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Stiffness and energy dissipation across the superficial and deeper third metacarpal subchondral bone in Thoroughbred racehorses under high-rate compression

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ABSTRACT

Subchondral bone injury due to high magnitude and repetition of compressive loading is common in humans and athletic animals such as Thoroughbred racehorses. Repeated loading of the joint surface may alter the subchondral bone microstructure and initiate microdamage in the bone adjacent to the articular cartilage. Understanding the relationship between microdamage, microstructure and mechanical properties of the subchondral bone adjacent to the articular cartilage is, therefore, essential in understanding the mechanism of subchondral bone injury. In this study, we used high-resolution μCT scanning, a digital image-based strain measurement technique, and mechanical testing to evaluate the three-dimensional pre-existing microcracks, bone volume fraction (BVF) and bone mineral density (BMD), and mechanical properties (stiffness and hysteresis) of subchondral bone (n = 10) from the distopalmar aspect of the third metacarpal (MC3) condyles of Thoroughbred racehorses under high-rate compression. We specifically compared the properties of two regions of interest in the subchondral bone: the 2 mm superficial subchondral bone (SSB) and its underlying 2 mm deep subchondral bone (DSB). The DSB region was 3.0 ± 1.2 times stiffer than its overlying SSB, yet it dissipated much less energy compared to the SSB. There was no correlation between structural properties (BVF and BMD) and mechanical properties (stiffness and energy loss), except for BMD and energy loss in SSB. The lower stiffness of the most superficial subchondral bone in the distal metacarpal condyles may protect the overlying cartilage and the underlying subchondral bone from damage under the high-rate compression experienced during galloping. However, repeated high-rate loading over time has the potential to inhibit bone turnover and induce bone fatigue, consistent with the high prevalence of subchondral bone injury and fractures in athletic humans and racehorses.

1. Introduction

Subchondral bone injury due to high magnitude and repetition of compressive loading is common in humans (Devas, 1958; Matcuk et al., 2016) and athletic animals such as Thoroughbred racehorses (Barr et al., 2009; Riggs et al., 1999). Repeated loading of the joint surface may alter the subchondral bone microstructure and initiate microdamage in the bone adjacent to the articular cartilage (Muir et al., 2008; Stepnik et al., 2004). Microdamage may stimulate both modelling and remodelling responses in subchondral bone and can lead to the development of stress fractures and/or osteoarthritis in the joint (Burr et al., 1985; Kawcak et al., 2001; Muir et al., 2006; Norrdin and Stover, 2006). Specifically, altered subchondral bone mechanics due to microdamage can influence how joint surface load is transferred to the underlying trabecular bone, and thus trigger changes in bone modelling and remodelling which are highly load dependent. (Burr, 2004; Shirazi and Shirazi-Adl, 2009; Whitton et al., 2010). Understanding the relationship between microdamage, microstructure and mechanical properties of the subchondral bone adjacent to the articular cartilage is, therefore, essential in understanding the mechanism of microdamage initiation and propagation across the whole joint.

The metacarpophalangeal joint in racehorses is subjected to extremely high compressive loads during galloping (Harrison et al., 2014). The palmar metacarpal subchondral bone, particularly the bone within 2–3 mm of the cartilage-calcified cartilage interface is a common site of fatigue-induced microdamage (Muir et al., 2006), which highlights the importance of investigation of subchondral bone mechanical/structural properties at this site. Previous studies investigated the

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relationship between the microstructure and mechanical properties of equine subchondral bone from the metacarpal condyles under low-rate compression (Rubio-Martínez et al., 2008; Leahy et al., 2010). Rubio-Martínez et al. (2008) observed a gradient of stiffness across the subchondral bone depth; superficial 6 mm cubes of dense subchondral bone were less stiff than deeper 6 mm cubes of trabecular bone. Additionally, variations in the mechanical properties of the trabecular bone specimens from condylar regions were associated with structural measures derived from micro-computer tomography (μCT) but similar associations were not observed for the more superficial subchondral bone specimens.

In the present study, we focused on the subchondral bone area closest to the joint surface, i.e. subchondral bone plate in Rubio-Martínez et al. (2008), to increase the resolution of the previous measurements by investigating two regions of interest (ROI) within the equine third metacarpal (MC3) subchondral bone: the superficial subchondral bone (SSB: 0.5–2.5 mm) and its underlying deep subchondral bone (DSB: 2.5–4.5 mm). We used high-resolution μCT and a digital image-based strain measurement system to evaluate the relative microstructure and mechanical properties (stiffness and energy absorption) of the two ROIS under high-rate compression. The image-based strain measurement technique allows for the investigation of tissue strain while avoiding artefacts associated with the free trabecular end of specimens, and their non-uniform thickness (Malekipour et al., 2013). The specific objectives of this study were to: 1) investigate the microstructure of the subchondral bone and identify pre-existing microcracks using μCT scanning, 2) determine the relative stiffness and shock-absorbing abilities (normalized hysteresis) of the SSB and DSB under experimental high-rate compression similar to one cycle of compression during galloping, and 3) investigate the relationship between the mechanical/structural properties of SSB and DSB. We hypothesized that the superficial 2 mm of subchondral bone would be less stiff, and dissipate more energy under high rate loading than the deeper 2 mm of bone, with associated differences in density and bone volume fraction gradient.

2. Methods

2.1. Specimen preparation

Cartilage-bone plugs (Ø = 6.5 ± 0.2 mm) were extracted from the distopalmar aspect of fresh frozen (−20 °C) third metacarpal condyles of n = 10 racehorses using a diamond core drill (Starlite Industries, Rosemont, PA) under continuous irrigation. Metacarpal condyles were collected from Thoroughbred racehorses of median age of 3.5 years (range, 3–7 years) that died or were euthanatized on racetracks in Victoria, Australia between May 2011 and March 2014. Specimens were harvested from both medial (n = 7) and lateral (n = 3) condyles.

During harvesting, the axis of the drill was oriented perpendicular to the cartilage surface. Bone was trimmed down on the proximal end of each specimen by using a self-irrigated diamond saw (Isomet, Buehler, Ltd., Lake Bluff, IL) to create a flat bone surface perpendicular to the longitudinal axis of the cartilage-bone plugs. The final trimmed bone thickness was 9.68 ± 0.79 mm with an average cartilage thickness of 0.46 ± 0.06 mm. To keep the integrity of the subchondral plate adjacent to the articular cartilage, we preserved the cartilage in situ. Subsequently, the cartilage-bone plugs were trimmed perpendicular to the cartilage surface to create a flat surface on the longitudinal side of each specimen (Fig. 1A). Prior to loading, this longitudinal surface was stained with fine graphite particles to create a random speckle pattern, which was necessary for the later image processing to calculate bone strains. The graphite particles were generated by rubbing pencil lead with sand paper, a technique which has been validated in a previous study (Malekipour et al., 2013).

2.2. Micro-CT scanning and bone microarchitecture

Prior to loading, cartilage-bone plugs were scanned using a μCT scanner (μCT50; Scanco Medical, Switzerland) at an isotropic resolution of 4 μm using 70 kVp tube voltage, 200 μA tube current, and 1050 ms integration time. A phantom was used to convert CT grayscale intensity values (Hounsfield units, HU) into equivalent bone mineral density (BMD, g/cm^3 HA). A strong correlation has been demonstrated between quantitative CT and mineralisation and ash density in bone from the equine distal metacarpus (Drum et al., 2009). We used a voxel size of 4 μm, which was the highest resolution of the scanner that achieved good quality images for our specimens, to identify pre-existing microdamage in the subchondral bone. Following the examination for microdamage (ImageJ, 1.48 v, National Institute of Health, USA), images were down-sampled to 40 μm for further morphology processing (Simpleware, v5.1, UK). Damage was identified by visual examination of all μCT slices assessing for linear lucencies or linear areas of increased mineralisation (Williamson et al., 2017). Examination of profile lines and images confirmed that this down-sampling affected the density and bone volume fraction (BVF) measurements no more than 0.06% and 0.39%, respectively. Mineralised bone was segmented using a consistent set of threshold grey values. SSB and DSB regions corresponding to the experimental ROIs were identified based on their distances from the cartilage-bone interface and thicknesses in μCT images. BVF of each ROI and the entire bone was calculated by dividing the volume of bone tissue in that region by its apparent volume. BMD of each ROI and the entire bone were specified as the average BMD over that region.

2.3. Mechanical testing

The experimental procedure was similar to that of a previous study (Malekipour et al., 2013). Cartilage-bone plugs were glued to a base plate using a thin layer (several micrometres) of cyanoacrylate glue on the proximal end of the bone (Burgin and Aspden, 2008). Vertical displacements were applied to the articular surface of cartilage via a stainless steel compression plate on a mechanical testing machine (Instron, 8874, UK). A time to peak strain of 0.05 s was chosen, similar to the time to peak bone strain during galloping of a racehorse (Davies and Mccarthy, 1994; Swanstrom et al., 2005). Displacements were applied to the cartilage surface using half-sine waveforms with magnitudes of 0.225 ± 0.018 mm at 5 Hz. The displacement magnitudes were calculated to apply 50–60% of equine subchondral bone yield strain (Rubio-Martínez et al., 2008). The stained flat surface of cartilage-bone plugs faced a microscope lens. A high-speed camera (MotionProY3, 1280 × 1024, USA) attached to a stereomicroscope (SZX7, Olympus, Japan) recorded the experimental deformation of each specimen at 1000 frames per second. Microscopic magnification was chosen that allowed imaging at a resolution of 170 pixels/mm (1024 pixels within 6 mm) in the vertical direction. Recorded images of each specimen were processed using a Matlab-based digital image correlation (DIC) code (Jones, 2013) to calculate the average displacement of bone at three different levels (Lines 1–3, Fig. 1B). A respective subset and grid size of 71 and 20 pixels was found to accurately calculate the displacement within ROIs. Using these parameters, the software measured the displacement of the Instron actuator with an accuracy of 1.89%. The ROIs were selected at a 0.5 mm distance from the cartilage-bone interface. Above this distance cartilage folding under loading hindered clear images, and an accurate displacement measurement of the bone. To remain unaffected by the cutting process and the presence of free edges, ROIs were specified away from the edges. In a preliminary study, the width of ROIs were reduced from the full width until the calculated stiffness and energy dissipation varied no more than 1.41 ± 1.35% and 1.18 ± 0.72%, respectively in the central 2 mm (Fig. 1B). The displacement data were then processed (MATLAB R2016a, MathWorks, USA) to calculate the overall strains of the two ROIs, i.e. SSB and DSB,