BONE MINERAL DENSITY CHARACTERISTICS OF THE THIRD METACARPAL/ METATARSAL DISTAL EPIPHYSIS OF THOROUGHBRED HORSES

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Abstract

This thesis includes two studies using non-invasive imaging techniques to quantify, in detail, the bone mineral density (BMD) characteristics of the distal third metacarpal (Mc3) and metatarsal (Mt3) epiphyses of Thoroughbred horses associated with exercise exposure and condylar fracture. Additionally, the relationship between the bone structure of the distal Mc3/Mt3 epiphysis and incurred cyclic loading, as well as techniques for imaging the area non-invasively, are reviewed.

Mt3 bones from fourteen trained or untrained Thoroughbred horses and Mc3 bones from fourteen Thoroughbred racehorses with or without condylar fracture were scanned using peripheral quantitative computed tomography (pQCT) at a site on the distal epiphysis. The relative proportions of volumetric bone mineral density (BMD_V) and the spatial distribution of BMD_V were quantitatively assessed using conventional and ArcGIS software. The relative proportion of voxels within nine threshold categories of BMD_V and spatial statistics of BMD_V distribution were compared for regions of interest in the palmar/plantar epiphysis between respective treatment groups; trained vs. untrained controls or fractured vs. non-fractured controls.

In study one, trained horses had a significantly higher ($P \leq 0.006$) proportion of high BMD_V voxels and a significantly lower ($P \leq 0.006$) relative proportion of low BMD_V voxels than controls in the central condylar regions of the plantar Mt3 epiphysis. In other regions of the plantar epiphysis the trained horses also had a significantly higher ($P \leq 0.006$) relative proportion of high BMD_V voxels than controls; however, there were no significant differences for the relative proportion of low BMD_V voxels. These relationships were also evident with multiple correspondence analysis. There was strong to marked clustering of high BMD_V voxels in the central condylar region of all of

the trained horses (I = 0.64 - 1.0, P = 0.01) and no clustering of low BMD_V voxels. In contrast, half of the control horses had clustering of high BMD_V voxels, which was weak to strong (I = 0.64 - 1.0, P = 0.01) and there was weak to moderate clustering of low BMD_V voxels in the lateral and medial central condylar regions (I = 0.45-0.62, P = 0.01 and I = 0.45-0.57, P = 0.01, respectively).

In study two, there were no significant differences between the median age (P = 0.7), number of race starts (P = 0.5), the relative proportion of BMD_V voxels, or the spatial distribution of BMD_V voxels in regions of the palmar Mc3 epiphysis between the fractured and control groups.

The results of this thesis suggest that the response of bone to exercise is specific in relation to anatomical site, the thresholds of BMD that change, and the spatial distribution of BMD. In both studies the exercise exposure was responsible for much of the variation in the relative proportions and the spatial distributions of BMD_V.

The clinical relevance of these findings are that detailed quantification of previous exercise exposure needs to be considered when determining if a BMD response of the Mt3/Mc3 epiphysis is part of a physiological or pathological finding.

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List of abbreviations

BMD	Bone mineral density
BMD_V	Volumetric bone mineral density
CT	Computed tomography
GIS	Geographic information systems
Mc3	Third metacarpal bone
MCA	Multiple correspondence analysis
MRI	Magnetic resonance imaging
MSI	Musculoskeletal injury
Mt3	Third metatarsal bone
POD	Palmar/plantar osteochondral disease
pQCT	Peripheral quantitative computed tomography
PSBs	Proximal sesamoid bones
ROI	Region of interest
SCB	Subchondral bone

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CHAPTER 1

Introduction

Thoroughbred horse racing is a large global industry. Internationally in 2010, 88 billion Euros was wagered on 260,000 Thoroughbreds, which ran in 160,000 races (IFHA 2010). Domestically in 2011, racing generated $1.6 million in value added to the New Zealand economy, which is approximately 0.9% of GDP (NZRB 2010). Wagering contributes to 86% of net revenue to support stakes, administration and development of the racing industry (NZRB 2011), and there are approximately 6,000 horses that run in 3,000 races annually (IFHA 2010; NZRB 2010). Therefore, public affinity for, and confidence in, the racing industry is essential to maintain revenue.

A major welfare and economic issue that the racing industry faces is wastage, which is defined as any injury or disease that results in involuntary loss of a horse's ability to train or race (Bailey *et al.* 1997b). Wastage is an issue of importance domestically and internationally; furthermore, the maintenance of high welfare standards is a key objective for both international and national racing organisations, which have established committees to investigate welfare issues (BHA 2012; IFHA 2010). As part of the focus on welfare, and to decrease wastage, multi-million dollar collaborative research projects have been funded by racing industries to investigate racehorse injuries and deaths so that measures to prevent their occurrence can be initiated (Stover and Murray 2008).

Despite global efforts, musculoskeletal injury (MSI) continues to be a major cause of wastage in the Thoroughbred racing industry. In New Zealand 78.8% of days off training were attributed to MSI (Perkins *et al.* 2005a) and 83% of racehorse deaths in

California were due to MSI (Johnson *et al.* 1994). The deaths associated with injury have emotional impact on those involved in the industry as well as the wagering public, while days of lost training have been found to significantly affect return on racehorse owner investment (Hernandez and Hawkins 2001).

Both catastrophic and non-catastrophic MSI most commonly occur in the distal limb of racehorses; in particular, the bone and soft tissue structures of the metacarpo/metatarsophalangeal (fetlock) joint are affected (Johnson *et al.* 1994; Perkins *et al.* 2005a; Rossdale *et al.* 1985). Condylar fractures of the third metacarpal (Mc3) or metatarsal (Mt3) epiphysis are an example of catastrophic bone injury and represent 8-10% of all fractures sustained during racing or training (Ramzan and Palmer 2011; Verheyen and Wood 2004). Like the majority of MSIs incurred by racehorses, condylar fractures are thought to be the end point of stress-induced bone changes due to cyclic loading (Parkin *et al.* 2006; Radtke *et al.* 2003; Stover and Murray 2008; Zekas *et al.* 1999a).

Examination of the loading on bone structure and the risk factors for condylar fracture suggest that the Mc3/Mt3 distal epiphysis adapts to exercise to withstand the high stresses encountered that could result in fracture (Firth *et al.* 2005; Parkin *et al.* 2004a, 2005). An increase in bone mineral density (BMD) is the most significant change in the Mc3/Mt3 distal epiphysis to occur with exercise. These responses have been measured over the entire epiphysis and alterations in BMD spatial distribution have been described qualitatively (Brama *et al.* 2009; Easton and Kawcak 2007; Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). Determining BMD response to exercise in specific regions of the epiphysis and developing a quantitative technique for assessing BMD spatial distribution would allow objective comparison of BMD parameters. Quantitative comparisons could be made between individuals, or changes in BMD response could be monitored over time.

The spatial distribution of BMD and the degree of very high density bone have also been associated with microdamage and condylar fracture (Norrdin and Stover 2006; Riggs *et al.* 1999a; Stepnik *et al.* 2004; Tranquille *et al.* 2012; Whitton *et al.* 2010). However, these studies have used invasive microscopic techniques that cannot be used in live horses, or have described the spatial distribution qualitatively. A quantitative approach for the assessment of images obtained using non-invasive imaging techniques could permit a non-biased assessment of bone change in live horses with condylar disease.

The purpose of the current thesis is to review the existing literature on the relationship between the structure of the distal Mc3/Mt3 epiphysis and cyclic loading, and to quantitatively describe and compare the BMD characteristics of the epiphysis in relation to exercise exposure and condylar fracture. The studies in this thesis will add detailed, specific, quantifiable data to the existing understanding of the BMD characteristics of the distal Mc3/Mt3 epiphysis in relation to exercise exposure and condylar fracture. Additionally, the development of quantitative methods to analyse specific BMD changes of the Mc3/Mt3 epiphysis could have future practical application to allow non-biased comparison of horses in clinical or research settings.

Wastage associated with orthopaedic disease in the Thoroughbred racing industry

Wastage is defined as any injury or disease that involves an interference with the training schedule of a horse, resulting in lost days in work, a prolonged spell or retirement from racing (Bailey *et al.* 1997b). In a survey of 1,022 Thoroughbreds in Britain, 39% failed to start in a race by two years old and 24% by three years old (Wilsher *et al.* 2006). Not only do many horses fail to start, but 40% of exits from racing in a group of New Zealand horses were found to be due to events that resulted in the retirement of horses within the first three years of entering training (Perkins *et al.* 2005a). In the United Kingdom, 10% of all two year olds that did not continue racing as three year olds were destroyed due to injury or an inappropriate temperament for racing (Wilsher *et al.* 2006). Across all age groups the destruction due to injury was estimated to be 1.05/1000 racing starts and 0.39/1000 training starts in Canada (Cruz *et al.* 2007). In the United Kingdom a higher rate of racing fatalities for any reason were found to occur in 2.9/1000 race starts (Williams *et al.* 2001) and fatal fractures to occur in 0.4/1000 race starts (Parkin *et al.* 2004c).

Not only does wastage have psychological effects on both people involved in the industry and the general public, it also causes a quantifiable economic loss. In a study of Thoroughbreds that were bought as untrained yearlings and sold as trained two year olds, the return on investment was $23,000 less for horses that had 13-108 days of lost training compared with only 1-11 days of lost training (Hernandez and Hawkins 2001). In the same study, horses that had surgery to correct minor abnormalities in their first year of training were found to yield $10,000 less return on investment compared to horses that did not have surgery. Injury can also result in impaired performance with 64% of horses that returned to racing after condylar fracture found to having decreased total earnings post-injury (Zekas *et al.* 1999b).

MSIs are a major cause of involuntary wastage in flat-racing Thoroughbreds. In an Australian study, 40% of racehorses were found to sustain MSI during their first fast preparation (Cogger *et al.* 2006) and MSI contributed to 78.8% of lost training days in New Zealand horses (Perkins *et al.* 2005a). In Australia, 42% and 25% of lost training days were attributed to shin soreness and fetlock problems respectively, compared to non-musculoskeletal conditions, which resulted in 32% of lost training days (Bailey *et al.* 1999a). Similar results were found in British racehorses; the proportion of lost training days due to MSI was found to be 68% compared to 22% for respiratory conditions (Rossdale *et al.* 1985).

Unlike voluntary forms of wastage, MSI can necessitate permanent loss of any future use, or destruction of the horse. In the United Kingdom a perceived lack of ability accounted for 18% of two year old and 28% of three year old Thoroughbreds that were retired from racing, compared to veterinary problems that accounted for 4% and 9% of retirements, respectively (Wilsher *et al.* 2006). Likewise, voluntary exits from racing have been found to occur frequently (65% of exits) in New Zealand racehorses (Perkins *et al.* 2005a). Even so, MSI is a major cause of fatality in racehorses around the world, contributing 0.03% of an 0.04% fatality incidence rate in Australian racehorses during racing (Bailey *et al.* 1997a) and 83% of deaths associated with racing or training in Californian racehorses (Johnson *et al.* 1994).

MSIs most commonly affect the limbs of racehorses, in particular the bones and soft tissue structures that support the fetlock joint (Clegg 2011; Stover 2003). Injuries that involved the limbs made up 96.8% of MSIs that forced horses to stop racing or be destroyed in a cohort study of New Zealand horses in their first three years of racing (Perkins *et al.* 2005a). Additionally, in flat-racehorses in Britain, 77.5% of clinical conditions were found to be due to MSI of the limbs (Williams *et al.* 2001). Specifically, both catastrophic and non-catastrophic injuries most commonly occur in the distal half of the forelimb (Johnson *et al.* 1994; Rossdale *et al.* 1985). Recently, non-catastrophic fetlock injuries were found in 102/165 horses during the first two years of training compared to 82/165 for carpal injuries (Reed *et al.* 2012); while catastrophic injuries of Mc3 and the suspensory apparatus were found to most commonly result in fatality (Hernandez and Hawkins 2001; Johnson *et al.* 1994). Bone injury due to cyclic loading, such as shin soreness and fractures of Mc3/Mt3, are common with a significantly higher rate of shin soreness found in younger horses than older horses (Perkins *et al.* 2005a). Furthermore, just over a quarter of all fractures sustained during racing or training have been found to be Mc3 or Mt3 fractures (Verheyen and Wood 2004).

Risk-factors for the development of MSI have been investigated, but it is difficult to give specific recommendations to prevent injury because they appear to be multifactorial and may differ with injury type or the methods used to investigate them. Risk factors for MSI reported in several studies are the trainer (Cogger *et al.* 2006; Perkins *et al.* 2005b), occurrence during a race (Perkins *et al.* 2005a), the racecourse location (Boden *et al.* 2007), horse age (Bailey *et al.* 1997a; Perkins *et al.* 2005a), the track type (Kristoffersen *et al.* 2010; Parkin *et al.* 2004a) and track condition (Boden *et al.* 2007; Parkin *et al.* 2005). However, these risk factors can be multifactorial and relate to the amount and intensity of loading that the horse encounters. For example, in America there was a 1.7 times increased risk for catastrophic injury to occur on turf compared to dirt tracks; however, most races on turf were 66 times more likely to be a long race (Hernandez *et al.* 2001). Additionally, there was a higher risk of MSI when the track surface was firm or hard compared to soft (Parkin *et al.* 2004b, 2005; Williams *et al.* 2001).

Risk factors associated with the training histories of racehorses suggest that the tissues adapt to specific loading conditions in order to withstand the exercise intensity demanded; however, tissues can become damaged if loading is excessive. Studies by Parkin *et al.* (2004a, 2005) reported that horses had a higher risk of sustaining catastrophic distal limb and condylar fractures if they had done no gallop work in their first year of training or had not started racing until three or four years of age. However, increasing the distance or percentage of time spent training at maximum speed was found to be associated with the occurrence of MSI in two year old Thoroughbred racehorses (Cogger *et al.* 2006). These results reflect the finding that cumulative training was at first protective for MSI; then, after a plateau effect, increased the occurrence of MSI (Perkins *et al.* 2005b).

It is clear that wastage is a major cause of loss in Thoroughbred racing industries globally; however, the losses would decrease if MSIs were prevented. Risk factors for MSI have been identified, yet their specific effects on musculoskeletal tissues are not fully understood. It is likely that the interactions between risk factors and tissue responses are complex and verge between being necessary for physiological adaption and inducing pathologic change. It is likely that research needs to relate the specific risk factors that have been associated with MSI to specific tissue responses in order to build a more cohesive basis on which impending injuries can be recognised.

Structure and function of the equine fetlock joint

The fetlock joint is a high range of motion joint that is driven by energy stored in closely associated tendinous structures (Back *et al.* 1995; Clayton *et al.* 1998), and the articulating bones have adapted to allow for high energy function (Thomason 1985b). The fetlock joint is a synovial joint, composed of the Mc3 or Mt3 bone, the proximal phalangeal bone and the proximal sesamoid bones (PSBs) (Figure 1.1). The palmar surface of Mc3 is the most loaded part of the joint during motion due to the force exerted by the PSBs when elastic energy is stored in the tendinous structures on the palmar/plantar aspect of the limb (Clayton *et al.* 1998). These forces that occur have been found to reach maximum stresses of 40-50 MPa in the flexor tendons and 18-25 MPa in the suspensory ligaments during gallop (Biewener 1998). Repeated very high pressure on the palmar/plantar surface of Mc3/Mt3 is thought to greatly contribute to the occurrence of lesions in the palmar/plantar region of Mc3/Mt3 through structural changes in the bone (Norrdin *et al.* 1998; Riggs *et al.* 1999a).

The suspensory apparatus and digital flexor tendons allow the horse to store energy during motion with virtually no net generation or loss of energy (Clayton *et al.* 1998), which decreases the muscular effort needed for locomotion. Kinetic and kinematic gait analysis has indicated that 36% of stored elastic energy can be converted to mechanical work during gallop (Biewener 1998). In the distal limb, the suspensory ligament, superficial digital flexor tendon and deep digital flexor tendon are composed of elastic but strong tendinous tissue made of helical type 1 collagen. The proximal and distal ends of the suspensory apparatus are anchored to the bone of proximal Mc3/Mt3 and the proximal phalanx respectively (Dyce *et al.* 2002). The two components are divided by the PSBs that articulate with Mc3/Mt3. The flexor tendons run palmar to the PSBs and suspensory ligament (Dyce *et al.* 2002), compressing them against the palmar/plantar surface of Mc3/Mt3 (Figure 1.1). Together, these components form a catapult structure (Harrison *et al.* 2010); the elastic energy stored in the tendinous tissue structures during the stance phase of gait is released as kinetic force (Biewener 1998), pushing against the palmar/plantar surface of Mc3/Mt3, and contributing the larger part of the work to the subsequent movement of the distal limb (Clayton *et al.* 1998; Harrison *et al.* 2010).

The pressures within the fetlock joint are high during motion and increase with exercise intensity (Biewener 1998; Brama *et al.* 2001; Harrison *et al.* 2010), acting directionally within the joint (Clayton *et al.* 1998). The forces acting in the fetlock joint are higher than those acting on other joints in the distal forelimb and have been found to be higher during gallop 45.9±0.9 Nkg^{-1} compared to walk 20.6±2.8 Nkg^{-1} (Harrison *et al.* 2010). The forces concentrate on the palmar/plantar surface of the joint (Clayton *et al.* 1998), between the PSBs and Mc3/Mt3, due to elastic forces acting in the flexor tendons and suspensory ligament (Harrison *et al.* 2010). The resulting action creates a “nut cracker” effect, which crushes as well as pressurises the epiphysis of Mc3/Mt3 (Thomason 1985a).

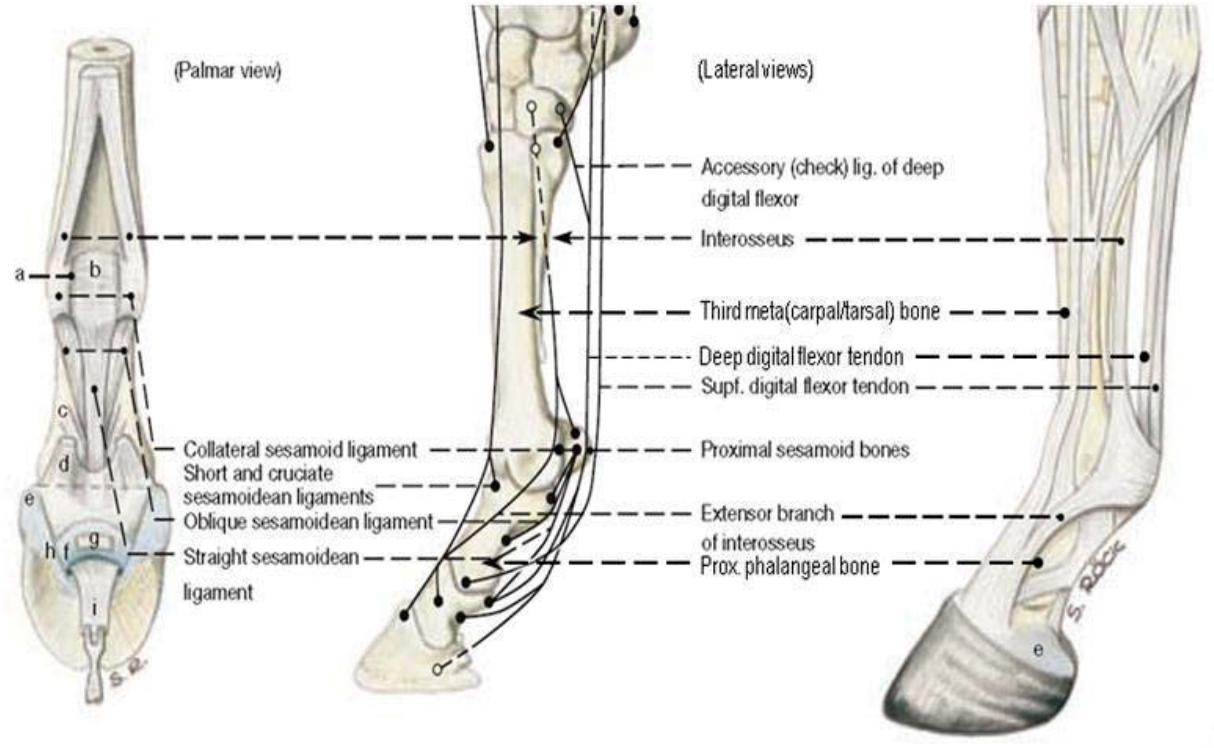


Figure 1.1: Anatomy of the bones and soft tissue structures of the equine fetlock joint. The Mc3/Mt3, PSBs and proximal phalangeal bone articulate to form the fetlock joint. The joint is closely associated with tendinous structures of the suspensory apparatus (suspensory/ interosseus ligament and distal sesamoidean ligaments) and the superficial and deep digital flexor tendons. Adapted from Budras *et al* (2009)

The structure of the distal Mc3/Mt3 epiphysis

The gross architecture of Mc3/Mt3 has adapted to disperse and withstand the high forces exerted on its distal articular surface during locomotion (Thomason 1985a, b). Mc3 and Mt3 are the long weight bearing bones in the distal limb of the horse; they have been found to be compressed axially during gait with specific vector forces acting on the palmar and dorsal surfaces of the epiphysis that are exerted by the proximal phalangeal bone and the PSBs (Biewener *et al.* 1983). Grossly, the articular surface of the distal end of Mc3/Mt3 is rounded and the weight-bearing medial and lateral condyles are separated by the sagittal ridge (Figure 1.2). The bone structure is made of an outer layer of cortical bone, including the subchondral bone (SCB), which surrounds trabecular bone. The SCB is stronger than the underlying trabecular bone and protects the trabecular structure from detrimental compressive forces (Rubio-Martinez *et al.* 2008a). In the palmar and dorsal regions of the condyles the subchondral cortical bone is thickened, has a greater density, and merges with the trabeculae of the epiphysis that are initially dense and thickened, but become thinner toward the centre of the epiphysis (Figure 1.2) (Boyde *et al.* 1999; Riggs *et al.* 1999b; Thomason 1985a).

The trabecular arrangement has evolved to redistribute the high forces encountered due to ungulate movement from the articular surface to the strong diaphysis (Thomason 1985b). The trabeculae from the palmar and dorsal surfaces are orientated toward the central epiphysis, where they transmit forces to central longitudinal trabeculae, which eventually join with the cortical bone of the diaphysis (Figure 1.2) (Thomason 1985a). In this way, the high forces during loading are efficiently dispersed from weaker to stronger areas of the bone.

The microstructure of bone within the Mc3/Mt3 distal epiphysis also contributes to the overall strength and elasticity of the bone. On a microscopic level, bone is made from

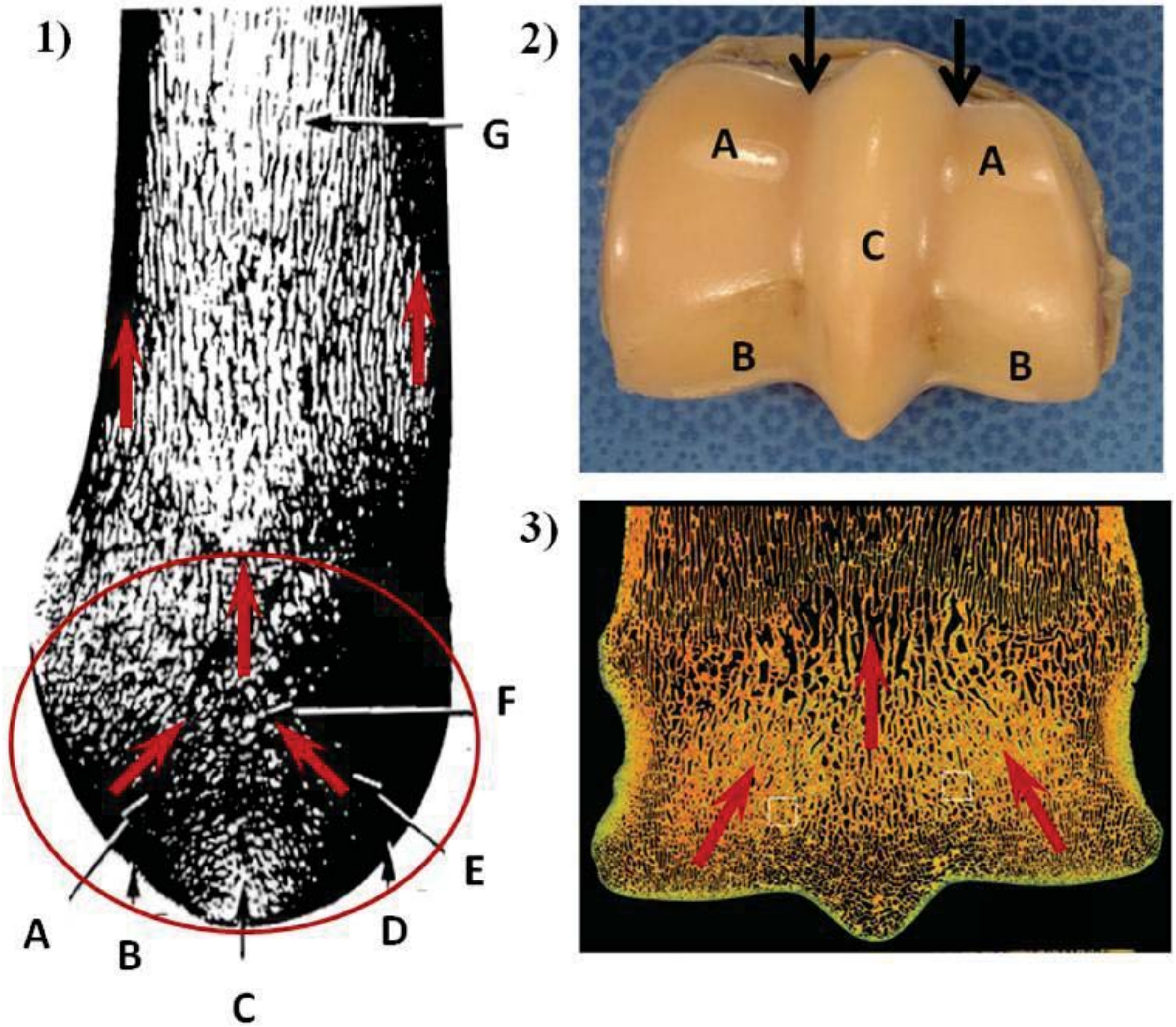


Figure 1.2: Anatomy of the trabeculae and articular surface of the Mc3/Mt3 distal epiphysis.

1) A parasagittal section through the central condylar region of the distal Mc3/Mt3 epiphysis (circled) shows the orientation of the trabeculae within the trabecular bone (↑) to direct forces from the distal articular surface (B,D), to the central epiphysis (F) and then to the diaphyseal region of the bone (G). The distal articular surface is curved (B, D) and there are dense regions of bone in the palmar/plantar (A) and dorsal (E) regions that leave a central region (C) of less dense trabeculae. Adapted from Thomason (1985a).

2) The distal articular surface of Mt3 is shown with the sagittal ridge (C) between the condylar surfaces, which are separated into dorsal (A) and palmar/plantar (B) sections. The parasagittal grooves are shown (↓).

3) The orientation of trabeculae is shown in a longitudinal section of the distal Mc3/Mt3 epiphysis (↑). Adapted from Boyde and Firth (2005).

sheets of mineralised collagen fibres called lamellae that can form a number of arrangements. The lamellae can either wrap concentrically around a hollow canal to form an osteon, such as in cortical bone, or stack in thick layers to form plate or rod-like structures, such as in lamellar bone (Rho *et al.* 1998). In cortical bone, which includes SCB, the osteons have been found to orientate parallel with each other in the direction of compressive force (Smit *et al.* 2002). When the osteons are all aligned in the same direction the bone has been found to have optimum strength (Claes *et al.* 1995). In comparison, trabecular, or cancellous, bone is composed of an irregular, winding arrangement of lamellae. In the distal Mc3 epiphysis, the trabeculae are arranged in sagittal parallel sheets with weaker horizontal cross-struts (Boyde *et al.* 1999). Such an arrangement would allow more strength when the bone is compressed compared to when it is bent.

It is clear that the function of the fetlock joint causes both bone and soft tissue structures to be exposed to high forces during locomotion. Heavily loaded regions of Mc3/Mt3 have been structurally adapted to withstand and disperse these high loads. However, further changes to the structure and composition of the bone occur in response to exercise that could further protect the bone from the detrimental effects of cyclic loading.

The response of bone to cyclic loading

Bones have evolved through time for their specific function; Thomason (1985b) described the way that the structure of the Mc3/Mt3 epiphysis has evolved over millions of years for high speed ungulate motion. However, bone can also change its structure during its lifespan in response to cyclic loading during exercise. Changes in BMD and morphology have been found to occur in both the appendicular and axial skeletons of a range of species (Bradney *et al.* 1998; Gomez-Cabello *et al.* 2012; Hasselstrøm *et al.* 2007; Mosekilde *et al.* 1994; Wolff *et al.* 1999; Yeh *et al.* 1993) and alter the material properties of the bone. The specific changes that occur depend on the location of the bone, the maturity of the animal and the peak strain incurred during exercise.

In long bones of the appendicular skeleton, the diaphyseal bone responds differently to exercise than the epiphyseal or metaphyseal bone. Diaphyses are composed of cortical bone and have been found to respond to exercise by predominantly increasing the cortical bone thickness in humans (Haapasalo *et al.* 2000), horses (Firth *et al.* 2005) and rats (Mosekilde *et al.* 1994). The increased size is attributed to a large amount of periosteal (Haapasalo *et al.* 2000) or endosteal (MacKelvie *et al.* 2004) modelling, or both (Firth *et al.* 2005) that are induced by exercise. The diaphyses of long bones can increase the cortical bone thickness without affecting articulations or impeding on areas of trabecular bone; in contrast, bones involved in articulations have been found to have relatively small increases in cortical thickness, such as the carpal bones (Firth *et al.* 1999a), PSBs (Cornelissen *et al.* 1999) and distal or proximal ends of the equine Mc3 (Firth *et al.* 2005; McCarthy and Jeffcott 1992).

An increase in cortical thickness was primarily associated with increased strength rather than changes in BMD in the Mc3 (Firth *et al.* 2011a) and tibial (Nicholson and Firth 2010) diaphyses of Thoroughbred horses that had been subjected to exercise from a young age. Likewise, the humeral diaphysis of tennis players was found to increase strength by increases in cortical bone thickness rather than by BMD increases (Haapasalo *et al.* 2000).

Diaphyseal bone can also respond to exercise on a microstructural level by influencing the orientation and size of osteons, which are the building blocks of cortical bone.

Osteons will orientate in line with the greatest forces acting on bone (Petrtyl *et al.* 1996). Additionally, osteon size decreases with increased bone strain (Frost 1990), which has been described in the Mc3 diaphysis of 17 month old Thoroughbred horses that had been given 13 weeks of pre-race training (Firth *et al.* 2005) as well as in areas of tension in the calcaneus of mule deer (Skedros *et al.* 2001). The influence of osteon organisation on bone strength has been demonstrated in different areas of the equine Mc3 diaphysis; the ability to withstand fatigue was associated with a high density of recently formed osteons with dense cement lines (Gibson *et al.* 2006; Gibson *et al.* 1995). Additionally, the orientation of osteons has been found to influence bone strength in healing cortical bone of sheep tibial diaphyses (Claes *et al.* 1995).

In contrast to diaphyseal bone, epiphyseal and metaphyseal bone is composed of mainly trabecular bone. These regions of bone respond to exercise by predominantly increasing the BMD, which is the amount of mineral content per unit area of bone tissue (Blood and Studdert 1999). The trabecular bone of the epiphysis has been found to increase BMD by 37% in response to exercise compared to a 1.62% increase in BMD of diaphyseal cortical bone after 13 weeks of training in Mc3/Mt3 bones of 18 month old horses (Firth *et al.* 2005). Similarly, in a randomised exercise trial of girls exposed to jumping exercise there were significant BMD increases in the femoral neck and intertrochanteric region of the femur, but there was no BMD change in the femoral diaphysis (Petit *et al.* 2002).

Increased BMD has been found to strengthen the femoral neck region ($r = 0.507$, $p<0.0002$) in rats (Ammann *et al.* 1996) and has been found to be related to the elasticity of human and bovine trabecular bone (Ashman and Rho 1988). BMD is used as a predictor of strength in human medicine with each standard deviation decrease found to increase the risk of hip fracture in aged women by 2.6-2.7 times (Cummings *et* *al.* 1994). However, very high levels of bone mineral content have been found to reduce the ability of equine Mc3 cortical bone to deform in response to loading (Les *et al.* 2002), which could predispose to tissue damage.

In areas of trabecular bone, like the epiphysis and metaphysis of long bones, the arrangement and size of the trabeculae can also influence the strength of the tissue. Trabecular connectivity was demonstrated to determine elasticity and strength using a finite-element model (Kinney and Ladd 1998), but a later study that physically tested the mechanical properties of bone found that the bone volume fraction determined 84-94% of the mean and maximum bone strength (Kabel *et al.* 1999). The thickness of trabeculae has been determined to be an important determinant of strength, being more correlated with the bending moment ($r=0.63$, $P<0.05$) and stiffness ($r=0.68$, $P<0.05$) in the femoral necks of gastrectomised rats than a BMD decrease of 21% (Stenstrom *et al.* 2000). Additionally, an increased number and thickness of trabeculae was found to increase the strength in the vertebral bodies of rats (Mosekilde *et al.* 1994).

The peak strains and strain frequency are key determinants for the degree of bone change that occurs in response to an exercise program (Smith and Goodship 2008). There were significant differences in the diaphyseal cortical area, stress-strain index, BMD and the epiphyseal BMD of Mc3 in horses that had galloped compared to cantered or untrained controls (Firth *et al.* 2005). Additionally, in the tarsometatarsal diaphysis of young roosters both endosteal (+370%) and periosteal (+40%) bone formation was increased in response to jumping compared to walking exercise (Judex and Zernicke 2000). It has also been demonstrated in the trabecular bone of the PSBs that higher peak compressive forces attributable to dirt tracks were associated with significantly thicker trabeculae than control or wood-chip track trained horses (Young *et al.* 1991). Only a short exposure to peak strain was needed to induce a maximal bone response in avian bone sections (Rubin and Lanyon 1984b) and, through the analysis of training data collected from racehorses with fatigue injury of Mc3, Boston and Nunamaker (2000) have proposed exercise regimes that use short distance gallops, rather than long distance gallops, to prevent MSI in young horses.

Studies by Firth *et al.* (1999a; 1999b) found comparable BMD and cortical bone thickness in equine carpal bones from horses that had been exposed to similar peak strains for either 18 weeks or 18 months, which demonstrates that no further bone responses occur if a strain level is maintained, irrespective of the duration of the exercise program. Although Parkin *et al.* (2004a, 2005) found that the exposure to peak strain was protective against bone tissue failure in the form of condylar fracture in Thoroughbred racehorses, a high frequency of loading cycles at canter or gallop has been found to be associated with condylar fracture (Verheyen *et al.* 2006). The importance of recovery for ensuring optimum bone response was also demonstrated in rat ulna bones when bone strength was enhanced due to the division of cyclic loading into four bouts per day, compared to being delivered in one bout per day (Robling *et al.* 2002).

The absence of peak strain to maintain changes associated with exercise has been found to reduce the BMD of trabecular bone by 51.5% and cortical bone by 32.7% in the distal and proximal ends of the tibia in human spinal injury patients over the first two years of injury (Hangartner and Rodgers 1994). Additionally, a total depletion of BMD gained during training was measured in the Mc3 diaphysis of racehorses after the discontinuation of training and a period of pasture rest (Firth *et al.* 2011a). Consequences of absent exercise levels may persist long term as a study of International Space Station crew members found that trabecular BMD of the hip had recovered to

93% of the pre-flight BMD after one year and after five years was lower than expected (Carpenter *et al.* 2012).

The site specific effects of strains on bone morphology have been demonstrated on a total body level by the type of physical activity undertaken; male weight lifters have been found to have significantly higher humeral BMD than runners of the same age and weight (Hamdy *et al.* 1994). Alterations have also been observed in different regions of a bone; for example, a study of tibial BMD in 4 week old rats found that exercise increased BMD of the distal tibial metaphysis by 43.4% compared with a 24% increase in the proximal tibial metaphysis (Iwamoto *et al.* 1999). Additionally, the Mc3/Mt3 epiphysis of horses has been found to have a zone of sclerosis in the load path of the PSBs that changed significantly with exercise; however, no significant differences were seen in response to exercise in non-loaded regions of the epiphysis (Firth *et al.* 2005).

Specific characteristics of bone response to exercise are also determined by the stage of maturity of the subject. A reduction of endosteal resorption was responsible for an increase in cortical size in diaphyseal cortical bone for humans (Bradney *et al.* 1998), rats (Mosekilde *et al.* 1994) and horses (Firth *et al.* 2011a) that were exposed to exercise during periods of growth, but a periosteal response increased the cortical size when mature humans (Haapasalo *et al.* 2000) and post-pubertal horses (McCarthy and Jeffcott 1992) were exercised. Human studies have suggested that there is a critical period during early puberty in which exercise will have a peak effect on BMD accrual because of hormonal changes (MacKelvie *et al.* 2002). Exercise could be crucial for ensuring that at least 26% of total adult bone mineral content is accrued during the 2 years of accelerated growth around puberty (Bailey *et al.* 1999b). Structural changes that occur to bone during growth have been demonstrated to persist long term in rodents with increased cortical thickness and fatigue life persisting into adulthood, despite a reduction in exercise level (Warden *et al.* 2007).

However, a study by Cornelissen *et al.* (1999) proposed that very high exercise demands early in life could result in a detrimental effect on the bone. In the study, foals that were subjected to sprint training in the first 5 months of life had significantly higher BMD of PSB trabecular bone compared to foals that were boxed or pasture reared. However, when the foals were subsequently turned out in pasture from 5-11 months of age, the sprint trained group had a significantly lower BMD than the boxed group. As yet, the effects of differing exercise intensities at a range of ages or maturity levels in horses have not been compared.

Structural changes of the distal Mc3/Mt3 epiphysis in response to exercise

Structural changes occur due to cyclic loading in the mineralised tissues of the Mc3/Mt3 epiphysis, which could be an adaptive mechanism to withstand the high loads encountered during motion. Under the influence of exercise, the thickness of both the cortical SCB and trabecular bone increases causing a BMD increase in the region (Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). These structural changes could alter the material properties of the epiphysis, allowing the structure to withstand the particular compressive loads attributed to the exercise intensity that the horse is subjected to.

The mineralised tissues of the epiphysis consist of articular calcified cartilage, SCB and trabecular bone. The three tissues respond to exercise differently, due to their differing bone types. Exercise has been found to have little effect on the structural or mechanical properties of articular calcified cartilage (Doube *et al.* 2007; Ferguson *et al.* 2008).

However, the SCB, which is the cortical bone component of the Mc3/Mt3 epiphysis, responds to load by thickening, therefore increasing the BMD in the region of bone adjacent to it (Brama *et al.* 2009; Dykgraaf *et al.* 2008; Easton and Kawcak 2007; Kawcak *et al.* 2000; Riggs and Boyde 1999). Trabecular bone of the Mc3/Mt3 epiphysis responds to exercise by increasing the number of trabeculae as well as their thickness, by depositing new bone around existing trabeculae, in what appears microscopically to be a rapid response (Boyde 2003; Boyde and Firth 2005). These responses of the epiphyseal SCB and trabecular bone to exercise are observed as an increase in BMD of the Mc3/Mt3 epiphysis due to new bone deposition (Boyde and Firth 2005; Firth *et al.* 2005).

The BMD increase of the trabecular and SCB in the central condylar regions of the palmar/plantar epiphysis is related to the peak strains encountered during exercise (Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). There was no difference in BMD response in young horses given long term light conditioning exercise compared to controls at pasture (Dykgraaf *et al.* 2008; Firth *et al.* 2011a); however, there was a significant response in horses that were given race training or treadmill exercise compared to controls (Firth *et al.* 2005; Kawcak *et al.* 2010; Riggs and Boyde 1999). It is only at relatively high loads, such as canter and gallop, that the Mc3 epiphysis contacts the articular PSB surfaces sufficiently to transmit pressure between them (Brama *et al.* 2001). As exercise intensifies, increasing the load on the limb, more pressure is exerted on the central condylar regions of Mc3/Mt3, not only by the PSBs, but also due to the compressive effect of the proximal phalanx and PSBs squeezing together (Merritt *et al.* 2010). As a result, the BMD increase involves the palmar/plantar SCB and epiphyseal bone of both condyles but not the sagittal ridge region of the epiphysis (Riggs and Boyde 1999).

The spatial distribution of high BMD relates directly to the load path created during exercise by the PSBs (Easton and Kawcak 2007; Firth *et al.* 2005). In the condyles, the spatial distribution of increased BMD extends from the distopalmar aspect of the epiphysis obliquely across to the dorsoproximal surface, with no effect on the parts of the epiphysis that are not in the load path created by the PSBs (Firth *et al.* 2005). The size of the area of increased BMD is determined by the load that the epiphysis is subjected to during the stance phase of gait (Easton and Kawcak 2007; Firth *et al.* 2005). For example, the size of areas of increased BMD were larger in horses that had galloped, because gallop increases the pressure between the PSBs and Mc3, and larger in Mc3 compared to Mt3 because more weight is distributed over the forelimbs than the hindlimbs (Brama *et al.* 2001; Firth *et al.* 2005). Also, Mt3 was found to have a larger area of high density bone in the lateral condyle compared to the medial condyle ($P = 0.007$) in exercised (Firth *et al.* 2005) and non-exercised (Riggs *et al.* 1999b) horses, which could be a reflection of potentially asymmetric loading of the condyles in the hind limbs. Specific characteristics of the BMD spatial distribution have yet to be linked to specific loading conditions; however, understanding how the BMD spatial distribution responds to loading could provide a baseline to predict the load that an epiphysis has been subjected to, or if the response is typical for a given load.

The specific BMD responses that occur in the distal Mc3/Mt3 epiphysis to exercise could be adaptive by altering the mechanical properties of the bone so that it can withstand the compressive loads that it is exposed to. Microscopic examination of areas that gain BMD in response to exercise, have shown that new bone deposited around existing trabeculae has a lower degree of mineralisation, which could potentially be more elastic in relation to focal loads or the increase in total bone material could make the epiphyseal structure stronger (Boyde and Firth 2005). It has been found that condylar bone is stiffer, stronger and tougher than the sagittal ridge bone ($P < 0.001$) (Rubio-Martinez *et al.* 2008a), yet the change in material properties as new bone is deposited in the Mc3 epiphysis in response to exercise has not been determined.

Bone diseases of the Mc3/Mt3 epiphysis associated with cyclic loading

Bone structural abnormalities occur even though the purpose of structural bone alterations in response to exercise could be an adaptive response to protect the bone from being damaged by cyclic loading. The abnormalities could be due to an inappropriate response of the tissue to loading, or a result of tissue fatigue. Three common diseases of the distal Mc3/Mt3 epiphysis; osteoarthritis, palmar/plantar osteochondral disease and condylar fracture, are linked to abnormal loading of, or bone response in, the epiphysis and are associated with structural changes in the bone tissue. In this section, the characteristics, bone structural changes and theories as to the aetiology of Mc3/Mt3 condylar fracture are discussed in detail because fracture represents a total and catastrophic failure in Mc3/Mt3 structure and function.

Osteoarthritis

Osteoarthritis is the failed repair of damage caused by excessive mechanical stress on joint tissues (Brandt *et al.* 2008). Osteoarthritis of the fetlock joint has been observed in 33% of Thoroughbred racehorses aged 2-3 years old (Neundorf *et al.* 2010). Inflammation and swelling of the affected joint can cause lameness, with lameness being found to be the primary cause of lost training days in racehorses (Hernandez and Hawkins 2001; Perkins *et al.* 2005a; Rossdale *et al.* 1985; Wilsher *et al.* 2006) . Structural changes in the bone tissue of the Mc3/Mt3 epiphysis have been found to occur in horses with osteoarthritis of the fetlock joint in response to abnormal loading. Early in the investigation of human osteoarthritis, trabecular thickening and clustering of the immediately adjacent trabecular bone resulted in BMD increase of the SCB (Layton *et al.* 1988). Additionally, a higher percentage of very high BMD in the palmar/plantar regions of the Mc3/Mt3 condyles in horses with osteoarthritis has been observed (Young *et al.* 2007). Abnormal joint biomechanics, such as increased loading, have been found to reactivate ossification centres causing bone thickening and subsequent thinning of articular cartilage through its replacement with bone (Burr and Radin 2003). These thickened regions of bone have also been linked to microcracking in human osteoarthritis patients (Burr and Radin 2003), and lytic lesions were found within palmar regions of thickened bone in horses with osteoarthritis (Young *et al.* 2007).

Palmar/plantar osteochondral disease

Palmar/plantar osteochondral disease (POD) is characterised by lesions of the SCB that can progress to collapse of the bone and ulceration of the overlying cartilage (Barr *et al.* 2009). Also named "traumatic overload arthrosis", POD is believed to occur due to an inability of the Mc3/Mt3 palmar/plantar condyles to withstand excessive loading (Pool 1996). The condition affects a high number of Thoroughbred racehorses with a prevalence of 67% found in 3-10 year old horses (Barr *et al.* 2009). Although lameness or decreased performance could be associated with the disease, POD is commonly an incidental finding, indicating that horses are still able to race with the condition (Barr *et al.* 2009; Norrdin and Stover 2006; Tranquille *et al.* 2012). However, this could potentially be detrimental as it infers that horses could be racing on structurally abnormal bone tissue. Horses with POD have been found to have abnormally high BMD of epiphyseal trabecular bone, which has the same density and mechanical properties as the SCB, and becomes stronger than the SCB in more severe stages of disease (Rubio-Martinez *et al.* 2008a). This contrasts to horses without POD that have denser (Firth *et al.* 2005; Rubio-Martinez *et al.* 2008a, b), stiffer and tougher SCB compared to the underlying trabecular bone (Rubio-Martinez *et al.* 2008a). POD is also associated with microcracking, bone resorption and microfracture within areas of thickened subchondral and trabecular bone that result in tissue failure of the SCB (Norrdin *et al.* 1998; Norrdin and Stover 2006).

Mc3/Mt3 condylar fracture

Fractures involving Mc3/Mt3 have been found to contribute to 25.7% of fractures in British flat racehorses, of which a third were condylar fractures (Verheyen and Wood 2004). Condylar fractures represent a disease process in which structural bone changes, as a response to cyclic loading, ultimately lead to catastrophic failure of the bone. Condylar fractures have been suggested to be an example of a stress induced overload injury as they occur in specific locations, have a specific morphology and have been associated with prior pathologic changes (Parkin *et al.* 2006; Radtke *et al.* 2003; Stover and Murray 2008; Zekas *et al.* 1999a). Although theories on the pathogenesis of condylar fracture have varied over the years, it is now thought that condylar fractures are a result of underlying microstructural changes in the bone of the palmar/plantar Mc3/Mt3 epiphysis (Riggs 1999; Stepnik *et al.* 2004; Whitton *et al.* 2010).

Characteristics of condylar fractures

The locations of condylar fractures are similar between the populations of racehorses that have been studied. Mc3 is more commonly affected than Mt3 (Bassage and Richardson 1998; Ellis 1994; Parkin *et al.* 2006; Rick *et al.* 1983; Zekas *et al.* 1999a) and the lateral condyle is more commonly affected than the medial condyle (Bassage and Richardson 1998; Zekas *et al.* 1999a); however, some studies have found that medial condylar fractures could predominate in the hindlimbs (Ellis 1994; Riggs 1999).

Most condylar fractures have been observed within the region from the sagittal ridge midline to the central condyle (Zekas *et al.* 1999a). Specifically, a large proportion of fractures have been observed to occur around the parasagittal groove region, with distances from the sagittal ridge midline found to be 11 mm (Parkin *et al.* 2006) to 12-15 mm (Rick *et al.* 1983) in two studies. Two studies have found more left forelimb and more right hindlimb fractures than the associated contralateral limbs (Ellis 1994; Rick *et al.* 1983), perhaps as a result of training on a counter-clockwise track, yet other studies of a similar size have found no differences between right and left limbs (Bassage and Richardson 1998; Parkin *et al.* 2006; Zekas *et al.* 1999a). The predictable nature of fracture location is an indicator of stress induced injury that could cause specific points of weakness to occur.

The morphology of the fracture line is also similar between individuals with condylar fracture, which could be due to the structure of the bone and underlying pathologic changes that cause specific areas of weakness. The underlying structural architecture of the trabecular bone in the distal Mc3/Mt3 has been found to determine the propagation of the fracture line; initially deviating axially and then abaxially, between parallel sheets of lamellar bone, to either involve (complete) or not involve (in-complete) the cortical bone of the metaphysis (Boyde *et al.* 1999). By following the natural abaxial curvature of the trabeculae, the fracture lines consistently end between 50 and 135 mm proximal to the distal articular surface of the bone (Rick *et al.* 1983). Additionally, fractures that continue to elongate axially have been found to form spiral fractures (Ellis 1994) as they follow the trabecular orientation of the opposite side from which they originate.

Articular fragments that cause comminution of the fracture line have also been observed in several studies (Ellis 1994; Parkin *et al.* 2006; Rick *et al.* 1983; Zekas *et al.* 1999a). They could be related to the degree of loading that the limb incurred or could represent areas of prior bone lesions that cause focal areas of structural weakness. Significantly more articular comminution was seen in the forelimbs compared to the hindlimbs of racehorses with fracture (Parkin *et al.* 2006) and 23% of fractures that originated from the parasagittal groove to the mid-condyle had articular comminution, compared to 3% of fractures that originated from the sagittal ridge mid-line to the parasagittal groove (Zekas *et al.* 1999a). It is unknown if the differences are a consequence of the distribution of forces that could differ between limbs and regions of the Mc3/Mt3 epiphysis when fractures occur, or if the fragments represent areas of prior pathologic change that caused a weakening of the bone at the time of fracture. This concept needs further investigation as the presence of an articular fragment with condylar fracture has been found to lower the prognosis for return to racing (Zekas *et al.* 1999b).

Both gross and microscopic pathologic changes have been found in and around the articular origins of condylar fractures. Gross pathologic changes found concurrently with condylar fracture in Thoroughbred racehorses include significantly more severe cartilage wear lines on the injured limb ($P<0.01$) and a branching array of cracks on the SCB of the condylar groove at the origin of the fracture line (Radtke *et al.* 2003). Additionally, fracture lines have been found to pass through POD lesions (Kaneko *et al.* 1993); however, these lesions have been found irrespective of fracture (Pool and Meagher 1990). Microscopic pathologic changes such as microcracks and microfracture have also been found around condylar fracture lines of Thoroughbred racehorses (Stepnik *et al.* 2004) and fracture lines have been observed to pass through focal lytic defects in the SCB and articular calcified cartilage of the condylar grooves (Riggs *et al.* 1999a). It is possible that some of these pathologic changes observed are instrumental in the pathogenesis of fracture; however, these must be distinguished from pathologic findings that occur irrespective of fracture formation.

Theories on fracture formation

Numerous theories have been proposed to explain the aetiology of condylar fracture. Theories range from suggesting that condylar fractures are "spontaneous" to theories that build a picture of incremental bone tissue changes that occur in response to exercise and weaken focal areas of the condyles eventually resulting in fracture propagation.

Early theories on condylar fracture aetiology ranged from insinuating spontaneous structural causes to developmental condylar disease. An early structural theory, supporting spontaneous fracture, was that the orientation of the articular surfaces of the proximal phalanx and Mc3/Mt3 could be arranged abnormally during locomotion by the proximal phalanx remaining stationary in relation to Mc3/Mt3 as it rotated in preparation to protract, resulting in excessive shear force across the condyles and fracture (Rooney 1974). However, there has been no specific experimental evidence that this occurs and it has been further proposed that injuries to the sagittal ridge would be observed if the theory was correct (Riggs 1999). In contrast, it has been suggested that developmental osteochondrosis induces osteonecrosis and osteosclerosis under the influence of exercise, leading to fracture (Kaneko *et al.* 1993). However, other studies found that developmental osteochondrosis was of no consequence to the development of fracture (Pool and McIlwraith 1995) and suggested that biomechanical forces result in the failure of skeletal tissues at predictable sites (Pool and Meagher 1990). This has led to further investigations of the response of the distal Mc3/Mt3 to cyclic loading.

The failure of bone structure due to incremental microstructural changes caused by cyclic loading is currently the most likely aetiology for condylar fracture. Significant increases in epiphyseal BMD have been observed in response to cyclic loading, such as gallop exercise on a treadmill or track, in Thoroughbred racehorses (Firth *et al.* 2005;

Kawcak *et al.* 2000; Riggs and Boyde 1999). Such BMD changes alone have been suggested to cause condylar fracture, due to a gradient formed between the dense condylar and the less dense sagittal ridge bone that could result in concentrations of focal stress (Riggs *et al.* 1999b). However, fracture lines have recently been found to pass through areas of very dense bone (Whitton *et al.* 2010) where abnormal microscopic changes in bone structure have been found to occur (Norrdin *et al.* 1998; Norrdin and Stover 2006). Additionally, microcracking, microfracture (Norrdin and Stover 2006) and evidence of osteoclastic remodelling (Norrdin *et al.* 1998) have been found to occur within areas of very dense bone in the palmar region of the condyles, and have also been found at the origin of existing condylar fractures (Riggs *et al.* 1999a; Stepnik *et al.* 2004). Microfracture could weaken the subchondral and adjacent trabecular bone to allow fracture propagation.

Other microstructural changes, characterised by osteolysis, could be associated with the pathogenesis of condylar fracture. Focal lytic areas of SCB and articular calcified cartilage have been found at sites that fractures commonly occur in the parasagittal grooves (Firth *et al.* 2009; Riggs *et al.* 1999a) as well as around the origin of parasagittal groove fractures (Riggs *et al.* 1999a; Whitton *et al.* 2010). Although these findings were largely in racehorses, one study (Firth *et al.* 2009) found lytic lesions in lightly exercised 17 month old Thoroughbreds with histologic evidence of mineralisation failure (Doube *et al.* 2007). The evidence of such a microstructural abnormality before the initiation of high cyclical loading could be incidental, such as osteochondrosis was found to be (Pool and McIlwraith 1995), or it has been suggested that it could contribute to the pathogenesis of condylar fracture (Firth *et al.* 2009). Additionally, lytic changes in the parasagittal grooves could contribute to the pathogenesis of parasagittal groove fractures, while microfractures within areas of high density bone could lead to mid-condylar fractures.

It is clear that the bone of the Mc3/Mt3 epiphysis not only alters its structure in response to loading, but is damaged under the influence of excessive loads. Osteoarthritis, POD and condylar fracture of the distal Mc3/Mt3 epiphysis are all characterised by changes in BMD and microstructure. However, instead of the bone changes being adaptive to the cyclic load, they result in detrimental mechanical properties such as areas of weakness or brittleness that can eventually result in tissue failure. It is currently unknown exactly what changes in bone structure or microstructure contribute to the pathogenesis of each disease process or how these changes progress from a physiologic response to a pathologic response during training. Prospective longitudinal studies in live horses using non-invasive techniques to monitor changes in the distal Mc3/Mt3 epiphysis are needed to further understand how and why the bone responses progress from being physiologic to pathologic.

Investigating the response of bone in the Mc3/Mt3 epiphysis

A number of techniques have been used to investigate the responses of bone to exercise including non-invasive imaging and invasive microscopy techniques. The techniques have been used in both diagnostic and research settings; however, although invasive techniques such as scanning-electron microscopy (Norrdin *et al.* 1998; Stepnik *et al.* 2004), microradiography (Riggs *et al.* 1999a) and high resolution peripheral quantitative computed tomography on bone sections (Whitton *et al.* 2010) have detected features associated with condylar bone lesions, they cannot be used in live horses. As a result, non-invasive imaging techniques are currently being investigated for their ability to detect bone changes of the Mc3/Mt3 epiphysis in live horses to predict tissue failure.

However, current studies have focused on a qualitative rather than a quantitative method of image analysis (Morgan *et al.* 2006; Tranquille *et al.* 2012). This could introduce observer error through bias and could also be time expensive as a skilled observer must observe and score each image. Quantitative methods of image analysis would decrease such errors and make image analysis time efficient as well as allow direct quantitative comparison between images.

Non-invasive imaging techniques

Non-invasive imaging techniques such as radiography, nuclear scintigraphy, magnetic resonance imaging (MRI) and computed tomography (CT) are routinely used for the diagnosis of orthopaedic injury in equine veterinary practice; however, their specific use to predict condylar injury has not yet been ascertained.

Radiography (x-ray) and nuclear scintigraphy are used commonly in the diagnosis of orthopaedic injuries in equine patients, but they are not sensitive enough to detect subtle bone changes of early damage. Conventional radiography allows bone structures to be imaged by the variable penetration of x-rays through the tissues onto a digital or film screen allowing regions of high and low density bone to be detected as variations in radio-density and radio-opacity respectively, as tissue density determines the penetration of the x-rays(Butler *et al.* 2000). Radiography is the most common imaging technique used in equine veterinary practice for the diagnosis or screening for pathologic changes of bone. However, while it has been found to be good or excellent for the detection of bone lesions, it was not sensitive enough to detect pathologic small cracks that were detected with CT imaging (Morgan *et al.* 2006); additionally, lesions of the SCB were less frequently detected using radiographs when compared with CT or MRI (O'Brien *et al.* 2011). Further, bone remodelling may not be apparent on x-ray for approximately two to three weeks after it first occurs (Butler *et al.* 2000).

In comparison, early changes in bone remodelling that are not detectable on radiographs can be apparent using nuclear scintigraphy (Biggi *et al.* 2009). Nuclear scintigraphy of bone detects the fixation of polyphosponate molecules labelled with technetium in bone sites that are undergoing active remodelling (Ueltschi 1997). When used for diagnostic purposes, significant subjective differences in increased radio-isotope uptake have been found between epiphyseal regions in lame and non-lame horses (Biggi *et al.* 2009). Additionally, ratios of increased radio-isotope uptake between Mc3 epiphyseal and diaphyseal cortical bone have been found to be significantly higher in horses affected with SCB lesions and lameness, respectively, compared to controls (Parker *et al.* 2010). However, the technique is indicated for determining the location of bone that has an increased rate of turnover, rather than detecting specific pathologic processes; therefore, imaging techniques such as MRI or CT are often used subsequently.

In equine veterinary practice MRI is currently used to diagnose lesions that cannot be detected using radiography, and in research it has been used to investigate tissue changes that could be linked to the occurrence of condylar fracture. MRI uses a magnetic field to align protons in the tissues that then produce different signals when they release applied radiofrequency energy (Edelman and Warach 1993). Because the rate of the release of energy is different for particular tissues, MRI results in superior soft tissue contrast; however, differences in bone tissues can also be seen (Edelman and Warach 1993). Specifically, on T1- and T2-weighted images, variations in bone mineralisation, as well as areas of fluid that represent the initial response of bone to injury, can be seen (Dyson and Murray 2007). High-field circular MRI machines with a stronger magnetic field produce higher quality images than low-field open MRI machines (Murray *et al.* 2009). However, low-field MRI is cheaper because images can be obtained in standing sedated horses, whereas they must be under general anaesthesia when the high-field circular MRI machines are used. MRI is useful to detect SCB injuries that are not able to be diagnosed with radiography or scintigraphy (Gonzalez *et al.* 2010). However, when MRI was compared with CT, there were no differences for the detection of SCB lesions, and neither could detect cracking of calcified cartilage (O'Brien *et al.* 2011). MRI has also been investigated in the detection of bone changes related to condylar fracture (Tranquille *et al.* 2012). The study found that there were significant differences in the signal intensities of the condylar regions between fractured and non-fractured lateral condyles. However, the determination of signal intensities was qualitative, differences were between consecutive grades, and the effects of different exercise intensities as a confounder on the spatial distribution of sclerosis was not considered.

While used in the clinical setting for the diagnosis and assessment of orthopaedic disease, CT has also been used in multiple studies to investigate BMD changes associated with fracture in both humans and horses (Marshall *et al.* 1996; Riggs *et al.* 1999b; Sheu *et al.* 2011; Whitton *et al.* 2010). CT produces an image through the quantification of x-ray beams by a series of detectors passing through a slice of an anatomical area (Andre and Resnick 1996). The technique produces an image with high anatomical detail because it can detect slight differences in x-ray absorption of the tissues (Kraft and Gavin 2001). Like MRI, CT can be expensive when circular human machines are used as horses are required to be anaesthetised. However, although peripheral quantitative computed tomography (pQCT) has been used in equine research, a standing pQCT machine has been developed (Stratec medical, Pforzheim, Germany) and is currently being used with success in two centres in Germany (Van Weeren and Firth 2008).

The benefit of pQCT is that it measures volumetric bone mineral density (BMD_V) directly, rather than relating the x-ray absorption to a pre-formed scale as is the case for other CT techniques. Additionally, pQCT is able to isolate trabecular from cortical bone; therefore, it has been found to be more sensitive to changes in BMD that occur in early post menopausal women than non-quantitative CT (Reinbold *et al.* 1986). For these reasons, and the apparent reproducibility obtained on subsequent measurements of human long bones (Rinaldi *et al.* 2011), pQCT is commonly used for the assessment of human BMD parameters.

An additional benefit when pQCT is used to image bone, is that it has been found to give an accurate estimation of bone strength non-invasively (Jamsa *et al.* 1998; Wachter *et al.* 2001). Parameters measured using pQCT such as mineral content, cross-sectional area and indices of bone strength were found to be predictive for the fracture risk of the tibia and radius in men (Sheu *et al.* 2011). However, in a meta-analysis of studies of women, although the predictive ability of BMD_V for fractures was similar to, or better than, that of blood pressure for stroke, or cholesterol for cardio-vascular disease, the pQCT measurements could not predict what individuals would fracture (Marshall *et al.* 1996).

Specific characteristics as to the amount and how the spatial distribution of BMD alters in response to exercise in the equine Mc3/Mt3 epiphysis have been investigated with pQCT; however, the spatial distribution of BMD was analysed qualitatively rather than quantitatively (Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). Further investigations using pQCT to relate specific exercise levels with physiologic and pathologic bone changes are warranted.

Investigating the structural characteristics of bone with non-invasive imaging

Studies that have quantitatively analysed BMD changes of the distal Mc3/Mt3 epiphysis in response to exercise using non-invasive imaging techniques have calculated total BMD change, while the BMD spatial distribution has been described qualitatively (Cornelissen *et al.* 1999; Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). Additionally, sclerotic bone changes observed on MRI images that were associated with condylar fracture were described using a qualitative scale (Tranquille *et al.* 2012). Average BMD values taken over the entire epiphysis do not describe the structural/ spatial orientation of the BMD response, which could be useful to monitor as it could determine if a bone is responding normally or abnormally to a given exercise load. Additionally, qualitative analysis of images introduces observer bias, as well as being time expensive, for the analysis of large data sets in a research setting. Therefore, the development of quantitative techniques to assess alterations in the spatial distribution of BMD on images would be useful.

On software made for non-invasive imaging techniques, such as CT, there are quantitative methods for analysing BMD characteristics from sections of an image using pre-defined regions of interest. However, information can only be obtained on how much of a particular BMD level is present, rather than the spatial distributions of BMD values. In contrast, geographic information systems (GIS) are computer systems used to analyse geographic data for the study of geomorphology and are able to classify, understand relationships between and detect changes in features on a map; therefore enabling the spatial distribution of features to be described using spatial statistics (Oguchi and Wasklewicz 2011). The statistical calculations measure probabilities for the clustering or dispersion of the features (Mitchell 2005).

GIS assigns each point on an image an x,y,z co-ordinate in space and therefore is able to calculate spatial statistics to describe their relationships both discreetly and over a period of time (deSmith *et al.* 2011). It has been found that multiple images taken over time can be overlaid for reliable comparison (Gares *et al.* 2006; Hill and Turnispeed 1989) and then spatial statistics can be applied to these changes (Staley and Wasklewicz 2006; Takagi *et al.* 2007). The measures of spatial statistics calculate the probability of a particular condition to occur within a set location, and provide a level of significance associated with the group of points selected for analysis (Mitchell 2005). Particular spatial statistics that can be applied to data with x,y,z point attributes and measure the degree of clustering or dispersion of points on a group or individual level are the Moran's I correlogram, the average nearest neighbour test and the Getis-Ord test (deSmith *et al.* 2011).

Spatial autocorrelation determines if there is underlying geographic clustering of data based on both the data's location and value. It is measured using the Moran's I formula and should be used when the data has an attribute associated with it that may influence the degree of clustering (Mitchell 2005). On images of BMD, spatial autocorrelation could be used to determine if different values of BMD were arranged in a clustered, random or dispersed manner (Allen 2009; Griffith 2009). The coefficient produced by the calculation, called the Moran's index (I), describes the degree of clustering from +1.0, indicating clustering, to -1.0, indicating dispersion. Within this range, weak clustering (0.25-0.5), moderate clustering (0.5-0.7), strong clustering (0.7-0.9) and a marked degree of clustering (0.9-1) are described numerically (Griffith 2009).

Using spatial statistics, the relationship between individual points and neighbouring points of the same value can be investigated to determine if points of the same value are evenly distributed, or occur in cluster formations (Mitchell 2005). The formula used is the average nearest neighbour test. The location of the points is the only input into the equation: it compares the distance between each individual point to its nearest neighbouring point to determine if the points are closer than the distance that would be expected if all of the points were in a dispersed situation (Allen 2009). On images of BMD, the average nearest neighbour test could be used to determine if units of particular BMD value are evenly dispersed or aggregated compared to each other. In this measure a number is produced to indicate a completely aggregated (0), random (>0, <1) or an evenly (>1) spaced distribution (Clark and Evans 1954).

Preferential clustering of values can also be determined using spatial statistics. The Getis-Ord General G statistic considers the value of points in an area then determines if similar values of high value or of low value are clustering more. This statistic could be used for the analysis of non-invasive images to see what values preferentially cluster in a region of interest. For this statistic a G value is given for the defined area; positive numerical values indicate a dominance of high value clustering, whereas negative numerical values indicate a dominance of low value clustering (Getis and Ord 1992).

It is clear from current research that non-invasive imaging has the potential to reliably produce data that is associated with both structural bone changes in response to exercise, as well as those associated with condylar disease. Detailed quantitative analysis of images is needed so that the images can be reliably compared between patients, as well as for individuals over time. It is also clear that the responses of bone are specific to their given load. The exact changes that occur in the bone of the Mc3/Mt3 epiphysis to specific loads have, as yet, not been quantified; however, longitudinal studies using a group large enough to have statistical power are expensive. Even so, attempts to allow quantitative comparison between non-invasively obtained images would be useful because eventually it would help clinicians to determine if a patient had a bone response typical or atypical for its given exercise history. The recognition of atypical changes could allow the patient to be monitored to see if, or what, condylar disease occurs. If such studies are not conducted, it is likely that we will never know if bone changes, such as the degree sclerosis observed in fractured condyles, are typical of the given exercise level or true atypical pathologic findings.

CHAPTER 2

Quantification of exercise induced bone mineral density response in the distal epiphysis of the equine third metatarsal bone

Abstract

The aim of this study was to describe and compare the regional proportion and spatial distribution of volumetric bone mineral density (BMD_V) in the plantar epiphysis of the third metatarsal bone (Mt3) between trained and untrained Thoroughbreds, and to determine if BMD_V was associated with gross cartilage or subchondral bone (SCB) lesions of the plantar articular surface.

Archived left Mt3 distal epiphyses from 14 Thoroughbred fillies, which had been raised under the same conditions except for either undergoing pre-race training (n = 7) or paddock rest (n = 7) for 13 weeks, were scanned using peripheral quantitative computed tomography (pQCT). The relative proportion of BMD_V in nine threshold categories from 400-1200 mg/cm^3 for six regions of the plantar epiphysis were compared using non-parametric ANOVA, and the spatial distribution of BMD_V in the central condylar regions quantitatively assessed using ArcGIS software.

In the central condylar regions, trained horses had a significantly higher ($P \leq 0.006$) proportion of voxels from 1000 mgcm^3 to ≥1200mgcm^3 and a significantly lower ($P \leq 0.006$) relative proportion of voxels from 500 mgcm^3 to 700 mgcm^3 than control horses. In other regions of the plantar epiphysis differences were for the proportion of voxels ≥1200mgcm^3 ($P \leq 0.006$). The BMD_V differences of the central condylar regions were reflected in the analysis of the entire epiphysis. Multiple correspondence analysis

reflected these findings. In the central condylar regions of all of the trained horses there was strong to marked clustering of BMD voxels >800 mg/cm^3 (I = 0.64 - 1.0, P = 0.01) and no clustering of BMD voxels <600mg/cm^3. In contrast, half of the control horses had weak to strong clustering (I = 0.64 - 1.0, P = 0.01) of BMD voxels >800 mg/cm^3, and weak to moderate clustering of BMD voxels <600mg/cm^3 in the lateral and medial central condylar regions (I = 0.45-0.62, P = 0.01 and I = 0.45-0.57, P = 0.01, respectively).

The responses of BMD_V to exercise are very specific to the threshold categories of BMD_V that change, the anatomic site and how the spatial distribution of BMD_V alters. However, the resulting effects of these changes on the material properties of the bone need to be investigated further through the study of sites that have incurred structural damage.

Introduction

Bone responds to the peak strains incurred during exercise by changing its mineral content and structure (Firth *et al.* 2005; Judex and Zernicke 2000; Rubin and Lanyon 1984b; Smith and Goodship 2008). Specifically, in the distal epiphyses of the third metacarpal bone (Mc3) and third metatarsal bone (Mt3) there is a profound bone mineral density (BMD) increase due to new bone deposition around pre-existing trabeculae of the trabecular bone (Boyde 2003; Boyde and Firth 2005; Firth *et al.* 2005). The BMD response is associated with the load path exerted by the proximal sesamoid bones (PSBs) on the palmar/plantar Mc3/Mt3 epiphysis during the stance phase of gait, and has been found to be more prolific in response to gallop than canter (Firth *et al.* 2005).

Such bone responses that occur during exercise could be a functional adaptation to allow the epiphysis to withstand compressive forces during high speeds. Training at the same intensity of exercise encountered during competition has been found to be important for reducing bone injury in horses (Boston and Nunamaker 2000). Horses that were galloped in the first year of training had a reduced risk of sustaining condylar fracture than horses that were not galloped (Parkin *et al.* 2004a, 2005). However, condylar diseases that are linked to cyclic loading; including osteoarthritis, palmar/plantar osteochondral disease (POD) and condylar fracture, are common in racehorses (Barr *et al.* 2009; Neundorf *et al.* 2010; Verheyen and Wood 2004).

Characteristics associated with excessive cyclic loading, such as exercise programs with long distances at high speeds, have been linked to a higher risk of developing condylar fracture (Verheyen *et al.* 2006). Additionally, these injuries are preceded by microstructural changes, such as microfractures, that occur in the palmar regions of the Mc3/Mt3 epiphysis in regions of high density bone (Burr and Radin 2003; Norrdin *et al.* 1998; Norrdin and Stover 2006; Riggs 1999; Stepnik *et al.* 2004; Whitton *et al.* 2010; Young *et al.* 2007). However, it is currently unknown at what specific point in training such damage occurs, or if the horses that develop condylar disease have abnormal structural changes that precede microdamage.

The majority of studies that have investigated early bone damage have used techniques that are only possible post-mortem on bone sections, such as scanning-electron microscopy (Norrdin *et al.* 1998; Stepnik *et al.* 2004), microradiography (Riggs *et al.* 1999a) and high resolution peripheral quantitative computed tomography (Whitton *et al.* 2010). Studies that have investigated BMD response to exercise using non-invasive imaging have measured BMD changes of the whole epiphysis or in large regions of interest (Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999); while those that have described the spatial distribution of BMD associated with condylar fracture or exercise did so qualitatively (Firth *et al.* 2005; Morgan *et al.* 2006; Tranquille *et al.* 2012). Peripheral quantitative computed tomography (pQCT) allows quantitative data capture of BMD and the images can be exported to image analysis programs for further assessment. Programs commonly used to assess spatial relationships between geographic structures can be used to calculate spatial statistics on pQCT images; therefore, they could provide a detailed, quantitative assessment of the BMD changes that occur in the Mc3/Mt3 epiphysis, and a greater understanding of how the epiphysis responds to exercise. The information could then be used in the future to assess if there are differences between diseased and healthy condyles, or what features within exercise programs have the greatest effect on adaptive BMD changes.

The aim of the current study was to develop a method using non-invasive imaging to quantitatively describe changes that occur in the Mc3/Mt3 distal epiphysis in response to exercise. Specifically, the aim was to describe and compare the regional proportion and spatial distribution of volumetric bone mineral density (BMD_V) values within loaded regions of the plantar Mt3 epiphysis between exercised and non-exercised control 2 year old Thoroughbred horses without condylar disease or limb lameness. Furthermore, the spatial distribution of gross cartilage and subchondral bone (SCB) lesions were investigated to determine if BMD_V was associated with the presence of lesions in the given regions of interest (ROIs).

It was hypothesised that there would be no difference between the exercised and control groups for the BMD_v proportions in all plantar regions of the Mt3 epiphysis, or between the region that contacts the PSBs during the stance phase of gallop and regions that do not. Additionally, it was hypothesised that there would be no difference in the spatial distribution of BMD_v values in loaded regions of the Mt3 epiphysis between exercised and control horses. For the pathology of articular cartilage and SCB, it was hypothesised that there would be no difference in the presence of lesions between the exercised and control horses, and that lesions would not be in regions with a large proportion of high BMD_V.

Materials and Methods

Sample collection

Archived distal left Mt3 bones from fourteen 2 year old Thoroughbred fillies (7 controls and 7 exercised) that had been subjected to a previous controlled exercise trial were available for analysis (Firth *et al.* 2004) The details of the study design including breeding, raising and training of the horses have been described in detail by Firth *et al* (2004). In summary, the dams of the fillies had been managed under the same conditions from conception until parturition and the fillies had been reared under the same conditions until they were selected non-randomly by a trainer to be either exercised by race training (n = 7) or to be yard rested controls (n = 7), as pairs, in grass paddocks 25 x15 m in size. At the onset of race training, the fillies were an average age of 662 days (SD 26 days) and 652 days (SD 34 days), and had mean bodyweights of 391 (SD 13) and 358 (SD 21) kg, for trained and untrained groups, respectively. Training protocols were consistent with programs used to prepare Thoroughbreds for trials prior to spring racing in the Southern Hemisphere and did not differ from the protocols used to prepare the trainer's commercial racehorses at the time (Firth *et al.* 2004) (Martin Johnson, personal communication, December 14 2011). The trained group were exercised 6 days a week for a 13 week period using an exercise protocol consisting of three stages: weeks 1-4 slow canter (mean velocity 7.54 SE 0.05 m/sec), weeks 5-8 medium canter (mean velocity 8.90 SE 0.05 m/sec) and weeks 9-13 fast canter (mean velocity 8.40 SE 0.05 m/sec) with gallops twice a week on Wednesdays and Saturdays (mean velocity 14.62 SE 0.12 m/sec). Training was consistently performed in a counter-clockwise direction on an oval grass or sand track with a short warm up of walk and trot. At the completion of the training phase all horses were euthanised and tissues fixed in 10% formalin for a series of musculoskeletal and histological studies (Firth *et al.* 2005).

Gross pathologic analysis of limbs:

Division of the articular surface

The distal articular surface of each left Mt3 was divided into six plantar ROIs. The regions were bordered on the dorsal margin by the transverse ridge and the plantar margin by the plantar extent of the articular surface. The area was bisected in the sagittal plane using the sagittal ridge, and the medial and lateral sections were divided into three equal sections (Figure 2.1).

Gross examination of articular surface lesions

Each limb was examined grossly by an observer (SHB) blinded to the exercise history of the horses and each ROI scored for lesions using a scoring system modified from Barr *et al* (2009) (Table 2.1).

Image acquisition

Digital photographs of each Mt3 distal articular surface were obtained (Stylus Tough 6020, Olympus Imaging Corp, Centre Valley, PA, USA), at a focal distance of 40cm under controlled illumination, to allow a magnified confirmation of lesions.

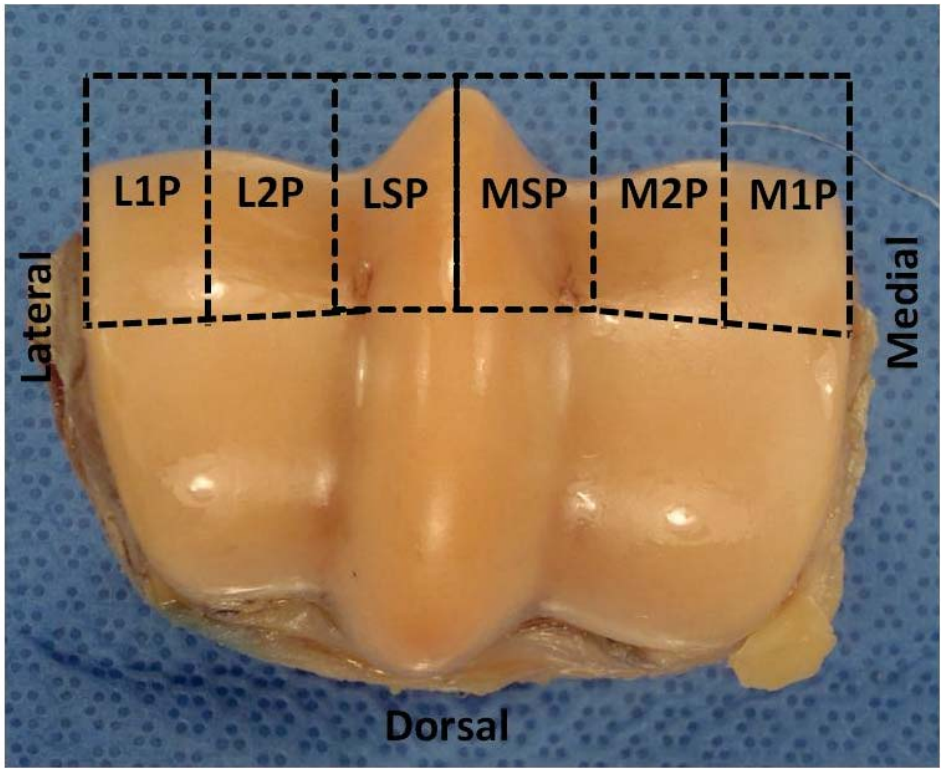


Figure 2.1: ROIs of the plantar articular surface used for pathologic analysis. Three equal divisions were created either side of the sagittal ridge mid-line: L1P, L2P, LSP, MSP, M2P and M1P.

Table 2.1: Adapted scoring system for pathology of the distal Mc3/Mt3 articular surface (Barr *et al.* 2009)

Lesion type	Score	Description
Plantar osteochondral disease	0	No evidence of POD
	1	Discolouration (bruising) of SCB only, no or minimal disruption of overlying articular cartilage.
	2	Discolouration (bruising) with mild to moderate disruption of articular cartilage.
	3	Established POD lesions. Discolouration and disruption/collapse of articular surface.
Wear lines in cartilage	0	Wear lines absent.
	1	Partial thickness wear lines in cartilage.
	2	Full thickness wear lines in cartilage.
Cartilage loss	0	No evidence of cartilage loss.
	1	Partial thickness cartilage loss (fibrillation).
	2	Full thickness cartilage loss (ulceration) with minor exposure of calcified cartilage/SCB.
	3	Extensive full thickness cartilage loss with exposure of the SCB over an area >5 mm in diameter.
Linear fissures (within parasagittal grooves)	0	No evidence of linear fissures.
	1	Faint groove with intact cartilage visible along length.
	2	Well defined groove with partial thickness split in cartilage.
	3	Well defined groove with full-thickness split in cartilage.
Marginal remodelling	0	Marginal remodelling absent.
	1	Marginal remodelling present.

Indian ink Staining

Using a modified method of Cantley et al (1999), Indian ink was applied to each Mt3 articular surface and scored for cartilage excoriation. Briefly, Indian ink was applied to the joint surface and left for 4 minutes then rinsed off with 100ml physiologic saline. Each ROI was scored by a single observer (SHB) and graded on a scale of 1-3 (Table 2.2).

Table 2.2: Modified scoring system for the evaluation of Indian ink uptake (Cantley *et al.* 1999)

Staining score	Description of lesion
0	No evidence of stain uptake by the cartilage
1	A fine line of stain greater than 1 mm long or grey staining up to 2 mm in width
2	More than one strongly stained line or black staining up to 3 mm wide
3	A strong, black stain 3-5 mm wide or more than one black stained area (up to 5 mm in width)

Quantitative computed tomography

Fifteen contiguous cross-sectional pQCT images (2mm slice thickness) were made of the epiphysis of each of the left Mt3 bones (XCT 2000; Stratec Medizin Technic, Pforzheim, Germany). A standard phantom calibration was performed at the start of each group of measurements and the images were made from the most distal point of the sagittal ridge in a proximal direction. All specimens were positioned so that the Mt3 diaphysis was horizontal within the centre of the scanner with the plantar surface upward. The uniformity of slices was ensured by verifying obtained images against a panel of standard images.

Regions of interest

The sixth sequential pQCT image of each condyle was selected, representing a transverse section 10-12 mm from the distal aspect of the sagittal ridge. Each condyle was divided into six plantar ROIs to correlate with the division of the articular surface for pathologic analysis, and to account for the anatomic locations of condylar fractures (Zekas *et al.* 1999a). Each pQCT image was divided with a line running in the sagittal plane from the dorsal to the plantar apex of the sagittal ridge and a line segment bisector perpendicular in the transverse plane. The plantar half created was divided into six equal sagittal divisions, three either side of the sagittal mid-line. The medial and lateral margins for the plantar divisions were the most medial and most lateral points of the plantar epiphysis. The ROIs were outlined using the software for XCT 2000; the ROIs were the entire epiphysis (ALLEPI), the abaxial half of the lateral plantar condylar surface (L1P), the axial half of the lateral plantar condylar surface (L2P), the lateral sagittal ridge and parasagittal groove (LSP), the abaxial half of the medial plantar condylar surface (M1P), the axial half of the medial plantar condylar surface (M2P) and the medial sagittal ridge and parasagittal groove (MSP) (Figure 2.2).

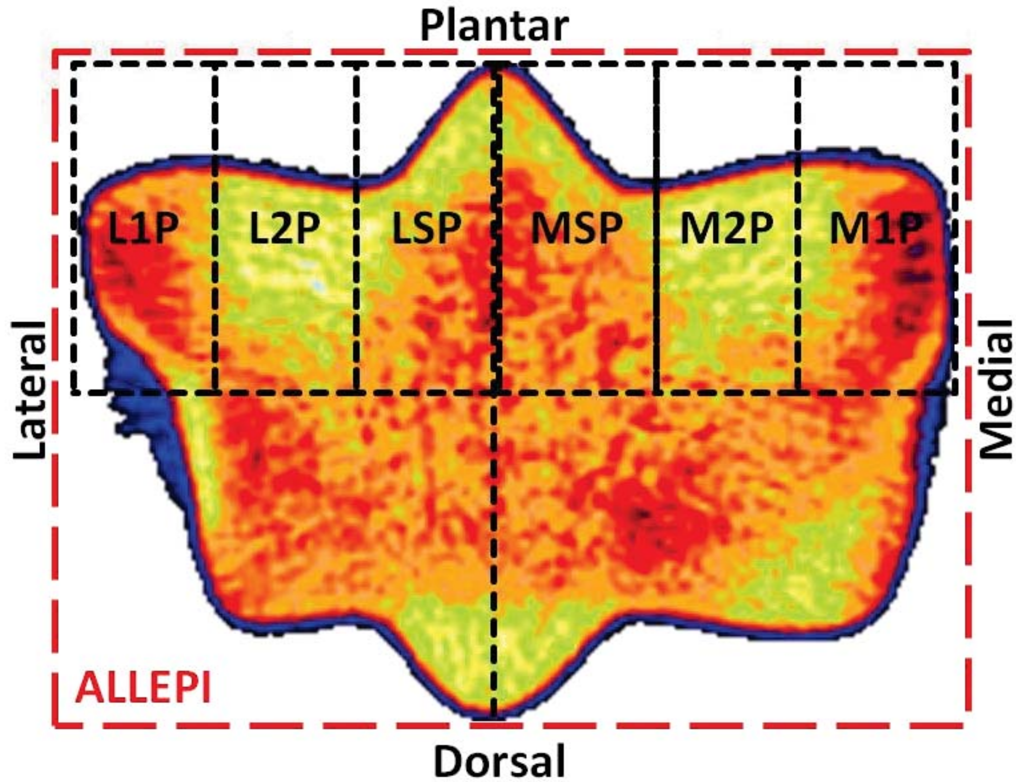


Figure 2.2: ROIs of the plantar epiphysis used for pQCT image analysis. Three equal divisions were created either side of the sagittal ridge mid-line: L1P, L2P, LSP, MSP, M2P and M1P.

pQCT image analysis

For each slice the BMD_V data (mg/cm^3) were extracted for each ROI and the proportion of voxels within each of the following BMD_V thresholds were calculated: 400-499, 500-599, 600-699, 700-799, 800-899, 900-999, 1000-1099, 1100-1199, ≥1200 mg/cm^3. For the calculation of relative BMD_V proportions, the area represented by ≥400 mg/cm^3 was used as the entire bone mass of the ROI.

Statistical analysis of gross lesions and BMD_V

Data were initially structured for analysis and screened for errors in Microsoft Excel (2007). Data without variation were subsequently excluded from statistical analysis. Data for pQCT image analysis and the lesion scores were imported into Stata 8 (StataCorp LP, College Station, TX, USA) for analysis. Multiple correspondence analysis (MCA) was used as an exploratory technique to project the variables of interest (treatment group, BMD_V threshold and/or lesion scores) on a two-dimensional plot using Stata 11.1 (StataCorp LP, College Station, TX, USA). The analyses were adjusted to account for the over-inflation of the inertia values along the diagonal of the matrix and the technique allowed description of how strongly and in which way variables were interrelated (Greenacre 2007).

Analysis of gross lesion scores

Fisher's exact tests were used to analyse the presence of each lesion score in each ROI between exercised and control horses. A Bonferroni correction was applied to adjust for the multiple types of lesions that were analysed. The level for statistical significance was $P \leq 0.02$. MCA was used to examine associations between exercise and the lesions observed by classifying each lesion type as present (1) or absent (0). The associations between gross lesion scores and thresholds of BMD_V in each ROI were examined using

MCA by plotting the lesion score and BMD_V values categorised as above (1) or below (0) the population median for each BMD_V threshold.

Analysis of BMD_V data

The distributions of initial BMD_V data in each ROI were examined using histograms and descriptive statistics. Comparison of exercised versus control horses for the median proportion of voxels within each density threshold were examined using the Kruskall-Wallis non-parametric ANOVA. A Bonferroni correction was applied to adjust for multiple comparisons and the level for statistical significance was $P \leq 0.006$. MCA was performed on the BMD_v data for each ROI by categorising each horse as either above (1) or below (0) the median value within each BMD_V threshold. The categories of BMDv and exercise group were then analysed using MCA.

GIS spatial analysis

pQCT images were imported as ASCII format files into the GIS analysis program ArcMap (ArcGIS 9.3, ESRI, Redlands, CA, USA) for spatial analysis. The imported images were reclassified by pixel value to represent low (<600 mg/cm^3), medium (≥600 <800 mg/cm^3) and high (≥800 mg/cm^3) BMD_V. These BMD_V thresholds were determined using the threshold categories that had shown significant differences between exercised and control horses in the ROIs L2P and M2P on the initial analysis of BMD_V data. The upper threshold was decreased to 800 mg/cm^3 due to an insufficient number of pixels with values >1000 mg/cm^3.

Smaller ROIs were created in ArcMap within the original ROIs L2P and M2P, in order to analyse the plantar Mt3 epiphysis that is close to the point of contact between Mt3 and the PSBs during the stance phase of gallop. The ROI created was [0.2 x length of line from the plantar apex of the sagittal ridge to the dorsal apex of the sagittal ridge]^2 to account for the variation in condylar size between individuals. The plantar border of the ROI extract was aligned with the inner margin of the articular calcified cartilage on the plantar border of the condyle, and centred within the lateral and medial borders of the ROI M2P or L2P (Figure 2.3).

Clustering and spatial relationships for each BMD_V threshold were tested using the Global Moran's I, Average Nearest Neighbour and Getis-Ord General G tests. Statistics could not be generated if there were few or no pixels within the threshold being analysed, and results with a level of statistical significance below $P \geq 0.01$ were excluded from interpretation. Additionally, a polygon was drawn around the outer margin of high BMD_V pixels and around the margin of low BMD_V pixels within each ROI. The spatial relationship between low and high BMD_V clusters was recorded as: low BMD_V cluster outside the margins of the high BMD_V cluster (1) or low BMD_V cluster inside the margins of the high BMD_V cluster (0).

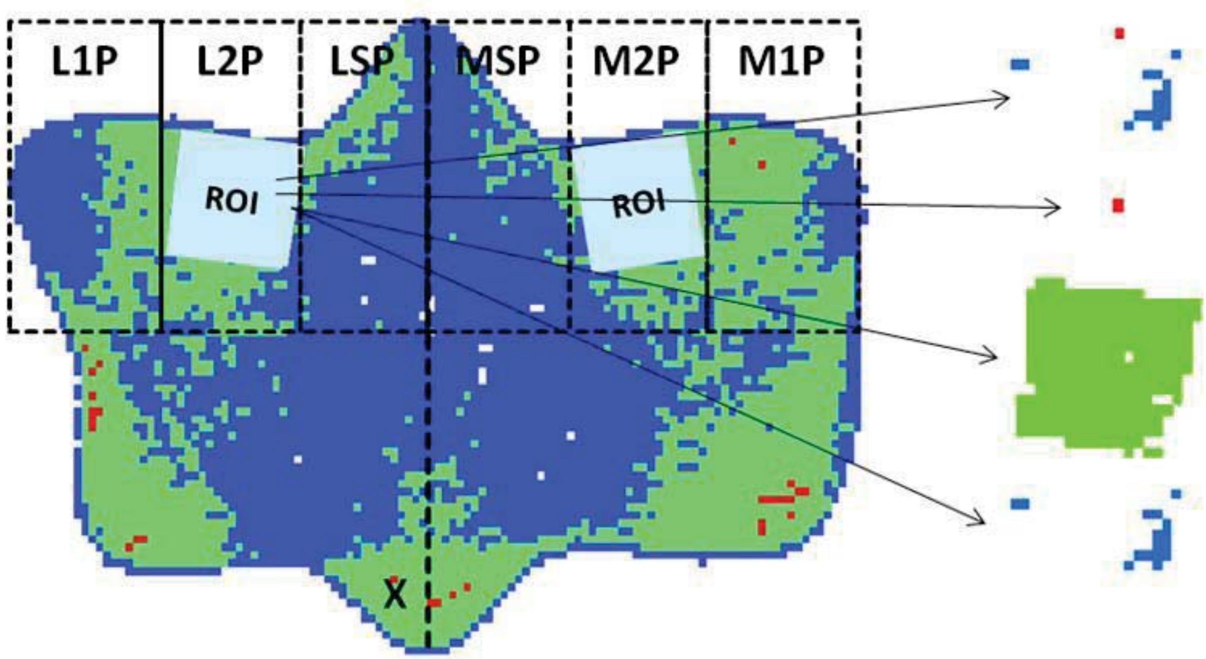


Figure 2.3: Reclassification of pQCT images and the ROIs for used for spatial analysis of BMD_V in ArcMap. Voxels were reclassified into low BMDv <600 mg/cm^3 ( ), medium BMDv 600-800 mg/cm^3 ( ) and high BMDv >800 mg/cm^3 ( ) thresholds. A square ROI was constructed using 0.2 x the length of the line X and pixels for each density were extracted for spatial analysis (right).

Results

The effect of training on gross lesion score

There was no evidence of POD, wear lines in the cartilage or marginal remodelling for any of the horses studied. All horses had both medial and lateral linear fissures in the parasagittal grooves. There were varying degrees of mild cartilage loss associated with the central condylar regions L2P and M2P; there were significantly more control horses with cartilage loss in M2P ($P = 0.005$). There were no other significant differences in lesions between exercised and control horses.

The effect of training on distribution of BMD_V within different ROIs

There were significant differences in the median proportion of voxels between exercised and control horses in all of the ROIs studied except MSP. In the ROIs L2P and M2P, exercised horses had a significantly higher relative proportion of high BMD_V (categories from 1000-1099 to ≥1200 mg/cm^3), and a significantly lower relative proportion of low BMD_V values (categories 500-599 and 600-699 mg/cm^3), compared to control horses. In the ROI ALLEPI, exercised horses also had a significantly higher relative proportion of high BMD_V (categories from 1000-1099 to ≥1200mg/cm^3) and a lower relative proportion of low BMD_V (category 500-599mg/cm^3). In contrast, for the ROIs L1P and M1P, exercised horses only had a significantly higher relative proportion of BMD_V than controls in the category ≥1200mg/cm^3. For all ROIs there were no significant differences between exercised and control horses in the middle BMD_V categories between 700 and 999 mg/cm^3.

The distributions of the BMD_V values on histograms showed similarities between contralateral ROIs (L2P and M2P, L1P and M1P, LSP and MSP) and dissimilarity between the distributions of BMD_V in ipsilateral ROIs (L1P, L2P and LSP, M1P, M2P and MSP). The distributions of the sagittal ridge ROIs had an overall right shift for the exercised group, but no shifts in distribution were observed for the condylar ROIs (Figure 2.4).

Multiple correspondence analysis

In the ROIs L1P and M1P, dimension one explained 79% and 69% of the variance, respectively. In the ROIs L2P, LSP and M2P, 92%, 96% and 90% of the variance was explained by the dimension one. For the ROI L2P, the dimension one was categorised by the treatment group and all BMD_V categories except for 400, 800 and 900 BMD_V categories. I n L2P, training was clustered with greater than the median 1200 BMD_V category and below the median 600 BMD_V category (Figure 2.5). For the ROI M2P, training was clustered with greater than the median 1200 BMDv category and below the median 700 BMD_V category (Figure 2.5). In both ROIs L2P and M2P, the trained group differed from the average profile in an extreme opposite position compared to the control group.

Spatial analysis using ArcGIS

Moran's I test:

All of the exercised horses showed a strong to marked clustering of high BMD_V in the L2P ROI (I = 0.64 - 1.0, P = 0.01); however, only three of the control horses showed clustering that was weak to strong (I = 0.39 - 0.89, P = 0.01). The clustering of high BMD_V in M2P was similar with five of the exercised horses showing a moderate to marked clustering (I = 0.64-0.94, P = 0.01) but no significant clustering in the control group.

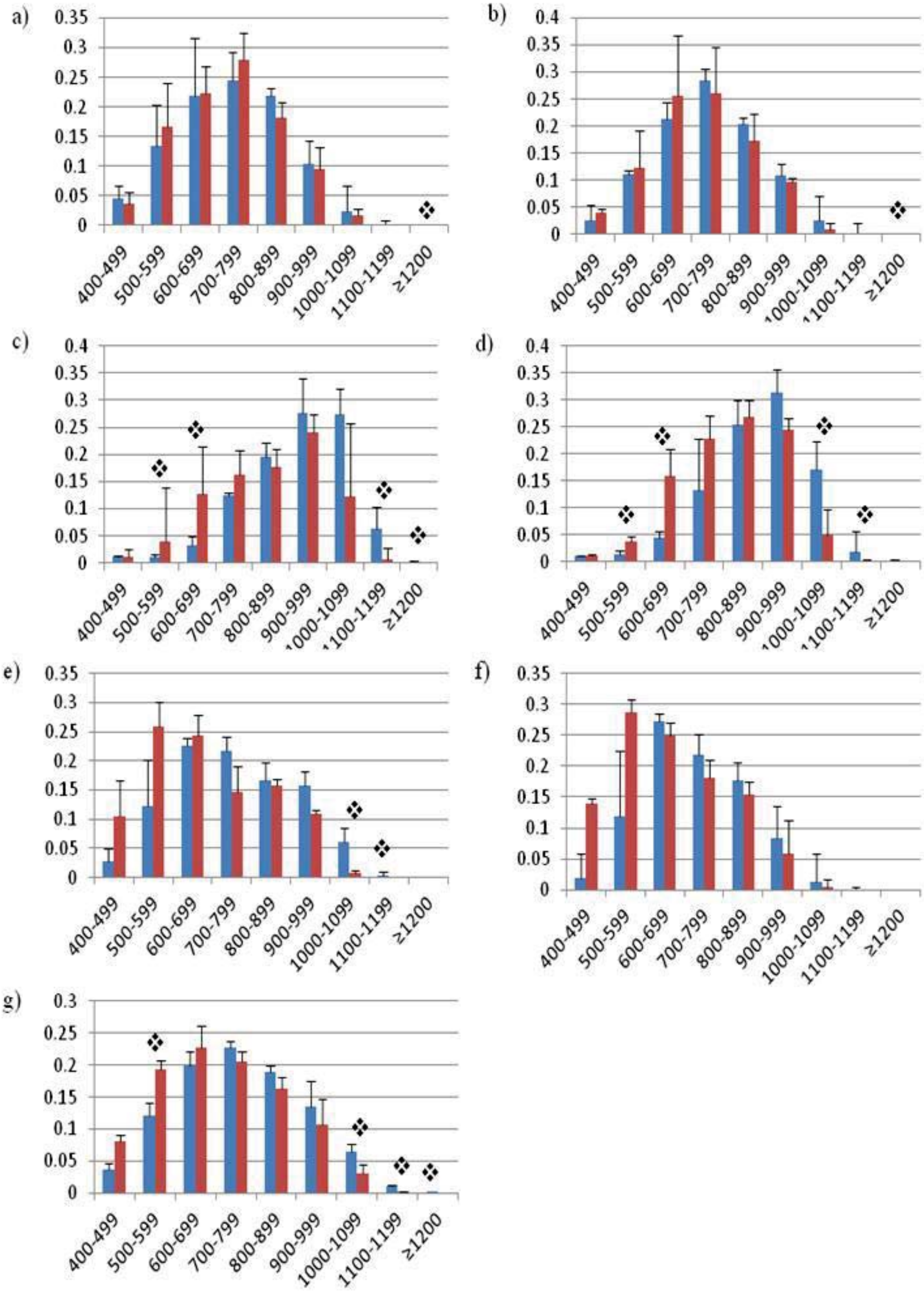


Figure 2.4: The median relative proportion of voxels (y) for BMD_V thresholds (mg/cm^3) (x) in ROIs of the plantar distal epiphysis for exercised (■) and control (■) groups. Error bars represent the upper quartile range of the related bone density threshold. Significant results are represented by (❖) at P = 0.006. (a) L1P, (b) M1P, (c) L2P, (d) M2P, (e) LSP, (f) MSP and (g) ALLEPI.

a) b)

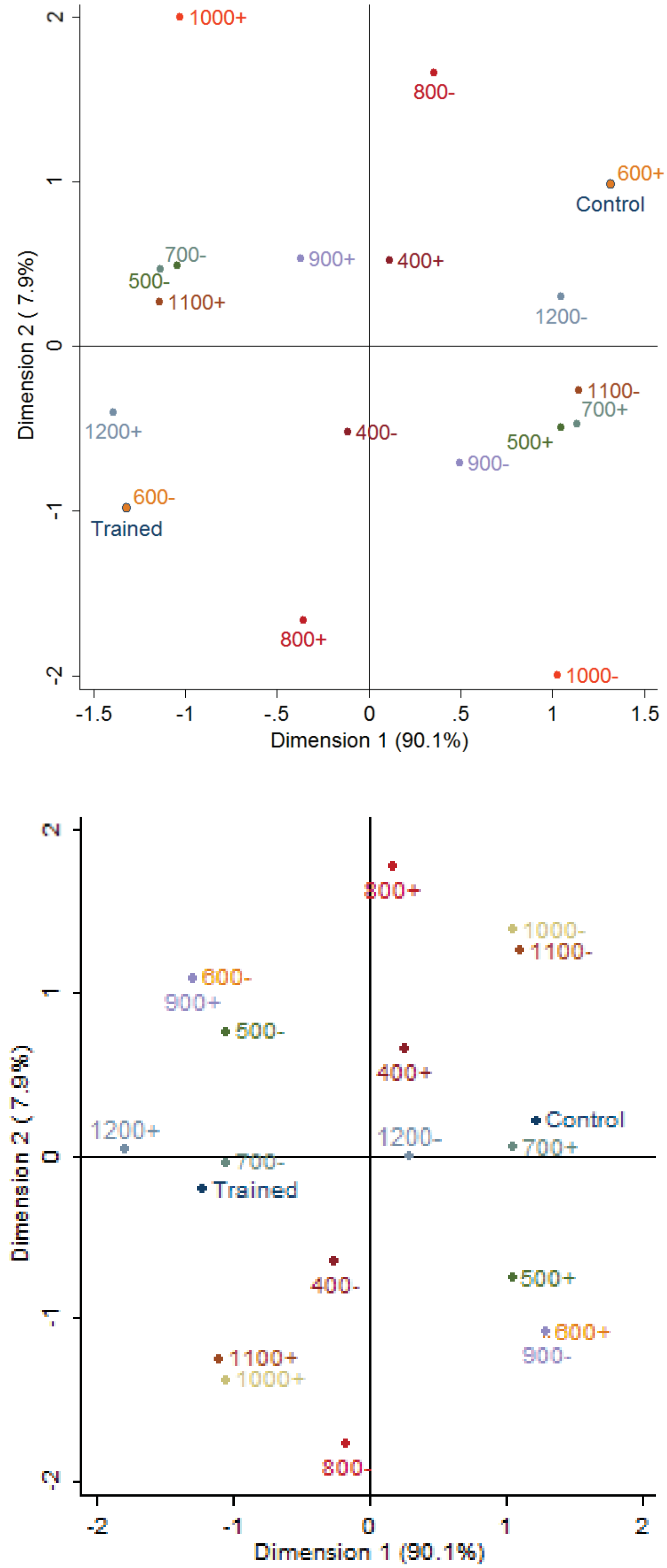


Figure 2.5: MCA plots for BMD_V thresholds and the exercised variable for the ROIs L2P (a) and M2P (b). Above (+) and below (-) median values for each BMD threshold are shown (number represents the start value of the threshold).

Discrepancies were also observed between the exercised and control group in the analysis of low BMD_V. There were no significant measures of clustering in the exercised group, but in the control group there were two significant results in the L2P ROI and three significant results in M2P that both showed weak to moderate clustering (I = 0.45-0.62, P = 0.01 and I = 0.45-0.57, P = 0.01 respectively).

Medium BMD_V had uniform clustering for exercised and control horses. All horses had significant strong to marked clustering of medium BMD_V in L2P ($I_{Exercise}$ = 0.96-1.02, $I_{Control}$ = 0.85-0.99, P = 0.01) and in M2P ($I_{Exercise}$ = 0.89-0.97, $I_{Control}$ = 0.88-0.95, P = 0.01).

Average nearest neighbour test:

Significant results for the nearest neighbour test were all >1, indicating an evenly spaced distribution of pixels in each BMD_V threshold cluster.

Getis-Ord General G and arrangement of high and low BMD_V clusters

Clusters of high BMD_V dominated over clusters of low BMD_V for both exercised and controlled horses in both ROIs. Only a small number of horses had significant test values due to either absent or minimal pixels in the high (control) or low (exercised) BMD_V thresholds.

Horses in both groups that had both low and high BMD_V pixels present in either the M2P or L2P ROIs did not have low value clusters positioned within high density value clusters.

Discussion

This study aimed to develop a method to quantitatively describe changes detected by pQCT that occur in the bone of the Mc3/Mt3 distal epiphysis in response to exercise, as well as to determine if the spatial distribution of BMD_V was associated with lesions of the articular cartilage and SCB.

This study utilised specimens from a population of horses that had been reared and exercised in a controlled manner for a previous study. The two major limitations due to using specimens from this group were the small sample size and that the left Mt3 condyles were the only specimens available for analysis. However, having detailed exercise, health and management records was important to the integrity of the study, and the response of the left Mt3 epiphyseal bone to exercise is likely to be reflective of the response of other Mt3 and Mc3 epiphyseal bones.

The quantitative results obtained in this study reflect qualitative results from a previous study using Mc3 bones of the same group of horses (Firth *et al.* 2005). Additionally, both fracture and SCB lesions also occur in the hind limbs (Barr *et al.* 2009; Bassage and Richardson 1998; Ellis 1994; Parkin *et al.* 2006; Rick *et al.* 1983; Zekas *et al.* 1999a), and there is evidence that there is no difference between the occurrence of fracture between the left and right sides (Bassage and Richardson 1998; Parkin *et al.* 2006; Zekas *et al.* 1999a). These findings suggest that Mc3 and Mt3 bones incur similar stresses during exercise and respond similarly to load, even though the magnitude of the load may differ between front and hind or left and right limbs.

Another limitation of the study population was that all horses were young and were given initial racing preparation over a short period of time. The age of the horses could have affected the observed BMD_V response to exercise; however, the intensity level of the exercise is likely to be a stronger determinant of the observed BMD_V response. In humans, it has been found that the BMD response is greatest at the specific maturity level that occurs in the two years around puberty (Bailey *et al.* 1999b). Therefore the BMD_V responses observed in this study could be partially due to the age of the subjects, and not be applicable to horses at other ages. Currently the specific age of accelerated BMD increase in response to exercise for trabecular bone of the Mc3/Mt3 epiphysis is unknown; however, a number of studies suggest that the intensity level of exercise is likely to be a strong driver of BMD rather than age. Moderate exercise in horses from 4 days to 17 months of age had no effect on BMD_V in the distal Mc3 epiphysis (Firth *et al.* 2011b), whereas significant differences in BMD have been observed with higher intensity exercise in studies using horses from birth to 2 years of age (Cornelissen *et al.* 1999; Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). In the current study, the exercise intensity level was sufficient to produce a significant difference between exercised and non-exercised horses, and the response is likely to be reflective of horses at different maturity levels; however, studies of BMD_V response at different maturity levels are needed to validate this assumption.

To the author's knowledge, the use of ArcMap for the spatial analysis of BMD distribution within the Mt3 epiphysis is novel; however, it did have limitations when used for this purpose. The exact placement of the ROI when calculating spatial statistics was a source of error. This is because the spatial distribution of increased BMD_V can vary over a small distance; therefore, any differences in placing the ROI, or in condylar size, can result in random error. The study design attempted to mitigate this source of error by placing the ROIs in ArcMap in a defined position and having the ROI size proportional to the size of the overall CT image.

Additionally, the study design could have resulted in a decreased sensitivity to spatial distribution because only three ranges of BMD_V were used for the analysis, which could mask subtle changes in BMD_V spatial distribution. However, analysing only these three thresholds of BMD_V was considered an adequate indicator of the change in spatial distribution because the ranges were defined by values that had shown significant differences in the relative proportion of voxels between exercised and control limbs in response to exercise on the initial BMD_V analysis.

The results of the current study suggest that bone responds to exercise specifically by changing the relative proportion of BMD_V at low and high values, rather than changing uniformly over all BMD_V values. In all ROI's there were no significant changes in the mid-range BMD_V categories; however, the majority of ROI's showed significant changes at high BMD_V values or at high and low BMD_V values. Highly loaded regions of the Mt3 epiphysis changed at both high and low BMD_V thresholds. Regions of low density bone in the Mt3 epiphysis represent the porosity formed by marrow cavities between trabeculae (Boyde 2003).

In trained horses it has been observed microscopically that "fronds" of cellular, mineralised bone that act as a scaffold for the deposition of lamellar bone, form around existing trabeculae during exercise; therefore, decreasing the size of the marrow cavities (Boyde and Firth 2005). This could explain why the low density thresholds that represented marrow cavities decreased. The high density thresholds could be representative of the newly deposited bone and therefore explain why high density thresholds increased with exercise. The medium density thresholds that did not change significantly may be representative of the pre-existing trabeculae. Increasing high density bone in response to exercise could act to reinforce the parts of the epiphysis that are under focal loads, while reducing the porosity of the marrow cavities could increase the global stiffness of the epiphysis.

Alterations of BMD_V in response to exercise occurred due to 13 weeks of moderate to high intensity pre-race training, suggesting that the response is a fast, adaptive response to exercise. The response is likely to occur because it could be faster than osteoclastic remodelling of the region and could be more energy efficient. Although the average BMD_V level of equine trabecular bone increases with higher intensity exercise and remains unchanged if the exercise intensity remains the same (Firth *et al.* 1999a; Firth *et al.* 1999b; Firth *et al.* 2005); it is unknown how the threshold categories and spatial distribution of BMD_V would respond if the same intensity level of exercise was continued over a longer period of time, or if the intensity of exercise was increased further. Perhaps there would be changes to the medium density thresholds in response to longer term exercise through osteoclastic remodelling of the lamellar bone of the trabeculae. Alternatively, the architectural structure of the trabeculae may change in an adaption to longer term exercise. For these reasons, longitudinal studies are needed that investigate not only BMD response, but also the architectural changes that occur in the Mc3/Mt3 epiphysis over time.

The current study found that the BMD_V response to exercise was very site specific. The finding that the central condylar regions respond the most to exercise is consistent with the site specificity described in other studies (Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). However, the current study adds an understanding as to the specific nature of BMD_V responses in different regions of the distal Mt3/Mc3 epiphysis, which could have implications on the material properties of the epiphysis.

Currently, the material properties at points across the Mc3/Mt3 epiphysis have not been compared; however, sagittal ridge BMD_V increases uniformly to cause an overall shift to higher BMD_V values in comparison to the central condylar regions, which increases BMD_V within specific thresholds instead of an overall shift. This could result in stiffer sagittal ridge bone and more compliant central condylar bone. A degree of compliancy could enable the central condylar regions to withstand the high compressive load from the PSBs incurred during motion, and avoid the region from becoming too stiff. An adaptation to maintain a degree of compliancy was suggested from microscopic observations that found a lower mineralisation of newly deposited bone in central condylar regions in response to exercise; potentially increasing the elasticity of the tissue (Boyde and Firth 2005). In contrast, very high levels of ash density have been found to cause bone to become brittle and deform easily (Les *et al.* 2002), which would not be appropriate for the central condylar regions of the epiphysis. Furthermore, discrepancy between the material properties of adjacent bone regions could support the proposal that a gradient in elastic modulus exists between the central condylar region and the sagittal ridge, causing a concentration of shear force, and could contribute to the formation of condylar fractures (Riggs and Boyde 1999). Site specific changes to the material properties of Mt3/Mc3 epiphyseal bone that occur due to exercise need to be analysed further to understand the reasons why there is a site specific response of the bone to exercise and the impact that the alterations have on structural integrity.

This study was the first to quantitatively investigate the alterations in the spatial distribution of BMDv in response to exercise. In comparison, earlier studies have described alterations in the spatial distribution qualitatively (Cornelissen *et al.* 1999; Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). The central condylar regions were investigated due to the significant specific changes that were observed to occur in the initial analysis of BMD_V relative proportion. It was found that high density bone clustered strongly in a uniform manner when it increased with exercise, whereas low density bone was clustered weakly in a uniform manner in control horses. In contrast, the spatial distribution of medium BMD_V was similar between exercised and control horses. These findings support the reasoning for the specific changes in BMD_V threshold categories that occur with exercise discussed previously; the medium BMD_V may represent the structural trabecular bone that does not change in response to exercise, whereas the high BMD_V is deposited around it, filling in areas of low BMD_V.

Clustering of high value BMD_V in the central condylar regions of the epiphysis that contact the PSBs and incur the highest strains during loading, is likely to be related to a cellular response, as osteocytes are activated by strains in bone tissue (Skerry *et al.* 1989) and have been found to communicate with osteoblasts that form new bone (Doty 1981; Taylor *et al.* 2007; Vatsa *et al.* 2007). The strong clustering of high density bone could be important to maintain strength in the central condylar region because if areas of high density bone were not clustered, there could be a sharp gradient of elastic modulus that could lead to weakness. Such is the case in palmar osteochondral disease where areas of radiodense and radiolucent bone have been found to occur at adjacent sites (Norrdin and Stover 2006). Clusters of low density bone were absent in the exercised horses and even in the control horses these were only weakly clustered. Tight clustering of low density bone would cause areas of weakness that could cause damage to the SCB or entire Mt3 structure. As these horses were exercised in a moderate manner without sustaining injury, it can be proposed that alterations of the spatial distribution of BMD_V described in this study are designed to maintain the structural integrity of the Mt3 epiphysis under loading conditions that are not excessive.

In this study there was no association between BMD_V and articular cartilage or SCB lesions, which is consistent with other findings (Drum *et al.* 2007; Young *et al.* 2007). The only significant pathological finding was that exercise was associated with less cartilage fibrillation, which may be due to the protective effect that exercise has on the cartilage matrix. However, due to the low numbers used in this study the result could be due to chance.

In conclusion, the response of BMD_V to exercise is very specific to threshold categories of BMD_V, the exact anatomical site across the plantar articular surface, and also in the spatial distribution of the BMD_V response. It is clear that the BMD response is targeted so as to have a particular effect on the material properties in the areas that are loaded during exercise. The resulting material properties that the BMD_V alterations induce in the bone tissue and how they alter the interaction between adjacent sites of bone need to be investigated further by investigating sites that have already incurred structural damage.

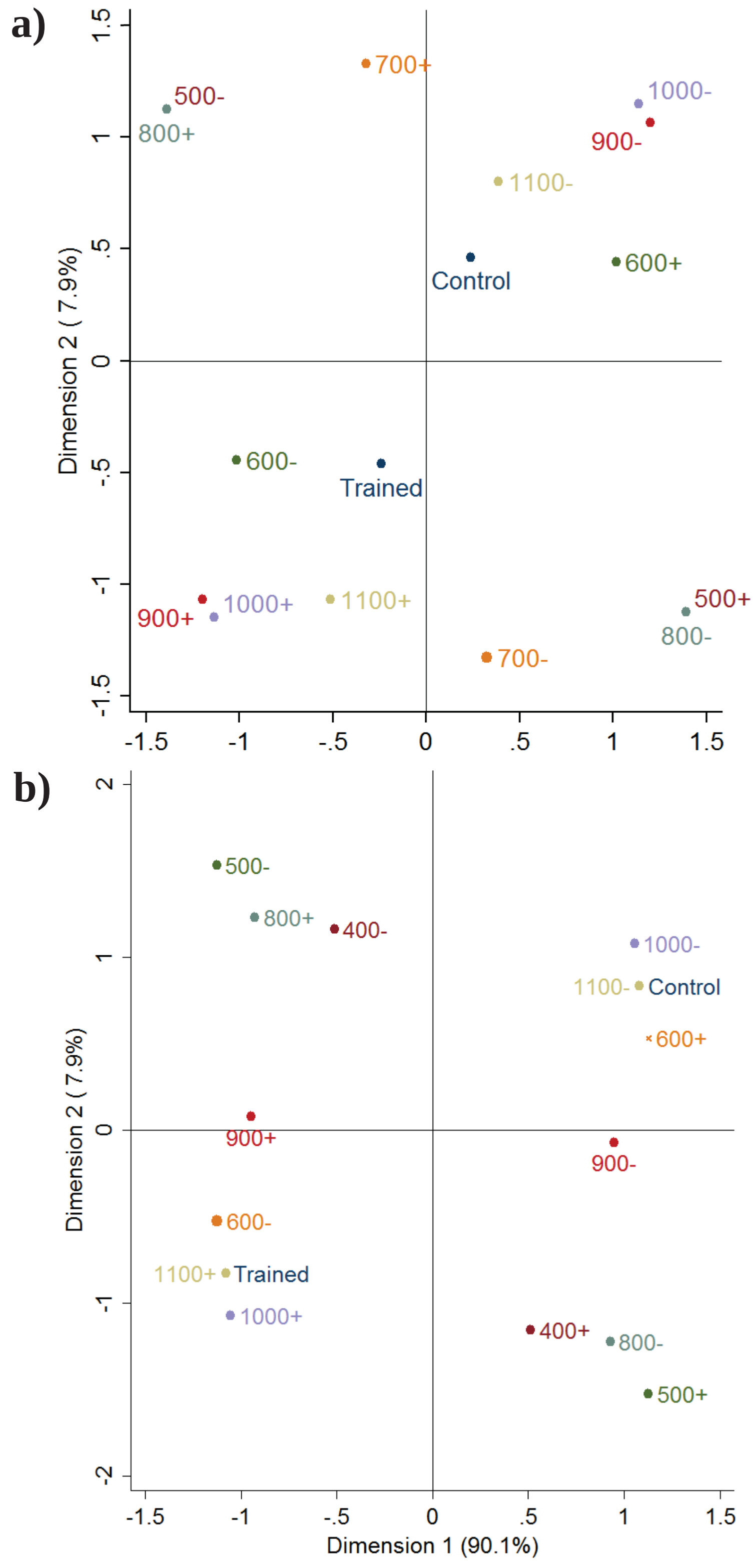
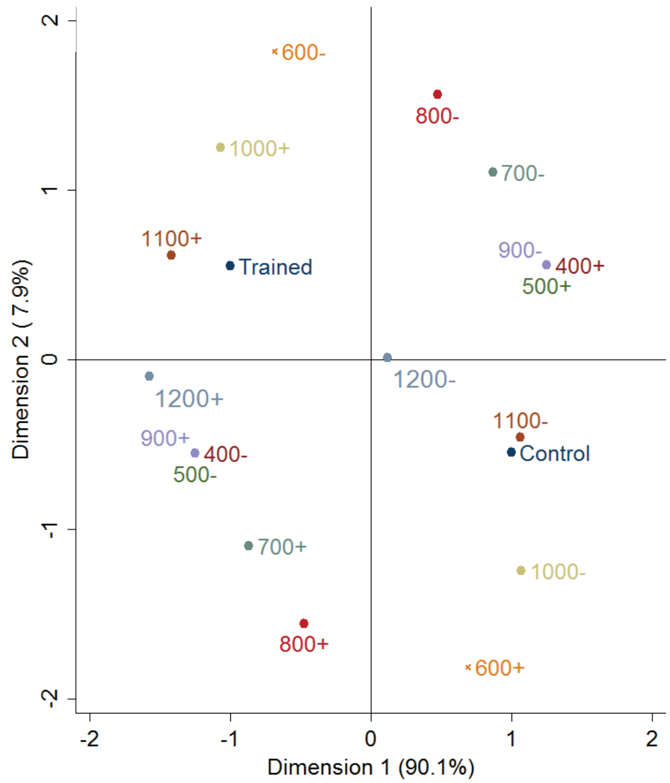


Figure A1.1: MCA plots for BMD_V thresholds and the exercised variable for the ROIs L1P (a) and M1P (b). Above (+) and below (-) median values for each bone density threshold are shown (number represents the start value of the threshold).

a)



b)

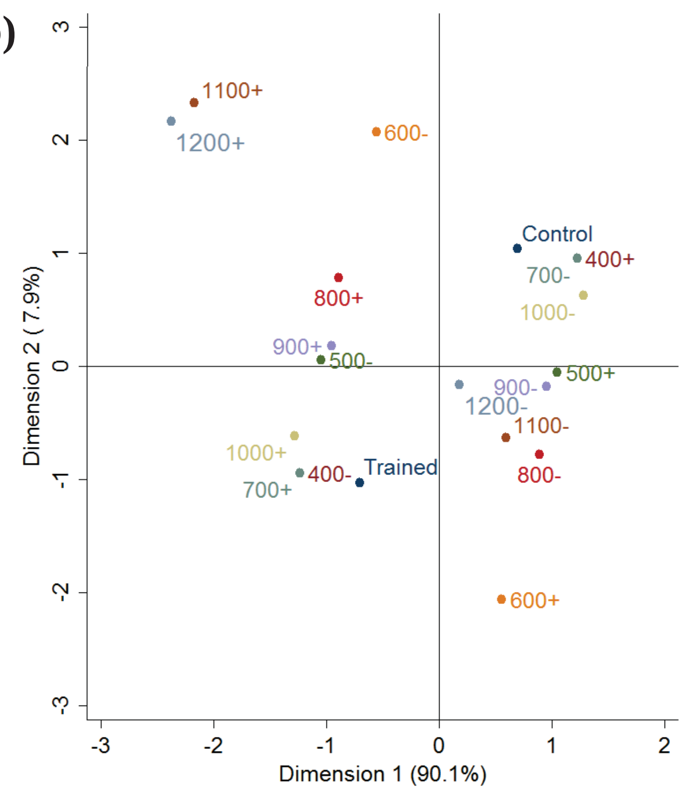


Figure A1.2: MCA plots for BMD_V thresholds and the exercised variable for the ROIs LSP (a) and MSP (b). Above (+) and below (-) median values for each bone density threshold are shown (number represents the start value of the threshold).

CHAPTER 3

Quantitative comparison of the bone mineral density characteristics of third metacarpal distal epiphyses with and without condylar fracture

Abstract

The aim of this study was to compare the regional proportion and spatial distribution of volumetric bone mineral density (BMD_V) of the palmar region of the third metacarpal (Mc3) distal epiphysis between limbs from Thoroughbred racehorses with condylar fracture and non-fractured limbs.

The distal epiphysis of cadaver Mc3 bones from six limbs with condylar fracture and eight non-fractured control limbs were scanned using peripheral quantitative computed tomography (pQCT) and the BMD_V levels and spatial distributions quantitatively assessed using conventional and ArcGIS software. The relative proportion of voxels within nine threshold categories of BMD_V, as well as spatial statistics of the BMD_V distribution were compared between the fractured and non-fractured groups and associations between the BMD_V threshold categories and the presence of fracture were investigated.

There were no significant differences between the fractured and control groups for sex (P = 0.6), age (P = 0.7), total starts (P = 0.5) or leg side (P = 0.7). There were no differences between the groups for the BMD_V characteristics investigated, although a non-significant trend toward the fractured group having lower BMD_V values in the thresholds 700-799 mg/cm^3 (P = 0.02) and 800-899 mg/cm^3 (P = 0/007) but higher BMD_V in the threshold 1000-1099 mg/cm^3 (P = 0.01) were found for the central

condylar region of the lateral condyle. There were also no relationships between the fractured condyles and BMD_V thresholds on multiple correspondence analysis, although above median high BMD_V thresholds were clustered with below median low BMD_V thresholds. There were no significant differences in the spatial distribution of BMD_V between fractured and control groups.

There were no significant differences in the BMD_V characteristics of the distal Mc3 epiphysis between limbs with and without condylar fracture. The exercise exposure explained most of the BMD_V response in the epiphyses. A holistic approach to relate the specific features of exercise programs with specific changes in bone morphology and the occurrence of fracture is necessary to begin to understand why catastrophic injuries occur.

Introduction

Catastrophic injuries of the third metacarpal bone (Mc3) and the suspensory apparatus are the most common cause of fatality in Thoroughbred racehorses (Hernandez and Hawkins 2001; Johnson *et al.* 1994) and just over a quarter of all fractures sustained during racing or training are Mc3 or third metatarsal bone (Mt3) fractures (Verheyen and Wood 2004). For horses that are not euthanised as a result of condylar fracture, impaired performance causes economic loss; 64% of horses that returned to racing had decreased total earnings post-fracture (Zekas *et al.* 1999b).

Condylar fractures most commonly involve the lateral condyle of Mc3/Mt3; however, they can involve the medial condyle (Bassage and Richardson 1998; Ellis 1994; Parkin *et al.* 2006; Rick *et al.* 1983; Riggs *et al.* 1999a; Zekas *et al.* 1999a). A large proportion of fractures have been observed to occur in proximity to the parasagittal grooves, with distances from the sagittal ridge midline found to be 11 mm (Parkin *et al.* 2006) to 12-

15 mm (Rick *et al.* 1983). It has been proposed that condylar fractures represent a disease process in which cyclic loading causes incremental structural bone changes that ultimately lead to catastrophic failure of the tissue (Riggs 2002; Stover 2003). It is suggested that condylar fractures are an example of a stress induced overload injury as they occur in specific locations, have a specific morphology and have been associated with prior pathologic changes (Parkin *et al.* 2006; Radtke *et al.* 2003; Stover and Murray 2008; Zekas *et al.* 1999a).

Although theories on the pathogenesis of condylar fracture have varied over the years, it is now thought that condylar fractures are a result of underlying microstructural changes in the bone of the palmar/plantar Mc3/Mt3 epiphysis (Riggs 1999; Stepnik *et al.* 2004; Whitton *et al.* 2010). Such changes could be induced by excessive cyclic loading during long distance training (Verheyen *et al.* 2006), or if the bone has not previously been exposed to maximal loading conditions (Parkin *et al.* 2004a, 2005).

The Mc3 epiphysis has been found to respond dynamically to exercise with significant increases in the volumetric bone mineral density (BMD_V) of trabecular bone (37%), in contrast to an observed 1.6% increase in diaphyseal cortical bone BMD_V (Firth *et al.* 2005). Such changes are likely to be an adaptive response of the bone to withstand the forces encountered during exercise. However, areas of very high mineral content have been demonstrated to be brittle (Les *et al.* 2002) and fracture lines have been found to pass through areas of very dense bone (Whitton *et al.* 2010). Additionally, abnormal microscopic features such as microcracking, microfracture and evidence of osteoclastic remodelling have also been found in dense regions of the palmar Mc3 epiphysis (Norrdin *et al.* 1998; Norrdin and Stover 2006).

Peripheral quantitative computed tomography (pQCT), and the use of spatial statistics determined by geographic information systems (GIS) software, enabled a detailed quantitative description of the BMD_V responses to exercise in young Thoroughbred horses in study one. It was demonstrated that the BMD_V increase of the Mc3/Mt3 epiphysis in response to exercise is specific in relation to anatomic site, BMD_V values and spatial distribution. However, it is unknown if the BMD_V characteristics differ between horses that sustain condylar fracture and those that do not. Therefore, the aim of the current study was to compare the regional proportion and spatial distribution of BMD_V within loaded regions of the palmar Mc3 epiphysis between fractured and non-fractured control Thoroughbred racehorses.

It was hypothesised that there would be no difference in BMD_V proportions between the fractured and control groups in all palmar regions of the Mc3 epiphysis. Additionally, it was hypothesised that there would be no difference in the spatial distribution of BMD_V between fractured and control horses in palmar regions of the Mc3 epiphysis.

Materials and Methods

Sample collection

Archived limbs with condylar fracture (n = 6) and controls without condylar fracture of Mc3 (n = 8) were available for analysis. In the fractured group, all horses had been euthanised within 24 hours of sustaining condylar fractures and all limbs had been frozen within 24 hours after euthanasia. Six fractured limbs were obtained from five horses and radiographs confirmed five lateral displaced fractures and one medial non-displaced condylar fracture with all fractures originating in proximity to the respective parasagittal groove. Non-fractured contralateral limbs were available for two of the fractured horses and six other control limbs were chosen matched on age, sex, limb and the number of starts in the fractured limb group. The populations were compared using

Fisher's exact test and Kruskall-Wallis non-parametric ANOVA for age, sex, limb side and number of starts. The metacarpophalangeal joints of the control horses were disarticulated and each joint examined (SHB) to confirm that there were no subchondral bone lesions of the distal Mc3 articular surface (Barr *et al.* 2009).

Quantitative computed tomography

Twenty contiguous cross-sectional pQCT images (2mm slice thickness) were made of the epiphysis of each of the Mc3 bones using pQCT (XCT 2000; Stratec Medinzin Technic, Pforzheim, Germany). A standard phantom calibration was performed at the start of each group of measurements. The scan started at the most distal aspect of the sagittal ridge in a proximal direction. All specimens were positioned so that the Mc3 diaphysis was horizontal within the centre of the scanner with the palmar surface upward. The uniformity of slices was ensured by verifying obtained images against a panel of standard images.

Regions of interest

A panel of standard pQCT images was used to select the sixth pQCT image proximally from the distal aspect of the sagittal ridge, which represented a transverse section 10-12 mm from the distal point of the sagittal ridge. Each CT image was divided into six palmar regions of interest (ROIs) to account for the contact area of the PSBs on Mc3 and for the anatomic locations of condylar fractures (Zekas *et al.* 1999a). Briefly, each CT image was divided with a line running in the sagittal plane from the dorsal to the palmar apex of the sagittal ridge and a perpendicular bisecting line in the transverse plane. The palmar half created was divided into six equal sagittal divisions, three either side of the sagittal mid-line. The medial and lateral margins for the palmar divisions were the most medial and most lateral points of the palmar epiphysis. The resulting

ROIs were; the entire epiphysis (ALLEPI), the abaxial half of the lateral palmar condylar surface (L1P), the axial half of the lateral palmar condylar surface (L2P), the lateral sagittal ridge and parasagittal groove (LSP), the abaxial half of the medial palmar condylar surface (M1P), the axial half of the medial palmar condylar surface (M2P) and the medial sagittal ridge and parasagittal groove (MSP) (Figure 3.1).

pQCT image analysis

For each condyle, the BMD_V values (mg/cm^3) were extracted for each ROI and the proportion of voxels within each of the following BMD_V thresholds calculated: 400-499, 500-599, 600-699, 700-799, 800-899, 900-999, 1000-1099, 1100-1199, ≥1200 mg/cm^3. For the calculation of relative BMD_V proportions, the area represented by ≥400 mg/cm^3 was used as the entire bone mass of the ROI.

Statistical analysis of BMD_V

Data were initially structured for analysis and screened for errors in Microsoft Excel (2007). Data without variation were subsequently excluded from statistical analysis. Data for pQCT image analysis were imported into Stata 8 (StataCorp LP, College Station, TX, USA) for analysis. Multiple correspondence analysis (MCA) was used as an exploratory technique to project the variables of interest (treatment group and BMD_V threshold) on a two-dimensional plot using Stata 11.1 (StataCorp LP, College Station, TX, USA). The analyses were adjusted to account for the over-inflation of the inertia values along the diagonal of the matrix and the technique allowed description of how strongly and in which way variables were interrelated (Greenacre 2007).

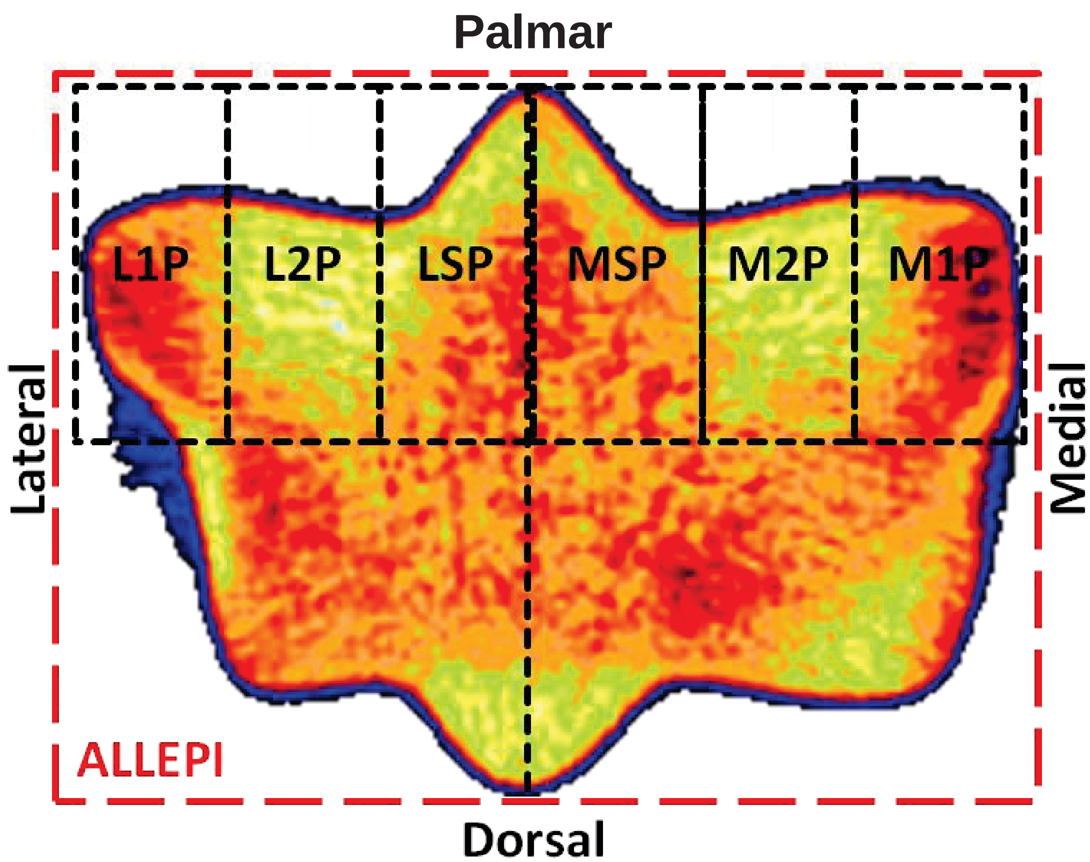


Figure 3.1: ROIs of the palmar epiphysis used for pQCT image analysis. Three equal divisions were created either side of the sagittal ridge mid-line: L1P, L2P, LSP, MSP, M2P and M1P.

Analysis of BMD_V data

The distributions of initial BMD_V data in each ROI were examined using histograms and descriptive statistics. Comparison of fractured versus control horses for the median proportion of voxels within each density threshold were examined using the Kruskall-Wallis test non-parametric ANOVA. A Bonferroni correction was applied to adjust for multiple comparisons and the level for statistical significance was $P \leq 0.006$. MCA was performed on the BMD_V data for each ROI by categorising each horse as either above (1) or below (0) the median value within each BMD_V threshold. The categories of BMD_V and fracture group were then analysed using MCA.

GIS spatial analysis

pQCT images were imported as ASCII format files into the GIS analysis program ArcMap (ArcGIS 9.3, ESRI, Redlands, CA, USA) for spatial analysis. The imported images were reclassified by pixel value to represent low (<600 mg/cm^3), medium (≥600 <800 mg/cm^3) and high (≥800 mg/cm^3) BMD_V. These BMD_V thresholds were determined to change significantly in response to exercise in study one.

Smaller ROIs were created in ArcMap within the original ROIs L2P and M2P, in order to analyse the palmar Mc3 epiphysis that contacts the sesamoid bones during the stance phase of gallop. The ROI created was $[0.2 \times \text{length of line from the palmar apex of the sagittal ridge to the dorsal apex of the sagittal ridge}]^2$ to account for the variation in condylar size between individuals. The palmar border of the ROI was aligned with the inner margin of the articular calcified cartilage on the palmar border of the condyle, and centred within the lateral and medial borders of the ROI M2P or L2P (Figure 3.2).

Additionally, ROIs were created over the sites of fracture at the medial and lateral palmar parasagittal groove regions in both fractured and control horses. These ROIs were [0.1 x length of line from the palmar apex of the sagittal ridge to the dorsal apex of the sagittal ridge]^2 to specifically target the sagittal groove area. For sites that had sustained fracture, the ROI was halved and each half was placed either side of the fracture line to avoid error created by voxel sharing within the fracture gap (Figure 3.2).

Clustering and spatial relationships were tested using the Global Moran's I, Average Nearest Neighbour and Getis-Ord General G tests. Statistics could not be generated if there were no pixels within the threshold being analysed and statistics that fell below the level of statistical significance of $P \leq 0.01$ were excluded from interpretation. Additionally, a polygon was drawn around the outer margin of high BMD_V pixels and the around margin of low BMD_V pixels within the ROI. The spatial relationship between low and high BMD_V clusters was recorded as: low BMD_V clusters outside the margins of the high BMD_V cluster (1) or low BMD_V clusters inside the margin of the high BMD_V cluster (0).

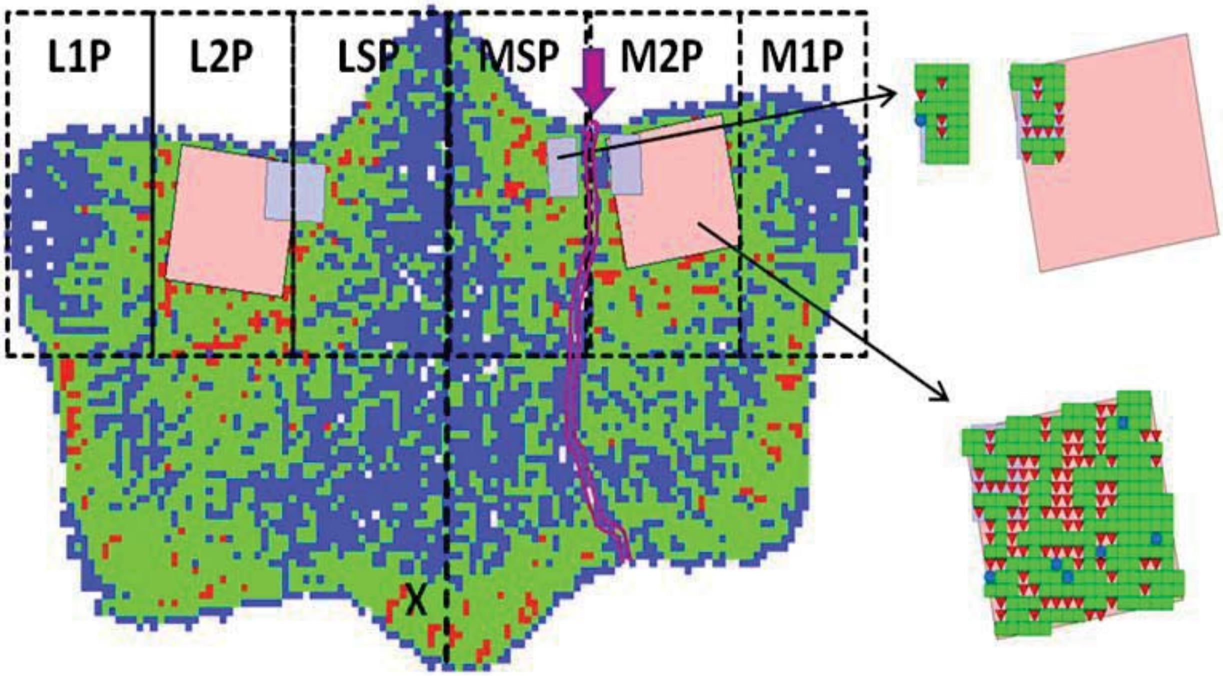


Figure 3.2: Reclassification of pQCT images and the ROIs for used for spatial analysis of BMD_V in ArcMap. Pixels were categorized as low ( ), medium ( ) or high ( ) BMD_V. ROIs were created for both M2P and L2P each with an area of [0.2 x length X] ( ) as well as over the medial and lateral parasagittal grooves each with an area of [0.1 x length X] ( ). The pixels were extracted as points (right) to enable the calculation of spatial statistics. In this image the fracture line (==) divides the ROI over the medial parasagittal groove.

Results

Sample analysis

There were no significant differences found between the fractured group (n = 6) and control group (n = 8) of Mc3 condyles for sex (P = 0.6), age (P = 0.7), total starts (P = 0.5) or leg side (P = 0.7). The median number of race starts was 6 (range 0-18) and median age was 4 years old (range 2-6). In the control group of limbs there were no subchondral bone lesions; however, all horses had partial thickness cartilage loss in the central condylar regions of the palmar epiphysis.

The effect of training on distribution of BMD_V within different ROIs

There were no significant differences in the median proportion of voxels between the fractured and control groups in all of the ROIs studied. The ROI L2P had non-significant trends toward the fracture group having lower BMD_V in the thresholds 700-799 and 800-899 mg/cm^3 (P = 0.02 and 0.007, respectively) and higher BMD_V in the 1000-1099 mg/cm^3 (P = 0.01) threshold (Figure 3.3).

Multiple correspondence analysis

In most ROIs, ≥80% of the inertia was explained by dimension one, except for ROI LSP where 75% was explained by dimension one. The MCA graphs for all ROIs showed that the variables for greater than the median high BMD_V thresholds (900-1200 mg/cm^3) were clustered together, deviating from the average profile (centre of the graph) in the same direction. Overall, the MCA graphs for each of the ROIs showed little clustering of the fracture variables with BMD_V categories or number of race starts (Figure 3.4).

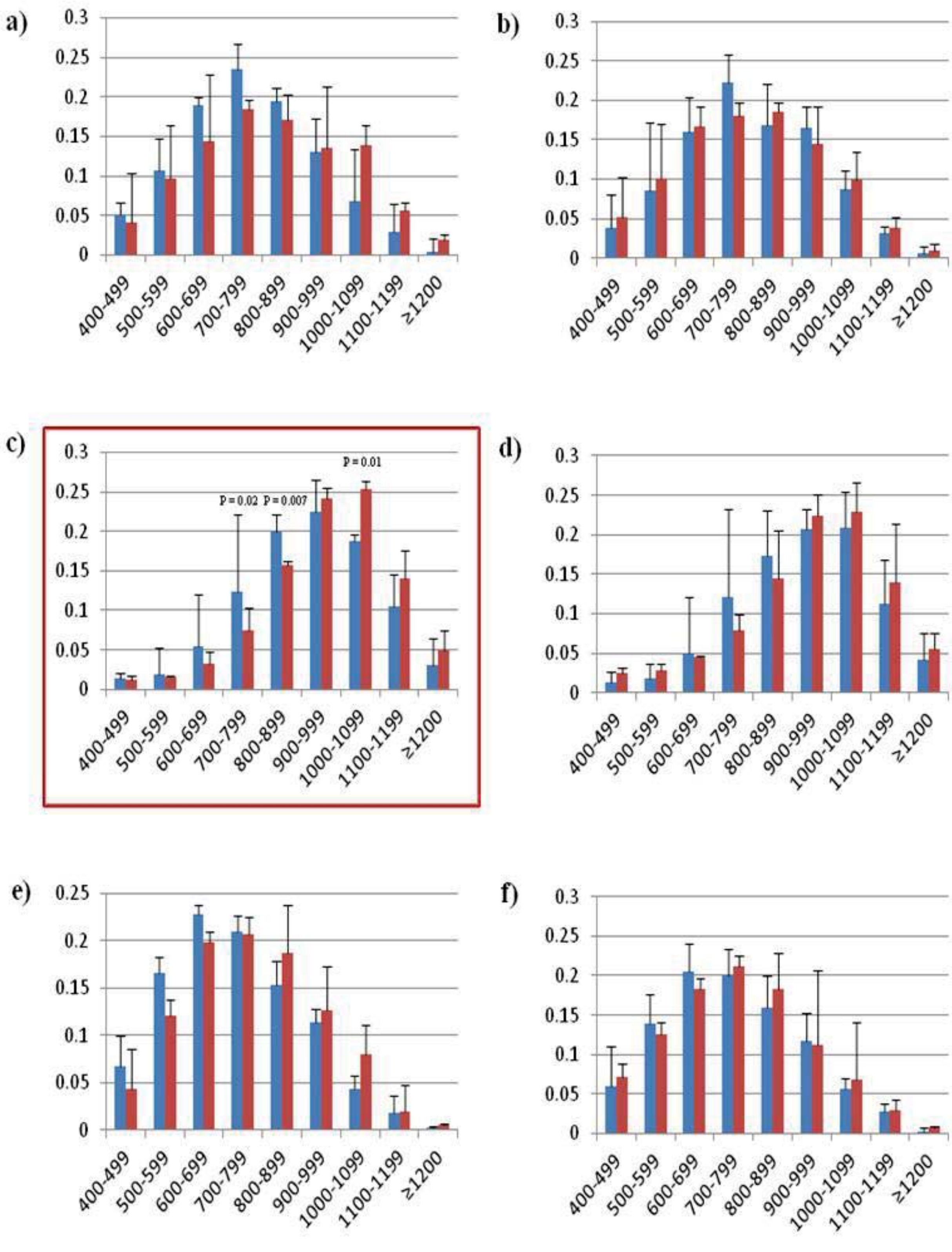


Figure 3.3: The median relative proportion of voxels (y) for BMD_V thresholds (mg/cm^3) (x) in ROIs of the palmar distal epiphysis for non-fractured ( ) and fractured ( ) groups. Error bars represent the upper quartile range of the related BMD_V threshold. (a) L1P, (b) M1P, (c) L2P, (d) M2P, (e) LSP and (f) MSP.

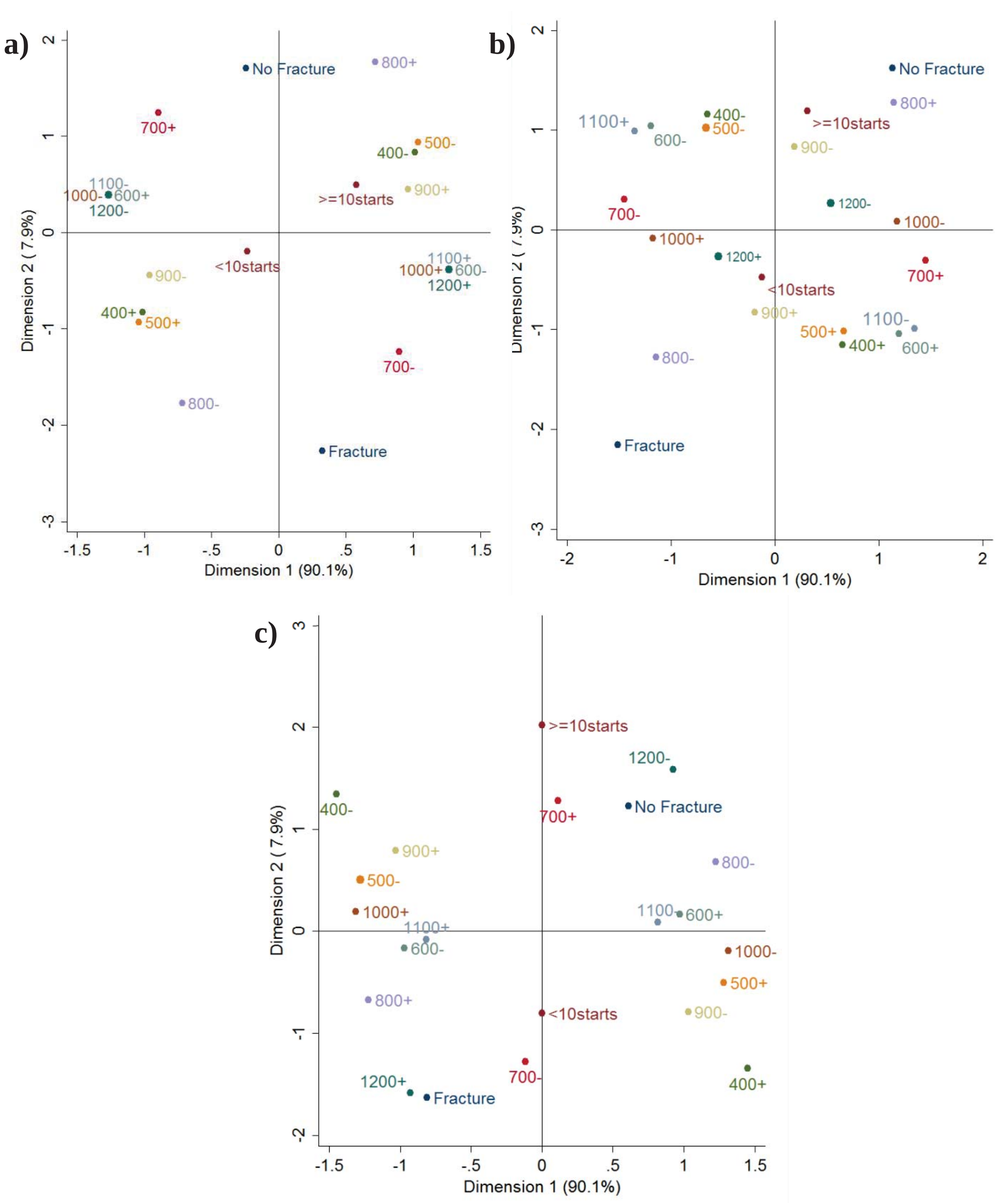


Figure 3.4: MCA plots for BMD_V thresholds and the fracture variable for the lateral ROIs L1P (a), L2P (b) and LSP (b). Above (+) and below (-) median values for each BMD threshold are shown (number represents the start value of the threshold).

Spatial analysis using ArcGIS

There were no differences between the fracture and control groups for any of the spatial analysis statistics generated by ArcMap.

Moran's I test

In the ROI within L2P there was moderate to strong clustering of high BMD_V in all of the fractured (range I = 0.69 – 0.89, $P \leq 0.01$) and six of the control (range I = 0.75 – 0.85, $P \leq 0.01$) condyles. There was strong to marked clustering of medium BMD_V for all of the fractured and control condyles (ranges I = 0.86 – 0.92 and I = 0.79 – 0.96, $P \leq 0.01$ respectively). There were only two significant values for clustering of low BMD_V that were both in the control group, and showed moderate to strong clustering (0.60, 0.79, $P \leq 0.01$).

In the ROI within M2P there was strong to marked clustering of high BMD_V in all of the fractured condyles (range I = 0.75 – 0.93, $P \leq 0.01$) and weak to strong clustering of high BMD_V in seven of the control condyles (range I = 0.39 – 0.90, $P \leq 0.01$). There was strong to marked clustering of medium BMD_V for all condyles in both the fractured and control groups (ranges I = 0.85 – 0.98 and I = 0.81 – 0.95, $P \leq 0.01$ respectively) but only two significant results for low BMD_V in the same two control horses that also had strong clustering in the L2P ROI (0.76, 0.90, $P \leq 0.01$).

For the lateral parasagittal groove ROI there was moderate to strong clustering of high BMD_V in four of the fractured (range I = 0.63 – 0.84, $P \leq 0.01$) and four of the control condyles (range I = 0.53 – 0.82, $P \leq 0.01$). There was strong to marked clustering of medium BMD_V for the all of the fractured and control condyles (ranges I = 0.73 – 0.91 and I = 0.76 – 0.91, $P \leq 0.01$ respectively). There were no significant values for clustering of low BMD_V in either of the fractured or control groups.

For the medial parasagittal groove ROI there was weak to strong clustering of high BMD_V in five of the fractured (range $I = 0.39 – 0.97$, $P \leq 0.01$) and five of the control (range $I = 0.43 – 0.87$, $P \leq 0.01$) condyles. There was strong to marked clustering of medium BMD_V for the all of the fractured and control condyles (ranges $I = 0.79 – 0.95$ and $I = 0.72 – 0.91$, $P \leq 0.01$ respectively). One horse from the control group had significant moderate clustering (0.52, $P \leq 0.01$) of low BMD_V.

Nearest neighbour test

Significant results for the nearest neighbour test were all >1, indicating an evenly spaced distribution of pixels in each BMD_V threshold cluster. For the high and low BMD_V thresholds not all horses had significant results due to a minimal number or absence of pixels in these thresholds.

Getis-Ord General G and arrangement of high value and low BMD_V clusters

High BMD_V clusters dominated over low BMD_V clusters in both fractured and control condyles for all ROIs. There were only a small number of significant test values due to either absent or minimal pixels of high or low BMD_V.

Both fractured and control groups had horses with low BMD_V clusters located inside high BMD_V clusters; however, this appeared to occur less frequently than low BMD_V clusters located outside high BMD_V clusters for both the fracture and control groups. The fractured group appeared to have more occurrences of low BMD_V clusters occurring within high BMD_V clusters for all ROIs; however, due to the low number of condyles analysed, it is unknown if there was a true difference.

Discussion

This study aimed to compare the regional differences in the amount and spatial distribution of BMD_V on the palmar Mc3 epiphysis between fractured and non-fractured condyles.

The main limitations of this study are related to the study population. As well as the study population being small, non-fractured contralateral limbs were available for only two of the fractured limbs so it was not possible to make a comparison of fractured with non-fractured limbs that had exactly the same exercise and management exposure. It is unknown if asymmetric loading occurs between fractured and contralateral limbs prior to fracture formation, due to either underlying disease, or as a result of training. Two studies found a greater prevalence of left forelimb than right forelimb fractures, which could have been due to training on a counter clockwise track distributing more mass over the left forelimb (Ellis 1994; Rick *et al.* 1983). Using limbs from horses that had no significant orthopaedic disease in either forelimb could have reduced any confounding factors related to asymmetric loading or differences in underlying bone lesions between fractured and contralateral limbs.

The greatest limitation in this population was that we did not know the exact training history for each horse. Our assumption was that horses with more starts were exposed to more exercise; however, not only were there a wide range of race starts (range 0-18), but there are also inherent faults with this assumption. Racing records are a very approximate measure of exercise exposure and specific aspects of training protocols have been found to be related to the risk of fracture occurrence (Cogger *et al.* 2006; Perkins *et al.* 2005b). This is because even small exposures to peak strain cause maximal bone responses (Rubin and Lanyon 1984b) and exposure to gallop for short durations during training was found to be protective against condylar fracture (Parkin *et* *al.* 2004a, 2005) and shin splints (Boston and Nunamaker 2000). In contrast, previously untrained horses exposed to more than 44km at canter (≤14ms^{-1}) or 6km at gallop (>14ms^{-1}) in a 30 day period have been found to increase the risk of fracture (Verheyen *et al.* 2006). It has not been determined what bone structural changes were present under these circumstances immediately prior to fracture; therefore, detailed exercise data must be considered in relation to structural changes observed in bone.

It is also unknown if, or for how long, the horses used in the current study were rested or worked prior to fracture or euthanasia. BMD_V of the Mc3 epiphysis has been demonstrated to decrease to pre-training levels after a rest period of a few months (Firth *et al.* 2011a). On subsequent consideration of the population, it was found that three horses that had raced in the fractured group had been euthanised within a month of their last recorded race. This contrasted to the non-fractured population in which, of the three horses that raced, two had been euthanised two years after their last recorded race and one within a year of its last recorded race. It is unknown what the exact exercise exposures were between the last race and euthanasia; however, there were no differences in BMD_V characteristics between the fractured and non-fractured groups.

The placement of the ROIs in ArcMap, either side of the fracture line, could have decreased the sensitivity of the results. In studies that have examined microstructural changes around fracture lines, small microcracks and areas of lysis have been found very close to the articular origin of the fracture line (Riggs 1999; Stepnik *et al.* 2004; Whitton *et al.* 2010). However, the ROIs were placed just outside the margin of the fracture line so as to avoid having parts of the fracture gap included in the analysis. This method was used as there were low BMD_V artefacts resulting from a voxel sharing effect within the fracture gaps on all images. Gaps at the articular margin of fracture lines have been described as pathologic using micro-CT (Whitton *et al.* 2010) and diagnostic computed tomography (Cruz and Hurtig 2008). However, it is unknown if voxel sharing contributed to the appearance of the described lesions, or if the lesions were pathologically related to fracture.

Findings in the current study suggest that exercise is associated with BMD_V characteristics rather than fracture. There were no significant differences between the BMD_V proportions or spatial distributions of BMD_V between the fractured and non-fractured horses. This could have been due to the two groups having no significant differences in exercise levels, causing similar BMD_V responses in each group. Bone mineral density (BMD) has a greater response to exercise that induces high strain in the bone tissue (Arasheben *et al.* 2012; Cornelissen *et al.* 1999; Firth *et al.* 2005; Hasselstrøm *et al.* 2007) and there were significant differences in the BMD_V characteristics in study one of this thesis, between horses that had been exposed to differing exercise levels.

A recent study found differences in the size and intensity of the sclerotic region in the palmar area of the Mc3 epiphysis between fractured and non-fractured horses (Tranquille *et al.* 2012). However, in the study the amount of exercise exposure that the horses had been subjected to was not considered. It is possible that the fractured group had been exposed to more exercise than the non-fractured group and therefore confounded the results. Although features of training regimes such as speed and distance have been related to condylar fracture (Verheyen *et al.* 2006), the direct effects on specific bone changes as training parameters are adjusted have not been investigated.

From the literature it is clear that a high frequency of loading cycles is detrimental to the health and strength of bone tissue (Boston and Nunamaker 2000; Robling *et al.* 2002; Verheyen *et al.* 2006). This could be because a high frequency of loading does not allow remodelling to occur, or allows the formation of microdamage to the bone. It has been demonstrated in study one that the higher peak strains induced by exercise are associated with a more pronounced BMD_V response, especially in the upper and lower BMD_V thresholds; however, it is unknown if the spatial distribution of the BMD_V response is different between condyles that are loaded with high or low frequency. Although there was a higher risk of fracture with 880 loading cycles of gallop and 7700 loading cycles of canter within a 30 day period in racehorses (Verheyen *et al.* 2006), it is unknown if detrimental changes in the structure of bone occur before this time and after how many loading cycles they begin to occur. Prospective longitudinal studies are warranted that investigate the structural properties of bone in relation to the cumulative number of loading cycles that the condyle has been exposed to.

BMD alterations of the Mc3/Mt3 distal epiphysis that occur in response to training are likely to be an adaptive response and allow the bone to withstand the cyclic loading demanded. This is because bone responds to dynamic loading by changing its structure in order to ensure that the forces encountered are within a physiologic threshold of strain (Rubin and Lanyon 1984a). The BMD response has been found to be greater in horses that had galloped than horses that had cantered (Firth *et al.* 2005) and horses that had galloped during their race training period were less likely to fracture than those who had done no gallop work (Parkin *et al.* 2004a, 2005). Once an adaptation has occurred, it is maintained for as long as exposure to the load is continued (Firth *et al.* 1999a; Firth *et al.* 1999b), but will be altered if the peak strain is decreased (Firth *et al.* 2011a) or increased (Firth *et al.* 2011b). Additionally, it is evident that training needs to be specifically aimed at the type and degree of competition in order to elicit an appropriate bone response and aid in injury prevention (Boston and Nunamaker 2000; Nunamaker 2002; Parkin *et al.* 2004a).

It has been demonstrated that specific training factors that influence peak strain are associated with specific changes in bone morphology. For example, different track surfaces can influence specific alterations in cortical or trabecular thickness (Nunamaker 2002; Young *et al.* 1991) and there is a greater bone density response to gallop than to canter (Firth *et al.* 2005). However, it is unknown if increasing peak strain using harder track surfaces induces the same structural bone changes as increasing the peak strain by training at different galloping speeds, or if the horse was to carry extra weight during training. If the relative effects of training factors on bone structural change were known, it would aid in the development of training programs that balance the factors that elicit protective structural bone changes for ongoing exercise demands, without causing structural damage.

In conclusion there were no differences in the BMD_V characteristics of the distal Mc3 epiphysis between Thoroughbred racehorses with condylar fracture and those without condylar fracture. A holistic approach to relate the specific features of exercise programs with specific changes in bone morphology and the occurrence of fracture is necessary to begin to understand why catastrophic injuries occur.

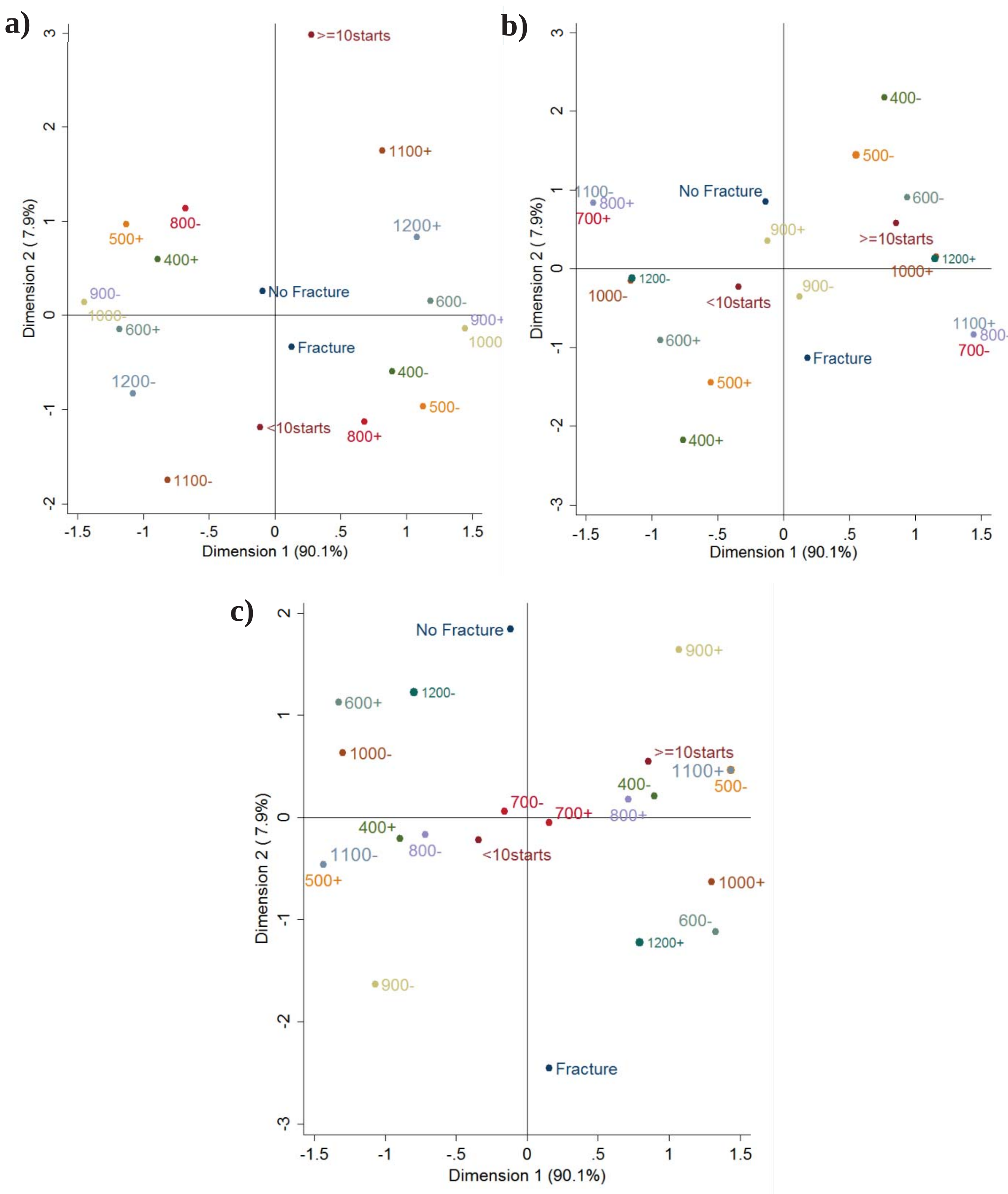


Figure A2.1: MCA plots for BMD_V thresholds and the fracture variable for the medial ROIs M1P (a), M2P (b) and MSP (b). Above (+) and below (-) median values for each BMD threshold are shown (number represents the start value of the threshold).

Chapter 4

General discussion

The primary aim of this thesis was to quantitatively describe and compare the bone mineral density (BMD) responses of the distal third metacarpal (Mc3) and third metatarsal (Mt3) epiphyses in relation to exercise exposure and condylar fracture. Previous studies had measured the BMD response of the entire epiphysis and qualitatively described the spatial distribution of the BMD response (Brama *et al.* 2009; Easton and Kawcak 2007; Firth *et al.* 2005; Kawcak *et al.* 2000; Riggs and Boyde 1999). Additionally, studies that had investigated the BMD characteristics of fractured condyles had not considered that the differences between fractured and control groups could have primarily been due to differences in exercise exposure and could have been within a physiological range (Tranquille *et al.* 2012; Whitton *et al.* 2010). To the author's knowledge, this was the first study to quantitatively describe the regional BMD response using spatial statistics and to describe the changes that occur within narrow BMD thresholds.

The results from this thesis indicate that the BMD response to exercise is extremely specific in anatomical location, the BMD values that increase, and the spatial distribution of the BMD response. The findings from the first study were consistent with previous qualitative findings in the Mc3 epiphyses of the same group of horses (Firth *et al.* 2005). Other specific responses of bone have been found in other regions of the distal limb in relation to particular alterations in training regimen such as track type (Nunamaker 2002; Young *et al.* 1991) and the speed of training (Firth *et al.* 2005; Nunamaker 2002). As the specific responses are likely to be an adaptation to protect the bone from particular stresses induced by exercise, it is important that training exposes bone tissue to the same peak stress that will be encountered during competition so that the tissue can withstand the higher exercise demand (Boston and Nunamaker 2000; Parkin *et al.* 2004a, 2005).

The study found that the overriding factor affecting BMD response was the amount of exercise exposure, with no differences found between the BMD characteristics of fractured and non-fractured condyles. Areas of compromised bone surrounding mid-condylar fractures on computed tomography scans have been observed, but it has been suggested that parasagittal condylar fractures could have a different aetiology, without the presence of prior bone degeneration (Cruz and Hurtig 2008). The fractures in this study were all located in proximity to the parasagittal grooves; therefore, the finding that there was no difference in BMD characteristics between fractured and control condyles supports the suggestion that parasagittal condylar fractures could occur without prior bone degeneration. However, mid-condylar fractures were not available for analysis and areas of potential voxel sharing within and around the fracture gap were deliberately excluded, which could have reduced the ability to detect areas of compromised bone adjacent to the fractures.

A main limitation of these studies was the small sample size. In earlier studies that have assessed the BMD response to exercise, the sample size has also been small (Kawcak *et al.* 2000; Riggs and Boyde 1999), whereas studies that have used non-invasive imaging to compare limbs with condylar fracture to non-fractured limbs have had large sample sizes (O'Brien *et al.* 2011; Tranquille *et al.* 2012). However, a point of difference for the current study was that the racing records were taken into consideration when selecting the control population.

The other main limitation of these studies was related to the exercise exposure of the horses. In study one, the horses had only been exposed to the training required for a trial start, which is less than a racehorse in training or a horse that had possibly been exposed to excessive cyclic loading resulting in fracture. However, the controlled exposure to exercise and management provided results with greater integrity than if the limbs from racehorses without known exercise and management histories had been analysed.

In study two, the exact exercise histories of the racehorses were unknown. Previous studies that have matched fractured limbs with non-fractured limbs on a case-control basis have also not known exact exercise histories of the horses (O'Brien *et al.* 2011; Radtke *et al.* 2003; Tranquille *et al.* 2012). Fatal distal limb fracture has a relatively low incidence in flat racing of 0.4/1000 race starts (Parkin *et al.* 2004c); therefore, fractured limbs must be sourced from a large population of racing Thoroughbreds at the time that they occur. Detailed exercise histories that are reliable are difficult to obtain due to the reliance on gaining study material from many different sources. In comparison, racing records from racing institutions are a reliable source of data, although they are an approximate measure of exercise load. The study design could have been improved by collecting more detailed training records and using a collaborative approach with epidemiologists to compare fractured and non-fractured populations. In the future, detailed data capture from all industry participants is required, rather than on select cohorts willing to provide data; otherwise, the low incidence of fracture will continue to limit the number of cases with known exercise histories.

Catastrophic injuries have been investigated by collaborating veterinary, training and post-mortem records through the California Post-mortem Program that started in 1990 to investigate the deaths of racehorses in California (Stover and Murray 2008). Epidemiological studies produced from training data have found specific risk factors for catastrophic injury such as age, sex (Estberg *et al.* 1996b; Estberg *et al.* 1998b), shoe type (Kane *et al.* 1996) and cumulative exercise distances at speed (Estberg *et al.* 1998a; Estberg *et al.* 1995; Estberg *et al.* 1996a). Additionally, studies that have examined specimens have found gross and microscopic bone changes could underlie tissue failure (Norrdin and Stover 2006; Stover and Murray 2008). Although these findings have increased our understanding of what specific factors increase the risk of bone failure, and how they could be affecting the bone before it fails, temporal relationships such as at what point tissues change from withstanding cyclic loading to being damaged and what hallmarks could signify early damage are not fully understood.

Further investigation of the temporal relationships between risk factors and different types of musculoskeletal injury are warranted because catastrophic injury and the injuries that might precede them result in a significant amount of wastage and economic loss in Thoroughbred racing industries globally (Bailey *et al.* 1997b; Clegg 2011; Cruz *et al.* 2007; Hernandez and Hawkins 2001; Perkins *et al.* 2005a; Stover 2003; Wilsher *et al.* 2006; Zekas *et al.* 1999b). Multiple risk factors contribute to injury, but it is not clear what environmental or tissue level factors determine what type of injury a particular horse will incur or if the injury types are related. For example microcracking has been found in both horses with condylar fracture (Stepnik *et al.* 2004) and palmar/plantar osteochondral disease (POD) (Norrdin and Stover 2006); however, a recent study suggested that having POD could somehow be protective of condylar fracture, perhaps because horses with POD were less likely to gallop as fast (Tranquille *et al.* 2012). Additionally, it has been demonstrated that risk factors, such as high speed exercise, can have a cumulative effect that is time dependent (Estberg *et al.* 1998a;

Estberg *et al.* 1996a). The investigation of how bone tissue responds to specific risk factors over time could provide insight into the temporal relationships between different types of musculoskeletal injuries and their risk factors.

To move forward in our understanding of condylar fracture, and other stress-induced orthopaedic diseases, environmental and tissue related risk factors need to be considered mutually. The solution is for research not to become broader, but rather to consider how specific elements interact over time. As the risk factors are multifactorial and temporally associated, an ongoing collaborative research approach is warranted. This could be achieved by researchers pooling funds and establishing the co-operation of the racing industry to medically screen horses throughout their careers, collect detailed exercise records, and collect specimens for post-mortem evaluation. However, a more practical method would be to initiate national data-bases, with future research aims in mind, to achieve detailed archives of training, racing, clinical and post-mortem records.

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