.

Studies have found that horses use their left thoracic limb as their leading limb in stressful situations, which according to some researchers indicates the dominant role of the right hemisphere in the control of the behavior of horses under stress (31).

One of the studies investigating left-right differences in limb bones in horses has confirmed the presence of femoral asymmetry (28). Post-mortem measurements of left and right femurs from 11 horses showed statistically significant differences with regard to five parameters, which were higher in left femurs. According to the authors of the study, this may be due to the greater loading of the left side of the body resulting from the curvature of race tracks (28). In turn, measurements performed on the X-rays of 40 clinically healthy Thoroughbred horses confirmed the dominance of the right thoracic limb (6).

One study on bone mineral density (BMD) and bone mineral content (BMC) in bilateral MC3 bones and proximal phalanges was conducted by Tóth et al. (34). The bones were examined from four directions using dual-energy X-ray absorptiometry (DXA). The authors of the study found no statistically significant differences in bone mineral density and bone mineral content between the left and right proximal phalanx (34). Similarly, a study by Dzierzęcka and Charuta (9) found that there were no statistically significant

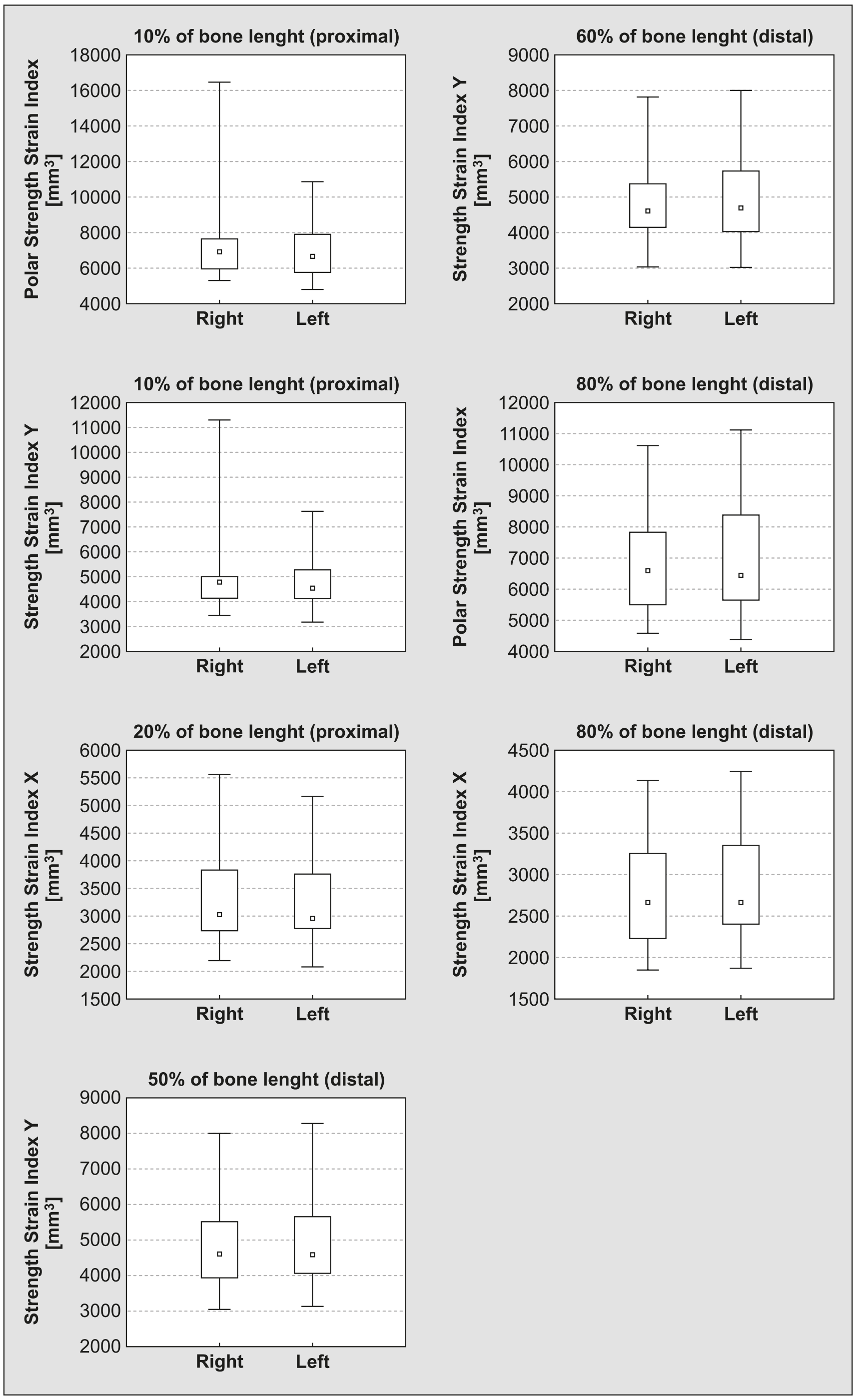

Fig. 2. Compare the Strength Strain Indexes (SSI) of the right and left MCIII bones at individual locations

Explanations: size of the box represents variability occurring in 50% of the animals (25-75% range of bone length – proximal), whiskers represent Min. and Max.

differences in bone mineral density and bone mineral content between the right and left forelimb proximal phalanges analysed.

Another study compared densitometric and geometric parameters between the left and right forelimb proximal phalanges of horses at 15%, 50% and 85% of the bone length using peripheral quantitative computed tomography (pQCT). The statistical analysis showed that there were no statistically significant differences between the right and left proximal phalanges in any of the parameters measured at 50% and 85% of the bone length. In turn, the values of the parameters measured at 15% of the bone length, i.e. at the proximal metaphysis, were statistically significantly higher in the left forelimb proximal phalanx, which indicates that the strength of the left proximal phalanx in the proximal metaphyseal region is higher (10). Other researchers have found that the loading of the equine radius and tibia by exercise-induced forces results in higher values of the strength strain index (25).

Our study showed that the values of the parameters analysed were significantly higher in the right bones at: 10% (SSI_polar, SSI_Y), 20% (SSI_X) and 50% (SSI_Y), i.e. at the proximal metaphysis and at the proximal diaphysis. Given that the bone adapts to the loading, it turns out that the proximal end of the MC3 bone is stronger, hence more resistant to fractures, in the right bones. However, the strength parameters measured at 60% (SSI_Y) and 80% (SSI_X) of the diaphyseal length, i.e. at the distal diaphysis, were significantly higher for the left bones. This may indicate that the MC3 bones are not only loaded axially (up-down), but that during the animal's movement the directions of the exerted forces are more complex and varied depending on the bone section. The forces exerted on the MC3 bones in horses pertain to the X plane, which in a standing horse denotes the front-back movement. Furthermore, there are forces exerted in the Y plane, which denotes transverse movement, and forces associated with torsional movements.

The higher the SSI value, the higher the bone's resistance to fractures in a given section. However, each "type" of SSI indicates the resistance of the bone in a different plane/direction. Thus, SSI_polar is a parameter describing the expected bone resistance to torsion fractures, i.e. those with a rotation (rotation of the distal end of the bone relative to its proximal end); SSI_X is a parameter describing the expected bone resistance to fractures in the X plane, i.e. in the transverse (front–back) plane; SSI_Y is a parameter describing the expected bone resistance to fractures in the Y plane, i.e. in the transverse (side – side) plane.

Our results are in line with the observations presented in the fundamental work by Currey (3), who found that the yield strain is determined by the bending strength. However, in our study the yield strain was not the same for all the sections of the right and left MC3 bones, as demonstrated by the statistically significant differences in the SSI values for the bone sections described above. Furthermore, as shown by Mouloodi et al. (23) who used an artificial neural network in their study, bones are a material which is difficult to properly comprehend. This is because biological structures, including bones, are characterised by individual variability associated particularly with the distribution and density of trabeculae.

Studies on the MC3 bone have also been conducted by Piotrowski et al. (29). These authors demonstrated that the middle third of the bone is characterised by the largest cross-sectional area, which then gets smaller at both ends. At the same time, they found that the distal end of the principal axes (which are usually within 15 degrees of the anatomical axis) is internally rotated in relation to their proximal end.

Interestingly, the bending stiffness in the antero-posterior plane at midshafts, i.e. the plane which we described as X, was about 2/3 of the stiffness in the medio-lateral plane, i.e. the Y plane in our study. These observations are consistent with our findings. At 50% of the bone length, both in the right and left MC3 bones, SSI Y was higher than SSI_X. The SSI_X value constituted about 70% of the SSI_Y value (Tab. 1). Additionally, we demonstrated that SSI_Y was significantly higher in the right MC3 bone.

As reported by Piotrowski et al. (29), the torsional stiffness is highest proximally. However, these authors did not determine differences between bilateral MC3 bones. Our study showed that SSI_Y, describing the expected bone resistance to fractures in the Y plane, i.e. in the transverse side-side plane, as well as SSI_polar, describing the expected bone resistance to torsion fractures, were significantly higher in the right MC3 bone at 10% of the bone length from the proximal end. It is interesting to note that at 60% and 80% of the bone length, i.e. at the distal end, the SSI Y value was significantly higher in the left MC3 bone. However, relative to SSI_polar, it was significantly higher at the distal end of the right bone. Thus, our observations show that, except for the distal diaphysis, where SSI_Y is significantly higher in the left MC3 bones, the SSI_X, SSI_Y and SSI_polar parameters are significantly higher in the right MC3 bones.

It has been found that mechanical loading during growth intensifies the normal increase in the bone diameter that occurs in long bone shafts (7). It is important to gain a deeper understanding of the phenomenon, known as the functional adaptation of bone, in order to determine the physiological threshold that cannot be exceeded when working with a young horse. As a result of excessive mechanical strain, the resorption of bone caused by microcracks may prevent optimum new bone formation. A change in the proportion between those two stages of bone regeneration results in negative remodeling, leading to fatigue cracks. Therefore, knowledge about the strength parameters of the MC3 bone is of key importance. A comparison between the findings from our study and literature data may provide a better insight into the strain behavior of equine bones. Our results confirm the observation that mechanisms that modulate MC3 bone density and microstructure can cause significant changes in the bone at a certain level, leaving the remaining bone length unaffected. MC3 bones undergo the most substantial changes in microstructure in the vicinity of proximal metaphysis and proximal diaphysis and were significantly higher for the right MC3 bones. However, strength parameters measured at the distal diaphysis were significantly higher for the left MC3 bones. The small sample size was a limitation of the study. The reason for this was the difficulty of gathering homogeneous research material – Polish Coldblood horses came from the same region, worked on flat, not mountainous terrain. The authors are cognizant of the potential impact of those limitations on their findings. There was the absence of mechanical test to failure data in our work. It was difficult to determine, and this is also a limitation of our research. Our observations suggest that this type of research should be continued within a larger population.

There were significant differences in strength parameters between the left and right MC3 bone in the Coldblood horses studied, depending on the site of measurement. In most cases, the parameters were significantly higher in the right MC3 bone. The higher values of strength parameters in the right MC3 bone at the level of shaft where the most intense bone remodeling occurs may indicate the dominance of the right thoracic limb in this species.

Further studies on the parameters of the MC3 bone in horses should focus on the proximal and distal metaphyseal region. This could help draw practical conclusions with regard to the fracture risk assessment in these bones and confirm the dominance of a given limb in this species.

Ethics approval: bones of animals were obtained from slaughterhouses. This study did not require official or institutional ethical approval.

Prior publication: Data have not been published previously.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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