Quantitative comparison of bone mineral density characteristics of the distal epiphysis of third metacarpal bones from Thoroughbred racehorses with or without condylar fracture

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OBJECTIVE
To compare regional proportions and spatial distributions of volumetric bone mineral density (BMDv) of the palmar aspect of the distal epiphysis of the third metacarpal bone (McIII) in limbs with or without a condylar fracture from Thoroughbred racehorses.

SAMPLE
McIIIs from cadavers of Thoroughbred racehorses with (n = 6 bones) and without (8) a condylar fracture.

PROCEDURES
BMDv and spatial distributions of BMDv in peripheral quantitative CT images of the distal epiphysis of McIIIs were quantitatively assessed with spatial analysis software. Relative proportions of voxels within 9 threshold categories of BMDv and spatial statistics for BMDv distribution were compared between fractured and nonfractured limbs.

RESULTS
No significant differences in BMDv characteristics were identified between fractured and nonfractured limbs, although fractured limbs had a lower proportion of voxels in the BMDv thresholds 700 to < 800 mg/cm³ and 800 to < 900 mg/cm³ but a higher proportion of voxels in the BMDv threshold 1,000 to < 1,100 mg/cm³ for the central condylar region of the medial condyle. Results of spatial analysis reflected the response of bone to race training rather than differences between fractured and nonfractured limbs. In both limb groups, uniform clusters of low BMDv with areas of high BMDv were identified.

CONCLUSIONS AND CLINICAL RELEVANCE
BMDv characteristics of the distal epiphysis of McIIIs reflected training load, and fracture characteristics were subtle. Serial imaging techniques in conjunction with detailed training data are required to elucidate the onset of the pathological response to load in horses. (Am J Vet Res 2016;77:32–38)
The distal epiphysis of McIII responds dynamically to exercise in Thoroughbreds, with significant increases in BMDv of trabecular bone (37%), in contrast to an observed 1.6% increase in BMDv of diaphyseal cortical bone. Such changes are likely an adaptive response of the bone to withstand the forces encountered during exercise. However, regions of high mineral content can be brittle and fracture lines can pass through areas of highly dense bone. Additionally, abnormal microscopic features such as microcracking, microfracture, and evidence of osteoclastic remodeling have also been found in dense regions of the palmar aspect of the epiphysis of McIII.

The combination of pQCT and spatial statistics has been used to provide a detailed quantitative description of BMDv responses to exercise in young Thoroughbreds. Response of the epiphysis of MtIII to exercise is highly specific in relation to anatomic site, BMDv, and spatial distribution of BMDv. Quantitative description of the response to training load with serial CT scans is not only an interesting research approach but also an opportunity to provide evidence of serious early structural (pathological) change, which could prove useful in a clinical environment. However, whether this approach could be used to differentiate BMDv characteristics between horses that sustain condylar fracture of the McIII and those that do not is unknown. Therefore, the purpose of the study reported here was to compare regional proportions and spatial distributions of BMDv within loaded regions of the palmar aspect of the distal epiphysis of the McIII between Thoroughbred racehorses with or without condylar fractures of that bone.

Materials and Methods

Samples

Archived limbs from Thoroughbred racehorses with a condylar fracture (n = 6 limbs) and control limbs from Thoroughbred racehorses without a condylar fracture of McIII (8) were available for analysis. All horses with a condylar fracture that provided limbs had been euthanized within 24 hours after sustaining the fracture; and all limbs had been frozen within 24 hours after euthanasia. The 6 fractured limbs were obtained from 5 horses with fractures (ie, 1 horse provided 2 limbs). Radiography confirmed that 5 of these limbs had a displaced fracture of the lateral condyle and the sixth had a nondisplaced fracture of the medial condyle, with all fractures originating proximal to the respective parasagittal groove. The 8 nonfractured limbs consisted of 2 nonfractured contralateral limbs from 2 of the 5 horses with fractures and 6 other nonfractured limbs collected from horses euthanized for nonorthopedic reasons and frozen within 24 hours after euthanasia. Donors of nonfractured limbs were chosen to match donors of fractured limbs on the basis of age, sex, limb, and number of starts. Race information for individual horses was extracted from an online database. Metacarpophalangeal joints were disarticulated and each joint examined by 1 investigator (SHB) to confirm the absence of subchondral bone lesions of the distal articular surface of the McIIIs.

Quantitative CT

Methods used to obtain pQCT images of the distal epiphysis with a pQCT scanner have been described in detail elsewhere. Prior to scanning each limb, the scanner was calibrated with a cone phantom provided by the scanner manufacturer. During scanning, 20 contiguous scans (voxel size, 0.5 mm) were obtained from the most distal aspect of the epiphysis of each McIII. A 2-mm transverse slice representing a section 10 to 12 mm from the most distal aspect of the epiphysis of each McIII was chosen for analysis.

Each CT image was then divided into 6 palmar ROIs to account for the contact area of the proximal sesamoid bones on McIII and for the anatomic locations of condylar fractures. Briefly, this division was performed with a line running in the sagittal plane from the dorsal to the palmar apex of the sagittal ridge and a perpendicular bisecting line in the transverse plane (Figure 1). The palmar half created was divided into 6 equal sagittal divisions, with 3 on either side of the sagittal midline. The medial and lateral margins for the palmar divisions were the most medial and most lateral points of the palmar aspect of the epiphysis. The resulting ROIs were the entire epiphysis and the L1P, L2P, LSP, M1P, M2P, and MSP.

For each condyle, BMDv data were extracted for each ROI and the proportion of voxels within each of the following thresholds was calculated: 400 to < 500, 500 to < 600, 600 to < 700, 700 to < 800, 800 to < 900, 900 to < 1,000, 1,000 to < 1,100, 1,100 to < 1,200, and ≥ 1,200 mg/cm³. For calculation of relative BMDv proportions, the area represented by ≥ 400 mg/cm³ was used as the entire bone mass of the ROI.

Figure 1—Illustration of ROIs used for pQCT image analysis of the palmar aspect of the distal epiphysis of the McIII in limbs from Thoroughbred racehorses with or without a condylar fracture. Three equal divisions were created on either side of the sagittal ridge midline: L1P, L2P, LSP, M1P, M2P, and MSP. ALLEPI = Entire epiphysis.
Statistical analysis

Exported pQCT data were initially structured for analysis and screened for errors. Data were then imported for analysis. Multiple correspondence analysis was used as an exploratory technique to plot the variables of interest (fracture group, number of races, and BMDv threshold) on a 2-D graph. Analyses were adjusted to account for the overinflation of the inertia values along the diagonal of the matrix, and the technique allowed description of how strongly and in which way variables were interrelated.

Distributions of initial BMDv data pertaining to each ROI were examined for normality by means of histograms and descriptive statistics. Comparison of fractured versus unfractured limbs for the median proportion of voxels within each density threshold was performed with the nonparametric Kruskal-Wallis test. A Bonferroni correction was applied to adjust for multiple comparisons, and values of $P \leq 0.006$ were considered significant. Multiple correspondence analysis of the BMDv data was performed for each ROI by categorizing each horse as either above (1) or below (0) the median value within each BMDv threshold. Categories of BMDv, number of races, and fracture group were then analyzed by means of MCA.

The pQCT images were imported as ASCII format files for spatial analysis in a geographic information system. Import images were reclassified by pixel value to represent low ($< 600 \text{ mg/cm}^3$), medium ($\geq 600 \text{ to } < 800 \text{ mg/cm}^3$), and high ($\geq 800 \text{ mg/cm}^3$) BMDv on the basis of results from a preliminary study.

Smaller ROIs were created in a geographic information system within L2P and M2P to analyze the palmar aspect of the epiphysis of McIII that contacts the sesamoid bones during the stance phase of gallop (Figure 2). To account for the variation in condylar size among horses, the ROI was created as follows:

$$\text{ROI} = (0.2 \times \text{length of line from the palmar apex of the sagittal ridge to the dorsal apex of the sagittal ridge})$$

The palmar border of the ROI was aligned with the inner margin of the articular calcified cartilage on the palmar border of the condyle and centered within the lateral and medial borders of M2P or L2P. Additionally, ROIs were created parallel to the palmar border of the condyle over the sites of fracture at the medial and lateral palmar parasagittal groove regions in both fractured and unfractured limbs. To specifically target the sagittal groove area, these ROIs were created as follows:

$$\text{ROI} = (0.1 \times \text{length of line from the palmar apex of the sagittal ridge to the dorsal apex of the sagittal ridge})$$

For sites that had sustained fracture, the ROI was halved and each half was placed on either side of the fracture line to avoid error created by voxel sharing within the fracture gap.

Clustering and spatial relationships of BMDv were tested with the global Moran $I$ test, nearest neighbor analysis, and Getis-Ord general $G$ test. Statistics could not be generated when no pixels existed within the threshold being analyzed, and statistics with a value of $P \geq 0.01$ were excluded from interpretation. Additionally, a polygon was drawn around the outer margin of high BMDv pixels and around the margin of low BMDv pixels within the ROI. The spatial relationship between low and high BMDv clusters was recorded as low BMDv clusters outside the margins of the high BMDv cluster (1) or low BMDv clusters inside the margin of the high BMDv cluster (0).

Results

Samples

No significant differences were identified between limbs with $(n = 6)$ and without $(8)$ condylar fractures of McIII with respect to sex $(P = 0.60)$, age $(P = 0.70)$, total starts $(P = 0.50)$, or limb side $(P = 0.70)$ of the associated horses. Median number of race starts for the horses was $6$ (IQR, $0$ to $16$), and median horse age was $4$ years (IQR, $4$ to $5$). No subchondral bone lesions were identified in the nonfractured limbs. All limbs had some partial-thickness cartilage loss in the central condylar regions of the palmar articular surface.

Effect of fracture on distribution of BMDv within various ROIs

No significant differences were identified in the median proportion of voxels in all ROIs between fractured and unfractured limbs. For the M2P ROI, fractured limbs had a lower, albeit not significantly, proportion of voxels in the thresholds $700 \text{ to } < 800 \text{ mg/cm}^3 (P = 0.02)$ and $800 \text{ to } < 900 \text{ mg/cm}^3 (P = 0.007)$ and higher, albeit not significantly $(P = 0.01)$, BMDv in the $1,000 \text{ to } < 1,100 \text{ mg/cm}^3$ threshold (Figure 3).
**MCA**

In most ROIs, $\geq 80\%$ of the inertia was explained by dimension 1, except for the LSP ROI, where $75\%$ was explained by dimension 1. The MCA for all ROIs revealed that the variables for greater than the median high BMDv thresholds (900 to 1,200 mg/cm$^3$) were clustered together, deviating from the mean profile (center of the graph) in the same direction. Overall, MCA results for each of the ROIs revealed little evidence of clustering of the fracture variables with BMDv categories or number of race starts (Figure 4).

**Spatial analysis**

No significant differences were identified between fractured and nonfractured limbs for any spatial analysis statistic.

**Moran I test**

For the M2P ROI, significant ($P \leq 0.01$) moderate to strong clustering of high BMDv was identified in the condyles of all fractured ($I = 0.69$ to 0.89) and 6 of 8 nonfractured ($I = 0.75$ to 0.85) limbs. There was significant ($P \leq 0.01$) strong to marked clustering of medium BMDv for all fractured ($I = 0.86$ to 0.92) and nonfractured ($I = 0.79$ to 0.96) limbs. Only 2 values were significant ($P \leq 0.01$) for moderate to strong clustering ($I = 0.60$ and 0.79) of low BMDv, and both pertained to the nonfractured limbs.

For the L2P ROI, significant ($P \leq 0.01$) strong to marked clustering ($I = 0.75$ to 0.93) of high BMDv was identified in all fractured condyles, and significant ($P \leq 0.01$) weak to strong clustering ($I = 0.39$ to 0.90) of high BMDv was identified for 7 nonfractured condyles.

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*Figure 3*—Median relative proportion of voxels for BMDv thresholds in the ROIs M1P (A), L1P (B), M2P (C), L2P (D), MSP (E), and LSP (F) of the palmar aspect of the distal epiphysis of the McIII in nonfractured (n = 8; black bars) and fractured (n = 6; gray bars) limbs from Thoroughbred racehorses. Error bars represent the upper quartile range of the related BMDv threshold. *Indicated value differs significantly ($P < 0.05$) between limb groups.
There was significant ($P \leq 0.01$) strong to marked clustering of medium BMDv for all fractured ($I = 0.76$ to 0.90) and nonfractured ($I = 0.81$ to 0.95) condyles, but only 2 significant ($P \leq 0.01$) results for low BMDv in the same 2 nonfractured condyles that also had strong clustering ($I = 0.76$ to 0.90) for M2P.

For the medial parasagittal groove ROI, significant ($P \leq 0.01$) moderate to strong clustering of high BMDv was identified in 4 fractured ($I = 0.63$ to 0.84) and 4 nonfractured ($I = 0.53$ to 0.82) condyles. There was significant ($P \leq 0.01$) strong to marked clustering of medium BMDv for all fractured ($I = 0.73$ to 0.91) and nonfractured ($I = 0.76$ to 0.91) limbs. No significant values were obtained for clustering of low BMDv in fractured or nonfractured limbs.

For the lateral parasagittal groove ROI, significant ($P \leq 0.01$) weak to strong clustering of high BMDv was identified in 5 fractured ($I = 0.39$ to 0.97) and 5 nonfractured ($I = 0.43$ to 0.87) condyles. There was significant ($P \leq 0.01$) strong to marked clustering of medium BMDv for all fractured ($I = 0.79$ to 0.95) and nonfractured ($I = 0.72$ to 0.91) limbs. One horse from the nonfractured group had significant ($P \leq 0.01$) moderate clustering ($I = 0.52$) of low BMDv.

**Nearest neighbor analysis**

Significant results for the nearest neighbor analysis were all $> 1$, indicating an evenly spaced distribution of pixels in each BMDv threshold cluster. For the high and low BMDv thresholds, not all horses had significant results owing to a minimal number or absence of pixels in those thresholds.

**Getis-Ord general G and arrangement of high value and low BMDv clusters**

High BMDv clusters dominated over low BMDv clusters in both fractured and nonfractured condyles for all ROIs. Only a small number of significant test values were achieved owing to absent or minimal pixels of high or low BMDv.

Both fractured and nonfractured limb groups contained limbs with low BMDv clusters located inside high BMDv clusters; however, this appeared to occur less commonly than low BMDv clusters located outside high BMDv clusters for both limb groups. The fractured group appeared to have more occurrences of low BMDv clusters within high BMDv clusters for all ROIs than did the nonfractured group.

**Discussion**

Fracture in racehorses is a multifactorial event involving the interaction of the peak strain, strain rate, number of load cycles, temporal relationship of the application of load, and training surface. Therefore, it is important to minimize variation of these factors when attempting to associate anatomic changes with fracture. The horses from which fractured and nonfractured McIIIs were obtained for the present study represented a fairly homogeneous group with respect to age and number of race starts, but we were lacking in data to describe the training program and the temporal pattern between periods of rest and accumulated fast exercise. Similarity in age and racing exposure was reflected in the similarities between fractured and nonfractured limbs for many of the BMDv and spatial variables examined. Consistency of the racing and training environment within New Zealand may have also helped reduce between-horse variation.

Previous work by our group has revealed the rapidity of bone response to race training stimuli and the sensitivity of pQCT to detect these changes in horses. The location and nature of the response in the distal epiphysis of McIII are dependent on the magnitude and frequency of the training load. Serial

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**Figure 4**—Multiple correspondence analysis plots for BMDv thresholds and variables (number of race starts on record and fracture status) for the ROIs L1P (A), L2P (B), and LSP (C) of the palmar aspect of the distal epiphysis of the McIII in nonfractured ($n = 8$) and fractured ($6$) limbs from Thoroughbred racehorses. Thresholds are represented in categorized groups as greater than (+) and less than (−) median values for each BMDv threshold (number represents start value of threshold). Variables clustered together spatially were considered similar, whereas those widely dispersed were rarely associated with each other. Variables clustered around the center of the plot represent the mean profile with regard to the categories of BMDv. A lack of clustering for fractured and nonfractured limbs is highlighted by the opposite positions of these groups on the plot. Each color represents a pair of variables.
noninvasive imaging techniques such as pQCT may allow characterization of such specific and rapid bone responses throughout training in young and mature horses and investigation of the point when the bone response deviates from what is expected given the degree of training. Differences are likely to exist in bone response characteristics that represent the likelihood of fracture early in training (ie, within the first 12 weeks in 2- and 3-year-old horses), compared with that in older horses with previous training. Young horses need short periods of exposure to peak forces to obtain optimal bone strength owing to an increased risk of fracture with accumulation of training at canter only, whereas fractures can occur in areas previously remodeled high density bone, which may exist in older horses with accumulated training.\textsuperscript{11,12} The horses in the present study were all \textgreek{>} 3 years of age and had all raced several times, which was reflected in the skewed distribution of BMD\textsubscript{v} in the regions that had been under compression from the sesamoid bones (L2P and M2P). Within the fractured limb group, there was a mild right shift in BMD\textsubscript{v} distribution, although this association was not evident in the MCA plots.

Placement of the ROI on either side of the fracture line in the study reported here could have decreased the sensitivity of the results. In studies\textsuperscript{12,15} in which microstructural changes around fracture lines were examined in equine limbs, small microcracks and areas of lysis were identified close to the articular origin of the fracture line. However, in the present study, ROIs were placed just outside the margin of the fracture line to avoid having parts of the fracture gap included in the analysis. This method was used to avoid low BMD\textsubscript{v} artifacts resulting from a voxel-sharing effect within the fracture gaps on all images of fractured condyles. Gaps at the articular margin of fracture lines as identified via micro-CT have been described as pathological,\textsuperscript{12,27} with a voxel size approximately half that used with pQCT. However, there is a trade-off of voxel size with scan time when considering how pQCT could be used for serial collection of data.

Focal sites of porosity would appear as low BMD\textsubscript{v} clusters within high BMD\textsubscript{v} clusters. These focal sites of porosity were observed in both limb groups of the present study, although they were more common for the fractured group. Given the age and racing exposure of the study horses, sites of focal remodeling would be expected to be identified in both groups. The greater number of these sites identified in the fractured group supported an association between focal porosity and fracture.\textsuperscript{12} This spatial technique may provide an opportunity to quantify the risk of fracture in relation to sites of focal porosity, but evaluation of a greater number of samples would be required to statistically confirm this association.

A high frequency of loading cycles is detrimental to the health and strength of bone tissue.\textsuperscript{11,28} This could be because a high frequency of loading does not permit remodeling to occur or predisposes bone to the formation of microdamage. Higher peak strains induced by exercise are reportedly associated with a more pronounced BMD\textsubscript{v} response than lower peak strains, particularly in the upper and lower BMD\textsubscript{v} thresholds\textsuperscript{13}; however, whether the spatial distribution of the BMD\textsubscript{v} response is different between condyles that are loaded with high or low frequency is unclear. Although a high likelihood of fracture has been identified in racehorses with 880 loading cycles of gallop and 7,700 loading cycles of canter within a 30-day period,\textsuperscript{11} it is unknown whether detrimental changes in the structure of bone occur before this point and the number of loading cycles after which they begin to occur. Prospective longitudinal studies are warranted to investigate the structural properties of bone in relation to the cumulative number of loading cycles to which the condyle of an McIII has been exposed.

Specific training factors that influence peak strain are associated with specific changes in bone morphology. For example, various track surfaces can influence specific alterations in the thickness of cortical or trabecular bone.\textsuperscript{29,30} and a greater bone density response occurs with galloping than with cantering.\textsuperscript{15} However, it is unknown whether increasing peak strain with harder track surfaces induces the same structural bone changes as increasing the peak strain by training at various galloping speeds or with horses carrying extra weight during training. If the relative effects of training factors on bone structural change were known, then this information would aid in the development of training programs that balance factors known to elicit protective structural bone changes for ongoing exercise demands, without causing structural damage.

In the study reported here, BMD\textsubscript{v} characteristics in the distal epiphysis of limbs with or without condylar fractures of McIII appeared to be primarily exercise-related responses. Spatial analysis identified clusters of low BMD\textsubscript{v} within high BMD\textsubscript{v}, which may have represented focal areas of porosity in both limb groups. Training data and serial imaging would be important to obtain a greater understanding of the interrelationship of load, bone response, and fracture risk.

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**Footnotes**

b. Stratec XCT200 pQCT, Stratec Medizin Technik, Pforzheim, Germany.
c. Stata, version 11.1, StataCorp LP, College Station, Tex.

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