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# Impact of race training on volumetric bone mineral density and its spatial distribution in the distal epiphysis of the third metatarsal bone of 2-year-old horses

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## ABSTRACT

The aim of the study was to use spatial and multiple correspondence analysis (MCA) to describe and compare the regional proportion and spatial pattern of volumetric bone mineral density ($BMD_v$) values within loaded regions of the plantar metatarsal epiphysis of young horses in race training. A single 2 mm transverse peripheral quantitative computed tomography 'slice', 10 mm proximal from the distal limit of the sagittal ridge of the distal metatarsal epiphysis was obtained from 14 2-year-old Thoroughbred fillies (7 exercised and 7 controls). Six regions of interest were generated and examined for relative $BMD_v$ using MCA. The spatial distribution of $BMD_v$ was statistically examined at two sites loaded by the proximal sesamoid bones using geographical information software.

The $BMD_v$ response was focal with distinct regional differences in relation to load. Deposition of new bone within existing high density bone contributed to a greater bone fraction and the distinct profile of clusters of uniformly distributed high density bone as well as a lower proportion of lower density bone in exercised horses. The MCA and spatial analysis provided statistical techniques to quantify and describe non-invasively the exercise induced changes in bone that had previously been described using microradiography of thin slices and by block-face imaging. These statistical techniques may prove useful in quantifying spatial patterns of response to load.

## Introduction

After lack of athletic ability, musculoskeletal injury is the most common reason cited for the loss of horses from racing, or for interruptions from racing (Perkins et al., 2004; Bolwell et al., 2012). Most of the conditions responsible for this loss are focussed on the lower limbs (distal third metacarpal [McIII]/metatarsal [MtIII] bone and fetlock joint in particular), and include osteoarthritis, and fractures (of the condylar and proximal sesamoids) (Verheyen et al., 2003; Perkins et al., 2005; Parkin et al., 2006; Reed et al., 2012).

During high speed activities, such as race training and racing, the distal McIII epiphysis is subjected to large compressive forces (Harrison et al., 2010), which have been documented to result in cartilage loss, significant remodelling of the epiphysis and localised increases in volumetric bone mineral density ($BMD_v$) (Riggs, 2002; Firth et al., 2005) that often contribute to lameness (Tull and Bramlage, 2011). These bone and cartilage responses are often site-specific and focal, reflecting the heterogeneity of load on the distal McIII epiphysis. Within affected bones, the localised sclerosis is greatest at the palmer aspect of the condyles and least at the sagittal ridge, creating the possibility for development of shear along planes of different densities, in this case primarily at the axial margin of the condyles (Riggs, 2002). The focal and site-specific responses observed in the distal Mc3III epiphysis are thought to be related to cyclic loading of the joint and are associated with fractures (Whitton et al., 2010). If data could be captured serially, such as via computed tomography (CT), and numerical quantification of the responses provided, there would be an opportunity to refine our understanding of the distal McIII epiphyseal response to the cyclical loading associated with race training.

A parameter referred to as the stress–strain index (SSI), which weights the $BMD_v$ of each pixel with its relationship from the centre of the bone cortex, has been used to quantify the resistance of bone diaphyses to torsion and bending (Firth et al., 2005). In a cohort of 2-year-old Thoroughbred fillies this measure was sensitive enough to differentiate mid McIII diaphyseal bone responses between horses

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that had galloped <1800 m from those that had galloped 4450 m (Firth et al., 2005). The SSI has also been used in association with other parameters derived from serial CT scans to quantify the nature of the diaphyseal response to early exercise during growth and race training (Firth et al., 2011, 2012). However, given the non-cylindrical shape of the McIII epiphysis, and its inability to alter size in response to load, the SSI is not an appropriate measure of response in this epiphysis to exercise.

The statistical technique of spatial analysis has been extensively used in the discipline of epidemiology (Pfeiffer et al., 2008), but to date has not been widely used to quantitatively describe tissue (bone) responses to exercise (Rose et al., 2012). This method facilitates quantitative description of what previously has been described qualitatively such as the high levels of clustering of certain BMD$_v$ pixels, whether such clusters are arranged in uniform or random patterns, and if the dispersion of pixels is related to the BMD$_v$ of neighbouring pixels. The aim of this study was to use spatial analysis to quantify differences in BMD$_v$ in clearly defined populations of control and race-trained 2-year-old Thoroughbred fillies (Firth and Rogers, 2005).

## Materials and methods

### *Horse selection and training schedules*

The left distal MtIII of 14 2-year-old Thoroughbred fillies from a previous controlled exercise trial were available for analysis (Firth et al., 2004; Firth and Rogers, 2005). In brief, 14 fillies raised under similar conditions were selected for either race-training or yard rest (controls) in a grass paddock (dimensions, 25 × 15 m). The fillies were 662 (±26) and 652 (±34) day-old for the trained and untrained groups, respectively, at the start of the 13 week training period, and were trained on either grass or sand tracks at a commercial training complex. Training protocols were consistent with conventional flat race training programmes for 2-year olds in New Zealand (Bolwell et al., 2010). The training programme consisted of three phases: weeks 1–4 ($7.54 \pm 0.05$ m/s), weeks 5–8 ($8.90 \pm 0.05$ m/s) and weeks 9–13 ($6.33 \pm 0.06$ m/s) with gallops twice weekly on Wednesdays and Saturdays, respectively ($14.62 \pm 0.12$ m/s) (Rogers and Firth, 2004). At the completion of the training phase, all horses were euthanased and tissues were sampled for a series of musculoskeletal and histological studies (Firth and Rogers, 2005). The study was approved by the Massey University Animal Ethics Committee.

### *Peripheral quantitative computed tomographic scanning*

Left distal MtIII bones, stored in formalin, were scanned using axial peripheral quantitative computed tomography (pQCT; XCT 2000, Stratec Medical). Fifteen 2 mm contiguous scans, with a pixel size of 0.5 mm, were taken from the most distal point of the sagittal ridge. From each scan series, a 2 mm 'slice' representing 10–12 mm from the most distal point of the sagittal ridge was extracted using the manufacturer's software. This site was chosen as it represented the midsection of where sclerosis was occurring due to impact of the sesamoids on the bone's plantar surface and was analogous to sites previously examined (Firth et al., 2005, 2011). The images were checked against a panel of standard images for uniformity and data were used for subsequent analysis.

The BMD$_v$ data (mg/cm$^3$) were extracted using the scanner's proprietary software for the entire slice and for the six regions of interest (ROI) (Fig. 1), representing regions subject to different loads during training (Riggs et al., 1999; Harrison et al., 2014). The ROI were: the abaxial half of the lateral plantar condyle (L1P); the axial half of the lateral plantar condyle (L2P); the lateral sagittal ridge and groove (LSP); the abaxial half of the medial plantar condyle (M1P); the axial half of the medial plantar condyle (M2P); and the medial sagittal ridge and groove (MSP). The extracted data permitted examination of the proportion of pixels within a given density range and their spatial location. The BMD$_v$ data were examined as 100 mg/cm$^3$ threshold categories starting at 400 mg/cm$^3$ with a final category of ≥1200 mg/cm$^3$.

### *Statistical analysis*

Data were initially structured for analysis and screened for errors using the Excel program (Microsoft). The distribution of the data was examined using histograms and descriptive statistics. To examine the effect of training on the distribution of BMD$_v$ within different ROI, the median proportion of pixels within the defined BMD$_v$ ranges in the trained and control horses were compared using the non-parametric Kruskall–Wallis test. For this analysis the critical level for assigning statistical significance ($P < 0.05$) was adjusted for multiple comparisons ($n = 8$) using a Bonferroni correction, which resulted in a level for statistical significance set at $P \le 0.006$. The level of significance used for spatial analysis was $P < 0.05$.

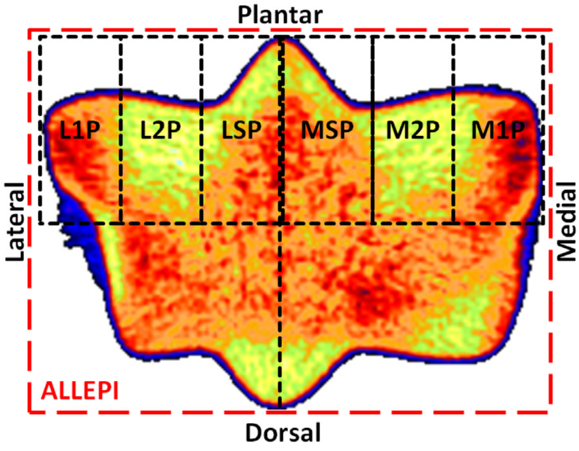

**Fig. 1.** Regions of interest (ROI) were selected dividing the plantar half of the peripheral quantitative computed tomographic image into three sections on both lateral and medial aspects of the midline: L1P, most lateral ROI; L2P, central lateral ROI; LSP, lateral aspect of sagittal ridge; MSP, medial aspect of sagittal ridge; M2P, central medial ROI; and M1P, most medial ROI.

Multiple correspondence analysis was used to visualise the relationships between categorical variables of interest (treatment group and categories of BMD$_v$) on a two-dimensional plot, allowing spatial clusters to be identified. This method of analysis is an exploratory technique that uses a data matrix to describe how strongly and in which way variables are interrelated (Greenacre, 2007). Variables that are clustered together spatially are considered similar to each other, whereas those plotted furthest apart are rarely associated with each other. Those variables clustered around the centre of the plot represent the average profile with regards to the categories of BMD$_v$ (Greenacre, 2007). Variables that are furthest away from the centre, and those in extreme opposite positions (for example top left and bottom right of plot), are considered different to the average profile (unique characteristics). The median proportion of pixels was calculated for each BMD$_v$ category and horses were coded as being below '0' or ≥ the median value '1'. The statistical analysis and associated plots were produced using Stata 11.1 (StataCorp LP).

For the spatial analysis of BMD$_v$, the pQCT data images for each scan were exported as ASCII format files and imported into ArcMap (ArcGIS 9.3, ESRI). The L2P and M2P RPIs were chosen for further investigation and a new square ROI was created to avoid boundary effects with the calcified cartilage layer (Fig. 2). The plantar border of this square ROI was then aligned with the margin of the calcified cartilage on the plantar border of the condyle, and centred within the lateral and medial borders of M2P or L2P, respectively. The pixels of the imported images were reclassified into three BMD$_v$ categories: low (<600 mg/cm$^3$), medium (600–800 mg/cm$^3$), and high (>800 mg/cm$^3$). Clustering and spatial relationships were tested using Moran's I test, nearest neighbour distance test and the Getis–Ord general G test. Moran's I test determines the degree of grouping of pixels based on their value and the size of the

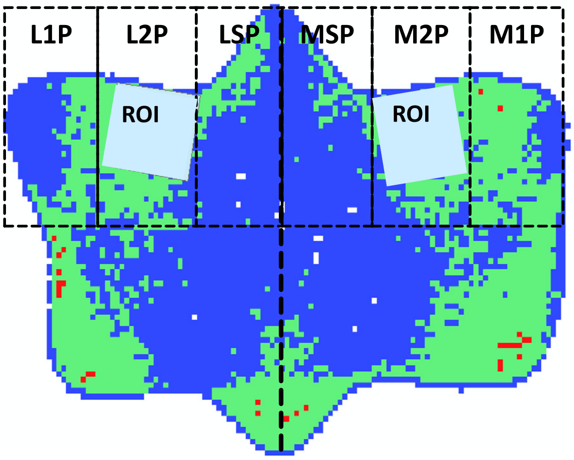

**Fig. 2.** Stylised representation of the regions of interest (ROI) for spatial analysis and classification of pixels into low (<600 mg/cm$^3$ 🟦), medium (600–800 mg/cm$^3$ 🟩), and high (>800 mg/cm$^3$ 🟥) volumetric bone mineral density (BMD$_v$).