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11 **Microstructural properties of the proximal sesamoid bones of Thoroughbred racehorses**
12 **in training**

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18

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20 microcomputed tomography

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28

29 Summary

30 **Background:** Proximal sesamoid bone fractures are common catastrophic injuries in
31 racehorses. Understanding the response of proximal sesamoid bones to race training can
32 inform fracture prevention strategies.

33 **Objectives:** To describe proximal sesamoid bone microstructure of racehorses and to
34 investigate associations between microstructure and racing histories.

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

36 **Methods:** Proximal sesamoid bones from 63 Thoroughbred racehorses were imaged using
37 micro-computed tomography. Bone volume fraction (BTV) and bone material density
38 (BMD) of the whole bone and four regions (apical, midbody dorsal, midbody palmar and
39 basilar) were determined. Generalised linear regression models were used to identify
40 associations between bone parameters and race histories of the horses.

41 **Results:** The mean sesamoid BTV was 0.79 ± 0.08 and BMD was 806.02 ± 24.66 mg
42 HA/ccm. BTV was greater in medial sesamoids compared to lateral sesamoids (0.80 ± 0.07
43 vs 0.79 ± 0.08 ; $P < 0.001$) predominantly due to differences in the apical region (medial -
44 0.76 ± 0.08 vs. lateral - 0.72 ± 0.07 ; $P < 0.001$). BTV in the midbody dorsal region (0.86 ± 0.06)
45 was greater than other regions (midbody palmar - 0.79 ± 0.07 , basilar - 0.78 ± 0.06 and apical -
46 0.74 ± 0.08 ; $P < 0.001$). BTV was greater in sesamoids with more microcracks on their
47 articular surface (Coef. 0.005; 95% CI 0.001, 0.009; $P = 0.01$), greater extent of bone
48 resorption on their abaxial surface (Grade 2 - 0.82 ± 0.05 vs. Grade 1 - 0.80 ± 0.05 or Grade 0 -
49 0.79 ± 0.06 ; $P = 0.006$), in horses with a low (0.82 ± 0.07) or mid handicap rating (0.78 ± 0.08)
50 compared to high rating (0.76 ± 0.07 ; $P < 0.001$), in 2 to 5 year old horses (0.81 ± 0.07)
51 compared with younger (0.68 ± 0.08) or older horses (0.77 ± 0.08 ; $P < 0.001$), and in horses that
52 commenced their racing career at less than four years of age (0.79 ± 0.08 vs. 0.77 ± 0.07 ;
53 $P < 0.001$). BMD was greater in the midbody dorsal (828.6 ± 19.6 mg HA/ccm) compared to
54 other regions (apical - 805.8 ± 21.8 , midbody palmar - 804.7 ± 18.4 , basilar - 785.0 ± 17.1 ;
55 $P < 0.001$), in horses with a handicap rating (low - 812.1 ± 20.0 , mid - 821.8 ± 21.3 and high -
56 814.6 ± 19.4) compared with those with no rating (791.08 ± 24.4 , $P < 0.001$), in females

57 (806.7±22.0) and geldings (812.2±22.4) compared to entires (792.7±26.2; P=0.02), and in
58 older horses (<2 year old – 763.7±24.8 vs. 2-5 year old – 802.7±23.4, and 6-12 year old –
59 817.8±20.0; P=0.002).

60 **Main limitations:** Data were cross-sectional.

61 **Conclusions:** Densification of the proximal sesamoid bones is associated with the
62 commencement of racing in younger horses and the presence of bone fatigue related
63 pathology. Lower sesamoid bone volume fraction was associated with longevity and better
64 performance.

65

66 **Introduction**

67 Proximal sesamoid bone fracture is a common injury of racehorses. In Thoroughbred horses,
68 55 per cent of catastrophic bone fractures in Hong Kong and 30 to 55 per cent of catastrophic
69 musculoskeletal injuries in California are due to proximal sesamoid bone fractures [1; 2]. In
70 Australia and the United Kingdom, proximal sesamoid bone fracture accounts for up to 21
71 per cent of catastrophic fractures [3-5]. Non-catastrophic injuries involving the proximal
72 sesamoid bones are also a major cause of lost training days and premature retirement of
73 racehorses [6; 7].

74 Most fractures in racehorses are due to repeated or cyclic loading resulting in fatigue failure
75 of bone [8]. Epidemiological evidence that cumulative distance at high speed puts horses at
76 higher risk of proximal sesamoid fracture supports other research evidence that bone fatigue
77 is a cause [9; 10]. However, microcracks, a typical feature of fatigue injury, are rare in the
78 trabecular bone of fractured and non-fractured proximal sesamoid bones leading to a
79 suggestion that fatigue may not play a role [11]. Nevertheless, focal areas of bone resorption
80 have been observed adjacent to the articular surface and on the palmar surface of proximal
81 sesamoids that are consistent with targeted remodelling in response to focal fatigue injury and
82 these sites have not been examined for microdamage [12; 13].

83 In the condyles of the third metacarpal bone, bone volume fraction (BTVF, bone volume as a
84 proportion of total tissue volume and therefore the inverse of porosity) increases with training
85 and age and in association with microdamage accumulation [14; 15]. The data available on
86 the effect of training on the microstructure of the proximal sesamoid bones shows a similar
87 response with decreased porosity observed in horses in race training [16]. Given these

88 associations it is not surprising that fractured proximal sesamoid bones are denser than
89 unfractured controls as intense cyclic loading, microdamage of bone and bone microstructure
90 are all related [12; 17].

91 The aim of this study was to investigate associations between proximal sesamoid bone
92 microstructure and racing histories of Thoroughbred racehorses in order to better understand
93 the relationship between the bone's loading history, bone modelling and injury. We
94 hypothesised that proximal sesamoid bone volume fraction would increase with career
95 progression as well as with evidence of proximal sesamoid subchondral bone injury.

96

97 **Materials and methods**

98 The lateral and medial proximal sesamoid bones were obtained from one forelimb of 63
99 Thoroughbred horses undergoing postmortem examination for reasons unrelated to this study,
100 excluding those with proximal sesamoid fractures. Thirty-six were euthanased due to a
101 catastrophic musculoskeletal injury and 27 for non-musculoskeletal reasons. The racing
102 history for each horse was obtained from the official repository for race records (Racing
103 Australia, <http://racingaustralia.horse>). This race history data includes the dates of each race
104 and official trial, number of races, wins and places, and horse-level and race-level
105 information (race distances, track conditions, handicap rating – a combined measure of
106 success and longevity). From this information we calculated racing intensity (e.g. days
107 between each start) and cumulative distances raced (Table S1 and S2).

108 *MicroCT imaging*

109 Two-dimensional transverse images of whole proximal sesamoid bones were obtained using
110 micro-computed tomography (μ CT; Scanco μ CT50¹) at a resolution of 24.2 μ m and X-ray
111 settings of 70 kVp, 200 μ A, 14 W and 0.5 mm aluminium filter. Image segmentation was
112 carried out for BTV and bone mineral density (BMD, a measure of the amount of mineral
113 in the bone material) calculations in the regions of interest (ROI) drawn for whole proximal
114 sesamoid and four bone regions (apical, midbody dorsal, midbody palmar and basilar). The
115 method for defining the ROI for the regions is presented in **Figure S1**.

116 *Proximal sesamoid bone pathology*

117 Bone microcracks were identified as bright linear areas in the calcified cartilage and
118 subchondral bone on μ CT images and confirmed across three planes of section (Figure 1), as

119 described previously [15]. The number of microcracks on the articular surface were counted
120 for each sesamoid on the frontal plane. The degree of lysis at the abaxial border was graded
121 as follows: 0 grade for no lysis, 1 for one small area of lysis (< 2 mm) and 2 for a large area
122 (> 2 mm) or multiple areas of lysis (Figure 2A). Additional pathology that was graded
123 included subchondral bone lysis and focal lysis at the palmar aspect of the bone (Figure 2B
124 and C). Palmar osteochondral disease (POD) lesions on the distal third metacarpal condyles
125 of the articulating fetlock joint were also graded using guidelines previously described [14].

126 *Data Analysis*

127 Normality of continuous variables was assessed by examining histograms and using the
128 Shapiro-Wilk test. Scatter plots and box-and-whisker plots were generated to initially assess
129 the relationship between the outcome bone parameters (BTV and BMD) and continuous
130 and categorical predictor variables, respectively. To investigate the association between the
131 outcome bone parameters and other bone material properties and race history factors, we
132 generated univariable generalised linear regression models. With n=63 samples and assuming
133 power of 80% at a significance level of $P < 0.05$, we expect to be able to detect small to
134 medium effect sizes of our predictor variables ($\delta = 0.13$ for one predictor variable;
135 $\delta = 0.24$ for six predictors fitted to our model). Variables that were $P < 0.20$ in univariable
136 modelling were fitted into a multivariable model and retained if $P < 0.05$ using the backwards
137 stepwise approach. Two-way interaction terms between study factors remaining in the final
138 model were assessed. Models were adjusted for clustering on horse to account for multiple
139 within horse observations at the region and sub-region level. Model diagnostics included
140 assessment of linearity between continuous outcome and predictor variables, identifying
141 outliers and variables with high leverage or influence, normality of residuals, the link test to
142 identify model specification error, examination of tolerance (> 0.1), and the variance inflation
143 factor ($VIF < 10$). Regression coefficients (coef.) and their 95% confidence intervals (95% CI)
144 are presented. All statistical analyses were conducted using Stata, version 15.1².

145

146 **Results**

147 The mean age of the horses was 4.5 years (s.d. 2.2; range 1 to 11). Of the 63 horses in this
148 study, 46 had raced previously and 17 were unraced. Fifty-two horses were in active race
149 training while 11 were resting from training at the time of death. The samples included 28 left
150 and 35 right limbs collected from 14 females, 16 entire males and 33 geldings.

151 *Relationship between BVTV and BMD*

152 The mean sesamoid BVTV was 0.79 ± 0.08 (range 0.52 to 0.96) and mean BMD was
153 806.02 ± 24.66 (range 677.06 to 874.12) mg HA/ccm. BVTV and BMD were positively
154 correlated across all regions ($P < 0.001$) except in the apical region ($P = 0.05$). The sesamoid
155 BVTV and BMD categorised by study factors are presented in **Table S1** and **S2** respectively.

156 *Bone pathology*

157 Bone microcracks were observed in 67/126 (53.2%) proximal sesamoid bones in 47/63
158 (74.6%) horses. Microcracks were observed in 38 (60.3%) medial sesamoids compared to 29
159 (46%) lateral sesamoids with more microcracks present in medial sesamoids than lateral
160 bones ($P < 0.002$). Areas of resorption of bone on the abaxial surface were observed in 43/126
161 (34.1%) bones (16 lateral, 27 medial) in 20/63 (31.7%) horses, and focal subchondral bone
162 resorption was observed in 35/126 bones (27.8%; 16 lateral, 19 medial) in 28/63 (44.4%)
163 horses. Areas of focal resorption adjacent to the palmar border of the bone were only
164 observed in 5/126 (4.0%) bones (3 lateral, 2 medial) in 4/63 (6.3%) horses.

165
166 *Associations with bone volume fraction*

167 Univariable associations between BVTV and study factors are presented in **Table S1**. In
168 multivariable analysis (Table 1), sesamoid BVTV was higher in the medial sesamoids than
169 the lateral sesamoids for whole bones, but this was due to differences in the apical region
170 (Figure 3A) as there was no difference between medial and lateral bones for basilar, midbody
171 dorsal and midbody palmar regions. When BVTV was compared between regions, the
172 midbody dorsal region was highest, followed by the midbody palmar, basilar then apical
173 region (Figure 3A). Sesamoids with more microcracks and a higher grade of abaxial
174 resorption had greater BVTV. Lower BVTV was observed in horses with no handicap rating
175 or with a higher rating (Figure 3B), horses that were younger than 2 years or older than 5
176 years (Figure 3C), and horses which were four years old at their first career start compared to
177 those that first started at a younger age or were unraced (Figure 3D).

178 *Associations with bone material density*

179 Univariable associations between BMD and study factors are presented in **Table S2**. In
180 multivariable analysis (Table 1), BMD was not significantly different between the medial and

181 lateral sesamoids but did differ by region (Figure 4A). BMD was greatest in the midbody
182 dorsal, followed by the apical, midbody palmar, then basilar subregion. BMD was higher in
183 horses that had handicap rating compared to those with no rating (Figure 4B). BMD was
184 higher in females and geldings than entires (Figure 4C) and in older horses.

185

186 **Discussion**

187 The distribution of bone material in the proximal sesamoid bones of racehorses is not
188 uniform with the highest BVTV and BMD in the midbody dorsal region, the bone that
189 underlies the articular surface. Higher BVTV was associated with higher BMD across all
190 except the apical region. Rather than a simple increase with career progression, sesamoid
191 BVTV increases with the commencement of a racing career, being lower in horses that are
192 younger than two years of age and those without a handicap rating. However, for experienced
193 racehorses sesamoid BVTV is lower in older horses, those with a higher handicap rating, and
194 in horses that were older when they had their first start. As found in other areas of the
195 skeleton subjected to repeated high loads in the galloping horse, microdamage was observed,
196 but only in the articular surface of the sesamoids and as hypothesised was associated with
197 higher BVTV.

198 The sesamoid BVTV values obtained in this study were lower than those measured in
199 fractured and unfractured sesamoids in the USA using the same technique [17]. This disparity
200 in BVTV values may be due to different training or racing practices between geographical
201 locations leading to different patterns of joint loading. For example, horses predominantly
202 race on dirt surfaces in the USA whereas turf racing is most common in Australia [18; 19].
203 There are limited data available on proximal sesamoid BMD values in racehorses for
204 comparison. Our finding that the midbody dorsal region has the greatest BVTV and BMD is
205 consistent with previous findings that porosity was lower and relative mineral density
206 determined by qualitative assessment of μ CT images was higher in the subchondral bone
207 region of sesamoid bones from horses with fractured sesamoids [12; 20]. Bone adapts to the
208 loads applied to it and the articulations between the proximal sesamoids and the palmar
209 aspect of the condyles of the third metacarpal bone are subjected to very high magnitude
210 compressive force during galloping exercise [21-23]. The loading environment of the rest of
211 the sesamoid bone is unknown. However, based on our bone density results, lower loads
212 would be expected in the basilar, palmar and apical regions. Interestingly when comparing

213 medial and lateral sesamoids, only the apical region had a higher BVTV in medial sesamoids,
214 whereas the loads on the articular surface of medial sesamoids are higher than on lateral
215 bones during high speed exercise [24].

216 Our finding that BVTV is higher in the proximal sesamoid bone of horses once they
217 commence race training is consistent with previous evidence that densification of proximal
218 sesamoid bones occurred in response to treadmill exercise in 2-year-old horses [16].
219 Similarly, in the distal metacarpus, a marked increase in BVTV is associated with the
220 commencement of race training [25-28]. This adaptation to training likely increases the
221 resistance to fatigue failure under cyclic loading as has been observed in the distal metacarpal
222 subchondral bone [29].

223 In response to high magnitude cyclic loading, along with subchondral bone densification we
224 expected to observe evidence of microdamage. Microdamage is typically found at sites prone
225 to fatigue fractures [15; 30]. Sesamoid fractures are predominantly a racehorse injury and
226 their epidemiology is consistent with bone material fatigue playing an important role, as
227 fractures are associated with cumulative galloping distance, and greater exercise intensity [9].
228 We observed oblique mineralised subchondral bone microcracks in approximately one third
229 of the sesamoids examined but they were less prevalent and less extensive than we have
230 observed in the subchondral bone of the distal metacarpus [15]. Consistent with this,
231 sesamoid fractures are less prevalent than metacarpal condylar fractures in Victorian
232 racehorses [31]. The common site where microcracks were observed in the current study
233 corresponded to that where focal subchondral bone discolouration and resorption has been
234 observed in association with sesamoid bone fractures [13]. Similar to previous findings in the
235 distal metacarpus, increased subchondral bone microcracks were associated with higher
236 BVTV in sesamoid bones [32; 33]. This is consistent with both being a result of high
237 magnitude cyclic loading.

238 To our knowledge this is the first study to show an association between the microstructure of
239 any bone and performance. Less successful horses over time (low or mid handicap ratings)
240 had higher BVTV in the proximal sesamoid bones compared to those with no rating or with
241 higher rating (success over a sustained period). Because high BVTV is associated with
242 intense training exercise and microdamage it is possible that affected horses are overtrained
243 and/or suffering from conditions associated with intense training. Scintigraphic uptake of
244 radiopharmaceutical in the condyles of the third metacarpus and metatarsus, consistent with

245 subchondral bone injury, was associated with poor race performance in horses [34]. Overload
246 of the suspensory apparatus during training is also associated with poorer race performance
247 [35].

248 Entire male horses are at greater risk of fatal sesamoid fracture than geldings or females [9].
249 It has been speculated that this may be due to differences in how entire male horses are
250 managed, and their greater body size and mass [9]. Our finding of lower sesamoid BMD in
251 entire males than in females and geldings indicates that there are intrinsic sex differences in
252 sesamoid bone material which may account for the difference in fracture risk. Two-year-old
253 colts have higher levels of markers of bone formation and resorption than two-year-old fillies
254 suggesting higher bone turnover in young male horses which is likely to lead to lower BMD
255 [36]. Metacarpal subchondral bone with lower BMD is less stiff and although when bone
256 samples were tested at a single load magnitude there was no association between fatigue life
257 and BMD [29], with varying load magnitudes higher BMD is associated with longer fatigue
258 life [37]. Therefore, lower BMD in entire male horses' sesamoid bones could be a
259 mechanism for their higher fracture risk.

260 Our results show that cyclical loading of bone with the introduction of race training is
261 associated with bone densification. Training and racing have the potential to lead to fatigue
262 related changes in proximal sesamoid bones with the accumulation of bone microcracks and
263 formation of focal areas of bone resorption. Consistent with cyclic loading being the cause of
264 densification and injury, fractured sesamoid bones have been shown to have higher BVTV
265 than controls. Additionally, higher BVTV is reported for sesamoid bones of racehorses from
266 countries with higher rates of sesamoid fracture [17]. The risk of sesamoid bone fracture is
267 also greater in more experienced racehorses with higher cumulative gallop distances or more
268 intense exercise during training [9; 10]. This pattern is similar to the fatigue related changes
269 contributing to increased fracture risk in the third metacarpal bone where increased BVTV,
270 the accumulation of microdamage, and fracture are associated with advanced racing career
271 and higher cumulative distance covered [14; 15; 38]. Therefore, contrary to the speculation
272 that sesamoid fracture is not likely to be fatigue related [11], evidence from our study is
273 consistent with bone material fatigue in sesamoids contributing to fracture. The finding that
274 horses with better performance and greater longevity have less dense sesamoids suggests that
275 these horses were not subjected to excessive galloping early in their career and that longevity
276 and success of racehorses could be improved by subjecting younger horses to less intense
277 racing.

278 The limitations of this study include the use of cross-sectional data and therefore changes in
279 sesamoid microstructure over time with workload and performance were not assessed. The
280 estimation of bone microdamage was based on the presence of mineralised linear
281 microcracks in the subchondral bone. As shown by a previous study of the distal metacarpus
282 [15], these are only a proportion of microcracks so do not represent the total microcracks
283 present in the subchondral bone.

284 **Conclusion**

285 The proximal sesamoid bones of horses in race training have microstructural changes in the
286 subchondral bone similar to those observed in association with training and fatigue related
287 changes of the metacarpal condyles. Prevention of sesamoid injuries in racehorses is
288 therefore likely to involve managing training and racing intensity.

289

290 **Authors' declaration of interests**

291 No competing interests have been declared.

292 **Ethical animal research**

293 The use of animal tissues met the requirement of the University of Melbourne Animal Ethics
294 Committee (Ethics ID:14001).

295 **Informed consent**

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

297 **Data accessibility statement**

298 The data that support the findings of this study are available on request from the
299 corresponding author. The data are not publicly available due to privacy or ethical
300 restrictions.

301 **Source of funding**

302 This project was funded by Racing Victoria, the Victorian Racing Industry Fund of the
303 Victoria State Government, and The University of Melbourne under the Equine Limb Injury
304 Prevention Program

305 **Authorship**

306 R.C. Whitton and E.J. Mackie were responsible for the study concept and design, and
307 obtaining funding. Acquisition of data was by B. Ayodele, A.S.M. Wong and R.C. Whitton

308 and analysis by B. Ayodele, P.L. Hitchens, A.S.M. Wong and R.C. Whitton. All authors were
 309 involved in interpretation of the data, drafting the article, critical revision and final approval.

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313

314 **Manufacturers' addresses**

315 ¹Scanco, Scanco Medical AG, Basserdorf, Switzerland.

316 ²Stata 15.1, StataCorp, College Station, Texas, USA.

317 **Tables**

318 **Table 1:** Multivariable associations between the sub-regional BVTV and BMD with other
 319 bone material properties and race history study factors.

Variable	BVTV		BMD	
	Coef. (95% CI)	P-value	Coef. (95% CI)	P-value
Main effects				
Region				
Lateral	Reference (Ref)			
Medial	0.03 (0.02, 0.04)	<0.001		
Sub-region				
Apical	Ref		Ref	
Basilar	0.05 (0.04, 0.07)	<0.001	-20.7 (-24.26, -17.22)	<0.001
Midbody palmar	0.06 (0.05, 0.08)	<0.001	-1.01 (-4.4, 2.40)	0.6
Midbody dorsal	0.13 (0.12, 0.15)	<0.001	22.80 (19.52, 26.09)	<0.001
Medial sub-regions				
Medial apical	Ref			
Medial basilar	-0.03 (-0.04, -0.02)	<0.001		

Medial midbody palmar	-0.04 (-0.05, -0.03)	<0.001		
Medial midbody dorsal	-0.04 (-0.05, -0.02)	<0.001		
Number of microcracks	0.005 (0.001, 0.009)	0.013		
Abaxial resorption grade				
Grade 0	Ref			
Grade 1	0.006 (-0.02, 0.03)	0.56		
Grade 2	0.04 (0.01, 0.06)	0.001		
Handicap rating				
No rating	Ref		Ref	
<75 (low) rating	0.07 (0.042, 0.09)	<0.001	14.97 (9.05, 20.88)	<0.001
75 - 99 (mid) rating	0.04 (0.01, 0.07)	0.007	22.90 (15.41, 30.40)	<0.001
100 - 175 (high) rating	0.03 (-0.009, 0.08)	0.123	12.28 (3.67, 20.88)	0.005
Sex				
Entire			Ref	
Gelding			7.45 (1.46, 13.44)	0.02
Female			10.33 (2.06, 18.61)	0.01
Age at death (years)				
1 year [§]	Ref		Ref	
2 to 5 years	0.11 (0.05, 0.18)	<0.001	24.12 (0.71, 47.54)	0.04
6 to 12 years	0.07 (-0.002, 0.14)	0.059	32.84 (8.64, 57.03)	0.008
Age at first start				
Unraced	Ref			
<3 years old	-0.04 (-0.07, -0.003)	0.032		
3 years old	-0.04 (-0.07, -0.007)	0.015		
>3 years old [§]	-0.11 (-0.15, -0.08)	<0.001		

320

321 **Figure Legends**

322 **Figure 1:** Two dimensional μ CT images of a proximal sesamoid bone showing bone
323 microcracks. Articular surface microcracks (indicated by arrows) are shown on the same
324 sesamoid bone in different planes of section. Insets are high magnification images of white
325 box areas.

326 **Figure 2:** (A-C) Two dimensional μ CT images of proximal sesamoid bones showing bone
327 lesions. A lesion is indicated by arrows on the same sesamoid bone in different planes of
328 section: (A) abaxial surface bone resorption (grade 2), (B) focal subchondral bone resorption
329 (>2 mm area of lysis), (C) palmar surface bone resorption. (D) 3D image of a proximal
330 sesamoid bone showing location of transverse slices in A, B and C (black lines).

331 **Figure 3:** Proximal sesamoid bone volume fraction (BVTV), in different regions by lateral
332 and medial bones (A): BVTV was greater in midbody dorsal, followed by midbody palmar,
333 basilar and apical regions (margins plot with 95% CI, $P<0.001$); and in medial than lateral
334 sesamoids within apical region ($P<0.001$). BVTV by handicap ratings (B): horses with no
335 handicap rating or with a higher rating had lower BVTV ($P<0.001$). BVTV by racing age (C):
336 BVTV was higher in 2 to 5-year-old horses ($P<0.001$). BVTV by age at career start (D):
337 Horses with career start at 2 years of age had higher sesamoid BVTV ($P<0.001$).

338 **Figure 4:** Proximal sesamoid bone mineral density (BMD) in different regions (A): BMD
339 was highest in midbody dorsal, followed by apical, midbody palmar, and basilar regions
340 (margins plot with 95% CI, $P<0.001$). BMD by handicap ratings (B): horses with a handicap
341 rating have higher BMD than those without ($P<0.001$). BMD by horse sex (C): BMD was
342 lower in entire males than in geldings and females ($P=0.02$).

343

344 **Supporting information**

345 **Figure S1:** (A) Sagittal μ CT section of a sesamoid bone showing the regions of interest
346 selected. Sections equivalent to 6.25% of the image sets were excluded between regions and
347 at the ends of image sets. (B) Transverse μ CT section of a sesamoid taken at midbody (dotted
348 line in A). Two lines were drawn perpendicular to the articular surface at the axial and

349 abaxial regions (dotted lines) extending from the dorsal to the palmar surface. A line through
350 the midpoints (red dots) was then used to divide the bone into the dorsal and palmar halves
351 (white line).

352 **Table S1:** Univariable associations between the outcome variable (sub-regional BTVV) with
353 other bone microstructural characteristics and race history study factors.

354 **Table S2:** Univariable associations between the outcome variable (sub-regional BMD) with
355 other bone microstructural characteristics and race history study factors (N=504
356 observations).

357

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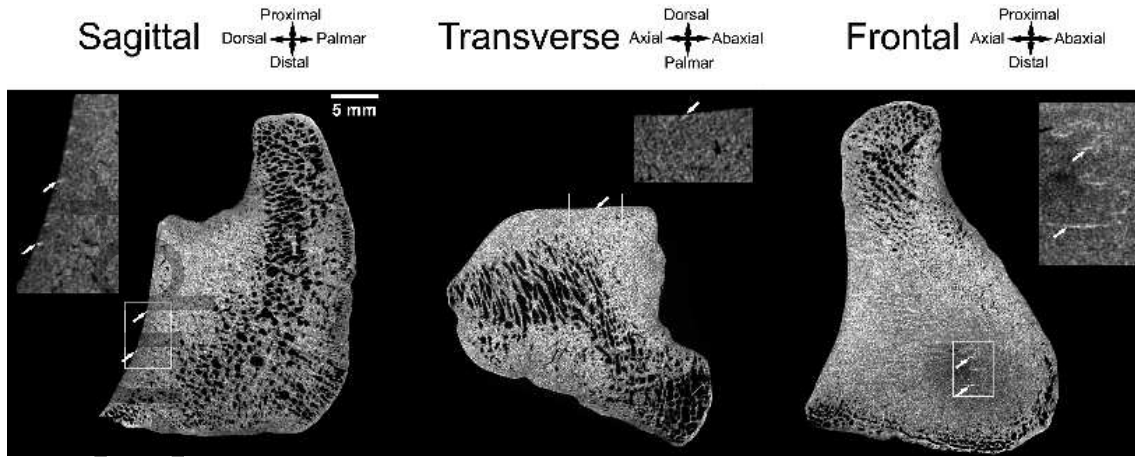
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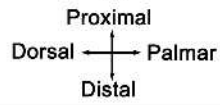


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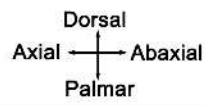
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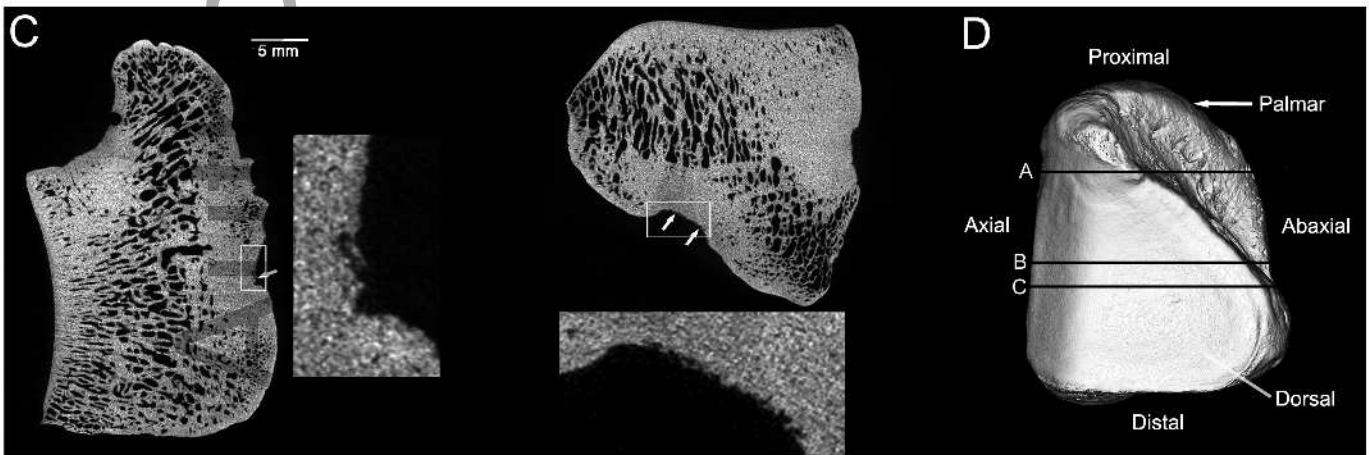
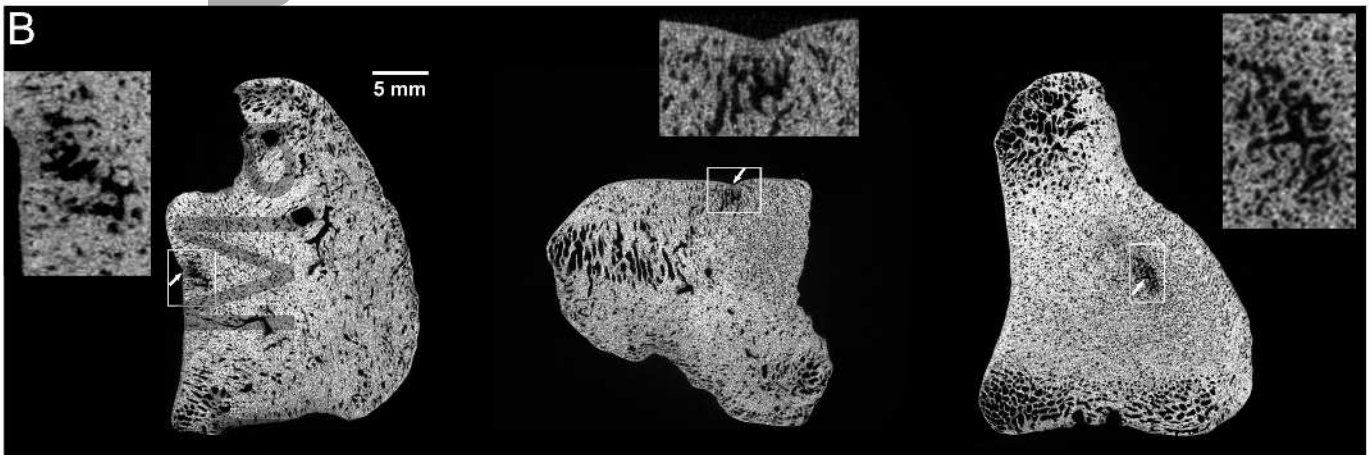
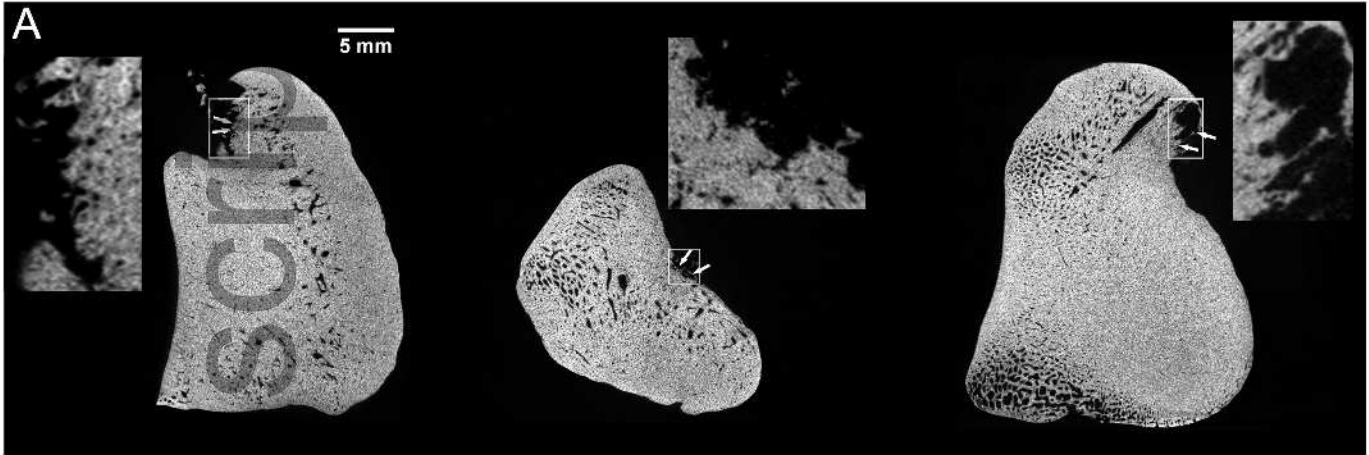
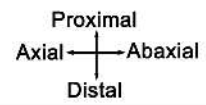
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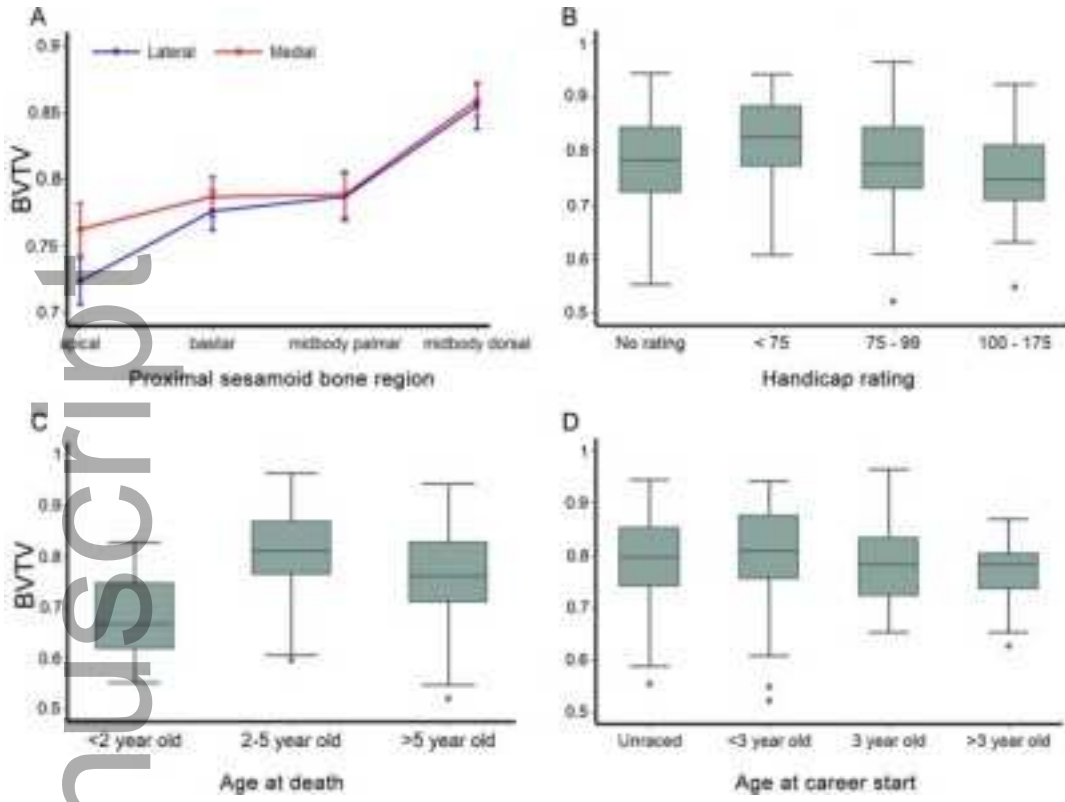
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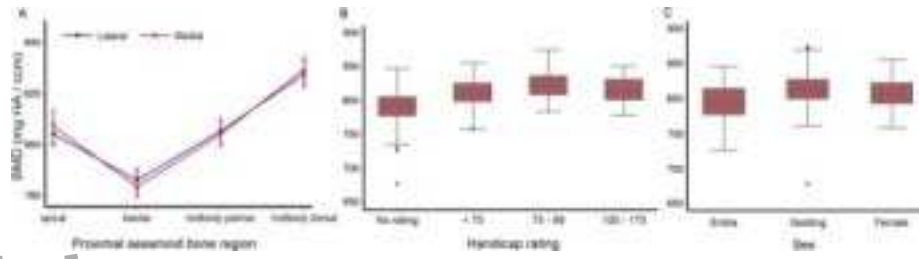
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