Comparison of macrostructural and microstructural bone features in Thoroughbred racehorses with and without midbody fracture of the proximal sesamoid bone

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Objective—To compare macrostructural and microstructural features of proximal sesamoid bones (PSBs) from horses with and without PSB midbody fracture to gain insight into the pathogenesis of PSB fracture.

Sample Population—PSBs from 16 Thoroughbred racehorses (8 with and 8 without a PSB midbody fracture).

Procedures—Parasagittal sections of fractured and contralateral intact PSBs from horses with a PSB fracture and an intact PSB from age- and sex-matched control horses without a PSB fracture were evaluated for visual, radiographic, microradiographic, histologic, and histomorphometric differences in bone porosity, vascular channels, heme pigment, trabecular anisotropy, and pathological findings.

Results—Fractured PSBs and their contralateral intact PSBs had more compacted trabecular bone than did control PSBs. Focal repair or remodeling was evident in the palmar aspect of many fractured and contralateral intact PSBs. Fracture coincided with microstructural features and propagated from the flexor to the articular surface.

Conclusions and Clinical Relevance—Fractured PSBs had adapted to high loading but had focal evidence of excessive remodeling and porosity that likely predisposed the horses to complete fracture and catastrophic injury. Development of diagnostic imaging methods to assess porosity of PSBs may help to identify at-risk horses and allow for modifications of training and racing schedules to reduce the incidence of PSB fracture in Thoroughbred racehorses. (Am J Vet Res 2010;71:755–765)

Fractures of the PSB are one of the most common fatal musculoskeletal injuries incurred by Thoroughbred racehorses during racing and training. Thirty to fifty percent of California Thoroughbred racehorse deaths have been attributed to PSB injuries. Similarly, PSB fractures are also commonly incurred during racing at venues in Kentucky, Florida, and New York. Eighty percent of a sample of California horses that died because of a PSB fracture had fracture of both the medial and lateral PSB bones (ie, biaxial fractures) within the same limb. Biaxial PSB fractures are 1 manifestation of suspensory apparatus failure or loss of metacarpophalangeal joint (fetlock) support. Catastrophic injuries that affect horses in high-profile races commonly occur in the region of the metacarpophalangeal joint. Proximal sesamoid bone injuries accompanied by ligamentous suspensory apparatus injury or third metacarpal condylar fracture also account for many non-catastrophic, career-ending injuries. Measures that prevent catastrophic and career-ending PSB fractures have the potential to improve equine welfare and public perception of the racing industry.

The pathogenesis of PSB fractures is poorly understood. In a postmortem study of Thoroughbred racehorses, those with PSB osteophytes or large vascular channels were less likely to die because of PSB fracture

ABBREVIATIONS

<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>ASMC</td>
<td>Age- and sex-matched control</td>
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<td>CILC</td>
<td>Contralateral intact limb control</td>
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<td>HR</td>
<td>High resolution</td>
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<td>MIL</td>
<td>Mean intercept length</td>
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<td>PSB</td>
<td>Proximal sesamoid bone</td>
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<td>ROI</td>
<td>Region of interest</td>
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than racehorses without evidence of these features on palmarodorsal contact radiographs. These findings support the theory that PSB fractures are acute phenomena related to a single overload event or multiple excessive loading events in a short interval.

In contrast, several risk factors associated with PSB fracture indicate that genesis of such fractures may be related to training and racing activities that occur over several months. Fatal PSB fractures reportedly occur in horses that accrue higher cumulative distances, have longer periods of continuous activity, and have higher exercise intensities (particularly during the 1, 2, and 6 months before death) than horses that die for reasons other than PSB fracture. Supporting evidence that training affects the likelihood of PSB fracture was found through in vitro biomechanical studies of cadaver limbs from horses with different recent training histories. Limbs from horses that were in active training failed by PSB fracture, whereas limbs from horses that were not in active training failed by suspensory ligament disruption. The investigators proposed that active training may strengthen the suspensory ligament, leaving the PSBs as the weakest point of the suspensory apparatus and predisposed to fracture. An alternative explanation is that changes may develop within PSBs from horses in active training that increase susceptibility to fracture.

Preexisting changes are present in other bones of Thoroughbred racehorses that completely fracture during racing or training. Periosteal callus and an incomplete fracture line precede complete fractures of the humerus. Similarly, periosteal callus precedes scapular, pelvic, and tibial complete fractures and vertebral facet fractures. Cartilage fissures, erosion, and discoloration; linear defects in mineralized articular cartilage; and densification of subchondral bone precede parasagittal condylar fractures of the third metacarpal bone. To the authors’ knowledge, there is no direct evidence of preexisting damage as a precursor of PSB fracture.

Because chronic and intensive training and racing, but not osteophytosis, increase the risk of fatal PSB fracture in Thoroughbred racehorses, we hypothesized that microstructural, tissue-level changes predispose Thoroughbred racehorses to acute, complete PSB fractures. Our primary objective for the study reported here was to compare microstructural features of PSBs from horses with PSB fracture to microstructural features of PSBs from ASMC horses without PSB fracture by means of gross, microscopic, radiographic, and micro-radiographic examination of parasagittal PSB sections to provide insight into the pathogenesis of equine PSB fracture.

**Materials and Methods**

**Horses and sample selection**—A subset of a convenience sample of PSBs from Thoroughbred racehorses described in other reports was included in the study. In the initial convenience sample of horses, 109 of 221 horses had PSB fractures and midbody PSB fractures were the most common fracture configuration. Eight case horses for the present study were selected from horses with a PSB midbody fracture (ie, a simple transverse fracture within the middle proximodistal third of the PSB, as determined from palmarodorsal contact radiographs). Case horses were randomly chosen from subsets of 2- to 7-year-old horses, with consideration for inclusion of females, geldings, and sexually intact males.

Each fractured bone (fracture group) from a case horse had 2 sets of intact, control bones. The first set of control bones consisted of the comparable (medial or lateral) nonfractured PSB from the contralateral limb of the respective case horses (CILC group). This contralateral set was likely to have the same stage of disease or adaptive change, but without fracture, as the fractured bones because the right and left forelimbs have the same loading history with similar loading circumstances during racing and exercise.

The second set of control bones was drawn from a different group of 8 ASMC (2 to 7 years of age; female, gelding, or sexually intact male) horses selected to provide insight into nonpathological, age- or sex-related changes in forelimb PSBs of deceased racehorses. Each horse in this group contributed the same PSB (ie, limb and bone) as the fractured bone for its ASMC horse. These horses had sustained and were euthanatized because of musculoskeletal injuries other than forelimb PSB fracture (third metatarsal bone lateral condylar fracture, scapular fracture, humeral fracture, pelvic fracture, hind limb first phalangeal fracture, and rupture of a hind limb suspensory ligament). The case and control horses were not matched by fatal event (racing, training, or unrecorded workout) or exercise and racing histories. In all, 24 forelimb PSBs from 16 horses were included in the study.

**Bone processing**—The PSBs were removed from the suspensory apparatus of each limb and trimmed of soft tissues to within approximately 5 mm of bone margins. The intersesamoidean ligament was transected longitudinally, separating the lateral and medial bones. Each selected PSB was embedded in dental methylmethacrylate and cut into approximately ten 3-mm-thick serial parasagittal slices by use of a circular diamond band saw. Digital color images were taken of both cut surfaces for all slices. Lateromedial contact HR radiographs taken in a cabinet radiograph unit by use of HR mammography film (3 mA, 9 minutes, and 10 kVp exposure time) were digitized.

A single 3-mm-thick parasagittal slice from each PSB with a midbody fracture was selected for further processing. Selection was primarily based on detection of ≥ 1 of the following HR radiographic features: prominent vascular channels, fracture margin irregularities or lucencies, and irregular silhouettes of the bone palmar flexor margin. When multiple slices had these features, slices near the apex of the bone were chosen instead of bone slices that were axial or abaxial to the apex to maximize the volume of bone available for examination, and slices with midbody fractures that divided the bone volume equally were preferred instead of slices that had small proximal or distal bone fragments. Because of the roughly pyramidal shape of the PSB, as parasagittal serial slices proceeded from the slice containing the apex...
toward the abaxial surface, the proximal fracture fragment diminished relative to position along the slope of the abaxial surface where the medial and lateral branches of the suspensory ligament insert. Serial slices for control bone specimens were selected from the same location as for the matched fractured bones.

Selected slices were stained en bloc with basic fuchsin, then dehydrated in graded ethanol solutions and infiltrated and embedded in methylmethacrylate. Serial parasagittal sections, approximately 300 to 500 \( \mu \)m thick, were cut with a circular diamond band saw and ground by hand with increasing fine-grit sandpaper to a specimen thickness of 100 \( \mu \)m. Contact microradiographs included an aluminum step-wedge and were produced with a system of regional locations that captured bone material in different functional locations.

**Histopathologic description**—Initial subjective description included a regional anatomic overview of features visible on a parasagittal section of each PSB. Microradiographic images were assessed with a light microscope at 5X magnification for evidence of bone microstructural damage, bone remodeling, fracture margin linear quality, and resorption cavities and surfaces. Gross specimens and HR radiographs were assessed visually for evidence of preexisting damage or repair, such as cracks or radiolucencies. Close-up evaluation and photographic imaging of gross surface lesions, if present, were performed with a dissecting microscope.

**PSB regional map**—To determine whether bone features had a regional distribution, each PSB was conceptually divided into 9 regions that captured subchondral, medullary, and palmar flexor bone materials and regions with and without ligamentous attachments (midbody vs apical or basilar) for data collection. The parasagittal section of each PSB was divided into dorsal-palmar and proximodistal thirds. Proximodistal regions of the bone were designated as apical, midbody, and basilar, whereas dorsal-palmar regions were assigned as subchondral, medullary, and palmar flexor (Figure 1).

**Measurements and data categorization**—Subjective assessment of features visible on the PSB parasagittal surface included characterization of compact bone tissue, vascular channels, heme pigment, subchondral sclerosis, and trabecular orientation. Bone tissue was considered cancellous when trabeculae could be visually discerned on gross images and on HR radiograph images of 3-mm-thick sections and when intertrabecular spaces subjectively occupied \( \geq 30\% \) of tissue area on microradiographic images. Otherwise, the tissue was characterized as compact bone.

Vascular channels on palmarodorsal radiographs of PSBs are visible as linear lucencies within the body of the PSB and are continuous with abaxial funnel-shaped lucencies at the superimposed abaxial margin of the bone. Cross sections of vascular channels on gross, parasagittal plane images were identified as isolated, distinct, round (\( \geq 2 \) mm in diameter) to oval (\( \geq 2 \) mm in length) cavities in the bone surface. Vascular channels on HR radiographs and microradiographic images were identified as round to oval radiolucencies corresponding to the same location and size as the vascular channels seen on gross surfaces.

Subjective characterization of heme pigment (presumptively blood, marrow space, or small capillaries) was performed on gross surface images only. The pigment was classified by size and shape as punctate for small, nearly pinpoint spots of red; filled cancellous when red color was localized in intertrabecular spaces; diffuse focal for foci of diffuse red staining; and diffuse global for red coloration of the entire region.

Figure 1—Map of parasagittal section regions for tabulating features on gross surface images, HR radiographs, and microradiographic images of PSBs obtained from Thoroughbred racehorses with and without PSB fractures. The 9 regions consist of apical subchondral (ApSub), apical medullary (ApMed), apical palmar flexor (ApFlx), midbody subchondral (MidSub), midbody medullary (MidMed), midbody palmar flexor (MidFlx), basilar subchondral (BasSub), basilar medullary (BasMed), and basilar palmar flexor (BasFlx). The bone in this image is the fractured left medial PSB from a 2-year-old gelding.
The presence or absence of trabecular arrays that appeared to diverge from the center of rotation of the third metacarpal bone condyle through the PSB was recorded for HR radiographic and microradiographic images. The presence or absence of a radiodense subchondral crescent-shaped feature was recorded for HR radiographic and microradiographic images. This feature was not included in the regional tally because it only existed within the subchondral spongiosa when present. The presence or absence of groups of longitudinal trabeculae, oriented in a proximodistal direction, was also recorded.

Quantitative histomorphometry—Bone porosity and bone anisotropy index between fracture, CILC, and ASMC groups were examined by means of quantitative histomorphometry. Three ROIs (3.35 × 2.5 mm) were standardized for measurements from microradiographs. The 3 ROIs were centered in subchondral, medullary, and palmar flexor regions within the middle third of the body of the PSB adjacent to the fracture margin in the fractured bones. All ROIs were captured in tagged image file format with a light microscope digital microscopy unit at 5X magnification.

Bone porosity, expressed as a percentage, was calculated with the aid of a custom data analysis program. The custom program extracts a binary image with only black or white colors from an original grayscale image with 256 possible intensity values by use of an intensity threshold. Porosity and anisotropy index were determined from the black (void) pixels in the binary image. Manual point counting was used to establish a binary threshold value that closely captured the estimated percentage porosity. One ROI was selected from each set of matched images for point counting. A transparent grid with ≥300 total intersections was overlaid on a printed image to be used for point counting. The intersections that fell on the dark or black area of bone were counted as void space, and all other intersections were counted as bone. Only full-thickness porosities were included in the point counts. The percentage porosity was calculated from the ratio of dark or black intersections to total intersections, multiplied by 100. Varying thresholds were applied to the custom program until a binary threshold approximated the porosity values derived from the point counting method (r = 0.92) for 19 images. The same threshold value was used for all images. Bone porosity was calculated as the percentage of total pixels that occupied void spaces.

Bone anisotropy index was determined with the MIL method. The MIL was derived by rotating a set of uniformly spaced parallel lines, at an interval of every other pixel, upon the binary image. The number of the bone-porosity intercepts upon each parallel line, at a given angle θ, was counted and normalized to the length of standardized lines, measured in pixels, by dividing the total number of intercepts by the total length of standardized lines. The image was rotated 360° in 10° increments, and the bone intercepts measure was repeated for every 10°. The MIL of each given angle was computed as the ratio between the total number of bone pixels and half of the normalized bone intercepts. The MIL data were then fit to an ellipsoid shape. Briefly, the locus of the MIL vector endpoints emanating from a common center was plotted, and a multivariate, linear least squares fitting technique fits these points to an ellipse. The resulting coefficients, as anisotropy tensors, provided the predominant orientation of trabeculae within that section relative to a 2-D polar coordinate system. The ratio of the length of the long axis and the short axis was used to indicate the structural symmetry in trabecular bone, with a value of 1.0 indicating isotropy and 2.0 indicating anisotropy.

Statistical analysis—Geographic regional differences in characteristics of compact bone, vascular channels, heme pigment, radial trabeculae, and longitudinal trabeculae among fracture, CILC, and ASMC groups were examined by use of the McNemar test for correlated proportions, matching the groups in a pairwise manner. Separate analyses were performed for gross surface images, HR radiographs, and microradiographs. The proportion of regions within a PSB (number of regions/9) that had compact...
bone tissue, vascular channels, heme pigment, radial trabeculae, subchondral crescent, and longitudinal trabeculae were compared among groups by use of the Wilcoxon signed rank test. Differences in bone porosity and anisotropy index for each ROI between fractured, CILC, and ASMC PSB groups were compared by use of Kruskal-Wallis nonparametric 1-way ANOVA. Kruskal-Wallis ANOVA was chosen because the data were not normally distributed. Significance for all tests was set at a value of $P < 0.05$.

**Results**

**Horses**—The 8 horses selected for the control group had a biaxial, unilateral PSB simple transverse midbody fracture. These included one 2-year-old gelding (left forelimb, medial bone), one 2-year-old sexually intact male (right forelimb, medial bone), one 3-year-old gelding (left forelimb, lateral bone), one 4-year-old female (left forelimb, lateral bone), one 5-year-old female (right forelimb, medial bone), one 5-year-old gelding (right forelimb, lateral bone), one 6-year-old female (left forelimb, medial bone), and one 7-year-old gelding (right forelimb, lateral bone).

All PSB midbody fractures existed as part of a biaxial fracture set, in which a fracture also existed in the other PSB from the same limb (medial or lateral). Four of the biaxial sets had midbody fractures, and 4 had basilar PSB fractures within the other (nonexamined) bone.

**General anatomic observations**—Fractured and intact PSBs were composed primarily of densely compacted cancellous bone that formed thick trabeculae of varying widths and osteons (Figure 2). On parasagittal sections, the PSBs did not have a periosteum, a cortical zone, or a well-demarcated medullary cavity. The apical and basilar regions of the subchondral spongiosa often had wider intertrabecular spaces than the subchondral spongiosa of the midbody region, which was densely compacted, forming a radiodense crescent shape visible on the HR radiographs and microradiographs. The intertrabecular spaces of the medullary spongiosa ranged from narrow and densely filled-in to wide, broad openings. Sixty-three percent of all PSBs had medullary trabeculae arranged in a radial pattern as evident on HR radiographs and microradiographs.

The palmar flexor spongiosa just palmar to the medullary spongiosa contained a consistent region of trabeculae that were oriented longitudinally and a focus of transversely oriented osteons, palmar to the longitudinal trabecular field, adjacent to the palmar flexor insertion of the intersesamoidean ligament. There were also densely compacted longitudinal trabeculae located in the area of the abaxial fossa (apical subchondral and medullary spongiosa), most likely associated with insertion of suspensory ligament Sharpey fibers. Large vascular channels (> 2 mm in diameter), when present, were most often located in the apical medullary and midbody medullary spongiosa (10/24 bones).

**Subjective assessment of gross specimens, HR radiographs, and microradiographs**—Contour irregularities and fragment fracture margin incongruities were examined on microradiographs. Six of the 8 fractured bones had an irregular contour within the palmar flexor region, whereas the contours of the subchondral and medullary regions were mostly smooth, linking the fracture plane through the intertrabecular spaces. When digitally apposed, fracture margins were not congruent within the palmar region and there were gaps between the PSB fragments (Figure 3).
Shallow, linear defects in a transverse plane, measuring < 1 mm in width and < 2 mm in length, adjacent to the palmar flexor bone margin and parallel to the fracture plane, were evident in several serial levels of

Figure 5—Gross surface (A and C) and HR radiographic (B and D) images of a palmar focal defect (arrow) in the intact medial PSB from a 6-year-old female Thoroughbred racehorse, the contralateral PSB of which was fractured. Bar = 5 mm in panels A and B; bar = 1 mm in panels C and D.

Figure 6—Microradiographic images of PSBs with midbody fractures from various Thoroughbred racehorses. A—Remodeling (arrow) aligned with the orientation of Sharpey fibers from the site of suspensory ligament insertion is evident in the apical region of the PSB of a 2-year-old gelding. B—A subchondral crack (arrow) is visible in the PSB from a 5-year-old gelding. C—Narrow intertrabecular channels are visible sprouting from wider intertrabecular spaces in the PSB from a 2-year-old gelding. D—Resorption-like cavities are evident in the left medial PSB from a 6-year-old female. Bar = 2 mm in panels A–C; bar = 1 mm in panel D.

Table 1—Median (range) number of map regions with compact bone tissue, vascular channels, heme pigment, radial trabeculae, and longitudinal trabeculae in gross specimens and HR radiographic and microradiographic images of fractured (n = 8), CILC (8), and ASMC (8) PSBs from Thoroughbred racehorses.

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<th>Bone characteristic</th>
<th>Gross specimens</th>
<th>HR radiographs</th>
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*Only 1 bone had global heme pigment visible on the sagittal surface.
Fx = Fracture. NA = Not applicable because the feature was not visible for measurement or quantification.
*Different superscript letters indicate a significant (P < 0.05) difference between groups within a method.
parasagittal slabs from the medial PSB in 2 horses. A 2-year-old gelding had a linear palmar defect distal to the fracture margin in the fractured medial PSB (Figure 4), and a 6-year-old female had a linear palmar defect in the CILC medial PSB (Figure 5). Palmar defects were not seen in the ASMC group.

Additional features—The 2 horses with the linear palmar defect had an additional defect not seen in any other horse. This defect was located within the spongiosa of the abaxial fossa, where the medial and lateral branches of the suspensory ligament insert, at the level of the axial border of the abaxial fossa (suspensory ligament insertion site). The defects appeared as a focal sequence of irregular, arched resorption cavities and were found in both the fractured medial PSB and the contralateral bone (Figure 6). Several fractured PSBs had sharp, small cracks subjacent to the subchondral spongiosa fracture margin that were not present on the matched CILC or ASMC PSB microradiographs. Clusters of narrow intertrabecular channels could be seen sprouting from wider intertrabecular spaces. Intertrabecular spaces often resembled resorption cavities with partial-thickness porosities forming rough cavity margins.

Subjective analyses—The predominant bone tissue in each of the 9 geographic regions was categorized as compact or cancellous to determine whether location of compact bone tissue was different between fractured PSBs and control bones without fracture (Table 1). Group differences in the number of regions with compact bone tissue were also determined.

More fractured bones had gross evidence of compact bone tissue in the apical medullary (P = 0.041) and basilar medullary (P = 0.041) regions than did ASMC bones. The fractured and CILC PSBs had more regions of compact bone tissue than ASMC PSBs (P = 0.039 and P = 0.016, respectively). The number of regions with compact bone tissue was not significantly (P = 0.938) different between fractured and CILC PSBs (Table 1). All fractured and CILC PSBs had compact bone tissue in the midbody palmar flexor region, whereas 5 of 8 ASMC PSBs had compact bone in the same region.

Among all groups, no difference in compact bone tissue within regions was evident on HR radiographs. The CILC PSBs had more regions of compact bone tissue than did ASMC PSBs (P = 0.031). The number of regions with compact bone tissue was not significantly (P = 0.039 and P = 0.563) different between fractured PSBs and ASMC PSBs (P = 0.156) or between fractured PSBs and CILC PSBs (P = 0.461).

More fractured PSBs had compact bone tissue in the apical palmar flexor geographic region than ASMC bones on microradiographic images (P = 0.041). Fractured PSBs had more regions of compact bone tissue than ASMC PSBs (P = 0.031), and CILC PSBs had marginally insignificantly (P = 0.055) more regions of compact bone tissue than ASMC PSBs. The number of regions with compact bone tissue was not significantly (P = 0.363) different between fractured PSBs and CILC PSBs.

When PSBs were examined for vascular channels to detect differences between fractured bones and con-
trol bones, no significant differences were detected among groups by region or by number of bones with vascular channels. This was true for all examinations of parasagittal surface images, HR radiographs, and microradiographs. When the nature of heme pigment distribution (punctuate, filled, diffuse focal, or diffuse global) was assessed on the gross surface images, no differences were detected among groups by region or by bone on gross examination of parasagittal surface images. Regions with global heme pigment distribution were found only on ASMC PSBs.

Radial trabecular arrays were visible on HR radiographs and microradiographic images. Among all groups, no regional differences in radial trabecular were found. On HR radiograph images, CILC and ASMC PSBs had more regions with radial trabeculae than did fractured PSBs \( (P = 0.031) \) each, whereas the number of regions with radial trabeculae was not different between CILC PSBs and ASMC PSBs \( (P = 0.375) \). However, on microradiographs, CILC PSBs had more regions with radial trabeculae than did PSBs \( (P = 0.031) \), whereas there was no difference in number of regions between fractured PSBs and ASMC PSBs \( (P = 0.688) \) or between CILC PSBs and ASMC PSBs \( (P = 0.250) \).

Subchondral crescent figures were recorded as evident or not evident on HR radiographs and microradiographs and not by region. Three fractured bones, 4 CILC bones, and 6 ASMC bones had visible subchondral crescent figures on HR radiographs. Four fractured bones, 5 CILC bones, and 4 ASMC bones had visible subchondral crescent figures on microradiographs. The total number of PSBs with subchondral crescent figures was not significantly different among fractured, CILC, and ASMC bones for either type of imaging.

Longitudinal trabeculae were assessed on microradiographs. Among all groups, no regional differences in the number of regions with longitudinal trabeculae were evident on microradiographs. Fractured PSBs had more regions with longitudinal trabeculae than did ASMC PSBs \( (P = 0.047) \). There was a marginally nonsignificant \( (P = 0.063) \) increase in the number of regions with longitudinal trabeculae in fractured PSBs, compared with the number in CILC PSBs, and there was no significant difference \( (P = 0.813) \) between CILC PSBs and ASMC PSBs.

Quantitative histomorphometry—Bone porosity and anisotropy index were compared for subchondral, medullary, and palmar flexor ROIs between fractured CILC, and ASMC groups (Table 2). Percentage bone porosity for all ROIs in each PSB was compared among the case and control groups. Percentage bone porosity in the ASMC group was significantly \( (P = 0.013) \) greater than that in the fractured and CILC groups. However, porosity was not significantly \( (P = 0.789) \) different between fractured and CILC groups.

Bone porosity of individual ROIs ranged from 0.7% to 42.0% (Table 2). When the percentage porosity was compared among ROI locations (subchondral, medul- lary, and palmar flexor), it was significantly greater in the medullary versus subchondral locations \( (P = 0.001) \) and palmar flexor \( (P = 0.016) \) locations, but percentage porosity was not different between subchondral and palmar flexor locations \( (P = 0.312) \).

Percentage porosity was compared between individual ROI locations within PSB case and control groups. In the ASMC group, medullary locations had a significantly greater percentage porosity, compared with subchondral \( (P = 0.016) \) and palmar flexor \( (P = 0.021) \) locations, but percentage porosity in subchondral and palmar flexor locations was not significantly \( (P = 1.00) \) different. In the CILC group, medullary locations had significantly greater porosity, compared with the subchondral \( (P = 0.027) \) locations, whereas porosity in the palmar flexor location was not significantly different from that in the medullary location \( (P = 0.674) \) or the subchondral location \( (P = 0.115) \). Percentage porosity was not significantly different among ROIs in the fractured PSB group.

The percentage of bone porosity differed by ROI locations among case and control groups. In the medullary ROI, percentage porosity in the ASMC group was significantly greater than that in the fractured \( (P = 0.012) \) and CILC \( (P = 0.016) \) groups. The remaining ROI locations did not differ significantly in porosity among fractured, CILC, and ASMC PSBs (Figure 7).

The mean anisotropy index, a measure of the proportion of trabecule that were aligned along a preferential direction, was not significantly different among the fractured, CILC, and ASMC PSBs or among subchondral, medullary, and palmar flexor ROI locations. Anisotropy index ranged from 1.0 to 1.86.

Discussion

In the study reported here, we examined PSBs from Thoroughbred racchorses with a PSB midbody fracture, the CILC PSB from the same horse, and intact PSBs from horses matched by age and sex to each horse with a fracture to find evidence of microstructural features that might predispose horses to acute PSB fractures. Fractured and CILC PSBs had compaction of trabecular bone and lower bone porosity than did ASMC PSBs, particularly in the medullary region of the bone. Fracture margin incongruity was evident on the palmar aspect of the fracture margins in 6 of 8 horses with PSB fracture. An additional focal defect, consistent with bone remodeling, was found in fractured medial and CILC PSBs in 2 of the 6 horses with fracture margin incongruity. The fracture appeared to initiate from sites of previous remodeling in the palmar flexor region and propagate toward the articular surface.

Fracture margin incongruity in the palmar flexor region likely represented preexisting regional pathologic change characterized by focal bone resorption. Incongruities between reapposed fracture fragments within the palmar flexor region were suggestive of focal loss of bone tissue. The rounded, scalloped surface contours on fracture fragments in this region are typical findings in association with bone resorption bays (Howship lacunae) and are less consistent with straighter contours typical of artifactual fragmentation of bone margins during grinding of undercalcified tissues during processing. However, bone tissue adjacent to the focal area of bone resorption was likely damaged or malacic because bone tissue in this region disintegrated from several bone sections during the final stages of hand polishing to the 100-µm thickness.
The bilateral limb distribution of focal regions of bone remodeling observed in horses with a midbody fracture of the PSB supported the role of repetitive, overuse activities in the pathogenesis of PSB fracture. The left and right legs of racehorses experience similar conditions during racing. Similar to stress fractures in racehorses, catastrophic complete fracture may occur unilaterally but the underlying disease is bilateral and the circumstances that preceded the complete fracture are present in the CILC bone.13 Bilaterally, PSBs likely experience similar stresses that induce bone damage and stimulate repair. Both sets of forelimb PSBs sustain sufficient pathological change to promote a complete fracture, and when fractures occur in 1 forelimb, the CILC PSBs contain the pathological events that preceded the fracture. In addition, the palmar linear defect detected in the fractured medial PSB with fracture margin incongruity was distal to the fracture margin. This suggested that there may be >1 palmar site that underwent microstructural changes and that the fracture propagated through the site that provided the least resistance.

Fracture likely initiates in the palmar flexor region and propagates dorsally. The palmar flexor region is loaded in tension by the ligamentous attachments of the suspensory apparatus, whereas the subchondral and medullary regions sustain compression caused by articulation with the metacarpal condyle.25 Fracture propagation from palmar to dorsal is consistent with the anisotropically mechanical behavior of bone material. Bone material is weaker in tension than compression.24 Consequently, fracture initiation on the palmar surface is consistent with fracture of bone material in tension. Crack propagation direction is transversely oriented relative to the longitudinally oriented osteons in the palmar flexor region, which inhibit crack propagation by diverting cracks at osteonal cement line boundaries.23 Cracks might damage bone material and stimulate bone remodeling, which initiates bone resorption.24 Bone resorption results in loss of bone material and a weaker structure, allowing for continued stable crack propagation in the region of longitudinally oriented osteons.26-27

The incongruous fracture margins in the palmar flexor region were consistent with bone resorption or osteonal pullout. Two factors likely promote unstable crack propagation and complete PSB fracture. First, as the crack progresses from palmar to dorsal in the PSB, there is less material to resist loading while stresses are concentrated at the tip of the so-called bone resorption crack. Eventually, local stresses exceed strength of the remaining bone tissue and result in catastrophic crack propagation. Second, the crack tip approaches and reaches the region of radially oriented trabeculae (transversely oriented relative to the PSB), which has few morphological structures to inhibit crack propagation.35-37 Consequently, the crack propagates rapidly to the articular surface. The smooth nature of the subchondral and medullary margins of the fracture are consistent with unstable crack propagation. Hyperextension of the metacarpophalangeal joint probably plays a role in PSB fracture. Forces acting upon PSBs that promote separation of palmar regional bone tissue include longitudinal tension from the suspensory apparatus and transverse compression from the third metacarpal bone condyle.26 Forces would be greatest during metacarpophalangeal joint hyperextension when the suspensory ligament and distal sesamoidean ligaments serve to distract the apical and basal regions of the PSBs about the metacarpal condylar fulcrum. Repeated metacarpophalangeal joint hyperextension associated with intense exercise during racing and race training is likely to create focal regions of microdamage in the palmar flexor spongiosa, promoting resorption of damaged bone tissue and focal osteoporosis that weakens bone tissue and enhances the likelihood of complete PSB fracture.

Palmar linear defects were detected in medial PSBs exclusively. However, it is unclear whether there is a medial or lateral PSB predilection for development of remodeling sites that are predisposed to PSB fracture because of the small sample size histomorphometrically evaluated in the present study. Although PSB fractures have historically been reported to be more prevalent in the medial PSB,24,30 within a sample of deceased Thoroughbreds in one of our prior studies,13 lateral and medial fractures were distributed equally. It is possible that injury to the soft tissue attachments of the PSBs could attenuate individual PSB loads. For example, if there is weakening or gradual disruption of the lateral suspensory ligament branch, additional load may be diverted to the medial PSB through the medial suspensory ligament branch. A prior study1 of the forelimbs of 7 Thoroughbred cadavers revealed that partial transection of the medial branch of the suspensory ligament led to a significant increase in lateral condylar surface strain of the third metacarpal bone.

The sampling strategy used in the present study likely enhanced the ability to find a defect that could predispose a horse to PSB fracture. Without any a priori knowledge of where a defect exists, systematic sampling would be more likely to miss the defect than find the defect because the defect is likely to be small relative to the bone volume given that defects are not recognized clinically on radiographs. Practically, the location of the defect does not matter as much as the fact that it occurs because any defect could predispose horses biomechanically to fracture. It is also unlikely that the defect occurs randomly in location because bone stresses and strains are not homogeneous but vary by location and loading conditions. Consequently, selection of the specific 3-mm parasagittal bone slice for further processing was based on any presence of morphological findings that could be consistent with a defect to optimize the chances of finding a defect. Any bias in the analysis was minimized by sampling the CILC and the ASMC bones in the same location as in the fractured bone.

The prevalence of preexisting pathological change in PSBs of horses in the present study was probably underestimated. The case horses in this study had 2 fractured PSBs (ie, both the medial and lateral PSBs were fractured) in 1 forelimb and 2 intact PSBs (medial and lateral) in the contralateral limb. Only 2 of the fractured PSBs and only 1 of the CILC PSBs from horses with a PSB fracture were evaluated. As part of our initial study design, only 1 bone was chosen from the fractured set to enter the full processing procedure and these bones were equally chosen from 4 lateral and 4 medial frac-
tured PSBs. Because preexisting lesions were found in 6 of 8 horses with a PSB fracture, the likelihood of selecting a PSB with preexisting lesions if only 1 PSB is actually affected with preexisting lesions is 50%. It follows that, if the nonevaluated fractured PSBs had a lesion, then most, if not all, horses with a PSB midbody fracture would have had preexisting lesions. This proportion may actually be larger because the palmar defects did not extend the entire distance from the medial to the lateral borders of the PSB. Therefore, use of the parasagittal sections for detailed examination may have resulted in omission of affected bone tissue from examination.

Compaction of trabecular bone may be an adaptive response to exercise that could also facilitate pathological bone fracture. Density of PSBs is known to increase with race training.13 In the present study, compacted bone material was more evident in PSBs from horses with PSB fracture than PSBs from horses without PSB fracture. An increase in bone density is expected to increase tissue material properties and whole bone strength. However, high bone density could make affected PSBs more brittle and more likely to completely fail when loading exceeds the bone's yield strength.13 Presence of vascular channels and presence of heme pigment were not associated with PSB fracture. It is possible that some features interpreted as vascular channels were large intertrabecular spaces. Soft tissue cavities with a shape consistent with that of the course of a blood vessel were subjectively interpreted as vascular channels, but the presence of blood vessels could not be determined with the methods we used. As defined in the present study, vascular channels were commonly found in the apex or midbody medial region, but similar to findings in one of our previous studies,3 vascular channels were found more often in bones without PSB fractures. In the present study group, fractures crossed through visible vascular channels but were not diverted to follow vascular channels. Vascular channel walls may have additional matrix supportive tissues that provide rigid but flexible walls that protect the blood vessel.34 Similarly, heme pigment was not associated with PSB fracture.

The clinical importance of these findings is relevant to early injury detection and major injury prevention. Consistent with findings of our previous study,3 it is unlikely that apical or basilar osteophytes would be associated with the linear palmar lesions, whereas large vascular channels visualized on clinical radiographs are typically present in the abaxial or middle region of the PSB. However, we speculate that horses sustain and attempt to repair damage in the bone tissue of the palmar flexor cortex. Affected horses are unlikely to have overt swelling, heat, or pain related to soft tissues, radiographic bone lesions, or metacarpophalangeal joint distention. These horses may have subtle behavioral or clinical signs of PSB disease, and signs of disease may be masked by analgesic or anti-inflammatory medication. Detection of early pathological changes would facilitate appropriate rehabilitation and injury prevention. Signs of focal osteoporosis, bone edema, and bone remodeling may be visible through computed tomography, magnetic resonance imaging, and nuclear scintigraphy, respectively.

In the study reported here, preexisting pathological changes were detected in the palmar flexor region of fractured and CILC medial PSBs from horses with PSB midbody fracture. Proximal sesamoid bones that retained porous medullary radial trabeculae were less likely to fracture than PSBs with compacted medullary trabeculae. Knowledge of the location and morphological features of these findings provides an opportunity to develop techniques for early detection of mild injuries and prevention of PSB fractures.

References

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