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Compressive fatigue life of subchondral bone of the metacarpal condyle in thoroughbred racehorses

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ABSTRACT

In racehorses, fatigue related subchondral bone injury leads to overt fracture or articular surface collapse and subsequent articular cartilage degeneration. We hypothesised that the fatigue behaviour of equine subchondral bone in compression follows a power law function similar to that observed in cortical and trabecular bone. We determined the fatigue life of equine metacarpal subchondral bone *in-vitro* and investigated the factors influencing initial bone stiffness. Subchondral bone specimens were loaded cyclically in compression [54 MPa (n = 6), 66 MPa (n = 6), 78 MPa (n = 5), and 90 MPa (n = 6)] until failure. The fatigue life curve was determined by linear regression from log transformed number of cycles to failure and load. A general linear model was used to investigate the influence of the following variables on initial Young's Modulus: age (4–8 years), specimen storage time (31–864 days), time in training since most recent rest period (6–32 weeks), limb, actual density (1.6873–1.8684 g/cm^3), subchondral bone injury grade (0–3), and cause of death (fatigue injury vs. other). Number of cycles to failure was (median, range) 223,603, 78,316–806,792 at 54 MPa; 69,908, 146–149,855 at 66 MPa; 13204, 614–16,425 at 78 MPa (n = 3); and 4001, 152–11,568 at 90 MPa. The fatigue life curve was $\sigma = 112.2\text{–}9.6 \log_{10}N_f$, ($R^2 = 0.52$, $P < 0.001$), where N_f is number of cycles to failure and σ is load. Removal of the three horses with the highest SCBI grade resulted in: $\sigma = 134.2\text{–}14.1 \log_{10}N_f$, ($R^2 = 0.72$, $P < 0.001$). Initial Young's Modulus (mean ± SD) was 2500 ± 494 MPa (n = 22). Actual density (ρ) was the only variable retained in the model to describe initial Young's Modulus (E): $E = -8196.7 + 5880.6\rho$, ($R^2 = 0.34$, $P = 0.0044$). The fatigue behaviour of equine subchondral bone in compression is similar to that of cortical and trabecular bone. These data can be used to model the development of SCBI to optimize training regimes.

Introduction

Up to 80% of Thoroughbred racehorses suffer from subchondral bone injury (SCBI) [1–4]. This condition is due to fatigue damage caused by repetitive extreme loading of joint surfaces when galloping. Microcracks develop in the subchondral bone in areas of high load and follow one of two pathways: (1) they grow to form an overt fracture which may be fatal or (2) they remain contained locally, resulting in focal bone necrosis, subsequent collapse of the subchondral bone plate and degeneration of articular cartilage [1,5–7]. Affected horses require a prolonged resting period, have fewer starts and earn less prize money than unaffected horses [8–10]. Understanding the fatigue limits of subchondral bone is essential to develop training strategies that reduce the risk of injury.

The development of fatigue injury in bone is a complex process which depends on the rates of damage accumulation, bone adaptation and damage repair [11–14]. The equine metacarpal condyle responds to race training with increased bone apposition and reduced resorption leading to local thickening of the subchondral bone plate [15–18]. Other factors affecting bone fatigue include its material properties and architecture [19–22]. Bone stiffness directly influences fatigue life as it determines strain at a given load [23–25]. Mathematical modelling of fatigue injuries allows a better understanding of how these many processes interact, and fatigue life is a critical input into these models [26]. Fatigue life of cortical and trabecular bone follows a power law function of the applied load [23,27,28]. The fatigue life of equine subchondral bone is unknown despite the widespread acknowledgement that SCBI in athletic horses is a fatigue injury.

In this study we investigated the fatigue life of equine subchondral bone *in-vitro* through cyclic loading of small samples of the palmar aspect of the metacarpal condyle from mature Thoroughbred racehorses in training. We aimed to determine (1) the parameters of the compressive fatigue life curve and (2) the stiffness of the subchondral bone. We also investigated whether horse related factors and specimen storage time influenced subchondral bone stiffness. We hypothesised that

Abbreviations: SCBI, Subchondral bone injury.

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(1) the fatigue behaviour of subchondral bone can be described with a power law function; and that (2) subchondral bone stiffness increases with the duration of training since the horse's most recent rest period and actual bone density.

Materials and methods

Bone specimen

Horses

Metacarpal condyles were collected from a convenience sample of Thoroughbred racehorses in training that died or were euthanized on racetracks in Victoria, Australia, during racing or training between October 2009 and March 2012. Horses were included if they were four years of age or older, did not meet inclusion criteria for other studies which required the metacarpi, and staff were available to collect samples. There were 15 males (13 castrated) and eight females. Causes of death or euthanasia were musculoskeletal stress injury [n = 10; tibia fracture (n = 1); carpal fracture (n = 1); suspensory ligament failure or proximal sesamoid bone fracture (n = 3); fracture of the lateral metacarpal condyle and ipsilateral proximal phalanx (n = 3); metatarsal condylar fracture (n = 2)]; sudden death or exercise induced pulmonary haemorrhage (EIPH) (n = 5); combination of fracture and EIPH (n = 2); fall at a hurdle (n = 2); trauma unrelated to racing and training (n = 2); and gastrointestinal or respiratory disease (n = 2).

Bone collection

Specimens were collected within 24 h of death. The palmar aspect of the distal metacarpal condyle was dissected free of soft tissue and removed with a bandsaw (HT Barnes, BMSS Butchers Machinery, North Coburg, VIC, Australia) by cutting in a plane 55° to the frontal plane in a palmaroproximal to dorsodistal direction through the centre of rotation of the condyle. Specimens were wrapped in gauze soaked with normal saline (0.9% sodium chloride, Baxter, Old Toongabbie, NSW, Australia; or Compound Sodium Lactate [Hartmann's], Fresenius Kabi, Friedberg, Germany), enclosed in ziplock bags, and stored in plastic containers at −20° Celsius until further use and between steps of preparation. One leg per horse was selected for the study using a random number allocator function (Microsoft Excel 2007) as there is no evidence for side predilection for SCBI [1,3]. In horses with a current (n = 3) or healed (n = 1) metacarpal condylar fracture the opposite leg was used. The right leg of one horse was missing at the time of leg allocation and the left leg was used instead.

Specimen preparation

Specimens were thawed at room temperature. The cartilage of the lateral condyle was removed with a scalpel blade. Only the lateral condyle was used as the lateral condyle is less commonly and less severely affected by SCBI than the medial condyle [3,8,9].

A cylindrical specimen with a diameter of approximately 6.7 mm was cut using a diamond coated core drill bit (#102075, Starlite Industries Inc, Rosemont, PA, USA) mounted on a drill press (3/4 HD 16 Speed Bench Drill Press, Carba-Tec Melbourne Pty Ltd, Springvale, VIC, Australia) rotating at 210 revolutions per minute. Bone cylinders were located 3–5 mm palmar to the transverse ridge and centred in the middle third of the condyle with the cylinder's long axis perpendicular to the joint surface. The cylinders were cut to a length of 7–8 mm using a diamond coated wavering blade mounted on a low speed saw (IsoMet, Buehler, Lake Bluff, Illinois, USA). The ends of the cylinders were wet ground manually until planoparallel using electrocoated silicon carbid abrasive paper (P600, KMCA, Bunnings, Hawthorn, VIC, Australia) and a custom jig with two parallel surfaces and several holes (diameter between 6.5 and 7.0 mm) perpendicular to them [29]. According to the manufacturers' instructions tap water at room temperature was used at all times for cooling and hydration during drilling and cutting. The amount of Ca^{++} leakage from the specimens into the tap water was considered minimal due to the relatively short time used for drilling (20 to 40 min) and cutting (5 to 10 min) as opposed to storage for months to years and mechanical testing for hours to days.

Specimen surfaces were dried with lint free paper wipes (Kimwipes, Kimberly-Clark Professional, Milson Point NSW, Australia) and weighed six times on a precision scale (Model MS204S/01, Mettler-Toledo Ltd., Port Melbourne, VIC, Australia) to determine total specimen mass (m). Diameter and length of the specimens were measured six times each with digital callipers. The mean values of total specimen mass, diameter and length were used to calculate the specimen surface area, volume (v), and actual density ($\rho = m/v$) [30]. Specimens were allocated to four different load groups using a random number table while blocking for age and actual density as these were considered potential confounding factors [31]. The degree of subchondral bone sclerosis, and therefore apparent density, varies between horses and apparent density is positively correlated with bone stiffness [32]. Actual density was chosen as a substitute for apparent density as it can be determined without altering samples which is essential before mechanical testing [30]. We blocked for age because half of the horses were 4 years old and the effect of age on fatigue life is unknown.

Mechanical testing

All tests were performed at room temperature (mean 24 °C, range 21 °C to 28.5 °C) on a hydraulic material testing machine (Instron 8874, Instron, Bayswater, VIC, Australia). Specimens within a load group were tested in random order using a random number table [31]. High load groups were tested first for logistical reasons. Specimens were thawed for 30 min in Ca-buffered 0.9% saline (0.154 M NaCl, 1.381 mM $CaCl_2$) at room temperature prior to potting into custom built stainless steel fixtures Fig. 1 [33].

A 2 cm high Perspex ring was tightly fitted into a slot in the periphery of the lower platen. The Perspex ring covered the entire height of the specimen and the lower half of the upper platen to enable immersion of the sample in Ca-buffered 0.9% saline (0.154 M NaCl, 1.381 mM $CaCl_2$) during testing. The upper platen was fastened in series to the 10 kN load cell (Dynacell, Instron, Bayswater, VIC, Australia) on the actuator of the material testing machine.

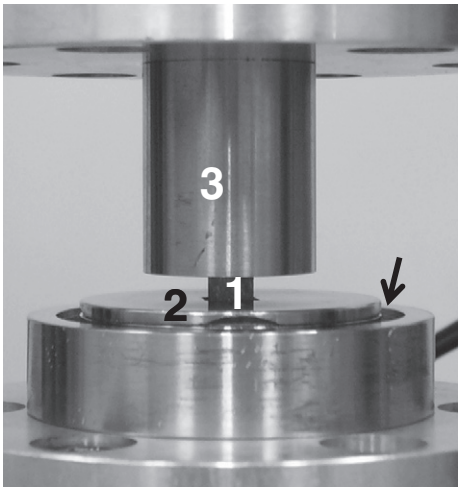

Fig. 1. Specimen of equine metacarpal subchondral bone (1) in custom built stainless steel fixtures for compression–compression fatigue testing. The slot in the periphery of the lower platen (arrow) accommodates a 2 cm high Perspex ring that covers the entire height of the specimen and the lower half of the upper platen thus enabling submersion of the specimen in fluid during testing. The cover plate (2) is held in place by two holding pins and can be lifted to facilitate specimen removal after testing. The upper platen (3) is mounted in series with the inbuilt load cell of the Instron material testing machine.