



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Lloyd, KA;Ayodele, BA;Hitchens, PL;Beck, C;Mackie, EJ;Whitton, RC

Title:

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

Date:

2020-09-01

Citation:

Lloyd, K. A., Ayodele, B. A., Hitchens, P. L., Beck, C., Mackie, E. J. & Whitton, R. C. (2020). Associations between the radiographic appearance of vascular channels in proximal sesamoid bones, their microstructural characteristics and past racing performance in Thoroughbreds. *Equine Veterinary Journal*, 52 (5), pp.670-677. <https://doi.org/10.1111/evj.13239>.

Persistent Link:

<https://hdl.handle.net/11343/275446>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

DR. KRISTEN LLOYD (Orcid ID : 0000-0002-4834-5222)

DR. PETA LEE HITCHENS (Orcid ID : 0000-0002-7528-7056)

DR. CHRIS WHITTON (Orcid ID : 0000-0003-0012-4065)

Article type : General Article

Editorial reference code: EVJ-GA-19-236.R3

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

Kristen A. Lloyd, Babatunde A. Ayodele, Peta L. Hitchens, Cathy Beck, Eleanor J. Mackie and Robert C. Whitton

Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Werribee Victoria, Australia

*Corresponding author email: cwhitton@unimelb.edu.au

Keywords: horse; sesamoiditis; proximal sesamoid bone; radiograph; microcomputed tomography

Running head: Microstructural analysis of the proximal sesamoid bone

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/EVJ.13239](https://doi.org/10.1111/EVJ.13239)

This article is protected by copyright. All rights reserved

24 **Summary**

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

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

33 **Study design:** Cross-sectional.

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

39 **Results:** All PSBs had at least one vascular channel (mean 3.6 ± 0.89) observed on μ CT
40 originating from the abaxial border, yet in only 63.6% (75/118) were channels observed
41 radiographically. Proximal sesamoid bones with a higher bone volume fraction (odds ratio
42 [OR] 1.08; 95% confidence interval [CI] 1.01-1.15; $P = 0.03$) and wider channel diameter
43 (mm) on μ CT (OR 20.67; 95% CI 3.29-130.00; $P = 0.001$) were more likely to have vascular
44 channels identified on radiographs. Greater radiographic channel number (OR 0.96; 95% CI
45 0.92-1.00; $P = 0.04$) and channel diameter (mm; OR 0.96; 95% CI 0.92-1.00; $P = 0.04$) were
46 associated with fewer career placings.

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

49 **Conclusions:** The ability to identify vascular channels radiographically indicates widening of
50 channels and densification of the PSB. More radiographic channels and greater channel
51 diameter were associated with similar or poorer measures of past performance, suggesting
52 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

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

90 **Materials and Methods**

91 *Study Population*

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

95 *Sample Preparation*

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

103 *Sample Imaging*

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

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

115 *Data Collection*

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

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

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

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

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

155 *Data Analysis*

156 The minimum sample size required for a two-tailed test to ensure adequate repeatability
157 between radiographic and μ CT assessments was determined. Assuming 80% power, an
158 estimated prevalence of visible vascular channels of 0.3 and a null hypothesis of 0.5, a
159 minimum of 77 observations to detect a kappa coefficient of 0.8 as statistically significant
160 was required [15], which was below the number of samples used for analysis in this study (n
161 = 118).

162 Intra-observer reliability between the novel and existing systems were calculated using
163 weighted Cohen's kappa coefficient (κ) with 95% confidence intervals (CI). Agreement was
164 assessed using previously described scales ('poor' at a kappa statistic of <0.00, 'slight' at κ =
165 0.00 - 0.20, 'fair' at κ = 0.21 - 0.40, 'moderate' at κ = 0.41 - 0.60, 'substantial' at κ = 0.61 -
166 0.80 and 'almost perfect' at κ = 0.81 - 1.00) [16].

167 Univariable generalised linear models accounting for clustering at the horse level were
168 generated to investigate associations between radiographic and μ CT variables, and between
169 radiographic and performance variables. Binary logistic regression and ordered logistic
170 regression models were fitted for outcome variables with two or more than two categories
171 respectively. All models were adjusted for clustering on horse identification number to
172 account for within horse measurements from both lateral and medial PSBs. To compare
173 differences in outcomes between the lateral and medial proximal sesamoid bones, equality
174 tests on matched data were performed via the Wilcoxon sign-rank test. Odds ratio (OR) and
175 95% CI were recorded. For the ordered logistic regression models, a proportional odds
176 assumption test was used to assess whether the relationship between each outcome category
177 was the same. Instances where the proportional odds assumptions were not met were due to
178 low category sample numbers. Model diagnostics, including assessing goodness-of-fit,
179 Pearson and Deviance residuals, checking for multicollinearity, and leverage of influential
180 observations were performed. Predictors with $P < 0.2$ from the univariable analyses were
181 entered into a multivariable model, removed in a backwards stepwise process, and retained if
182 $P < 0.05$, with potential confounding variables (such as age) included if they modified the
183 coefficients of other covariates by greater than 20%.

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

188 **Results**

189 *Study Population*

190 One hundred and eighteen PSBs were collected from 59 horses (left fore from 26 horses,
191 right fore from 33 horses). Age ranged from 16 months to 12 years, with a mean (\pm s.d) of 4.4
192 ± 2.4 years. Fourteen horses were female, 29 horses were geldings, and 16 horses were entire
193 males. Nine horses were resting from training and 50 horses were in race training at the time
194 of death.

195 Of the 42 horses that had started in at least one race meeting the inclusion criteria for
196 performance analyses, six were entire males, 24 were geldings and 12 were female. The mean
197 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
198 racing. The mean number of career starts was 22.6 ± 25.1 . Thirty-eight horses were in
199 training at the time of death, and four were resting. Prizemoney earned was \$AUD $265,260 \pm$
200 801.51×10^3 .

201 *Repeatability Testing*

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

210 *Radiographic and Microstructural Analysis*

211 Radiolucent lines originating from the abaxial margin interpreted as vascular channels were
212 present radiographically in 63.6% (75/118) of PSBs, whereas on μ CT all PSBs had clearly
213 defined channels originating from the abaxial margin and traversing the bone in a dorsal and
214 distal direction, with a mean channel number of 3.60 ± 0.89 per sesamoid bone. The mean
215 channel diameter on μ CT was 1.45 ± 0.37 mm, with the minimum diameter recorded at
216 0.8mm and maximum of 3.6mm. All PSBs had at least one channel with coning at the abaxial
217 margin on μ CT, most commonly involving the most proximal channel (Figure 1).

218 Mean whole bone BV/TV was $78.8 \pm 5.6\%$, with a minimum of 62.9% recorded in an 18-
219 month-old colt in pre-training, and maximum of 90.6% in a three-year-old filly in race
220 training (Figure 2). Medial PSBs were found to have proportionally more bone tissue than
221 lateral PSBs (BV/TV $79.9 \pm 5.1\%$ vs. $78.3 \pm 6.0\%$; $P < 0.001$). Vascular channel number ($P >$
222 0.9) and diameter ($P = 0.06$) were also compared between medial and lateral PSBs on μ CT
223 and not found to be different.

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

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

241 *Performance Analysis*

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

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

256 Discussion

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

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

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

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

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

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

304 With career progression (indicated by greater number of career seconds, places, starts,
305 years racing and age), horses were found to have more severe radiographic changes
306 consistent with ‘sesamoiditis’. Similarly, the severity of palmar/plantar osteochondral disease
307 (POD), a condition also associated with increased subchondral BV/TV and prominent
308 vascular channels [19], is associated with cumulative race starts irrespective of horse age
309 [21]. Therefore, it is likely that these combined radiographic changes in proximal sesamoid
310 bones are signs of accumulated intense cyclic loading and that skeletal injury often associated
311 with such a history may be the reason performance is somewhat compromised. The presence

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

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

341 In conclusion, the ability to observe proximal sesamoid bone vascular channels of any
342 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.

348

349 **Authors’ declaration of interests**

350 No competing interests have been declared.

351

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).

355

356 **Owner informed consent**

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

358

359 **Sources of funding**

360 This study received funding from Racing Victoria Limited, the Victorian Racing Industry
361 Fund of the Victorian State Government, and The University of Melbourne as part of the
362 Equine Limb Injury Prevention Program.

363

364 **Acknowledgements**

365 The authors would like to thank Kane Wilson for his assistance with sample imaging.

366

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

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

375

376 **Manufacturers' addresses**

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

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

379 ³Scanco 50, SCANCO medical AG, Bruttisellen, Switzerland

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

381 StataCorp LP

382

383 **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

384 **Table 1:** Agreement measures for categorical data in the assessment of proximal sesamoid

385 bone vascular channel morphology using previously published and a novel radiographic

386 grading system. [CI = Confidence interval].

387

Radiographic Outcome

Variable	μ CT 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		
Channel shape (Scale 0-4) ^b	Parallel and irregular	3.49	1.5, 7.80	0.002
	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
Radiographic composite score ^b		<i>Coefficient</i>		
	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

388

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

393

Radiographic Outcome Variable	Performance Predicting Variable	OR	95% CI	p-value
-------------------------------	---------------------------------	----	--------	---------

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

394

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

399

400 List of Figure Legends

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

407 **Fig 2:** Oblique radiographs (A, B) and sagittal and transverse plane images from μ CT
 408 sequences (C, D) showing the left fore lateral PSB with the minimum BV/TV of 63% in an
 409 unraced 18-month-old colt in pre-training (A, C) and right fore lateral PSB with the
 410 maximum BV/TV recorded of 91% in a three-year-old filly in race training (B, D). Scale =
 411 10mm.

412

413 **Supporting Information**

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

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

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

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

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

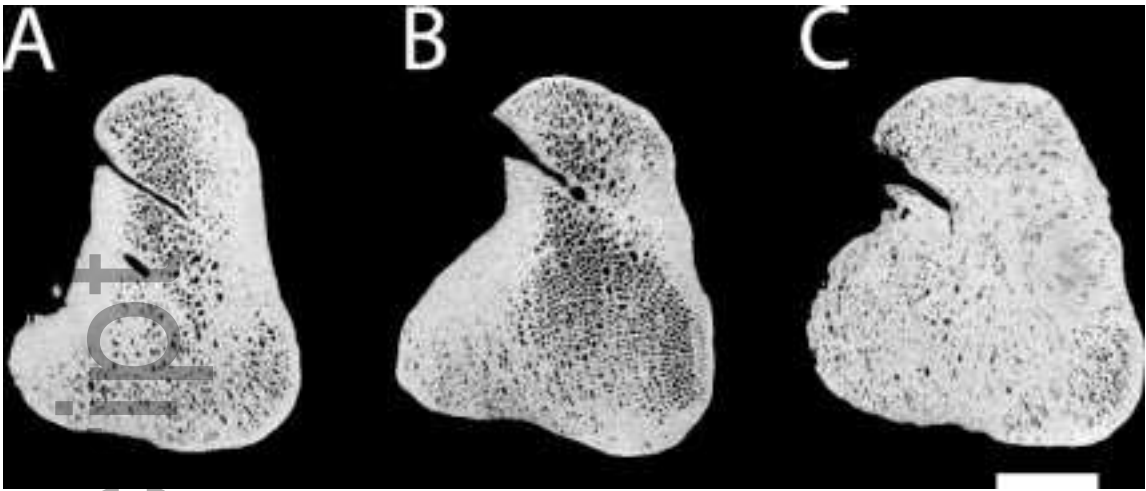
425

426 **References**

- 427 1. Cornelissen, B.P.M., Rijkenhuizen, A.B.M. and Barneveld, A. (1996) The arterial shift
428 features in the equine proximal sesamoid bone. *Vet. Quart.* **18**, 110-116.
- 429 2. Hardy, J., Marcoux, M. and Breton L. (1991) Clinical relevance of radiographic findings
430 in proximal sesamoid bones of two-year-old Standardbreds in their first year of training.
431 *J. Am. Vet. Med. Assoc.* **198**, 2089-2094.
- 432 3. Grondahl, A.M., Gaustad, G. and Engeland, A. (1994) Progression and association with
433 lameness and racing performance of radiographic changes in the proximal sesamoid
434 bones of young Standardbred trotters. *Equine Vet. J.* **26**, 152-155.
- 435 4. McLellan, J. and Plevin, S. (2014) Do radiographic signs of sesamoiditis in yearling
436 Thoroughbreds predispose the development of suspensory ligament branch injury?
437 *Equine Vet. J.* **46**, 446-450.

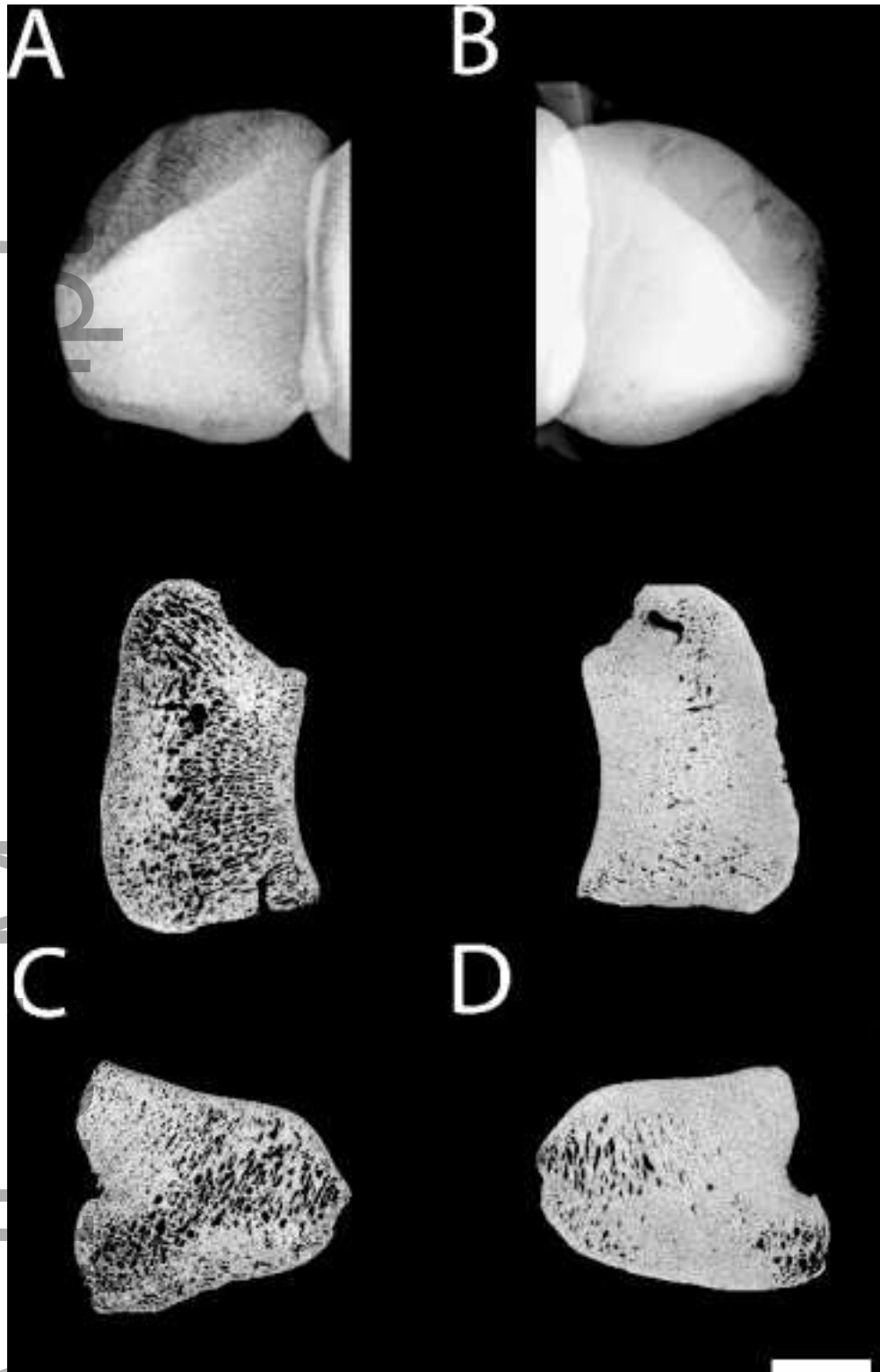
- 438 5. Spike-Pierce, D.L. and Bramlage, L.R. (2003) Correlation of racing performance with
439 radiographic changes in the proximal sesamoid bones of 487 Thoroughbred yearlings.
440 *Equine Vet. J.* **35**, 350-353.
- 441 6. Kane, A.J., McIlwraith, C.W., Park, R.D., Rantanen, N.W., Morehead, J.P. and Bramlage,
442 L.R. (2003) Radiographic changes in Thoroughbred yearlings. Part 1: Prevalence at the
443 time of the yearling sales. *Equine Vet. J.* **35**, 354-365.
- 444 7. Kane, A.J., McIlwraith, C.W., Park, R.D., Rantanen, N.W., Morehead, J.P. and Bramlage,
445 L.R. (2003) Radiographic changes in Thoroughbred yearlings. Part 2: Associations with
446 racing performance. *Equine Vet. J.* **35**, 366- 374.
- 447 8. O'Brien, T.R., Morgan, J.P., Wheat, J.D. and Suter, P.F. (1971) Sesamoiditis in the
448 Thoroughbred: a radiographic study. *J. Am. Vet. Radiol. Soc.* **12**, 75-87.
- 449 9. Jackson, M., Vizard, A., Anderson, G., Clarke, A., Mattoon, J., Lavelle, R., Lester, N.,
450 Smithenson, T. and Whitton R.C. (2009) A prospective study of presale radiographs of
451 Thoroughbred yearlings. *Rural Industries Research and Development Corporation.*
452 *Publication*, 9-82.
- 453 10. Lin, H.H., Peng, S.L., Wu, J., Shih, T.Y., Chuang, K.S. and Shih, C.T. (2016) A novel
454 two-compartment model for calculating bone volume fractions and bone mineral densities
455 from computed tomography images. *IEEE Trans. Med. Imag.* **36**, 1094-1105.
- 456 11. Martig, S., Hitchens, P.L., Lee, P.V.S. and Whitton, R.C. (2019) The relationship
457 between microstructure, stiffness and compressive fatigue life of equine subchondral
458 bone. *J. Mech. Behav. Biomed. Mater.* **101**, 103439.
- 459 12. Plevin, S., McLellan, J. and O'Keeffe, T. (2016) Association between sesamoiditis,
460 subclinical ultrasonographic suspensory ligament branch change and subsequent clinical
461 injury in yearling Thoroughbreds. *Equine Vet. J.* **48**, 543-547.
- 462 13. Racing Victoria Limited (2018) Racing Victoria. <<http://www.racing.com>> [accessed
463 November 2017-July 2018].
- 464 14. Racing Victoria Limited (2018) Racing Victoria: Handicapping.
465 <<https://www.racingvictoria.com.au/the-sport/racing/handicapping>> [accessed
466 November 2017-July 2018].
- 467 15. Sim, J. and Wright, C.C. (2005) The kappa statistic in reliability studies: use,
468 interpretation and sample size requirements. *Phys. Ther.* **85**, 257-268.

- 469 16. Landis, J.R. and Koch, G.G. (1977) The measurement of observer agreement for
470 categorical data. *Biometrics* **1**, 159-174.
- 471 17. Bender, I.B. (1982) Factors influencing the radiographic appearance of bony lesions. *J.*
472 *Endodont.* **8**, 161-170.
- 473 18. Poulos, P.W. (1988) Radiographic and histologic assessment of proximal sesamoid bone
474 changes in young and working horses. *Proc. Am. Assoc. Equine Practnrs.* **34**, 347-358.
- 475 19. Williamson, A.J., Sims, N.A., Thomas, C.D.L., Lee, P.V.S., Stevenson, M.A. and
476 Whitton, R.C. (2018) Biomechanical testing of the calcified metacarpal articular surface
477 and its association with subchondral bone microstructure in Thoroughbred racehorses.
478 *Equine Vet. J.* **50**, 255-260.
- 479 20. Young, D.R., Nunamaker, D.M. and Markel, M.D. (1991) Quantitative evaluation of the
480 remodelling response of the proximal sesamoid bones to training-related stimuli in
481 Thoroughbreds. *Am. J. Vet. Res.* **52**, 1350-1356.
- 482 21. Pinchbeck, G.L., Clegg, P.D., Boyde, A., Barr, E.D. and Riggs, C.M. (2013) Horse-,
483 training- and race-level risk factors for palmar/plantar osteochondral disease in the racing
484 Thoroughbred. *Equine Vet. J.* **45**, 582-586.
- 485 22. Hitchens, P.L., Hill, A.E. and Stover, S.M. (2018). Relationship between historical
486 lameness, medication usage, surgery, and exercise with catastrophic musculoskeletal
487 injury in racehorses. *Front. Vet. Sci.* **5**, 217. doi: 10.3389/fvets.2018.00217.
- 488 23. Bani Hassan, E., Mirams, M., Ghasem-Zadeh, A., Mackie, E.J. and Whitton, R.C. (2016)
489 Role of subchondral bone remodelling in collapse of the articular surface of
490 Thoroughbred racehorses with palmar osteochondral disease. *Equine Vet. J.* **48**, 228-233.
- 491 24. Kim, W., McArdle, B.H., Kawcak, C.E., McIlwraith, C.W., Firth, E.C. and Broom, N.D.
492 (2013) Histomorphometric evaluation of the effect of the early exercise on subchondral
493 vascularity in the third carpal bone of horses. *Am. J. Vet. Res.* **74**, 542-549.
- 494 25. Cresswell, E.N., McDonough, S.P., Palmer, S.E., Hernandez, C.J. and Reesink, H.L.
495 (2018) Can quantitative computed tomography detect bone morphological changes
496 associated with catastrophic proximal sesamoid bone fracture in Thoroughbred
497 racehorses? *Equine Vet. J.* **51**, 123-130.
- 498 26. Davis, A.M., Fan, X., Shen, L., Robinson, P. and Riggs, C.M. (2017) Improved
499 radiological diagnosis of palmar osteochondral disease in the Thoroughbred racehorse.
500 *Equine Vet. J.* **49**, 454-460.



evj_13239_f1.tif

Author Manuscript



evj_13239_f2.tif