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Bone adaptation to exercise in Arab horses practicing enduro equestrian 120-160km – quantitative and functional analysis

[*Adaptação óssea ao exercício em cavalos árabes praticantes de enduro equestre de 120-160 km - análise quantitativa e funcional*]

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ABSTRACT

Seeking the improvement of equine sports medicine, the present work aimed to determine quantitative data from different bone structures. Therefore, the objective of this work was to quantify the thickness of the cortex in a comparative way to determine the bone densitometry and also the bone density of the bone trabeculae of the spongy substance of the radius, calcaneus, and metacarpal III bones of horses practicing equestrian enduro long-distance running tests. Six thoroughbred Arabian horses and long-distance competitors (120 and 160km) were evaluated for metacarpal III, calcaneus, and accessory carpal bone. Long bones have a different micro functional structure than short bones. The sites that received and dissipated energy varied in distal, proximal epiphysis, and diaphysis according to the bone. The calcaneus and accessory carpal bone had a lower transverse thickness of the compact substance and greater density of the same, this increase was related to the improvement of local resistance. The density of the spongy substance varied with different bones. Thus, it was concluded that stress directly influenced the quantity and quality of the compact and spongy substance.

Keywords: comparative, equine sports medicine, densitometry

RESUMO

O presente trabalho teve como objetivo buscar o aperfeiçoamento da medicina equina esportiva, mediante a determinação de dados quantitativos, de diferentes estruturas ósseas. Para tanto, quantificou-se, de forma comparativa, a espessura do córtex, determinou-se a densitometria óssea e ainda a densidade óssea das trabéculas ósseas da substância esponjosa dos ossos rádio, calcâneo e metacárpico III de equinos praticantes de prova de longa distância de enduro equestre. Foram avaliados seis equinos, da raça Puro Sangue Árabe, competidores de longa distância (120 e 160km). Ocorreu adaptação dos ossos rádio, metacárpico III, calcâneo e osso acessório do carpo, por influência do esforço ao longo da vida atlética de equinos da raça Árabe que percorreram longas distâncias em provas de enduro equestre. Os ossos longos apresentaram estrutura microfuncional diferente dos ossos curtos. Os locais que receberam e dissiparam energia variaram em epífise distal, proximal e diáfise, de acordo com o osso. O calcâneo e o osso acessório do carpo apresentaram menor espessura transversal de substância compacta e maior densidade dela; esse aumento foi relacionado à melhora da resistência local. A densidade da substância esponjosa variou de acordo com os diferentes ossos. Desse modo, concluiu-se que o estresse influenciou diretamente sobre a quantidade e a qualidade da substância compacta e esponjosa.

Palavras-chave: comparativa, medicina equina esportiva, densitometria

INTRODUCTION

Equestrian Enduro is considered the second largest equestrian modality practiced in the world and is considered a modality with high speeds for a long period (Nagy *et al.*, 2012). The horse has a musculoskeletal structure adapted to maximize locomotion efficiency. This was achieved by decreasing limb weight by reducing distal muscle mass and the number of bones (Firth *et al.*, 2011).

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Bone mineral density (BMD) is the result of a dynamic process of formation and resorption of bone tissue called remodeling. When the animal is in the growth phase, formation exceeds resorption and skeletal mass increases. In general, it is known that in horses, exercise leads to an increase in BMD after months of training (Firth *et al.*, 2011).

Stress is necessary for the maintenance of bone mass and to maximize load capacity during exercise, however, the bone's ability to adapt to stress decreases with advancing age and may take months to become complete (Marlin and Nankervis, 2008).

Among the various techniques developed for bone evaluation, there is optical densitometry in the radiographic image, which can be used for sequential analysis of bone mass in animals undergoing field training, at a lower cost than other methodologies, having, as a preponderant factor, the precision (Vulcano *et al.*, 2003), This is a viable way to monitor bone adaptation to exercise.

In the search for the optimization and improvement of equine sports medicine, the present work has as a practical aim to establish radiographic parameters, through the determination of quantitative data of different bone structures. Therefore, the objective of this work was to quantify, in a comparative way, the thickness of the cortex, to determine the bone densitometry and the bone density of the bone trabeculae of the spongy substance of the radius, calcaneus, and metacarpal III bones of horses practicing equestrian enduro long-distance running tests.

MATERIAL AND METHODS

Six horses were evaluated, two castrated males and three females, of the Purebred Arabian breed, from the same training center, submitted to the same handling, work regime, and feeding. The age of these animals ranged from eight to ten years, and all had already completed at least one long-distance competition (120 and 160km) of equestrian endurance, endorsed by the International Equestrian Federation (FEI) throughout 2016.

Only animals that did not claudicate and did not deviate from uprightness were submitted to the experiment. To verify their health status, a specific clinical examination of the locomotor system was performed, as described by Keegan *et al.* (2010).

X-rays of the right and left thoracic and pelvic limbs were taken using a digital radiography system (Xamaru ® DR), with a focus-film distance of 60 cm, measured by the focus of the emitter projector (Orange®). Images were captured in the dorsal-palmar and latero-medial projections of the third metacarpal bone (proximal and distal epiphyses and diaphysis) and latero-medial projections of the radius (distal epiphysis and diaphysis) accessory carpus and calcaneus (base and body) bones (Table 1). For comparison, the bones were divided into two groups according to anatomy, long bones, including the radius and third metacarpal bone, and short bones, represented by the calcaneal bone and accessory carpal bone. The images obtained were captured and stored in DICOM format for later analysis.

Table 1. The radiographic technique is used to capture images according to the different bones evaluated

Bone	Technique	
	dorsal-palmar	Latero-medial
Metacarpal III	74 kv / 3.20 mAs	74 kv / 2.50 mAs
Radio	-	76 kv / 4.00 mAs
accessory of the carpus	-	76 kv / 4.00 mAs
calcaneus	-	74 kv / 3.20 mAs

The images captured in the latero-medial projections were processed in the ProgRes program CapturePro (Version 2.5). To standardize the images, the "*measure*" tool in mm was used to standardize the width of the penetrometer (made of a specific aluminum alloy, with 25 steps, measuring 5x18mm/each). This standardization was necessary so that the film focus distance did not influence the reading of the data. From this, measurements of the compact substance of the different bones were performed.

To perform the radiographic optical densitometry examination, a penetrometer made of a specific aluminum alloy was used, with 25 steps, measuring 5x18mm (frontal area) each. Numbered from 1 to 25, with the first step being 1mm thick, then add 1mm for each step, up to the twenty-fifth. The penetrometer was positioned medially on the dorso-palmar images and dorsally on the lateral-medial images.

The captured images were processed using Adobe Photoshop CS6 (version 6.0, Adobe Systems Inc., San Jose, CA, USA). An area of interest for the analysis was marked on the image, in a standardized size of 20x10 pixels. The histogram tool was used, which provided the mean optical density in pixels, of the selected region of the bone and the penetrometer step with the mean closest to the analyzed region (Figure 1). Both the averages of the points analyzed and the corresponding step in the penetrometer were compared. The selections of the bone regions were made from the latero-medial images, where 6 different points of the compact substance of the diaphysis of the radius bone and metacarpal bone III, 4 of the calcaneal bone, and 3 points of the accessory carpal bone were selected for analysis. Thus, 72 points of the compact substance of the radius and metacarpal bone, 48 of the calcaneal bone and 36 of the accessory carpal bone were analyzed.

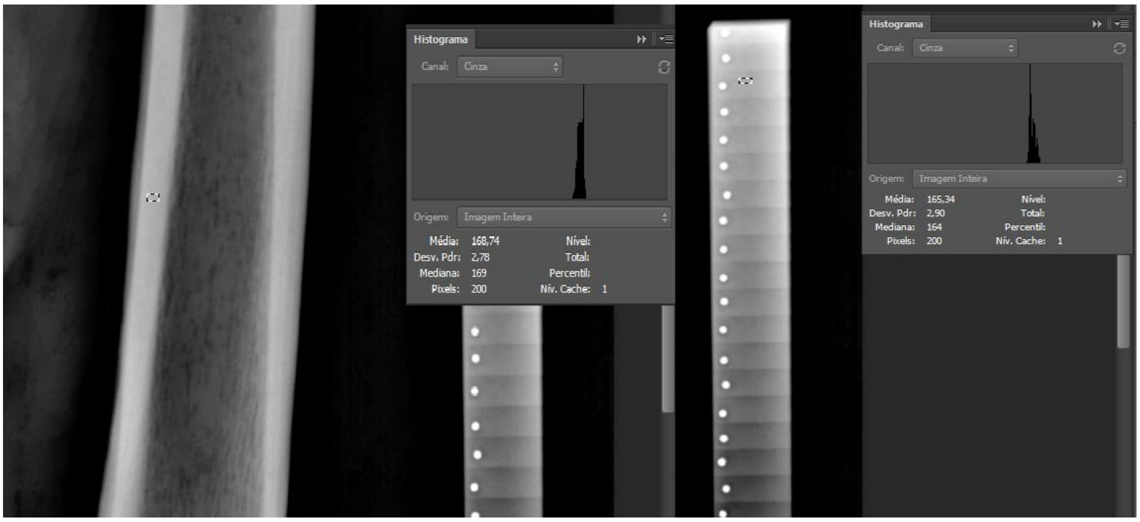

Figure 1. Digitalized radiographic image of the diaphysis of the radius bone in Adobe Photoshop, showing the area of the diaphysis and the penetrometer selected for measurement and the histogram graphics providing the average radiographic optical density of the analyzed region.

The captured images were processed using Adobe Photoshop CS6 (version 6.0, Adobe Systems Inc., San Jose, CA, USA). Using the latero-medial projections for the radius, calcaneus, and accessory bones and the dorso-palmar projections for the metacarpal bone III, sections were made that represented the epiphyseal region of the long bones and the spongy substance of the short bones. The images obtained were processed using the Image Pro Plus program. Employing the tool "*Perform Segmentation*", which differs from the gray tones of the image, and with the histogram tool it was possible to determine the averages of the trabeculae of the analyzed section.

To evaluate the measurements of the animals, a tape suitable for calculating weight and height was used. Weight was assessed from the thoracic perimeter in the widest region of the thorax with the measuring tape positioned between the spinous processes arranged between the fifth and sixth thoracic vertebrae, passing through the intercostal space of the fifth and sixth ribs, caudally to the olecranon of the ulnar bone. The tape used estimates weight based on the thoracic perimeter using the formula: $P= T^3 x80$, where P= Body weight in Kg and T= thoracic perimeter in meters. Height was measured from the highest point of the interscapular region, located in the space defined by the spinous processes arranged between the fifth and sixth thoracic vertebrae, to the ground.

Descriptive analysis was applied to obtain the mean and standard deviation. Normality was tested using the Kolmogorov-Smirnov test. Before each parameter was evaluated, the one-way test, ANOVA, with Dunn's post-test was applied. The Wilcoxon test evaluated a certain parameter for each evaluated bone. The *r* values found from the application of Pearson's correlation test were obtained by analyzing the height/weight/density/thickness of compact and spongy substances from different bones. Statistics were performed using the Sigma-Stat 3.5 program and adopting $p \leq 0.05$ as significant.

RESULTS

The long bones had a thickness of the compact substance 49.09% greater than the evaluated short bones (Figure 2). Comparing the thickness of the compact substance of the epiphyses and diaphysis separately, an increase of 11.74% in the average of the distal epiphyses, about the proximal one, of the radius bone and a decrease of 3.5% in the metacarpal bone III can be seen (Figure 3).

The short bones presented an increase of 41.55% in the compact substance density, compared to the long bones. No pattern was observed between short and long bones in the evaluation of spongy substance density, with the radius bone having the highest average and the metacarpal bone III the lowest.

Densitometry showed a decrease of 60.89% in the average density of the compact substance of the distal epiphysis, compared to the proximal one of the radius bones. In metacarpal bone III, the diaphysis obtained the highest average and the distal epiphysis had an average increase of 38.10% compared to the proximal one (Figure 3).

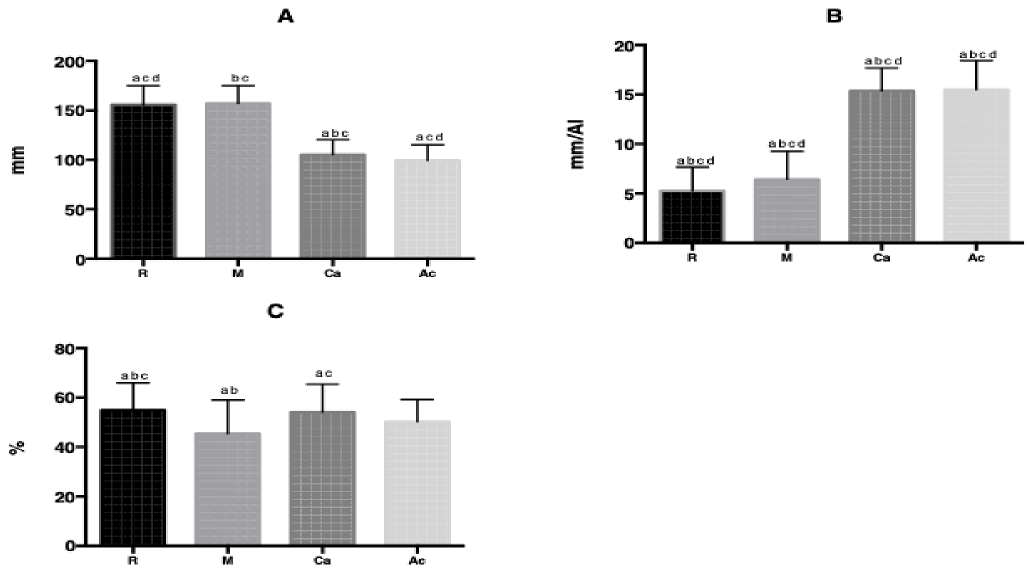

Figure 2. Representative graphs (A) thickness (mm) of the compact substance; (B) Density (mm/Al); (C) Density (%) of radius (R), third metacarpal III (M), calcaneus (Ca) and accessory carpal (Ac) bones. Equal letters express the statistical difference between columns ($p \leq 0.05$).

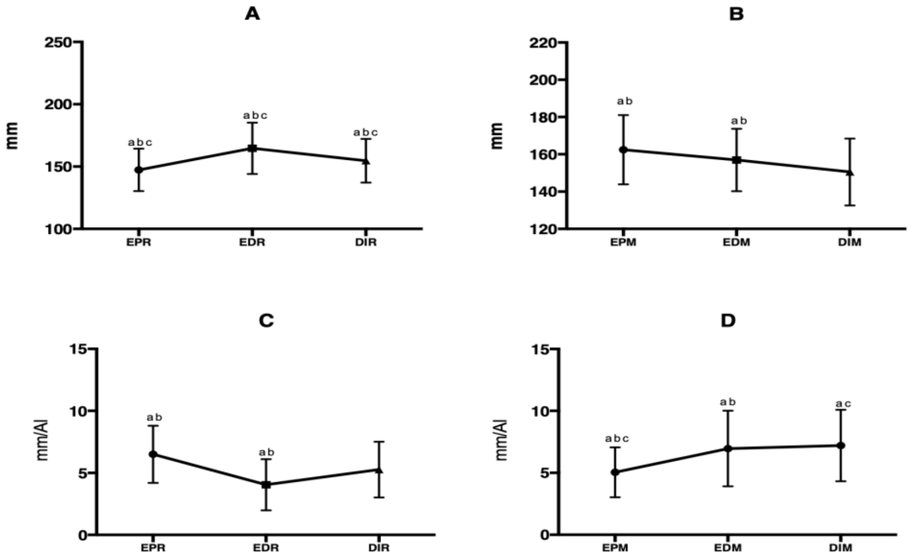

Figure 3. Representative graphs (A) thickness (mm) of the different regions of the radius bone – proximal epiphysis of the radius bone (RPE); distal epiphysis of the radius bone (DRE); diaphysis of the radius bone (DIR). (B) Thickness (mm) of the different regions of the III metacarpal bone – proximal epiphysis of the III metacarpal bone (EPM); distal epiphysis of the third metacarpal bone (EDM); diaphysis of the third metacarpal bone (IMD). (C) Densitometry (mm/Al) of the different regions of the radius bone – proximal epiphysis of the radius bone (RPE); distal epiphysis of the radius bone (DRE); diaphysis of the radius bone (DIR). (D) Densitometry (mm/Al) of the different regions of the III metacarpal bone – proximal epiphysis of the III metacarpal bone (EPM); distal epiphysis of the third metacarpal bone (EDM); diaphysis of the third metacarpal bone (IMD). Equal letters express the statistical difference between columns ($p \leq 0.05$).

When Pearson's correlation test was applied, different r values were obtained, not revealing an evidenced pattern between weight, height, and thickness regarding the densities of the compact and spongy substances (Table 2).

Table 2. Correlation of the Pearson(r) coefficient between weight, height, and the compact (cs) and spongy substance (ss) of the radius, metacarpal III, calcaneus, and accessory carpal bones

		Thickness				Density			
		Height		Weight		Height		Weight	
		r	P	r	P	r	P	r	P
cs	Radio	-0.29	0.58	-0.60	0.21	-	-	-	-
	Pastern	-0.46	0.36	0.22	0.67	-	-	-	-
	Calcaneus	-0.42	0.41	-0.37	0.47	-	-	-	-
	Accessory	-0.93	0.24	-0.84	0.36	-	-	-	-
ss	Radio	-	-	-	-	0.34	0.51	0.74	0.09
	Pastern	-	-	-	-	-0.47	0.35	0.13	0.80
	Calcaneus	-	-	-	-	-0.41	0.42	0.29	0.58
	Accessory	-	-	-	-	0.48	0.34	-0.05	0.92

DISCUSSION

The choice of bones considered the positioning, morphology, and type of ossification, endochondral or epiphyseal, in addition to accessibility for imaging (Skedros *et al*., 2009; Firth *et al*. 2011). According to Skedros *et al*. (2009), the radius bone represented those long-curved bones, which were subject to torsion or some flexion, the third metacarpal bone represented long straight bones that suffer some torsion and the calcaneal bone was considered a short bone and underwent some flexion. Vulcano *et al.* (2003) efficiently used the accessory carpal bone to measure bone mineral density. The present study was based on these motivations, and used the metacarpal III and accessory carpal bones, which were chosen as the standard for long bones and short bones, respectively, for the application and interpretation of the statistical analysis due to their dimensions having a certain proportionality, therefore being objects of interest for the comparative establishment, in the or functional aspect of the bones for the animals of this study.

In general, it is already known that physical exercise leads to bone adaptations. (Firth *et al.*, 2011). Specifically, it was observed that in endurance horses, long bones had a greater thickness of their compact substance, when compared to short bones.

The differences found in the different regions of the distal and proximal epiphyses and diaphysis of the long bones corroborate the data obtained by Firth *et al.* (2011) when they verified an increase in bone strength in the proximal epiphysis of the third metacarpal bone in exercised Thoroughbred foals. Regional variations were verified in the morphology of osteons in the radius and metacarpal bones of horses and calcaneus of sheep, which could be explained by the difference in the form of energy absorption for each of the different bones used (Van Oers *et al.*, 2006; Skedros *et al*., 2009), still suggesting that the thickness of the compact substance varied between the different regions in the compact substance of long bones (Hiller *et al.*, 2003).

For Skedros *et al.* (2009), two main reasons modify and adapt the constituent microstructures of bones, the first reason would be that the compact substance has different mechanical properties, and therefore suffers constant micro-injuries, accumulating and exposing itself to excessive tension, compression and load and recurrent; The second is that throughout the load peak, distinct regional variations of bone matrix adaptation are active, promoting changes in the stressed area. Therefore, in endurance horses that covered 120-160km the thickness of the compact substance, in bones considered as long, the morphology of each of the analyzed bones acted actively in the variation of this parameter. It is also relevant that the variations in the thickness of the epiphyses of the radius bones and metacarpal bones III, were different, based on the regions that were subject to a greater initial load, and smaller dimension where there is energy dissipation, thus emphasizing both the functional as well as the architectural roles of bones.

Based on mechanical and physical stimuli, bones may undergo a certain degree of increase in mass and cross-sectional architecture (Firth *et al.*, 2011). Therefore, bone mineral density was understood as being the result of a dynamic process of formation and reabsorption of bone tissue (Firth *et al.*, 2011), from which there was a functional adaptation and, consequently, the participation of osteoclasts and osteoblasts of the already existing bone matrix. In the evaluated high-performance endurance horses, a greater density of the compact substance of the short bones and an even greater density of the smaller cross-sectional areas of the long bones were evidenced. For (Firth *et al.*, 2011) this process occurred throughout the entire life of the animal and is still an important form of renewal and maintenance of bone mass, as these characteristics of bone remodeling were controlled, among other things, primarily by microlesions. caused in regions subject to intense stress.

Boyde (2003) in a study with metacarpal bone III of racing horses and femur of humans concluded that the greatest change caused by exercise was the increase in the amount of bone deposited in the medullary space, thus acting as a form of compensation in the exercised group, compared with control. The size and quantity of osteons produce a variation in the regional structure, depending on whether the site receives tension or compression (Van Oers *et al.*, 2006). For Hiller *et al.* (2003), exercise inhibits the absorption of osteoclasts, forming a compensatory mechanism for the body to deposit more and lose less bone mass, found in endurance animals mainly in the regions of the spongy substance of the radius bone.

For horses that practice equestrian endurance, weight and height were not determining factors in the increase in density of the compact and spongy substance. For humans, the increase in body mass was associated with a higher density of vertebrae and ribs (Salamat *et al.*, 2013). Still, in humans, a correlation was observed between the increase in bone density and weight, but this was not only associated with weight but also with the percentage of lean mass, leading to the belief that the data obtained could be influenced by other factors such as level of training, muscle strength, hormonal regulation, and sex (Cadore *et al.*, 2005). Because of these authors, and still based on the data obtained from the application of the Pearson correlation test, in the endurance animals investigated it was not possible to affirm that the weight and height of the animals interfered with the data related to the densities of the compact and spongy substances.

CONCLUSION

The effort throughout the athletic life of Arabian horses that ran long distances in equestrian endurance events influenced the adaptation of the radius, metacarpal III, calcaneus, and accessory carpal bones. Long bones have a different micro-functional structure than short bones. For long bones, the transversal thickness of the compact substance was greater and the density smaller, according to the place that received tension or compression. Still, within this perspective, the sites that received and dissipated energy varied in the distal, proximal epiphysis, and diaphysis, according to the bone. The calcaneus and accessory carpal bone had a lower transverse thickness of the compact substance and greater density of the same, this increase was related to the improvement of local resistance. The density of the spongy substance varied with different bones. Thus, it was concluded that stress directly influenced the quantity and quality of the compact and spongy substance in the bones of horses practicing 120 and 160 km events, according to the biomechanical function and anatomy of the evaluated bones.

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