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Determination and padronization of normal values of bone mineral density (BMD) of the acessory carpus bone in young Thoroughbred horse using optical densitometry in radiographic image

Determinação e padronização dos valores normais da densidade mineral óssea (DMO) do carpo acessório de eqüinos em crescimento da raça puro sangue inglês (PSI) por meio da densitometria óptica em imagem radiográfica

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Abstract

The bone mineral density (BMD) of the accessory carpus bone was measured (in aluminum milimeters - mmAl) in 12 female and 12 male thoroughbred horses aging from 12 months old until the moment of complete closure of the radio distal epiphysis, using optical densitometry in radiographic images. Evaluation was made using a software specially developed for optical density measurement in X-ray films, containing the radiographic image of the accessory carpus bone, the region of soft parts adjacent to the accessory carpus and the degrees of an aluminum stepwedge, used to measure BMD value (average of the interest region established on the accessory carpus bone corresponding to the value on the stepwedge [in milimeters]). BMD values did not show differences between sexes (p=0.86). BMD value (in mmAl) was 3.109 + 0.056 x age (in months) at this age interval.

Key-words:
Horse.
Bone mineral density.
Accessory carpus.

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Introduction

Horses raised for sport activities, work and leisure are constantly submitted to physical conditioning since birth, requiring a permanent qualitative and quantitative evaluation of their development. For more accurate qualitative evaluation of this development, an evaluation of the bone constitution by mineralization or by the mineral density of the compact bone is needed.

Studies carried out in humans have shown a direct correlation between the incidence of fractures and the bone mineral content.[1] Previous studies have also suggested that the stress due to training leads to different values of bone mineral.[2]

The use of *in vivo* measurement techniques is needed to evaluate the mineralization or the bone mineral density (BMD) of growing animals. Several methods and techniques have been studied and developed, aiming to find the one that best fits the conditions of work and has an accuracy as good as the Direct Absorption by Photons[3], the Analysis of Activation by Neutrons[4], the Computerized Tomography[5], the Radiographic Photometry[6] and the Optical Densitometry in Radiographic Image , which can be used for a more accurate sequential analysis of the bone mass and with a lower cost than other methods.[7,8,9,10,11,12,13,14,15,16]

The densitometry of radiographic images is a methodology able to detect slight

differences in bone mineral content, when standardized radiographic images are used. This method was recommended to be used *in vivo* in the evaluation of the consolidation of fractures.[17]

The growth of foals raised in the traditional way, breast-fed up to the 4th month was compared to other ones raised with commercial milk. Weight, height and radiographic optical density of the 3rd metacarpus was used as criteria to evaluate growth.[18] Using the same methodology, was analyzed the correlation between bone density and weight through radiographic image, concluding that there was a positive correlation between these values.[19]

The utilization of digital radiographic images using a video camera or a scanner has improved the use of the densitometric methodology, making a more accurate and elaborated analysis of this technique possible. This evaluation of density through digital image derives, from the radiographic optical densitometry.[20]

The digital bone densitometry was used to calculate the bone mineral content of the 3rd lumbar vertebrae, the femoral lap and the 2nd metacarpus in distinct human groups.[20] The results were compared to the ones obtained through DPA (Dual Photon Absorptiometry), observing that the shapes of the curves and the precision and reproduction index of this method suggest that it can be used to detect and observe bone mineralization of patients, with moderate cost. This method was independent from sensitization and processing parameters of the radiographic film.

The use of *in vitro* studies regarding to optimization of the optical densitometry technique in radiographic images of bone pieces, concluded that the sectoring of the characteristic curves and the correction of the irregular effect of X-radiation provided a significant increase in the sensitivity of the radiographic densitometry method used, once they allowed the identification of the extraction of up to 12% of calcium in bone pieces[10].

The objective of this work was to develop and standardize a technique for the determination of normal values of bone mineral density (BMD) of the accessory carpus bone in young Thoroughbred horses, thus making possible an appropriate and efficient evaluation of the normal bone structure in these animals, using optical densitometry in bone radiographic images.

Materials and Methods

Twelve female and 12 male Thoroughbred horses, aging from 12 months old, belonging to the Eqüilia Horse Breeding Farm, in the region of Avaré, in the state of São Paulo, were used. All animals were clinically healthy and kept under the same conditions of handling, fed with balanced food and supplementation with green alfalfa, hay and coast-cross grass.

All animals had conventional training from 18 months old on, when they were submitted to guidance training on the ring for a month, riding with trot on the ring for one more month and riding on the track with trot and gallop, with two laps on a 1.300m track until the moment of complete closure of the radio distal epiphysis, when the study was finished.

The radiographic images of the animals were obtained from 12 months old (moment M1), followed for intervals of 120 days (M2, M3 and M4). From moment M4 on, at the beginning of closure of the radio distal epiphysis of some animals, they started to be monthly evaluated in moments M5 and M6, when the complete closure of the radio distal epiphysis of all remaining animals of both groups occurred. (Tables 1 and 2)

The radiographic image of the accessory carpus bone of the left limb on the medial side projection was chosen to be used in the study of the bone mineral density in horses, using a 24x30cm frame containing a radiographic film inserted into a 3-mm wood frame, to which an aluminum [Alloy 6063 – ABNT (Brazil)] stepwedge (*phantom*) was attached and used as a densitometry

reference, composed by 29 steps, being the first step 1.00 ± 0.03mm wide and varying from 1.00 ± 0.03mm from one step to the other, up to the twenty-ninth step. Each step had an area of 5x15 mm. The values in millimeters of each step of the aluminum stepwedge were checked with a micrometer and the values obtained from each step, maintaining a maximum variation of ± 0.03mm of one step in relation to the next upper or lower step. The values (in mmAl) of each step of the stepwedge were used as input for the computer program developed to serve as reference for the bone mineral density (BMD) of the accessory carpus bone.

The craniocaudal projection was used to follow up the complete closure of the radio distal epiphysis, with the central radius placed on the physeal region of the distal portion of the left radio.

An FNX JOCKEY 90 (FNX – Distribuidora de Equipamentos Médicos Ltda.) portable x-ray machine was used to obtain the radiographic images, using a focus-film distance of 70cm. The kilovoltage (kVp) was standardized at 56kVp and the milliamperage/second (mAs) was variable, depending on the bone development of the animals. A BRAF (BRAF – Kodak Brasileira Com. e Ind. Ltda.) film was used and the development and fixation processes were carried out in a Macrotec (MACROTEC – Ind. e Com. de Equipamentos Ltda.) automatic processor.

The radiographic images of the accessory carpus bone of the animals and the phantom were digitalized using an HP ScanJet 6c (Hewlett Packard) scanner, with an HP ScanJet 6c (Hewlett Packard) transparency adaptor.

A software (ATHENA – SAI – Sistema de Inteligência Avançada Com. Imp. Exp. Ltda. – SJC/SP) containing the radiographic image of the accessory carpus bone, the region of soft parts around the accessory carpus bone and the steps of the aluminum phantom was developed to measure the optical density in X-ray films, making the measurement of the bone mineral density possible, which, in this study, is the average of the region of interest established on the accessory carpus bone corresponding to the millimeters of the step on the aluminum stepwedge.

The statistical analysis was carried out using regression analysis with auto-

Table 1
Values of bone mineral density (BMD) in aluminum millimeters (mmAl) of the accessory carpus bone of 12 Thoroughbred mares in M1, M2, M3, M4, M5 and M6, with the respective ages (in months) until the final closure of the radio distal epiphysis (CRDE) with averages (x) and standard deviations (s). Avaré, SP. 2001

	MOMENTS													
	M1		M2		M3		M4		M5		M6			
Nº	**BMD**	**age**	**BMD**	**age**	**BMD**	**age**	**BMD**	**age**	**BMD**	**age**	**BMD**	**age**	**BMD**	**CRDE**
01	3,50	12,26	3,96	16,43	4,07	20,43	4,59	24,56	4,45*	25,46*			4,45	25,46
02	3,47	11,70	-	-	4,57	19,86	4,30*	24,00*					4,30	24,00
03	4,00	12,66	4,02	16,83	4,61	20,83	4,74*	24,96*					4,74	24,96
04	3,48	12,83	4,85	17,00	4,59	21,00	4,30	25,13	5,28*	26,10*			5,28	26,10
05	3,60	12,46	3,87	16,63	4,07	20,63	4,39	24,76	4,19*	25,73*			4,19	25,73
06	3,95	12,70	3,83	16,86	4,50	20,86	4,41*	25,00*					4,41	25,00
07	4,05	11,86	3,73	16,03	4,40	20,03	4,51*	24,16*					4,51	24,16
08	3,30	9,86	4,04	14,03	4,29	18,03	4.30	22,16	4,06*	23,13*			4,06	23,13
09	3,70	12,56	4,02	16,73	4,08	20,73	4,34*	24,86*					4,34	24,86
10	3,50	11,80	3,57	15,96	4,02	19,96	3,57	24,10	4,74*	25,06*			4,74	25,06
11	3,99	10,70	4,33	14,86	4,66	18,86	4,68	23,00	4,86	23,96	5,32*	24,86*	5,32	24,86
12	4,48	12,63	4,92	16,80	3,81	20,80	4,74	24,93	4,39	25,90	4,35*	26,08*	4,35	26,08
X	**3,75**	**12,00**	**4,10**	**16,20**	**4,31**	**20,16**	**4,41**	**24,30**		**25,04**			**4,56**	**24,95**
S	**0,34**	**0,90**	**0,43**	**0,94**	**0,29**	**0,90**	**0,31**	**0,90**		**1,02**			**0,40**	**0,87**

(*) Moment of the closure of the radio distal epiphysis (CRDE) and final reading of the bone mineral density (BMD) of the accessory carpus bone.

Table 2
Values of bone mineral density (BMD) in aluminum millimeters (mmAl) of the accessory carpus bone of 12 Thoroughbred males in M1, M2, M3, M4, M5 and M6, with the respective ages (in months) until the final closure of the radio distal epiphysis (CRDE) with averages (x) and standard deviations (s). Avaré –SP., 2001

	MOMENTS													
	M1		M2		M3		M4		M5		M6			
Nº	BMD	idade	BMD	idade	BMD	idade	BMD	idade	BMD	idade	BMD	idade	BMD	CRDE
13	4,01	11,63	4,45	15,80	4,28	19,80	4,40	23,96	4,50*	24,90*			4,50	24,90
14	4,30	10,76	3,83	14,93	4,13	18,93	4,14*	23,06*					4,14	23,06
15	3,78	12,76	3,63	16,93	3,62	20,93	3,66*	25,06*					3,66	25,06
16	3,37	12,10	3,17	16,26	4,05	20,26	4,60	24,40	5,11*	25,36*			5,11	25,36
17	3,81	12,56	4,16	16,73	4,27	20,73	4,82	24,86	4,74*	25,83*			4,74	25,83
18	3,77	11,30	4,30	15,46	3,80	19,46	4,43*	23,60*					4,43	23,60
19	3,69	12,26	4,11	16,43	3,87	20,43	4,39	24,56	4,48*	25,53*			4,48	25,53
20	3,43	11,30	4,21	15,46	4,58	19,46	4,56	23,60	4,81	24,56	4,75*	25,46*	4,75	25,46
21	4,11	13,06	4,24	17,23	4,96	21,23	4,38	25,36	4,52*	26,33*			4,52	26,33
22	3,68	11,16	3,89	15,33	4,03	19,33	4,38	23,46	4,60	24,43	4,56*	25,33*	4,56	25,33
23	3,81	12,10	3,83	16,26	4,07	20,26	4,21	24,40	4,46	25,36	4,53*	26,26*	4,53	26,26
24	3,73	12,56	3,53	16,73	4,12	20,73	4,15*	24,86*					4,15	24,86
X	**3,80**	**11,96**	**3,94**	**16,12**	**4,15**	**20,12**	**4,34**	**24,32**		**25,28**			**4,46**	**25,13**
S	**0,26**	**0,72**	**0,37**	**0,72**	**0,35**	**0,72**	**029**	**0,60**		**0,41**			**0,36**	**0,96**

(*) Moment of the closure of the radio distal epiphysis (CRDE) and final reading of the bone mineral density (BMD) of the accessory carpus bone.

correlation. An effect of sex and an effect of interaction between sex and time were included in the model to compare the lines of males and females. The significance of these effects was tested by the *t* statistics. The analysis was made using the SAS software (SAS Inc., 1996), by using PROC MIXED.

The effects of sex on the average of the carpus bone mineral density (BMD) at the moment of closure of the radio distal epiphysis and on the average time of closure of the radio distal epiphysis were investigated using the *t* test.

Results

The regression lines for bone mineral density values (in aluminum millimeters) of the accessory carpus bone of Thoroughbred males and females in function of time (age) were given by line equations BMD = 3,099+0,058 x age (Figure 1) and BMD = 3,162+0,052 x age (Figure 2), respectively. The adjustments of these lines showed no evidence that growth is different between sexes (p=0,86). Therefore, only one line was employed, joining both sexes.

The adjusted line equation was BMD = 3,109 + 0,056 x age (Figure 3).

Figure 1
Regression curve of bone mineral density, in mmAl, of the accessory carpus bone of female Thoroughbred horses in function of time (age).

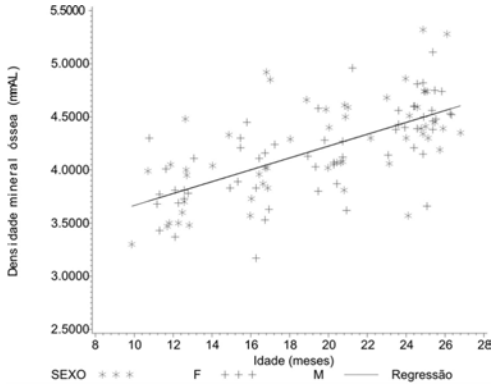
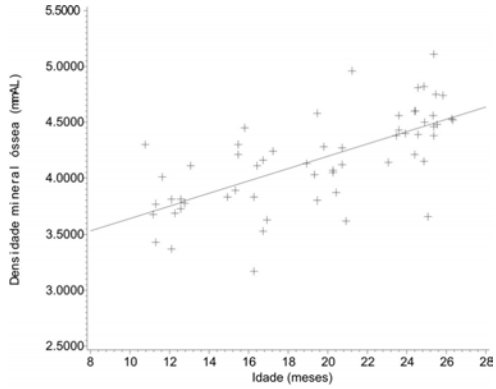


Figure 2
Regression curve of bone mineral density, in mmAl, of the accessory carpus bone of male Thoroughbred horses in function of time (age).



The line inclination was significant (p=0,0001), indicating that values of bone mineral density (in aluminum millimeters) of the accessory carpus bone of the animals increased, in average, 0,056 ± 0,007 mmAl per month, until the moment of closure of the radio distal epiphysis.

Comparing average values of the bone mineral density (BMD) of the accessory carpus bone of males and females at the moment of closure of the radio distal epiphysis by means of the *t* test, there was no significant statistical difference between them (p=0,5544), as well as between the averages of the time for closure of the radio distal epiphysis of females and males (p=0,7608).

Discussion and Conclusions

This study tried to develop and standardize a technique that evaluated the normal bone mineral density (BMD) of Thoroughbred horses in an efficient, practical and economical way, allowing a more efficient analysis of the sport potential of these animals and the evaluation of the physical conditioning, which has been routinely carried out in sport animals nowadays. The evaluation of bone mineral density in Thoroughbred horses is based on the optical densitometry in radiographic image, which can be used for a sequential analysis of the bone mass, with low cost in relation to the other methodologies and having precision as its main point.[7,8,9,10,11,12,13,14,15,16]

This research allowed the evaluation of the bone mineral density of the accessory carpus bone in Thoroughbred horses from 12 months old until the moment of complete closure of the radio distal epiphysis, when the animals start more intense work, demanding a higher effort of the bone-muscular system.

The technique used was based on setting a calibration curve obtained from the values of the radiographic optical density of the degrees of the aluminum stepwedge (*phantom*), made up with 29 degrees from 1 to 1 mm, varying from one degree to the other not more than ± 0,03 millimeters, which was used as a densitometric reference standard in function of the wideness of the degrees. Thus, the radiographic image of each animal had its curve traced in function of the transference of the values of the optical densitometry from the radiographic images of the degrees in millimeters of the aluminum stepwedge. These values were then correlated to the average of the optical densitometry of the region of interest of the

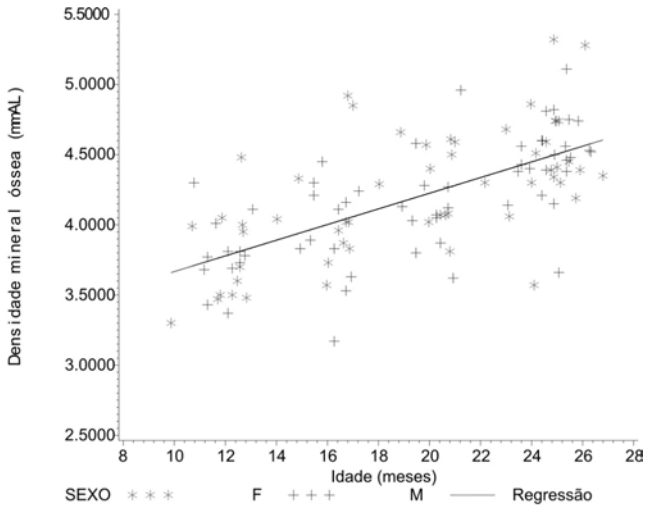


Figure 3
Regression curve of bone mineral density, in mmAl, of the accessory carpus bone of the set of female(***) and male(+ + +) Thoroughbred horses in function of time (age).

accessory carpus in the radiographic image, expressed in comparison to the values obtained from the aluminum stepwedge (in mmAl). Reproducibility was obtained using this methodology with 5% of error in the evaluation of 30 radiographic images of a same patient, without the support of the computer resources available nowadays which provide even more precise results.[21]

To overcome technical problems in relation to the radiographic technique[22], we have standardized a fixed value of 56 kVp for the kilovoltage. We have also maintained the accessory of the carpus and the phantom parallel to each other and centered along the flow of the primary radiation, where the geometric problems of the image of the anodic effect are bypassed[6], making results reproductive, reminding that every technical variation affects both image values of the radiographic image and phantom, and allows reliability of the results when kVp and focus-object distance are maintained constant.

A high reproducibility with an error around 1%, was reported using this technique indicating that it's sensitive for the evaluation of the bone mineral density and allowing a follow up of the variation of the bone mineral content of patients individually.[23]

The use of the method of digital radiographic optical densitometry to estimate the bone mineral content of the accessory carpus of equines adopted in this experiment has been already evaluated, when the results of this method was compared to the ones obtained through DPA (Dual Photon Absorptiometry)[20], concluded that the precision and reproducibility index of the method makes it qualified to be used to detect and observe the mineralization of patients with moderate cost, regardless sensitization and film development parameters, as confirmed in this study, where it was possible to observe the mineralization of the accessory carpus bone during the growth of the animals.

The software used for determination of the bone mineral density by means of optical densitometry in digital radiographic images with the support of the ATHENA SAI company – Sistema de Inteligência Avançada Com. Imp. Exp. Ltda., for standardization of the values of bone mineral density of the acessory carpus bone of growing Thoroughbred horses, from 12 months old until the complete closure of the radio distal epiphysis, showed that females, from the beginning of the study until the average age of 12 $\pm$ 1 month old, showed average values of bone mineral density of 3.75 $\pm$ 0.34 mmAl. At the moment of closure of the radio distal epiphysis, which occurred at the average age of 25 $\pm$ 1 month old, average values of bone mineral density was 4.58 $\pm$ 0.40 mmAl, with an average variation of 0.83 mmAl. For the 12 males, with the average age of 11.96 $\pm$ 0.72 months old, an average value of bone mineral density of 3.80 $\pm$ 0.26 mmAl was observed and, at the moment of closure of the radio distal epiphysis, which occurred at the average age of 25.13 $\pm$ 0.96 months old, average value for the bone mineral density was 4.46 $\pm$ 0.36 mmAl, with an average variation of 0.66 mmAl. This slight variation in the values of bone mineral density of the accessory carpus between the beginning and the end of the study, for both males and females, is probably due to the fact that horses, from 12 months old on, have their bone mineral constitution almost totally structured, with slight variations in the bone mineral density of the skeleton from 12 to 25 months old.

It may also be affirmed that the little increase in the bone mineral density of the accessory carpus bone during the development of the study will allow the evaluation of the bone age of horses through the radiographic optical densitometry, as reported in the study growth and development of bone in children.[24] In our study, the radiographic conditions were standardized, allowing the evaluation of the bone mineral density (in mmAl) of the accessory carpus bone of Thoroughbred horses between 12 and 25 months old, using the software developed.

There was no significant statistical

difference between sexes during the growth of the animals, so only one line was adjusted for the evaluation of the animals of both sexes, given by the line equation of bone mineral density in mmAl (BMD mmAl = 3.109 + 0.056 x age).

In this study, it was possible to develop of software to evaluate the bone mineral density by means of optical densitometry in radiographic image, determining normal values of bone mineral density, in mmAl, of the accessory carpus bone of male and female Thoroughbred horses.

Resumo

Em 12 fêmeas e 12 machos da raça Puro Sangue Inglês com idade média de 12 meses, avaliou-se os valores normais da densidade mineral óssea do carpo acessório em milímetros de alumínio (mmAl) até o momento do fechamento completo da epífise distal do rádio, por meio do método de densitometria óptica em imagens radiográficas. A avaliação foi realizada por meio de um programa computacional (software) especialmente desenvolvido para medida de densidade óptica em filmes de raios-X, o qual contém a imagem radiográfica do osso carpo acessório, região de partes moles adjacente ao carpo acessório e os degraus de uma escala de alumínio (phatom), que permitiu a medida de densidade mineral óssea, sendo esta a média aritmética da região de interesse determinada no osso carpo acessório correspondente ao valor em milímetros da escala. Os valores da densidade mineral óssea em mmAl do acessório do carpo em função da idade não apresentaram diferenças entre os sexos (p=0,86) permitindo que uma equação de reta fosse ajustada para ambos os sexos (densidade mineral óssea (DMO) mmAl = 3.109 + 0,056 x idade em meses), na faixa etária estudada.

Palavras-chave:
Eqüinos.
Densidade mineral óssea.
Acessório do carpo.

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