

Tutorial Article

Osteochondral injury and joint disease in the athletic horse

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Introduction

Joint disease is common in horses. The careers of many performance horses are impaired or prematurely curtailed as a consequence of lameness due to arthritides. Osteoarthritis, characterised by progressive and permanent disease of articular cartilage and associated bone and soft tissues of the joint, represents one end-stage of a series of pathological processes, which may be initiated by joint trauma (McIlwraith 1996). In other cases, more acute injuries, such as osteochondral fracture, may arise as a consequence of weakening of the tissues of the joint following chronic, cyclical loading (Pool 1996). The fact that it is not possible to predict reliably the pathological outcome of similar injuries to different joints reflects our incomplete understanding of the disease processes involved.

The diarthrodial joint can be considered as a complex organ in which several different tissue components, ligaments (intra- and extra-articular), fibrous joint capsule, synovial membrane, hyaline cartilage (nonmineralised and mineralised) and bone, form a functional unit. It is increasingly recognised that there are complex biomechanical and biochemical interactions between all the components of the joint and the large number of permutations that can result from varying degrees of involvement of each tissue may partially account for the unpredictable pathological consequences of joint injuries. The complex interaction of different tissues is also a confounding factor in experimental studies designed to investigate mechanisms of joint disease, especially those conducted *in vitro*. Despite these limitations, significant advances have been made over recent years in understanding the pathological processes involved following joint injury.

The purpose of this article is to review some of the more important pathological processes involved following traumatic injury to the osteochondral tissues of a joint, highlighting some outstanding issues which require further investigation.

Aetiology

Joint injuries arise as a consequence of physical disruption of tissues due to mechanical stress. This may occur due to the

joint being exposed to loads that exceed physiological limits, either as a consequence of excessive magnitude, excessive number of cycles or abnormal direction of the load. Alternatively, loads within the normal physiological range may cause injury when the mechanical integrity of the tissues has been compromised by concurrent disease (**Fig 1**).

Abnormal loads: normal joint

Supraphysiological strains may arise as a consequence of an accident, such as a fall or collision, and may result in gross disruption of articular tissues including chondral/osteochondral fracture, partial or complete erosion of articular cartilage and ligament or capsule tears (Pool 1996). Significant injury may arise even in the absence of gross pathology and a proportion of cases of 'idiopathic' or primary osteoarthritis in man (and probably horses) may have their origins in a single episode of trauma months or even years earlier (McIlwraith 1996; Nuki 1998). A more frequent cause of joint injury is fatigue damage of osteochondral tissues through repetitive loading, particularly at high speed. Both cartilage and bone are viscoelastic materials, i.e. their mechanical properties vary with the rate of loading, and both become more brittle (prone to fracture) with high strain rates (Morrel and Quinn 2004). Consequently, impact loads are much more damaging to joint tissues than loads of similar magnitude applied in a more gentle fashion (Radin *et al.* 1973). Both strain magnitude and strain rate of bones of the appendicular skeleton increase linearly with speed (Rubin and Lanyon 1982). This is consistent with the clinical observations that osteoarthritis of the knee is more common in human runners (Gelber *et al.* 2000) and injuries of the fetlock are frequent in racehorses (Pilsworth 2003). Repetitive high-speed exercise resulting in cyclical impact loading of a joint is most likely to result in maximum injury. After each cycle of load there is a lag phase as the cartilage regains its relaxed dimensions which is associated with redistribution of fluid within the matrix (Mow and Hung 2003), and a high rate of cyclical loading, as achieved by the galloping horse, may interfere with full function of this mechanism. Equally, there are reparative mechanisms that take days or weeks to complete (Burr 2003; Hunziker and Tyler

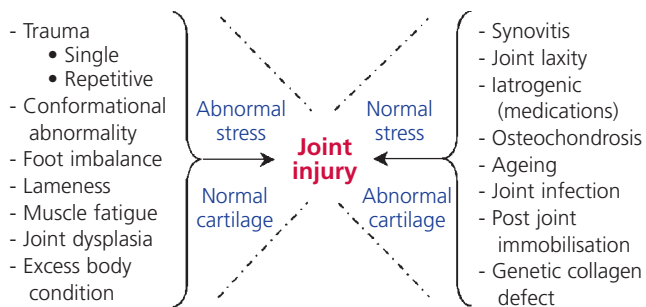


Fig 1: Summary of factors considered important in the aetiology of joint injury.

2003). Repeated impact loading on a daily basis is more likely to overwhelm such mechanisms, leading to cumulative damage. Other factors likely to affect the rate of loading of the limbs, such as track surface and shoeing, can also be expected to influence the incidence of joint injury.

Conditions that result in joint instability and/or loading in abnormal planes are a risk factor for joint injury (Fig 1). It is increasingly recognised that both the cartilage and bone forming a joint have heterogeneous biochemical and biomechanical properties, possibly reflecting physiological adaptation of the tissues to prevailing loads in youngsters (Brama *et al.* 2000a). Such mechanisms may increase the resilience of the tissues to 'normal' loads but render them more prone to injury by 'abnormal' loads. In addition, uncoordinated or asymmetrical loading of a joint may result in high focal stresses as congruence of the apposing joint surfaces is disrupted. Joint dysplasias are rare in the horse, although malformation of the glenoid of the scapula in Shetland ponies has recently been associated with osteoarthritis of the shoulder joint in this breed (Boswell *et al.* 1999). Conformational abnormalities, including poor foot balance, particularly in the mediolateral plane, are recognised as risk factors for joint disease in the distal limb of the horse (Turner and Anderson 1996). 'Back-at-the-knee' conformation is thought to predispose Thoroughbred and Standardbred racehorses to osteochondral fragmentation of the carpus (Ross 2003), although a quantitative study has failed to substantiate this hypothesis (Barr 1994). Ligamentous injuries are frequently associated with, and implicated in, the aetiopathogenesis of osteochondral injuries of associated joints (Sanders-Shamis *et al.* 1988; Wright 1995). The pattern of loading of the limbs, and hence joints, is altered by lameness (Buchner 2001) and, therefore, continued exercise of a lame horse may be considered a risk factor for joint injury. Fine neuromuscular control is important for normal joint function, not only for achieving correct limb placement and loading during exercise but also for supporting and absorbing energy transmitted across the joint (Radin 1999). Selective denervation of a joint can rapidly lead to the development of osteoarthritis (Salo *et al.* 2002), and neurological disorders associated with prominent loss of proprioception in man are frequently associated with the development of joint disease, e.g. Charcot's arthropathy (Salo 1999). Physiological fatigue of musculotendinous units associated with extreme exercise

will lead to joint instability and degradation of their ability to absorb energy, thereby increasing the severity of impact loads on the joint (Radin 1999).

Normal/abnormal loads: abnormal joint

Articular tissues compromised by existing disease are more prone to physical damage due to a structurally weakened matrix and/or impaired ability to initiate reparative processes due to cell death or dysfunction (McIlwraith 1996). Intra-articular infection, developmental joint abnormalities, iatrogenic toxic insult following intra-articular injection and prolonged periods of immobilisation of a joint within a rigid cast have all been implicated as causes of joint disease (Videman *et al.* 1981; McIlwraith 2002). Inappropriate levels of exercise during the recovery phase from these diseases will significantly increase the risk of joint injury.

Disease syndromes

Significant advances have been made in recent years in understanding the pathological mechanisms involved in and following injury to a joint. The close physical and biochemical associations between the different tissue components of the joint complicate analysis of these mechanisms; attempts to reduce such analysis to individual tissues may be misleading. Therefore, relevant information will be reviewed as it relates to recognised disease syndromes rather than individual tissue types.

Synovitis and capsulitis

Synovitis is common as a primary condition in athletic horses and is frequently the precursor of more widespread and chronic joint disease (McIlwraith 1996). Irritation through repeated mechanical trauma is presumed to be the major cause of synovitis, which is reflected in the observation that high-motion joints, such as the metacarpophalangeal, metatarsophalangeal and carpal joints, are most frequently affected (McIlwraith 1996). Synovitis may also arise secondarily to primary osteochondral injury, through the effects of inflammatory mediators, cartilage wear fragments or free proteoglycans within synovial fluid (Evans *et al.* 1982; Boniface *et al.* 1988). Infection and intra-articular injection are well-recognised causes of synovitis (Bertone 2003a,b). There are several pathological consequences of synovitis, as described below.

Synovial effusion

Increased blood flow, increased permeability of the endothelium and synovial intima as a consequence of inflammation and reduced joint movement due to pain result in net movement of fluid into the joint cavity. Intra-articular pressure of normal equine joints is usually subatmospheric (-1.25 mmHg), but positive pressures of 28.33 mmHg have been recorded in joints affected by synovitis, and 51 mmHg when complicated by concurrent capsulitis (Strand *et al.* 1998). A negative intra-articular pressure enhances functional stability

of joints and, consequently, this mechanism is disrupted in the presence of synovitis. In addition, increased intra-articular pressures may impede vascular perfusion of joint tissues, and in extreme cases may be sufficiently high to rupture the joint capsule (Bertone 2003b). An increased volume of fluid will reduce contact between synovial membrane and exposed cartilage, thereby increasing the distance for diffusion between cartilage and blood vessels and hence reducing nutritional exchange to cartilage. Also, hyaluronan becomes more dilute as fluid volume increases, which will have a detrimental effect on intra-articular lubrication (Bertone 2003b).

Inflammation

Synoviocytes are a rich source of inflammatory mediators and proteinases implicated in cartilage degeneration (McIlwraith 1996; Caron 2003). This is discussed further below and the reader is guided elsewhere for a detailed review of the subject (McIlwraith 1996).

Cartilage injury and osteoarthritis

Loss of articular cartilage is the major cause of joint dysfunction and disability in joint disease. Severe trauma may cause gross disruption of the joint surface with complete erosion of cartilage and possible involvement of subchondral bone. More commonly the insult results in more subtle pathology, the extent and consequences of which may not be immediately apparent. Injury may involve articular cartilage directly or secondarily through biochemical or biomechanical affects of adjacent, damaged tissues. Cellular recovery and synthetic processes may be sufficient to repair the tissue and restore homeostasis. However, if compromise of cellular function or damage to the matrix is beyond a threshold limit and/or if the traumatic insult is maintained then catabolic processes may exceed anabolic capacity and progressive disease ensues, ultimately leading to osteoarthritis.

Pathogenesis

Injury to articular cartilage may arise as a consequence of any one or combination of the following pathological processes:

- a. Direct mechanical damage of matrix (Thiabault *et al.* 2002).
- b. Destruction of matrix through effects of locally produced (cartilage, synovium, bone) catabolic enzymes (McIlwraith 1996).
- c. Damage to chondrocytes (Aigner *et al.* 2002) resulting in:
 - Death (due to necrosis or apoptosis).
 - Altered synthesis of selective matrix molecules (up- and down-regulation) and of destructive enzymes and inflammatory mediators.
 - Phenotype modulation, associated with an overall altered gene expression profile.
- d. Increased risk/rate of cartilage damage through change in mechanical environment (subchondral bone sclerosis; Radin and Rose 1986).

Direct damage of cartilage matrix: Disruption of the fibrillar collagen network occurs following joint trauma (Pool 1996). High strain rates associated with impact loads can cause fluid pressures within the cartilage matrix that exceed the restraining capacity of the collagen network, causing its tensile failure (Morrel and Quinn 2004). Mechanical tests of cartilage disc explants immediately before and just after cyclical loading at high physiological strain rates demonstrated a weakening of the collagen network and an increased hydraulic permeability due to the cyclical loading. These changes were linked to an increase in denatured type II collagen in the discs, which was not associated with any demonstrable collagenase activity (Thiabault *et al.* 2002). The loading intensity was insufficient to cause cartilage fissures or loss of cell viability, but did result in loss of proteoglycans and collagen fragments from the loaded disc to the culture medium in the days following loading, suggesting further biochemical events secondary to mechanical disruption of the tissue. Mechanical induction of collagen denaturation may occur by direct stretching and disruption of fibrillar structure or could be partly related to heating via viscous dissipation during loading (Thiabault *et al.* 2002). In addition to having biomechanical consequences, unwinding of the collagen fibrils facilitates the action of collagenases by providing more favourable access to their target substrates (Patwari *et al.* 2003) and may expose the collagen to the actions of relatively nonspecific enzymes, which may, therefore, have a more important role in collagen remodelling than was previously thought (Thiabault *et al.* 2002).

Impact studies on cartilage *in vivo* and on *in vitro* samples in which a layer of bone was left attached to the cartilage demonstrated a decreasing propensity to matrix damage with depth of tissue (Patwari *et al.* 2001; Lewis *et al.* 2003). This gradation is lost when experiments are performed on cartilage samples in which all bone has been removed and under these circumstances there is more diffuse damage throughout the samples (Torzilli *et al.* 1999; Patwari *et al.* 2001). These findings illustrate a 'protective' effect of the subchondral bone, which may partly be accounted for by it acting as a shock absorber (Radin 1999) and partly by it physically anchoring the deeper layers of cartilage, thereby limiting the degree of deformation and hence damage to a given load (Lewis *et al.* 2003). The orientation of collagen fibres in cartilage, with transverse fibres in the superficial layers, will maximise resistance to lateral deformation at the surface where tension in this plane is greatest. However, when splits in the cartilage do penetrate the transverse layer there is little resistance to deeper progression, where fibres are progressively orientated more perpendicular to the joint surface.

Destruction of matrix through effects of locally produced catabolic enzymes: Up-regulation of catabolic processes is a fundamental aspect of cartilage injury and disease (McIlwraith 1996). Chondrocytes and synoviocytes are a rich source of inflammatory mediators and enzymes capable

TABLE 1: Proteinases involved in cartilage degradation

Proteinases	
Matrix metalloproteinases (MMPs)	
MMP 1, 8, 13	Collagenase 1, 2, 3
MMP 2, 9	Gelatinase A, B
MMP 3,10	Stromelysin 1, 2
MMP14, MMP17	Membrane type
A disintegrin & MMP with thrombospondin-like motifs (ADAMTSs)	Aggrecanase-1 (ADAMTS 4)
Cysteine proteinases	
Serine proteinases	
Aspartic proteinases	

of degrading the extracellular matrix (**Table 1**). Under physiological conditions, chondrocytes regulate a dynamic metabolic steady state in which anabolic and catabolic processes are balanced; there is a steady turnover of molecules, which is essential for maintenance of a healthy matrix (Kuettner and Thonar 2001). Regulation of enzyme activity is at 3 different levels. Synthesis/secretion of proteinases is altered by inflammatory mediators, principally the cytokines interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α). Enzymes are secreted as latent proenzymes and have to be activated extracellularly. Enzyme activity is modulated by tissue inhibitors of metalloproteinases (TIMPS), which are produced by a variety of cell types, including chondrocytes and synoviocytes (Clegg *et al.* 1998). Following joint injury there is up-regulation of synthesis of IL-1 and TNF- α , increased synthesis of enzymes and exhaustion of TIMPS (McIlwraith 2002; Caron 2003). Considerable effort has been invested in identifying the various enzymes involved in cartilage digestion in disease and factors which influence them (McIlwraith 1996). There is an accumulating body of evidence that collagenase 3 (MMP13) is responsible for initiating collagenolysis and aggrecanase (principally ADAMTS5) destruction of aggrecan in articular cartilage (Sandy 2003; Stanton *et al.* 2005).

Matrix pathology: One of the early identifiable events in joint disease is swelling of the cartilage matrix, indicating disruption of the collagen fibre network (Heinegard *et al.* 2003). The degree to which this is due to the effects of collagenases or direct physical damage remains unknown. Reduced concentration of aggrecan in the tissue is also detected early in cartilage disease, as demonstrated by decreased intensity of staining with metachromatic and/or cationic dyes. This may result from loss of aggrecan or its dilution as a consequence of matrix overhydration (Heinegard *et al.* 2003). Increased release of proteoglycan fragments is routinely detected in synovial fluid in early disease (Dahlberg *et al.* 1992). In addition, recent studies show alterations in the metabolism of various minor molecules as an early event in cartilage disease (Heinegard *et al.* 2003). These molecules, such as COMP and fibronectin, have important roles in organising the detailed architecture of cartilage; hence, imbalance in their concentration in the matrix may have

significant mechanical implications for cartilage (Heinegard *et al.* 2003). These early morphological changes to cartilage following injury are associated with concurrent changes in its mechanical properties; decrease in tensile, dynamic shear and compressive moduli and failure stress are all recorded in the first 6–12 weeks in the canine anterior cruciate transection model (Mow and Hung 2003). Similar patterns of biochemical and biomechanical change are noted in cartilage from naturally occurring osteoarthritis in human individuals in which there is detectable fibrillation of the cartilage surface (Akizuki *et al.* 1986). Interestingly, these mechanical properties were found to be degraded in all cartilage adjacent to damaged areas, even if it had a normal gross appearance (Mow and Hung 2003). Continued mechanical loading of compromised cartilage results in increasing deformation of and consequent injury to the tissue. Gross morphological abnormalities develop, such as surface fissures followed by erosion and, finally, complete ulceration of the matrix (Bullough 2001).

A similar progressive range of gross and histological changes is seen in cartilage from horses with joint disease (Pool 1996). One of the more common abnormalities identified in equine joints, particularly in the metacarpo-/tarsophalangeal joints of performance horses, is the presence of wear lines. These gross pathological features are associated with chondrocyte death, loss of proteoglycans from the matrix and disruption of collagen fibres in the superficial cartilage layer (Pool 1996). Brama *et al.* (2000b) demonstrated a significant correlation between a reduction in hydroxylysylpyridinoline (HP) crosslinks and the presence of wear lines in the proximal articular surface of the equine first phalanx. The level of pathology was significantly more advanced in a group of 2-year-old horses given strenuous exercise in the 13 weeks prior to euthanasia than in a control group that were confined to yards over a similar time. Brama *et al.* (2000b) suggested that the reduction of HP crosslinking, and associated increase in cartilage water content, were due to microdamage and loosening of the collagen network, which subsequently lead to loss of proteoglycan aggregates.

In an unrelated equine study, synovial biomarkers for aggrecan synthesis, proteoglycan release and for type II collagen degradation were significantly elevated in equine mid-carpal joints in horses following commencement of a strenuous exercise programme (Frisbie *et al.* 2003). The levels of these markers were increased significantly further still in cases in which an experimental carpal joint lesion had been created (Frisbie *et al.* 2003).

Damage to chondrocytes: The outcome of trauma to a joint will, to a large extent, depend on the ability of chondrocytes to mount a reparative response to the cartilage component of the injury. Chondrocytes respond positively to dynamic loading within the physiological range, by increasing synthesis of proteoglycans and collagen directly and exhibiting an enhanced response to insulin-like growth factor-I (Bonnasari *et al.* 2001). For instance, an increased rate of proteoglycan synthesis was detected in equine cartilage obtained from

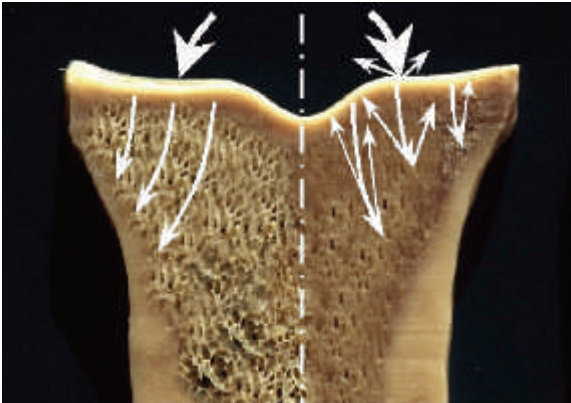


Fig 2: Diagrammatic illustration of the putative effects of bone sclerosis on the distribution of force from the articular surface. Frontal section through the proximal half of the first phalanx. The right half of the image has been digitally enhanced to emphasise the effects of bone sclerosis.

fetlock joints of horses that had undertaken moderate exercise compared with controls (Bird *et al.* 2000a). However, chondrocytes are sensitive to excessive mechanical deformation and even solitary loads of sufficient magnitude will severely impair chondrocyte metabolism or cause cell death. The consequences of loss of chondrocytes are compounded by the facts that proliferative activity of remaining chondrocytes is very poor (Aigner *et al.* 2001) and the cell clusters which do form are unlikely to add significantly to matrix anabolism (Aigner *et al.* 1997).

Chondrocyte death has attracted significant research attention in recent years. While a proportion of chondrocyte demise is due to necrosis, apoptosis is increasingly recognised as a significant feature, particularly in cartilage loaded below the threshold required to cause detectable damage to the matrix (Patwari *et al.* 2001; Aigner *et al.* 2002). In experimental studies, the extent of chondrocyte death following cartilage trauma is a function of the magnitude, rate and duration of applied load (Patwari *et al.* 2001; Chen *et al.* 2003). Cells in the superficial layer are more prone to injury (Chen *et al.* 2003) and chondrocyte death is most intense in the vicinity of cracks in the matrix (Lewis *et al.* 2003). This may explain why traumatic fissures in cartilage are often considered an irreversible event in the progression of cartilage injury (Pritzker 2003). Various mediators of apoptosis are under investigation; nitrous oxide, a putative regulator of apoptosis in a number of cell systems (Chung *et al.* 2001) is elevated in diseased cartilage (von Rechenberg *et al.* 2000; Clegg and Mobasher 2003), although it has also been demonstrated to protect against cartilage matrix degradation under certain circumstances (Bird *et al.* 2000b) and its role in cartilage remains uncertain. Interleukin-1 and TNF- α are potential inducers of cell death, although their role in this capacity in cartilage is still to be elucidated (Aigner *et al.* 2002). Chondrocytes are dependent on their biophysical relationship with the surrounding extracellular matrix and damage to this, which is common following joint trauma, can induce apoptosis (Aigner *et al.* 2002).

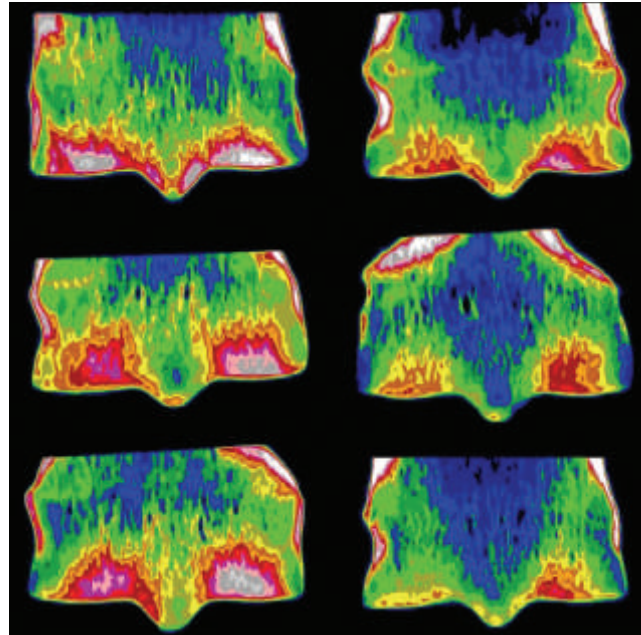


Fig 3: Computer-generated 1 mm thick tomographic slices in the frontal plane through the palmar aspect of the distal condyles of third metacarpal bones from experimental Thoroughbreds. The 3 images on the left are from horses that undertook a 19-week exercise programme on a high-speed treadmill, while those on the right are from control animals restricted to walk over a similar period. Density maps have been colour-coded: white = most radiodense, dark blue/black = least radiodense. Note increased radiodensity of the subchondral bone in the condylar region of bones from exercised horses compared with those from controls.

Even if they survive an episode of acute trauma, the metabolic activity of chondrocytes can be severely impaired for days or more (Patwari *et al.* 2001). Chondrocytes from bovine explants, which had been loaded to 50% strain for 5 mins 3 days earlier, lost their ability to respond positively to dynamic compression at physiological loads. Cells in control explants, which had not received the injurious load, showed significant increase in uptake of ^{35}S -sulfate, as a measure of proteoglycan synthesis, while loaded cells did not (Patwari *et al.* 2001).

There is evidence that some traumatised chondrocytes may undergo phenotypic modulation leading, ultimately, to inappropriate terminal differentiation (Westacott 2003). This process includes expression of markers, which lead to endochondral bone formation, and mineralisation of the surrounding matrix (Hashimoto *et al.* 1998; Kirsch *et al.* 2000).

Agents which inhibit or retard the process of apoptosis represent one potential new therapeutic strategy for reducing the risk of cartilage degeneration following trauma (Goggs *et al.* 2003). Interestingly, hyaluronan and polysulphated glycosaminoglycan both may have a protective influence on chondrocytes by inhibiting apoptosis (Goggs *et al.* 2003).

Increased risk/rate of cartilage damage through change in its mechanical environment (subchondral bone sclerosis): Subchondral bone is an integral component of the synovial joint; it supports the articular cartilage, maintains shape of

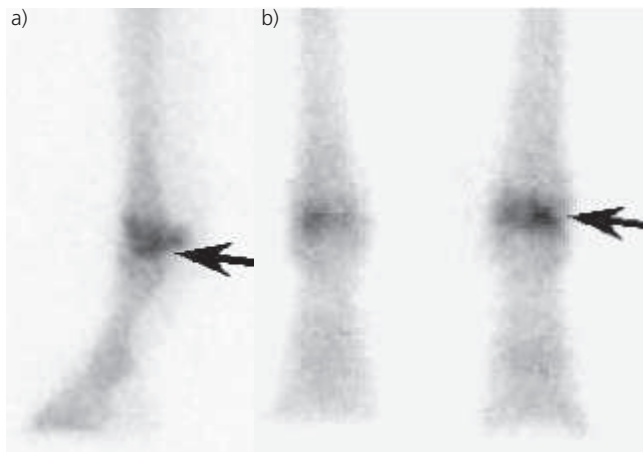


Fig 4: Scintigraphic images of the distal forelimbs of a Thoroughbred racehorse. There is intense focal uptake of radiopharmaceutical in the lateral condylar region (arrows), localised to the palmar aspect on the lateral view (a).

the bearing surface and transmits loads from the joint to the rest of the bone. It is well established that subchondral bone is an important focus of pathology in long-standing joint disease (hence the term 'osteoarthritis') and changes to this region are often identified radiographically as a characteristic feature of joint abnormalities in the horse (Butler *et al.* 1993). However, the exact nature and range of pathology that occurs in subchondral bone in response to injury, the significance of that pathology in relation to the function of the joint as a whole and its temporal relationship to pathology of other tissues in the development of osteoarthritis are important questions which remain unresolved, despite close scrutiny in recent years (Kawcak *et al.* 2001).

The importance of subchondral bone in relation to disease of articular cartilage revolves around 2 central issues:

- That changes in the material characteristics of subchondral bone result in altered strains in the overlying cartilage, leading to increased incidence and rate of damage to cartilage.
- That increased metabolic activity in subchondral bone may have direct biological effects on metabolic activity of the cartilage, for example through increased cytokine production. In addition, pathology of subchondral bone may potentially result in disturbance of nutritional avenues to deeper layers of joint cartilage.

Due to its narrowness, articular cartilage absorbs only a small proportion of energy associated with loads transmitted across a joint (1–3%); the majority is transmitted to the underlying subchondral bone and thence to the deeper spongiosa (30–50%), and the rest is largely accounted for by surrounding soft tissue structures (Radin *et al.* 1970). Ability of a material to absorb energy is determined, to an extent, by the amount it deforms to a given stress: an object which deforms more, particularly in an inelastic manner, absorbs more energy.

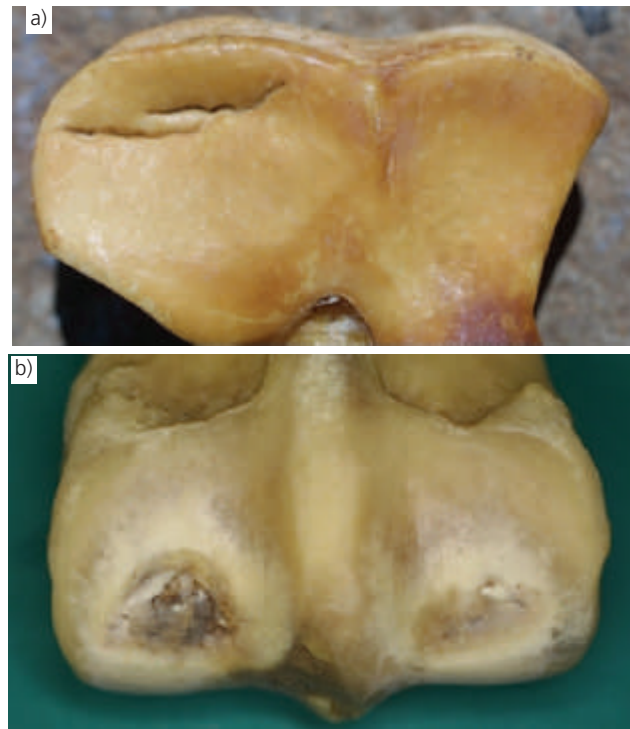


Fig 5: Gross pathology visible in the mineralised cartilage and subchondral bone in the a) radial articular facet of the third carpal bone and b) palmar aspect of the lateral condyle of the third metacarpal bone. Macerated specimens from 2 separate Thoroughbred racehorses.

Change in the material properties of bone lying beneath a joint will affect the relative proportion of energy absorbed at its surface, the cartilage (Fig 2). Depending on the extent of change in the bone and/or the presence of concurrent pathology in overlying cartilage this may have significant negative effects on the health of the cartilage, especially in impact loading. In addition, it has been suggested that normal joint function relies on deformation of the whole joint surface to achieve better congruence under load. Under light loads the points of contact between apposing surfaces are restricted, but when load is increased these regions deform, thereby bringing progressively more of the joint into apposition (Bullough 1980, 2001). This is hypothesised to provide an additional mechanism of shock absorption, to direct loads more effectively to the bone's diaphyseal cortex and to improve fluid flow through the cartilage (Bullough 2001; Kawcak *et al.* 2001). Excessive change in the material properties, through change in the bone density, will have a negative impact on this mechanism, resulting in less shock absorption of the joint as a whole and increased risk of high stress, point contact areas in the joint. Attempts to provide experimental confirmation of these hypotheses are fraught with difficulty. However, texture correlation, a technique used to provide trabecular strain information in cadaver specimens, demonstrated that artificial subchondral stiffening of subchondral bone using polymethylmethacrylate in sections from the human tibia doubled trabecular strain in the juxta-articular region (McKinley and Bay 1999).

An association between increased density of subchondral bone and joint disease has been suggested for many years in man. Patients with osteoarthritis of the hip have increased bone mass of the proximal femur compared with those without joint disease (Hannan *et al.* 1993) and osteoporosis is generally seen as a protective factor against osteoarthritis (Verstraeten *et al.* 1991; Dequeker *et al.* 1995). Work in experimental animals has demonstrated increase in apparent density of subchondral bone in response to impact loading which is associated with cartilage degeneration (Simon *et al.* 1972; Radin *et al.* 1973, 1984). Radin *et al.* (1984) hypothesised that it is the change in bone density (increased bone per unit volume, *Vf*) that leads to cartilage disease (Radin and Rose 1986). In an experimental model of impact loading using the rabbit stifle, increased bone remodelling determined scintigraphically *in vivo* and density (*Vf*) determined at *post mortem* preceded subsequent cartilage degeneration (Radin *et al.* 1984). Conversely, other studies, principally those which have used the canine anterior cruciate transection model to study the pathogenesis of osteoarthritis, have demonstrated significant reductions in *Vf* in the weeks and months following surgery, while cartilage pathology is reported to occur (Boyd *et al.* 2000, 2002). It is important to distinguish the primary cause of joint injury in the two groups, which may explain the difference in findings relating to subchondral bone. The impact-loading model used by Radin *et al.* (1984) will stimulate bone formation while the joint instability model used by Boyd *et al.* (2002) is likely to result in a degree of disuse osteoporosis, secondary to reduced limb loading.

Studies in the horse have shown regional increase in the density of subchondral bone of the proximal sesamoid bones (Young *et al.* 1991a), bones of the carpus (Young *et al.* 1991b; Firth *et al.* 1999; Goodship *et al.* 1999; Kawcak *et al.* 2000) and the distal condyles of the third metacarpal bone (Riggs and Boyde 1999; Kawcak *et al.* 2000) in response to increased levels of exercise (**Fig 3**). These are regions prone to joint disease (Pool 1996), although severe changes in the subchondral bone may occur despite the presence of cartilage pathology (see below).

Analysis of osteochondral tissues from 2 strains of guinea-pig showing different susceptibility to osteoarthritis shed a different light on the interaction of bone and cartilage in joint disease (Huebner *et al.* 2002). Strain 13 guinea-pigs have a lower basal rate of bone turnover and, at 2 months of age, thicker subchondral bone in bones of the femorotibial joint compared to the Hartley strain. However, by age 12 months Hartley animals develop more severe osteoarthritis coincident with a phase of rapid subchondral bone formation (Huebner *et al.* 2002). The implication from that study is that it is the process of subchondral bone thickening rather than the absolute thickness that is associated with the development of cartilage disease. This finding is consistent with scintigraphic studies in man, which indicated that increased subchondral bone metabolic activity was predictive of progressive osteoarthritis (Dieppe *et al.* 1993). It also provides a basis for the potential use of bisphosphonates in the management of osteoarthritis.

One explanation for the correlation between bone remodelling activity and the onset of cartilage disease is that inflammatory mediators originating from bone may be influencing chondrocytes and cartilage matrix. *In vitro* co-culture system devised to determine the effects of osteoblast-like cells on cartilage metabolism showed that cells derived from bone of osteoarthritic patients had the potential to increase glycosaminoglycan release from cartilage, whereas similar cells from nonarthritic patients did not (Westacott *et al.* 1997). This effect may be mediated by direct release of proteinases by osteoblasts or indirectly through release of proinflammatory cytokines. Given that cartilage destruction occurs primarily at the surface in osteoarthritis and that superficial chondrocytes are more sensitive to cytokines, the cytokine route seems more likely (Westacott 2003). It is conceivable that transfer of mediators of matrix destruction and cellular activity occur in the opposite direction, from cartilage to bone. There is increasing evidence that altered mechanical loading plays a major role in chondrocyte differentiation to hypertrophy and subsequent mineralisation of surrounding matrix (Westacott 2003). Transfer of factors involved in this process to adjacent subchondral bone may well have direct effects on osteoblast activity and subsequent bone density. While under normal circumstances there is no direct communication between bone and cartilage, small cracks have been identified in mineralised cartilage, extending into subchondral bone (Sokoloff 1993), providing a hypothetical route for exchange of inflammatory mediators between these 2 tissues.

Subchondral bone disease

Injury to subchondral bone is increasingly recognised as an important primary cause of joint pain in racehorses. A syndrome involving lameness associated with pain localised to the distal condyles of the third metacarpal/metatarsal bones (McIII/MtIII) and intense focal uptake of radiopharmaceutical in the palmar/plantar aspect of the condyles without other signs of joint disease has been described in the Thoroughbred racehorse (Shepherd and Pilsworth 1997; Arthur *et al.* 2003; Pilsworth 2003; **Fig 4**). Similarly, pain localised to the mid-carpal joint, associated with radiographic evidence of sclerosis of the third carpal bone and intense radiopharmaceutical uptake by this bone but few other findings, has been well documented (Arthur *et al.* 2003). The positive scintigraphic findings suggest a state of increased bone remodelling activity and have encouraged the use of the term 'nonadaptive remodelling' for this condition (Arthur *et al.* 2003).

Gross pathology of subchondral bone is commonly identified at both of these anatomical locations in horses subjected to repetitive, high-speed exercise (Hornof *et al.* 1981; O'Brien *et al.* 1985; Pool 1996; **Fig 5**). Characteristic lesions occur in the palmar/plantar aspect of the distal condyles of McIII/MtIII that show a spectrum of severity, ranging from blue discolouration of subchondral bone, visible through the overlying cartilage, to severe osteochondral ulcers (**Fig 6**). The lesions occur at the surface of a volume of severely

sclerotic subchondral bone, occupying the palmar/plantar distal quadrant of the affected condyle, and involve an area of avascular bone necrosis in the immediate subchondral region (Hornof *et al.* 1981; Norrdin *et al.* 1998; **Fig 7**). Fragmentation of necrotic bone may extend several millimetres below the cartilage in some cases (Norrdin *et al.* 1998). The overlying cartilage is generally viable (Hornof *et al.* 1981; Norrdin *et al.* 1998). Microradiographic studies indicate increased mineral density of the necrotic bone, immediately below the mineralised cartilage (Riggs *et al.* 1999a). Similar changes have been reported underlying articular cartilage of the radial articular facet of the third carpal bone (Pool 1996).

The correlation between clinical signs of subchondral bone disease and the pathology described above has yet to be fully elucidated. Clinical cases in which there is acute collapse of the osteonecrotic ulcer in the palmar/plantar aspect of the condyles of McIII/MtIII are usually associated with severe lameness, joint pain and clearly visible findings on appropriate radiographic projections. However, early cases, which may also be associated with severe lameness, may have only mild bruising of the subchondral bone visible at *post mortem* (Pilsworth 2003). Conversely, horses affected by quite severe pathology may show only mild lameness.

Magnetic resonance imaging (MRI) performed *post mortem* on a horse in which an osteochondral defect of the palmar aspect of McIII had previously been identified arthroscopically was able to demonstrate loss of articular cartilage associated with sclerosis of the underlying bone (Martinelli *et al.* 1996). Recent studies with more refined equipment using axial short tau inversion recovery (STIR) and dual echo (T2-weighted and proton density) protocols demonstrated regions of high signal intensity at the margins of intense bone sclerosis in the distal condyles of the MtIII in 2 horses which had pain localised to this region (Zubrod *et al.* 2004). The medial condyle was affected in both MtIII in one case and the lateral condyle of the left MtIII in the other. Similar MRI findings are frequently reported in man in association with subchondral bone disease and are commonly referred to as representing bone bruising or bone oedema (Rangger *et al.* 1998; Rubin *et al.* 2000; Sowers *et al.* 2003). In one study, histological examination of biopsy material retrieved from the centre of such MRI lesions in 3 separate patients who had suffered recent, acute trauma revealed microfractures of cancellous bone, oedema and bleeding in the fatty marrow (Rangger *et al.* 1998). Another study of material obtained from regions of bone with hyperintense MRI STIR signal (Zanetti *et al.* 2000) found bone marrow necrosis, bone marrow fibrosis and trabecular abnormalities, but no evidence of significant bone marrow oedema. Patients in the latter study were all suffering from chronic, advanced joint disease and it may be that intraosseous bleeding and oedema are genuine findings in acute disease.

One study of 41 people with early or mid-stage degenerative arthrosis and osteonecrotic lesions in the subchondral bone and metaphyseal region of the proximal tibia documented a spectrum of MRI findings that were associated with severe acute or subacute pain (Lotke *et al.* 2000). Smaller lesions tended to resolve while larger areas were associated

with rapid deterioration in clinical signs. The authors speculated that oedema in the closed metaphyseal space was a cause of pain and if pressures became high enough may cause cell death. Pedersen *et al.* (1989) recorded significantly elevated intraosseous pressures in the subchondral region of hip bones in patients with idiopathic osteonecrosis of the femoral head, but not in patients suffering from just arthrosis of this joint. Idiopathic osteonecrosis of the medial condyle of the femur is a well-recognised and extremely painful condition in man (Lotke *et al.* 2002). Vascular compromise and ischaemia of the bone is the most commonly accepted explanation for this condition, and is often associated with a generalised coagulopathy or thrombophilia (Lotke *et al.* 2002). Trauma is the second most common explanation (Lotke *et al.* 2002). Blockage of vascular canals has been demonstrated in osteonecrotic bone in the palmar/plantar aspect of the distal condyles of McIII/MtIII in the horse (Hornof *et al.* 1981; Norrdin *et al.* 1998). However, it is currently not possible to determine whether this is the cause of associated bone pathology or a result of it. The density of viable osteocytes in subchondral bone from the radial carpal and third carpal bones and the distal condyles of McIII from experimental horses was significantly lower in horses which had undergone an exercise programme in the 6 months prior to euthanasia than from a similar group which had been rested (Kawcak *et al.* 1999). It is conceivable that bone cells are killed (necrosis) or die (apoptosis) as a consequence of trauma. Remodelling may then be inhibited and microdamage would accumulate that, in time, may lead to disruption of vascular supply to the bone (**Fig 8**). Further work is required to substantiate this hypothesis. High-resolution MRI will be a useful modality for such studies.

Fatigue fractures

Articular-based fractures are common in Thoroughbred racehorses (Johnson *et al.* 1994a,b; Parkin *et al.* 2000; Verheyen and Wood 2004). The majority of these fractures fit the clinical criteria for inclusion as stress fractures (Riggs 2002):

- Associated with high-speed work.
- Occur in the absence of a specific traumatic event.
- Arise in consistent locations.
- Follow a predictable course through the bone.
- Show progressive characteristics (incomplete fractures are commonly identified at similar locations).

Recent work has provided histopathological evidence to support a fatigue-based mechanism for one of the more widespread fractures in the racing Thoroughbred (Boyde *et al.* 1999; Riggs *et al.* 1999a,b). Parasagittal fractures of the distal condyles of McIII/MtIII account for more than 25% of all fatal fractures in racehorses (Johnson *et al.* 1994a) and have a total incidence of approximately 1.3% among horses in training (Bathe 1994). In common with other 'spontaneous-type' fractures, the morphology of parasagittal fractures of the distal condyles of McIII and MtIII is highly consistent. They typically involve longitudinal fissures, which originate in the distal

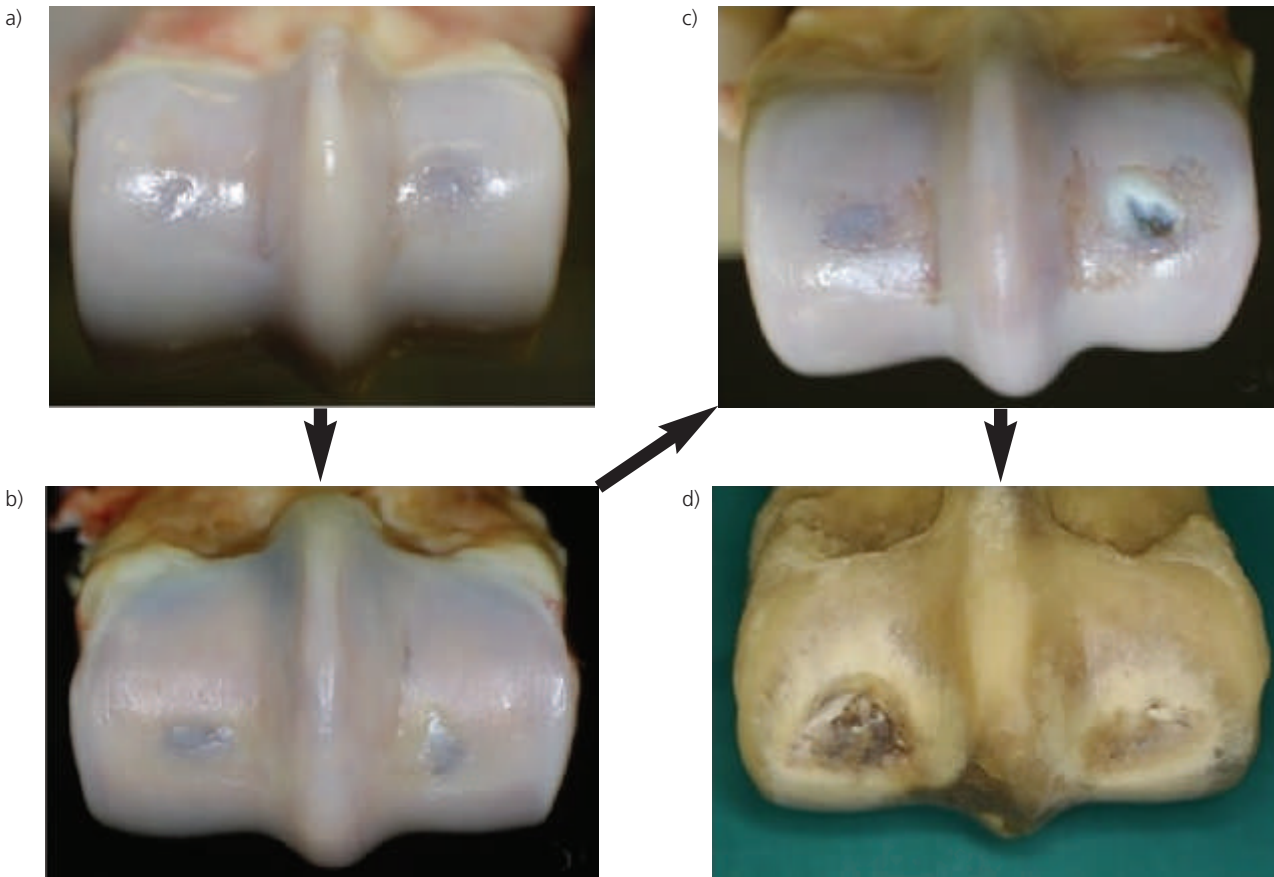


Fig 6: Gross view of the palmar aspect of the distal condyles of the third metacarpal (a, b, d) and metatarsal (c) bones from Thoroughbred racehorses, illustrating the spectrum of severity of osteochondral disease. a–c) Fresh specimens, lateral to the right; d) macerated specimen, lateral to the left.

articular surface of the bone near the junction of the condyle and the sagittal ridge (Ellis 1994; Zekas *et al.* 1999; **Fig 9**). The fractures are oriented in the sagittal plane and either curve abaxially as they propagate, so that they exit the lateral cortex between 50 and 135 mm proximal to the distal articular surface of the bone (Rick *et al.* 1983), or spiral proximally up the diaphysis of the bone (Richardson 1984; Ellis 1994).

Macro- and micromorphological studies of the distal condyles of McIII/MtIII demonstrated characteristic patterns of sclerosis of the subchondral bone in Thoroughbred racehorses. Bone forming the palmar aspect of the condyles was significantly more dense than that of the sagittal ridge (Riggs *et al.* 1999b). This pattern correlated well with the predicted distribution of loads on the articular surface and, presumably, represented an adaptive response to them. Foci of intense remodelling activity (manifesting as resorption cavities and endosteal new bone) in subchondral bone at the junction of the condyles and the sagittal ridge were frequent findings in bones from horses which had been in training at the time of death (Riggs *et al.* 1999a). These changes were often associated with narrow linear defects in the overlying subchondral bone plate, orientated around the circumference of the palmarodistal aspect of the condyles (Stover *et al.* 1994; Riggs *et al.* 1999a; **Fig 10**). The defects were in the same anatomical location as the site where parasagittal fractures of the condyles originate. Subsequent inspection of the articular

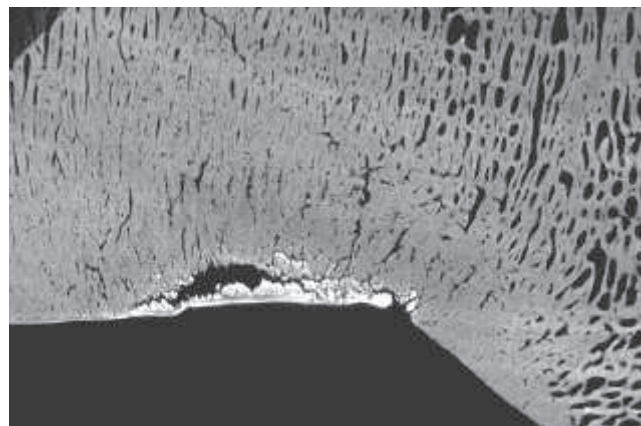


Fig 7: Microradiograph of a 100 µm section through the palmar aspect of the distal condyles of the third metacarpal bone from a 4-year-old Thoroughbred racehorse. Note the increased radiopacity of mineralised cartilage and immediate subchondral bone and collapse of deeper bone tissue. Field width = 15 mm.

margin of clinical fractures revealed that they originated from defects in the subchondral bone plate and passed through areas of intense focal resorption in underlying bone (Riggs *et al.* 1999a). Therefore, the pattern of changes is similar to that seen in 'classic' stress fractures that affect the shafts of long bones

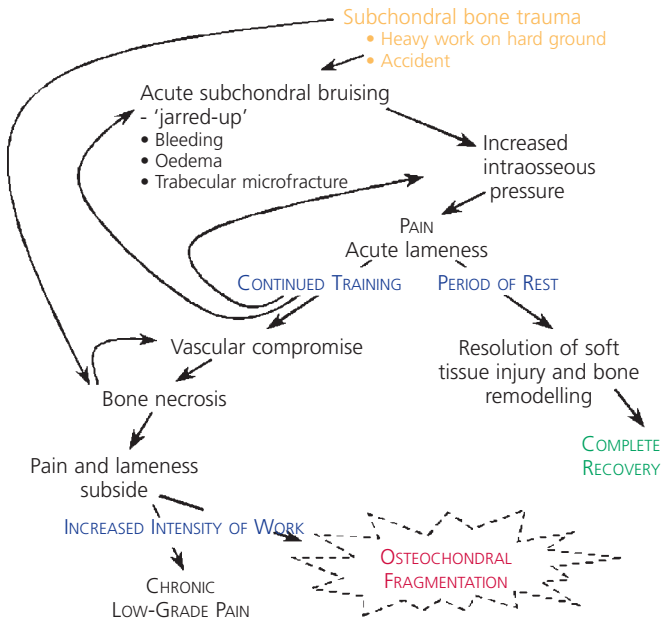


Fig 8: Flow diagram illustrating hypothetical pathogenesis of osteonecrosis of the distal condyles of the third metacarpal and metatarsal bones.

(Riggs 2002). It was hypothesised that sclerosis of the condyles but not the sagittal ridge creates a density and, hence, stiffness gradient which results in concentration of stress at the axial margin of the condyles. This, in turn, is likely to increase the intensity of local microdamage, which will stimulate focal bone resorption, thereby significantly weakening the bone at this specific location. Continued cyclical loading of the bone in the face of these changes is likely to lead to catastrophic fracture. Similar sclerotic changes have been described in the third carpal bone of Thoroughbred racehorses (Young *et al.* 1991b).

In both the distal condyles of McIII/MtIII and the third carpal bone, functionally adaptive increases in density of the subchondral bone in focal regions of high joint loading appear to be essential for the initiation of disease. The importance of osteonecrosis in the progression of pathology to fracture has not yet been determined. However, these fractures do appear to be the end-stage of a process which occurs over weeks or months, and which it may be possible to identify with appropriate screening methods. Novel serum biomarkers for osteochondral disease and/or diagnostic imaging may facilitate such an approach in the future.

Conclusions

Repetitive high-speed activity is well recognised as a significant risk factor for the development of osteoarthritis. It is increasingly apparent that the pathological effects of the trauma associated with high-impact loads, which may ultimately lead to osteoarthritis, are varied and occur in all tissues of the joint. Some of these effects are immediate while others are expressed over time, as the consequences of disruption of normal homeostatic mechanisms become manifest.



Fig 9: Typical example of a complete displaced parasagittal fracture of the lateral distal condyle of the third metacarpal bone from a Thoroughbred racehorse.

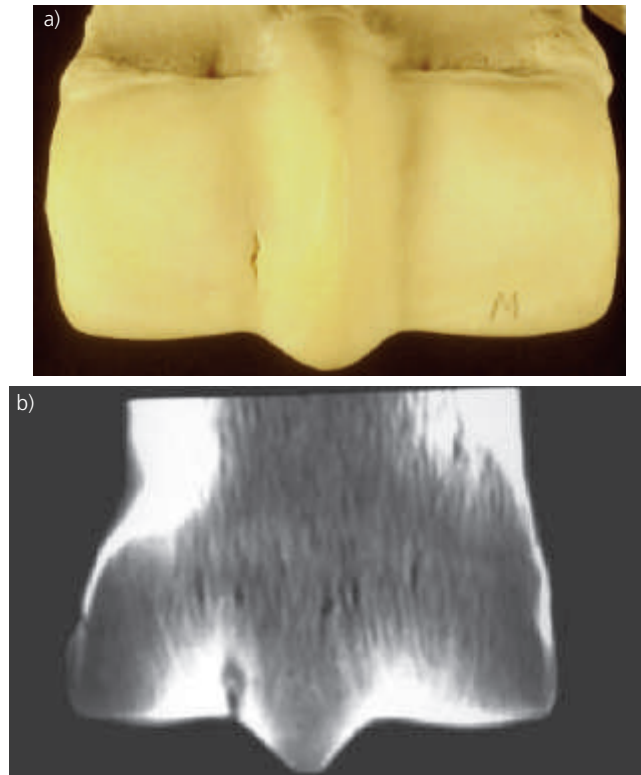


Fig 10: a) Palmar surface of the distal condyles of a third metacarpal bone (McIII) from a 6-year-old Thoroughbred racehorse after digestion of nonmineralised cartilage. Note the deep linear cleft in the mineralised cartilage and subchondral bone in the lateral condylar groove (lateral is to the left). b) Computer-generated 1 mm thick tomographic slice in the frontal plane through the palmar aspect of the distal condyles of McIII from a 12-year-old Thoroughbred racehorse. Note the defect in the subchondral bone underlying the lateral condylar groove (lateral is to the left).

Better understanding of the response of tissues of the joint to trauma paves the way for the development of strategies which may prevent progression to disease, e.g. modified training regimes, development of more sensitive techniques with which to diagnose disease earlier and formulation of novel treatments, targeted specifically at the most relevant tissues.

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