Musculoskeletal injuries account for the overwhelming majority of fatalities in racehorses, and are also a common cause of poor performance and premature retirement. Catastrophic fractures are often subsequent to cumulative stress-related bone injury, and occur at consistent anatomic locations. Stress-related bone injury creates focal areas of weakness within the bone structure that make affected bones highly susceptible to complete fractures during high-intensity exercise. The metacarpo-/metatarsophalangeal joints are commonly affected by stress-related bone injury in racing Thoroughbreds. Early recognition of such injuries is vital to facilitate timely treatment and/or management changes to prevent irreversible damage.

Positron emission tomography (PET) is a nuclear medicine imaging technique with several additional benefits when compared with planar scintigraphy commonly used in horses. PET provides cross-sectional (3-D) imaging, improved spatial resolution, and a greater lesion-to-background radiopharmaceutical uptake ratio. Used with the bone-specific radiotracer $^{18}$F-sodium fluoride ($^{18}$F-NaF), PET imaging is more sensitive for the detection of bone remodeling in the equine fetlock, and has better interobserver agreement when compared with planar scintigraphy. A recent pilot study demonstrated that areas of $^{18}$F-NaF uptake in the fetlock correspond to focal regions of bone resorption with increased vascularity and active osteoblastic activity based on post-mortem histology. $^{18}$F-NaF PET detected lesions not identified with other imaging techniques—in particular, in the palmar aspects of the metacarpal condyles and in the proximal sesamoid bones at sites commonly involved in catastrophic breakdowns.

OBJECTIVE
To assess the repeatability of equine $^{18}$F-sodium fluoride ($^{18}$F-NaF) positron emission tomography (PET) findings, and to evaluate the ability of PET to monitor the progression of areas of increased radiopharmaceutical uptake (IRU) in the fetlocks of Thoroughbred racehorses.

ANIMALS
25 racehorses with clinical signs related to fetlock injuries.

PROCEDURES
This study is a prospective, longitudinal clinical study. Twenty-five racehorses (54 fetlocks) underwent three $^{18}$F-NaF PET scans 6 weeks apart. The first $^{18}$F-NaF PET scan was performed at the start of a 12-week period of rest from racing (lay-up). Areas of IRU in the fetlock joints were quantified using maximal standardized uptake values (SUVmax) and were graded by 2 experienced observers. Statistical comparisons were made between scans to detect changes in IRU grade and SUVmax over time.

RESULTS
Standing PET findings were repeatable, with 131/149 (88%) areas of IRU identified on the initial scans seen again at the 6-week follow-up scan. The palmar/plantar condyles were the sites most commonly presenting with IRU, followed by the proximal sesamoid bones. Overall, 65% of fetlocks demonstrated improvement in IRU grade during the 12-week period of rest from racing. Areas of higher IRU grade took longer to resolve than the lower graded areas.

CLINICAL RELEVANCE
Standing PET findings in the racehorse fetlock were repeatable. The SUV-based grading system may be helpful when determining appropriate lay-up duration for Thoroughbred racehorses. PET may be used to monitor areas of the fetlock involved in catastrophic breakdown injuries in Thoroughbred racehorses.
Recently, a PET scanner designed specifically for imaging the distal limbs of standing horses has been developed that permits serial PET imaging without the risks associated with repeated general anesthetic episodes. The goals of this study were to assess the repeatability of PET findings on sequential scans over time and to evaluate the ability of PET to detect progression of areas of increased radiopharmaceutical uptake (IRU) in the fetlocks of Thoroughbred racehorses. The specific aims of this study were to acquire standing PET scans of the fetlocks of racehorses with lameness or performance issues over a 12-week lay-up period, quantify any areas of IRU using maximal standardized uptake values (SUVmax), and make comparisons between scans to detect changes in SUVmax and IRU grade over time. Our hypotheses were that PET findings would be repeatable, and that SUVmax and IRU grades would decrease significantly during a 12-week period of rest from training and racing.

Materials and Methods

Animals
Twenty-five Thoroughbred racehorses presenting for bone scintigraphy as part of a diagnostic workup for lameness or poor performance, at the referral imaging center of the Equine Hospital of the Southern California Equine Foundation at Santa Anita Park, between December 2019 and June 2020 were recruited for this longitudinal prospective equine PET study. Horses were selected for the PET study based on clinical or scintigraphic evidence of fetlock-related issues. Lameness examination had been performed by the horse’s attending veterinarian. History and clinical data were requested from the attending veterinarian, but a specific lameness examination was not performed as part of this study. All horses underwent a standing PET scan within 3 weeks of bone scintigraphy. The cost of the PET imaging was covered within the provisions of the study. Trainers committed to present the horses for follow-up PET scans 6 weeks and 12 weeks after the initial scan. The comparison between the scintigraphic and initial \(^{18}\)F-NaF PET findings have been reported elsewhere. The protocol was approved by the Animal Care and Use Committee and Clinical Trial Review Board of the University of California, School of Veterinary Medicine. A consent form was signed by the trainers of the included horses. All horses had been in active race training until the first scan in the study. All horses were subsequently withdrawn from training and racing during the 12-week period between the initial and final scans. Clinical and lameness examinations had been performed prior to the initial scan by the treating veterinarian for each horse.

PET acquisition
PET images were acquired with a dedicated standing equine PET scanner (MILEPET, LONGMILE Veterinary Imaging). This scanner comprised a horizontal, freely openable ring of detectors, installed on a wheel platform. The inner diameter of the ring was 25 cm, the detector height was 8 cm, and the scanner had a field of view adjustable from 8 to 22 cm, with translation of the detectors during image acquisition. Jugular catheters were placed aseptically and each horse was injected with 555 MBq (15 mCi) of \(^{18}\)F-NaF IV 30 minutes prior to imaging. Horses were sedated with detomidine (0.01 mg/kg IV) and butorphanol (0.01 mg/kg IV). The imaging protocol involved an initial 1-minute pilot scan over a 16-cm field of view, which was then used to plan a subsequent 3- to 5-minute scan over a 12-cm field of view. The actual duration of the 12-cm field-of-view scan was based on the number of counts obtained during the 1-minute pilot scan. PET images were reconstructed on a 300 × 300 × 288 grid, using 25 iterations of an iterative maximum likelihood expectation maximization algorithm with a 0.8-mm isotropic voxel. The reconstructed PET data were converted for display using an SUV scale. Attenuation correction was not applied.

Image interpretation
PET images were reviewed by an equine surgeon (JP) under the supervision of a board-certified veterinary radiologist (MS) using a DICOM viewer (Horos). Ten specific areas of the fetlock were evaluated for IRU: lateral and medial dorsal third metacarpal/tarsal bone, lateral and medial palmar/plantar metacarpal/tarsal condyles, lateral and medial proximal sesamoid bone, lateral and medial proximal phalanx subchondral bone, and lateral and medial dorsoproximal articular proximal phalanx.

For each area with IRU, the nonattenuation-corrected SUVmax was measured. SUVmax was defined as the tissue concentration of tracer divided by the decay-corrected injected activity and body weight. The Horos 2-D planar region-of-interest (ROI) tool was used on transverse plane images to position manually an ROI centered on an area of the fetlock with subjective IRU. A second, similar ROI in shape and size was drawn where subjective “normal” uptake was present on the same image (referred to as “background”). An SUVmax ratio was calculated for each area of IRU compared to background uptake (ie, IRU SUVmax/background SUVmax). Radiopharmaceutical uptake associated with each area was graded as normal (grade 0), mild (grade 1), moderate (grade 2), or marked (grade 3) based on the ratio between the SUVmax of an IRU site and the SUVmax of the bone background. A grade 1 IRU was defined as an IRU-to-background ratio < 2, a grade 2 IRU as ≥2 but < 3, and a grade 3 IRU as ≥ 3.

Statistical analysis
Statistical analysis was performed using GraphPad Prism (version 9.2.0). The association between the grades of IRU and the different scans, as well as the association between IRU grade at the original and follow-up scans were assessed with the
χ² test. The SUVmax of IRU sites, background, and IRU/background ratios were compared between the different scans using paired analysis. Normality of the distribution of the data was assessed with the Shapiro-Wilk test. The Friedman test was used for non-normally distributed data. Horses with missing data were excluded from the statistical analysis. Statistical significance was set at α = 0.05.

Results

Population

Twenty-five Thoroughbred racehorses with a median age of 3 years (range, 2 to 5 years) were included in this study. This cohort included 13 mares, 7 geldings, and 5 stallions.

All horses were in full training at the time of inclusion. Six horses had never raced, but all others had raced at least once. The median number of starts prior to the initial PET scan was 4 (range, 0 to 18 starts). The initial PET scan was performed at a median of 23 days (range, 1 to 88 days) after the last work, and 116 days (range, 17 to 285 days) after the last race in which the horses had competed. Horses were included either after lameness was localized to the fetlock using diagnostic analgesia and/or based on abnormal uptake in the fetlock on scintigraphy.

Both front fetlocks were scanned in 19 horses, both hind fetlocks in 4 horses, and all 4 fetlocks in 2 horses. This resulted in 54 fetlocks being scanned in total: 42 front fetlocks and 12 hind fetlocks. Of the 25 horses recruited for this study, 21 presented for both follow-up scans, 1 horse missed the 6-week follow-up scan but represented for the 12-week scan, and 3 horses were sold and lost to follow-up after the 6-week scan. The standing PET scan procedure was well-tolerated by all horses and no adverse incidents were reported.

Repeatability and grade evolution of IRU sites

A total of 149 areas of IRU were identified on the initial scans. This included 36 grade 3, 54 grade 2, and 59 grade 1 areas. One hundred thirty-one of 149 areas (88%) of IRU identified on the initial scan were seen again at the second scan. Of the 18/149 areas (12%) of IRU not seen again on follow-up scans, 12/18 (67%) were grade 1 on the initial scan.

The evolution of the grades of IRU identified on the first scan is illustrated (Figure 1). The majority of the grades decreased on follow-up scans, but a grade increase was seen for 23.4% (34/145) and 6.2% (8/129) of the areas of IRU between the initial and 6-week scans, and between the 6-week and 12-week scans respectively. There was a significant association between grade distribution and timing of the scans (χ², P < .001). The number of grade 3 areas of IRU decreased from 36 on the initial scan to 18 and 1 at the 6- and 12-week scans respectively. There was also an association between the original grades and the grades observed at the 6- and 12-week scans (χ², P = .029 and P = .009 respectively). At the 12-week scan, the complete resolution of IRU (grade 0) was 71.1% (32/45), 40.8% (20/49), and 22.9% (8/35) for IRU sites with initial grades 1, 2, and 3 respectively.

A total of 34 sites of IRU were detected on follow-up scans, but were not present on the original scan. A total of 6 new sites of IRU were associated with placement of transcondylar screws between the original and follow-up scans. Of the 28 noniatrogenic sites of IRU that developed after the first scan, 21 (75%) were detected at the 6-week scan, whereas only 7 (25%) appeared on the 12-week scan. The

Figure 1.—Evolution for all sites of ¹⁸F–sodium fluoride increased radiopharmaceutical uptake (IRU) observed in 54 fetlocks from 25 racehorses at 6 and 12 weeks after an initial positron emission tomography (PET) scan. Each column represents a different scan (scan 1, initial scan; scan 2, 6-week scan; and scan 3, 12-week scan). Each row corresponds to a specific site of IRU. A 4-level grading system based on the ratio between maximal standardized uptake value (SUVmax) of the IRU site and the bone background SUVmax was used. Grade 0 was used in absence of IRU and is displayed as dark red. Grade 1 IRU was defined as an IRU-to-background SUVmax ratio < 2 (displayed as bright red), grade 2 IRU as ratio ≥ 2 but < 3 (orange), and grade 3 IRU as ratio ≥ 3 (yellow). The white areas correspond to missing data resulting from horses lost to follow-up.
majority of these new sites of IRU were grade 1 (n = 17, 60.7%), whereas there were 8 (28.6%) grade 2 sites and 3 (10.7%) grade 3 sites.

**SUVmax comparisons**

The SUVmax of the IRU sites and background, as well as the ratios, for each of the 3 scans are presented (Figure 2). No significant differences were found for IRU SUVmax between the initial scan (median, 9.5; range, 2.9 to 31.7) and the 6-week scan (median, 9.6; range, 1.1 to 25.0; P > .999), but significant differences were observed with the 12-week scan (median, 6.1; range, 3.0 to 15.9; P < .001 for both comparisons). The background SUVmax was significantly greater at the 6-week scan (median, 4.9; range, 1.1 to 6.4) compared with both the initial scan (median, 4.5; range, 1.5 to 7.4; P < .001) and the 12-week scan (median, 4.35; range, 2.4 to 6; P < .001). When the ratios IRU SUVmax-to-background SUVmax ratios were used, significant differences were observed among all 3 scans. The median IRU/background ratios were 2.3 (range, 1.2 to 6.2), 2.0 (range, 1.0 to 5.1), and 1.35 (range, 1.0 to 3.9) at the initial, 6-week, and 12-week scans respectively. The P value was .002 between the initial scan and the 6-week scan, and < .001 for the other 2 comparisons.

**Distribution of IRU sites**

The distribution of sites and grades of IRU identified on the initial scans, and their evolution at 6 and 12 weeks are presented (Figure 3). The palmar/plantar condyles were the sites presenting most commonly with IRU, with 74.1% and 66.7% of the medial and lateral condyles affected respectively. The medial palmar/plantar condyles also presented the highest frequency of grade 3 IRU, with 27.8% of the condyles involved. The frequency of IRU decreased over time for all areas, with the medial palmar/plantar condyles remaining the most commonly affected. The only grade 3 IRU site persisting at the 12-week scan involved a medial palmar condyle. The sesamoid bones were the next sites presenting the most frequent IRU, with 42.6% and 22.2% of the medial and lateral sesamoid bones, respectively. Of the 28 sites that developed IRU after the initial scan, the dorsal distal metacarpus/tarsus was the most common site involved, with IRU in 5 lateral and 6 medial dorsal metacarpal ROIs respectively. These dorsal metacarpal/tarsal new sites of IRU included the full range of grades from 1 to 3. IRU appearing in the palmar/plantar condyles after the initial scan only occurred in 3 lateral and 2 medial condyles; all were grade 1.

**Evolution of highest grade per fetlock**

In 20/54 fetlocks (37%), the highest IRU grade on the initial scan was 3. In 24/54 fetlocks (44%), the highest IRU grade was 2. Nine of 54 fetlocks (17%) had
the initial scan and the 12-week follow-up, 65% of the fetlocks showed improvement in the highest grade IRU detected on the initial scan, 29% remained at the same grade, and 6% worsened. At the 12-week scan, only 1 fetlock (2%) still had a grade 3 area of IRU; the most severe IRU grades were grade 2 and grade 1 in 15 (31%) and 21 (44%) fetlocks respectively. Eleven fetlocks (23%) did not present any site of IRU. Examples of fetlock improvement over time are provided in Figures 4 and 5.

**Discussion**

Standing PET images of the fetlocks of 25 racehorses were acquired over a 12-week lay-up for lameness or poor performance. A total of 54 fetlocks were scanned, and 3 scans of each fetlock were acquired: initially at the start of the lay-up period, again after 6 weeks of rest, and, last, after 12 weeks of rest. On each fetlock scan, areas of IRU were quantified using the SUVmax. Subsequently, the areas of IRU were graded based on the ratio between the SUVmax of the area in question and the background SUVmax.

This study confirms the repeatability of 18F-NaF PET findings in the fetlocks of Thoroughbred racehorses on sequential scans over time. The majority of areas (88%) of IRU identified on the initial scans were observed again at the 6-week follow-up scans. Of the 12% of areas of IRU not seen again on follow-up scans, the majority were grade 1, and could plausibly have resolved by the time of the 6-week follow-up. The repeatability of PET findings demonstrated in this study indicates that PET may be a useful technique to monitor osseous lesions in the fetlocks of Thoroughbred racehorses over time. The ability to detect differences clearly in areas of IRU identified on scans performed 6 weeks apart is an illustration of the temporal resolution of PET imaging.

The results of this study highlight the ability of standing 18F-NaF PET to detect progression of areas of IRU in the fetlocks of Thoroughbred racehorses. PET demonstrated improvement in IRU in the majority of fetlocks (65%) with areas of IRU over the 12-week lay-up period. In previous work, areas of increased 18F-NaF uptake were associated histologically with bone resorption and active osteoblastic activity, indicating that sites of increased 18F-NaF uptake reflect areas only grade 1 IRU, and 1/54 (2%) had no detectable IRU (grade 0). Based on the highest grade area of IRU, 46% of fetlocks improved between the initial scan and the 6-week follow-up, 29% were static, and 25% worsened. Between the 6-week and the 12-week follow-up scans, 59% of the fetlocks improved, 35% remained at the same grade, and 6% worsened. Overall, between the initial scan and the 12-week follow-up, 65% of the fetlocks showed improvement in the highest grade IRU detected on the initial scan, 29% remained at the same grade, and 6% worsened. At the 12-week scan, only 1 fetlock (2%) still had a grade 3 area of IRU; the most severe IRU grades were grade 2 and grade 1 in 15 (31%) and 21 (44%) fetlocks respectively. Eleven fetlocks (23%) did not present any site of IRU. Examples of fetlock improvement over time are provided in Figures 4 and 5.
of bone modeling. The results of our study indicate that bone modeling activity in the fetlock may be ongoing in 50% of racehorses after 6 weeks of rest, and in 35% of racehorses after 12 weeks of rest. Previous studies suggested that adaptation to rest (ie, the point at which homeostasis in the processes of bone removal and replacement is reached) may take between 4 weeks and 4 months.\textsuperscript{1,4} The findings of our study suggest that a lay-up duration of 12 weeks or longer may be preferable for Thoroughbred racehorses. The duration of the rest period should also be guided by the grade of IRU of an area of interest, as the higher the initial grade of IRU, the lower the likelihood of that area of IRU being resolved in 12 weeks.

It is worth noting that 25% of areas of IRU demonstrated an increased grade between the initial scan and the 6-week follow-up. The reason for some areas of IRU failing to resolve, and new areas of IRU developing during lay-up, is unknown. Osteocyte mechanosensitivity is driven by hormonal regulators; thus, it may affect racehorses differently at differing stages of skeletal maturity.\textsuperscript{11} Furthermore, horses with a high initial bone volume fraction (ie, those that have undergone more intensive training) take longer to reach homeostasis in terms of bone turnover during rest than horses with an initially lower bone volume fraction.\textsuperscript{14} Last, the level of activity of horses during lay-up was not controlled or recorded; therefore, some horses may have had more exercise than others.

The background SUVmax was slightly greater at the 6-week follow-up scans than at the initial scans. This increase can be explained by the change in activity, with horses being removed from training. Increased bone resorption, associated with subchondral bone repair, has been reported in resting racehorses after a period of training.\textsuperscript{15} The increased osteoclastic activity is likely responsible for the increased \(^{18}F\)-\textsuperscript{NaF} uptake observed in our study at the 6-week follow-up scans.

The palmar/plantar condyles were the sites presenting most commonly with IRU in our study, and the highest frequency of grade 3 areas of IRU. This result concurs with a previous study\textsuperscript{16} describing patterns of radiopharmaceutical uptake in the fetlocks of Thoroughbred racehorses in planar nuclear scintigraphy.\textsuperscript{16} In a previous equine PET study\textsuperscript{8} published by our group, uptake in the fetlock correlated histologically with bone resorption, hypervascularization, and osteoblastic activity. These changes are proposed to represent subchondral bone modeling, which occurs commonly in the metacarpal/tarsal condyles of racehorses in training.\textsuperscript{17,18} Subchondral bone modeling reinforces the subchondral bone plate, enabling the bone to withstand the cyclic loading of training and racing.\textsuperscript{19,20} However, subchondral bone modeling has also been shown to precede the occurrence of microdamage in equine bone,\textsuperscript{19,21,22} which in some cases can progress to a stress fracture.\textsuperscript{23} There is evidence that \(^{18}F\)-\textsuperscript{NaF} PET may help differentiate between the effects of mechanical loading that do not produce damage, and fatigue loading that does result in bone injury.\textsuperscript{24} Although PET uptake may be associated with stress-related bone injury,\textsuperscript{24,25} further work is needed to define normal ranges of SUVmax in regions of the Thoroughbred fetlock, and to correlate IRU grades and SUVmax ratios with clinical outcomes such as lameness and reduced performance. With ongoing accumulation and analysis of data, it may be possible to predict the probability of certain lesions progressing to a performance-limiting or even catastrophic injury. For example, focal areas of PET uptake have been identified in the proximal sesamoid bones at sites where transverse fractures originate.\textsuperscript{8,22} Early detection of such changes may inform management strategies designed to mitigate further damage and reduce the risk of catastrophic breakdown injury. PET imaging could also be useful in detecting prodromal changes associated with palmar osteochondral disease, a condition notoriously difficult to detect prior to development of significant subchondral bone injury.\textsuperscript{22}

Our study also further demonstrates the validity of the lesion-to-background SUVmax ratio as a method for quantifying changes in radiopharmaceutical uptake in ROIs over time. The use of a ratio negates the effect of inter- and intrahorse variability in radiopharmaceutical uptake in the distal limbs of horses, which precludes direct comparison of SUVmax values in ROIs over time. This technique may be used to accrue objective data to monitor more accurately the progression of stress-related bone injuries in the fetlocks of Thoroughbred racehorses in both clinical and research settings. Further work correlating SUVmax ratios with clinical outcomes is needed before using this method as a prognostic tool.

The major limitations of our study are the small number of horses enrolled and the paucity of clinical information available for most horses. Because of these limitations, it was not possible to comment on the clinical significance of areas of IRU in individual horses. Further limitations include the small number of areas of IRU in some anatomic regions of the fetlock (eg, proximal P1 subchondral bone), which made it difficult to derive meaningful data regarding their progression over time. Last, the position of ROIs to obtain background SUVmax values was not standardized, but rather selected based on the observer’s opinion of an area of “normal” SUV. Attempts were made initially to standardize the background ROIs based on anatomic landmarks, but this method proved to be problematic. On some scans, the anatomic fixed areas coincided with representive background radiopharmaceutical uptake. However, on other scans, the fixed ROIs overlapped areas of IRU, or areas of poor radiopharmaceutical uptake, resulting in a misleadingly high or low background SUVmax. For this reason, basing the background ROI on fixed anatomic landmarks was eschewed in favor of selecting an area judged subjectively to be representative of background radiopharmaceutical uptake for each individual scan.

Standing \(^{18}F\)-\textsuperscript{NaF} PET detected areas of IRU in the fetlocks of Thoroughbred racehorses that were repeatable over time. Serial PET scans demonstrated temporal improvement in these areas of IRU in 65%
of fetlocks over a 12-week lay-up period. Higher graded areas of IRU take longer to resolve than lower graded areas—a finding that may be helpful when determining appropriate lay-up duration for Thoroughbred racehorses. Grades of $^{18}$F-NaF uptake based on SUVmax ratios appear to be a useful tool to measure and monitor bone turnover objectively in the Thoroughbred fetlock.

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