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Intracortical remodelling increases in highly-loaded bone after exercise cessation

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Resorption within cortices of long bones removes excess mass and damaged tissue, and increases during periods of reduced mechanical loading. Returning to high-intensity exercise may place bones at risk of failure due to increased porosity caused by bone resorption. We used microradiographic images of bone slices from highly-loaded (metacarpal, tibia, humerus) and minimally-loaded (rib) bones from 12 racehorses, 6 in active high-intensity exercise and 6 in a period of rest following intense exercise, and measured intracortical canal cross-sectional area (Ca.Ar) and number (N.Ca) to infer remodelling activity across sites and exercise groups. Large canals representing resorption spaces (Ca.Ar > 0.04 mm^2) were 5- to 18-fold greater in number and area in the third metacarpal bone from rested than exercised animals (p = 0.005–0.008), but were similar in number and area in ribs from rested and exercised animals (p = 0.575–0.688). A weaker, intermediate relationship was present in tibia and humerus, and when resorption spaces and partially-infilled canals (Ca.Ar > 0.002 mm^2) were considered together. The mechanostat may override targeted remodelling during periods of high mechanical load by enhancing bone formation, reducing resorption and suppressing turnover, but both systems may work synergistically in rest periods to remove excess and damaged tissue.

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1. Introduction

Osteoclastic resorption of bone serves several critical functions. It is essential in the mechanism for expression of skeletal adaptation to load: it facilitates reduction of bone mass as a response to reduced loads on the skeleton [1], moulds the surfaces of bones to refine their geometric properties in response to altered strain [2–6] and is the first stage in the process to replace bone with one microstructure [7] or fibre orientation [8–12] with that of another [13]. It also facilitates the repair of bone tissue by removing damaged or dead bone matrix to make way for its replacement with healthy tissue [14–16]. Furthermore, resorption of bone is important for mineral homeostasis, providing a mechanism to release mineral ions bound in bone into circulation [17].

While osteoclastic resorption is an ongoing process in the bones of most mammals there is evidence to support focal targeting of resorption in bones of the appendicular skeleton in regions where the matrix has suffered microdamage as a consequence of mechanical overload or repetitive stress injury [18,19]. Non-targeted or stochastic remodelling may occur as a homeostatic mechanism that has evolved to maintain the material health of the skeleton by reducing the effects of fatigue damage of bones in the long term [20,21]. The suppression of both targeted and stochastic remodelling by experimental administration of bisphosphonates provides support for this hypothesis [22]. Resorption, the essential first stage of the repair process, creates additional porosity that may relate to higher strain energy density [2], decreased elastic modulus [23] and diminished toughness [24–26].

The loss of strength and stiffness caused by increased porosity may predispose bone to accelerated accumulation of microdamage and monotonic failure in the face of ongoing cyclical loads [20,27]. Bone must strike a balance between the risks of tissue deterioration occurring through the accumulation of microdamage with the risks of failure due to increased porosity incurred during remodelling. Previous studies indicate that the balance falls in favour of allowing microdamage to accumulate [27] rather than attempting repair by remodelling while high loads continue. Histological examination of bone from experimental horses [28] and from post mortem specimens from racehorses [29] suggests that osteoclastic resorption in the third metacarpal bone is suppressed in horses that are consistently subjected to a high exercise workload compared to those forced to rest. A physiological control system may have evolved to favour the safest way to manage skeletal integrity in the face of ongoing mechanical loading, but evidence for this hypothesis is weak. The purpose of the present study is to develop the evidence base to better understand bone's response to periods of intense exertion and subsequent rest.

The aim of this study was to measure bone remodelling, using the density and size of resorption canals as a proxy, in selected regions of bones from horses in active training and from horses subject to enforced rest. Horses are a unique model system in which to study bone dynamics. Their bones are large and support plentiful secondary osteonal remodelling [8,28,30–32], which does not normally occur in laboratory rodents or other mammals smaller than about 2 kg body mass [33]. Material for detailed histological study is available post-mortem from elite equine athletes with much greater regularity and control than from elite sportspeople. The exercise history of the horses in the current study is known with high confidence because all the animals reside, train and race within one organisation that keeps records of all interventions, which is an unusual situation among sports animals.

2. Materials and Methods

(a) Study material

Bone was obtained post-mortem from Thoroughbred racehorses ($n = 12$) at the Hong Kong Jockey Club (HKJC) that had been euthanised for reasons unrelated to the study (Table 1).

Horses were assigned to one of two groups: "exercised" ($n = 6$) and "rested" ($n = 6$). The "exercised" group was restricted to horses that had been in regular training for racing and had

Table 1. List of horses in the study.

group	ID	age (y)	intense exercise (d)	exercise-death interval (d)	cause of death
exercised	01	5	8	0	drug anaphylaxis
	02	6	8	0	tendon rupture
	03	6	6	3	collapse during racing
	04	5	4	1	colic
	05	5	6	0	collapse during racing
	06	4	6	0	collapse during fast work
rested	07	8	5	35	advanced osteoarthritis
	08	6	5	91	tendon rupture
	09	8	5	82	chronic lameness
	10	8	4	45	advanced osteoarthritis
	11	5	5	79	unsuitable temperament
	12	6	6	94	chronic lameness

Table 2. Summary statistics of horses in the study.

variable		exercised	rested
age (y)	mean	5.17	6.83
	std. dev.	0.75	1.33
	minimum	4	5
	maximum	6	8
days with intense exercise (d)	mean	6.33	5.00
	std. dev.	1.51	0.63
	minimum	4	4
	maximum	8	6
days between last intense exercise and death (d)	mean	0.67	71.00
	std. dev.	1.21	24.84
	minimum	0	35
	maximum	3	94

undertaken intense exercise within seven days of their death. "Intense exercise" was defined as a race, barrier trial (practice race), training gallop or training canter and "regular" as this level of exercise occurred on at least four occasions in the preceding 30 days. The "rested" group was restricted to horses that had previously been in race training (at which stage they had undertaken similar intense exercise to that of the "exercised" group) but that were undergoing a period of rest and had not undertaken any intense exercise for 30–120 days prior to euthanasia. Details on each horse's daily exercise were sourced from The Hong Kong Jockey Club's Racing Information database [34]. Classification of the study horses are presented in Table 1 and summary of relevant statistics are presented in Table 2.

(b) Bone processing

Bone sections were cut from bones of the appendicular skeleton the right third metacarpal bone, right tibia, and left humerus, at locations that are common sites for fatigue fractures in Thoroughbred racehorses [35]. Sections were also cut from the mid-body of the tenth left rib

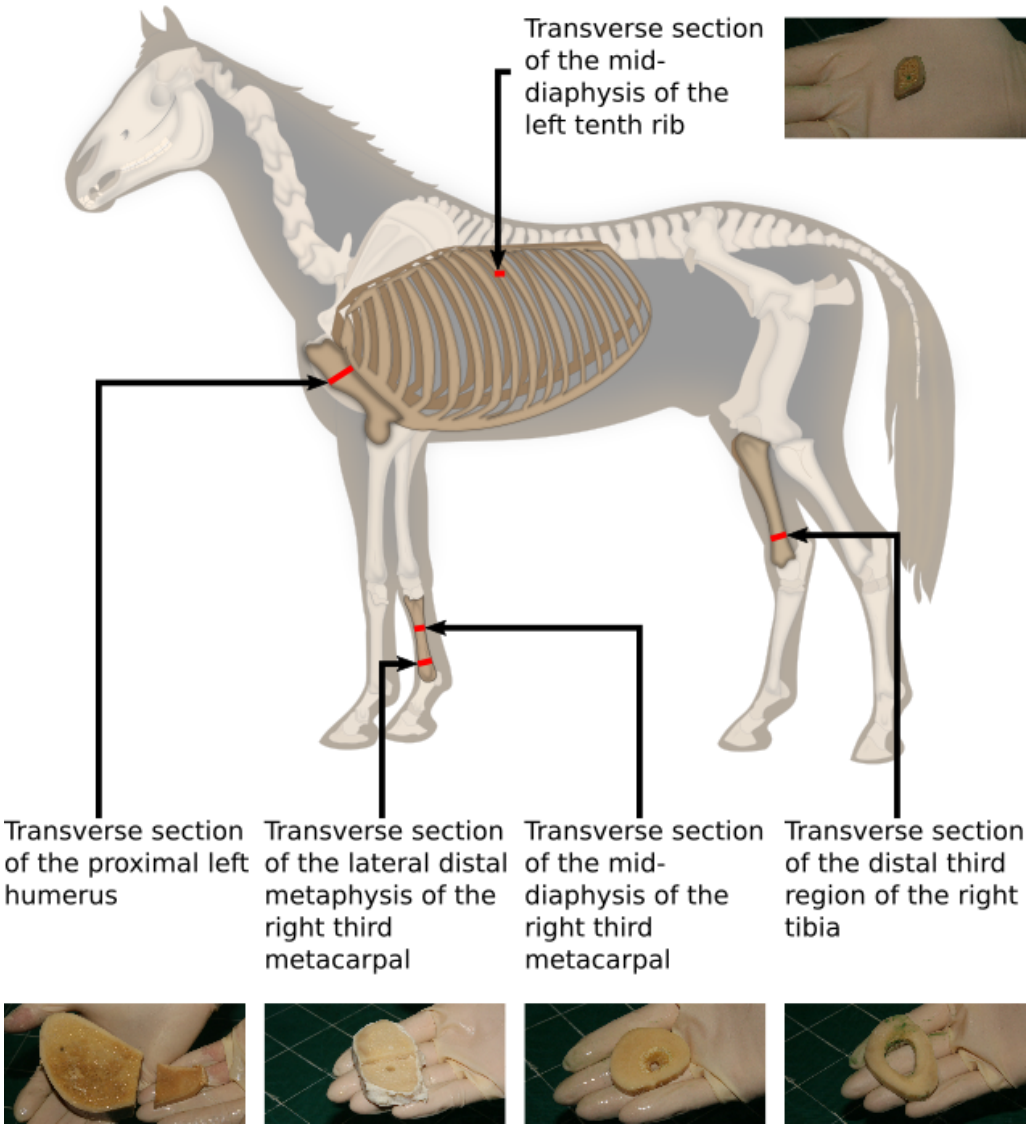

Figure 1. Sample collection sites. This work is a derivative of "Horse anatomy.svg" by Wikipedian Prolific and Wilfredor used under Creative Commons Attribution-Share Alike 3.0 Unported license.

to represent bone of the axial skeleton that is not subject to significantly altered loads during locomotion (Figure 1).

The bones were dissected in their entirety immediately post mortem. Transverse blocks of 20 mm thickness were cut from the specified region of each bone using a band saw (ST-WBS 180). The blocks from the rib, tibia and mid diaphysis of third metacarpal included the entire cross section of the bone. The blocks of the proximal humerus and distal metaphysis of third metacarpal included only the lateral half of the entire cross section. Blocks were dissected free of soft tissue including fat and periosteum before being placed in a bacterial pronase detergent (Tergazyme 10% alkaline pronase detergent, Alconox Inc, NY, USA) at 37 °C for five days to digest remaining soft tissue. Blocks were then fixed in 70% ethanol for seven days. Plane-parallel sections 250 µm and 100 µm thick were subsequently cut from each block in a plane at right angles to the long axis of the bone using an annular saw (Leica Microsystems SP1600). Sections were stored in

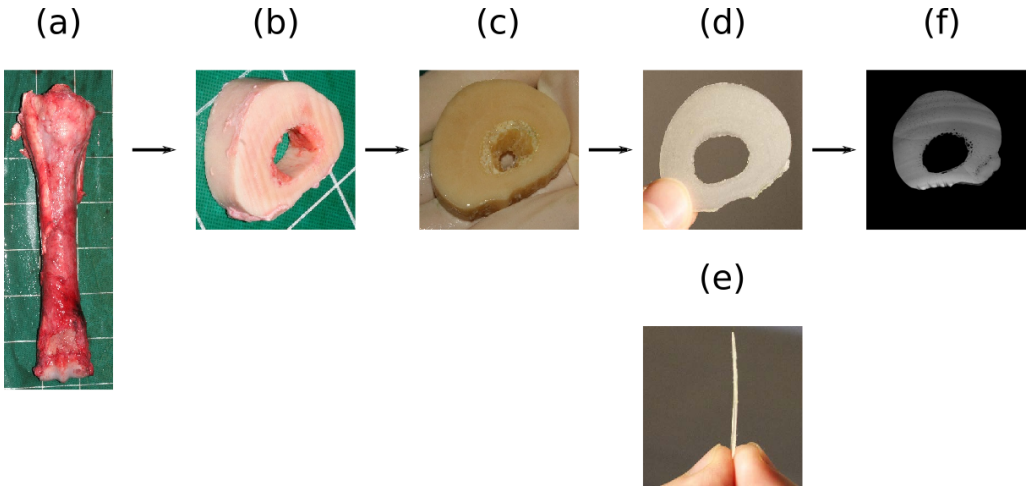

Figure 2. Bone processing from post-mortem collection to microradiograph: (a) dorsal view of the right third metacarpal bone immediately post mortem, (b) transverse 20 mm thick slice cut with a band-saw, (c) slice after five days of immersion in bacterial pronase detergent and subsequent fixation for seven days in 70% ethanol, (d) proximodistal and (e) lateral view of 250 µm thick sections cut with an annular saw, (f) microradiograph of 250 µm thick section. Dorsal to the top, medial to the right (d, f), medial to left (c)

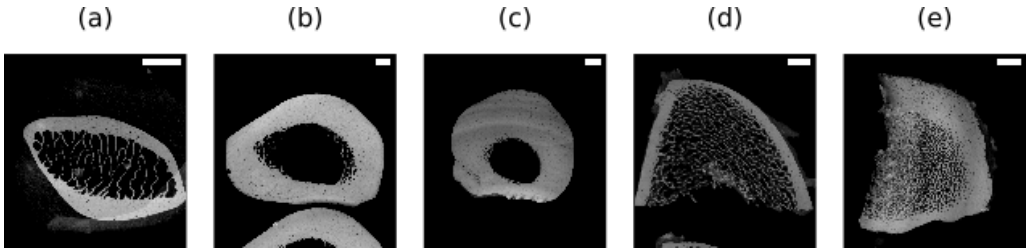

Figure 3. Example microradiographs obtained from each section: (a) transverse section of the mid-diaphysis of the left tenth rib (5× magnification), (b) transverse section of the distal third region of the right tibia (2× magnification), (c) transverse section of the mid-diaphysis of the right third metacarpal (2× magnification), (d) transverse section of the proximal left humerus (3× magnification), (e) transverse section of the lateral distal metaphysis of the right third metacarpal (3× magnification) (scale bars 5 mm). (a) Medial to top, cranial to left; (b) cranial to top, medial to right; (c, e) dorsal to top, medial to left; (d) lateral to top, dorsal to right

glycerol (Figure 2). The blocks of the distal third region of the right tibia and of the mid-diaphysis of the right third metacarpal bone of horse 11 were severely damaged during cutting and could not be used.

(c) Microradiography

Microradiographs (Figure 3) were obtained from each section by point projection digital microradiography at 26 kV (Faxitron, QADOS, Sandhurts, Berkshire, UK) and stored in 8-bit tagged image file format (TIFF). Raw images are available from figshare (see Data Availability statement).

Table 3. X-ray magnification, pixel spacing and minimum resolvable canal diameter (Ca.D) information for each sample site. The humerus from horse 5 was imaged at lower resolution than the rest of the humerus sections. Magnification was the maximum that allowed the entire specimen to be imaged in a single frame.

bone section	magnification	pixel spacing (µm)	minimum Ca.D (µm)
rib	5	10.0	11.28
humerus	3	16.7	18.84
humerus (ID 5)	2	25.0	28.21
tibia	2	25.0	28.21
metacarpal diaphysis	2	25.0	28.21
metacarpal metaphysis	3	16.7	18.84

(d) Image and Statistical Analysis

Microradiographs of each section were analysed using Fiji 1.53c [36–38] to quantify non-mineralised areas of the section (porosities) that appear as small radiolucent spots surrounded by radiopaque cortical bone matrix. Cortical bone was segmented by manually drawing along the endosteal and periosteal boundaries. A triangle method threshold [39] was calculated for each image and applied to the greyscale microradiographs, to assign each pixel to one of 'bone' or 'pore' binary phases. ImageJ's Analyze Particles function was used to identify individual pores (Figure 4). Particles were filtered using Python to retain those with circularity between 0.3 and 1 and area > 0.002 mm^2. Large, smooth pores at the endosteal margin of the cortex in each section that reflected transition to cancellous bone were manually excluded. Remaining pores were assumed to represent osteonal canals at the cutting cone (Ca.Ar > 0.04 mm^2) and closing cone (Ca.Ar = 0.002-0.04 mm^2) stages (Figure 4). The sizes were selected to coincide with diameters associated with mature Haversian canals (Ca.Dm, 20–30 µm in the horse) and varying stages of resorption/infilling of resorption canals [33].

The diameter of each canal (Ca.Dm, µm) was calculated from Ca.Ar assuming a circular cross section and recorded. The minimum canal diameter was slightly higher than the limit of resolution of the images (Table 3).

Cortical bone area (Ct.Ar, μm^2), count of individual canals (N.Ca, unitless), total canal area (Tt.Ca.Ar, μm^2), canal numerical density (N.Ca/Ct.Ar, μm^{-2}), and canal area fraction (Tt.Ca.Ar/Ct.Ar, unitless), were calculated for each section [40].

Differences in the measurements between exercised and rested groups were tested for using the Mann-Whitney U test, implemented in SciPy [41]. Full details of the image processing and statistical analysis are available at figshare (See Data Availability statement).

3. Results

Where groups differed, samples from the rested group contained more canals in the cutting and closing cone size ranges than samples from the exercised group (Figure 5, 6; Table 4, 5, 6, 7), which was most pronounced in the metacarpal sites and for the large canals that represented cutting cones and other resorption spaces (Ca.Ar > 0.04 mm^2). Numbers of canals (N.Ca/Ct.Ar) and overall porosity (Tt.Ca.Ar/Ct.Ar) were around two- to eighteen-fold greater in rested than exercised horses' metacarpal diaphysis and metacarpal metaphysis, but were not different between rested and exercised horses in the rib, humerus or tibia (Table 4, 6). A greater number of large canals was evident in the dorsal cortex of the rested metacarpal diaphysis (Fig. 5i) and the caudal tibia (Fig. 5f), while a higher number of diffusely-located large canals was evident in the rested metacarpal metaphysis (Fig. 5j).

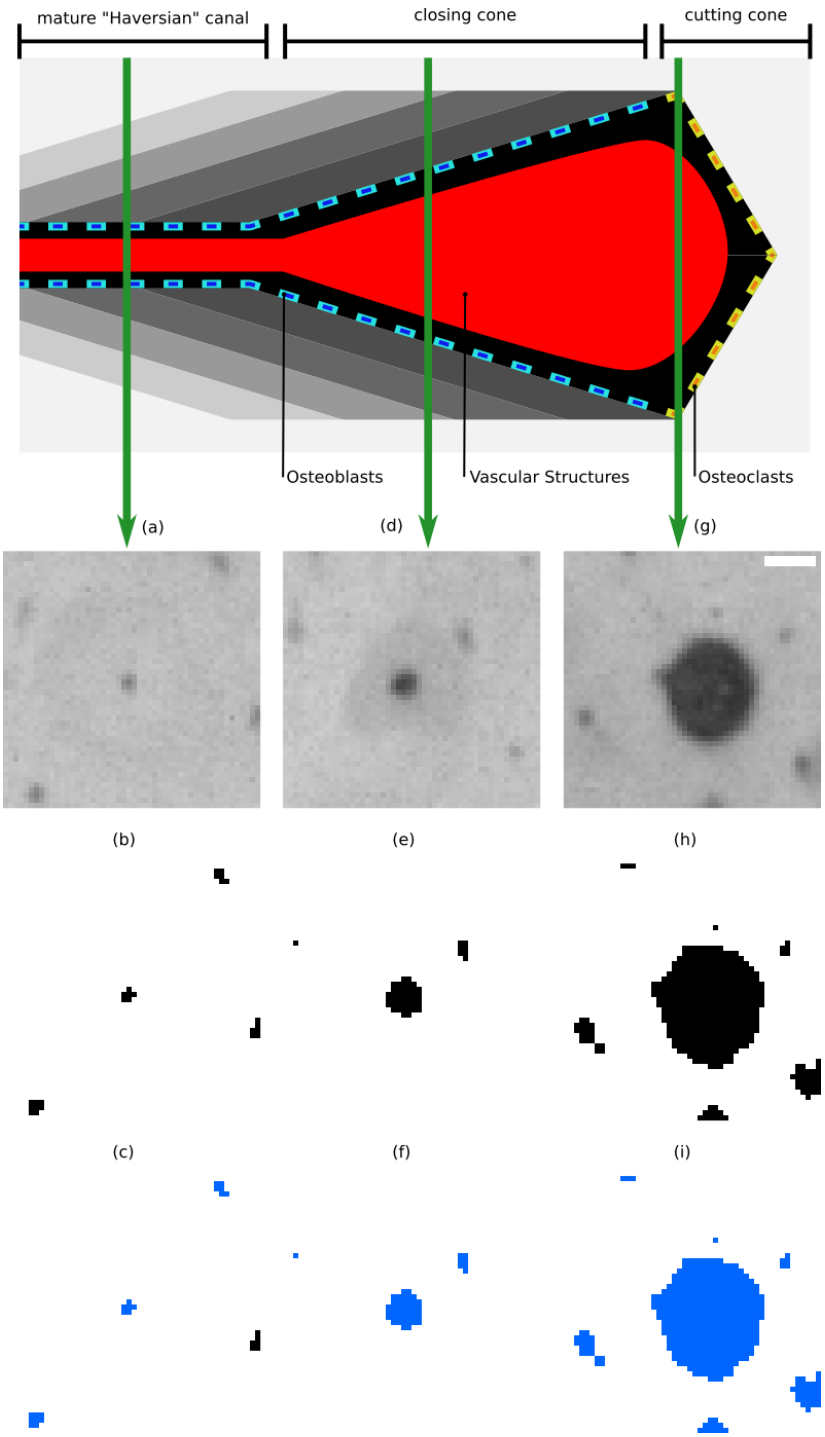

Figure 4. Illustration of remodelling in cortical bone with magnified view in region of interest from images used in this study and workflow used to select canals with Fiji's Analyze Particles function. Selected canal are in blue. (a) magnified view of mature "Haversian" canal in raw microradiography, (b) after threshold filter, (c) canal selection. (d) magnified view of closing cone in raw microradiography, (e) after threshold filter, (f) canal selection. (g) magnified view of cutting cone in raw microradiography, (h) after threshold filter, (i) canal selection. (scale bar 100 µm).

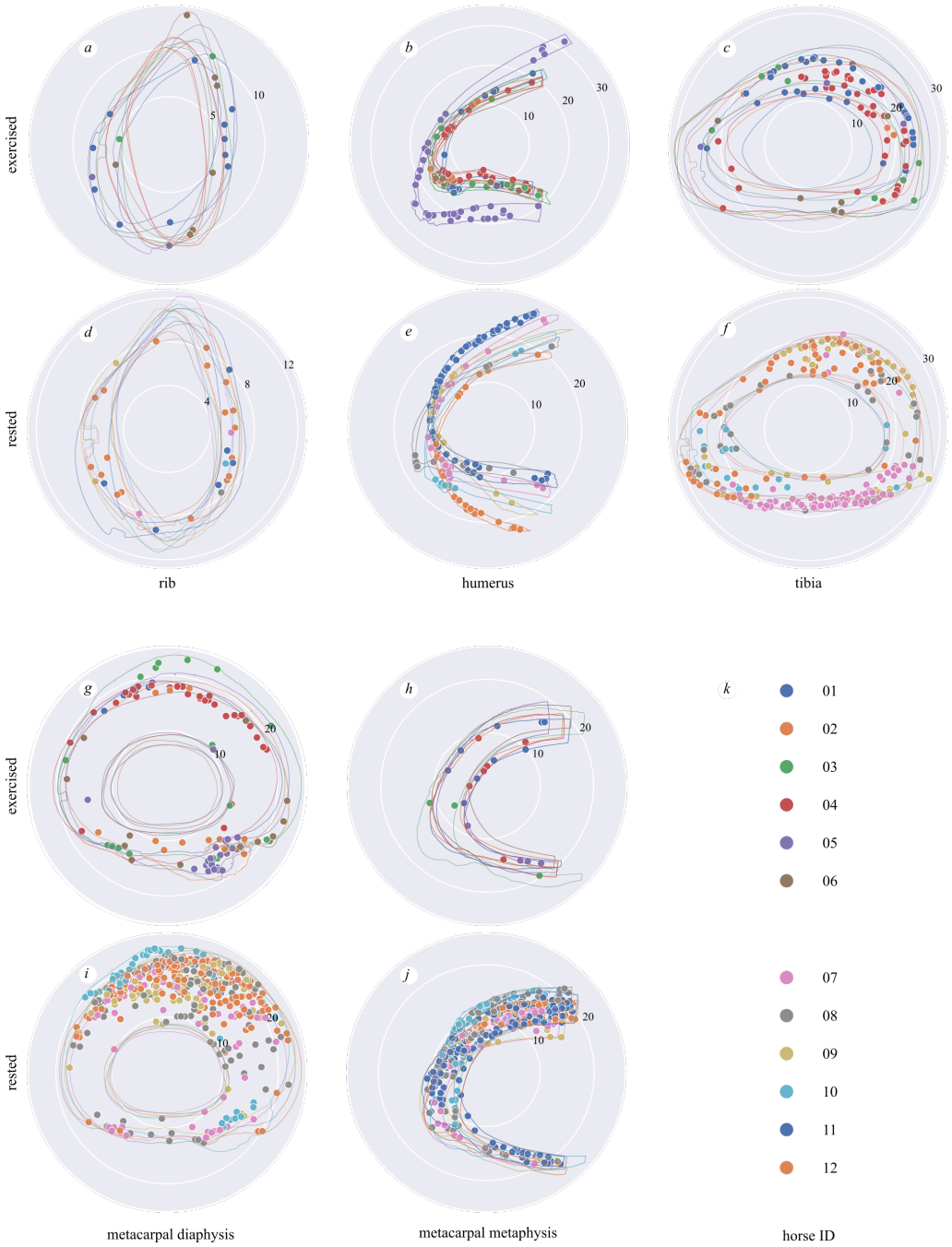

Figure 5. Polar plots of the largest canals representing resorption spaces or cutting cones (Ca.Ar > 0.04 mm^2) in bones from the exercised (a, b, c, g, h) and rested (d, e, f, i, j) groups, centred on each sample's centre of mass. Canals are indicated by solid dots, and periosteal and endosteal contours are indicated by outlines, all colour coded by horse ID (k). Note the larger number of large canals in the rested metacarpal and tibial sites and their anatomical distribution in the caudal tibia, dorsal metacarpal diaphysis and throughout the metacarpal metaphysis. Cranial / dorsal to the top, medial to the right. Scale in mm given by figures on each plot.

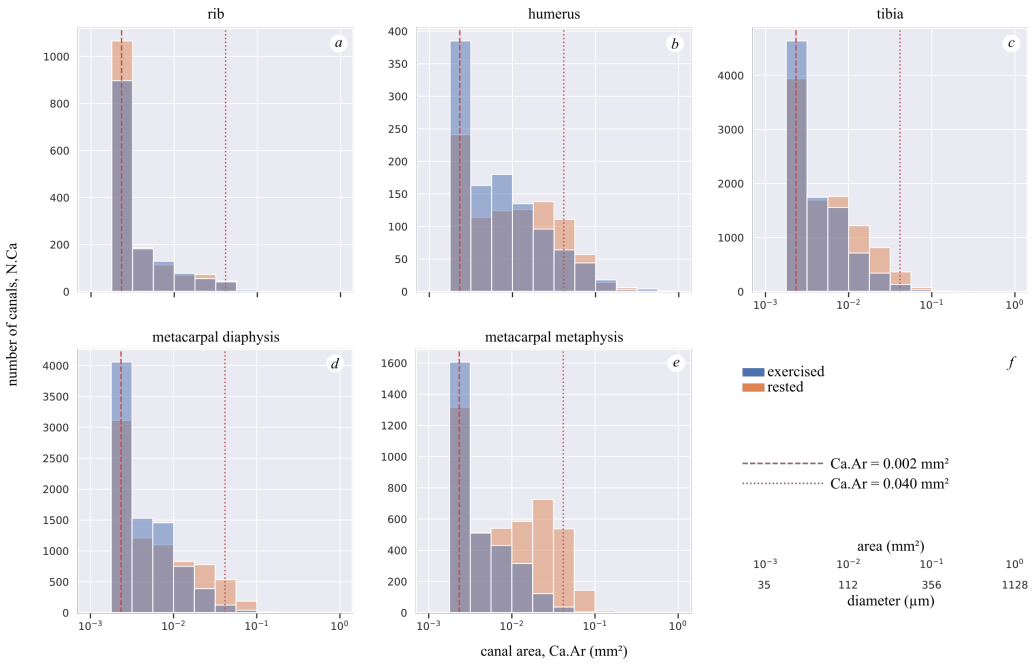

Figure 6. Canal number (N.Ca) summed over all samples within exercise and rested groups versus canal area (Ca.Ar) for canals with Ca.Ar > 0.002 mm^2, for each sample site. Note the greater number of large canals in the rested metacarpal metaphysis (e), and the similar distributions of canal sizes in rested and exercised ribs (a). Vertical dashed line at 0.04 mm^2 indicates the cutoff value between large (resorption spaces; illustrated in 5) and small (partially infilled) canals. Vertical dashed line at 0.002 mm^2 indicates the cutoff value between completely infilled mature Haversian canals and closing cones. Conversion between area and diameter assuming a circle provided in f.

Microradiographs of the humerus have considerable variation in shape and size of the bone (Figure 5c, d). Additionally, some samples presented extensive resorption in the region of the lateral cortex (Figure 7) where it was difficult to differentiate cortical bone from trabecular bone.

4. Discussion

We found significantly more and larger canals of a diameter that matches secondary osteonal remodelling events (i.e. the wide bore of cutting and closing cones) in the cortices of distal limb bones in resting horses than in horses undergoing high-intensity exercise. Assuming that the canals reflect resorptive activity, the finding provides further evidence to support the hypothesis that a physiological mechanism has evolved to limit or suppress resorption in the face of ongoing, high cyclical loads on bone. No relationship between osteonal remodelling and exercise group was noted in the rib, which does not experience elevated mechanical load during exercise. The magnitude of resorption appeared to increase with increasing distance along the limb, with the greatest resorption occurring in the bone that experiences very high strains and is the most fracture-prone, the third metacarpal.

An important function of osteoclastic resorption of bone is the repair of bone, through the replacement of damaged matrix with new, healthy tissue. Evidence that osteoclasts are recruited preferentially to target areas of microdamage or osteocyte necrosis in cortical bone [18,19,21] supports this concept. Accumulation of microdamage probably has little impact on mechanical properties of cortical bone until it becomes severe [19,21,27,42], while suppression of remodelling by half may result in 3 times the accumulation of microdamage [43,44]. In some cases, microdamage may improve the toughness of bone [45,46]. Bone repair without resorption

Table 4. Summary of canal dimensions by site and exercise group (mean ± s.d.) for cutting and closing cones (Ca.Ar > 0.002 mm^2), normalised by cortical area (Ct.Ar; identical to Table 6 because they are the same specimens).

bone	group	Ct.Ar (mm^2)	N.Ca	Tt.Ca.Ar (mm^2)	N.Ca/Ct.Ar (mm^{-2})	Tt.Ca.Ar/Ct.Ar
rib	exercised	80.77 ± 4.96	231.7 ± 101.5	1.360 ± 0.970	2.822 ± 1.091	0.0164 ± 0.0108
	rested	79.63 ± 6.61	259.8 ± 129.8	1.488 ± 0.801	3.273 ± 1.649	0.0186 ± 0.0098
humerus	exercised	141.2 ± 48.6	182.0 ± 51.88	2.879 ± 1.425	1.383 ± 0.517	0.0200 ± 0.0056
	rested	104.6 ± 24.2	155.5 ± 46.54	3.386 ± 1.678	1.544 ± 0.607	0.0325 ± 0.0142
tibia	exercised	823.5 ± 19.9	1527.3 ± 678.4	9.257 ± 4.786	1.855 ± 0.817	0.0112 ± 0.0057
	rested	897.8 ± 81.1	1975.8 ± 573.5	17.36 ± 4.117	2.212 ± 0.667	0.0193 ± 0.0043
metacarpal	exercised	782.7 ± 73.0	1391.8 ± 561.2	9.055 ± 3.328	1.770 ± 0.693	0.0116 ± 0.0046
diaphysis	rested	816.1 ± 58.9	1552.4 ± 850.2	17.44 ± 5.802	1.920 ± 1.108	0.0213 ± 0.0069
metacarpal	exercised	253.6 ± 27.2	504.8 ± 83.77	3.026 ± 0.863	2.028 ± 0.493	0.0120 ± 0.0037
metaphysis	rested	250.1 ± 36.9	729.5 ± 171.4	11.42 ± 3.522	2.913 ± 0.581	0.0457 ± 0.0113

Table 5. Results of Mann-Whitney comparisons between exercised and rested groups for canals with Ca.Ar > 0.002 mm^2. U values tend towards 0 as the overlap between the two groups' rank distributions decreases to 0. All groups have n = 6 except rested tibia and rested metacarpal diaphysis which have n = 5.

bone	Ct.Ar		N.Ca		Tt.Ca.Ar		N.Ca/Ct.Ar		Tt.Ca.Ar/ Ct.Ar	
	U	p	U	p	U	p	U	p	U	p
rib	21	0.689	15	0.689	16	0.810	17	0.936	14	0.575
humerus	25	0.298	22	0.575	15	0.689	16	0.810	9	0.173
tibia	6	0.121	8	0.235	4	0.055	10	0.411	5	0.083
metacarpal diaphysis	12	0.648	16	0.927	3	0.036	16	0.927	4	0.055
metacarpal metaphysis	19	0.936	6	0.066	0	0.005	5	0.045	0	0.005

Table 6. Summary of canal dimensions by site and exercise group (mean ± s.d.) for cutting cones (Ca.Ar > 0.04 mm^2), normalised by cortical area (Ct.Ar; identical to Table 4 because they are the same specimens).

bone	group	Ct.Ar (mm^2)	N.Ca	Tt.Ca.Ar (mm^2)	N.Ca/Ct.Ar (mm^{-2})	Tt.Ca.Ar/Ct.Ar
rib	exercised	80.77 ± 4.96	3.667 ± 4.412	0.201 ± 0.246	0.043 ± 0.051	0.0024 ± 0.0028
	rested	79.63 ± 6.61	5.000 ± 6.633	0.261 ± 0.332	0.062 ± 0.082	0.0032 ± 0.0041
humerus	exercised	141.2 ± 48.6	18.17 ± 10.15	1.586 ± 1.411	0.126 ± 0.051	0.0103 ± 0.0053
	rested	104.6 ± 24.2	23.00 ± 17.65	1.919 ± 1.356	0.221 ± 0.148	0.0179 ± 0.0105
tibia	exercised	823.5 ± 19.9	15.83 ± 15.78	0.923 ± 0.984	0.019 ± 0.019	0.0011 ± 0.0011
	rested	897.8 ± 81.1	50.20 ± 26.02	2.979 ± 1.872	0.056 ± 0.028	0.0033 ± 0.0020
metacarpal	exercised	782.7 ± 73.0	17.00 ± 9.252	1.058 ± 0.632	0.022 ± 0.014	0.0014 ± 0.0009
diaphysis	rested	816.1 ± 58.9	93.00 ± 44.07	5.469 ± 2.494	0.112 ± 0.048	0.0066 ± 0.0027
metacarpal	exercised	253.6 ± 27.2	3.667 ± 3.502	0.240 ± 0.201	0.014 ± 0.014	0.0009 ± 0.0008
metaphysis	rested	250.1 ± 36.9	66.00 ± 41.56	3.893 ± 2.585	0.262 ± 0.141	0.0154 ± 0.0087

Table 7. Results of Mann-Whitney comparisons between exercised groups for canals with Ca.Ar > 0.04 mm^2. *U* values tend towards 0 as the overlap between the two groups' rank distributions decreases to 0. All groups have $n = 6$ except rested tibia and rested metacarpal diaphysis which have $n = 5$.

bone	Ct.Ar		N.Ca		Tt.Ca.Ar		N.Ca/Ct.Ar		Tt.Ca.Ar/ Ct.Ar	
	U	*p*	*U*	*p*	*U*	*p*	*U*	*p*	*U*	*p*
rib	21	0.689	16	0.806	15	0.688	14	0.575	15	0.688
humerus	25	0.298	17	0.936	15	0.689	12	0.378	8	0.128
tibia	6	0.121	4	0.055	5	0.083	4	0.055	5	0.083
metacarpal diaphysis	12	0.648	0	0.008	0	0.008	0	0.008	0	0.008
metacarpal metaphysis	19	0.936	0	0.005	0	0.005	0	0.005	0	0.005

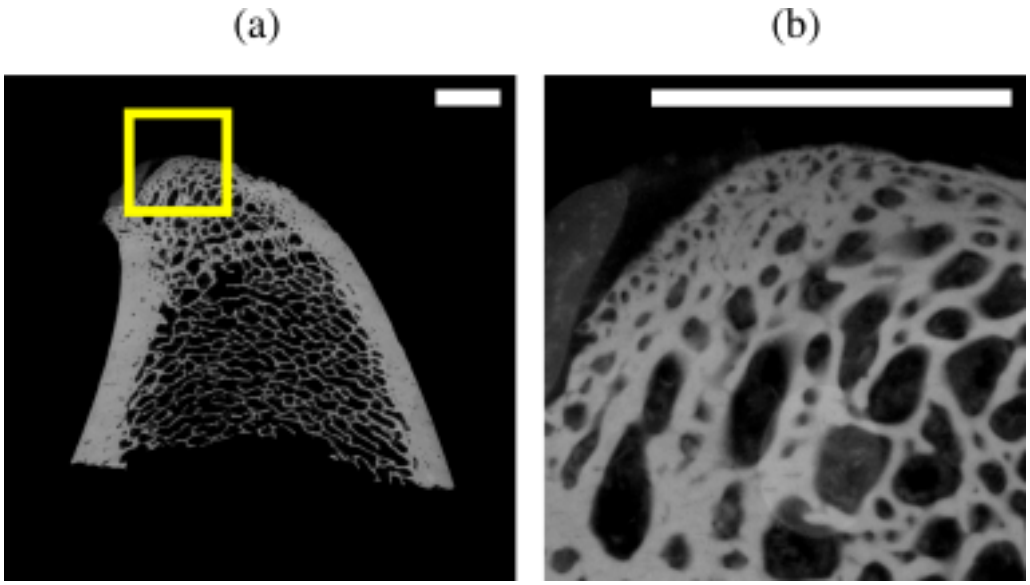

Figure 7. Illustration of lateral cortex where it was difficult to differentiate cortical bone from trabecular bone: (a) transverse section of the proximal left humerus (scale bar 5 mm), yellow boxed region in (a) enlarged in (b)

may occur by organic and inorganic substrates bridging and sealing cracks [30,47], which might be an inherent property of mineralised tissues occurring across all vertebrate taxa like bone remodelling is [48]. In vitro mechanical testing of bone specimens machined from the third metacarpal of Thoroughbred racehorses showed that the elastic modulus and yield strength of specimens were not significantly affected by prior fatigue loading equivalent to a lifetime of racing [49]. Conversely, there is good evidence that cortical porosity is negatively correlated with Young's modulus [25], compressive ultimate stress [50], and fracture toughness [45]. Changes in porosity account for more than 75% of the variability in the strength of the cortical bone [51], and fatigue-induced microdamage located near cortical pores may be more likely to lead to fracture than that located in regions of high mineral content [52].

In the present study, resorption was determined by mapping porosities, which were assumed to represent resorption spaces, partially-infilled canals and mature Haversian canals, whose number and size may be an accurate histological measurement of bone remodelling [21,53]. Resorption spaces in cortical bone may be the classical cutting cone passing through bone matrix, or resorption occurring on the surface of existing osteonal canals [3,54]. Porosities within cortical bone are typically present in the form of osteocyte lacunae ($< 5\,\mu$m diameter) with associated, tortuous interconnected canaliculi ($< 1\,\mu$m diameter), Haversian canals at the centre of osteons (c. $20\,\mu$m diameter) Volkmann's canals, running roughly perpendicular to Haversian canals; resorption canals, in varying stages of being infilled with new bone (between 10 and $400\,\mu$m). Haversian and resorption canals are roughly circular to ellipsoidal in cross section. The former two predominantly lie parallel with the long axis of the bone and so appear circular in transverse section of long bones. Conversely Volkmann's canals appear as tubular or elongated oval structures, depending on their precise orientation relative to the plane of section. Occasionally, larger ($>600\,\mu$m wide), irregular porosities are present close to the endosteal surface of long bone, representing a transition to trabecular bone [3]. We managed these by manually excluding them during segmentation. The presence of such features was particular common in the location studied in the proximal humerus.

Osteonal canals rapidly infill, and subperiosteal primary osteonal bone growth may occur early in training [28], conforming to the mechanostat model whereby increased strains lead to net bone deposition [55]. Our data broadly agree with the mechanostat in that the bone regions that experienced the most intense difference in loads (metacarpal diaphysis) responded the most markedly in terms of osteonal infilling (small canals) under exercise and bone resorption (large canals) during rest. Some resorption, indicated by a small number of large canals, may occur even in the most highly-loaded regions (Figure 6), consistent with previous findings [29].

The high number of large canals observed in the rested group has important implications for physical activity management [56–59], in particular the re-introduction of training after returning from a prolonged rest period. High porosity may be related to reduced bone yield stress and stiffness [25,60,61] and may also create stress risers allowing fracture propagation [62]. Physical readiness training, reduced marching, exercise on flat rather than hilly terrain, and adhering to an exercise protocol, reduces stress fractures of the lower limb bones in military recruits [63,64]. There is some epidemiological evidence that racehorses returning after an eight week rest period are at greater risk of sustaining catastrophic humeral fractures [65], however, it remains to be seen whether a gradual programme of exercise reintroduction is more bone-safe, and this study did not detect a strong bone remodelling signal in the humeral site examined. The time taken for completion of bone remodelling is unknown, however, osteoclasts proceed longitudinally through bone at a rate of about 40 $\mu m\, d^{-1}$ [66], while osteoblastic infilling starts at the same time radial resorption ends [67] and at 1.0–1.5 $\mu m\, d^{-1}$ [28,68] should take 60–90 days to infill from cement line (c. 100 µm radius) to finished canal (c. 10 µm radius) [33]. Completion of remodelling at the organ scale relies on a number of secondary osteons working asynchronously in different locations and is likely to be complicated by individual variations in bone loading, training and resting patterns. Gentle preconditioning exercise that stimulates infilling and that does not suppress targeted remodelling is desirable, but the specific training regimens required to achieve this remain obscure.

Healed fractures are very common among mammals having appeared early in vertebrate evolution [48], suggesting a survival advantage to risking fracture events because fractured bones have the capacity to heal well. We speculate that it may be a lesser cost to the organism to accumulate microdamage and defer remodelling during chronic high load periods, than to incur both high metabolic cost and increased fracture risk by resorbing highly-loaded bone.

While the most plausible biological explanation for the increased number and size of canals is associated with the remodelling process that occurs with low levels of mechanical loading, the association between duration of rest period and age is unknown. We selected a rest period of 4–16 weeks based on results of previous studies [69–71] in which bone resorption was detected between 4 and 16 weeks.

To the authors' knowledge, no previous studies have reported on the differences in fracture-free bone resorption between exercised and rested racehorses. Choosing non-fractured horses was paramount as horses with diagnosed catastrophic or non-catastrophic fractures may have had altered normal bone loading patterns and would not be truly representative of bone loading and resorption, although it was possible that other non-fracture related reasons may also have caused significant lameness that may have reduced the intensity of bone loading during high intensity exercise. Furthermore, this selection criterion resulted in a small sample size of 12 horses, which limits the power of the study to detect small differences between groups and increased the possibility of random error. Matching for age was not considered practical and would have further reduced case numbers; failure to do so here resulted in the exercised horses being on average younger than the rested horses (Table 2) [72]. We considered that restricting the selection criterion to high intensity exercise was appropriate because it has the advantage of reducing the confounding influence of training variation between horses, and is consistent with the design of other studies in the field [29,72,73]. The loss of a tibia specimen was unfortunate because the two- to three-fold difference in N.Ca/Ct.Ar and Tt.Ca.Ar/Ct.Ar for cutting cone-sized canals in the tibial site cannot be considered a robust result and is merely suggestive.

The numerical cutoffs used to classify canals into 'cutting cones' and 'closing cones' is a simplification that aids in interpretation. The microradiographic data presented here do not capture the physiological status of each canal, only their sizes. We are also aware that a hard cutoff does not necessarily represent the diversity of modes by which resorption and infilling may occur and their dynamic nature, including substantial resting periods where neither resorption nor deposition are occurring. We believe that it is helpful to separate the very largest canals from the moderately-sized and smallest canals because these categories give some insight into the overall status of the bone and in particular whether porosity that can have resulted only from resorption is more or less common depending on the exercise or rested status of the animal.

Variation in bone geometry was minimised by cutting bone blocks in pre-determined standardised locations and visualising canals relative to the bone's centre of mass (Figure 5). The relationship between bone remodelling and bone geometry was not within the scope of this study but is likely to be affected by differences in mechanical loading [74–76], and changes to the second moment of area [77,78] to minimise peak bone strains. It is therefore not unreasonable to assume that different training regimens may have influenced the geometry of some of the bone sections, and the relationship between bone resorption and bone geometry should be explored in future studies. The counting of canals was automated with Fiji [36–38] which should have reduced bias. A thresholding technique was used for porosity segmentation but did contend with substantial variation in background intensity due to uneven sample thickness. Given the aim of this study, two-dimensional microradiography was considered a suitable imaging technique to identify bone resorption, however, future studies should use three dimensional imaging techniques such as X-ray microtomography and incorporate other markers of bone remodelling such as intra-vital calcein labelling to evaluate bone formation rate [68].

5. Conclusion

We found in distal limb bones but not in ribs or proximal limb bones that total canal area and the number of large canals, representing resorption activity, were greater in rested than in exercised animals, and that large canals were concentrated in regions known to experience high strains during peak athletic loading. We surmise that the resorption phase of bone remodelling is significantly suppressed by high intensity exercise and is subsequently reactivated during a rest period to repair accumulated microdamage in a site-specific manner. During high intensity exercise periods, the balance between the mechanostat (increasing bone mass) and targeted remodelling (increased secondary osteonal cutting cone formation) appears to tilt towards the mechanostat. Targeted remodelling and the mechanostat may work synergistically in rest periods to remove damaged and excess bone tissue. Numerous large canals might exacerbate the risk of catastrophic failure should intense exercise be reintroduced prior to osteoblastic infilling that completes the bone remodelling cycle. Future studies should be directed at determining appropriate training regimens to protect athletes' bones when they return to exercise following an extended period of rest.

6. Ethical Statement

Post-mortem material was derived from racehorses that died for reasons unrelated to the current study. Permission to use residual tissues is granted by the Hong Kong Jockey Club ownership agreement. Racing and training information was taken from publicly available data.

7. Data accessibility

All raw images are available at figshare doi:10.6084/m9.figshare.c.5910674. Bone statistics from the raw images are available at figshare doi:10.6084/m9.figshare.19410431. Pore information from the raw images is available at figshare doi:10.6084/m9.figshare.19410452. Full details of the image processing and statistical analysis are available at figshare doi:10.6084/m9.figshare.19418933.

8. Authors' contributions

Raniere Gaia Costa da Silva: formal analysis, methodology, writing original draft, review and editing. Tsim Christopher Sun: methodology, writing original draft, review and editing. Ambika Prasad Mishra: writing review. Alan Boyde: methodology. Michael Doube: supervision, writing original draft, writing review and editing. Christopher Riggs: supervision, conceptualization, methodology, writing original draft, writing review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

9. Competing interests

We declare we have no competing interests.

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