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Associations between the radiographic appearance of vascular channels in proximal sesamoid bones, their microstructural characteristics and past racing performance in Thoroughbreds

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Running head: Microstructural analysis of the proximal sesamoid bone

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Summary

Background: Abnormalities in vascular channel appearance within the proximal sesamoid bone (PSB) are the most common findings in Thoroughbred yearling presale radiographs, and are often evaluated on radiographs of adult racehorses. Despite this, their pathogenesis and clinical significance are poorly understood, and associations with racing performance are inconsistent.

Objectives: To determine microstructural characteristics of the PSBs associated with the radiographic appearance of vascular channels using microcomputed tomography (μ CT), and to determine associations with past racing performance in mature horses.

Study design: Cross-sectional.

Methods: One pair of PSBs were isolated from a forelimb of 59 Thoroughbred racehorses undergoing post-mortem examination. Each PSB ($n = 118$) was radiographed, assigned a vascular channel grade using previously published and novel grading systems, then imaged using μ CT. Associations between radiographic, μ CT and performance variables were investigated with uni- and multivariable generalised linear models.

Results: All PSBs had at least one vascular channel (mean 3.6 ± 0.89) observed on μ CT originating from the abaxial border, yet in only 63.6% (75/118) were channels observed radiographically. Proximal sesamoid bones with a higher bone volume fraction (odds ratio [OR] 1.08; 95% confidence interval [CI] 1.01-1.15; $P = 0.03$) and wider channel diameter (mm) on μ CT (OR 20.67; 95% CI 3.29-130.00; $P = 0.001$) were more likely to have vascular channels identified on radiographs. Greater radiographic channel number (OR 0.96; 95% CI 0.92-1.00; $P = 0.04$) and channel diameter (mm; OR 0.96; 95% CI 0.92-1.00; $P = 0.04$) were associated with fewer career placings.

Main limitations: Radiographs of isolated bones avoided the normal superimposition of tissue encountered in the live horse.

Conclusions: The ability to identify vascular channels radiographically indicates widening of channels and densification of the PSB. More radiographic channels and greater channel diameter were associated with similar or poorer measures of past performance, suggesting that these changes are not desirable.

53 Introduction

54 The health status of the proximal sesamoid bone (PSB) within the metacarpophalangeal joint
55 of equine athletes is often assessed based on the presence and appearance of vascular
56 channels on radiographs. However, there is a lack of consensus on the clinical significance of
57 changes in PSB vascular channel morphology, commonly referred to as 'sesamoiditis'.
58 Theories for the pathogenesis of sesamoiditis include being secondary to circulatory
59 disturbances [1], as an adaptive (physiologic) [2] or maladaptive (pathologic) [3] response to
60 race training, or secondary to repeated trauma associated with hyperextension of the fetlock
61 joint, with or without concurrent suspensory ligament branch injury [4].

62 Definitions of what constitutes a 'normal' vascular channel in the current grading
63 systems are inconsistent, with authors divided on whether the coning of channels at the
64 abaxial margin observed in some channels is a normal variation [5] or abnormal change [6].
65 Similarly, the association between a radiographic diagnosis of sesamoiditis and performance
66 in the horse is poorly defined. In Thoroughbred yearling horses the presence of radiographic
67 vascular channels on radiographs has been described variously as an incidental finding [7],
68 the cause of clinically significant lameness [8] or as positively associated with measures of
69 racing performance [9]. As vascular channel morphology at the time of yearling sale is used
70 to predict future athletic ability, the lack of standardisation of what is radiographically
71 classified as normal or abnormal is a highly relevant issue.

72 Microcomputed tomography (μ CT) allows assessment of bone microstructure in three
73 dimensions and has been used to predict bone quality and mechanical properties. Bone
74 volume fraction (BV/TV) is a three-dimensional morphological parameter of bone that can be
75 measured with μ CT describing the amount of mineralised bone per unit volume, which is
76 correlated with bone loading ability and strength under both single and cyclic loading
77 [10,11].

78 The primary objective of this study was to determine the characteristics of PSB
79 microstructure associated with the radiographic appearance of vascular channels in
80 Thoroughbred racehorses through the use of μ CT, and to report the prevalence of
81 morphological variables including coning at the abaxial margin. The second objective was to
82 determine whether the presence, number and diameter of vascular channels had an

association with past racing performance in mature horses. We hypothesised that more prominent vascular channels on radiographs of proximal sesamoid bones would be associated with wider channel diameter as measured with μ CT and a higher BV/TV resulting in greater contrast between voids and surrounding bone. We also hypothesised that racehorses with better measures of past performance would have higher radiographic scores for vascular channels within forelimb PSBs when compared to those of less well performed horses, as was similarly found in a previous prospective study on yearlings in Australia [9].

Materials and Methods

Study Population

Criteria for sample collection were Thoroughbred yearlings in pre-training, mature Thoroughbreds in race training and those resting from training or retired from racing within a period of 12 months.

Sample Preparation

The paired PSBs from one randomly selected forelimb of horses presenting for post-mortem examination to the Melbourne Veterinary School, University of Melbourne, Victoria, Australia from 1 December 2008 to 31 December 2016 were isolated. The fetlock was disarticulated and the PSBs were dissected from soft tissue attachments, with the intersesamoidean ligament initially kept intact. The articular surfaces were photographed, lesions grossly described and drawn for reference before the samples were stored in 70% ethanol at room temperature.

Sample Imaging

The paired sesamoids were removed from ethanol, placed in sealed bags, and positioned on a computed radiography detector using foam padding to simulate the radiographic projections in the standing horse (dorso45°lateral-palmaromedial and dorso45°medial-palmarolateral oblique projections; 55 kV, 160 mA and 20 ms (3.2 mAs); generator: CPI CMP 200¹, tube: Varian Medical System². A steel ball calibration marker (25 mm) was used to facilitate correction for magnification.

Following radiography, the sesamoids were separated through the intersesamoidean ligament and individual whole bones were imaged with μ CT (Scanco 50³) at a resolution of 24 μ m using x-ray settings of 70 kVp, 200 μ A and 0.5 mm aluminium filter. Using the proprietary Scanco 50 μ CT software, the whole PSB was selected as the region of interest, image segmentation was performed and BV/TV and bone material density were determined.

Data Collection

A modified radiographic grading system for PSB changes was developed based on grading systems previously published to provide a more comprehensive and objective radiographic assessment of vascular channel morphology [4,5,7,12]. Binary variables (definitions provided in Table S1) including vascular channel presence, sclerotic vascular channel outline, focal lucencies, an irregular abaxial margin, osteophytes, enthesophyte, abnormal sesamoid shape, sesamoid elongation or fracture were allocated a score of zero (absent) or one (present). A scale of increasing grade with severity of changes observed was used for variables including the number of clearly visible radiographic channels (≤ 2 channels, > 2 channels), morphological appearance of the vascular channels (parallel for length, parallel with conical opening at the abaxial margin, combination of channels with parallel and non-parallel margins, all channels with irregular margins), vascular channel depth (channels extending either < 10 mm or > 10 mm from the abaxial margin) and vascular channel diameter (all channels < 2 mm, combination of channels < 2 mm and ≥ 2 mm, all channels ≥ 2 mm in diameter measured objectively on radiographs with correction for magnification). Each sesamoid was given a composite radiographic score calculated by standardising each individual grade for the variables channel number, diameter, opacity, shape and depth, summing the standardised scores and presenting them on a scale from 0 - 100.

A similar grading system for assessing the μ CT sequences was also developed. This included the same variables such as vascular channel number, shape and diameter (mm) as defined for the radiographic grading system. Additional microstructural variables included the nature of trabecular bone surrounding the channels, presence of cyst like structures, articular surface irregularities, focal and generalised subchondral bone resorption including generalised degree of porosity, and osteophytes. Continuous data recorded included BV/TV (as a percentage),

apparent and material density (mgHA/cm³). The radiographic and μ CT grading tables are provided in Tables S2 and S3 respectively.

Intra-observer repeatability testing to determine differences in reliability of the novel radiographic grading system compared with the existing systems (Table S4) between two assessments was performed by the primary author (equine surgical resident). These assessments were not blinded but were undertaken 6 months apart.

Horses that started in at least one race were included in the performance analyses (n = 42), with racing history for each recovered using Racing Victoria Limited's online database [12]. Horses excluded from performance analyses were in training but unraced at the time of death (n = 17). Continuous predictors of performance included age at first start (years), years racing, prizemoney (career total \$AUD), performance rating at last start (Victorian Handicap Rating [13]), career starts, career places, career wins, seconds and thirds, number of group or listed starts and number of group or listed places. Categorical variables included sex (filly/mare, colt/stallion or gelding), training status at time of death (resting, training), race distance at last start (sprint, mile, intermediate, long, extended) and whether the cause of death was due to catastrophic fracture.

Data Analysis

The minimum sample size required for a two-tailed test to ensure adequate repeatability between radiographic and μ CT assessments was determined. Assuming 80% power, an estimated prevalence of visible vascular channels of 0.3 and a null hypothesis of 0.5, a minimum of 77 observations to detect a kappa coefficient of 0.8 as statistically significant was required [15], which was below the number of samples used for analysis in this study (n = 118).

Intra-observer reliability between the novel and existing systems were calculated using weighted Cohen's kappa coefficient (κ) with 95% confidence intervals (CI). Agreement was assessed using previously described scales ('poor' at a kappa statistic of <0.00, 'slight' at κ = 0.00 - 0.20, 'fair' at κ = 0.21 - 0.40, 'moderate' at κ = 0.41 - 0.60, 'substantial' at κ = 0.61 - 0.80 and 'almost perfect' at κ = 0.81 - 1.00) [16].

Univariable generalised linear models accounting for clustering at the horse level were generated to investigate associations between radiographic and μ CT variables, and between radiographic and performance variables. Binary logistic regression and ordered logistic regression models were fitted for outcome variables with two or more than two categories respectively. All models were adjusted for clustering on horse identification number to account for within horse measurements from both lateral and medial PSBs. To compare differences in outcomes between the lateral and medial proximal sesamoid bones, equality tests on matched data were performed via the Wilcoxon sign-rank test. Odds ratio (OR) and 95% CI were recorded. For the ordered logistic regression models, a proportional odds assumption test was used to assess whether the relationship between each outcome category was the same. Instances where the proportional odds assumptions were not met were due to low category sample numbers. Model diagnostics, including assessing goodness-of-fit, Pearson and Deviance residuals, checking for multicollinearity, and leverage of influential observations were performed. Predictors with $P < 0.2$ from the univariable analyses were entered into a multivariable model, removed in a backwards stepwise process, and retained if $P < 0.05$, with potential confounding variables (such as age) included if they modified the coefficients of other covariates by greater than 20%.

Poisson regression (count outcome data) and linear regression (continuous outcome data) were used to investigate associations between selected μ CT (BV/TV, vascular channel number and diameter) and performance variables (age, years racing, career starts, places and prizemoney). Stata/SE (14.2)⁴ was used to perform all statistical analyses.

Results

Study Population

One hundred and eighteen PSBs were collected from 59 horses (left fore from 26 horses, right fore from 33 horses). Age ranged from 16 months to 12 years, with a mean ($\pm$ s.d) of 4.4 ± 2.4 years. Fourteen horses were female, 29 horses were geldings, and 16 horses were entire males. Nine horses were resting from training and 50 horses were in race training at the time of death.

Of the 42 horses that had started in at least one race meeting the inclusion criteria for performance analyses, six were entire males, 24 were geldings and 12 were female. The mean age at first start was 2.8 ± 0.7 years and age at death was 5.2 ± 2.3 years, with 2.2 ± 2.3 years racing. The mean number of career starts was 22.6 ± 25.1 . Thirty-eight horses were in training at the time of death, and four were resting. Prizemoney earned was \$AUD $265,260 \pm 801.51 \times 10^3$.

Repeatability Testing

Intra-observer agreement between assessments for each of the previously published and novel radiographic grading systems was greater than 90%. The strength of agreement (κ) was almost perfect for the dichotomous and modified Spike-Pierce & Bramlage scale, and substantial for the remaining grading scales assessed (Table 1). If the cumulative radiographic score was altered to only encompass the variables used in the existing scales i.e. radiographic vascular channel presence, number, diameter and shape, the agreement increased to 93.9% and $\kappa = 0.78$, which was still equivalent to substantial.

Radiographic and Microstructural Analysis

Radiolucent lines originating from the abaxial margin interpreted as vascular channels were present radiographically in 63.6% (75/118) of PSBs, whereas on μ CT all PSBs had clearly defined channels originating from the abaxial margin and traversing the bone in a dorsal and distal direction, with a mean channel number of 3.60 ± 0.89 per sesamoid bone. The mean channel diameter on μ CT was 1.45 ± 0.37 mm, with the minimum diameter recorded at 0.8mm and maximum of 3.6mm. All PSBs had at least one channel with coning at the abaxial margin on μ CT, most commonly involving the most proximal channel (Figure 1).

Mean whole bone BV/TV was $78.8 \pm 5.6\%$, with a minimum of 62.9% recorded in an 18-month-old colt in pre-training, and maximum of 90.6% in a three-year-old filly in race training (Figure 2). Medial PSBs were found to have proportionally more bone tissue than lateral PSBs (BV/TV $79.9 \pm 5.1\%$ vs. $78.3 \pm 6.0\%$; $P < 0.001$). Vascular channel number ($P > 0.9$) and diameter ($P = 0.06$) were also compared between medial and lateral PSBs on μ CT and not found to be different.

Findings from univariable analysis are provided in Table S5. Significant findings from the multivariable analysis between radiographic outcome variables and μ CT predicting variables are provided in Table 2. Bone volume fraction was a key predictor for identifying vascular channels on radiographs, for greater numbers of channels, those channels having a more varied shape, greater diameter and the PSB having a higher radiographic composite score. A greater channel diameter on μ CT was positively associated with the identification of channels radiographically, those channels having a greater radiographic diameter and the PSB having a higher cumulative radiographic composite score. A more varied channel shape on μ CT was positively associated with identifying a greater number of channels on radiographs and those channels having a more varied channel shape radiographically. Bone material density was negatively associated with identifying channels of a larger diameter radiographically and was associated with lower cumulative radiographic scores.

Whether the horse was euthanised due to catastrophic fracture was included in analysis, but no association with any radiographic variable was identified on univariable analysis and there was no evidence of confounding. Fracture as an outcome variable was also assessed univariably against μ CT variables including bone volume fraction and material density, with no association found ($P = 0.1$ and $P = 0.1$ respectively).

Performance Analysis

Findings from univariable analysis are provided in Table S5. Significant findings from the multivariable analysis between radiographic outcome variables and performance predicting variables are provided in Table 3. Career seconds, career places, career starts, years racing and age were all highly correlated with each other (Spearman's rank correlation coefficient [ρ] 0.82-0.96, $P < 0.001$), therefore only one of these variables remained in each of the multivariable models. Having more than two radiographic vascular channels, abnormal morphology and/or a wider channel diameter were associated with fewer career places, however the 95% CI's included an OR of 1, suggesting that this association was not very strong. Presence of vascular channels on radiographs and higher cumulative radiographic scores were associated with fewer career seconds, but no association was found with career places in either model ($P = 0.6$ and $P = 0.07$, respectively).

No significant association was found between the μ CT outcome variables BV/TV, vascular channel number or diameter and performance variables including horse age, number of career starts, career places or total prizemoney.

Discussion

We have shown that changes in bone volume fraction (BV/TV) and proximal sesamoid bone (PSB) vascular channel diameter are the microstructural variables most commonly associated with the ability to identify vascular channels, in greater numbers and of more abnormal shape on radiographs. More severe radiographic changes (consistent with various definitions of 'sesamoiditis' [1,5,7]) were associated with some indicators of poorer performance in the mature Thoroughbred racehorse such as career places. On μ CT, coning at the abaxial margin was observed in all sesamoids and the majority of vascular channels measured less than two millimetres in diameter.

The radiographic identification of vascular channels combined with an increased number of channels with a larger diameter or abnormal vascular channel shape were associated consistently with an increased whole bone BV/TV. On radiographs it has been suggested that a 30% loss of bone mineral is required for consistent visualisation of lysis, however this is based on osteoporotic bone and does not apply to denser bone where a greater than 6.6% bone mineral loss is consistently observable [17]. On an oblique projection, X-rays traverse a maximum width of 30 mm (although usually much less) at the abaxial margin of the PSB [18], therefore the range of vascular channel diameters potentially spans the 6.6% threshold. In addition, consistent with our findings, lytic areas within less dense bone are more difficult to observe due to lack of contrast [17].

The arbitrary 2mm maximum diameter for a 'normal' vascular channel [4,5,9,18] is consistent with our finding in that 96% of channels measured on μ CT were below this. However, based on findings from Williamson *et al.* that channel diameter in the distal metacarpal subchondral bone may change in response to race training and exercise, the classification of a channel as normal or abnormal based on its diameter alone may be problematic [19]. The characteristic coning of vascular channels at the abaxial margin of the PSB was observed in all samples on μ CT which supports this feature as being a normal

variation in channel morphology. In contrast, the radiographic grading system of Kane *et al.* classes sesamoids with this characteristic as abnormal [6].

Having radiographically visible vascular channels within the PSB, those channels being greater in number and with a more abnormal morphology were tentatively associated with fewer career placings, but with no effect on total career prizemoney. These results are similar to those of Spike-Pierce and Bramlage (2003) who found that yearling horses with evidence of prominent vascular channels at the time of sale had poorer outcomes for injury and performance, concluding that ‘sesamoiditis’ was negatively associated with future athletic ability [5]. Our study examined an older cohort of horses and it is well documented that vascular channel appearance on radiographs can change over time, making comparisons difficult [2,3,20]. Despite this, PSB vascular channel presence and morphology appear to be relatively poor predictors of performance given that few variables were weakly associated with performance in the current study.

On intra-observer repeatability testing of both the previously published radiographic grading systems compared with a novel system created by the authors, no differences were identified with all systems having good agreement. Although this suggests that no system is more reliable than another for the assessment of vascular channels on radiographs, it does not reflect how accurate the systems are for correctly identifying pathology. For example, although testing showed that identification of the coning of channels on radiographs has good repeatability, it gives no indication as to which system is correct in classing them as a normal variant or abnormal change. Given the radiographic assessment of channels within the PSB in yearlings may be used to predict future athletic performance, further investigation is required.

With career progression (indicated by greater number of career seconds, places, starts, years racing and age), horses were found to have more severe radiographic changes consistent with ‘sesamoiditis’. Similarly, the severity of palmar/plantar osteochondral disease (POD), a condition also associated with increased subchondral BV/TV and prominent vascular channels [19], is associated with cumulative race starts irrespective of horse age [21]. Therefore, it is likely that these combined radiographic changes in proximal sesamoid bones are signs of accumulated intense cyclic loading and that skeletal injury often associated with such a history may be the reason performance is somewhat compromised. The presence

of vascular channel morphological changes on radiographs could be used as an early indicator of skeletal stress and overload, however not associated with catastrophic fracture risk in this population, which can be associated with both under and over training [22]. Interestingly, BV/TV was not found to be associated with age or training history as has been identified in other regions of the equine skeleton [23,24]. This may reflect a modelling response to accumulating micro-damage within the sesamoid bone, as has been suggested in a previous study of PSB morphology [25] which found BV/TV to be greater in fractured PSBs compared with age- and sex-matched controls in a population of Thoroughbreds.

There are several limitations to this study. Only the forelimb PSBs were collected which did not allow for comparison with hindlimbs as previous radiographic studies have done [2,3,5,6]. Radiographs of isolated PSBs avoided normal superimposition of tissue encountered in the live horse, with some additional attenuation of X-rays occurring in the clinical situation reducing the ability to identify subtle features of the bone. However, the incidence of channel identification in this study (63.6%) was much lower than previously reported in observational studies of yearling radiographs (98%) [6,9], suggesting this effect was minimal. The cross-sectional study design meant that changes in sesamoid vasculature over time and workload with the effect of skeletal maturity were not assessed. The assessment of radiographic and μ CT variables was largely subjective and although the μ CT resolution was thought to be sufficient, voids less than 24 μ m were not identified, thus influencing measurements determined by proprietary software. Fetlock pathology other than that affecting the sesamoids was not included in the performance analysis, even though lesions such as palmar osteochondral disease have previously been associated with poorer performance [21]. However, lesions of the metacarpal condyles are challenging to assess radiographically [26] and it is most clinically relevant to determine if what observers can easily assess on radiographs can predict performance, such as changes consistent with 'sesamoiditis'. Also, differences between study populations for variables such as horse age, number of career starts and years racing makes comparisons with previous studies difficult, and the relatively small sample size and collinearity between predictors restricting the fitting of more informative multivariable models.

In conclusion, the ability to observe proximal sesamoid bone vascular channels of any morphology on radiographs suggests densification of the bone and widening of the channels

343 themselves. This observation is consistent with the response of bone to excessive cyclic
344 loading and microdamage. Radiographic evidence of ‘sesamoiditis’ referred to in this
345 instance as the presence of wide, irregular vascular channels in the mature racehorse may
346 therefore be a sign of skeletal overload which is consistent with our observation of associated
347 poor performance, although the associations with performance were tentative at best.

349 **Authors’ declaration of interests**

350 No competing interests have been declared.

352 **Ethical animal research**

353 Tissue collection was in accordance with the guidelines of The University of Melbourne’s
354 animal ethics committee (Ethics ID #14001).

356 **Owner informed consent**

357 Owners gave consent for their animals' inclusion in the study.

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367 **Authorship**

368 K. A. Lloyd and R. C. Whitton were involved in all aspects of study design, sample
369 collection, data analysis and manuscript preparation. B. A. Ayodele contributed to study
370 design and data collection. P. L. Hitchens contributed to study design and data analysis. C.
371 Beck contributed to study design and data collection. K. A. Lloyd, R. C. Whitton and P. L.
372 Hitchens had full access to all data in the study and take responsibility for integrity of data

and accuracy of analysis. E. J. Mackie contributed to study design and manuscript preparation. All authors approved the final manuscript prior to submission.

Manufacturers' addresses

¹CPI CMP 200, Georgetown, Ontario, Canada June 2004

²Varian Medical System, North Charleston, South Carolina USA June 2015

³Scanco 50, SCANCO medical AG, Bruttisellen, Switzerland

⁴Stata/SE 14.2, StataCorp 2015 *Stata Statistical Software: Release 14* College Station, TX: StataCorp LP

Tables

Scale Type	Agreement (%)	Weighted Kappa Coefficient [κ] (95% CI)	Standard Error
Dichotomous	95.00	0.89 (0.81, 0.98)	0.09
Spike-Pierce & Bramlage [5]	94.33	0.71 (0.66, 0.78)	0.06
Modified Spike-Pierce & Bramlage [4]	95.83	0.82 (0.74, 0.88)	0.07
Modified Kane [4]	90.42	0.77 (0.75, 0.84)	0.07
Plevin <i>et al.</i> [12]	94.58	0.75 (0.71, 0.89)	0.06
Cumulative Radiographic Score	91.61	0.74 (0.70, 0.74)	0.06

Table 1: Agreement measures for categorical data in the assessment of proximal sesamoid bone vascular channel morphology using previously published and a novel radiographic grading system. [CI = Confidence interval].

Radiographic Outcome

Variable	μCT Predicting Variable	OR	95% CI	p-value
Presence of vascular channels ^a	BV/TV (%)	1.08	1.01, 1.15	0.03
	Channel diameter (mm)	20.67	3.29, 130.00	0.001
>2 vascular channels ^b	BV/TV (%)	1.10	1.03, 1.17	0.004
	Varying channel shape			
	Parallel	1.00		
	Parallel and irregular	3.49	1.5, 7.80	0.002
Channel shape (Scale 0-4) ^b	BV/TV (%)	1.12	1.05, 1.20	0.001
	Channel diameter (mm)	4.22	1.83, 9.73	0.001
	Material density (mgHA/cm ³)	0.97	0.95, 0.99	0.002
Channel Diameter (Scale 1-3) ^b	BV/TV (%)	1.11	1.03, 1.20	0.009
	Channel diameter (mm)	10.64	2.50, 45.23	0.001
	Material density (mgHA/cm ³)	0.98	0.95, 0.99	0.02
	<i>Coefficient</i>			
Radiographic composite score ^b	BV/TV (%)	1.24	0.22, 2.26	0.02
	Channel diameter (mm)	30.52	14.21, 46.84	<0.001
	Material density (mgHA/cm ³)	-0.37	-0.69, -0.05	0.02

Table 2: Significant findings from multivariable analysis between radiographic outcome variables and μCT predicting variables: a) binary logistic regression model; b) ordered logistic regression model [μCT = microcomputed tomography; BV/TV = bone volume fraction; OR = odds ratio; CI = confidence intervals].

Radiographic Outcome Variable	Performance Predicting Variable	OR	95% CI	p-value
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Presence of vascular channels ^a	Career seconds	0.83	0.71, 0.97	0.02
>2 vascular channels	Career places	0.96	0.92, 1.00	0.04
Channel shape (scale 0-4) ^b	Career places	0.95	0.90, 0.99	0.02
Channel diameter (scale 0-2) ^b	Career places	0.96	0.92, 1.00	0.04
<i>Coefficient</i>				
Radiographic composite score ^b	Career seconds	-5.82	-9.5, -2.1	0.03

Table 3: Significant findings from multivariable analysis between radiographic outcome variables and performance predicting variables, with all models adjusted for sex of the horse, a) binary logistic regression model; b) ordered logistic regression model [OR = odds ratio; CI = confidence intervals].

List of Figure Legends

Fig 1: Examples of coning of vascular channels at the abaxial margin on dorsal plane images from different μ CT sequences, noting the proximal and similar location of each channel: A) right fore lateral PSB from a two-year-old colt; B) right fore lateral PSB from a two-year-old filly; C) right fore medial PSB from a three-year-old filly. All horses were unraced but in race training at the time of death. Scale = 10mm. [μ CT = microcomputed tomography; PSB = proximal sesamoid bone].

Fig 2: Oblique radiographs (A, B) and sagittal and transverse plane images from μ CT sequences (C, D) showing the left fore lateral PSB with the minimum BV/TV of 63% in an unraced 18-month-old colt in pre-training (A, C) and right fore lateral PSB with the maximum BV/TV recorded of 91% in a three-year-old filly in race training (B, D). Scale = 10mm.

Supporting Information

Table S1: Definitions of radiographic variables assessed using a binary scale during analysis; *Clearly present defined as a radiographic change being seen without windowing, filters, magnification etc. [PSB = proximal sesamoid bone]

Table S2: Novel radiographic grading system used to assess individual proximal sesamoid bones within the fetlock joint of horses. [PSB = proximal sesamoid bone].

Table S3: μ CT grading system used to assess individual proximal sesamoid bones within the fetlock joint of horses. [SCB = subchondral bone].

Table S4: Existing radiographic grading systems used for proximal sesamoid bone assessment and in repeatability analysis.

Table S5: Univariable analysis results for both radiographic outcome variables against μ CT predicting variables and against performance predicting variables.

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