Determination of correlation of proximal sesamoid bone osteoarthritis with high-speed furlong exercise and catastrophic sesamoid bone fracture in Thoroughbred racehorses

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OBJECTIVE
To examine whether proximal sesamoid bone (PSB) articular cartilage and bone osteoarthritic changes or palmar osteochondral disease (POD) scores were associated with exercise history and catastrophic PSB fracture in Thoroughbred racehorses.

SAMPLES
PSBs from 16 Thoroughbred racehorses (8 with and 8 without PSB fracture).

PROCEDURES
Exercise history was collected, and total career high-speed furlongs was used as the measure of total exercise per horse. At necropsy, medial and lateral condyles of the third metacarpus from each forelimb were assigned a POD score, followed by imaging with micro-CT for evaluation of osteophyte size. Three investigators that were blinded to the type of PSB (fracture or no fracture) used the Osteoarthritis Research Society International (OARSI) scoring system to evaluate acellularity, chondrocyte necrosis, cartilage fibrillation, chondrone formation, safranin O stain uptake, and tidemark advancement of 1 central sagittal tissue section/PSB (4 PSBs/horse). Cartilage thickness and bone necrosis were scored on the basis of histologic examination.

RESULTS
POD score, osteophyte size score, percentage of bone necrosis, tidemark advancement, chondrone formation, and total OARSI score were greater in horses with more accrued total career high-speed furlongs. Scores for POD, osteophyte size, fibrillation, acellularity, chondrone formation, and total OARSI were greater for horses with PSB fracture.

CONCLUSIONS AND CLINICAL RELEVANCE
OARSI scoring revealed that more advanced osteoarthritic changes strongly correlated with total career high-speed furlongs and PSB fracture. However, the effect of exercise was dominant, suggesting that exercise history will be important to include in future models that aim to assess risk factors for catastrophic PSB fracture. (Am J Vet Res 2021;82:467–477)

Fracture of the PSBs was the most common fatal musculoskeletal injury in horses on New York racetracks from 2013 to 2015, accounting for 35.3% of fatalities.1 Proximal sesamoid bone fracture was also the most overrepresented cause of death from 1994 to 2008 in other US racing jurisdictions, including California, Kentucky, and Florida.1-7 Radiography, CT, and MRI have been used to evaluate the metacarpophalangeal joints of cadaveric horses for evidence of OA or other preexisting joint disease that may have precipitated the fatal injury. A prior study8 reveals that racehorses that sustained catastrophic condylar fracture of the MC3 had MRI-confirmed bone marrow lesions in the MC3 condyle, suggesting that joint disease may precede fracture. Another study9 includes application of the OARSI grading system to demonstrate increased severity of osteochondral histopathologic changes in the MC3 of Thoroughbred racehorses with lateral condylar fractures. In horses that sustained catastrophic fracture of the PSBs, increased macroscopic OA scores and presence of PSB osteophytes are reported.10 However, data from other studies11,12 suggest that catastrophic fracture of the PSBs is not associated with greater radiographic or MRI evidence of OA in the fractured limb. For example, in a cohort of California Thoroughbred racehorses, the odds of PSB fracture were approximately 2 times the odds of fracture for those PSBs without radiographic evidence of osteophytes.11 Similarly, an MRI study12 of cadaveric Thoroughbred racehorses

ABBREVIATIONS
MC3 Third metacarpal bone
OA Osteoarthritis
OARSI Osteoarthritis Research Society International
POD Palmar osteochondral disease
PSB Proximal sesamoid bone
reveals that biaxial PSB fracture is associated with orthopedic disease in the MC3 of the contralateral limb, suggesting that horses with orthopedic disease in one limb may be at increased risk of catastrophic PSB fracture in the contralateral limb as a result of compensatory overloading of this limb.

Radiography, CT, and MRI provide superior assessment of bone versus cartilage and, thus, offer limited ability to assess articular cartilage integrity of the PSBs. Although MRI is best among these imaging modalities to characterize articular cartilage, it is still limited for evaluating the thin cartilage and highly curved articular cartilage surfaces of the metacarpophalangeal joints of horses and by the long acquisition times required for gradient echo sequences. Cartilage changes such as fibrillation and loss of chondrocyte viability could precede more advanced osteoarthritic changes such as osteophytosis and joint space narrowing.

Macroscopic and histologic evidence of preexisting cartilage damage is found in the condyles of MC3 from horses that sustained condylar fractures. Macroscopic changes include wear lines, linear cartilage defects, and ulceration of the distal palmar and plantar aspects of the condyle associated with POD. Interestingly, in 1 study, the degree of preexisting pathological changes in the parasagittal grooves of the medial and lateral condyles was not associated with a horse’s age, career duration, or number of career race starts. Although most studies focus on bone histology, Smith et al used the Mankin scoring system to demonstrate histologic evidence of severe cartilage damage in the palmar aspect of the MC3 from horses with clinical and radiographic evidence of OA, compared with control horses; however, whether any of these horses were racehorses is unclear. Preexisting articular cartilage damage associated with the MC3, including chondrocyte loss, glycosaminoglycan loss, and fibrillation, is positively correlated with the number of race starts in Thoroughbred racehorses; however, no correlation is noted between subchondral bone sclerosis and age or number of race starts. Macroscopic cartilage damage, including wear lines, is documented in the PSBs of horses, but no published studies have included objective histologic scoring of articular cartilage in the PSBs from racehorses that sustained catastrophic PSB fracture. A semiquantitative macroscopic grading system for the MC3 condyles and a microscopic grading system for articular cartilage are described as part of the OARSI histopathology initiative.

Therefore, the objective of the study reported here was to determine whether articular cartilage and osteoarthritic changes of PSBs were associated with exercise history and catastrophic PSB fracture in Thoroughbred racehorses. Specifically, the aims were to compare PSB OA scores in horses with and without PSB fractures by use of macroscopic, micro-CT, and histologic assessment of the PSBs and cartilage, and to determine whether correlations existed between macroscopic, micro-CT, or histologic assessment and total high-speed exercise and fracture. Horses that sustained a PSB fracture were hypothesized to have more advanced OA, compared with those that did not, and OA was hypothesized to correlate with total high-speed exercise.

Materials and Methods

Animals

Proximal sesamoid bones were collected from Thoroughbred racehorses that had been euthanized or that had died on New York racetracks and subsequently underwent necropsy. Bones were harvested from 8 racehorses that were euthanized because of PSB fracture in 1 forelimb (case group) and from 8 sex- and age-matched Thoroughbred racehorses that did not sustain a PSB fracture (control group; same specimens as those used in a previous report). Causes of death for 5 horses of the control group were acute incoordination and subsequent collision with the inside rail of the racetrack, apparent cardiovascular collapse, and suspected acute cardiac event; the other 5 horses were euthanized because of suspensory ligament failure, chronic postoperative laryngeal infection, traumatic cervical fracture, thoracic spinal process fracture and intracranial hemorrhage, and traumatic pectoral wound. Horses ranged in age from 3 to 10 years and included mares, geldings, and colts. When available, 2 PSBs were collected from each forelimb, yielding 15 fractured PSBs, 1 intact PSB in a fractured forelimb, and 16 intact PSBs from the contralateral forelimb of the case group and 30 PSBs from the control group for a total of 62 PSBs. All limbs were dissected by a board-certified anatomic pathologist (SPM).

Exercise history for each horse was provided by the New York State Gaming Commission and included all officially timed high-speed workouts (≤ 14 s/furlong). The total career high-speed furlongs (ie, the sum of race distances and the distance of all official timed workouts) was used as the measure of total exercise per horse. Other exercise history recorded included the total number of race starts, career duration, total rest weeks, and total work weeks. A rest week was defined as any week without high-speed workouts, including racing and training, and a work week was defined as any week with high-speed workouts, including racing and training. All exercise history data were treated as continuous with 1 observation/horse. Age and each exercise history variable were assessed for collinearity, and correlations were reported as Pearson product moment correlation coefficients.

POD and osteophyte size scores

Necropsy reports included macroscopic observations of joint disease, such as articular cartilage damage to the MC3 and PSBs and the presence of PSB os-
osteophytes, on the basis of a grading system described by Barr et al.\textsuperscript{20} Medial and lateral metacarpal condyles of the MC3 of each limb were assigned a grade for POD\textsuperscript{20} as follows: grade 0, no evidence of POD; grade 1, discoloration of subchondral bone without disruption of overlying articular cartilage; grade 2, discoloration of subchondral bone plus mild to moderate disruption of overlying articular cartilage; and grade 3, discoloration of subchondral bone plus collapse of overlying articular cartilage. The mean POD grade for each horse was calculated and reported as a categorical outcome ranging from 0 to 3. All PSBs were subsequently imaged with micro-CT\textsuperscript{3} and then cut with a band saw\textsuperscript{b} or wafering saw\textsuperscript{c} into 2- to 3-mm-thick sagittal sections. The central sagittal section of each PSB was processed for histologic examination.

Micro-CT images of whole PSBs were collected and processed as previously reported\textsuperscript{20} (voxel size, 50 X 50 X 50 \(\mu\)m; projections, 720; exposure time, 20 milliseconds; kV, 100; mA, 50). To remove soft tissue from the images, locally adaptive thresholding techniques were used to identify bone. The local threshold was determined on the basis of mean and SD within a 2.5-mm window with mathematical computing software.\textsuperscript{d} An image-processing program\textsuperscript{e} was used to generate 3-D reconstructions of the bone from images captured with micro-CT so that the entire bone could be seen. Osteophyte size at the apical and basilar margins was then manually scored on a 4-point scale as follows: 0, no osteophyte present; 1, small osteophyte; 2, medium osteophyte; and 3, large osteophyte. For each horse, osteophyte size was calculated as the mean of the scores of the 4 PSBs and reported as a categorical outcome ranging from 0 to 3. So that osteophyte size could be compared between case and control groups, median and interquartile ranges were also calculated.

### OARSI, cartilage thickness, and bone necrosis scores

The central sagittal section of each PSB was fixed in neutral-buffered 10% formalin for 6 days. Following fixation, each section was rinsed for 1 hour in tap water and then decalcified in a 1:1 (vol/vol) solution of 20% sodium citrate and 49% formic acid. Duration of decalcification (range, 3 to 9 days) was determined by daily assessment of the section for pliability and radiographic evidence of decalcification. After decalcification, each section was rinsed in cool running tap water for at least 6 hours and then stored in 50% ethanol until processed (approx 24 to 72 hours later). A tissue processor\textsuperscript{4} was used to dehydrate, clear, and infuse each section with paraffin wax.\textsuperscript{5} Each section was embedded into a block of paraffin wax and sliced into 6- to 8-\(\mu\)m-thick sections with a microtome; each section was then affixed to frosted microscope slides (25 X 75 mm) with slide adhesive. Slides were stained with H&E and with safranin O and fast green stains. Three trained investigators (ENC, SPM, and HLR) who were blinded to the group from which the tissues originated examined the stained slides and scored the cartilage changes using the OARSI scoring system (Figure 1).\textsuperscript{19} The pathologist (SPM) provided the other 2 investigators with training that consisted of evaluating and scoring several slides and comparing scores prior to beginning review of the slides for this study. Three regions per PSB (apical, midbody, and basilar cartilage) were scored 0 to 4 for chondrocyte necrosis, acellularity, chondrone formation (chondrocyte cell clusters), cartilage fibrillation, and safranin O stain uptake and were scored 0 to 2 for tidemark advancement, such that 0 indicated no histopathologic changes and 4 (or 2 for tidemark advancement) indicated severe histopathologic changes. The median was calculated from the investigators’ scores for each OARSI component in each of the 3 regions/PSB. Then, the median scores of the 3 regions were summed to obtain a per-bone value and treated as categorical data. Mean OARSI component scores per horse, calculated as the mean of 4 PSBs, were then calculated and treated as categorical data. Finally, the sum of the 6 OARSI component scores was reported as the total OARSI score per bone (range, 0 to 66) and treated as continuous data.

The same 3 regions of interest/PSB for all 4 PSBs/horse were captured from digitally archived\textsuperscript{20} H&E-stained slides and analyzed in an image-processing program\textsuperscript{f} by 1 investigator (ABT) to determine cartilage thickness. The perpendicular distance from the articular surface to the subchondral bone (total cartilage thickness) and the perpendicular distance from the articular surface to the first tidemark were measured. From these 2 measurements, the perpendicular distance from the first tidemark to the subchondral bone was calculated. Five independent measurements were made for each distance per region, and the mean of the 5 values/bone region was determined. The mean total cartilage thickness for each region of each PSB was obtained and reported as total cartilage thickness per bone.

Bone necrosis (ie, a decrease in viable osteocytes) for all 4 PSBs/horse was determined by analysis of digitally archived\textsuperscript{18} H&E-stained slides (Supplementary Figure S1, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.6.467). Two 20X fields (400 X 400 \(\mu\)m [approx 0.32 mm\textsuperscript{2}]) of the distopalmar one-third of the PSB were obtained,\textsuperscript{18} and the number of lacunae were counted.\textsuperscript{21} Approximately 80 to 250 lacunae/bone (median, 180) were counted as full (viable osteocytes) or empty (nonviable osteocytes) on the basis of H&E nuclear staining within the lacunae. Percentage bone necrosis was calculated by dividing the number of empty lacunae by the number of total lacunae per bone and multiplying by 100\%.\textsuperscript{22}

### Reliability of OARSI score

Intraclass correlation coefficients were used to determine the reliability of the OARSI scores for assessment of OA of the PSBs. Intraclass correlation coefficients were calculated among all 3 investigators.
Figure 1—Photomicrographs of decalcified central sagittal sections of PSBs obtained from 16 Thoroughbred racehorses representative of low, medium, and severe cartilage changes associated with each component that was included in the total OARSI histologic score for PSB cartilage. Safranin O and fast green stains. *H&E stain. Bar = 200 µm. †Safranin O and fast green stains. Bar = 500 µm.
for each of the 6 categorical OARSI component scores and the continuous total OARSI score per bone. One score per investigator per bone was used (total of 62 PSBs) in calculating the intraclass correlation coefficients. The sum of the scores in the 3 regions/bone for each OARSI component score was used. Total OARSI score was the sum of all component scores in all 3 regions/bone. Intraclass correlation coefficients ranged from 0 (low consistency in scores among investigators) to 1 (high consistency of scores among investigators).

Regression models

All categorical data, which included OARSI component (chondrocyte necrosis, acellularity, chondrone formation, cartilage fibrillation, safranin O stain uptake, and tidemark advancement), osteophyte, and POD scores, were reported as 1 observation/horse. The exception was that only 1 forelimb was available for 1 horse (age, 10 years) of the control group, in which scores were reported as the mean of the 2 PSBs only for that forelimb. Categorical data were analyzed by use of ordinal logistic regression models, with total career high-speed furlongs and group (case or control) as model effects and a total furlongs-by-group interaction term where significant. Logistic regression modeling was performed with and without data for the 1 horse of the control group for which data of only 1 forelimb were available.

All continuous data, which included the total OARSI score, percentage of bone necrosis, and 3 cartilage thickness measurements (total cartilage surface to subchondral bone, articular surface to first tidemark, and subchondral bone to first tidemark), were analyzed by a linear mixed-effects regression model. The dependent variables were total OARSI score, percentage bone necrosis, and cartilage thickness measurement. The independent variables were total career high-speed furlongs and group as fixed effects and PSB nested within forelimb and forelimb nested within horse as random effects to account for the hierarchic nature of the data. Linear mixed-effects regression modeling was performed with and without data for the 1 horse of the control group, and the model interaction term total furlongs-by-group was tested for significance.

Scatterplots of the data were created for the number of observations (16 for categorical data and 62 for continuous data) used for statistical analysis. Statistical analysis was performed with commercially available statistical software.1 Values of P < 0.05 were considered significant.

Results

Animals

Eight horses with fractured PSBs (case group) were sex matched and age matched as closely as possible with 8 unaffected horses (control group), with imprecise matching for older animals such that an 8-year-old mare and a 7-year-old gelding in the case group were matched with a 5-year-old mare and a 10-year-old gelding in the control group, respectively.10 Horses in the case group were those that had experienced a PSB fracture during racing or race training, including 1 horse that experienced a PSB fracture prior to entering a single race. Seven horses had fractured PSBs in the left forelimb, and 7 horses sustained biaxial PSB fractures. Seven fractures were basilar, 7 were midbody, and 1 was apical.

Exercise history variables (total career high-speed furlongs [r = 0.93], total number of race starts [r = 0.97], career duration [r = 0.96], total rest weeks [r = 0.94], and total work weeks [r = 0.93]) were highly collinear with one another and positively correlated with age. Because horses were sex and age matched, the total career high-speed furlongs was used as the exercise variable of interest for all models, and age was excluded because of its collinearity with total career high-speed furlongs (Supplementary Figure S2, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.6.467). Mean (SD) total career high-speed furlongs for the case group was 246 (200) and for the control group was 322 (289).

POD and osteophyte size scores

The POD score for MC3 was significantly (P = 0.02) positively correlated with total career high-speed furlongs and was significantly (P = 0.004) greater for the case group (median [interquartile 25th to 75th percentile] range); case, 0.75 [0.06 to 1.18]; control, 0.13 [0 to 0.50]; Figure 2). Also, osteophyte size in the PSBs was significantly (P = 0.001) positively correlated with total career high-speed furlongs and was significantly (P = 0.04) larger for the case group (case, 1.50 [0.56 to 2.09]; control, 0.50 [0.03 to 1.31]; Figure 3).
Total OARSI score was significantly \((P < 0.001)\) greater in horses with more accrued high-speed furlongs, with an increase of 2.4 points (95% CI, 1.1 to 3.6) for each additional 100 accrued high-speed furlongs, and total score was 3.0 points (95% CI, 0.1 to 6.0) greater \((P = 0.045)\) for the case group (Figure 4; Table 1; Supplementary Figure S3, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.6.467). Total career high-speed furlongs and group remained significant \((P = 0.02\) and \(P = 0.04\), respectively) effects in the total OARSI score model, even with the exclusion of the 1 horse in the control group for which only 1 forelimb was available for analyses. When compared with the unaffected contralateral limb of the horses in the case group and the paired forelimb of the horses of the control group, total furlongs and group effects remained significant \((P = 0.04\) and \(P = 0.03\), respectively). Cartilage thickness was similar between groups, except that the distance from subchondral bone to the first tidemark was 22 \(\mu\)m greater \((P = 0.02)\) in the case group (Figure 5; Table 2). Bone necrosis score significantly \((P = 0.02)\) increased by 13% with each additional 10 accrued total career high-speed furlongs but was not significantly \((P = 0.4)\) different between case and control groups.

Figures 3—Representative micro-CT images (A) and corresponding photomicrographs (B) of sagittal sections of PSBs of the horses in Figure 1 and scatterplot and linear regression lines of mean osteophyte size score versus total career high-speed furlongs for the case and control groups (C). A—An image-processing program was used to generate 3-D constructions of the bone as captured with micro-CT so that the entire bone could be seen. Osteophyte size at the apical and basilar margins was then manually scored on a 4-point scale (none [0], small [1], medium [2], or large [3]). B—Sections were stained with safranin O and fast green stains. Bar = 3 mm. C—Mean osteophyte size score was significantly \((P = 0.001)\) positively correlated with total furlongs and was significantly \((P = 0.04)\) greater for the case group. See Figure 2 for remainder of key.
Reliability of OARSI score

Intraclass correlation coefficients ranged from a low of 0.13 for chondrocyte necrosis to a high of 0.94 for cartilage fibrillation, with coefficients of 0.41 for acellularity, 0.75 for safranin O stain uptake, 0.76 for chondrone formation, 0.85 for tidemark advancement, and 0.62 for total OARSI score. Because of the low intraclass correlation coefficient for chondrocyte necrosis, it was considered the least reliable OARSI component score; however, because chondrocyte necrosis is described in the OARSI histopathology initiative, it was included in the calculation of the total OARSI score.

Regression models

Statistical analysis comparing each of the 6 categorical OARSI component scores that comprised the total OARSI score revealed that chondrone formation and tidemark advancement scores were greater with more total career high-speed furlongs for all horses (Figure 4; \( P < 0.001 \)). Fibrillation scores were greater in the control group and lesser in the case group with more total career high-speed furlongs, and greater in the case group and lesser in the control group with fewer total career high-speed furlongs, consistent with a significant \( (P = 0.004) \) interaction between total furlongs and group. Acellularity, chondrone formation, fibrillation and total OARSI scores were significantly \( (P = 0.03, P = 0.04, P = 0.04, \text{and } P = 0.045) \) greater in cases than controls. Tidemark advancement was not significantly \( (P = 0.8) \) different between groups. SaFO stain = Safranin O and fast green stains. See Figure 2 for remainder of key.

Table 1—Median and interquartile (25th to 75th percentile) range for total OARSI and each of the 6 OARSI component scores for the PSBs obtained from Thoroughbred racehorses with (case group; n = 8) and without (control group; 8) PSB fracture. Maximum total OARSI score per PSB was 66, with score range for each PSB of 0 to 4 for acellularity, chondrone formation, cartilage fibrillation, and safranin O stain uptake and 0 to 2 for tidemark advancement. Higher scores indicate greater severity of cartilage changes.

<table>
<thead>
<tr>
<th>OARSI score</th>
<th>Case group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>19.75 (18.69–26.38)</td>
<td>17.88 (8.81–24.81)</td>
</tr>
<tr>
<td>Acellularity</td>
<td>4.38 (4.00–5.19)</td>
<td>3.75 (1.38–4.75)</td>
</tr>
<tr>
<td>Chondrone formation</td>
<td>1.88 (0.56–4.94)</td>
<td>0.25 (0.06–2.63)</td>
</tr>
<tr>
<td>Cartilage fibrillation</td>
<td>4.00 (2.63–4.19)</td>
<td>1.75 (1.25–3.69)</td>
</tr>
<tr>
<td>Chondrocyte necrosis</td>
<td>4.13 (3.56–4.94)</td>
<td>4.25 (2.88–5.31)</td>
</tr>
<tr>
<td>Safranin O stain</td>
<td>4.00 (2.63–5.19)</td>
<td>3.75 (1.25–6.31)</td>
</tr>
<tr>
<td>Tidemark advancement</td>
<td>2.25 (1.19–3.75)</td>
<td>2.25 (1.06–4.31)</td>
</tr>
</tbody>
</table>

Discussion

Histopathologic changes of articular cartilage consistent with OA were noted in the PSBs of the Thoroughbred racehorses in the present study. Cartilage and bone changes indicative of more advanced OA were associated with increased exercise; as measured by total career high-speed furlongs, and OA was associated with catastrophic PSB fracture. Total OARSI scores for PSBs were greater in horses that had more accrued total career high-speed furlongs and that had sustained catastrophic PSB fracture. The OARSI component scores for chondrone formation and tidemark advancement were greater in horses with more total career high-speed furlongs, whereas fibrillation was greater in the control group with more total career high-speed furlongs. The
OARSI component scores for acellularity, chondrone formation, and fibrillation were greater for the case group. Osteophyte, POD score, and chondrones were greater in the case group and in horses with more accrued more total career high-speed furlongs, revealing an association between amount of race training and OA.

Findings from the present study, however, should be interpreted with caution because the Thoroughbred racehorses ranged in age from 3 to 10 years and total career high-speed furlongs ranged from 9 to 889. Even after excluding the 10-year-old horse of the control group for which only 2 PSBs could be evaluated and that had accrued 889 career high-speed furlongs, total furlongs still ranged from 9 to 570. Furthermore, this cohort of horses did not include any that were 2 years old; however, it did include one 3-year-old horse that experienced a PSB fracture during training prior to entering its first race. Therefore, unique risk factors may be associated with PSB fracture in horses that have accrued fewer high-speed furlongs, compared with horses that have accrued more high-speed furlongs. For example, OA changes may not be a prerequisite for PSB fracture in young horses with few high-speed furlongs but may be associated with more total career high-speed furlongs in older horses and subsequent PSB fracture. Age and various parameters of exercise history had high collinearity.

Previous work suggests that PSB fracture is more common in older horses (≥ 5 years) with an extensive race history (≥ 20 race starts). However, larger epidemiological studies are required to determine whether distinct mechanisms or risk factors for PSB fracture are dependent on age, exercise history, or other intrinsic and environmental factors. Horses that do no gallop work prior to racing are at greater risk of fatal limb fracture, likely because of failure of the bones to adapt to loads experienced under racing conditions. Long distances of high-speed galloping also increase the incidence of fatal injuries in Thoroughbred racehorses. Short distances of high-speed exercise, as short as 4 to 10 furlongs/wk, reduce the risk of a fatal fracture of the distal portion of a limb (ie, any bone distal to the radius or tibia that requires euthanasia of a horse). Furthermore, incorporating short distances of breezing (< 20 furlongs/wk) into a training program has a protective effect on the development of fatigue injury of the MC3 (ie, bucked shins). These studies show that the relationship between exercise and fracture is complex, and the ideal training regimen has yet to be determined. Short-distance, high-speed exercise is essential to allow bones to adapt to the rigors of racing; however, excessive long-distance galloping can place horses at greater risk of a fatal fracture.

In the present study, PSBs in the horses of the case group had higher acellularity, chondrone formation, fibrillation, osteophyte size, and POD scores, versus horses of the control group. The formation of chondrones is a histologic hallmark of cartilage associated with OA. Although the biological role of chondrones is poorly understood, available evidence suggests that chondrones may protect against cartilage damage by increasing cell proliferation and matrix production or contribute to the pathogenesis of OA by upregulating hypertrophic differentiation markers and proinflammatory molecules. Chondrone clusters localize at sites of high mechanical loading and at sites of increased cartilage fibrillation.

Figure 5—Scatterplots and linear regression lines of mean bone necrosis scores (A) and cartilage thickness (B) of PSBs (4 PSBs/horse) from the horses of Figure 2. A—Percentage bone necrosis was determined by counting the number of lacunae (approx 80 to 250 lacunae/bone) in two 20X fields of H&E-stained slides of specimens from the distopalmar one-third of each PSB. Then, the number of empty lacunae (nonviable osteocytes) was divided by the number of all counted lacunae (viable osteocytes) plus empty) and multiplied by 100%. Percentage bone necrosis significantly increased with total career high-speed furlongs but was not significantly different between case and control groups. B—Cartilage thickness, the distance from subchondral bone to the first tidemark for 3 regions of each PSB (apical, midbody, and basilar regions), was not significantly correlated with total furlongs but was significantly (P = 0.02) greater in the case group. See Figure 2 for key.

Table 2—Mean (SD) total thickness and thickness of portions of PSB cartilage in the horses of Table 1 and P values for the correlations between PSB cartilage thickness and total career high-speed furlongs and for comparison of PSB cartilage thickness between case and control groups.

<table>
<thead>
<tr>
<th>Cartilage portion</th>
<th>Case group</th>
<th>Control group</th>
<th>Effect of total No. of furlongs (P value)</th>
<th>Effect of group (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular surface to subchondral bone (μm)</td>
<td>935 (132)</td>
<td>858 (157)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Articular surface to first tidemark (μm)</td>
<td>682 (95)</td>
<td>635 (128)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>First tidemark to subchondral bone (μm)</td>
<td>255 (59)</td>
<td>223 (47)</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values of P < 0.05 were considered significant.
in late-stage OA. These data support the supposition that chondrone formation in the PSBs of racehorses may be a response to mechanical overloading.

Fibrillation of articular cartilage and loss of chondrocytes in the superficial and deep osteochondral layers of MC3 are correlated with lateral condylar fractures in Thoroughbred racehorses. In the present study, however, the observed fibrillation in the limb that sustained a PSB fracture may have represented an artifact from trauma that occurred during and following the fracture; yet fibrillation was more severe in the control group with more accrued high-speed furlongs, suggesting that fibrillation was associated with exercise. In Thoroughbred racehorses, fibrillation assigned higher Mankin histologic scores is associated with extrusion of mineralizable extracellular matrix, which could contribute to the fragmentation of the cartilage surface. According to the OARSI histopathology initiative, acellularity may indicate the progression of pathological changes from chondrocyte necrosis, where lacunae with necrotic nuclei are present, to the absence of lacunae or nuclei.

Tidemark advancement was a frequent finding in the PSBs of the fractured limb and contralateral nonfractured limb and was strongly correlated with total career high-speed furlongs. However, tidemark advancement was not significantly different between case and control groups. Muir et al. conclude that for condylar fractures in racehorses, multiple tidemarks indicate an advancement of the calcified cartilage toward the articular surface, which may be because of exercise-induced loss of hyaline cartilage at the articular surface of the high-load-bearing metacarpopophalangeal joint. Interestingly, although tidemark advancement OARSI component scores did not differ between the case and control groups in the present study, the calcified cartilage layer (subchondral bone to first tidemark) was thicker in the case group. The calcified cartilage layer can increase in thickness through the advancement of the calcified cartilage toward the articular surface or the retardation of endochondral ossification at the subchondral bone interface. Cartilage thickness has been previously reported to increase with moderate training in young horses; however, high-intensity training was associated with more fibrillation and chondrones in the calcified cartilage of the middle carpal bones. These data suggest that cartilage thickness, including the calcified cartilage layer, may be worth investigating as a more sensitive measure of tidemark advancement versus OARSI tidemark advancement scores.

Bone necrosis correlates with the accumulation of bone microdamage after fatigue loading and in subchondral bone localized to areas of cartilage injury. Because phenotypic changes in osteocytes, including increased osteocyte apoptosis, are associated with OA, the finding in the present study that bone necrosis increased with total career high-speed furlongs is not surprising. The accumulation of microdamage in PSBs could also contribute to fracture risk. Given that most studies include evaluation of PSBs with 2-D or 3-D imaging at a resolution that would not detect microdamage or with histologic examination of limited tissue sections, identification of PSB microdamage may be missed. Microdamage has been identified in the metacarpopalangeal joint, primarily in the MC3, of racehorses. Microdamage increases with racehorse age up to 5 years old and increases in density at locations of high-bone volume fraction. Additionally, MC3 fractures originate from linear defects in the calcified cartilage and underlying subchondral bone. Whether microdamage could act as a point of crack initiation or lead to crack propagation in catastrophic PSB fracture is unknown.

A strength of the present study was the ability to combine histopathologic findings and knowledge of exercise history. To the authors’ knowledge, the present study was the first to include an evaluation of PSB cartilage from horses that sustained a catastrophic PSB fracture for evidence of preexisting OA. The present study also contributed valuable information about the relationship between articular cartilage and bone changes and race-training exercise programs. The condyles of MC3 have been extensively studied even though PSB fractures occur nearly as frequently as MC3 fractures, and PSB fracture is the leading cause of fatal musculoskeletal injury of horses in the United States. The main limitation of the present study was the small sample size. Other limitations were that histologic examination only included 1 central sagittal bone section and that only PSBs were included in the histologic analysis because MC3 condyles were not available.

The present study revealed that OA of the PSBs correlated with increased total career high-speed furlongs and catastrophic PSB fracture. Larger osteophytes, thicker calcified cartilage, and higher acellularity, chondrone formation, fibrillation, and POD scores were detected in horses that sustained PSB fracture, compared with sex- and age-matched horses without PSB fracture. Future studies would ideally reveal whether fracture risk is associated with OA as a function of exercise history and age; however, more animals would be required. Monitoring for osteoarthritic changes such as osteocyte size with the advances in standing CT or cartilage thickness with contrast-enhanced CT or standing MRI could assist in the monitoring of the joint health of Thoroughbred racehorses.

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Footnotes

a. Explore 120 preclinical CT scanner, GE Healthcare, Barrington, Ill.
b. Butcher Boy Machines International LLC, Selmer, Tenn.
c. Micromech Manufacturing Corp, Roselle, NJ.
e. Aperio ImageScope, Leica Biosystems, Buffalo Grove, Ill.
g. Leica EG1160, Leica Biosystems, Buffalo Grove, Ill.
h. Olympus Cut 4060 E, Olympus Corp, Center Valley, Pa.
i. 670° freezer/mill, Spex SamplePrep, Metuchen, NJ.
j. D8 Advance ECO powder diffractometer, Bruker Corp, Billerica, Mass.
k. DiffraEVA and JADE software, Bruker Corp, Billerica, Mass.
l. JMP, version pro 12.0.1, SAS Institute Inc, Cary, NC.

References