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Thoroughbred horses in race training have lower levels of subchondral bone remodelling in highly loaded regions of the distal metacarpus compared to horses resting from training

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ABSTRACT

Bone is repaired by remodelling, a process influenced by its loading environment. The aim of this study was to investigate the effect of a change in loading environment on bone remodelling by quantifying bone resorption and formation activity in the metacarpal subchondral bone in Thoroughbred racehorses. Sections of the palmar metacarpal condyles of horses in race training ($n = 24$) or resting from training ($n = 24$) were examined with light microscopy and back scattered scanning electron microscopy (BSEM). Bone area fraction, osteoid perimeter and eroded bone surface were measured within two regions of interest: (1) the lateral parasagittal groove (PS); (2) the lateral condylar subchondral bone (LC). BSEM variables were analysed for the effect of group, region and interaction with time since change in work status. The means ± SE are reported.

For both regions of interest in the training compared to the resting group, eroded bone surface was lower (PS: 0.39 ± 0.06 vs. 0.65 ± 0.07 per mm, $P = 0.010$; LC: 0.24 ± 0.04 vs. 0.85 ± 0.10 per mm, $P < 0.001$) and in the parasagittal groove osteoid perimeter was higher (0.23 ± 0.04% vs. 0.12 ± 0.02%). Lower porosity was observed in the subchondral bone, reflected by a higher bone area fraction in the LC of the training group (90.8 ± 0.6%) compared to the resting group (85.3 ± 1.4%, $P = 0.0010$).

Race training was associated with less bone resorption and more bone formation in the subchondral bone of highly loaded areas of the distal metacarpus limiting the replacement of fatigued bone. Periods of reduced intensity loading are important for facilitating subchondral bone repair in Thoroughbred racehorses.

Introduction

Repeated cyclical loading of bone during intense exercise results in bone fatigue. Fatigued bone is bone that has undergone cyclic loading and consumed a proportion of its fatigue life (the number of cycles to failure). Bone fatigue results in microdamage, initially at the molecular level, before the development of microcracks, which can be detected microscopically (Burr et al., 1997; Muir et al., 2008). Fatigued bone is replaced by remodelling, which involves the coordinated actions of the bone-resorbing osteoclasts, and the bone-forming osteoblasts. The action of osteoclasts produces scalloped ('eroded') surfaces, which can be observed histologically, and increased bone porosity, which can be quantitated as measurements of void area in bone sections. Increased porosity is due to the normal delay in the onset of the bone formation phase following resorption, where osteoblasts deposit bone matrix (osteoid) on bone surfaces which then slowly mineralise (Boyde and Firth, 2005).

The remodelling process has been shown to vary within bones both temporally and regionally (Murray et al., 2001; Boyde and Firth, 2005). Fatigue fractures and subchondral bone injuries occur when microdamage accumulates faster than can be repaired by remodelling. Therefore, factors that alter remodelling rates affect the risk of injury (Riggs, 2002). Under conditions of high cyclic loading, remodelling activity decreases, an effect that is thought to be due to inhibition of osteoclast recruitment (Jee et al., 1991; Rubin et al., 1999). This reduction of remodelling activity may be inconsequential if the loading is of short duration. However, in circumstances of prolonged loading or in inadequately adapted bone, microdamage will accumulate (Whitton et al., 2010). Microdamage can also directly stimulate focal remodelling, even under high loading conditions (Burr et al., 1985; Whitton et al., 2013). Paradoxically, while this targeted remodelling serves to assist healing under moderate loading conditions, it may accelerate injury development in circumstances of intense loading (van Oers et al., 2011).

The Thoroughbred racehorse subjects the subchondral bone of the metacarpophalangeal joint to extreme habitual loading when in race training (Harrison et al., 2010, 2013). Training periods vary in duration but can exceed 20 weeks without interruption (Whitton

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et al., 2010). There is limited quantified information on subchondral bone turnover during this period. Previously observed reduced porosity and scant resorption surfaces within distal metacarpal subchondral bone of horses in training are suggestive of suppressed remodelling rates (Boyde and Firth, 2005; Whitton et al., 2010, 2013). The combination of low bone turnover and high repeated loading exposes this area to a high risk of fatigue damage, consistent with the large proportion of race horses that exhibit distal metacarpal subchondral bone injury (Barr et al., 2009; Pinchbeck et al., 2013). A better understanding of the influence of race training on subchondral bone remodelling is essential to understanding the pathophysiology of subchondral bone injury.

In this study, we measured remodelling activity in the subchondral bone of the distal metacarpus of Thoroughbred horses either in race training or resting from training. Two sites on the distopalmar aspect of the third metacarpal bone that are considered to undergo high loading were selected: (1) the lateral parasagittal groove where microdamage is commonly observed; (2) the lateral condyle where injury is least common (Muir et al., 2008; Pinchbeck et al., 2013). We hypothesised that bone remodelling activity would be lower in intensely training horses. Additionally, we aimed to explore the relationship between remodelling activity and time since a change in training status.

Materials and methods

Third metacarpal (MC3) bones were collected from 48 Thoroughbred horses that died or were euthanased in the period April 2007–August 2012 that underwent comprehensive post-mortem examination at the University of Melbourne. The use of animal tissues met the requirements of the University of Melbourne Animal Ethics Committee. Twenty-seven of these horses had been included in a previous study (Whitton et al., 2010). Horses were assigned to two groups: (1) training: in race training; or (2) resting controls: horses that had previously undergone race training but were not currently training. Training horses had been exercising regularly for 4 weeks or longer and had progressed to intense exercise (fast canter or gallop) in the current training period. Resting horses had been restricted to a stable or paddock and were not undergoing forced exercise.

The sex distribution was the same for both groups, with 6 females and 18 males, 14 of which were castrated. The mean age (years) ± SE was higher in the training group (4.9 ± 0.4) than the resting group (3.7 ± 0.3). Of the training group, the cause of death was fracture ($n = 15$ including two biaxial sesamoid fractures and three proximal phalanx fractures), other musculoskeletal condition ($n = 3$ including two suspensory apparatus ruptures), acute abdomen ($n = 2$) and other medical conditions ($n = 4$). Of the resting group, the cause of death was fracture ($n = 2$), other musculoskeletal condition ($n = 9$), acute abdomen ($n = 7$) and other medical conditions ($n = 6$). None of the fractures within either group were of the metacarpus.

The palmar–distal aspects of both MC3s were removed by cutting the bone at 55° to the frontal plane through the centre of rotation of the condyles prior to storing in 70% ethanol as previously described (Whitton et al., 2010). Racing and training history were obtained from race records and telephone questioning of trainers, cross-referenced with race records from an official database (Sirius, Racing Victoria). Length of current training period was defined as the length of time (weeks) that a horse had been continuously training with no rest period of more than 4 weeks. Information regarding any lameness history and current or previous treatment, housing status for horses resting from training (i.e. stable or paddock) was obtained from the trainer and the horse's regular veterinarian.

Following random selection (using a random number generator) of the left or right MC3 the specimen was serially dehydrated and embedded in methyl methacrylate. The condyles from the selected limb were then sectioned with a low speed saw (Isomet, Buehler). The cut surface was ground, polished, carbon coated and examined with a scanning electron microscope (FEI Quanta field emission gun 200) fitted with an annular solid-state backscattered electron detector. Consecutive overlapping micrographs of both the entire section and of specific regions of interest (ROI) were acquired at magnifications of ×70 and ×200. All micrographs of each ROI were transferred to a desktop computer and the whole ROI was reconstructed using photo stitching software (Adobe Photoshop Elements 3). For light microscopy thin (8 μm) sections were cut using a microtome (Polycut E sledge microtome, Leica Microsystems). Methyl methacrylate was removed with methoxyethylacetate and the tissue was dehydrated with ethanol and stained with a commercial Masson's Trichrome kit (Australian Biostain).

Assessment of bone activity was based on standard two-dimensional bone histomorphometry measurements (Parfitt et al., 1987). Bone surface (perimeter; Pm) measurements were performed as previously described (Whitton et al., 2013) in the lateral condylar subchondral bone (ROI a, Fig. 1) and the lateral parasagittal groove (ROI b, Fig. 1). Eroded bone surface (E.Pm) was measured on BSEM images whereas osteoid surface (O.Pm) was measured on Masson's trichrome images using open source imaging software (Image J, NIH). For measurement of bone area (B.Ar) and total tissue area (T.Ar), BSEM images were automatically thresholded using the 'minimum' algorithm available in the software and performed in the lateral and medial condylar subchondral and trabecular bone combined (ROI c plus d, Fig. 1), the lateral condylar subchondral and trabecular bone (ROI c, Fig. 1) and the two subchondral bone areas; the lateral parasagittal groove (ROI b, Fig. 1) and the lateral condylar subchondral bone (ROI a, Fig. 1). Values for eroded surface are presented both as a percentage of total bone surface (E.Pm/B.Pm) and relative to tissue area (E.Pm/T.Ar), and osteoid surface values are presented as a percentage of bone surface (O.Pm/B.Pm). Bone area values are presented as a percentage of total tissue area (bone area fraction; B.Ar/T.Ar).

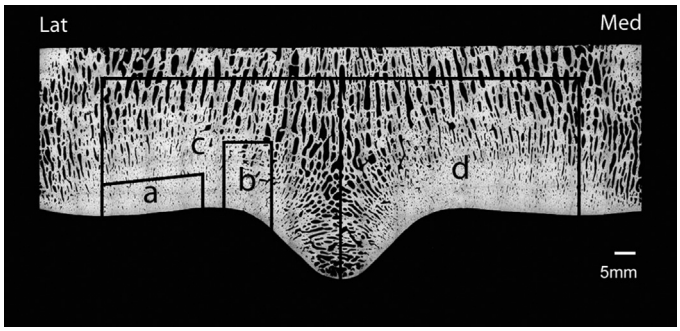

Fig. 1. Backscattered electron microscopy image of the distal palmar aspect of the third metacarpal bone of a Thoroughbred race horse in race training showing regions of interest (ROI) utilised in the study. (a) Lateral condylar subchondral bone, (b) lateral parasagittal groove, (c) lateral condyle, (d) medial condyle.

As bone resorption is the initial step in the remodelling cycle and formation can be due to both modelling and remodelling, we used eroded bone surface as our primary measure of remodelling. The mean calcified cartilage thickness for each ROI was determined by dividing the area of the calcified cartilage on the BSEM section by the length of the calcified cartilage for each ROI.

The number of microfractures per millimetre of articular surface was counted for each ROI on BSEM sections. To differentiate from artifactual fractures created by processing, microfractures were defined as discontinuity of the articular surface that also met one of the following criteria: (1) hypermineralised bone present immediately adjacent to the fracture line (Boyde, 2003); (2) direct association of the defect with bone fragmentation.

Statistical analysis

A power study determined that 26 bones were required for each group in order to detect a 20% difference in parameters of remodelling with a power of 80% and a probability of 0.05 (IBM SPSS SamplePower 3.0).

Statistical analysis was performed using IBM SPSS for Windows version 21. Equal variance between groups was confirmed by plotting the residuals. The effect of group on BSEM measures of remodelling was analysed with an independent samples *t* test. Comparisons of variables between ROI were assessed using a paired samples *t* test. A general linear model was used to test for an effect of 'time since change in exercise status' on BSEM variables within each group and the interaction between effect of 'time in training/rest' and variables in which an effect of training was found. For all analyses, age, sex, presence of a fracture, number of career starts and weeks in current training period were tested for significance or confounding. Despite the higher mean age of the training horses, age was not a confounder. Two-tailed significance was set at $P < 0.05$. All values are reported as mean (±SE).

Results

Lateral condylar subchondral bone sections (ROI a, Fig. 1) from both training and resting horses consisted predominantly of dense bone except for two of the unraced 2-year-olds, in which the bone was mostly trabecular. In contrast, sections from the parasagittal groove (ROI b, Fig. 1) contained areas of both dense and trabecular bone which was demonstrated by a difference in the percentage bone area fraction (B.Ar/T.Ar) between the two sites ($P < 0.001$, Table 1).

Measures of eroded surface were lower in training than resting horses in both subchondral bone ROI with the greatest differences observed in the lateral condylar subchondral bone (Table 1). This was accompanied by lower porosity in the subchondral bone of the