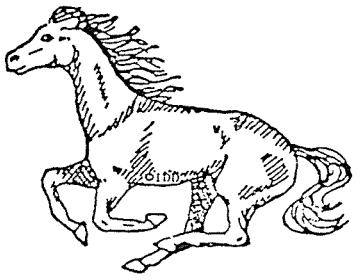
Investigation of naturally occurring osteoarthritis in the metacarpophalangeal joints of wild horses.



A thesis presented in partial fulfilment of the requirements for the degree of
Master of Veterinary Science
at Massey University

Charlotte Emily Louise Cantley
1997

CONTENTS

Chapter 2
Materials and Methods

Chapter 3
Results

Chapter 4
Discussion

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖

List of illustrations

Chapter 1

Chapter 2

Chapter 3

List of Tables

Chapter 1

Chapter 2

Chapter 3

Appendix

Acknowledgements

Thank you to **Professor Elwyn Firth** for imparting some of his great enthusiasm and knowledge to me. Elwyn provided central ideas and valuable guidance that helped create this thesis. **John Delahunt**, from Wellington Medical School, very kindly arranged permission for me to use the Lower Hutt Hospital DEXA scanner for this project. John also spent many long patient hours teaching me how to use the scanner and helped me on numerous occasions with both technical and theoretical problems. Thanks to **Keith Thompson** for sharing his histopathological wisdom with me and also for his constant encouragement and positive comments about my work. I am very grateful to **Dirk Pfieffer** who patiently and cleverly guided me through the statistical seas until I was fit to sail alone. My appreciation also goes to **Helen Hodge** who spent many hours helping me in the post-mortem room at the beginning of the study and to **Simone Holmes** who provided expert assistance in the preparation of the histological sections. I am thankful to **Kevin Stafford** who not only collected the horses' legs used in this study, but also provided information on the Kaimanawa horses and always made me laugh. Thank you to **Kathy Gibson** who assisted me greatly by constructively criticising this manuscript and also for giving me continuous encouragement. I really appreciated the help of **Lizzy Chambers, Roz Machon**, and **Adam Hittmann** who proof read the manuscript and offered me constant support and advice. Finally thanks **Gayle McKenna** for her help in printing this thesis.

Abstract

The purpose of this study was to assess the site, prevalence and characteristics of lesions affecting the proximodorsal aspect of the first phalanx (P1) in the forelimbs of wild horses.

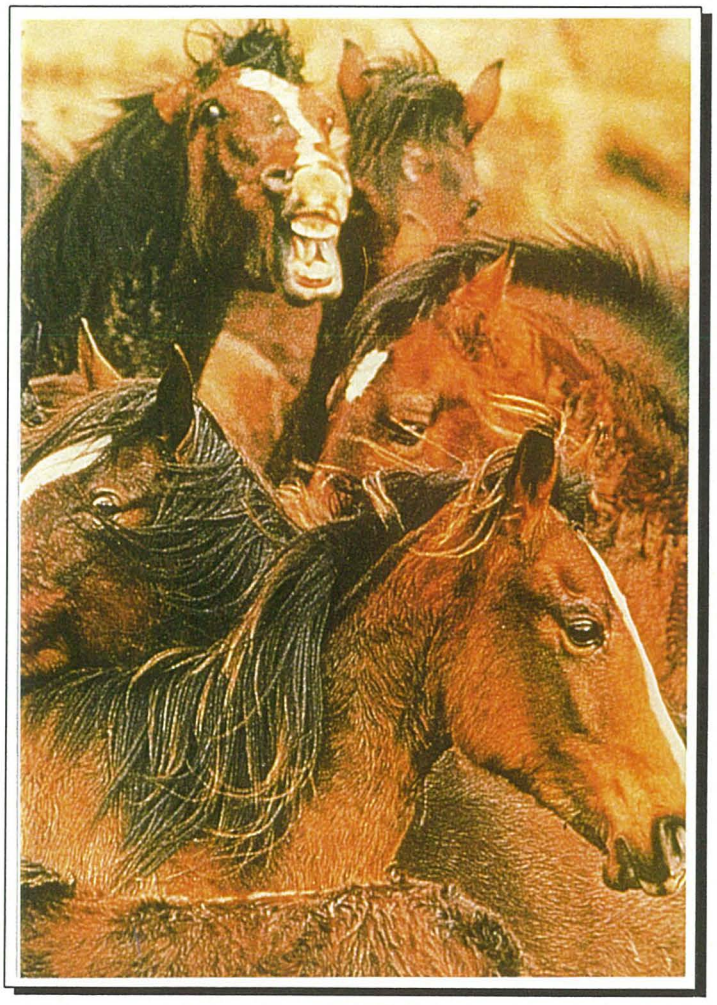
An investigation was made of the metacarpophalangeal joints of 22 wild New Zealand horses with a mean age of 7.36 + 3.27 years (range 2 - 14 years). The articular surfaces of the metacarpophalangeal joints were stained with Indian ink and macroscopic lesions on the medial and lateral eminences of P1 were graded. Radiographs were taken of 2mm thick sagittal bone slabs sawn from both the lateral and medial eminences of proximodorsal first phalanx. The subchondral bone mineral density for five regions on the proximo-dorsal aspect of each bone slab was determined using a Norland XR-26 bone densitometer. Histological sections of the bone slabs were then prepared and the articular cartilage lesions on the proximo-dorsal aspect of P1 were assessed using both subjective and objective scoring methods.

Subjective assessment of cabinet radiographs showed subchondral bone sclerosis to be greater in those horses with severe articular cartilage damage. The subchondral bone mineral density also increased with age and with increasing severity of lesions in the overlying articular cartilage. Ossicles with a distinct trabecular bone pattern were identified at the proximo-dorsal margin of P1 in eight specimens from 5 horses. The macroscopic and histological articular cartilage scores increased significantly with age and the lesions were more severe on the medial compared with the lateral eminence of P1.

The study demonstrated cartilage changes, wear lines and subchondral sclerosis, consistent with osteoarthritis in the metacarpophalangeal joint of wild horses. The

severity of the changes increased with age. There was a significant relationship between subchondral bone sclerosis and overlying cartilage changes in the proximo-dorsal aspect of P1. The observations represent an age-related osteoarthritic process that may be present in all horses.

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖



Chapter 1

Introduction

1.1 Osteoarthritis - its importance and background

Lameness is an important cause of wastage in the equine industry. In studies from England and North America the greatest loss from racing was caused by lameness (Goodman and Baker 1990; Rossdale, *et al.* 1982), and osteoarthritis was identified as a prominent reason for lameness. Osteoarthritis is defined as a progressive disease of diarthrodial joints and is characterised by progressive deterioration of articular cartilage and reactive changes at the joint margin and joint capsule (Caron 1992; Pool and Meagher 1990).

Osteoarthritis occurs in many species and is a major cause of lameness in horses and dogs, as well as being the single largest cause of locomotor disease in humans. Human and animal skeletal evidence show that the disease has been present since Neolithic times (Brothwell *et al.* 1969; Rogers *et al.* 1981). Habreden (1802) described osteoarthritic joints as the "nodes of Habreden", and in 1805 Haygarth wrote a detailed description of osteoarthritis, recognising that it could be a polyarticular disease. The advent of radiography allowed the various forms of the disease to be categorised into an 'atrophic' type, characterised by synovial inflammation with osteoporosis and erosion of the adjacent bone and cartilage, and a 'hypertrophic' type, featuring focal loss of articular cartilage, with hypertrophy of the underlying bone at the joint margins (Goldwraith 1897; Nichols *et al.* 1909). The atrophic or inflammatory type of arthritis was often polyarticular and more likely to affect juveniles. In some cases, hypertrophic osteoarthritis appeared to be linked to

preceding trauma and in others to ageing. This, combined with the central pathologic feature of 'degenerative' articular cartilage, led to the concept of an age-related, 'wear and tear' of synovial joints or a degenerative arthropathy.

In 1926, Ceil and Archer linked polyarticular osteoarthritis with menopause, and Kellgren and Morre (1957) classified this disorder. This information was helpful in the differentiation of two main types of osteoarthritis, the 'primary' form of the disease which mainly affects women, in which multiple joints, especially the hands, are affected and the 'secondary' form in which trauma or some other joint insult results in osteoarthritis of one or more joints.

The basic pathology of osteoarthritis was first outlined from post-mortem studies by Hein (1926) and Collins (1953). Subsequent studies, mainly involving the human hip joint, led to a more complex and dynamic concept of osteoarthritis (Byers et al 1970; Lloyd-Roberts 1953). Sokoloff (1969) reported that much of the pathology of osteoarthritis represents repair, rather than degeneration. The work of Trueta et al (1968) introduced the concept that osteoarthritis is a natural reaction of synovial joints to injury and is a product of normal remodelling or repair processes.

A five point radiographic scoring system of osteoarthritis was developed (Kellgren 1963) (Table 1.1), which was subsequently adopted by the World Health Organisation and became the gold-standard for assessment of human osteoarthritis. Kellgren (1963) identified a very poor relationship between radiographic changes and pain (Cobb *et al.* 1957; Lawrence *et al.* 1966).

Table 1.1 Radiographic grading system for human osteoarthritis

RADIOGRAPHIC GRADING SYSTEM FOR HUMAN OSTEOARTHRITIS		
Grade	**Classification**	**Description**
0	Normal	No features of osteoarthritis
1	Doubtful	Minute osteophyte
2	Minimal	Definite osteophyte
3	Moderate	Mild reduction in joint space
4	Severe	Greatly reduced joint space and subchondral bone sclerosis.

The studies over the last two decades have led to the current concept of human osteoarthritis, which is now viewed as an age-related, dynamic, reaction pattern of a joint responding to insult or injury. All tissues of the joint are involved, although the loss of articular cartilage and changes in adjacent bone remain the most striking features. It is now clear that the pathogenesis of osteoarthritis involves the interaction of intrinsic and extrinsic components and is initiated by many different factors.

The focus of osteoarthritic research has now swung towards biochemical and cellular investigations of the disease. The mechanisms controlling the biochemical and physical properties of articular cartilage was pioneered by Muir (1977) and Dieppe *et al.* (1991). This work has emphasised the central role of the chondrocyte in matrix synthesis, and of factors such as cytokines that influence chondrocyte activity. Biochemical research has also significantly contributed to the understanding of osteoarthritis (Mow *et al.* 1980; Pool 1993). The importance of physical forces on joints has been recognised in the pathogenesis of osteoarthritis for decades and recent technology has led to highly sophisticated investigations of biomechanical factors including the effects of physical force on the chondrocyte (Gray *et al.* 1988; Radin 1991).

In humans, osteoarthritis is strongly age related, with a peak of onset between 50 and 60 years and a predilection for the knees, certain joints of the hands, the hips and the small joints of the spine. In western populations, radiographic evidence of osteoarthritis occurs in approximately 65% of people of 65 years, about 80% of those aged over 75 years and is more common in women (Cushnaghan 1991). Pain is the most frequently observed clinical sign, most often related to use, but sometimes occurring at rest and at night. The range of joint movement is often restricted and joint motion commonly associated with pain and crepitus. There is usually a firm swelling of the affected joint on palpation.

Osteoarthritis is a commonly diagnosed condition in horses, but despite its immense importance to the equine industry it is not yet fully understood.

1.2 Normal structure and function of synovial joints

The synovial or diarthrodial joint comprises two or more articulating bones, the joint capsule, synovial fluid, joint cavity, the articular cartilage and in some joints, the meniscus.

The joint capsule, which is highly innervated and well vascularised, consists of an outer fibrous layer that is continuous with the periosteum, and an inner layer, the synovial membrane (fig. 1.1).

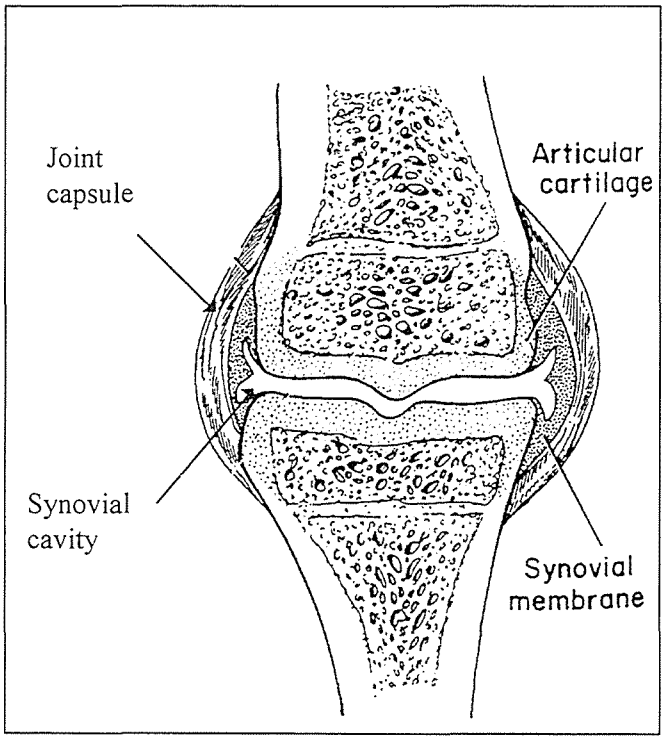


Figure 1.1 Diagrammatic representation of a normal metacarpophalangeal joint. *From Stashack T (1987) Adams Lameness in Horses (4th Edition), Lea & Febiger, Philedelphia.*

The synovial membrane consists of 2 types of cells (synoviocytes) loosely arranged in 3 - 4 layers, namely type A cells, which are mainly phagocytic, and type B cells which are responsible for synthesising hyaluronate (Ghandially 1978) (fig. 1.2). The functions of a normal joint are: free, painless movement of opposing articular surfaces within the required range of movement; the correct distribution of load across joint tissues that might otherwise be damaged by mechanical overloading (Palmoski 1980); maintenance of stability during use. The complementary shape of the opposing articular surfaces (one being convex and one concave) permits the range of motion, provides stability and ensures the most equitable loading during use.

No normal joint is truly congruent, because joints move in more than one direction and some are multiaxial (MacConaill 1950; Wormsley 1928). However synovial joints are designed such that the area of articular surface contact (congruency) increases with loading.

The different components of the normal synovial joint are discussed below, followed by a description of joint lubrication and innervation.

1.2.1 Synovial Fluid

Synovial fluid is an ultrafiltrate of plasma and contains hyaluronate, plasma electrolytes and plasma proteins in concentrations inversely proportional to their molecular weight. There are very few, if any, large molecules such as fibrinogen in synovial fluid. Hyaluronate is responsible for giving synovial fluid its viscosity and is the only glycosaminoglycan that is not sulphated.

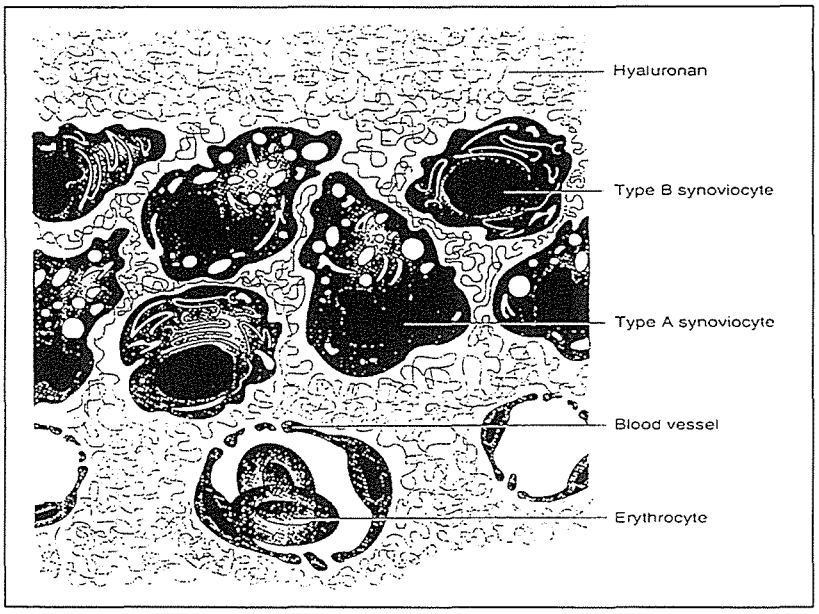


Figure 1.2 Diagrammatic representation of a histological section of synovial membrane with hyaluronic acid coating the surface. *(From: McIlwraith, C.W., and Trotter, G.W, (1996) Joint Diseases in Horses (ed) Saunders, W.B.).*

Hyaluronic acid is also an important factor in the boundary lubrication of the synovial membrane and fibrous joint capsule which is a major contributer of the frictional resistance in joint movement (Swann and Radin 1972; Swann 1978).

The concentration of hyaluronate in normal human synovial fluid is 2 to 3 mg/ml (Sunbald 1965) while normal equine synovial fluid contains 0.5 mg/ml (Saari *et al.* 1989).

1.2.2 Articular cartilage

Articular cartilage is hyaline cartilage that covers the part of the epiphysis which articulates with an apposing bone to form a joint. It is semi-transparent, smooth and blue-white in appearance. Some variation in articular cartilage colour, thickness and regularity of

contour may occur at particular sites, for example the triangular-shaped area of articulation between the equine metacarpus or metatarsus and the proximal sesamoid bones and synovial fossae.

Articular cartilage is composed of chondrocytes within a matrix, which is itself composed of water-soluble proteoglycans interspersed with collagen fibres. Articular cartilage has water holding and elastic properties (Freeman 1972), has no nerve supply, is avascular and has a high matrix-to-cell ratio.

Chondrocytes

Chondrocytes in general are responsible for articular cartilage metabolism and matrix synthesis. In articular cartilage these cells are arranged in four distinct zones or layers which are delineated from the calcified cartilage by the "tide mark". This is the junction between non-calcified and calcified cartilage evident on histologic section. The superficial tangential layer has the highest cell density. The chondrocytes in this layer are relatively small and flat (cigar shaped), and are orientated with their long axes parallel to the articular surface. The transitional layer contains round, immature cells containing very large nuclei and produce fine strands of collagen. In the radial zone the cells are bigger than the cells in the preceding zone, and arranged with their long axes perpendicular to the surface and calcified zones (Schenk *et al.*1988). In the calcified zone, the cells are smaller again with pyknotic nuclei, and lie in lacunae surrounded by calcium salts.

Collagen

The collagen fibrils in articular cartilage interact to form a woven mesh that restrains the proteoglycan aggregates. The formation of covalent bonds between collagen molecules adds to the overall tensile strength of the cartilage (Broom 1982). Using a chick model, Miller and Matsukas (1969) demonstrated that the collagen fibrils within matrix were all of Type II. Collagen fibrils account for about 75% of the dry weight of immature cartilage and decrease to about 50% in the mature animal (Kang 1981). The tangential layer collagen fibrils are small, tightly packed, and lie parallel to the surface. In the

transitional layer, the collagen fibrils are thicker, and are arranged in an oblique criss-cross fashion down to the calcified layer. This pattern enables the cartilage to withstand shearing stress superficially and compression more deeply.

Proteoglycans in articular cartilage

Proteoglycans are high molecular weight molecules (1000-3000 kDa) consisting of a central protein core to which many glycosaminoglycan side chains are covalently bonded. Most of the proteoglycans within the collagen framework of articular cartilage are bound non-covalently to hyaluronic acid molecules to form large proteoglycan aggregates (Hardingham, *et.al.* 1990; Stockwell 1979). Up to 90% of the total mass of the proteoglycan aggregates are glycosaminoglycans (carbohydrate polymers consisting of repeating disaccharide units). There are three types of glycosaminoglycans in articular cartilage, the proportions of which vary with age (Freeman 1979):

1. Chondroitin sulphate, which consists of a repeating glucuronic acid and N-acetylgalactosamine disaccharide unit. This glycosaminoglycan can exist as either chondroitin-6-sulphate or chondroitin-4-sulphate, depending on whether the sulphate group is bound to the sixth or fourth carbon atom of the galactose molecule. The proportion of chondroitin-6-sulphate increases with age (Freeman 1979; Neam 1993).
2. Keratan sulphate, which consists of a repeating galactose and N-acetylglucosamine disaccharide unit (Heinegard and Hascall 1974).
3. Hyaluronic acid, which is the only non-sulphated glycosaminoglycan. It is made up of repeating disaccharide units of N-acetyl glucosamine and glucuronic acid.

The proteoglycan monomers are composed of a core protein to which the glycosaminoglycans are covalently bonded (fig. 1.3). Hyaluronic acid is the nucleus of the proteoglycan aggregates in the extracellular matrix of articular cartilage. Each proteoglycan aggregate is made up of one molecule of hyaluronic acid and 50-100 proteoglycan monomers coupled to the hyaluronic acid by a linking glycoprotein (fig. 1.4). The polyionic charges of the glycosaminoglycan side chains repel each other and attract a hydration shell,

thereby providing the cartilage with its properties of selective permeability and compressive stiffness (Kempson *et al.* 1970).

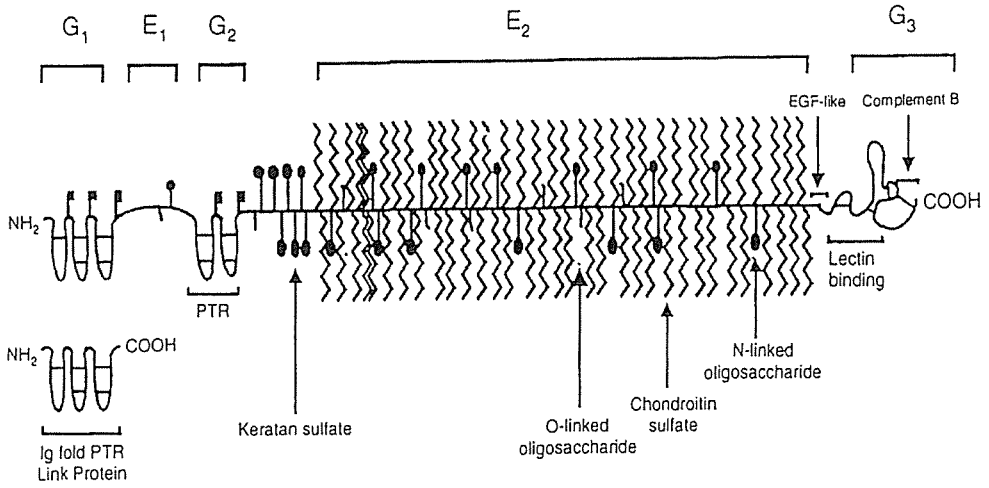


Figure 1.3 Schematic diagram of a proteoglycan monomer. (*From: Mcllwraith, C.W., and Trotter, G.W, 1996, Joint Diseases in Horses (ed) Saunders, W.B.)*

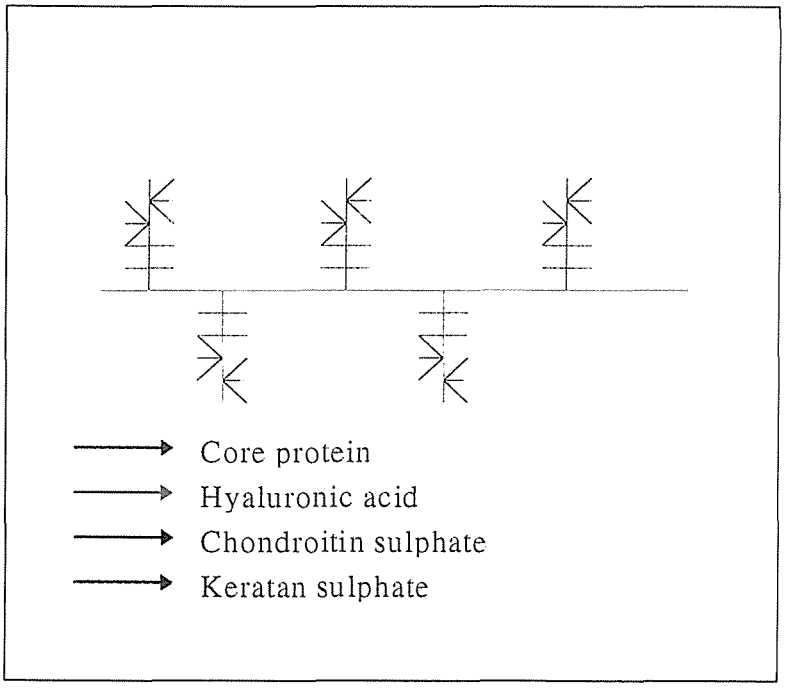


Figure 1.4 A simplified diagrammatic representation of a portion of a proteoglycan aggregate in articular cartilage.

Nutrition and metabolism of adult articular cartilage

Nutrition of articular cartilage is provided via the synovial fluid, and is dependent on the capillary blood supply to the synovial membrane. Nutrients flow freely through the synovial fluid, and diffuse through the articular cartilage matrix to the chondrocytes. Joint motion is necessary to circulate the synovial fluid and distribute the nutrients to, and remove the metabolites from the matrix (Maroudas 1973).

The relative rates of matrix synthesis and degradation are adjusted to achieve net growth, remodelling or equilibrium. Cartilage is degraded by proteases, and small low molecular weight proteins inhibit the degradation process (Howell 1969; Murphy 1991). It is possible that these low molecular weight proteins contribute to the normal equilibrium between anabolism and catabolism of cartilage. When this equilibrium is disturbed and catabolism predominates, osteoarthritis can be a consequence.

Age effects on articular cartilage

The significance of osteoarthritis in the older human population has prompted great interest in the effect of age on proteoglycan composition of articular cartilage. There is a higher proportion of keratan sulfate, and the chondroitin 6 - sulfate : chondroitin 4 - sulfate ratio is greater in adult compared to immature articular cartilage (Roughly *et al.* 1986). The normal changes that occur in the proteoglycans during ageing, however, are not necessarily the same as those that occur in osteoarthritic cartilage, and there are no similar data available for horses.

The relationship between ageing and articular cartilage fibrillation

Hein (1926) investigated the incidence of articular cartilage damage in relation to age in 1002 autopsies. Cartilage fibrillation became more common and severe with increasing age in several different synovial joints. The relationship between cartilage degeneration and age was confirmed in a number of subsequent studies of the knee, shoulder, elbow and hip joints (Bennett *et al.* 1942; Collins and Meachim 1961; Goodfellow and Bullough 1967). Byers *et al.* (1970) showed that histologically overt

fibrillation initially affects the periphery of the articular cartilage and local factors such as trauma may contribute to its progression (Meachim 1975).

Progressive and non-progressive articular cartilage fibrillation

Byers *et al.* (1970) classified the articular cartilage alterations in the hip on the basis of anatomical site, macroscopic appearance and radiography. Observations made on random femoral heads from a series of necropsies, were compared with those from hip resections conducted for the treatment of osteoarthritis. Femoral heads frequently developed age-related cartilage lesions which did not have an inherent potential to progress to clinical disease. These lesions were termed "non-progressive" cartilage lesions. A second type of cartilage alteration was identified on the femoral heads and was considered pathologically osteoarthritic in type. These cartilage lesions were termed "progressive" and were occasionally seen incidentally at necropsy and frequently seen on femoral heads resected during the treatment of osteoarthritis. Byers *et al.* (1970) considered the non-progressive and progressive articular cartilage processes to be independent of each other and proposed that articular cartilage changes in osteoarthritis may not simply be an exaggerated or accelerated form of the cartilage fibrillation that occurs during the 'normal ageing' process. A random survey of human synovial joints from 350 autopsies (Emery and Meachim 1973) showed that articular cartilage fibrillation spread across the cartilage surface, usually from a peripheral focus. Susceptibility to tangential spread varied between anatomical sites and between different individuals. The vertical spread of fibrillation into the articular cartilage, resulting in destructive thinning and potential exposure of bone, was also dependent on anatomical site and the subject.

1.2.3 Subchondral bone

The subchondral bone does not only provide structural support to the overlying articular cartilage. The undulating insertion of calcified cartilage on the subchondral bone plate creates the intimate relationship that results in the bone and cartilage acting as a unit rather than as independent entities. It has been proposed that an increase or decrease in

subchondral bone stiffness outside the physiological range may result in articular cartilage damage (Radin and Rose 1986). The bone mineral content (hydroxyapatite) contributes greatly to the stiffness of subchondral bone and enables the support of relatively high loads without substantial deformation. The inorganic component of bone is approximately 65% of the total bone matrix. The remaining 35% (of which 25% is water) is 95% collagen (predominantly type I) and 5% proteoglycans (Fisher and Hawkins 1987).

The subchondral bone plate and epiphyseal bone form an integral part of joint structure and are remodelled by bone-forming cells (osteoblasts), and bone resorbing cells (osteocytes). There is a close relationship between bone resorption and bone formation (Simmons and Grynpas 1990). The uncalcified extracellular matrix, osteoid, is secreted by osteoblasts. As soon as the osteoblast is surrounded by mineralised matrix, it is called an osteocyte (Hall 1990).

When a load is applied to a joint, the articular cartilage and bone undergo elastic deformation which bring the joint surfaces in contact with each other. Wolff's hypothesis states that bone density and bone architecture correlate with the magnitude and direction of applied load. It is believed that the trabeculae of subchondral bone undergo a self regulated re-modeling which maintains a joint shape capable of optimal load distribution (Ogoston 1976; Johnson 1964; Bullough 1983). The response of subchondral bone to repeated loading (training) is an increase in the inorganic component of bone, which increases the stiffness and appears radiographically as sclerosis. It has been demonstrated by force attenuation studies that bone and periarticular tissues, rather than articular cartilage, are the shock absorbers of the joint (Radin 1973).

1.2.4 Joint Lubrication

The normal diarthrodial joint has smooth, virtually frictionless motion, and can absorb and dissipate the load during the weight-bearing phases of the gait (Palmer and Bertone 1996). This function is performed by virtue of the properties of the synovial fluid, synovial membrane, articular cartilage, and subchondral bone. Articular cartilage is

lubricated by the complementary action of hydrostatic and boundary lubrication (*Radin 1973*).

Boundary lubrication

The joint capsule and surrounding tendons and ligaments provide the greatest resistance to joint movement, as the energy required to stretch soft tissues is almost 100 times greater than that required to overcome cartilage-on-cartilage resistance (Johns and Wright 1962). Reduction in the overall resistance is partly due to "boundary lubrication". Boundary lubrication contributes to the lubrication properties of synovial fluid by the adherence of lubricating glycoproteins (LPG-1) to the articular cartilage and synovial membrane, thereby decreasing the surface tension (Linn and Radin 1968; Hills 1989). Boundary lubrication is valuable in low speed, high-load situations such as standing and may play a role in surface contact, thereby contributing to overall joint stability (Simkin 1992). Under high-speed situations, however, boundary lubrication is less effective and the load is thought to be supported through hydrostatic (fluid film) lubrication.

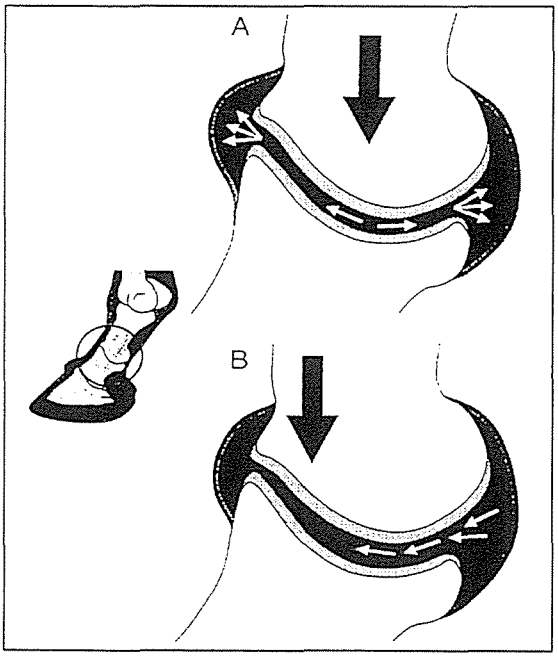


Figure 1.5 A. Squeeze film lubrication theory proposes that fluid is forced out of the cartilage into the synovial fluid. B. The hydrodynamic lubrication theory proposes that a wedge of fluid is formed in the unloaded space, creating a lifting pressure. (*From: Mcllwraith, C.W., and Trotter, G.W, 1996, Joint Diseases in Horses (ed) Saunders, W.B.)*

Hydrostatic lubrication (fluid film)

Currently, three types of hydrostatic (fluid-film) lubrications are thought to be functional in joints at high speeds: squeeze-film lubrication, hydrodynamic lubrication and elastrodynamic lubrication (fig. 1.5). Squeeze-film lubrication depends on the porosity of articular cartilage and occurs when two rigid surfaces move perpendicularly to each other. Fluid is exuded from the cartilage during

compression, and it is thought that a layer of fluid forms between the two opposing surfaces (weeping lubrication) (McCutheon 1959). The fluid is then apparently trapped and concentrated in pools on the surface of the cartilage which decreases the coefficient of friction (Walker 1968). Hydrodynamic lubrication occurs when two rigid surfaces move tangentially to each other to form a wedge of fluid in the unloaded space.

Elastrodynamic lubrication is a recently proposed joint lubrication mechanism that takes into account the visco-elastic properties of articular cartilage ignored in the fluid-film theory (Dawson 1986).

Hydrostatic lubrication is the important component of lubrication at high loads, and boundary lubrication is operative at low loads.

1.2.5 Innervation of synovial joints

The nerve supply of synovial joints originates from independent articular branches of peripheral nerves arising from related muscles (Dee 1978). The nerve fibres distributed to the fibrous joint capsule and associated ligaments are small, medium or large myelinated and unmyelinated fibres. The small fibres are pain-sensing (nocioceptive) and the medium fibres innervate mechanoreceptors which control proprioception and help to restrict joint motion within physiologic limits.

It has been shown that denervation of a joint results in the normal range of motion in the joint being exceeded. Mechanical trauma and subsequent degenerative changes can then occur (O'Connor and Palmoski, 1985).

1.3 Equine osteoarthritis

Osteoarthritis is an economically important musculoskeletal disease of domestic horses (Pool 1996). It is defined as a disease of diarthrodial joints characterised by variable degrees of articular cartilage destruction (Meachim 1969), subchondral bone sclerosis, marginal osteophyte formation and sometimes, in severe cases, eburnation (polished, exposed subchondral bone) (McIlwraith. 1982). Joint effusion and synovitis are often associated with the disease.

The joint most commonly affected with osteoarthritis in the horse is the metacarpophalangeal joint, and the problem is frequently observed in animals which have undergone training and racing at high speeds (McIlwraith 1982). This joint may be more susceptible to injury because it has a relatively small surface area and a wide range of motion in comparison to other limb joints (Pool 1991). Repeated over-extension of this joint at high speed results in impaction of the proximo-dorsal articular margin of the first phalanx (P1) on the distal end of the third metacarpal bone (Pool 1991) (fig. 1.6). This eventually causes articular cartilage lesions and chip fractures to the eminence located either side of the sagittal groove on the proximodorsal aspect of P1 (Colahan and Turner *et al.* 1987). The lesions tend to be more severe on the medial than the lateral eminence (McIlwraith 1987). Articular cartilage lesions have been found in horses at necropsy with no history of lameness (Rooney 1969; Sippel unpublished data 1942).

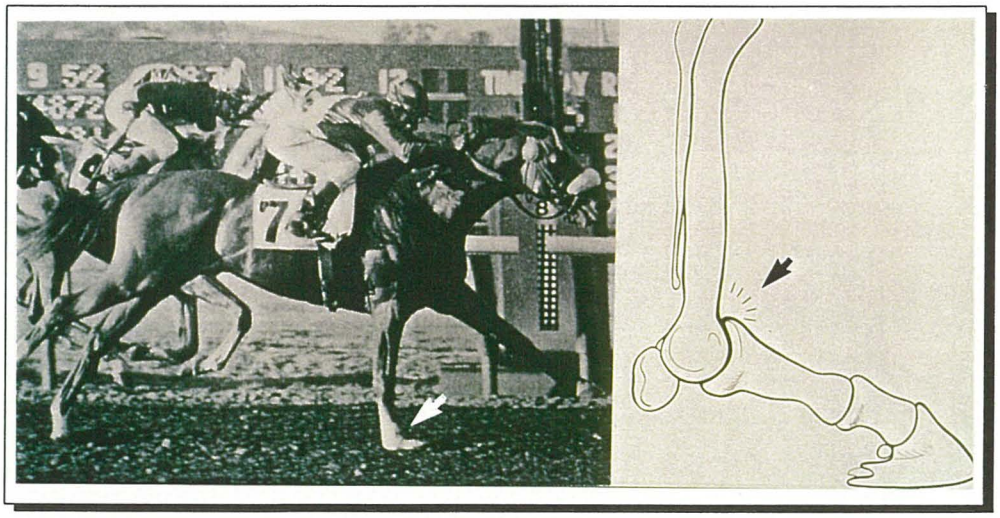


Figure 1.6 Over-extension of the metacarpophalangeal joint (arrows) occurs at high speed *(from: Stashak, T., Adams Lameness in Horses (1987) ed 4. Philadelphia, Lea & Febiger)*

1.3.1 Classification of osteoarthritis

Aetiology-based classification system

Osteoarthritis is conventionally divided in to primary and secondary varieties (Freeman and Meachin 1979). The term 'primary' osteoarthritis implies 'idiopathic' osteoarthritis where the cause or causes are unknown.

'Primary' (ideopathic) osteoarthritis develops in a joint previously free from disease or obvious mechanical abnormality. It seems likely that there may be several different forms of 'primary' osteoarthritis and that several aetiologic factors may be responsible (Kellgren 1961). This process may account for many of the apparently asymptomatic degenerative lesions found at necropsy in the paired joints of older horses.

The causes of 'secondary' osteoarthritis can be divided into four main categories: metabolic disorders, anatomic derangements, trauma and inflammation. Secondary osteoarthritis is more likely to occur in people or animals with a predisposition to the condition. In horses, secondary osteoarthritis may develop in previously normal joints following injury. The injury may occur at any age, secondary to factors such as osteochondrosis, poor conformation, angular limb deformities, sudden or repetitive trauma and joint infection (Pool *et al.* 1990). The pathological and clinical osteoarthritis that develops in the lower limb joints of horses following single or repetitive episodes of trauma is often referred to as traumatic osteoarthritis (Howard and Mcllwraith 1993; Rydell and Butler *et al.* 1970). It is likely that the repetitive insult of racing and training play a pivotal role in the initiation and progression of this form of the disease.

Classification based on biomechanical forces

Osteoarthritis can also be classified based on the effects of biomechanical forces exerted on normal and abnormal joints. There are two main categories in this classification system (Mitchell 1977) .

1. *The concentration of abnormal forces on a previously normal joint.*

Examples in horses include: intraarticular malalignment caused by delays in enchondral ossification of the long bone epiphysis, or misshapen cuboidal bones of the carpus and tarsus in foals; extra-articular malalignment due to ligamentous laxity and varus or valgus deformities; increased weight bearing on a limb to protect a painful contralateral limb; and increased work-load placed on joints before the structures have had time to accommodate to the training program. This category is a major cause of osteoarthritis.

2. *The concentration of normal forces on an abnormal joint.*

Examples of this mechanism in the horse include the normal forces of locomotion exerted on a joint damaged by osteochondrosis, infection or trauma. Normal forces acting on subchondral bone weakened by subchondral bone infection or cysts and the concentration of normal forces on normal articular cartilage overlying a stiffened subchondral bone plate are also classified in this category (Pool 1991).

Classification based on joint characteristics and predisposing factors

McIlwraith (1982), describes another classification of osteoarthritis in horses. The categories are: (1) osteoarthritis of high motion joints (such as the metacarpophalangeal joint) which is induced by trauma to the joint capsule after repetitive overextension of the joint; (2) osteoarthritis of low motion, high load joints, for example the proximal interphalangeal joint; (3) osteoarthritis secondary to predisposing disorders such as osteochondrosis, articular fractures, infectious arthritis, and (4) non-progressive, idiopathic cartilage erosions, observed as incidental findings in arthroscopic and necrospy examinations.

1.3.2 Pathology of osteoarthritis

Articular cartilage injury

In osteoarthritis, destruction of the articular cartilage is a major component in a series of events, some degenerative, and some regenerative, which ultimately affect all the tissues and structures of the joint. The extracellular matrix and chondrocytes of articular cartilage are continuously being replaced throughout life. Both in-vivo and in-vitro studies have demonstrated that changes in joint loading may lead to alterations in cartilage matrix and chondrocytes (fig. 1.7), (Bullough 1985; Thompson and Basset 1970). Low stress (below the physiological range) is associated with enhanced catabolic activity, but stress within the physiological range is associated with anabolic activity (Jones *et al.* 1982; Palmoski and Brandt 1984; Dwitt 1984). When excessive stress (supraphysiologic stress) is applied to articular cartilage, the chondrocytes are unable to adapt. There is a "window" of

physiological loading above and below which the chondrocytes do not synthesise or maintain an adequate functional matrix (Tammi *et al.* 1987; Van Kampen 1985).

At the gross pathological level, the articular cartilage first loses its normal lustre and consistency, becoming yellowish and soft (Van Pelt 1965). Blister formation on the articular surface is often an early change (Kelser 1938) that precedes pitting and superficial fraying of the cartilage (fibrillation) (Sokoloff 1979). Loss of articular cartilage is progressive and is characterised by superficial erosions, which are partial thickness lesions (Nilsson and Olsson, 1973), and full thickness erosions which may be localised or wide spread exposing the subchondral bone (Sokoloff 1979). Continued wear on the eburnated surfaces may result in grooving of the exposed subchondral bone.

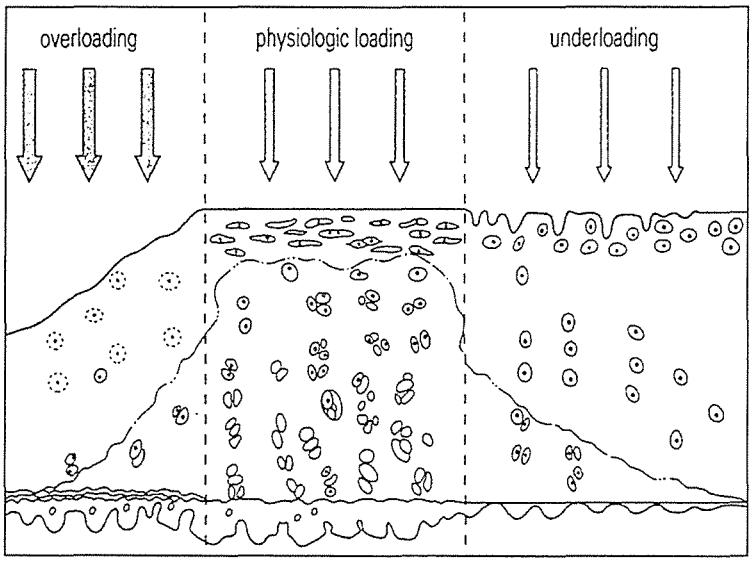


Figure 1.7 The effect of loading on articular cartilage. *(from: Rheumatology, 1994 (eds) Dieppe, P.A. and Kippel, J.H., Mosby Year book Europe Ltd)*

Grooves or wear lines in the articular cartilage in the direction of joint motion are a frequent finding (Kelser 1938; Rooney 1969) and are more prevalent in older animals.

They may be up to 3mm in width and superficial or deep. The cause and significance of wear lines is unknown. They have been observed in equine joints with no clinical evidence of dysfunction or disease, and also in joints with other signs of

osteoarthritis. Wear lines are represented histologically by varying levels of fibrillation (Nilsson 1973).

Histologically, articular cartilage damage in osteoarthritis is a progressive disruption along the planes of the collagen fibrils in the matrix. When the disruption is confined to the tangential layers of the matrix, the process is known as early fibrillation, and appears as cartilage discoloration at the gross pathologic level (Sokoloff 1976). When the process extends into the radiate layers it is called fibrillation which is represented at the gross pathologic level by superficial erosion, blisters and oedema, (Collins 1949; Kelser 1938; Sokoloff 1979). As fibrillation extends through the radiate layer, vertical clefts are formed and full thickness fragmentation and loss of articular cartilage can occur (full thickness erosion).

Cartilage fibrillation is associated with chondrocyte necrosis which is represented by empty lacunae (Meachim and Collins 1962). Histochemical staining indicates a proteoglycan depletion and an increase in water content of the cartilage matrix (chondromalacia) which results in subsequent softening of the cartilage (chondromalacia) (Mankin and Thrasher 1975; Mankin 1976). The biochemically altered articular cartilage is less resilient, more susceptible to trauma and therefore precedes fibrillation.

The effect of cartilage regeneration following injury can be identified microscopically as focal cell proliferation. Clumps of chondrocytes (chondrones) develop from the remaining viable chondrocytes which is considered to be a reactive response. There is often intense metachromasia around the chondrones, indicating an increased amount of sulfated glycosamines.

In early osteoarthritis, often only one of the opposed articular surfaces has evidence of articular cartilage damage. Therefore, fibrillation and other cartilage alterations cannot be attributed simply to abrasion.

Subchondral bone changes in osteoarthritis

Subchondral bone lesions that accompany articular cartilage changes include marginal lipping or osteophytosis and sclerosis (Kelser 1962; Raker 1968). In advanced

osteoarthritis, overt microfractures, osteoclastic bone resorption and new bone formation have all been observed in the subchondral bone plate. Eburnated subchondral bone often contains a proliferation of osteoblasts. The formation of new bone buttresses and strengthens the existing bone trabeculae, giving rise to the sclerosis seen on radiographic images (Christensen 1985). This sclerosis may also arise as repair of microfractures (Sokoloff 1982). Fibrocartilage may extend over previously denuded subchondral bone surfaces to form a more or less continuous layer of repair tissue (Storey 1971).

Subarticular cysts, common in human osteoarthritis, are seen only where the overlying cartilage is absent, but are rarely reported in equine osteoarthritis (O'Brien 1977). They are believed to result from the effect of intra-articular pressure on defects in the subarticular bony surface and marrow spaces of the subchondral bone (Landells 1953).

Separated fragments of subchondral bone and articular cartilage from a damaged joint may become incorporated in the synovial membrane or remain free as loose bodies in the joint cavity. These osteochondral fragments are frequently found in the metacarpophalangeal joints of athletic horses.

The soft tissues of the joint

The ligaments and capsular tissue surrounding an arthritic joint often show microscopic evidence of both degeneration and repair by scar tissue. It is difficult to determine if this damage preceeds or is a result of the osteoarthritis.

Microscopic examination of the synovial membrane in osteoarthritic joints usually demonstrates some degree of chronic inflammation (synovitis). The breakdown products of osteoarthritis are removed from the synovial fluid by the phagocytic cells of the synovial membrane. This inflammation results in villous hypertrophy of the synovial membrane and fibrosis of the joint capsule (Goldenberg 1982; Raker 1966; McIlwraith 1981). Extension of the hyperplastic synovium onto the articular surface of the joint (pannus) is sometimes present in osteoarthritic hip joints.

Osteoarthritic lesions in the high motion compared to the low motion equine joints

In the high motion joints, such as the metacarpophalangeal and carpal joint, the osteoarthritic changes are first seen near the joint margins (Raker 1966). There is a sequence of articular cartilage discoloration, fraying, erosion and ulceration in discrete areas, followed by subchondral bone sclerosis, marginal osteophyte formation and fibro-osseous proliferation. Synovitis and capsulitis are common findings in early osteoarthritis of high motion joints (Raker 1968). Eventually all the joint structures become affected (fig. 1.8).

Osteoarthritis of the low motion joints such as the interphalangeal, distal intertarsal and tarsometatarsal joints is characterised by periosteal proliferation which has a tendency to ankylosis in advanced cases (ring bone and bone spavin). The osteoarthritic changes that occur in the proximal interphalangeal joint are typically severe and include articular cartilage erosion, subchondral bone sclerosis, eburnation, marginal osteophytes and periosteal exostoses. Subchondral bone lysis is a typical feature of bone spavin and occasionally ringbone (Morgan 1968).

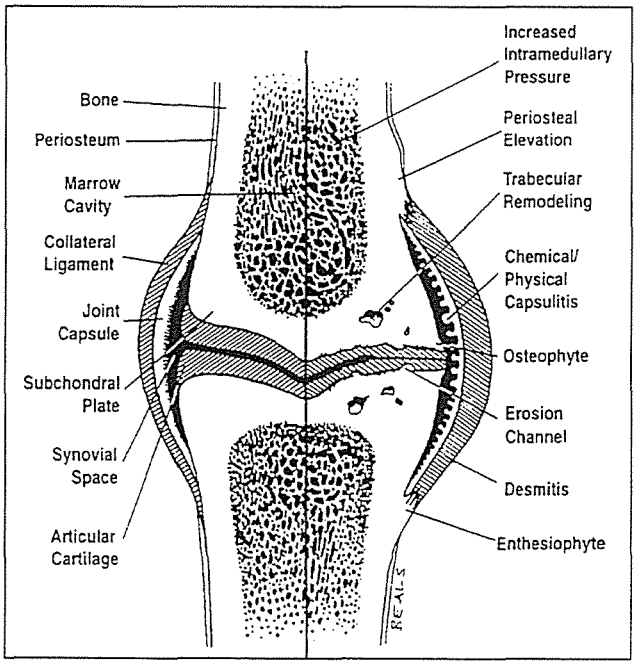


Figure 1.8 Comparison of normal articular anatomy (left) and the pathologic changes of osteoarthritis (right) in the metacarpophalangeal joint (*From: McIlwraith, C.W., and Trotter(ed), G.W, 1996, Joint Diseases in Horses Saunders., W.B.)*

1.3.3 Concepts of aetio-pathogenesis of equine osteoarthritis

Trauma is considered to be the most important factor in initiating osteoarthritis in the horse (Raker *et al.* 1966). It is likely that mechanical factors play a pivotal role in the initiation and progression of osteoarthritis, resulting in subchondral bone sclerosis, articular cartilage lesions and soft tissue inflammation. Research has shown that soft tissue inflammation has a primary role in the acute form of osteoarthritis in humans (Robinson *et al.* 1976) and there are often acute inflammatory changes in cases of osteoarthritis in the high-motion joints of race horses (Raker 1966).

It is not known if articular cartilage fibrillation precedes or follows subchondral bone sclerosis during the initiation of osteoarthritis and two contrasting hypotheses of the sequence of events have been proposed:

1. *Osteoarthritis begins as articular cartilage fibrillation that leads to secondary remodelling of the bony components of the joint.*

It has been postulated that articular cartilage lesions develop as a consequence of direct concussion when the range of motion is outside the physiological range (Rooney 1969). The ability to achieve perfect congruity at maximal loading is altered by speed, fatigue, poor conditioning, bad track surfaces, poor shoeing, and poor conformation. It has also been suggested that abnormal movement may also cause synovial fluid turbulence and joint lubrication defects, with resulting frictional wear (Rooney 1969). Radin (1972), however, disputed the hydrodynamic lubrication concepts and Davis (1977) showed that there were no deficiencies in the boundary-lubricating ability of synovial fluid from horses with osteoarthritis.

2. *Osteoarthritis is the consequence of changes in the subchondral bone.*

Radin *et al.* (1973, 1986) postulated that stiffening of the subchondral bone plate is the primary event in the development of osteoarthritis. The articular cartilage deteriorates as a result of stiffening of the subchondral bone, induced by repetitive trauma that causes

deformation and microfractures. The sclerotic subchondral bone has reduced shock absorption capacity resulting in increased stresses at the junction between the excessively stiff subchondral bone and the more compliant articular cartilage. This may lead to physical disruption of the cartilage structure, progressive loss of matrix and cells, synovitis and clinical progression of the disease.

The role of articular cartilage in osteoarthritis

Articular cartilage possesses an inherent ability to respond to applied stress. The stresses of racing and training in the equine athlete may, however, overwhelm the adaptive response. A single episode of trauma or repetitive trauma experienced by the joints during exercise may alter the sensitive biochemical composition of the matrix without causing visible injury (Radin and Paul 1971) thereby increasing the risk of potential damage to the cartilage. Further exercise may result in overt articular cartilage damage and subchondral bone thickening (Oettmeir *et al.* 1992). Osteoarthritic cartilage has been shown to have a decrease in one or more of the glycosaminoglycan and the decrease is in direct proportion to the severity of the disease (Mankin and Lippiello 1979; Mankin and Dorfman 1971). The proteoglycans have altered aggregation properties due to impaired interaction with hyaluronic acid (Mankin 1971). This could change the compressive stiffness of the articular cartilage, exposing the collagen to excessive flexion and tension forces (Meachim *et al.* 1965) and contributing to cartilage fibrillation (Meachim 1971).

The loss of proteoglycans and glycosaminoglycans from osteoarthritic articular cartilage is associated with degradation by lysosomal enzymes (Weissman 1966) and prostaglandins (Robinson *et al.* 1976). The lysosomal enzymes also appear to be associated with synovial membrane inflammation (Torbeck and Prieur 1979). The prostaglandins may exert their effect by both suppressing proteoglycan synthesis and by degradation of proteoglycans (Lippiello *et al.* 1978).

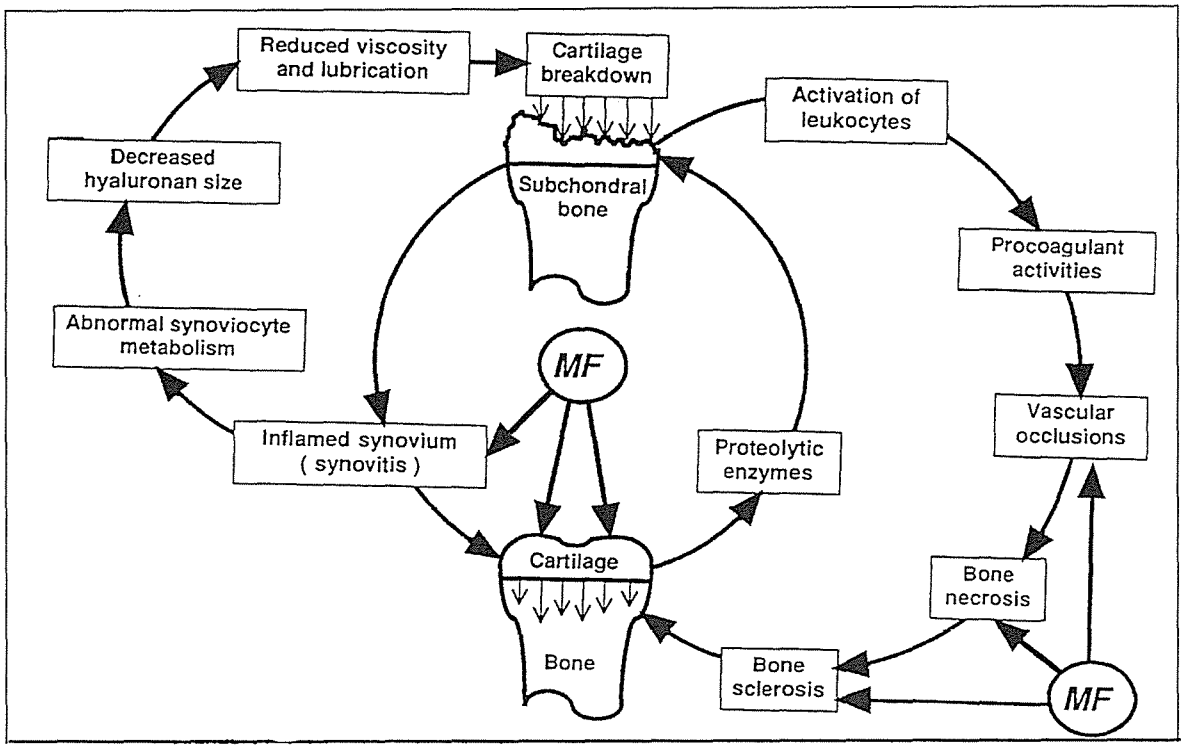


Figure 1.9 The interrelationship of pathologic changes in articular cartilage, subchondral bone, synovial membrane and synovial fluid. MF= mechanical factors. *(From: McIlwraith, C.W., and Trotter, G.W, 1996, Joint Diseases in Horses (ed) Saunders, W.B.)*

Direct damage to articular cartilage, as well as loss of proteoglycans and glycosaminoglycans, are probably important in the pathogenesis of osteoarthritis in the high motion joints such as the metacarpophalangeal joint (Maroudas *et al.* 1973; McIlwraith 1982). Events involving the articular cartilage, subchondral bone, synovial membrane and synovial fluid in osteoarthritic joints are shown in figure 1.9.

During an inflammatory episode, vascular permeability increases and production of synovial fluid exceeds its clearance, resulting in effusion, synovial membrane oedema, joint stiffness, and leakage of protein into the joint. The pressure within the joint is dependent on the fluid volume, the joint angle of the joint and compliance of the joint capsule. Increased intraarticular pressure results in increased subchondral bone pressure and hypoxia and subsequent subchondral bone changes (Levick 1990).

Subchondral bone in the initiation and progression of osteoarthritis

The ability of the subchondral bone to attenuate axial loads and spare the over-lying articular cartilage from damage was demonstrated by Radin (1970; 1973). The calcified articular cartilage zone assists in minimising the shear stress to the non-calcified articular cartilage. An increase or decrease in subchondral bone stiffness outside the physiological range may result in articular cartilage injury (Radin and Rose 1986). Morphometric analysis, radiographic assessment, and bone scintigraphy have demonstrated that repeated loading of a joint results in subchondral bone thickening and sclerosis which may be amplified by trabecular bone microfractures and subsequent bone healing (Christensen, 1985; Radin *et al.* 1984; Radin 1973; Noble and Alexander 1985). This activity-related subchondral bone sclerosis has been described in race-trained Thoroughbred horses, and was shown by morphometric and mechanical analysis of third carpal bones from race horses in treadmill training (Young *et al.* 1991). The consequence of subchondral bone thickening and stiffening results in increased transverse stress at the base of the calcified articular cartilage and the appearance of horizontal clefts in the deep zone of the cartilage, which may progress to the articular surface (Radin 1991). However, it was recently reported that there is no connection between the third carpal bone articular cartilage hexosamine content (a measure of proteoglycan content) of racehorses and the mechanical and morphological properties of subchondral bone (Richardson and Young *et al.* 1993). The production of osteophytes at the joint margins is a common finding in osteoarthritic joints. The osteophytes alter the joint conformation, change the pressure points and contact areas between the articulating bones and possibly result in further subchondral bone stress and sclerosis (Oettmeier and Arokoski *et al.* 1992).

1.3.4 Osteochondral fragmentation of the proximo-dorsal margin of the first phalanx.

Osteochondral fragmentation in the metacarpophalangeal joint is usually considered to be the result of trauma; compression of the dorso-proximal aspect of the proximal phalanx against the distal part of the third metacarpal bone when hyper-extension

of the fetlock joint occurs during racing or fast training (Copelan and Bramlage 1983; Haynes 1988). These fragments may be a secondary complication affecting joint margins affected by osteoarthritis. Osteochondral fracture fragments of the first phalanx have been considered an injury of race horses (Yovich and McIlwraith 1986), however, in a recent study rounded osteochondral fragments have been recognised in a group of non-racing horses (Kawcak and McIlwraith 1994).

Although chip fractures have often been considered acute injuries and maybe accompanied by acute clinical signs, it has been suggested relatively recently that some may be a secondary complication affecting joint margins previously altered by osteoarthritis (Pool and Meagher 1990). Chip fractures of the proximal first phalanx in the metacarpophalangeal joint arise from progressive subchondral bone remodelling induced by repetitive trauma of training, and eventually the sclerotic bone undergoes ischaemic necrosis and subsequent fragmentation.

A second abnormality that occurs at the proximo-dorsal margin of the first phalanx is the formation of a cartilaginous periarticular lip either side of the sagittal groove. The lip undergoes enchondral ossification and is known as an osteophyte. Overextension of the joint can result in separation of the osteophytes from the parent bone.

1.3.5 Diagnosis of osteoarthritis in the metacarpophalangeal joints

Clinical signs

Typical clinical signs are synovial effusion of the metacarpophalangeal joint, varying degrees of lameness, soft tissue swelling, and pain on flexion of the joint. The injury is usually associated with strenuous or fast exercise during which hyper-extension of the metacarpophalangeal joint occurs and the clinical signs are most prominent during the first few days after injury. Lameness may decrease with rest but can become apparent again with exercise.

Regional and intra-articular anaesthesia

Diagnostic anaesthesia is necessary to diagnose lameness when physical findings are minimal or equivocal. Intra-articular analgesia is more specific than a regional block as it isolates the source of pain to a particular articulation. The four point regional block (palmar and palmar metacarpal nerves) improves a lameness associated with the metacarpophalangeal joint, however it should be performed after a palmar nerve block at the level of the abaxial sesamoid bones in order to rule out the foot as a source of pain.

Synovial fluid analysis

Examination of the synovial fluid can provide valuable information in addition to that gained by clinical and radiographic examination.

Normal synovial fluid is pale yellow, clear and free of flocculent debris. Streaks of blood in the aspirate are indicative of haemorrhage from needle puncture. Uniformly diffuse haemorrhage represents an acute traumatic situation, whereas dark yellow (xanthochromic) samples represent previous haemorrhage and maybe associated with traumatic arthritis. The volume of synovial fluid is generally increased in association with active synovitis and often with osteoarthritis. Decreased synovial fluid volume could be correlated with fibrotic synovial membrane (McIlwraith 1996). Synovial fluid from diseased joints may form clots, and the rapidity of clotting and size of the clot have been considered roughly proportional to the severity of the synovitis. (McIlwraith 1996). The normal concentration of protein in equine synovial fluid is 1.81 $\pm$ 0.26 g/dl (Van Pelt 1974). Total protein in synovial fluid above 2.5 g/dl is considered abnormal and a concentration above 4 gm/dl represents severe inflammation (Perssons 1971). The viscosity of synovial fluid is directly related to the hyaluronic acid content and is considered to be a measure of the quantity and degree of polymerization of the hyaluronic acid. A decrease in viscosity is consistent with joint disease and inflammation. Precise viscosity measurements are made using a viscometer where the viscosity of synovial fluid is compared to that of distilled water (Van Pelt 1962). For practical use in the field a synovial fluid drop should stretch 2.5-5cm between thumb and finger. The total white cell count of normal synovial

fluid is approximately 167 ± 21 cells /mm^3 (Persson 1971) and the neutrophil content is generally less than 10%. Idiopathic synovitis and osteochondritis dissecans tend to have cell counts less than 1000 cells /mm^3. In osteoarthritis the synovial fluid cell count can vary tremendously depending on the amount of active synovitis present. Synovitis tends to be a prominent feature of equine osteoarthritis, resulting in counts of 5000-10,000 cells /mm^3. Cases of infectious arthritis have the highest white blood cell counts. In general, counts greater than 30,000 cells /mm^2 with predominantly neutrophils are suggestive of infection and counts greater than 100,000 cells /mm^3 are virtually pathognomic for infection. A "grey" area exists between traumatic arthritis with a high white blood cell count and infection with a low white blood cell count. White blood cell counts of up to 50,000 cells/mm^3 have been reported in cases of traumatic arthritis in humans (Graham 1978).

Microscopic cartilage fragments are often present in the synovial fluid of osteoarthritic joints. Particle analysis provides an indication of the degree of articular cartilage damage and involves the microscopic examination of metachromatically stained sediment after centrifugation (Van Pelt 1979). The correlation between identification of cartilage fragments and identifiable cartilage damage was reported as 100% (Tew 1980).

Radiography

The radiographic signs of metacarpophalangeal joint osteoarthritis include periarticular marginal osteophytes on the proximo-dorsal aspect of P1; sclerotic subchondral bone plate of proximal P1 and distal third metacarpus; narrowed joint space and periarticular enthesiophytes. Bone fragments associated the proximo-dorsal eminences of P1 may be identified, the medial eminence being affected more frequently than the lateral. Jeffcott (1983) radiographically detected small osteophytes in the metacarpophalangeal joints from a significant percentage of horses in regular work, and apparently free from lameness.

The metacarpophalangeal joint articular cartilage, synovial pad and the extent of the joint capsule and the synovial surface outline can be evaluated on arthrograms. This

technique is not routinely used in the diagnosis of metacarpophalangeal joint osteoarthritis but may be beneficial in cases with proliferation of the villonodular pad.

Bone scintigraphy

Bone scintigraphy is a technique that measures gamma ray emission following intravenous administration of a radionucleotide technitium-99m labelled methylene diphosphonate (^{99m} Tc-MDP). A gamma camera records the emission of gamma rays from the tissues and the image is recorded on film. The technique is much more sensitive than but less specific than conventional radiography. Within minutes, the ^{99m} Tc-MDP is distributed to the soft tissues giving the "soft tissue phase" and within 2-3 hours the nuclide binds to bone tissue. The distribution of ^{99m} Tc-MDP in bone is a function of blood flow and skeletal metabolism. Increased bone remodelling results in an increase in binding of the nuclide to the affected area and is recognised as a "hot spot" on the image (Lamb 1990). This technique is useful in the horse with a painful but radiographically normal metacarpophalangeal joint or to establish if a proximo-dorsal chip fragment is actively remodelling. In the study by Christensen (1985), bone scintigraphy was used to demonstrate that there was increased uptake of ^{99m} Tc-MDP, both at sites of osteophyte formation and in the subchondral bone of metacarpophalangeal joints with osteoarthritis.

Diagnostic arthroscopy

The diagnostic usefulnes of arthroscopy and the evaluation of equine joint disease has been documented in 1978 (McIlwraith and Fessler 1978). More recently, the term "chondroscopy" (diagnostic arthroscopy with a 2.7-mm arthroscope under local anaesthetic and recorded on a video tape) has been employed as a method for evaluating the articular cartilage lesions. Radiographic examination only demonstrates erosion of the articular cartilage when the erosion is sufficiently advanced that the joint space is narrowed or the subchondral bone shows radiographic change. The synovial fluid examination may demonstrate the presence of synovitis, but the degree of pathologic change in the synovial membrane is difficult to assess. Arthroscopy allows for a detailed examination of the

articular cartilage and synovial membrane and allows for a more accurate assessment of prognosis to be made.

Computed tomography (CT) and magnetic resonance imaging (MRI)

These two sophisticated diagnostic tools are not used in the diagnosis of metacarpophalangeal joint osteoarthritis in the live horse.

A CT scanner consists of an X-ray tube and sensors in a circular gantry. It is most useful when used in conjunction with a radiographic examination. CT can assist in defining complex intra-articular fractures, stress-induced subchondral bone sclerosis and other subchondral bone lesions such as cysts.

MRI produces sectional images, much the same as CT images. The images are proton images with high contrast and spatial resolution. The images provide excellent anatomic and pathophysiologic information of intra-articular and periarticular structures. MRI has the potential to provide information from images not available from any other imaging modality. Portable MRI units are now available for use in clinical situations.

Experimental methods of investigating osteoarthritis

Experimental animal models have been used to explore the early events that occur in the development of equine osteoarthritis and act as a bridge between *in vitro* research and clinical research (Crelin 1964; Dekel *et al.* 1978; De Palmer *et al.* 1958; Edwards *et al.* 1981; Evans *et al.* 1960,1981,1884; Garr 1973; Gritza *et al.* 1973; Gustafson *et al* 1992; Hall 1964; Hulth *et al.* 1970; Hurtig 1988; Ogata 1977; Telhag 1972; Tew 1981; Trotter *et al.* 1989; Troyer 1982; Vasan 1983; Williams 1984; Yovich *et al.* 1987).

Detailed assessment of the osteoarthritic changes present in post-mortem specimens includes: (1) gross assessment of articular surface using ink staining methods, (2) objective histological assessment of articular cartilage and subchondral bone, (3) biochemical parameters (proteoglycan content in articular cartilage), (4) mechanical and morphometric assessment of subchondral bone, and (5) radiography of the subchondral bone. The clinical and radiographic signs of osteoarthritis are often not closely related to

the severity of the articular cartilage changes, and conventional radiography is a very insensitive test of sclerosis. A more accurate and precise quantification of subchondral bone sclerosis would enable more subtle bone density changes within and between joints to be identified. Bone mineral density measurements using the dual energy X-ray densitometer allow a more sensitive and accurate assessment of subchondral bone sclerosis than conventional radiography.

1.4 Bone mineral density measurements

The majority of bone density studies in the horse have been concerned with the bone mineral density of cortical bone using non-invasive techniques in the standing horse (Jeffcott *et al.* 1987; 1988). A recent study reported subchondral bone density patterns of the first phalanx from a sound horse using computed tomography. The subchondral bone density was greater on the medial compared to the lateral aspect of proximal P1. This suggests that the medial aspect of the bone may have experienced higher loads than the lateral aspect based on Wolfs' law (Thompson *et al.* 1996).

1.4.1 Methods of non-invasive bone measurement

Conventional radiography

The assessment of bone density by radiography is imprecise as a change of 40% in bone mineral content is required for visual evaluation of sclerosis or osteolysis to be possible (Lachman 1955).

Radiographic photodensitometry

This method involves measuring the optical density from a radiograph. The technique is more accurate than radiography and changes in bone mineral content of 10-20% can be detected (Price *et al. 1983).*

Single Photon Absorpiometry

Single photon absorptiometry (SPA) (Cameron 1963) was the first practical method developed for non-invasive measurement of bone mineral content. The principle of the technique is to scan a bone with a narrow beam of low energy photons from a radionuclide source and to measure the degree of attenuation by bone, relative to its attenuation by tissue, by means of a scintillation detection system. One major disadvantage of SPA is that it provides the mineral content per unit length of bone scanned and takes no account of differences in bone size.

Dual photon Absorptiometry

This technique more accurately quantifies bone mineral content. It is considerably more expensive than SPA (Riggs *et al.* 1981). High activity gadolinium is the dual energy source of photons. This method is of limited value as it does not take bone volume into account.

Ultrasound Velocity

Ultrasound can be used to measure bone density because the velocity of sound through bone is directly related to the modulus of elasticity (stiffness) and density of bone (Katz 1980). Transmission ultrasound velocity involves passing an ultrasound beam through the bone from a transmitting to a receiving transducer.

Combined ultrasound velocity and photon absorptiometry

The bone mineral content measured by photon absorptiometry can be used to derive the bone mineral density if the cortical cross-sectional area is known. The ultrasound velocity technique provides an estimate of the cross sectional area and thus:
Bone mineral density = Bone mineral content/Cross sectional area (Greenfield *et al.* 1981).

Quantitative computed tomography

Quantitative computed tomography (QCT) is particularly useful for non-invasive measurement of bone density as it provides a quantitative assessment as well as an image.

The method measures trabecular bone at both peripheral and axial skeletal sites and is believed to be highly sensitive to disease and age-related bone loss. The procedure is expensive and the radiation dose for the patient is 30 times higher than for dual X-ray absorptiometry (Cann 1980).

Dual energy X-ray absorptiometry

The technique of dual energy X-ray absorptiometry allows rapid, non-invasive, safe, relatively inexpensive, precise measurement of bone mass or density at almost any part of the skeleton (Grier and Turner 1996). In human medicine, the clinical applications for assessment of bone density are well established, particularly in the evaluation of osteoporosis and metabolic bone disease (Griffiths and Zimmerman 1978). Dual energy X-ray absorptiometry is used most frequently to measure the spine and proximal femoral bone mineral density in human patients. It allows an estimation of the current rate of bone loss and the future relative risks of bone fracture (fig. 1.10).

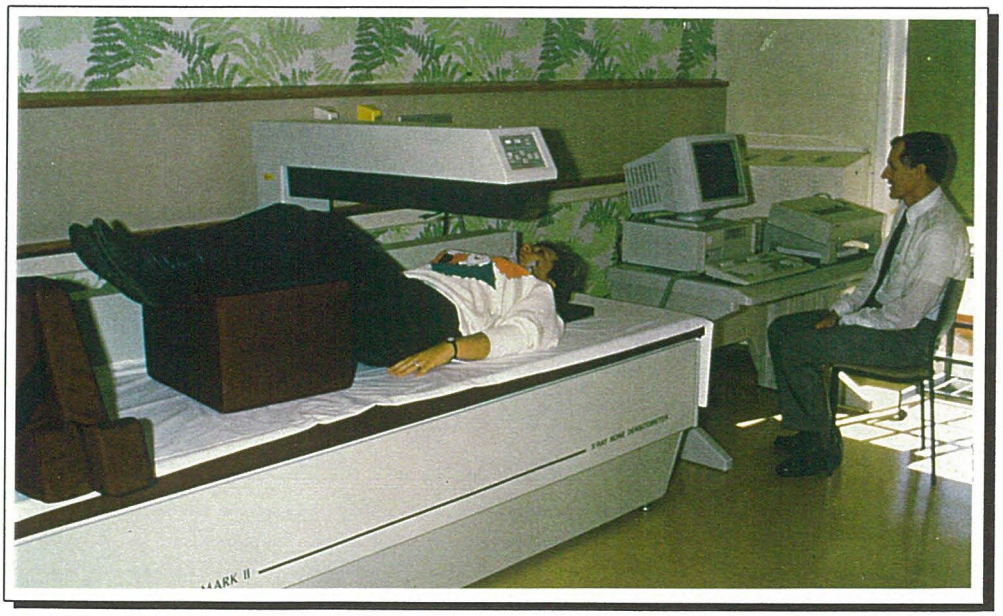


Figure 1.10. Norland XR-26 bone densitometer (Norland corp., Fort Atkinson, USA).

Dual energy X- ray absorptiometry is a significant improvement in the field of bone densitometry because the radiation source is relatively stable and there is a significant difference between the energy levels emitted, allowing high resolution (Sartoris and

Resnick 1990). By replacing the radio-isotope source used in photon absorptiometry with an X-ray tube, image resolution and precision have been improved, and scan times for humans have been reduced from 1 hour to 10 or 20 minutes (Wahner and Fogelman 1994).

The source and detector move in synchrony over the subject and the detector measures the intensity of X-rays that pass through the subject. The X-rays of two different energy levels are impeded by bone, fat and lean tissue. The X-ray properties of these materials are dissimilar because of the differing proportions of high atomic number elements. Bone mineral contains a large percentage of calcium and phosphorus, whereas soft tissue is composed almost entirely of hydrogen, carbon and oxygen. Algorithms are used to calculate the quantity and type of tissue scanned (Nord and Payne 1996).

The accuracy of dual energy X-ray absorptiometry measurements is usually determined by comparing the results to another independent method, such as measuring or scanning bone ash. The reproducibility (precision) of the DEXA scanner is best evaluated by measuring the coefficient of variation for a series of measurements on the same area (Rozenberg and Vandromme 1995). Evaluation of the accuracy and precision of the DEXA scanner is an essential part of densitometry studies and is important for the design of clinical trials and research projects.

Fundamentals of dual energy absorptiometry

Figure 11 is a schematic diagram of a DEXA scanner. There is an X-ray source with a collimator to direct the beam of X-rays through the body of the subject. There is an X-ray detector system which is capable of measuring the intensity of the transmitted X-ray beam. The measurements are made at two distinct X-ray energies. Finally there is a motorised drive system which can move the X-ray beam in a scanning pattern over the subjects body (or sample). The bone mineral density measurements are produced by the attenuation of the X-ray beam at two energies at every point in the scanned area. The results are presented in in an image form made up of many thousands of pixels (Nord *et al.* 1996).

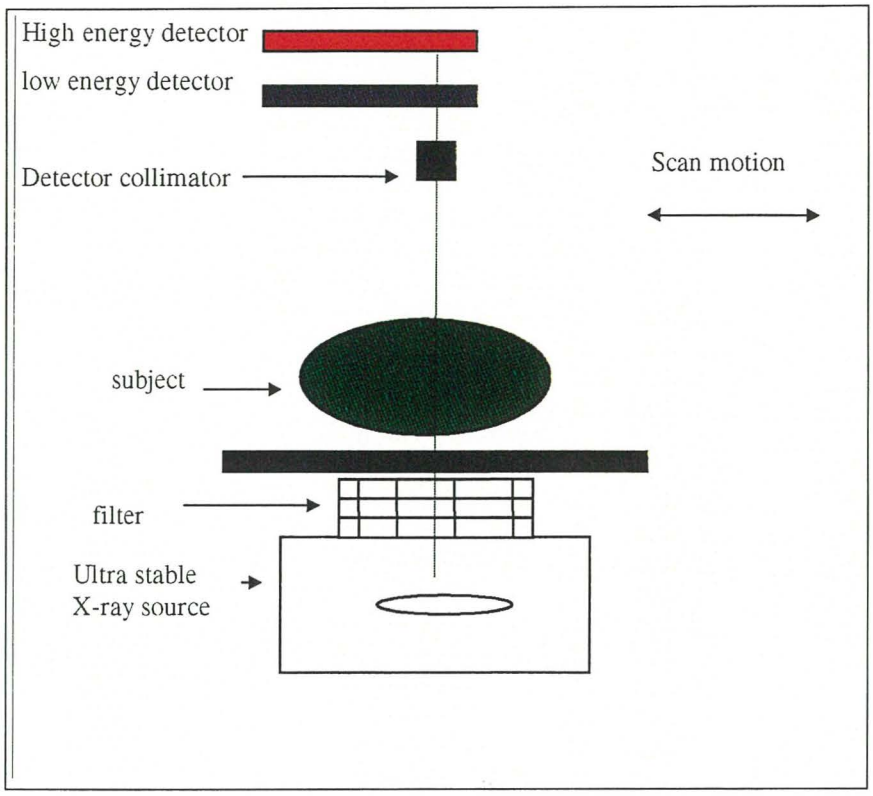


Figure 11. Diagram of a dual energy X-ray absorptiometry (DEXA) scanner

The use of dual energy X-ray absorptiometry on animal research models

Bone mineral measurements are used in animal models for the study of metabolic bone disease, nutrition research, fracture healing, response to prosthetic implants and drug effects on bone metabolism.

Certain factors must be addressed routinely when measuring bone mineral density in small animals or bone slabs. The small size of the subject or sample, means a different technique must be utilised. The method employed for small samples is an "ultra high resolution" software program, which increases the number of lines scanned and slows the speed of the scanning arm (Grier and Turner 1996). The resolution can be increased more than seven fold compared with a regular human scan (Griffin and Kimble 1993).

Rats are the most frequently used species in bone mineral density research because of their small size, short life span and the large amount of available information about their bone turn over. Rats have been used to evaluate the effects of: oestrogen deficiency

following ovariectomy, treadmill exercise, and immobilisation on bone. Rabbits have been used to study periprosthetic implants and as a model for post-menopausal osteoporosis. Dual energy X-ray absorptiometry has been used for *in vivo* measurements of bone mineral density in the lumbar vertebrae of cats with inherited lysosomal disease and mucopolysaccaroidosis. The similarity of canine bone remodelling to human bone remodelling makes the dog a popular model in human research. Sheep have been widely used as a model for post-menopausal osteoporosis by ovariectomy (Grier *et al.* 1996). There has been no published data on the use of the dual energy X-ray absorptiometry scanner to measure the subchondral bone mineral density in the proximal P1 of horses.

1.5 Research hypothesis and objectives

Osteochondral chip fragments are commonly found in the metacarpophalangeal joints of young race horses (Yovich and McIlwraith 1986) and similar, rounded, fragments have been found in non-racing domestic horses on proximo-dorsal P1 (Kawcak and MacIlwraith 1994). There is, however, no such published data for a natural population of wild.

In 1994, a group of wild horses was culled by the New Zealand Department of Conservation because the fragile Kaimanawa mountain plateau where they roam was unable to support their increasing numbers. Not only were the horses threatening certain species of native flora with extinction but many of the animals were suffering from malnutrition because of over grazing. Research in to controlling the numbers of wild horses by immuno-contraception is in progress (Stafford personal communication). This cull of Kaimanawa horses provided us with the opportunity to investigate the joints from a natural population of animals.

We were interested to find out if horses in a natural roaming existence have lesions in their metacarpophalangeal joints similar to those found in trained domestic horses.

When we discovered that the wild horses had lesions in the same areas of the metacarpophalangeal joint as domestic horses we decided to evaluate the affected articular

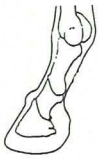
cartilage and the associated subchondral bone using various modalities. We proposed the two following hypotheses based upon our initial findings:

(1) that there is a naturally occurring age related osteoarthritic process in the front fetlock joints of wild horses that are not subjected to training or other human influences;

(2) that the bone mineral density of the subchondral bone of the proximodorsal eminencies of P1 increases as the overlying cartilage lesion becomes more severe.

The objectives of this study were to determine the site and prevalence of lesions affecting the proximo-dorsal margin of P1 in the fore limbs of wild horses and to assess the lesions using Indian Ink staining methods, radiography, dual energy X-ray absorptiometry, and histology. Dual X-ray absorptiometry, provides an accurate measure of bone mineral density and this study is the first to measure the subchondral bone mineral density of proximo-dorsal P1.

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖



Chapter 2

Materials and methods

2.1 Animals

Twenty two wild horses culled by the New Zealand Department of Conservation in 1994 were used in this study (fig. 2.1). This population of horses inhabits a mountainous plateau region of approximately 24,000 hectares in the central North Island of New Zealand. The horses are typically under 14.2 hands (1.47 m) in height and are therefore ponies by definition, but are genetically related to the British Thoroughbred. Age was assessed by inspection of their dentition by two veterinarians. The lower incisors were removed from the skull and examined in the laboratory against a set of teeth of known ages. Both fore-limbs were removed proximal to the carpus and the metacarpophalangeal joints were examined within 12 hours of death.

Figure 2.1 A group of Kaimanawa horses in a yard after a muster by the Department of Conservation.

2.2 Dissection of the metacarpophalangeal joint

The skin was removed from the limb and the metacarpophalangeal joint was isolated from the rest of the limb by sawing through the distal portion of the third metacarpal bone (McIII) and proximal first phalanx (P1) with a bandsaw. The proximo-dorsal portion of the joint capsule, collateral ligament and palmar joint capsule were sectioned allowing the joint to be fully opened. The relationship between P1, sesamoid bones and MCIII was maintained via the intact lateral collateral ligament or palmar joint capsule. The joints were identified by horse number, right or left limb, and medial or lateral aspect.

2.3 Articular surface staining

The joint surfaces were washed with isotonic saline at room temperature. The articular joint surfaces were stained with Indian ink, applied with a soft brush (fig. 2.2a), left in-situ for 4 minutes and washed off with isotonic saline (fig. 2.2b). The stained joint surfaces were photographed with identification labels (fig. 2.2c).

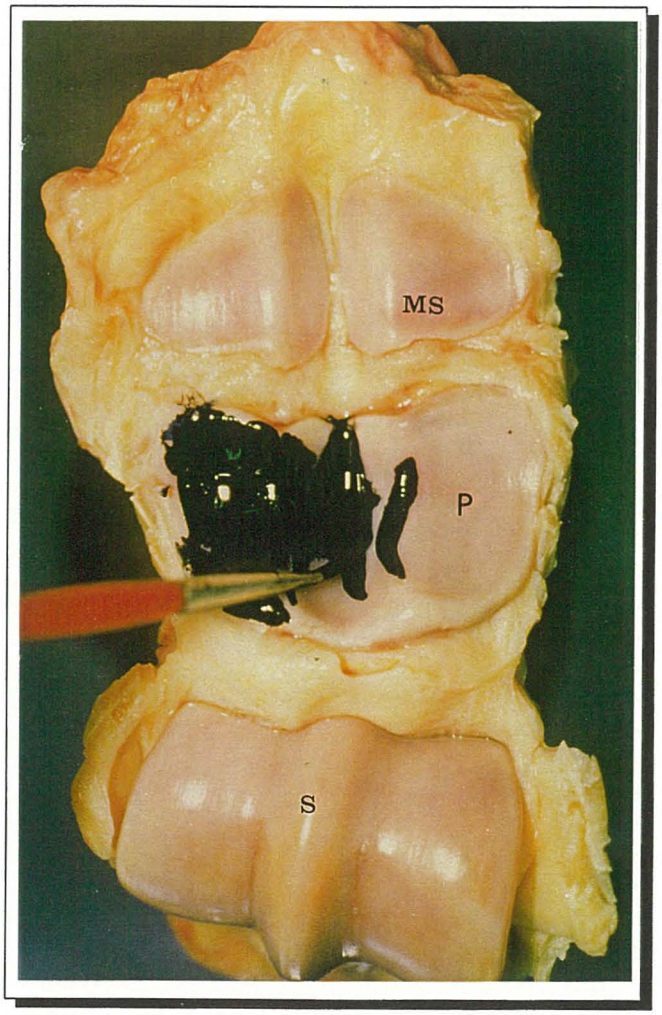


Figure 2.2a. Application of Indian ink to the joint surfaces using a soft brush.

P = proximal aspect of the first phalanx; MS = medial sesamoid; S = sagittal ridge of distal third metacarpal bone.

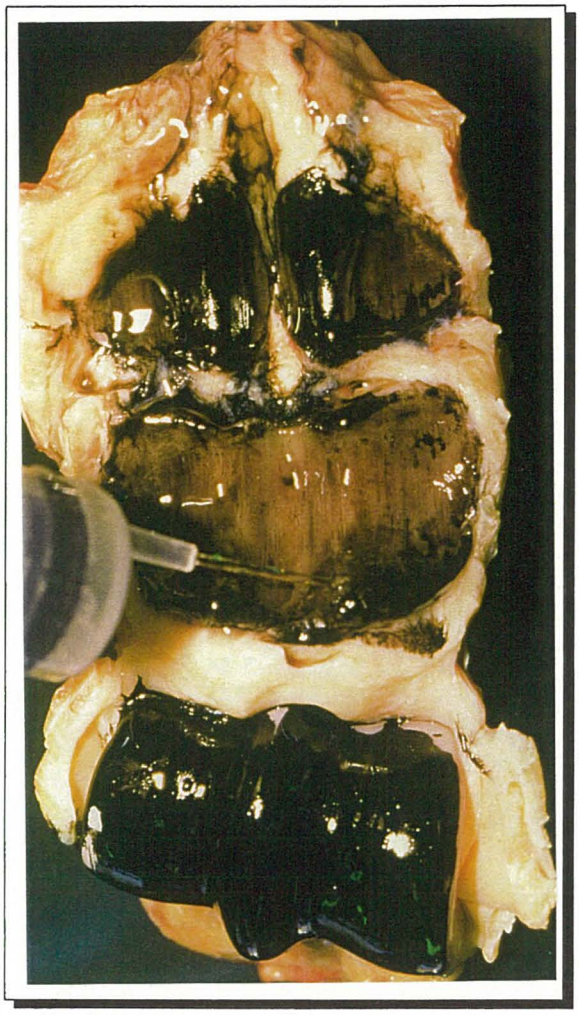


Figure 2.2b. The Indian ink is left in situ on the articular cartilage for 4 minutes before washing with isotonic saline.

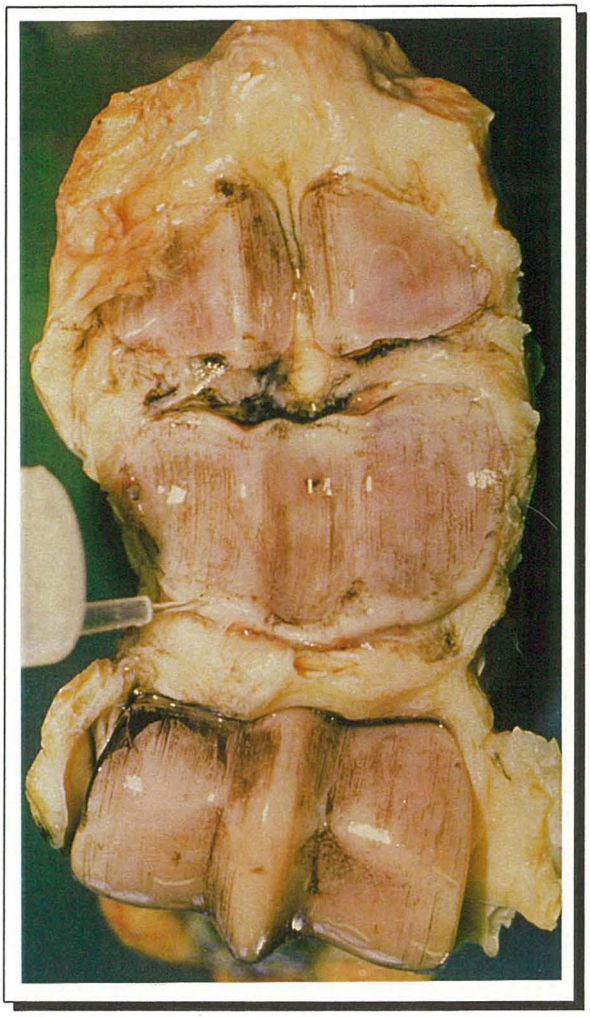


Figure 2.2c. The stained articular surface after washing is complete.

2.4 Macroscopic scoring of lesions on the medial and lateral eminences of P1

A grading system was established as described in Table 1.1. The grading system was applied separately to the medial and lateral proximal eminences of P1 after staining with Indian ink. The lesion length was measured from medial to lateral and the width was measured from dorsal to palmar aspect of the lesion using callipers. The amount of ink staining on the eminences was recorded: for example no stain, fine black lines or a strong black stained area. Any wear lines, cartilage flaps, ossicles or osteophytes were noted.

The combination of these features was used to produce a macroscopic lesion score between 0 and 5 for both medial and lateral eminences of P1. A macroscopic lesion score of 0 represented minimal articular damage and a score of 5 represented severe articular damage.

Table 2.1 Macroscopic scoring of the Indian ink stained articular surfaces.

Lesion score	Description of lesion
0	Either: No evidence of stain uptake by the cartilage. Or: A fine line stain 1mm or less in length and width.
1	Either: A fine line stain greater than 1mm long Or: Grey staining up to 2 mm in length and width
2	Either: More than one strongly stained line, with lines possibly overlapping and up to 5 mm in length. Or: An area stained grey/black up to 3 mm wide and 2 mm long
3	Either: A strong, black stain 2-4 mm long and 3-5mm wide. Or: More than one black stained area (1-4 mm in length and up to 5 mm in width) separated by either black or grey lines or no stain. Wear lines poorly stained and greater than 1.5 mm apart.
4	Either: The presence of a cartilage flap *(defined as an attached flap of articular cartilage that did not appear to be ossified)* and black or grey staining of the surrounding cartilage. Or: Black stain greater than 4 mm in length and width. The stained surface may appear irregular. Wear lines black stain 0.5 - 1mm apart.
5	The presence of an ossicle *(defined as a rounded, apparently ossified fragment, attached to the parent bone by fibrous tissue)* or osteophyte *(defined as a bony exostoses attached to the parent bone)* and/or grey or black staining of the surrounding cartilage.

2.5 Bone slabs

Single 2 mm-thick sagittal bone slabs were sawn from both the medial and lateral eminence of P1 using a circular saw with parallel diamond blades. The sites of the abaxial cuts were determined using an equation that was constructed by analysis and prospectively (figs. 2.3a and 2.3b).

(a) Distance, measured with slide callipers, from the medial to the lateral border of the proximal surface of P1.

(b) Distance between the medial border of P1 and the abaxial aspect of the medial P1 lesion.

(c) Distance between the lateral border of P1 and the abaxial margin of the lateral P1 lesion.

Distances (b) and (c) were expressed as a mean fraction of distance (a).

A = a x 0.275 B = a x 0.252 where:

(A) = the distance from the medial border to the abaxial saw line.

(B) = the distance from the lateral border to the abaxial saw line.

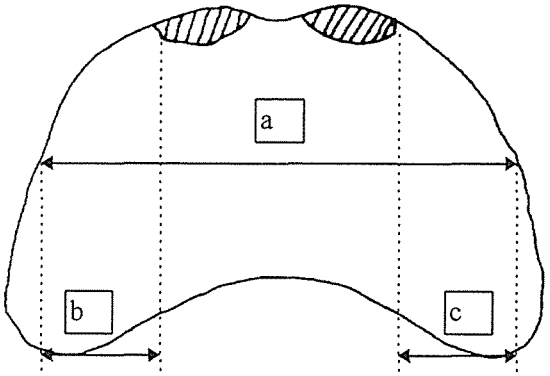


Figure 2.3a. Diagram of the proximal surface of P1 showing the measurements made on each bone slab that were used to create an equation for standardising the medial and lateral slab site.
a = distance from medial to lateral border of proximal P1.
b= distance from medial border of P1 to abaxial aspect of medial P1 lesion.
c = distance from lateral border of P1 to abaxial aspect of the lateral P1 lesion

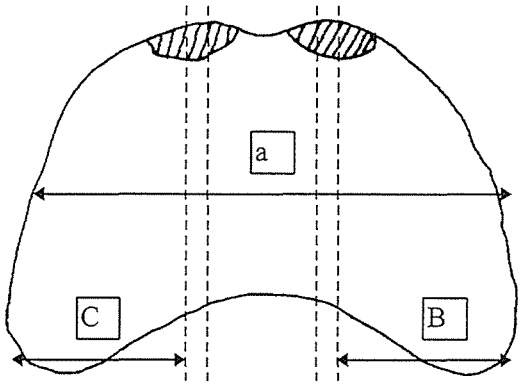


Figure 2.3b. Diagram of the proximal surface of P1 showing the position of the 2mm thick slabs taken through the medial and lateral proximo-dorsal eminencies of P1.
a = distance from medial to lateral border of proximal P1.
B = distance from the medial border of P1 to the medial abaxial saw cut.
C = distance from the lateral border of P1 to the lateral abaxial saw cut.

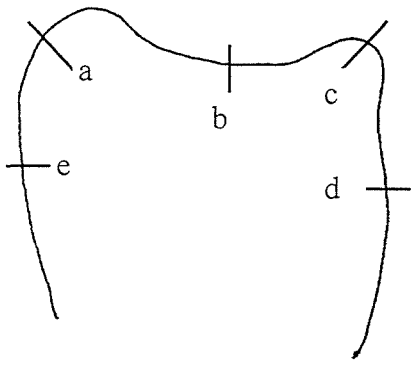


Figure 2.4. Diagram of a 2mm sagittal bone slab showing the thickness measurement sites made on each sample.
a = dorsal aspect ,
b = proximo-dorsal aspect,
c = mid proximal aspect,
d = proximo-palmar aspect,
e = palmar aspect.

The precise thickness of the bone slabs at five different sites on the proximal aspect of P1 (a-e) were determined using slide callipers (fig. 2.4).

2.6 Cabinet Radiographs

The bone slabs were radiographed in a Torren 150 cabinet X-ray machine (60 kV, 3 mA, 1 minute) using non-screen x-ray film (Fugi, HRG-30). The four bone segments (right medial, right lateral, left medial, left lateral) from each horse were placed on one film with the dorsal aspect of the bone slabs orientated in the same direction. The radiographs were subjectively assessed for subchondral bone sclerosis and trabecular bone pattern within the ossicles and osteophytes. Each bone slab was identified and fixed in 10 % buffered formalin.

2.7 Bone mineral density measurements

Norland XR-26 bone densitometer

The small animal research programme of a Norland XR-26 bone densitometer (Norland Corp., Fort Atkinson, WI) (DEXA scanner) was used to determine the bone mineral density of the slabs. This DEXA scanner has an ultra-stable X-ray tube source (Superior X-ray tube Co., Woodstock, IL) coupled with a multistage K-edge filter module fitted with eight filter combinations. The filter system allows the intensity of the X-ray beam to be optimised for subject thickness, while allowing the tube to operate at constant power. The source collimator is 1.9 mm in diameter, is situated above the filter assembly and directs a beam of X-rays through the body of the subject. The effective energies of the system are 46.8 and 80 KeV. There is a collimator attached to the X-ray detector system with aperture of 14 mm. The detector system consists of a low energy detector

and a high energy detector capable of measuring the intensity of the X-ray beam which has passed through the body of the subject. The measurement is made at two distinct energies. Finally there is a motorised drive system which can move the X-ray beam in a scanning pattern over the body or specimen (Sievanen 1994).

Quality assurance procedure

The scanner was calibrated routinely every day prior to use according to the recommendations of the manufactures. Subsequently, a dedicated lumbar spine phantom (Norland) was measured immediately after calibration. The uniformity of the beam intensity over the table top was assessed on a monthly basis by the rail alignment test software provided by the manufacturer. Measurement calibration and quality control phantom scanning was performed prior to each use of the machine. The coefficient of variation of the machine was determined for 3 different bone slabs. The background (paper or plastic) upon which the bone slabs were placed, the effect of air drying of the moist specimen and the effect of fixing in 10% formalin were shown to have no influence on the measurements.

Specific regions of interest for bone mineral density analysis

Five comparable 0.45mm^2 regions (A-E) on the proximo-dorsal aspect of each bone slab were assessed. The squares were positioned just within the visible edge of the bone. Area A was situated on the most proximo-dorsal aspect of the slab, areas B and C along the dorsal border of the bone slab, and areas D and E caudal to zone A, on the proximal surface of the slab (fig. 2.5).

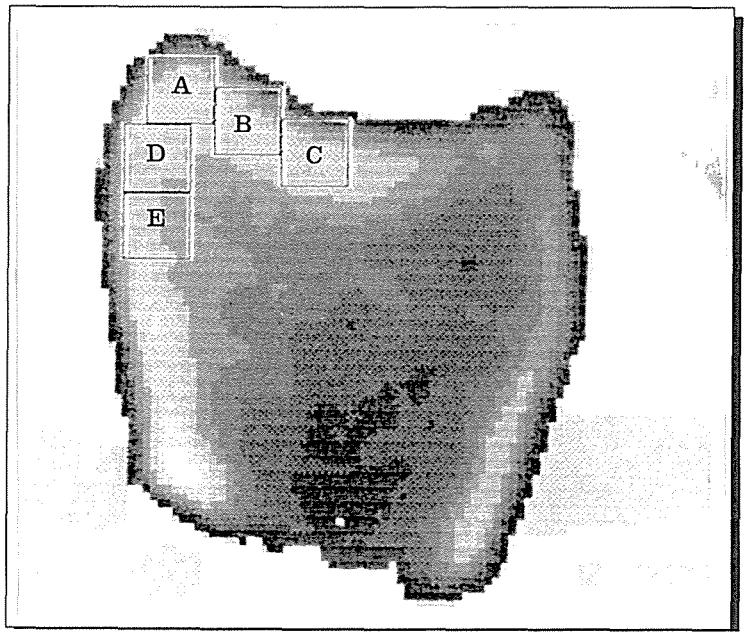


Figure 2.5 Bone slab showing areas A to E on the proximo-dorsal aspect of P1.

The median bone mineral density of areas A-E was related to the macroscopic lesion scores and the histological lesion scores. The individual bone mineral density measurements for areas A,B,C,D and E were compared with each other. The bone mineral density (g/cm^2) for each slab was corrected for variation in thickness. The bone mineral density measurements reported in this study are standardised to a 2mm thick slab (g/cm^2).

2.8 Preparation of the histological sections

The bone slabs were decalcified in 8N formic acid/1N sodium formate solution before preparation of 6μm histological sections.

The tissues were cycled through a series of treatments in a Shandon Elliot automatic tissue processor (Model SE400):

1. 70% ethanol 1 hour
2. Absolute ethanol 1 hour
3. Absolute ethanol 1 hour
4. Absolute ethanol 2 hours
5. Absolute ethanol 2 hours
6. Xylene 1 hour
7. Xylene 1 hour
8. Xylene 1 hour
9. Molten paraffin wax 2 hours
10. Molten paraffin wax 2 hours
11. Pastillated Paramat (mp.59°C)

The tissues were then transferred to a third paraffin bath and placed in a thermostat vacuum oven (Townson and Mercer Ltd, Croydon) for a further 2 hours, at 60°C and a vacuum of 600mmHg. The tissue embedding was completed using Tissue Tek thermal (60°C), dispensing (60°C) and cryo-consoles (-4°C).

Sections were cut using a sliding base-sledge microtome (Leitz & Wetzlar), at 6μm thickness. The prepared slides were stored in an oven (60°C) overnight before staining.

Staining of sections

A Haematoxylin and Eosin/Alcian Blue (H&E/Alcian Blue) staining procedure was employed using the following method:

1. Dewax in two changes of xylene (5 minutes).
2. Soak in absolute ethanol (until slides clear), 70% ethanol followed by a water rinse.
3. Stain in Alcian Blue for 8 minutes, followed by water rinse.
4. Stain in Mayers Haematoxylin for 10 minutes followed by water rinse.
5. Soak in Scott's Tap water (Magnesium sulphate 20g and Sodium bicarbonate 3.5g. dissolved in 1Lwater) for 2 minutes, followed by water rinse.
6. Stain in 1% Eosin for 2 minutes, followed by water rinse.
7. Differentiate and dehydrate in 70% ethanol and two changes of absolute ethanol.
8. Rinse in two changes of xylene.
9. Coverslip using mountant.

2.9 Histological scoring system

The proximo-dorsal aspect of articular cartilage in the histological sections was divided in to three zones 1, 2, and 3 (fig. 2.6). Zone 1 represented the area with the majority of the lesions. Zone three represented a control area with minimal lesions and zone two was the area between zones 1 and 3. The dorsal to palmar measurement of each zone was 6 mm. The histological appearance of articular cartilage in zones 1 and 3 were scored using the following criteria:

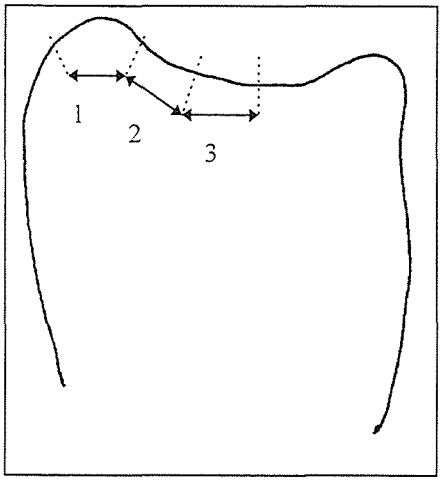


Figure 2.6 Diagrammatic representation of a sagittal bone slab showing the zones 1, 2 and 3 on the proximo-dorsal aspect of P1, which measured 6 mm in a dorso-palmar direction. Zones 1 and 3 were examined histologically.

Subjective histological score

The proximo-dorsal aspect of the section was assessed for evidence of degenerate cartilage. Fibrillation, partial or full thickness tears were identified and subjectively graded.

Grade 0 = smooth articular cartilage

Grade 1 = minor roughening

Grade 2 = moderate roughening (fig. 2.7)

Grade 3 = roughening plus cartilage tears (fig. 2.8)

Grade 4 = severe roughening plus full thickness tears

Grade 5 = severe roughening plus a cartilage flap or ossicle (fig. 2.9a and 2.9b)

Figure 2.7 Grade 2 articular cartilage fibrillation with Indian ink uptake in zone 1 showing empty lacunae (arrow) (H&E/alcian blue stain) (scale bar = 100 μ).

Objective histological scores

The objective histological scores were determined for zone 1 and zone 3. The objective histological score in zone 1 was comprised of six parameters, namely live chondrocyte number; chondrone number; characteristics of the articular cartilage tangential layer; the number of rows of chondrocytes in the tangential layer; cartilage thickness and number of empty lacunae. The objective histological score in zone 3 consisted of three parameters, namely: live chondrocyte number, tangential row number and cartilage thickness. Each parameter was given an individual score between 0 and 5, where 0 represented normality and 5 represented severe arthritic changes (table 2.2). The presence of an ossicle, osteophyte or cartilage flap was recorded. The counts in zone 1

were made adjacent to the palmar aspect of the lesion, or, if no lesions were present, at the most prominent point of P1.

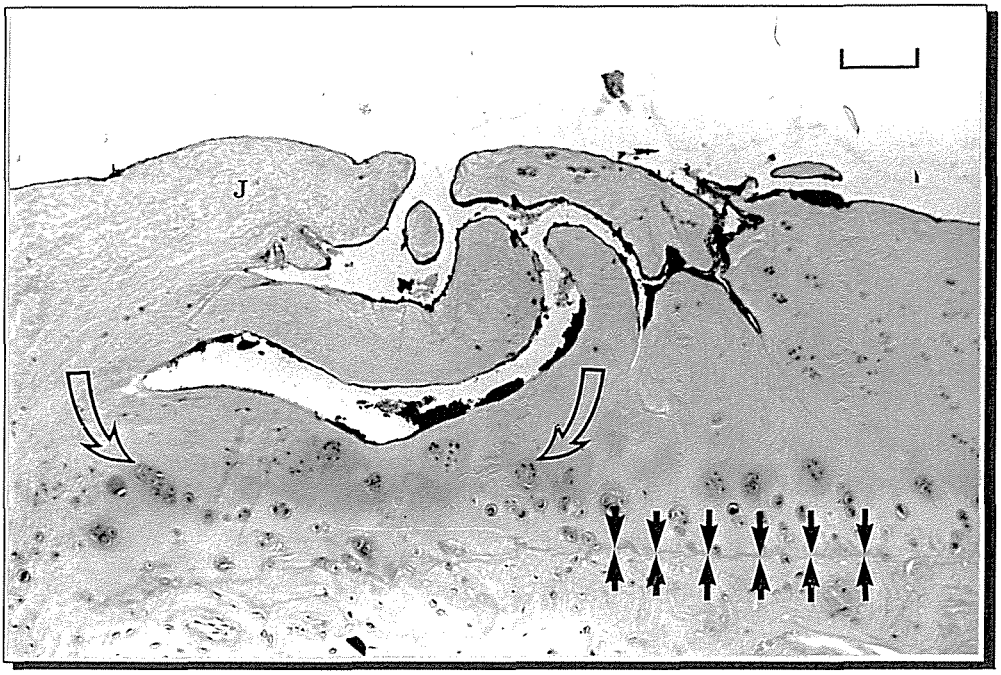


Figure 2.8 Grade 3 articular cartilage damage at the junction of the joint capsule (J), in zone 1. There are several chondrones (open arrows) deep to the fibrillated area and superficial to the "tide line" (solid arrows) (H&E/alcian blue stain) (scale bar = 100μ).

Table 2.2 Objective histological scoring system. Each category was graded between 0 and 5 where 0 represents normal articular cartilage and 5 represents severe changes.

Grade	Live chondrocyte number (zone 1&3)	Chondrone number	Tangential layer grade	Tangential row number (zone 1&3)	Cartilage thickness (zone 1&3)	Empty lacunae number
0	> 500	0	100% cigar	5	> 1mm	0
1	400-500	1-2	80% cigar, 20% round	4	0.8 - 1mm	1-5
2	300-399	3-4	60% cigar, 40% round	3	0.7 - 0.8mm	5-10
3	200-299	5-6	40% cigar, 60% round	2	0.6 - 0.7mm	10-15
4	100-199	7-8	20% cigar, 80% round	1	0.5 - 0.6mm	15-20
5	0-99	9-10	100% round	0	0.4 - 0.5mm	>20

1. *Live chondrocyte numbers in zones 1 and 3:* The number of live chondrocytes, including chondrones, in a 1 mm^2 area within the non-calcified portion of the articular cartilage, above the junction between the calcified and non-calcified cartilage ("tide line") (fig.

2.8). in zones 1 and 3, were determined using a x10 micrometer eye piece (Olympus) calibrated using a 0.001mm objective stage micrometer (Olympus).

2. *Chondrone number:* The number of chondrones (fig. 2.8), defined as clumps of two or more proliferating chondrocytes in a 1mm^2 area in zone 1, just palmar to the lesion were counted.
3. *Tangential layer grade:* The chondrocyte characteristics, (either cigar-shaped cells with dark nuclei and small amounts of cytoplasm arranged in horizontal rows, or more randomly arranged, circular shaped cells), were documented for the tangential layer of articular cartilage in zone 1.
4. *Tangential row number*: The number of chondrocyte rows in the tangential layer of articular cartilage in zone 1 and 3.
5. *Cartilage thickness in zones 1 and 3:* The thickness of the articular cartilage was measured, using the WF 10x micrometer (Olympus, Japan), from the "tide line" to the surface in zone 1 and zone 3.
6. *Empty lacunae:* The presence of empty lacunae (representative of dead chondrocytes) in a 2 mm^2 area in zone 1 was recorded.

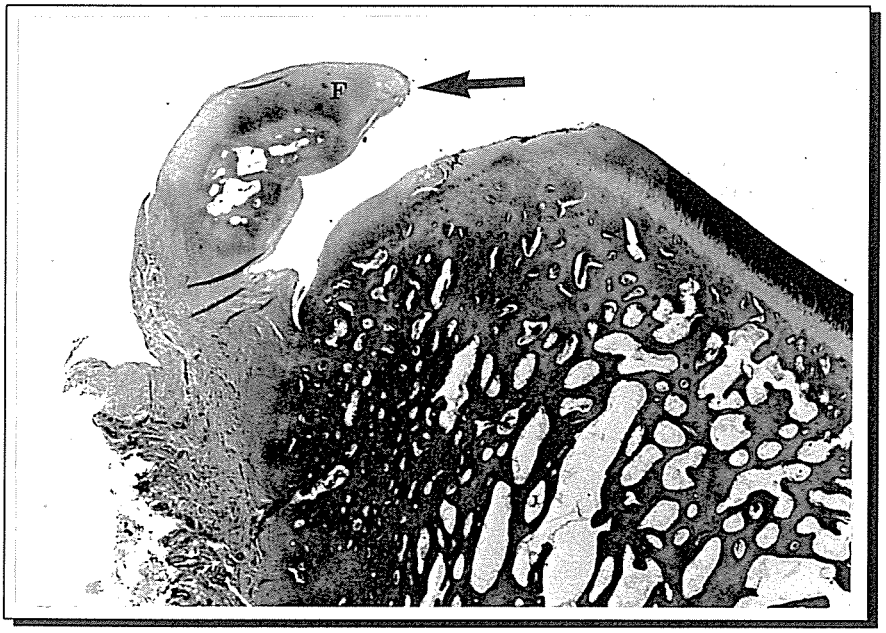


Figure 2.9a Ossifying articular cartilage flap (F) on the proximodorsal aspect of first phalanx. There is subchondral bone sclerosis (S) underlying the Grade 5 lesion (H&E/alcian blue stain) (magnification bar = 1mm).

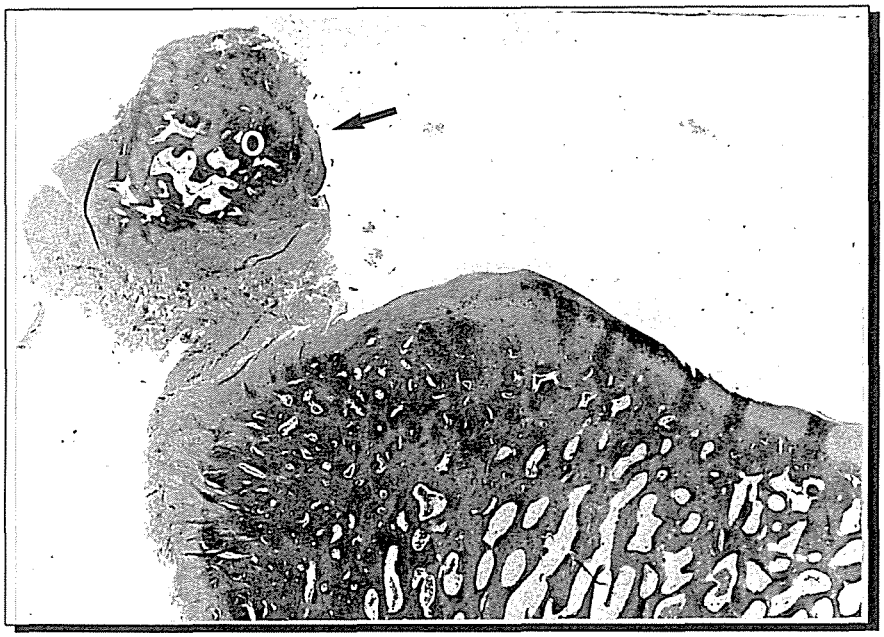


Figure 2.9b Ossicle (O) with a trabecular bone pattern and a fibrous attachment to the proximodorsal aspect of the first phalanx. There is subchondral sclerosis (S) under lying the grade 5 lesion. (H&E/alcian blue stain) (scale bar = 1mm).

2.10 Statistical analysis

Descriptive statistics were calculated for all variables. A Kruskal-Wallis one-way analysis of variance test was performed when a continuous variable was compared between different levels of nominal or ordinal variables with at least three categories, and Kruskal-Wallis Z-value multiple comparisons tests (Bonferroni) were used for comparing pairs of medians. The Mann-Whitney U test was used to compare between two groups of a continuous or ordinal variable with at least 5 categories, and Pearson's and Spearman's rank correlation coefficients were used for comparison between continuous variables. A probability (p) of less than 5% ($p < 0.05$) was considered significant in all cases. The results were presented graphically using box plots and error bar charts.

Box and whisker plots

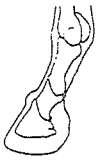
The top and bottom of the box represent the 25th and 75th percentiles enclosing the middle 50% of the data. The line through the middle of the box is the median. The

adjacent T-shaped lines that extend from the ends of the box represent the upper and the lower adjacent value.

Error bar charts

The means are shown as symbols and standard deviations as lines extending up and down from the symbol.

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖



Chapter 3

Results

3.1 Animals

The estimated maximum age of the horses used in this study was 14 years and the minimum age was 2 years. The mean age was 7.4 +/- 3.3. Three age group categories were established: group 1 represented animals younger than 5 years of age (n = 5), group 2 represented animals of 5-10 years of age (n = 11), and group 3 represented animals older than 10 years of age (n = 6).

3.2 Dissection of the metacarpophalangeal joints

Immediately after the joints were opened the articular surfaces were visually examined. Iatrogenic damage to the articular cartilage (scalpel incisions) were identified in several joints and were not confused with the natural lesions. The more severe lesions were obvious prior to staining with the Indian ink however the extent of the mild lesions were difficult to asses prior to staining.

3.3 Articular surface staining

Staining with Indian ink enhanced the lesions. Subtle lesions were more obvious, wear lines were easily identified and the full extent of severe lesions were apparent post staining.

3.4 Macroscopic observations

The lesions identified on the proximo-dorsal aspect of P1 included small (1-2 mm long) fine black stained lines, areas (1-4 mm in length and up to 5mm wide) of black

stained frayed articular cartilage, articular cartilage flaps, cartilaginous proliferation on the proximo-dorsal margin of P1, osteophytes and ossicles (fig. 3.1a-3.1e)

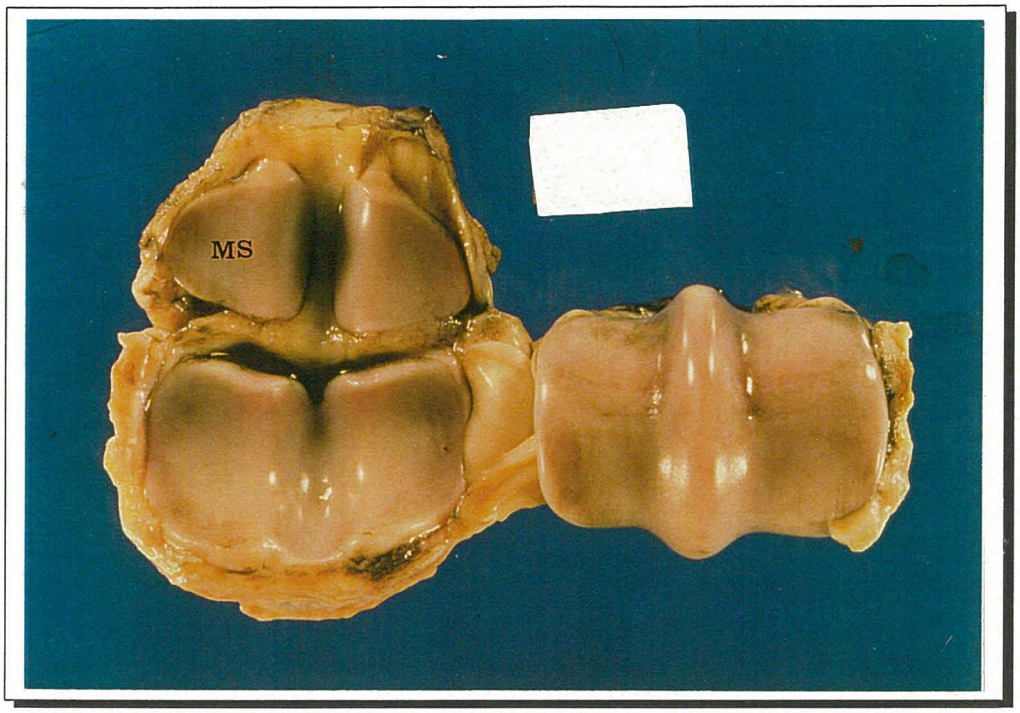


Figure 3.1a. A left metacarpophalangeal joint (age group 1) with a macroscopic lesion score of 1 on the lateral eminence and 1 on the medial eminence (*MS = medial sesamoidl).*

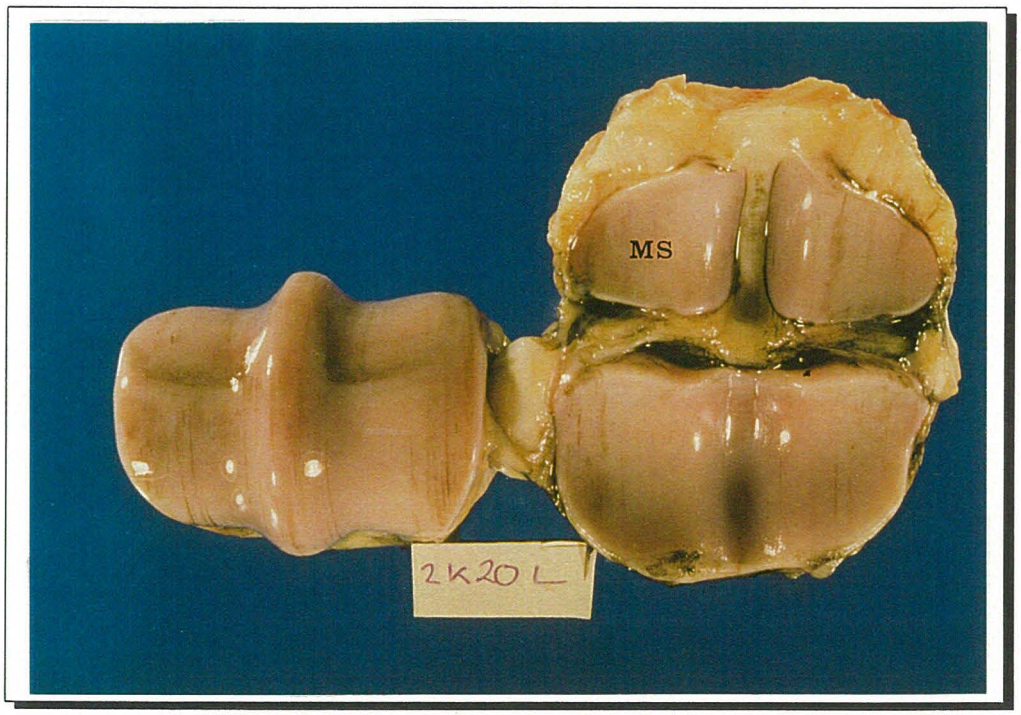


Figure 3.1b. A left metacarpophalangeal joint (age group 2) with a macroscopic lesion score of 2 on the lateral eminence and 3 on the medial eminence (*MS = medial sesamoid).*

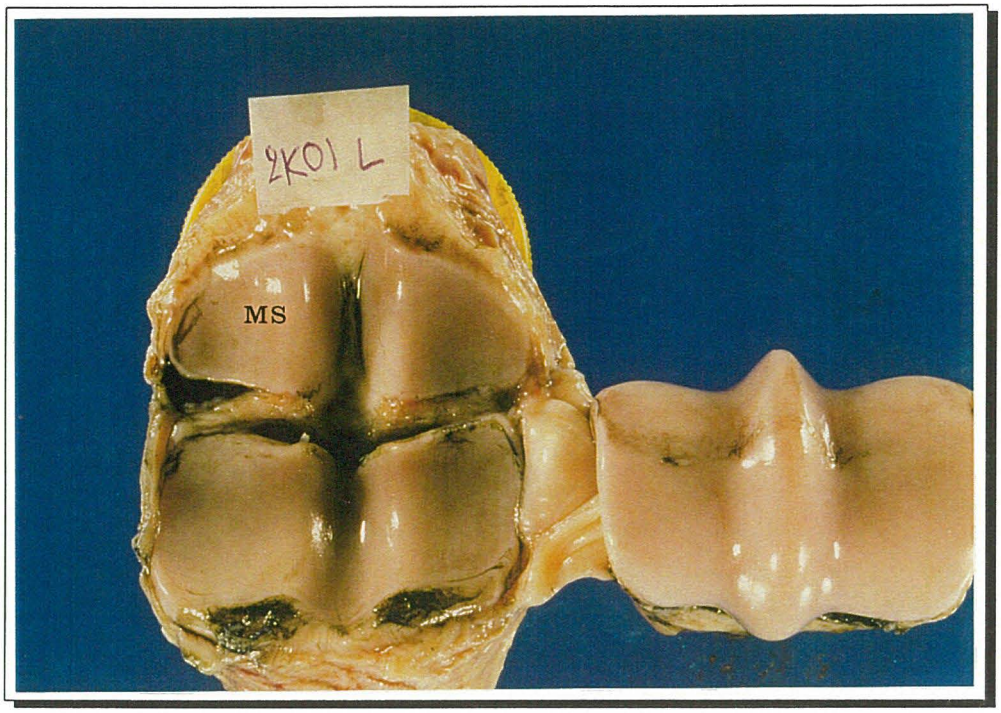


Figure 3.1c. A left metacarpophalangeal joint (age group 3) with a macroscopic lesion score of 3 on the lateral eminence and 4 on the medial eminence (*MS = medial sesamoid).*

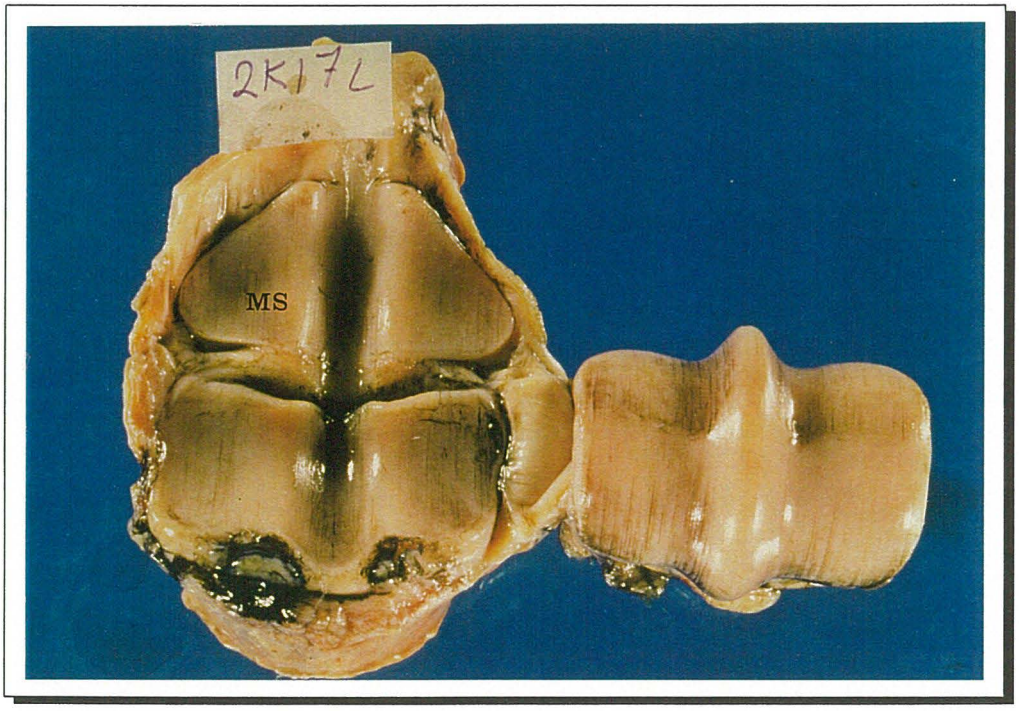


Figure 3.1d. A left metacarpophalangeal joint (age group 2) with a macroscopic lesion score of 4 on the lateral eminence and 5 on the medial eminence. Wear lines are visible on the articular surface (*MS = medial sesamoid).*

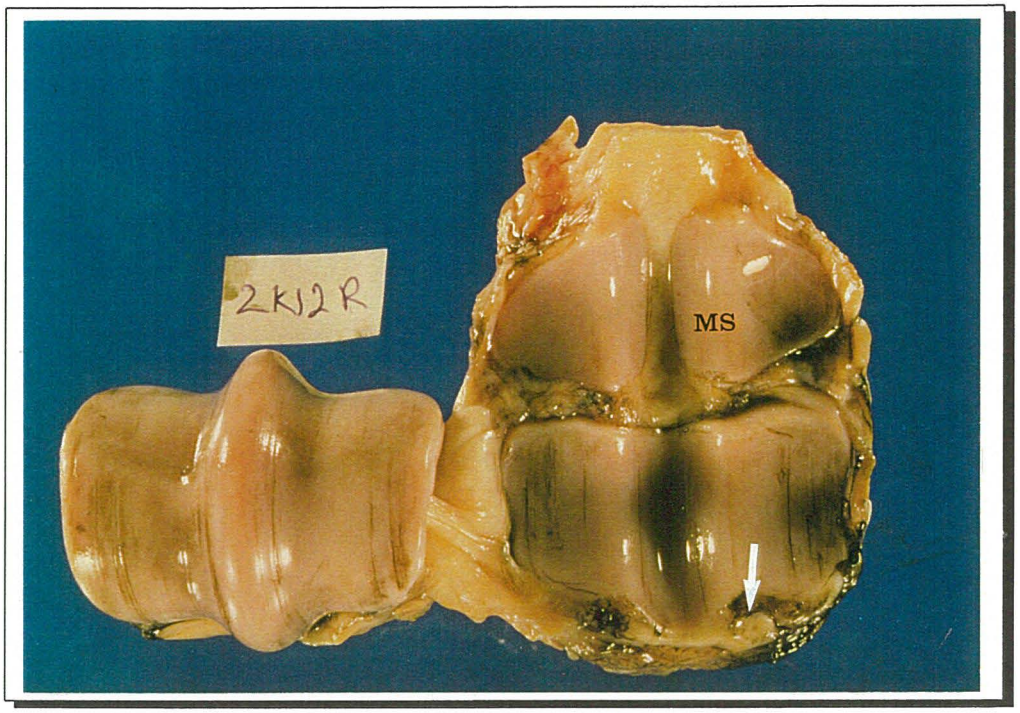


Figure 3.1e. A right metacarpophalangeal joint (age group 3) with a macroscopic lesion score of 4 on the lateral eminence and 5 on the medial eminence. There is an ossicle on the medial eminence (arrow) and there are wear lines on the articular surface (*M= medial).*

The median lesion score increased significantly with age group (Chi squared, corrected for ties (χ^2) = 157.20, degrees of freedom (DF) = 2, $p < 0.001$). Kruskal-Wallis multiple comparison Z-value tests (Bonferroni) showed that there was a significant difference between the lesion score medians of the different age group (fig. 3.2). The median lesion score was significantly greater on the medial compared with the lateral aspect of proximo-dorsal P1 (Mann-Whitney U Rank-sum test for difference in medians z = 3.46, $p < 0.0001$) (fig. 3.3).

3.5 Bone slabs

The thickness of the bone slabs varied between a maximum of 2.6mm and a minimum of 1.15mm. The palmar aspect of the slab was thinner (< 2mm) than the dorsal aspect for every slab. The palmar margin of one slab broke during the cutting process.

3.6 Cabinet radiographs

Subjective assessment of the radiographs showed that the subchondral bone in slabs with macroscopic lesion score 4 and 5 appeared more sclerotic, and the trabecular bone pattern less obvious compared to those with lower lesion scores. The thicker slabs appeared whiter than the thinner ones giving an impression of sclerosis. This was taken into account when assessing the radiographic images. Eight specimens from 7 joints of 5 horses had ossicles with a distinct trabecular bone pattern at the proximo-dorsal margin (figs. 3.4a-3.4e).

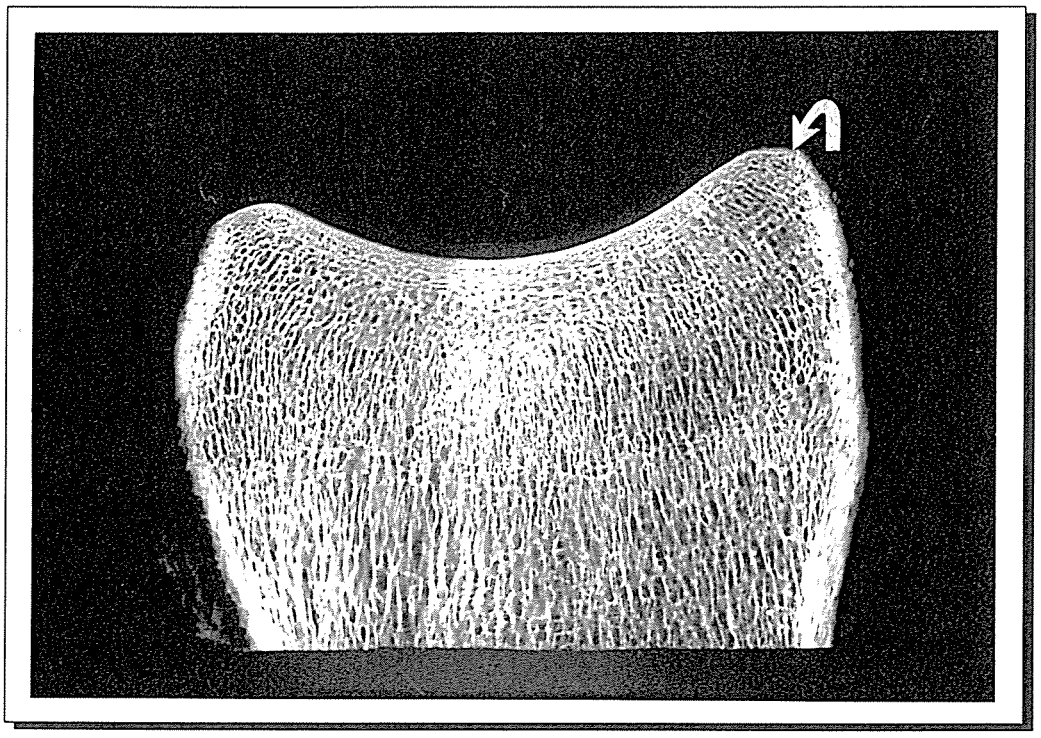


Figure 3.4a Cabinet radiograph of a 2mm thick sagittal bone slab from proximal P1. The proximo-dorsal eminence appears smooth and intact (arrow).

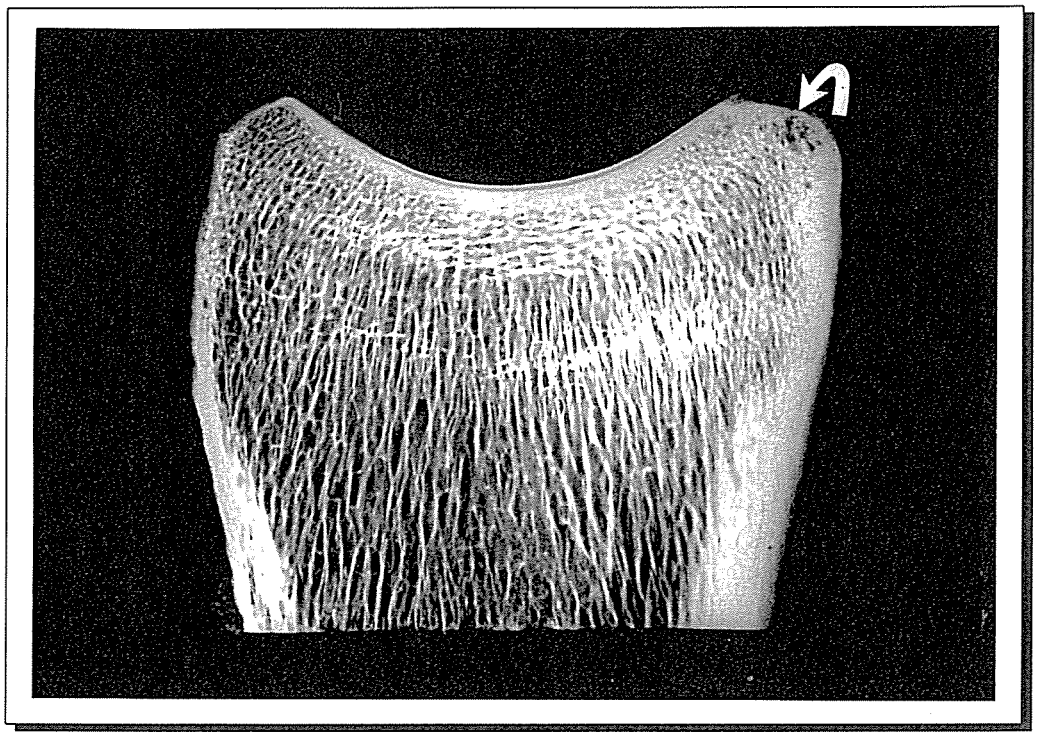


Figure 3.4b Cabinet radiograph of a 2mm thick sagittal bone slab from proximal P1. The proximo-dorsal eminence appears flattened with a radiolucent zone (arrow).

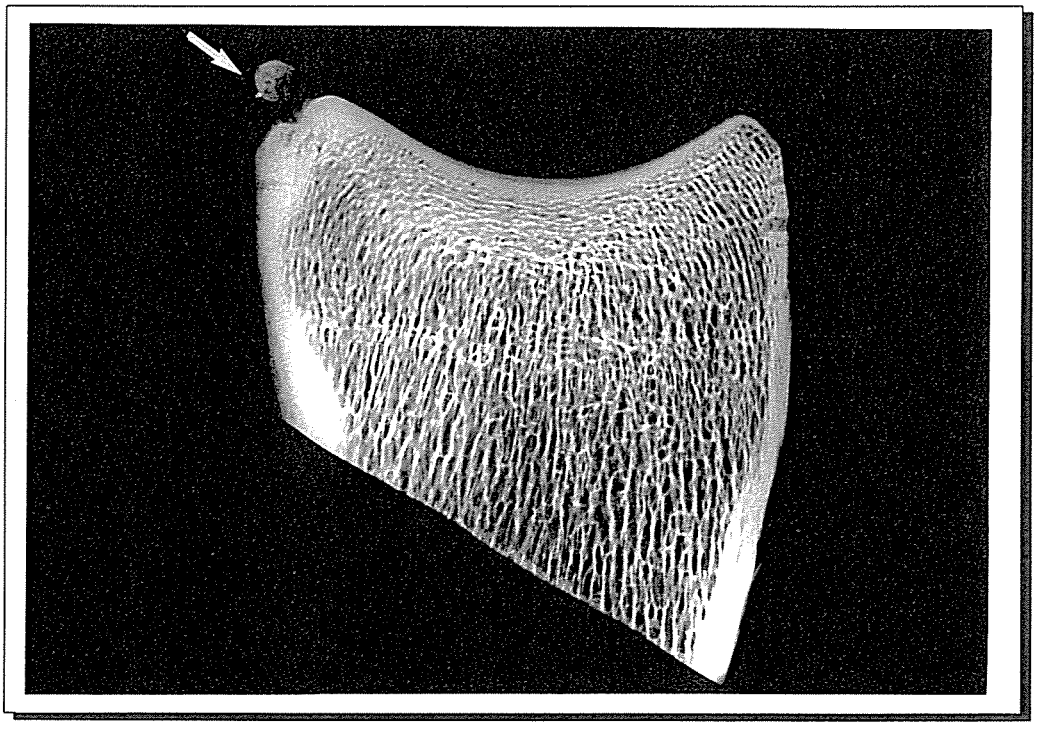


Figure 3.4c Cabinet radiograph of a 2mm thick sagittal bone slab from proximal P1. There is a small ossicle on the proximo-dorsal aspect of P1 and a corresponding defect in the parent bone.

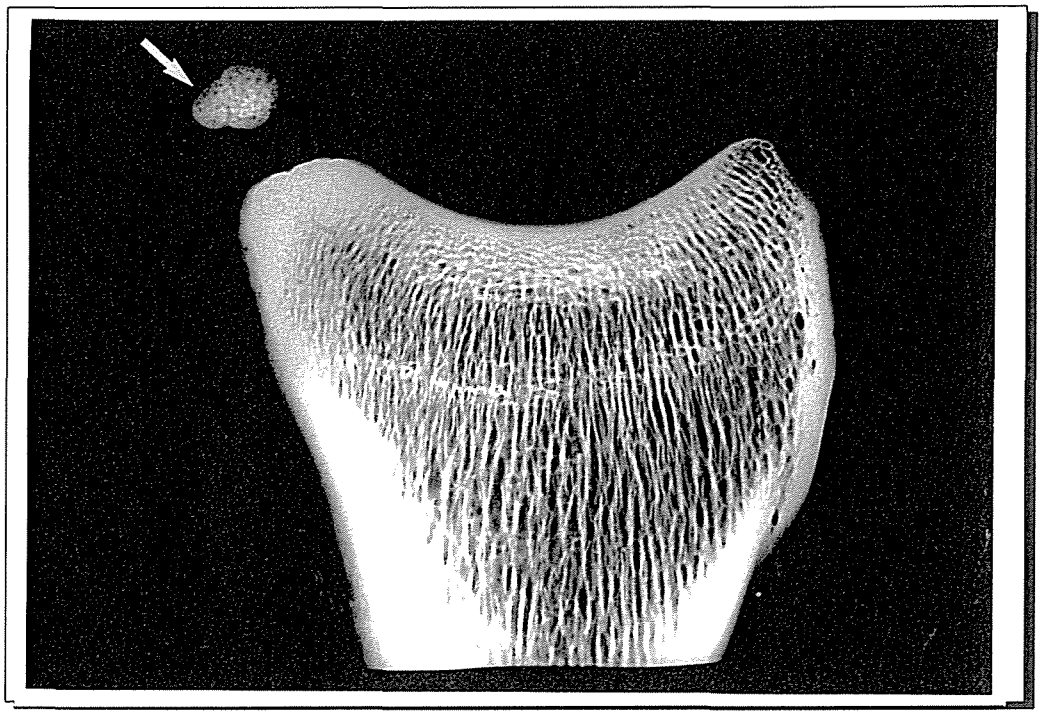


Figure 3.4d Cabinet radiograph of a 2mm thick sagittal bone slab from proximal P1. There is a large ossicle and sclerosis on the proximo-dorsal aspect of P1.

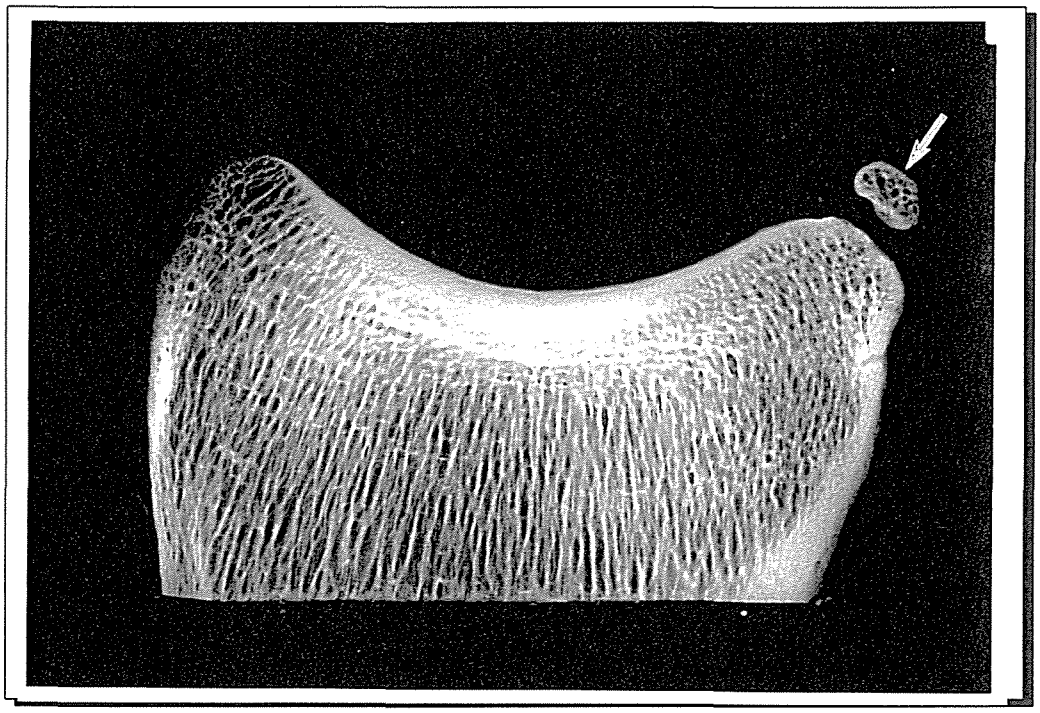


Figure 3.4e Cabinet radiograph of a 2mm thick sagittal bone slab from proximal P1. There is a large ossicle on the proximo-dorsal aspect of P1 clearly showing the trabecular bone pattern.

3.7 Bone mineral density observations

The bone mineral density images were composed of multiple coloured pixels. High bone mineral density was represented by a yellow colour and low bone mineral density by a purple colour. The proximo-dorsal aspect of P1 appeared to contain a greater proportion of yellow pixels than the proximo-palmar (fig. 3.5) The DEXA scanner was not sensitive enough to record the bone mineral density from the palmar aspect of several slabs that were less than approximately 1.8 mm thick. This did not affect the results as the area of interest was the proximo-dorsal aspect of the slab.

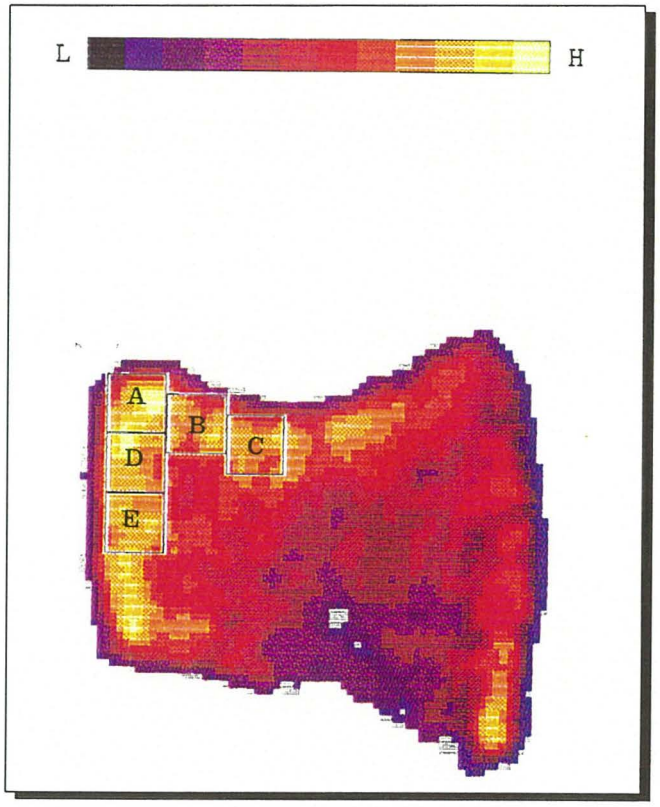


Figure 3.5 An example of DEXA image of a bone slab. The boxes A-E represent the areas of interest on the proximo-dorsal aspect of P1. Yellow = high bone mineral density, dark purple = low bone mineral density.

The bone mineral density range was 0.108 - 0.200 gm/cm^2 (corrected for thickness)with a mean of 0.1618 (SD +/- 0.01715). The median bone mineral density increased with age group of the horses (χ^2= 89.09, DF=2, $p < 0.001$). The Kruskal-Wallis multiple comparison Z-value test showed that the medians of the three age groups were significantly ($p < 0.001$) different from each other (fig. 3.6).

Specific regions of interest for bone mineral density analysis

The median bone mineral density increased between age groups consistently across areas A to E (χ^2 = 89.14, DF =2, p < 0.001), and the Kruskal-Wallis multiple comparison Z-value test showed that the median bone mineral density of area A was significantly greater than for all other areas. There was no significant difference in median bone mineral density between areas B,C,D, and E. (fig. 3.7). The median bone mineral density values increased with lesion score (χ^2= 137.2, DF=5, p < 0.001), although the Kruskal-Wallis multiple comparison Z value test showed that lesion scores 1 and 2 were not significantly different (fig. 3.8). There was no significant difference between the median bone mineral density of the medial and lateral aspect of P1 (Mann-Whitney U Rank-Sum test for comparison of medians, Z = 1.28, p = 0.194).

3.8 Histological observations *(objective and subjective scoring systems)*

The objective histological findings in articular cartilage from the proximo-dorsal lesions showed: the number of chondrocytes and cartilage thickness were decreased; the number of empty lacunae (fig. 3.9) and chondrones increased; the tangential layer was dominated by randomly arranged circular cells and the number of rows in this layer decreased. The articular cartilage was smooth and intact in horses with no lesions and became progressively more disrupted as the histological score increased (fig. 3.10a-3.10e).

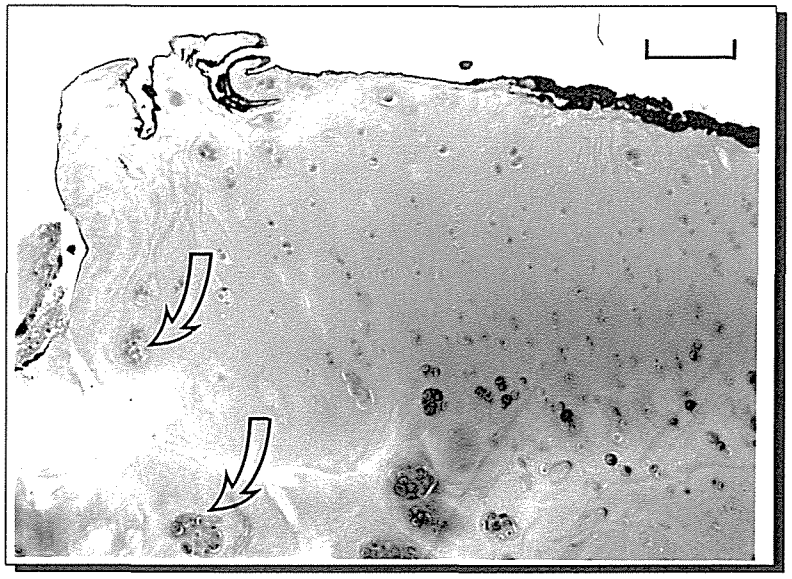


Figure 3.9 A high power histological section from the proximo-dorsal aspect of P1 stained with hematoxylin and eosin showing obvious cartilage fibrillation stained with black Indian ink and chondrones (arrows) (scale bar = 100μ)

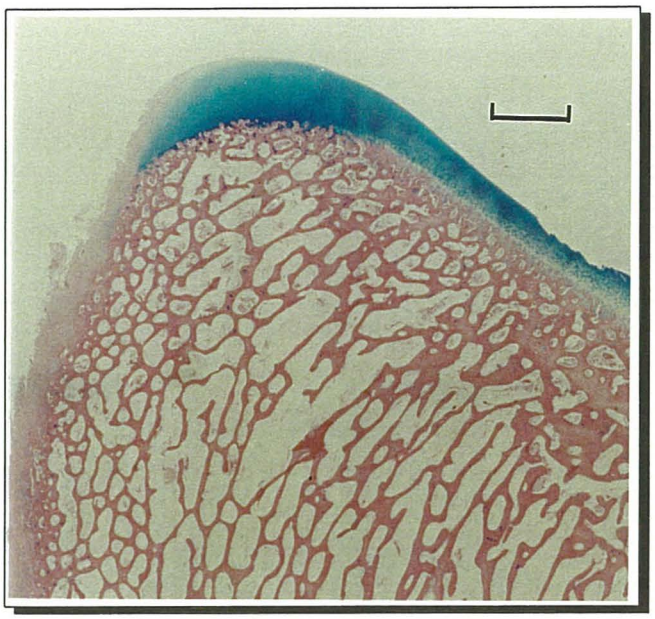


Figure 3.10a. A histological section from the proximo-dorsal aspect of P1 stained with H&E/alcian blue showing normal smooth articular cartilage. Subjective histological grade = 0. (scale bar = 1mm)

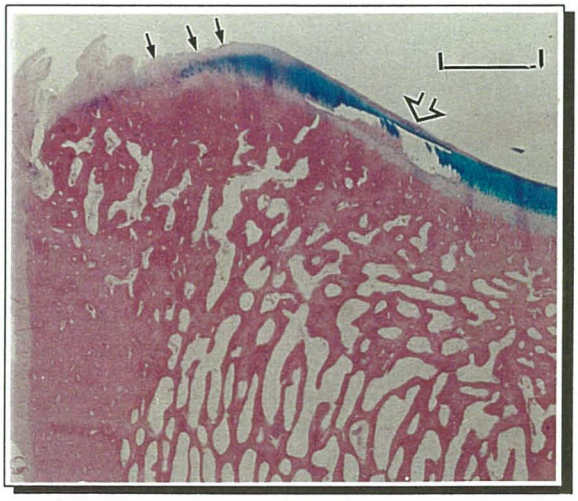


Figure 3.10b. A histological section from the proximo-dorsal aspect of P1 stained with hematoxylin and eosin and alcian blue. There is an irregular, disrupted articular cartilage surface with reduced up-take of alcian blue stain on the proximo-dorsal aspect of P1 (small arrows). Open arrow = artefact. Subjective histological grade = 2. (Scale bar = 1mm)

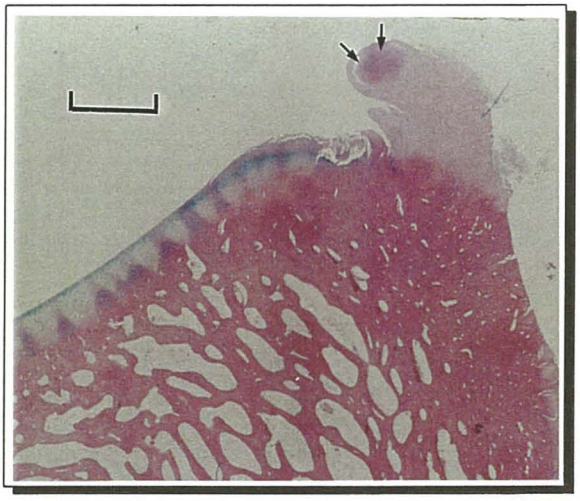


Figure 3.10c. A histological section from the proximo-dorsal aspect of P1 stained with hematoxylin and eosin and alcian blue. There is a catilage flap with a circular area of ossification at its apex on the proximo-dorsal aspect of P1 (small arrows). Subjective histological grade = 4. (Scale bar = 1mm)

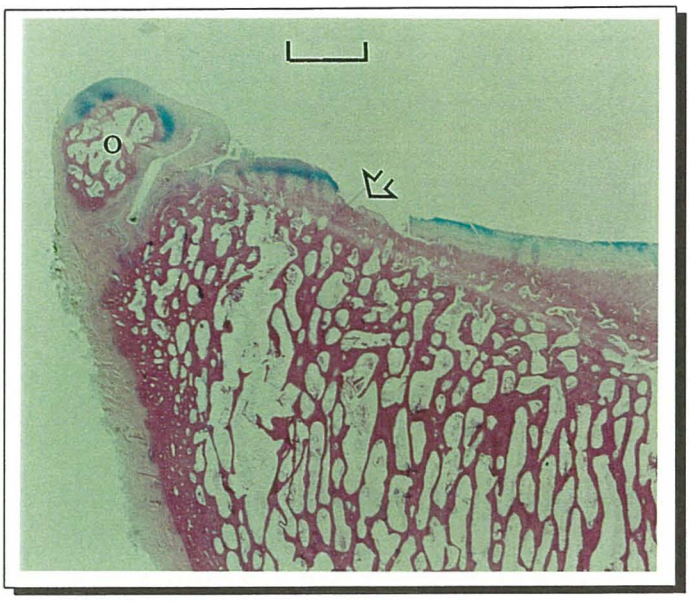


Figure 3.10d. A histological section from the proximo-dorsal aspect of P1 stained with hematoxylin and eosin and alcian blue. There is an ossicle covered in articular cartilage on the proximo-dorsal aspect of P1 (small arrows). Open arrow = artefact. Subjective histological grade = 5. (Scale bar = 2mm)

All six objective histological score categories in zone 1 (Table 3 and fig. 3.11), and all three objective histological score categories in zone 3 (Table IV), were significantly ($p < 0.001$) associated with the subjective histological score. The grades of the objective histological score categories in zone 3 were significantly ($p < 0.001$) lower than the corresponding grades in zone 1.

Table 3.1 The relationship between objective histological score categories in zone 1 and subjective histological score (Kruskal-Wallis multiple comparison, Z-value test).

Objective histological score variables in zone 1	Effect of increasing subjective histological score for each variable	Kruskall Wallis	Degrees of freedom	p value
Live chondrocyte number	Decreased	308.15	5	< 0.001
Chondrone number	Increased	314.86	5	< 0.001
Empty lacunae	Increased	291.68	5	< 0.001
Tangential layer grade	Increased	301.54	5	< 0.001
Rows in tangential layer	Decreased	176.51	5	< 0.001
Cartilage thickness	Decreased	234.46	5	< 0.001

Table 3.2 The relationship between objective histological categories in zone 3 and the subjective histological grade.

Objective histological score variables in zone 3	Effect of increasing subjective histological score for each variable	Kriskall Wallis	Degrees of freedom	p value
Live chondrocyte number	Decreased	290.87	5	< 0.001
Rows in tangential layer	Decreased	186.78	5	< 0.001
Cartilage thickness	Decreased	152.58	5	< 0.001

Subjective histological score compared with macroscopic lesion score

The median lesion score increased with subjective histological score ($\chi^2 = 310.4$, DF = 5, $p < 0.001$); however, the Kruskal-Wallis multiple comparison Z-value test showed that the medians of subjective histological score 0 and 1 were not significantly different (fig. 3.12).

Subjective histological score compared with age

The median age of horses increased with subjective histological score categories ($\chi^2 = 263.5$, DF = 5, $p < 0.001$). The Kruskal-Wallis multiple comparisons Z value test showed that the medians of subjective histological scores 4 and 5 were not significantly different from each other.

Subjective histological score compared to lesion site (medial/lateral) and side (left/right)

The median subjective histological score was significantly greater on the medial than the lateral aspect of P1 (Mann-Whitney U Rank-Sum test, Z = 4.35, $p < 0.001$). There was no significant difference between the median subjective histological score on the right and left legs (Mann-Whitney U Rank-Sum test, Z = 0.62).

Subjective histological score compared with bone mineral density

The median bone mineral density increased with subjective histological score ($\chi^2 = 131.15$, DF = 5, $p < 0.001$). The Kruskal-Wallis multiple comparison Z-value test showed that the medians of subjective histological scores 0 and 1 were not significantly different.

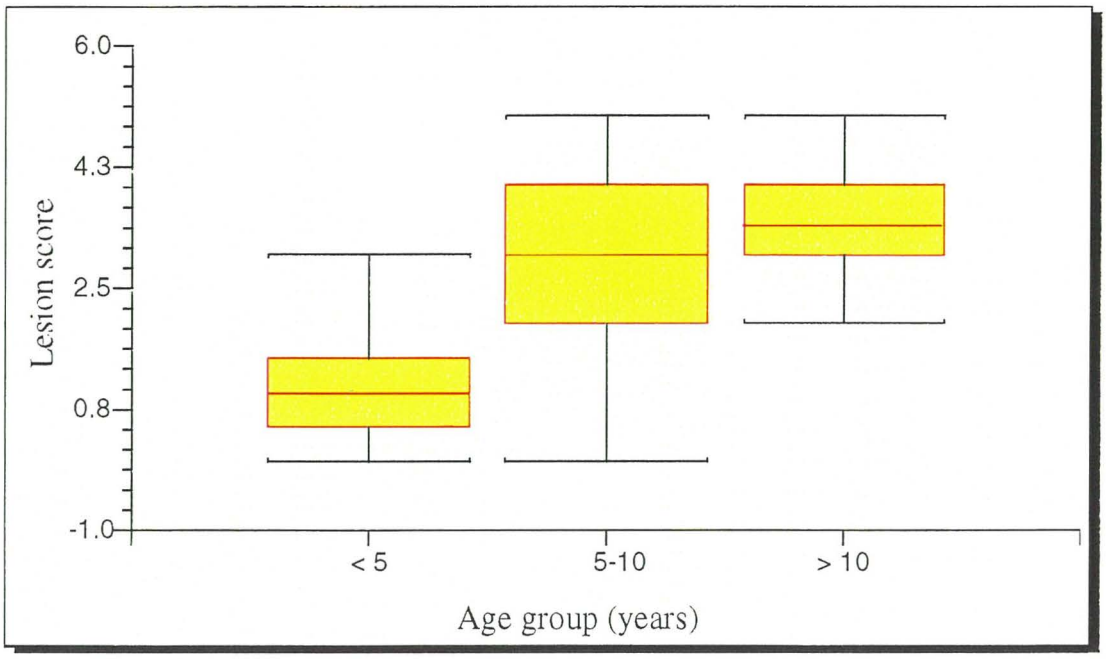


Figure 3.2 Box and whisker plot comparing lesion score and age group.

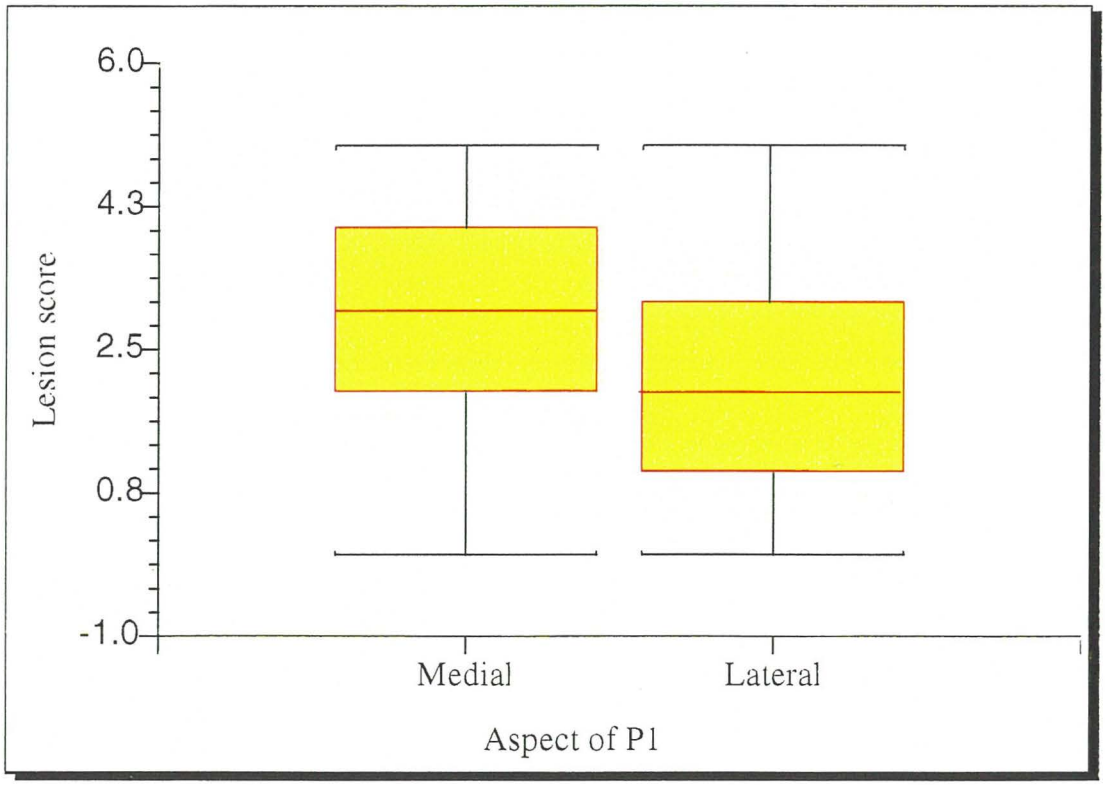


Figure 3.3. Box and whisker plot comparing the articular cartilage macroscopic lesion score between the medial and lateral eminences of the proximal first phalanx.

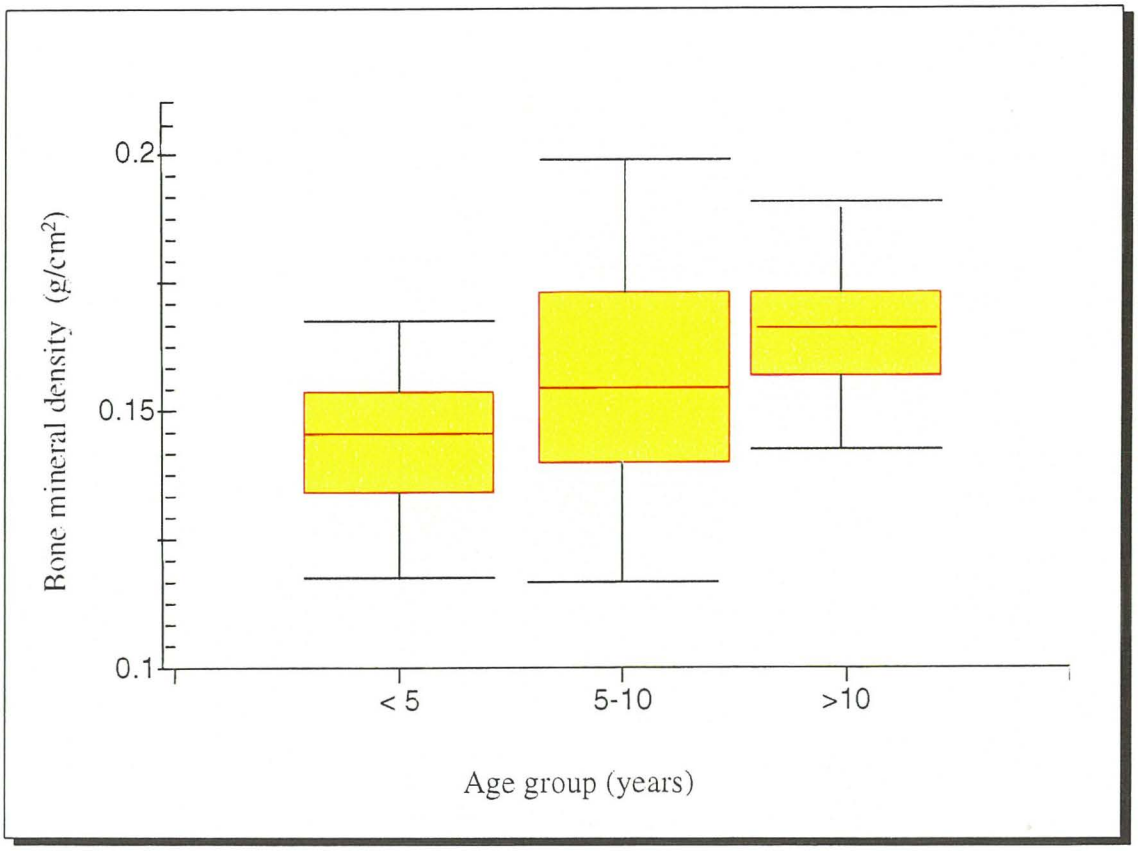


Figure 3.6 Box and whisker plot comparing bone mineral density in the proximo-dorsal aspect of P1 and age groups.

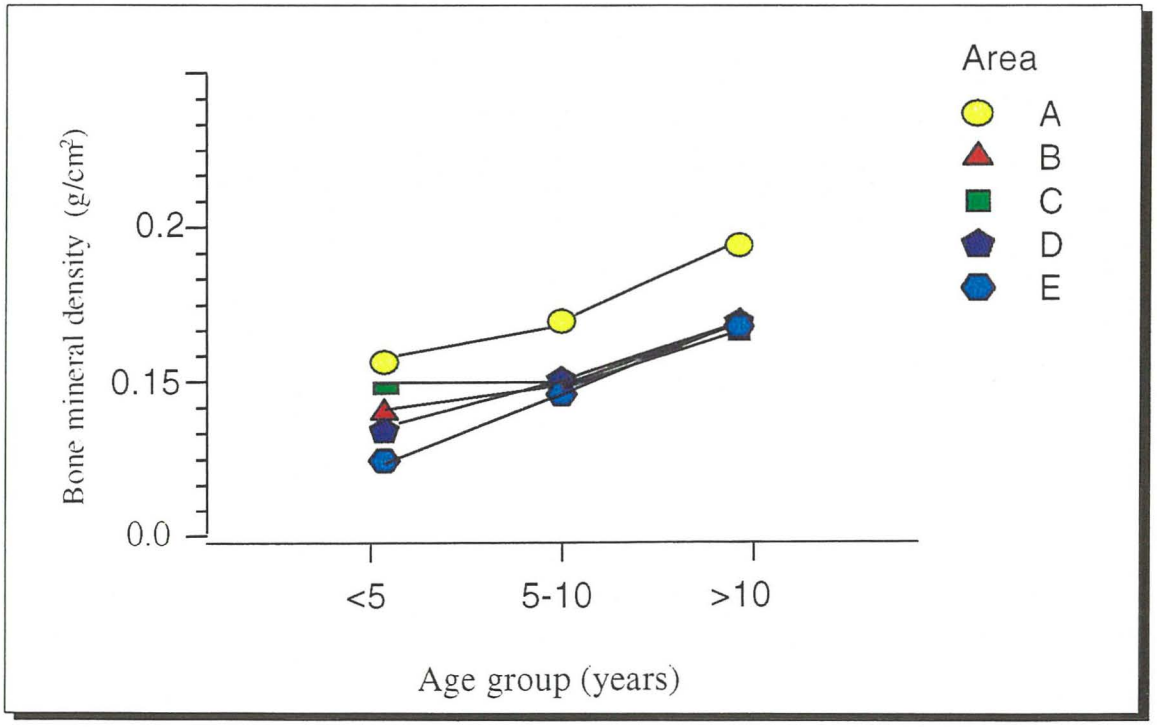


Figure 3.7 Comparison of median values of bone mineral density in areas A-E in the proximo-dorsal aspect of P1 and age group.

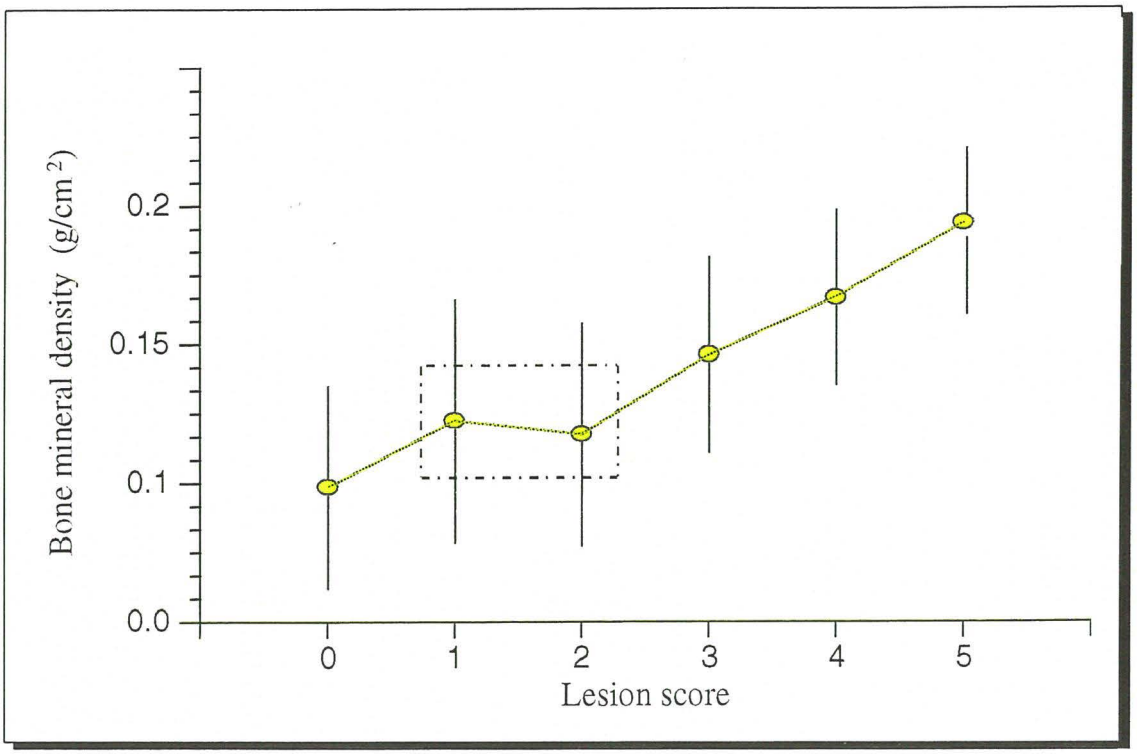


Figure 3.8 Error bar chart comparing bone mineral density in the proximo-dorsal aspect of P1 and the over lying articular cartilage macroscopic lesion score. (The dotted box encloses points that were not significantly different from each other).

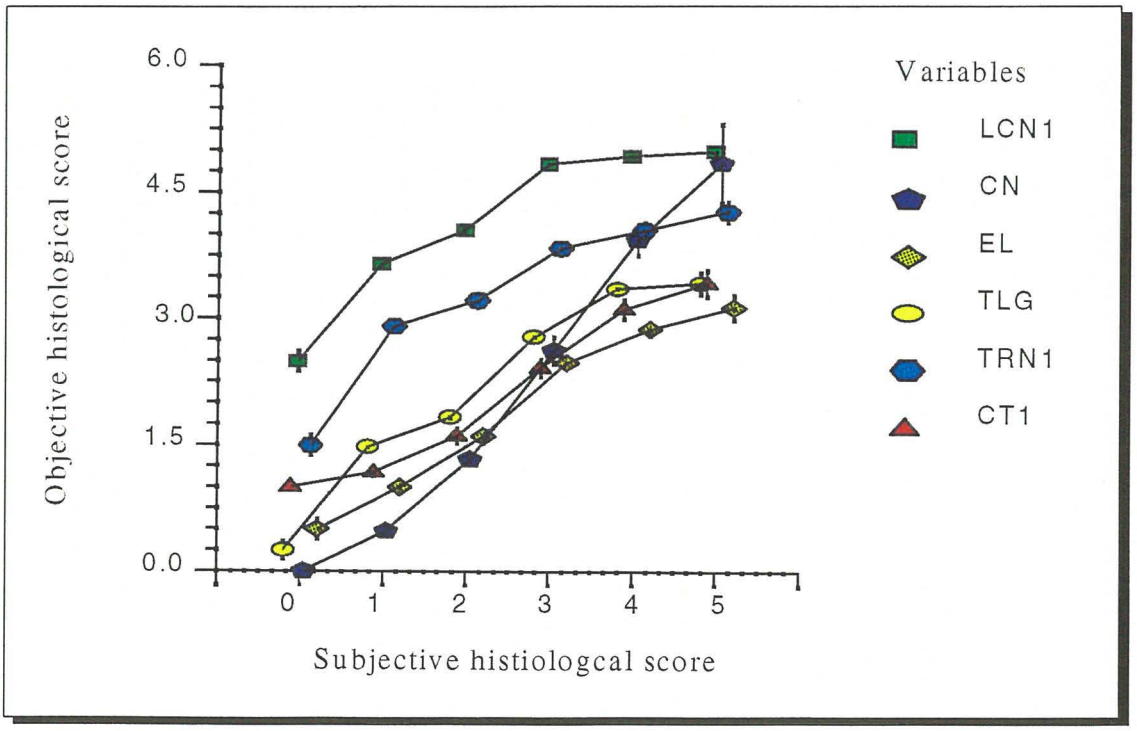


Figure 3.11 Error bar chart comparing the objective histological scores and subjective histological scores. (LCN = Live chondrocyte number in zone 1, CN = chondrone number, EL = empty lacunae, TLG = grade of tangential layer, TNR1 = number of rows in the tangential layer zone 1, CT1 = cartilage thickness zone 1).

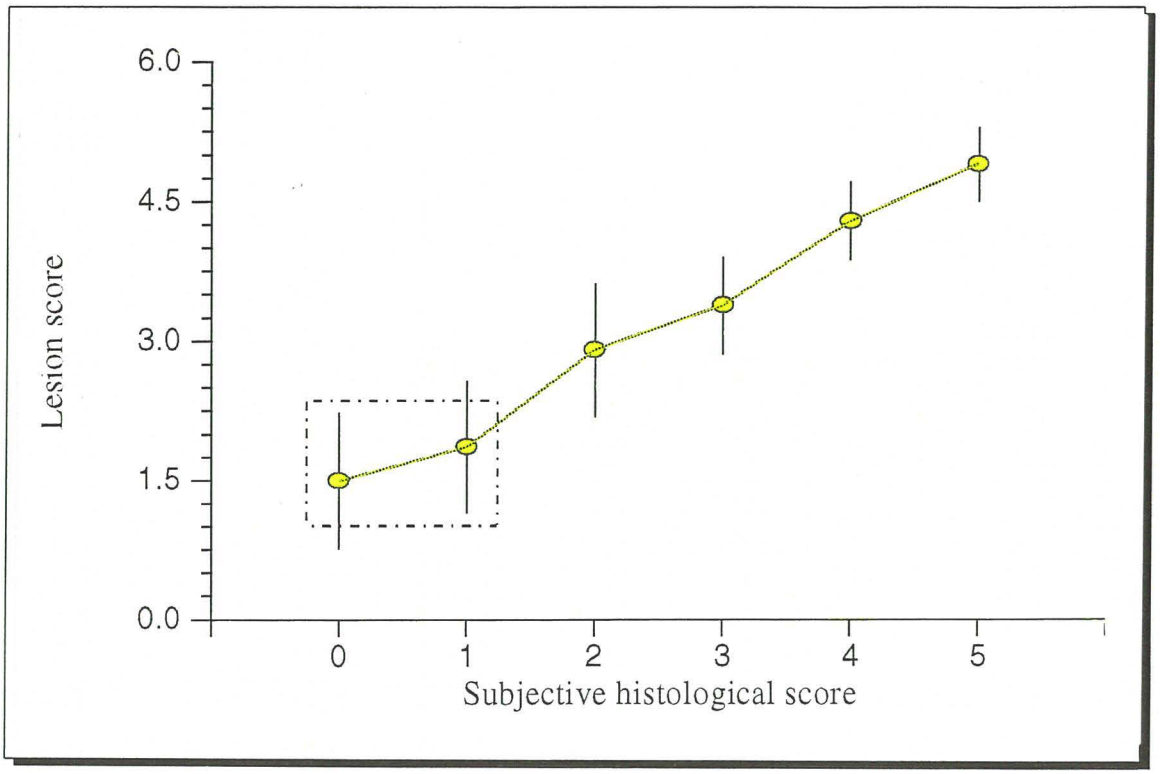


Figure 3.12 Error bar chart comparing lesion score and the subjective histological score. (The dotted box encloses points that were not significantly different from each other).

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖

Chapter 4

Discussion

The objective of this chapter is to consider each area individually, in the same order as in the materials and methods, and results sections. The findings, limitations or problems encountered within each area and the place of this study in relation to existing work on osteoarthritis will be discussed. Further research possibilities and the conclusions of the study will be presented at the end of the chapter.

4.1 Animals used in the study

The Kaimanawa wild horses used in this study represent a population of horses that has not been subjected to the physical stresses of training and racing. Genetic distance studies, based on the 17 loci that are routinely blood typed in horses for parentage verification, were performed on the Kaimanawa horses. The genetic markers identified in the Kaimanawa horses showed obvious similarity to Thoroughbreds (I.L Anderson, personal communication). Due to the genetic similarity between the wild horses and Thoroughbred horses, environmental rather than genetic factors were considered to be the major variable in this study.

The Kaimanawa herd, which has been in existence since the 1850's, consists of escaped military and stock horses and also deliberately released, unwanted horses. The herd was protected in 1981 and the population increased as there were no natural predators. Despite culls by the Department of Conservation of 250 horses in both 1993 and 1994, numbers had reached almost 1600 by the end of 1994.

Material for this study was randomly collected from the 1994 cull; however, the sample was not a truly random one as the foals and yearlings from the round up were not slaughtered. The horses were mustered using helicopters and were seen to gallop during the procedure. The lesions identified on the proximo-dorsal aspect of the first phalanx (P1) appeared to be chronic and were accompanied by neither sharp fracture lines nor haemorrhage, and were therefore unlikely to have been caused during the muster.

The clinical significance of the lesions could not be determined since no lameness history was available. This was because the tissue from the horses was collected directly from the slaughter house.

4.2 Ageing the horses

The ageing of horses from their dentition has been shown to be imprecise. Richardson *et al.* (1995) showed that most horses under 5 years of age were aged correctly to within one year of their true age; accuracy of age estimation was moderate for horses between 5 and 10 years of age and poor for horses older than 10 years of age. Although a reference collection of teeth was used to determine the ages of the horses in this study, the exact ages of the older horses was difficult, especially for horses greater than 10 years of age. The rough grass diet of the wild horses may result in more rapid tooth wear than occurs in the domesticated horse. This could mean that ageing the wild horses was even more inaccurate.

The horses were divided into the three age groups; young (less than 5 years), middle-aged (5 - 10 years) and old (greater than 10 years) because accurate ageing was not possible. It was the intention to have equal numbers of horses in each age group, but the young age group was under represented. Assuming that the horses in this study were correctly assigned to the age groups, the conclusions should not be significantly affected by inaccurate ageing.

4.3 Dissection of the metacarpophalangeal joint

The metacarpophalangeal joints were opened, the articular surfaces examined, washed with isotonic saline, graded, photographed and fixed in formalin within 24 hours of slaughter. This time lag is within the period accepted by others (Freeman and Meachim, 1979).

The articular surfaces were rinsed with isotonic saline rather than water to prevent osmotic alterations in the cartilage that may affect the subsequent histological staining process. Drying of the tissues was avoided during the examination prior to fixing.

The most severe lesions on the articular surfaces of the metacarpophalangeal joints were situated on the proximo-dorsal eminence of P1. Wear lines were also identified on the articular surface of P1 and the third metacarpal bone in some cases. The investigation centred on the focal proximo-dorsal P1 lesions, which were in a similar location to the P1 osteochondral fragments commonly identified in many kinds of competition horses of widely varying ages. There was considerable interest in the lesion on the proximo-dorsal aspect of P1 because it is one of the most common lesions, and possibly the site of earliest fibrillation, associated with osteoarthritis in the metacarpophalangeal joint.

4.4 Indian ink staining

Indian ink has been used to evaluate the articular cartilage of human femoro-patellar joints from necropsies. Mild articular cartilage change was more readily apparent in Indian ink-stained preparations than non-stained preparations, which was consistent with Meachim's findings (Meachim 1972a). The Indian ink preparations were helpful in the interpretation of histological sections. The particles of ink marked the articular surface and entered grooves that were in communication with the synovial joint space. This enabled artefacts created during the cutting of the histological sections to be distinguished from articular cartilage changes.

4.5 Macroscopic lesion scores

The macroscopic changes identified on the proximo-dorsal aspect of P1 after staining were consistent with osteoarthritic lesions. They included fibrillation (represented by fine black stained lines and areas of black stained articular cartilage), articular cartilage flaps, cartilaginous proliferation, osteophytes and ossicles. Wear lines were also identified on the proximal aspect of P1 and distal third metacarpal bone in some cases. Wear lines appear to be an age-related phenomenon and have been reported as frequent incidental findings in adult equine joints (Rooney 1969) and human joints (Byers *et al* 1970, Kelser *et al* 1938), but not in the joints of young children. The severity of the macroscopic lesions on the proximo-dorsal aspect of P1 increased with age, supporting the hypothesis that there is a naturally occurring, age-related osteoarthritic process in the metacarpophalangeal joints of wild horses. The lesions were more severe on the medial than the lateral eminence of proximal P1, consistent with McIlwraith's (1987) observations in athletic domestic horses.

Gross post-mortem change in articular cartilage has been categorised into non-progressive (age-related cartilage changes) and progressive (osteoarthritic changes) by Byers *et al.* (1970). Non-progressive degeneration consists of articular cartilage fibrillation that does not progress to clinical osteoarthritis, and progressive degeneration relates to overt osteoarthritis associated with clinical signs. Fibrillation is the earliest gross change seen on the articular surface in both categories of articular cartilage change and the boundaries between these categories are not, therefore, clearly defined (Sokoloff 1982). Byers (1970) classified cartilage changes in the human hip joint on the basis of the anatomical site, gross appearance and radiological features of specimens from random autopsies and compared them with findings from femoral heads resected in the treatment of patients with clinical osteoarthritis. The non-progressive changes were represented by superficial fibrillation, fissuring and flaking with no exposure of underlying bone, and the progressive changes were represented by severe fibrillation, cartilage flaps and eburnation. Osteophytes were classified as stage 1 when there was minimal projection of the osteophyte beyond the articular margin and stage 2 when there was obvious projection of the osteophyte. Stage 1 osteophytes increased in prevalence until 50 years of

age then become relatively constant. Stage 2 osteophytes became more frequent with increasing age and also accompanied the progressive articular cartilage changes (Byers 1970).

The gross articular cartilage changes identified in horses with lesion scores 0 - 3 were similar to the non-progressive, age-related articular cartilage changes identified by Byers *et al.* (1970), while the articular cartilage changes in the horses with macroscopic lesion scores 4 and 5 were similar to some of the progressive articular cartilage changes described in that report. Osteophytes similar to Byers (1970) stage II osteophytes were identified in animals with grade 5 lesion scores. There were animals within the old age group with lesion scores of 2 and 3 which may indicate that they had only age related changes. Using these criteria, it appears that wild horses may have both age-related and progressive osteoarthritic changes within the metacarpophalangeal joints.

Byers (1970) suggested that the age-related and progressive changes existed as separate entities. Freeman and Meachim (1979), however, proposed a contrary hypothesis for the development of the clinical form of primary osteoarthritis. They suggested that osteoarthritic changes result from exaggerated or accelerated development of the age-related, asymptomatic cartilage fibrillation which is encountered so frequently amongst the general human population at necropsy.

It could not be ascertained if the osteoarthritic changes demonstrated in this study represented a continuum of the age-related changes or were a separate incidental occurrence. Correlation of the macroscopic lesion scores to the lameness history would have been very valuable, but it was not possible to establish if the osteoarthritic changes were associated with clinical signs of the disease.

4.6 Bone slabs

The bone slabs cut with the parallel diamond blades were not of uniform thickness because the blades converged slightly as they moved through the bone. This resulted in the palmar aspect being consistently slightly thinner than the dorsal aspect. The precise thickness of the bone slabs (measured with sliding callipers) and the bone mineral density was corrected for thickness in order to compare sites and animals.

The palmar aspect of several bone slabs did not show on the scan, because the scanner threshold was not reached. This did not, however, affect the study as the area of interest was the proximo-dorsal aspect of P1, which was successfully scanned in all cases, after meticulous calibration before each use.

4.7 Bone mineral density

Radin (1986) proposed that the density and architecture of the subchondral bone can have a profound effect on both the initiation and progression of overlying articular cartilage changes. Bone mineral density accounts for about 70% of bone strength (Ott 1993) and bone fragility depends on bone density as well as bone quality (Schnitzler 1993). Bone mineral density is closely related to bone stiffness (Moris *et al.* 1995) and bone mineral density measurements are considered the most accurate predictor of fracture risk available (Hassager and Christiansen 1995). Although direct biomechanical investigation of the proximo-dorsal aspect of P1 has not been conducted, it is probably reasonable to assume that the increase in subchondral bone mineral density with age is associated with a corresponding increase in subchondral bone sclerosis, and stiffness. The increase in proximo-dorsal P1 sclerosis with age seen on the radiographs of the bone slabs, even though subjectively assessed, supported the bone mineral density findings.

The average subchondral bone mineral density of areas A-E within the proximo-dorsal aspect of P1 increased with articular cartilage lesion score. It is possible that the articular cartilage changes reported here are secondary to shear stresses created by an increase in the stiffness gradient between the subchondral bone and the overlying articular cartilage as proposed by Radin (1986). The bone mineral density, and presumably subchondral bone stiffness, was significantly greater in area A (situated immediately below the articular cartilage lesion) than in the adjacent areas. This increase in stiffness and abrupt difference in stiffness between areas may be important factors which determine the predisposition site of the lesion. Radin (1986) proposed that such stiffness gradient variations, induced by repetitive impact loading, create stresses in the articular cartilage and subsequent fibrillation. It was not, however, possible in this study

to determine if the bone mineral density change preceded the cartilage degeneration or vice versa.

Subchondral bone sclerosis is considered a feature of osteoarthritis. The increase in bone mineral density with age and the subjective radiographic findings support the hypothesis that naturally occurring, age related osteoarthritis occurs in wild horses.

4.8 Histology

The objective histological data provided further support for the first hypothesis that there is a naturally occurring, age-related osteoarthritic process in the metacarpophalangeal joints of wild horses. The histological features identified in this study were age-related and included reduced chondrocyte number, empty lacunae, chondrones, surface fibrillation and decreased articular cartilage thickness. These features are consistent with osteoarthritis and are similar to those reported by Meachim (1972b). The histopathological features of osteoarthritic articular cartilage described by Meachim (1972b) were articular cartilage thinning, multicellular rounded clusters of chondrocytes (chondrones), necrotic chondrocytes (empty lacunae), reduced staining of articular cartilage matrix and irregular articular surface with splits.

There were no chondrones or empty lacunae in zone 3, and the objective histological scores in zone 3 were lower than the corresponding scores in zone 1. This implies that the articular cartilage is affected by factors which are different and/or are different in extent, and/or elicit a different response in the two zones. The main factor differentially affecting the zones is likely to be the repetitive compressive strain from the distal third metacarpal bone (MCIII) upon the dorsal aspect of P1. Pool (1990) described how over-extension of the metacarpophalangeal joint can result in repetitive compression of the proximo-dorsal aspect of P1 by the disto-dorsal aspect of MCIII, and similar intrinsic trauma may occur in wild horses. The changes in zone 1 are possibly superimposed upon the less severe, 'normal-for-age' changes present in both zones.

The validity of the subjective histological score was supported by the close correlation between the objective and subjective histological scoring systems. This could be potentially useful in future studies on osteoarthritis as the subjective system is simple

and much quicker to use than the extensive objective system, and requires examination of a smaller area (zone 1). It was also easier to compare the single subjective histological score to other parameters such as age and bone mineral density measurements, than to employ the 6 score categories utilised in the objective histological scoring system.

There was a highly significant correlation between the macroscopic lesion score and the subjective histological score, which supports the validity of both the gross and the histological scoring systems.

4.9 General discussion

The nature of the intimate relationship between the articular cartilage and the underlying subchondral bone in the initiation and progression of osteoarthritis is not fully understood. One of the major theories of osteoarthritis is that articular cartilage deteriorates after there is a stiffening of the subchondral bone induced by repetitive loading, and experimental studies have tested this hypothesis (Radin 1984, Brown *et al.* 1984). Morphometric analysis, radiographic assessment and bone scintigraphy have demonstrated experimentally that repeated loading of a joint results in subchondral bone sclerosis in rabbits (Radin *et al.* 1984), guinea pigs (Simon and Radin 1972) and sheep (Radin and Orr 1982). Subchondral bone stiffness increased in the radial facet of third carpal bones of race-trained Thoroughbred horses as they underwent training (Young *et al.* 1991). A local subchondral osteogenic response has been documented in the third and radial carpal bones of Thoroughbreds exposed to galloping exercise on a treadmill (Firth *et al.* Submitted).

Adult subchondral bone appears to protect the overlying articular cartilage from damage by attenuating forces acting on the joint (Brown *et al.*1984*)*. Shear stress is also reduced by the calcified cartilage layer, which has a compliance between that of articular cartilage and the underlying subchondral bone (Redler 1975). Stiffening of the subchondral bone results in reduced joint congruity when a load is applied, minimising the contact area, and increasing the shear stresses experienced by the articular cartilage (Askew and Mow 1978). Radin (1986) reported that articular cartilage fibrillation on the human femoral head first appears in areas of steep stiffness gradients that cause stress

concentration. He also suggested that the stresses at the junction of excessively stiff subchondral bone and the more compliant articular cartilage lead to physical disruption of the articular cartilage structure, progressive loss of matrix and chondrocytes, and progression of the disease.

Richardson and Young (1996) found no significant correlation between the articular cartilage hexosamine content and the mechanical and morphologic properties of the underlying bone on the dorsal aspect of the third carpal bone. Also, the stiffness of the subchondral bone in pathological third carpal bones was greater than that of untrained Thoroughbred horses but not significantly greater than that of normal, trained horses. The conclusion was that there was no relationship between stiffening of the subchondral bone and degenerative change in the overlying articular cartilage in the third carpal bone of 3-4 year old Thoroughbred horses.

In contrast to the findings of Richardson and Young (1996), a relationship was demonstrated between subchondral bone mineral density and overlying articular cartilage changes (gross and histological) on the proximo-dorsal aspect of P1 of wild horses. The increase in subchondral bone mineral density was associated with histopathological abnormalities in the overlying articular cartilage. It was not possible to correlate subchondral bone mineral density with the amount and type of exercise in the wild horses. It is, however, highly likely that strain rate, and probably speed and exercise density, would have been much less in the wild horses than in Thoroughbred horses trained in the U.S.A. Despite this, there was an association of histological change and subchondral bone mineral density in the wild horses but not the Thoroughbreds. This leads to the possibility that total number of cycles, rather than strain rate or speed, induce bone mineral density changes. It is, however, possible that the lesions identified in the wild horses were more chronic than those described by Richardson and Young (1996) and may represent a later stage of the disease process. In any case, the stresses exerted on the proximo-dorsal aspect of P1 are different from those encountered on the radial facet of the third carpal bone, which may make comparison difficult and conclusions different.

Injury to the intercarpal joint of race horses is more likely due to chronic rather than acute strain (Bramlage 1987) and repetitive trauma has been considered to be the

main cause of proximo-dorsal osteochondral fractures of P1 in athletic horses (McIlwraith 1982). Repetitive cyclic loading results in fatigue damage, such as microfracture of the subchondral bone (Carter and Hayes 1977), which stimulates repair and remodelling in an attempt to cope with the stress demands (Lanyon 1982). If the stress and strain sustained during training are in excess of the bone's ability to remodel and adapt, then osteochondral fracture may occur (Bramlage 1987, Schneider 1987).

Metacarpophalangeal joint over-extension is a fatigue and/or speed related phenomenon in racing horses, but may also be activity related such as in show jumping horses. It has become almost axiomatic that over-extension is the cause of proximo-dorsal P1 lesions (Stashak 1987). Rooney (1969) proposed that over-extension of the metacarpophalangeal joint places stress on the dorsal aspect of proximal P1, and suggested that fractures occur more commonly on the medial than the lateral aspect, because the medial eminence is slightly more prominent than its lateral counterpart and is more prone to the intrinsic trauma associated with over-extension.

It is not known if the wild horses gallop at high speed, if they experience musculo-skeletal-ligamentous fatigue, or if their metacarpophalangeal joints sustain hyperextension. Recent osteochondral fractures were not identified on the proximo-dorsal aspect of P1 in the wild horses. The increase in bone mineral density and sclerosis with age may represent adaptation of the subchondral bone to the stresses sustained during a natural roaming existence, which if continued on altered subchondral bone may result in articular cartilage changes and osteophytosis, consistent with osteoarthritis. Perhaps the normal physiological range of metacarpophalangeal joint motion, together with a high total accumulation of cycles, resulted in the lesions observed in the wild horses.

Horses, like humans, may have a combination of non-progressive and progressive articular cartilage alterations occurring simultaneously in one joint. It may be that non-progressive changes in horses are age-related and represent the cartilage alterations, such as mild fibrillation and wear lines, that are frequent incidental findings at necropsy.

It is possible that the non-progressive articular cartilage changes in horses become progressive when another factor, such as the repetitive intrinsic trauma of race training, is imposed on the joint. It is also feasible that racing and training accelerates the naturally

occurring age-related changes and may also induce independent, progressive changes that account for clinically evident osteoarthritis. It is not clear, however, what the factors are in wild horses that might induce progressive articular cartilage changes. One possibility is that the susceptibility of articular cartilage to osteochondral change may be genetically determined.

4.9.1 Further research ideas

Further potential work in this area includes direct comparison of the metacarpophalangeal joint changes in trained thoroughbreds and wild horses of the same age.

An investigation of wild horse locomotion, gait analysis, distance travelled per day, lameness, and the degree of metacarpophalangeal joint dorsi-flexion in the natural habitat would be very valuable. Such information would assist in relating the changes identified in this study with exercise factors.

Another possibility for further research is a study of the proteoglycan content of articular cartilage from the wild horses, and correlation of this data to the bone mineral density of the underlying subchondral bone at various sites. Such information for the third carpal bone would enable Richardson's (1996) work on Thoroughbred horses to be compared with that on wild horses. It would also help to determine if subchondral bone change precede articular cartilage change or vice versa.

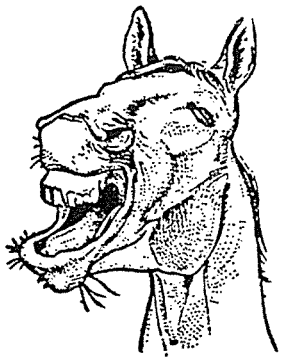
4.9.2 Conclusions

This study demonstrates that changes consistent with osteoarthritis, such as articular cartilage fibrillation, wear lines, subchondral bone sclerosis, osteophytosis and ossicles occur on the proximo-dorsal aspect of P1 in wild horses. There was a close relationship between each of the various features and between osteoarthritis severity and age. The lesions were grossly and histologically similar to some of those identified at post-mortem in the metacarpophalangeal joints of racehorses (Pool 1991). The clinical

significance of the lesions in the wild horses could not be determined as physical examination was not possible and lameness history was not available.

The age-related osteoarthritic process documented in this thesis may occur in all horses, and is therefore important in our understanding of the development of clinically evident osteoarthritis. These age-related changes in wild horses could be considered as a "background" of naturally occurring morphological abnormalities, upon which further changes may be superimposed.

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖



References

1. Adams ME, Billingham MEJ (1982): Animal models of degenerative joint disease. *Curr Top Pathol 71:265 - 297.*
2. Askew MJ, Mow VC (1978): The biomechanical functions of the collagen fibril ultrastructure of articular cartilage. *J Biomech Eng **100**:105-115.*
3. Bennett GA, Waine H and Bauer W (1942): Changes in the knee joint at various ages, with particular reference to the nature and development of degenerative joint disease. *Commonwealth fund, New York. Cited by* Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology Kippel JH, Dieppe PA (eds). Mosby Year book Europe Ltd.*
4. Blumel J (1960): Experimental immobilisation and remobilisation of rat knee joints. *J Bone Jt Surg (Am) **42**: 737 - 758.*
5. Bollet AJ (1969): An essay on the biology of osteoarthritis. *Arthritis Rheum Dis **48**:958-961.*
6. Bramlage LR, Schneider RK and Gabel AA (1987) A clinical perspective on lameness originating in the carpus. *Equine Vet J Orthopaedic supplement.*
7. Broom ND (1982): Abnormal softening in articular cartilage. *Arthritis and Rheum **25**:1209-1216.*
8. Brown TD, Radin EL, *et al.* (1984): Finite element studies of some juxtarticular stress changes due to localised subchondral bone stiffening. *J Biomech **17**:11-24.*
9. Bullough PG (1981): The geometry of diarthrodial joints, its physiologic maintenance and the possible significance of age related changes in geometry to load distribution and the development of arthritis. *Clin Orthop Rel Res **156**: 61-66.*
10. Bullough PG and Jagannath D (1983): The morphology of the calcification front in articular cartilage. *J Bone Jt Surg **65B**: 72-8.*

11. Bullough PG (1985): Topographical variations in the morphology and biochemistry of adult canine tibial plateau articular cartilage. *J Orthop Res 3:1-16.*
12. Byers PD, Contempoi J, O'Connor J (1970): A post-mortem study of hip joints. *Ann Rheum Disease; **29:**15-31.*
13. Cameron JR, Sorenson JA (1963): Measurement of bone mineral in vivo, an improved method. *Science **142**; 230-232.*
14. Cann CE, Genant HK (1980): Precise measurement of vertebral mineral content using computed tomography *J Comput Assist Tomogr **4**:493-500.*
15. Caron JP (1992): Understanding the pathogenesis of osteoarthritis. *Br Vet J **148:** 369-372.*
16. Carter DR and Hayes WC (1977): Compact bone fatigue damage - a microscopic examination. *Clin Orthop Rel Res. **127:**265-271.*
17. Ceil RL, Archer BH (1926): Classification and treatment of chronic arthritis. *JAMA **87**:741-746. Cited by* Dieppe PA (1994): In: Introduction to osteoarthritis; *Rheumatology Kippel JH, Dieppe PA (eds). Mosby Year book Europe Ltd*
18. Christensen SB (1985): Osteoarthritis: Changes in bone cartilage and synovial membrane in relation to bone scintigraphy. *Acta Orthop Scand **214**: 1-43.*
19. Clyne MJ (1987): Pathogenesis of degenerative joint disease. *Equine Vet J **19**(1):15-18.*
20. Cobb S, Merchange WR, Rubin T (1957): The relation of symptoms to osteoarthritis. *J Chronic Dis **5**:197-204,*
21. Colahan P, Turner TA, et al. (1987): *In proceedings of the 34th Annual convention of the Am Assoc Equine Pract, New Orleans, 689-699.*
22. Collins DH (1953): Osteoarthritis. *J Bone Jt Surg **35B**: 518-520.*
23. Collins DH (1938): The pathology of osteoarthritis. *Br J Rheumatol **14**:253-262*
24. Collins DH, Meachim G (1961): Sulphate fixation by human articular cartilage compared in knee and shoulder joint *Ann Rheum Dis **20**:117*-128.
25. Copelan R, Bramlage LR (1983): Surgery of the fetlock joint. *Vet Clin North Am Equine practice 2:221-231.*

26. Crelin ES, Southwick WO (1964): Changes induced by sustained pressure in the knee joint of adult rabbits. *Anat Rec* ***149****: 113 - 134.*

27. Cushnaghan J, Dieppe P (1991): Study of 500 patients with limb joint osteoarthritis. Analysis by age, sex and distribution of symptomatic joint sites. *Ann Rheum Dis* ***50****: 8-13.*

28. Davis WH, Lee SI, Sokoloff L (1978): Boundary lubricating ability in synovial fluid in degenerative joint disease. *Arthritis Rheum* ***21****:754-756.*

29. Dawson D, Jin ZN (1986): Micro-elastohydrodynamic lubrication of synovial joints. *Eng Med* ***15****:63-65.*

30. De Palma AF, Flynn JJ (1958): Joint changes following partial and total patellectomy. *J Bone Jt Surg (Am)* ***40****: 395-413.*

31. Dekel S, Weissman SL (1978): Joint changes after overuse and peak overloading of rabbit knees in vivo. *Acta Orthop Scand;* ***49****: 519 - 528.*

32. Dee R (1978): The innervation of joints. In: *Joints and synovial fluid.* Sokolff L (ed), *New York Academic press pp 177-204.*

33. Dieppe PA (1994): Osteoarthritis. *In: Rheumatology,* Kippel JH and Dieppe PA (eds), *Mosby Year book Europe Ltd.*

34. Dwitt MT, Handley CJ, *et al.* (1984): In vitro response of chondrocytes to mechanical loading. The effect of short term mechanical tension. *Connect Tis 12:97-109.*

35. Edwards GB (1982): Surgical arthrodesis for the treatment of bone spavin in 20 horses. *Equine Vet J* ***14****: 117 - 121.*

36. Edwards JCW, Sedgwick AD, Willoughby DA (1981): The formation of a structure with features of synovial membrane by subcutaneous injection of air. An in Vitro tissue culture system. *J Pathol* ***134****:147 - 156.*

37. Emery H and Meachim G (1973): Surface morphology and topography of patello-femoral cartilage fibrillation in Liverpool necropsies. *J Anat (Lond)* ***116****: 103-110.*

38. Evans CH, Mazzocchi RA, *et al.* (1984): Experimental arthritis induced by intraarticular injection of allogenic cartilagenous particles in to rabbit knees. *Arthritis Rheum* ***27****:200-207.*

39. Evans CH, Mears DC, Cosgrove JL (1981): Release of neutral proteinases from mononuclear phagocytes and synovial cells in response to cartilagenous wear particles in vitro. *Biochem Biophys Res Commun* ***677:287-294.***

40. Evans EB, Eggers GWN, *et al.* (1960): Experimental immobilisation and remobilization of rat knee joints. *J Bone Jt Surg(Am)* ***42****: 737 - 758.*

41. Fessler J (1984): Functional anatomy and physiology of diarthrodial joints. *In: The practice of large animal surgery.* Jennings PB (ed), *Philadelphia, WB Saunders, pp 687 - 715.*

42. Firth ECF, Goodship A, *et al.* (1997) Galloping exercise increases bone mineral density in the third and radial carpal bones of Thoroughbred horses. *Submitted for publication.*

43. Fisher LW, Hawkins G, *et al.* (1987): Purification and partial characterisation of small proteoglycans I and II from the mineral compartment of developing human bone. *J Biol Chem* ***262****: 9702-9708.*

44. Foland JW, McIlwraith CW, *et al.* (1994): Effect of betamethasone and exercise on equine carpal joints with osteochondral fragments. *Vet Surg* ***23****:369 - 376.*

45. Freeman MAR and Meachim G (1979): Ageing and degeneration. *In: Adult articular cartilage.* Freeman MAR (ed) *Pitman Medical. pp 487-545.*

46. Ghandially FN (1978): Fine structure of joints. *In: The joints and synovial fluid, Vol 1,* Sokoloff L (ed), *Academic press, New York. pp140-148.*

47. Garr EL, Davis W (1973): Degenerative changes following experimental patellectomy in the rabbit. *Clin Orthop* ***92****:296-304.*

48. Goldenberg DL, Egan MS, Cohen AS (1982): Inflammatory synovitis in degenerative joint disease. *J Rheumatol* ***9****:204-9.*

49. Goldwraith JE (1987): The treatment of disabled joints resulting from the so called rheumatoid diseases. *Boston med Surg J* ***136****; 79-84.*

50. Goodman NL, Baker BK (1990): Lameness diagnosis and treatment in the Quaterhorse race horse. *Vet Clin North Am (Equine practice)* ***6****:85-108.*

51. Goodfellow JW, Bullough PG (1967): The pattern of ageing of the articular cartilage of the elbow joint *J Bone Jt Surg* ***49B:*** *175-184.*

52. Graham J, Goldman JA (1978): Fat droplets and synovial leukocytosis in traumatic arthritis. *Arthritis Rheum **21**:76-80.*

53. Gray ML, Pizzanelli AM, *et al.* (1988): Mechanical and physiochemical determinants of the biochemical response. *J Orthop Res **6**: 777-792.*

54. Greenfield MA, Craven JD, *et al.* (1981): Measurement of the velocity of ultrasound in human cortical bone. *Inv Radiol **138**: 701-710.*

55. Grier SJ, Turner AS, (1996): The use of DEXA in animals. *Inv Radiol **31**:(1), 50-62.*

56. Griffin MG, Kimble W, *et al.* (1993): DEXA of the rat: accuracy precision and measurement of bone loss. *J Bone Miner Metab **8**:795-800.*

57. Griffiths HJ, Zimmerman RE (1978): The clinical applications of bone mineral analysis. *Skeletal Radiol 3:1-9.*

58. Gritza TL, Fry LR, *et al.* (1973): Deterioration of articular cartilage caused by continuous compression in a moving rabbit joint. *J Bone Jt Surg (Am) **55**: 1698 - 1720.*

59. Gustafson SB, Trotter GW, *et al.* (1992): Evaluation of intra-articularly administered sodium monoiodoacetate-induced chemical injury to articular cartilage of horses. *Am J Vet Res **53**:1193-1202.*

60. Hall BK (1990): The osteoblast and the osteocyte. *In: Bone (Vol 2),* Hall BK (ed), *West Caldwell, NJ, Telford press.*

61. Hall MC (1964): Articular changes in the knee of the adult rat after prolonged immobilisation in extension. *Clin Orthop **34**: 184 -195.*

62. Hardingham TE, Fosang AJ, Dudhia J (1990): Domain Structure of aggregating proteoglycans from cartilage *64th Biochem Soc Trans 64th, Bath **18**: 794-796. Cited in: Joint Diseases in the Horse*. McIlwraith CW and Trotter GW (eds), *Saunders WB company, Philadelphia.*

63. Hassager C, Christiansen C (1995): Measurement of bone mineral density. *Calcif Tissue Int. **57**:1-5.*

64. Haygarth J (1805): A clinical history of diseases: *II Nodosity of the joints. London: Gadell and Davies.* Cited by Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology Kippel JH, Dieppe PA (eds). Mosby Year book Europe Ltd*

65. Haynes PF (1988): Diseases of the metacarpophalangeal joint and the metacarpus. *Vet Clin North Am (Equine practice)* ***78****:5-74.*

66. Hein J (1926): Uber die arthritis Deformans. *Virchows Archi;* ***260****: 521-563*. Cited by Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology Kippel JH, Dieppe PA (eds). Mosby Year book Europe Ltd.*

67. Heinegard D, Hascall VC (1974): Aggregation of cartilage proteoglycans. *J Biol Chem* ***249****: 4250-4256.*

68. Hills BA (1989): Oligomellar lubrication of joints by surface active phospholipid. *J Rheumatol* ***16****:82-91.*

69. Howard, Mcllwraith, C.W, (1993), *Compend Contin Edu Pract Vet* ***15****(3).*

70. Howell DS, Pita JC, *et.al.* (1969): Demonstration of macromolecular inhibitors of calcification and nucleation factors in fluid from calcifying cartilage. *J Clin Invest;*. ***48****: 630*-641.

71. Hulth A, Linberg L, Telhag H (1970): Experimental osteoarthritis in rabbits. Preliminary report. *Acta Orthop Scand* ***41****: 522-530.*

72. Hurtig MB (1988): Use of autogeneous cartilage particles to create a model of naturally occurring degenerative joint disease in the horse. *Equine Vet J* ***20****(suppl 6): 19-22.*

73. Johns RJ, Wright V (*1962*): Relative importance of various tissues in joint stiffness. *J. Applied Physiol 17: 824-828.*

74. Johnson LC (1964): Morphologic analysis in pathology. The kinetics of disease and general biology of bone. *In: Bone biodynamics.* Frost HM (ed), *Boston: Little, Brown & Co pp 543-654.*

75. Jeffcott L (1983): Radiographic findings in the distal limb of normal horses. *Proceedings of the* 5^{th} *Bain-fallon memorial lectures; Equine lameness. AVA..*

76. Jeffcott LB, Buckingham SHW, *et al.* (1987): Non-invasive measurement of bone: A review of clinical research applications in the horse. *Equine Vet J suppl* ***6*** *: 71-79.*

77. Jeffcott LB, Buckingham SHW, *et al.* (1988): Non-invasive measurement of bone quality in horses and changes associated with exercise *In: Equine exercise and*

physiology II. Gillespie and Robinson (eds), *ICEEP publications, California. pp 615-630.*

78. Jones IL, Klamfeldt A, Sandstorm T (1982): The effects of continuous mechanical pressure upon the turn-over of articular cartilage proteoglycans in vitro. *Clin Orthop **165**:283-9.*
79. Kang AH (1981): Connective tissue, collagen and elastin. *In: Text book of Rheumatology, W.B. Saunders et al. Philadelphia, pp 221-238.*
80. Katz JL (1980): The structure and biomechanics of bone. *Soc Exp Biol Semin Ser **34**;137-168.*
81. Kawcak CE, McIlwraith CW (1994): Proximo-dorsal first phalanx osteochondral chip fragments in 320 horses. *Equine Vet J **26**:329-396.*
82. Kellgren JH, Moore R (1957): Generalised osteoarthritis and heberdens nodes. *Br Med J **1**;187-187.*
83. Kellgren JH (1963): The epidemiology of chronic rheumatism. In: *Atlas of standard radiographs. Oxford, Blackwell Scientific.*
84. Kellgren JH (1961): Osteoarthritis in patients and populations. *Br Vet Med J 2:1-16*
85. Kellgren JH, Lawrence JS (1957): Radiological assessment of osteoarthritis. *Ann Rheum Dis **16**:197-204.*
86. Kelser RA, Callender GR (1938): Equine degenerative arthritis. *Vet Med **38**:307-320.*
87. Kempson GE, Spivey CJ, *et al.* (1971): Correlation between stiffness and the chemical constituents of cartilage on the human femoral head. *J Biomech **4**(6):597-609.*
88. Kold SE, Hickman J (1986): An experimental study of the healing process of equine chondral and osteochondral defects. *Equine Vet J **18**:18-24.*
89. Lachman E (1955): Osteoporosis: The potentialities and limitations of its roentgenologic diagnosis. *Am J Roentgenol **74**:71-715.*
90. Lamb CR (1990): Non-skeletal distribution of bone seeking radio-pharmaceuticals. *Vet Radiol **31**:246-253.*
91. Landells JW (1953): The bone cysts of osteoarthritis. *J bone Jt Surg **35B**:643.*

92. Lanyon LE (1982) Mechanical function and bone remodelling. In: *Bone in clinical orthopaedics. Saunders WB, Philadelphia.*

93. Lawrence JS, Bremner JM, Bier F (1966): Osteoarthritis: prevalence in the population and X-ray changes. *Ann Rheum Dis* ***25****:1-24.*

94. Levick JR (1990): Hypoxia and acidosis in chronic inflammatory arthritis. *J Rheumatol* ***17****:570-581.*

95. Linn FC, Radin EL (1968): Lubrication in animal joints. *Arthrits and Rheum* ***25****:3-13.*

96. Lippiello L, Yamamoto K, *et al.* (1978): Involvement of prostaglandins from rheumatoid synovium in inhibition of articular cartilage metabolism. *Arthritis Rheum* ***21****:909-917.*

97. Lloyd-Roberts GC (1953): The role of capsular changes in osteoarthritis of the hip joint. *J Bone Joint Surg* ***35B:****627-642.*

98. MacConail MA (1950): The movements of bones and joints. *J Bone Joint Surg* ***32B****:244.*

99. Mankin HJ (1976): Biochemical changes in articular cartilage in osteoarthritis *In: American Academy of Orthopaedic Surgery Symposium on osteoarthritis St Louis, CV Mosby Co, pp 1-22.*

100. Mankin HJ, Dorfman H, *et al.* (1971): Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. Correlation of morphology, biochemical and metabolic data. *J Bone Jt Surg* ***53****:523-537.*

101. Mankin HJ, Thrasher AZ (1975): Water content and binding in normal and osteoarthritic human cartilage. *J Bone Jt Surg* ***57A****: 76-80.*

102. Mankin HJ, Lippiello (1979), The glycosaminoglycans of normal and arthritic cartilage. *J Clin Invest* ***50****:1712-1719.*

103. May SA, Hooke C, *et al.* (1996), unpublished data. Cited In: Current research relevant to equine joint disease, *Joint Diseases in the horse.* McIlwraith and Turner (ed) *pp 421- 436.*

104. Maroudas A (1973): Physico-chemical properties of articular cartilage. *In: Adult articular cartilage.* Freeman M.A.R. (ed), *New York. pp 131-170.*

105. Maroudas A, Evans H, *et al.* (1973): Cartilage of the hip joint. Topographical variation of glycosaminoglycan content in normal and fibrillated cartilage. *Ann Rheum Dis* ***32:1-9.***

106. Maroudas A (1979): Physicochemical properties of articular cartilage. *In: Adult articular cartilage.* Freeman MAR (ed) *Pitman Medical. pp 215-291.*

107. McIlwraith CW (1981): The use of arthroscopy, synovial fluid analysis and synovial membrane biopsy in the diagnosis of equine degenerative joint disease. *In: Equine medicine and surgery,* McAllister ES (ed), *Santa Barbara, Calif, Am. Vet. Pub. Inc.*

108. McIlwraith CW (1982): Current concepts in equine degenerative joint disease. *J Am Vet Med Assoc* ***180****: 239-250.*

109. McIlwraith CW, Fessler JF (1978): Arthroscopy in the diagnosis of equine joint disease. *J Am Vet Med Assoc* ***172****:263-268.*

110. McIlwraith CW, Fessler JF, *et al.* (1979): Experimentally induced arthritis in the equine carpus : Clinical determinations, *J Am Vet Med Assoc 40:11-20.*

111. McIlwraith CW and Trotter GW (1996): Clinical features and diagnosis of equine joint disease. In: Joint disease in the horse. McIlwraith CW and Trotter GW (eds) Saunders, Philadelphia. 120- 143.

112. Meachim G, Collins D (1962): Cell counts of normal and osteoarthritic articular cartilage in relation to uptake of sulphate in vitro. *Ann Rheum Dis* ***21****:45-49.*

113. Meachim G (1972a) Light microscopy of Indian ink preparations of fibrillated cartilage. *Ann Rheum Dis* ***31****:457-464.*

114. Meachim G (1972b): Articular cartilage lesions in osteoarthritis of the femoral head. *J Path* ***107****:199-210.*

115. Meachim G, Fergie IA (1975): Morphological patterns of articular cartilage fibrillation. *J Path (Lond.)****115****: 231-244.*

116. McCutheon CW (1959) Mechanisms of animal joints. Sponge hydrostatic and weeping bearings. *Nature* ***184****:1284-1285.*

117. Morgan JP (1968): Radiographic diagnosis of bone and joint diseases in the horse. *Cornell Vet* ***58:****(suppl):28-46.*

118. Mow VC, Lai WM (1980): Recent developments in synovial joint biomechanics *SIAM Review 22:275-317.*

119. Moris M, Peretz A, *et al.* (1995): Quantitative ultrasound bone measurements: Normal values and comparison with bone mineral density by dual X-ray absorptiometry. *Calcif Iissue Int.* ***57****:6-10.*

120. Muir IH (1977): Molecular approach to understanding osteoarthrits. *Ann Rheum Dis* ***36****:199-208.*

121. Muir IH (1979): Biochemistry. *In: Adult articular cartilage.* Freeman MAR (ed), *Pitman Medical. pp 145-215.*

122. Murphy G (1991): Current views on proteolytic events in matrix degradation. *In: Osteoarthritis current research and prospects for pharmatological intervention* Russell RGG, Dieppe PA (eds). *London IBC Technical services pp 85-91.*

123. Neam P (1993): Extracellular matrix cartilage; Proteoglycans. *In: Joint cartilage degrdation; Basic and clinical aspects.* Woesner JF (ed), *New York, Marcel Dekker, pp 109-138.*

124. Nichols EH, Richardson FL (1909): Arthritis deformans. *J Med Res* ***21****;149-223.* Cited by Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology.* Kippel JH, Dieppe PA (eds). *Mosby Year book Europe Ltd.*

125. Nickel R (1986): The locomotor system of domestic animals. *In: The anatomy of the domestic Animals (Vol.1), New York, Springer-verlag; pp 170.*

126. Nilsson G, Olsson SE (1973): Radiologic and patho-anatomic changes in the distal joints and phalanges of the Standardbred horse. *Acta Vet Scand (suppl)* ***44****:1-57.*

127. Nord RH, Payne RK (1996): Body composition by DEXA- A review of the technology. *J Clin Nutr (in press).*

128. O'Brein TR (1977): Diseases of the Thoroughbred fetlock joint: A comparison of radiographic signs with gross pathologic lesions, I*n Proceedings 23rd Annual Meeting Am Assoc Equine Pract, pp 367-380.*

129. O'Connor BL, Palmoski MJ *et al.* (1985): Neurogenic acceleration of degenerative joint lesions. *J Bone Jt Surg (Am.)****67****:562-572.*

130. Ogata K, Whiteside *et al.* (1977): The effect of varus stress on the moving rabbit knee joint. *Clin Orthop **129**:129 - 313.*

131. Ogoston A (1876): On articular cartilage. *J Anat Physiol **10:** 53-4.* Cited by Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology* Kippel JH, Dieppe PA (eds). *Mosby Year book Europe Ltd.*

132. Ott SM (1993) When bone mass fails to predict bone failure. *Calcif Tissue Int **53(suppl):S7-S13.***

133. Owen RH, Marsh JA, *et al.* (1984): Intra-articular corticosteroids and exercised induced arthropathy in horses. *J Am Vet Med Assoc **184**:302 - 308.*

134. Palmer JL, Bertone AL (1996): Joint biomechanics in the pathogenesis of traumatic arthritis. *In: Joint diseases in the horse.* McIlwraith CW and Trotter GW (eds), *Saunders WB company, Philadelphia.*

135. Palmoski MJ, Brandt, KD (1984): Effects of static and cyclic compressive loading on articular cartilage plugs in vitro. *Arthritis Rheum **27:**675-81.*

136. Palmoski MJ, Colyer RA, Brandt KD (1980): Joint motion in the absence of normal loading does not maintain normal articular cartilage. *Arthritis Rheum **23**:325-34.*

137. Perssons L (1971): On the Synovia in horses. *Acta Vet Scand Suppl **35**:1-77.*

138. Pool AR (1993): Cartilage in health and disease. *In: A text book of Rheumatology: Arthritis and Allied conditions.* McCarty DJ (ed). *Lea & Febiger, Philadelphia. pp 279-333.*

139. Pool RR and Meagher DM (1990): Pathologic findings and pathogenesis of race track injuries. *Vet Clin North Am (Equine Practice)**6**:1-30.*

140. Pool RR (1991): Pathology of secondary joint disease of the fetlock. *In: Athletic Injuries in the Performance horse. Proceedings of the 13th Bain-Fallon Lectures, AVA, Melbourne. pp 61-67.*

141. Pool RR (1996) Pathologic manifestations of joint disease in the athletic horse. In: Joint disease in the horse. *McIlwraith CW and TrotterGW (eds) Saunders, Philadelphia, 87-104.*

142. Price RI, Retallack RW, *et al.* (1983): Quantitative *in-vivo* determination of bone mineral using computerised roentgenographic densitometry. *Australas Phys Eng Sci ed* ***6****:128-137.*

143. Raker CW, *et al.* (1966), Pathophysiology of equine degenerative disease and lameness. *In: Proceedings 12th Annual. Meeting Am Assoc Equine Pract, pp 229-241.*

144. Raker CW, (1968): Clinical observations of bone and joint disease in horses. *Cornell Vet* ***58****:15-28.*

145. Radin EL, Paul IL (1970): Does cartilage compliance reduce skeletal impact loads? The relative attenuating properties of articular cartilage, synovial fluid, periarticular soft tissues and bone. *Arthritis Rheum* ***13****:139-144.*

146. Radin EL, Paul IL (1971): Response of joints to impact loading. *Arthritis Rheum* ***14****:356-362.*

147. Radin EL (1973): The physiology and degeneration of joints. *Semin Arthritis Rheum 2:245-257.*

148. Radin EL, Orr RB, *et al.* (1982): Effect of Prolonged walking on concrete on the knees of sheep. *J Biomech.* ***15****:487-492.*

149. Radin EL, Martin RB, *et al.* (1984) Effects of mechanical loading on the on the tissues on the rabbit knee *J Orthop Res 2:221*

150. Radin EL, Rose RM (1986): Role of subchondral bone in the initiation and progression of cartilage damage. *Clin Orthop* ***213****:34-40.*

151. Radin EL (1991): Biomechanical considerations *In: Osteoarthritis- diagnosis and management.* Moskowitz RW, Howell DS, *et al.* (eds), *Saunders WB, Philadelphia.*

152. Ray CS, Baxter GM, McIlwraith CW, *et al.* (1994): Development of subchondral cystic lesions following subchondral bone trauma in horses. *Vet Surg* ***23****: 414.*

153. Redler I, Mow VC, *et al.* (1975): The ultrastructure and biomechanical significance of the tide mark of articular cartilage. *Clin Orthop* ***112****:357-362*

154. Rico H, Revilla M, *et al.* (1995): Influence of weight and seasonal changes on radiogammetry and bone densitometry. *Calcif Tissue Int* ***5****:47-53.*

155. Richardson DW, Young DR, *et al.* (1996): The relationship of subchondral bone stiffness to overlying cartilage morphology and biochemistry. *Trans Orthop Res Soc. **18** (2), 720-725.*

156. Richardson JD, Cripps PJ, *et al.* (1995): An evaluation of the accuracy of ageing horses by their dentition: a matter of experience? *Vet Rec **137**:88-90.*

157. Riggs BL, Waher HW, *et al,* (1981), Differential changes in bone mineral density of the apendicular and axial skeleton with ageing. *J Clin Invest **67**:328-335.*

158. Robinson HJ, Granada JL, *et al.* (1976): Inflammatory and non-inflammatory osteoarthritis of the hip. *In: Proceedings. 22nd Annu. Meet Orthop Res Soc, pp 27-39.*

159. Rogers J, Dieppe Watt I (1981): Arthritis in Saxon and Medieval skeletons. *Br Med J **283**:668-671.*

160. Rooney JR (1969): Biomechanics of lameness in horses. *Williams and Wilkins Co. Balitmore.*

161. Rossdale PD, Hopes R, *et al.* (1985): Epidemiological study of wastage among race horses, 1982 -1983. *Equine Vet J **116**:66-70.*

162. Roughly PJ, Mort JS (1986): Ageing and the aggregating proteoglycans of human articular cartilage. *Clin. Sci **72**:337-344.*

163. Rozenberg S, Vandromme J, *et al.* (1995): Precision and accuracy of *in vivo* bone mineral density measurement in rats using DEXA. *Osteoporosis Proc Int Symp**5**:47-53.*

164. Saari H, Konttinen VT, *et al.* (1989): Concentration and degree of polymerization of hyaluronate in equine synovial fluid. *Am J Vet Res **50**:2060-2063.*

165. Sartoris DJ, Resnick D (1990): Current and innovative methods for non-invasive bone densitometry. *Radiol Clin North Am **28**:257-278.*

166. Schenk RK (1988) Articular cartilage morphology, *In: Articular cartilage biochenistry.* Kuttner K, Hascall E (eds), *New York, Raven press.*

167. Schnider RK (1987): Incidence, location and classification of 368 third carpal fracture in 312 horses. *Equine Vet J Orthopaedic supplement.*

168. Schnitzler CM (1993) Bone quality: a determinant for certain risk factors for bone fragility. *Calcif Tissue Int **53** (Suppl 1): 227-S31.*

169. Sedgewick AD, Al-Duij AY, *et al.* (1984): Studies in to the mode of action of NSAIDS using a model of facsimile synovium. *J Pharm Pharmacol* ***36****:171-174.*

170. Sedgewick AD, Lees P (1986): A comparison of air pouch, sponge and pleurisy model of acute carageenan inflammation in the rat. *Agents Actio* ***18****:439 - 446.*

171. Sievanen H, Oja P, Vuori I (1994): Scanner-induced variability and quality assurance in longitudinal dual energy x-ray absorptiometry measurements. *Med Phys* ***21:*** *(11), 1795-1805.*

172. Simkin PA (1992): Biology and function of synovium. *In: Biology and Biomechanics of the traumatized synovial joint.* Finerman GAM, Noyes FR (eds), *Am Acc Orthop Surgeons, pp 5-16.*

173. Simon SR, Radin EL, *et al.* (1972): The response of joints to impact loading - *in vivo* behaviour of subchondral bone. *J Biomech.****5****:267-272.*

174. Simmons DJ, Grynpas, MD (1990): Mechanism of bone formation in vivo; The osteoblast and the osteocyte. *In: Bone (Vol. 1).* Hall BK (ed), *The Telford press, pp 193-302.*

175. Sokoloff L (1969): The biology of degenerative joint disease. *Chicago University Press.*

176. Sokoloff L (1976): The general pathology of osteoarthritis. *In: American academy of orthopaedic surgery symposium on osteoarthritis. St Louis, CV Mosby Co, pp 22-33.*

177. Sokoloff L (1982): Pathology and pathogenesis of osteoarthritis. *In: Arthritis and allied conditions (9th edition).* McCartney DJ (ed). *Lea & Febiger, Philadelphia, pp 1135-1153.*

178. Sokoloff L (1982): Ageing and degenerative diseases affecting cartilage. *In: Arthritis and allied conditions (9th edition).* McCartney DJ (ed), *Lea & Febiger, Philadelphia, pp 111-135.*

179. Stockwell RA (1979): *In: Biology of cartilage cells.* Harrison RJ and McMinn RMH (eds), *Cambridge University press, London, pp 1-14.*

180. Stashak T (1987): Chip fractures of the proximal (first) phalanx in the metacarpophalangeal joint. *In: Adams Lameness in Horses (4th edition),* Lea & Febiger, Philadelphia, pp 568-573.

181. Storey GO, Landells JW (1971): Restoration of the femoral head after collapse in osteoarthritis. *Ann Rheum Dis* ***30****:406-12.*

182. Sunbald L (1965): Glycosaminoglycans and glycoproteins in synovial fluid. *In: The amino sugars (Vol* ***2A****).* Blancz EA, Jeanloz RW (eds), *New York Academic Press. pp 229-250.*

183. Swann DA (1978): Macromolecules of Synovial fluid. *In: Joints and synovial fluid,* Sokoloff L (ed.), *Academic press, New York. pp 407-435.*

184. Swann DA, Radin EL (1972): The molecular basis of articular lubrication; Purication and properties of a lubricationg fraction from bovine synovial fluid. *J biol Chem* ***274****:8069-8073.*

185. Tammi M, Paukkonen K, *et al.* (1987): Joint loading induced alterations in articular cartilage. *In: Joint loading. Wright (ed) Bristol. pp 64-88,*

186. Telhag H, Linberg L (1972): A method for inducing osteoarthritis changes in rabbit knees. *Clin Orthop* ***86:****214-223.*

187. Tew WP *(1980):* Synovial fluid particle analysis in equine joint disease. *Mod Vet Pract* ***61****:993-997.*

188. Tew WP, Hackett RP (1981): Identification of cartilage wear fragments in synovial fluid from equine joints. *Arthrits Rheum* ***24****:1491-1424.*

189. Thomas CC (1969): Skeletal evidence of Osteoarthritis. *In: Diseases in antiquity.* Brothwell D, Sanderson J (eds), *Springfield, Illinois.*

190. Thompson RC, Basset C.AL (1970): Histological observations on experimentally induced degeneration of articular cartilage. *J Bone Jt Surg* ***52A:*** *435-43.*

191. Thompson KN, Cheung TK, *et al.* (1996): Computerised bone density analysis or the proximal phalanx of the horse. *Equine practice* ***18****:26-29.*

192. Torbeck RL, Prieur DJ (1979): Plasma and synovial fluid lysozome activity in horses with experimental cartilage defects. *Am J Vet Res* ***40****:1531-1536.*

193. Trotter GW, Yovich JV, *et al. (*1989): Effects of intramuscular polysulfated glycosaminoglycan on chemical and physical defects in equine articular cartilage. *Can J Vet Res* ***53****:224-230.*

194. Troyer H, (1982): Experimental models of osteoarthritis: A review. *Semin Arthritis Rheum* ***11****:362 - 374.*

195. Trueta J (1963): Studies on aeitopathology of osteoarthritis of hip joints. *Clin Orthop Relat Res* ***31****:7-19.*

196. Van Pelt RW (1962): Properties of equine synovial fluid. *J Am Vet Med Assoc* ***141****:1051-1061.*

197. Van Pelt RW (1965): Comparative arthrology in man and domestic animals. *J Am Vet Med Assoc* ***147****:958-967.*

198. Van Pelt RW (1974): Interpretation of synovial fluid findings in the horse. *J Am Vet Med Assoc* ***165****:91-95.*

199. Van Kampen GPJ (1985): Cartilage response to mechanical force in high density chondrocyte cultures. *Arthritis and Rheum.****28****: 419-24.*

200. Vasan N (1983): Effects of physical stress on the synthesis and degradation of cartilage matrix. *Connect Tissue Res* ***12****:49-58.*

201. Walker PS, Dowson D, *et al.* (1968): "Boosted lubrication" in synovial fluid by fluid entrapment and enrichment. *Ann Rheum Dis* ***27****:512-520.*

202. Wahner HW, Fogelman I (1994): The evaluation of osteoporosis- DEXA in clinical practice. *Dunitz M (ed), Cambridge UK. Ltd.*

203. Weissman G (1966): Lysosomes and Joint disease. *Arthritis Rheum* ***10****:834-840.*

204. Wormsley T (1928): The articular mechanism of the diarthroses. *J Bone Joint Surg* ***10A****;40-48.* Cited by Dieppe PA (1994): Introduction to osteoarthritis. *In: Rheumatology Kippel JH, Dieppe PA (eds). Mosby Year book Europe Ltd.*

205. Williams JM, Brandt KD (1984): Immobilisation ameliorates chemically induced articular damage. *Arthritis rheum* ***27****:208-216.*

206. Young DR, Richardson DW, *et al.* (1991): Mechanical and morphometric analysis of the third carpal bone of thoroughbreds. *Am J Vet Res* ***52****: 402-409.*

207. Yovich JV, McIlwraith CW (1986): Arthroscopic surgery for osteochondral fractures of the proximal phalanx in the metacarpophalangeal and metatarsophalangeal joints in horses. *J Am Vet Med Assoc* ***188****:273-279.*

208. Yovich JV, Trotter GW, *et al.* (1987): Effects of polysulfated glycosaminoglycan on chemical and physical defects in equine articular cartilage. *Am J Vet Res* ***48****:1407-1414.*

❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖ ❖

Appendix

The data collected for the horses used in this study is presented in the following tables. The keys below refer to the tables of data in the following pages.

Group	Number	Horse	Age
1	1	2K10	4Y
1	2	2K15	4Y
1	3	2K19	3Y
1	4	2K06	4Y
1	5	2K22	2Y
2	1	2K07	5Y
2	2	2K04	6Y
2	3	2K20	8Y
2	4	2K14	8Y
2	5	2K12	9Y
2	6	2K08	5Y
2	7	2K17	7Y
2	8	2K09	8Y
2	9	2K05	6Y
2	10	2K11	8Y
2	11	2K21	9Y
3	1	2K18	11Y
3	2	2K16	10Y
3	3	2K03	12Y
3	4	2K13	10Y
3	5	2K01	12Y
3	6	2K02	14Y

Table A1 Key of horse groups, numbers, codes and ages

Abbreviation	Meaning
Leg 1	Right
Leg 2	Left
Aspect 1	Medial
Aspect 2	Lateral
MLS	Macroscopic lesion score
Area A-E	Area on proximo-dorsal bone slab
BMD	Bone mineral density
SHS	Subjective histological score
LCN (Z1)	Live chondrocyte number in zone 1
LCN (Z3)	Live chondrocyte number in zone 3
CH	Chondrone number
EL	Empty Lacunae
TLG	Tangential layer grade
TNR (Z3)	Tangential row number in zone 3
TRN (Z1)	Tangential row number in zone 1
CT (Z1)	Cartilage thickness in zone 1
CT (Z3)	Cartilage thickness in zone 3

Table A2 Key of abbreviations used in the data sheets (table A3)

Tables A3 Data sheets (over the page)

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
1	4	1	1	1	1	A	0.158	1	180	300	2	2	3	3	1	0.82	0.6
1	4	1	1	1	1	B	0.163	1	180	300	2	2	3	3	1	0.82	0.6
1	4	1	1	1	1	C	0.162	1	180	300	2	2	3	3	1	0.82	0.6
1	4	1	1	1	1	D	0.155	1	180	300	2	2	3	3	1	0.82	0.6
1	4	1	1	1	1	E	0.132	1	180	300	2	2	3	3	1	0.82	0.6
1	4	1	1	2	1	A	0.167	1	190	350	2	1	2	4	2	0.8	0.5
1	4	1	1	2	1	B	0.163	1	190	350	2	1	2	4	2	0.8	0.5
1	4	1	1	2	1	C	0.154	1	190	350	2	1	2	4	2	0.8	0.5
1	4	1	1	2	1	D	0.167	1	190	350	2	1	2	4	2	0.8	0.5
1	4	1	1	2	1	E	0.161	1	190	350	2	1	2	4	2	0.8	0.5
1	4	1	2	1	1	A	0.161	1	185	225	1	1	2	3	2	0.9	0.55
1	4	1	2	1	1	B	0.147	1	185	225	1	1	2	3	2	0.9	0.55
1	4	1	2	1	1	C	0.151	1	185	225	1	1	2	3	2	0.9	0.55
1	4	1	2	1	1	D	0.171	1	185	225	1	1	2	3	2	0.9	0.55
1	4	1	2	1	1	E	0.135	1	185	225	1	1	2	3	2	0.9	0.55
1	4	1	2	2	1	A	0.161	1	175	210	2	2	2	4	2	0.85	0.6
1	4	1	2	2	1	B	0.137	1	175	210	2	2	2	4	2	0.85	0.6
1	4	1	2	2	1	C	0.121	1	175	210	2	2	2	4	2	0.85	0.6
1	4	1	2	2	1	D	0.153	1	175	210	2	2	2	4	2	0.85	0.6
1	4	1	2	2	1	E	0.137	1	175	210	2	2	2	4	2	0.85	0.6
1	4	2	1	1	3	A	0.144	2	150	200	3	1	2	5	3	0.9	0.75
1	4	2	1	1	3	B	0.141	2	150	200	3	1	2	5	3	0.9	0.75
1	4	2	1	1	3	C	0.177	2	150	200	3	1	2	5	3	0.9	0.75
1	4	2	1	1	3	D	0.133	2	150	200	3	1	2	5	3	0.9	0.75
1	4	2	1	1	3	E	0.137	2	150	200	3	1	2	5	3	0.9	0.75
1	4	2	1	2	2	A	0.143	1	200	500	0	0	1	5	2	1	0.8
1	4	2	1	2	2	B	0.145	1	200	500	0	0	1	5	2	1	0.8
1	4	2	1	2	2	C	0.145	1	200	500	0	0	1	5	2	1	0.8
1	4	2	1	2	2	D	0.137	1	200	500	0	0	1	5	2	1	0.8
1	4	2	1	2	2	E	0.139	1	200	500	0	0	1	5	2	1	0.8
1	4	2	2	1	3	A	0.164	1	180	500	2	2	2	5	2	0.8	0.7
1	4	2	2	1	3	B	0.158	1	180	500	2	2	2	5	2	0.8	0.7
1	4	2	2	1	3	C	0.147	1	180	500	2	2	2	5	2	0.8	0.7
1	4	2	2	1	3	D	0.147	1	180	500	2	2	2	5	2	0.8	0.7
1	4	2	2	1	3	E	0.147	1	180	500	2	2	2	5	2	0.8	0.7
1	4	2	2	2	2	A	0.165	0	200	500	0	1	0	5	3	0.8	0.75
1	4	2	2	2	2	B	0.157	0	200	500	0	1	0	5	3	0.8	0.75
1	4	2	2	2	2	C	0.171	0	200	500	0	1	0	5	3	0.8	0.75
1	4	2	2	2	2	D	0.144	0	200	500	0	1	0	5	3	0.8	0.75
1	4	2	2	2	2	E	0.13	0	200	500	0	1	0	5	3	0.8	0.75
1	3	3	1	1	0	A	0.132	1	100	300	3	2	1	4	2	0.8	0.9
1	3	3	1	1	0	B	0.129	1	100	300	3	2	1	4	2	0.8	0.9
1	3	3	1	1	0	C	0.163	1	100	300	3	2	1	4	2	0.8	0.9
1	3	3	1	1	0	D	0.122	1	100	300	3	2	1	4	2	0.8	0.9
1	3	3	1	1	0	E	0.122	1	100	300	3	2	1	4	2	0.8	0.9
1	3	3	1	2	0	A	0.165	1	100	400	2	2	1	5	1	0.75	0.65
1	3	3	1	2	0	B	0.156	1	100	400	2	2	1	5	1	0.75	0.65

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
1	3	3	1	2	0	C	0.166	1	100	400	2	2	1	5	1	0.75	0.65
1	3	3	1	2	0	D	0.141	1	100	400	2	2	1	5	1	0.75	0.65
1	3	3	1	2	0	E	0.143	1	100	400	2	2	1	5	1	0.75	0.65
1	3	3	2	1	1	A	0.155	1	190	400	0	1	1	5	2	0.8	0.75
1	3	3	2	1	1	B	0.154	1	190	400	0	1	1	5	2	0.8	0.75
1	3	3	2	1	1	C	0.143	1	190	400	0	1	1	5	2	0.8	0.75
1	3	3	2	1	1	D	0.135	1	190	400	0	1	1	5	2	0.8	0.75
1	3	3	2	1	1	E	0.135	1	190	400	0	1	1	5	2	0.8	0.75
1	3	3	2	2	1	A	0.147	1	180	400	2	2	1	5	1	0.7	0.75
1	3	3	2	2	1	B	0.132	1	180	400	2	2	1	5	1	0.7	0.75
1	3	3	2	2	1	C	0.132	1	180	400	2	2	1	5	1	0.7	0.75
1	3	3	2	2	1	D	0.13	1	180	400	2	2	1	5	1	0.7	0.75
1	3	3	2	2	1	E	0.134	1	180	400	2	2	1	5	1	0.7	0.75
1	2	4	1	1	1	A	0.163	1	100	500	0	1	1	4	2	0.8	0.7
1	2	4	1	1	1	B	0.16	1	100	500	0	1	1	4	2	0.8	0.7
1	2	4	1	1	1	C	0.159	1	100	500	0	1	1	4	2	0.8	0.7
1	2	4	1	1	1	D	0.167	1	100	500	0	1	1	4	2	0.8	0.7
1	2	4	1	1	1	E	0.167	1	100	500	0	1	1	4	2	0.8	0.7
1	2	4	1	2	1	A	0.142	0	200	500	0	1	1	4	3	0.9	0.85
1	2	4	1	2	1	B	0.163	0	200	500	0	1	1	4	3	0.9	0.85
1	2	4	1	2	1	C	0.168	0	200	500	0	1	1	4	3	0.9	0.85
1	2	4	1	2	1	D	0.163	0	200	500	0	1	1	4	3	0.9	0.85
1	2	4	1	2	1	E	0.159	0	200	500	0	1	1	4	3	0.9	0.85
1	2	4	2	1	1	A	0.17	2	100	400	3	3	2	3	2	0.75	0.7
1	2	4	2	1	1	B	0.181	2	100	400	3	3	2	3	2	0.75	0.7
1	2	4	2	1	1	C	0.166	2	100	400	3	3	2	3	2	0.75	0.7
1	2	4	2	1	1	D	0.164	2	100	400	3	3	2	3	2	0.75	0.7
1	2	4	2	1	1	E	0.152	2	100	400	3	3	2	3	2	0.75	0.7
1	2	4	2	2	1	A	0.179	1	150	500	0	0	2	4	2	0.85	0.75
1	2	4	2	2	1	B	0.169	1	150	500	0	0	2	4	2	0.85	0.75
1	2	4	2	2	1	C	0.179	1	150	500	0	0	2	4	2	0.85	0.75
1	2	4	2	2	1	D	0.171	1	150	500	0	0	2	4	2	0.85	0.75
1	2	4	2	2	1	E	0.158	1	150	500	0	0	2	4	2	0.85	0.75
1	2	5	1	1	1	A	0.179	1	200	400	0	1	1	4	3	0.9	0.8
1	2	5	1	1	1	B	0.167	1	200	400	0	1	1	4	3	0.9	0.8
1	2	5	1	1	1	C	0.175	1	200	400	0	1	1	4	3	0.9	0.8
1	2	5	1	1	1	D	0.172	1	200	400	0	1	1	4	3	0.9	0.8
1	2	5	1	1	1	E	0.172	1	200	400	0	1	1	4	3	0.9	0.8
1	2	5	1	2	0	A	0.168	1	250	400	0	0	1	5	3	1	0.85
1	2	5	1	2	0	B	0.161	1	250	400	0	0	1	5	3	1	0.85
1	2	5	1	2	0	C	0.166	1	250	400	0	0	1	5	3	1	0.85
1	2	5	1	2	0	D	0.158	1	250	400	0	0	1	5	3	1	0.85
1	2	5	1	2	0	E	0.158	1	250	400	0	0	1	5	3	1	0.85
1	2	5	2	1	0	A	0.152	0	300	500	0	0	0	5	4	0.9	0.85
1	2	5	2	1	0	B	0.156	0	300	500	0	0	0	5	4	0.9	0.85
1	2	5	2	1	0	C	0.142	0	300	500	0	0	0	5	4	0.9	0.85
1	2	5	2	1	0	D	0.14	0	300	500	0	0	0	5	4	0.9	0.85

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
1	2	5	2	1	0	E	0.137	0	300	500	0	0	0	5	4	0.9	0.85
1	2	5	2	2	0	A	0.144	0	300	500	0	0	0	5	4	1	0.8
1	2	5	2	2	0	B	0.141	0	300	500	0	0	0	5	4	1	0.8
1	2	5	2	2	0	C	0.15	0	300	500	0	0	0	5	4	1	0.8
1	2	5	2	2	0	D	0.138	0	300	500	0	0	0	5	4	1	0.8
1	2	5	2	2	0	E	0.139	0	300	500	0	0	0	5	4	1	0.8
2	5	1	1	1	2	A	0.171	2	150	400	1	1	2	4	3	0.8	0.75
2	5	1	1	1	2	B	0.147	2	150	400	1	1	2	4	3	0.8	0.75
2	5	1	1	1	2	C	0.151	2	150	400	1	1	2	4	3	0.8	0.75
2	5	1	1	1	2	D	0.171	2	150	400	1	1	2	4	3	0.8	0.75
2	5	1	1	1	2	E	0.145	2	150	400	1	1	2	4	3	0.8	0.75
2	5	1	1	2	2	A	0.161	1	200	350	0	1	1	4	3	0.9	0.8
2	5	1	1	2	2	B	0.137	1	200	350	0	1	1	4	3	0.9	0.8
2	5	1	1	2	2	C	0.121	1	200	350	0	1	1	4	3	0.9	0.8
2	5	1	1	2	2	D	0.153	1	200	350	0	1	1	4	3	0.9	0.8
2	5	1	1	2	2	E	0.165	1	200	350	0	1	1	4	3	0.9	0.8
2	5	1	2	1	2	A	0.171	2	150	300	2	1	2	3	2	0.8	0.7
2	5	1	2	1	2	B	0.147	2	150	300	2	1	2	3	2	0.8	0.7
2	5	1	2	1	2	C	0.151	2	150	300	2	1	2	3	2	0.8	0.7
2	5	1	2	1	2	D	0.171	2	150	300	2	1	2	3	2	0.8	0.7
2	5	1	2	1	2	E	0.145	2	150	300	2	1	2	3	2	0.8	0.7
2	5	1	2	2	1	A	0.162	1	200	350	1	1	2	4	2	0.9	0.85
2	5	1	2	2	1	B	0.168	1	200	350	1	1	2	4	2	0.9	0.85
2	5	1	2	2	1	C	0.167	1	200	350	1	1	2	4	2	0.9	0.85
2	5	1	2	2	1	D	0.152	1	200	350	1	1	2	4	2	0.9	0.85
2	5	1	2	2	1	E	0.148	1	200	350	1	1	2	4	2	0.9	0.85
2	6	2	1	1	4	A	0.178	4	100	300	7	5	4	3	0	0.65	0.6
2	6	2	1	1	4	B	0.151	4	100	300	7	5	4	3	0	0.65	0.6
2	6	2	1	1	4	C	0.154	4	100	300	7	5	4	3	0	0.65	0.6
2	6	2	1	1	4	D	0.139	4	100	300	7	5	4	3	0	0.65	0.6
2	6	2	1	1	4	E	0.14	4	100	300	7	5	4	3	0	0.65	0.6
2	6	2	1	2	3	A	0.165	2	150	400	2	2	2	4	2	0.85	0.75
2	6	2	1	2	3	B	0.153	2	150	400	2	2	2	4	2	0.85	0.75
2	6	2	1	2	3	C	0.137	2	150	400	2	2	2	4	2	0.85	0.75
2	6	2	1	2	3	D	0.148	2	150	400	2	2	2	4	2	0.85	0.75
2	6	2	1	2	3	E	0.141	2	150	400	2	2	2	4	2	0.85	0.75
2	6	2	2	1	4	A	0.161	5	95	300	8	3	3	4	2	0.6	0.6
2	6	2	2	1	4	B	0.164	5	95	300	8	3	3	4	2	0.6	0.6
2	6	2	2	1	4	C	0.152	5	95	300	8	3	3	4	2	0.6	0.6
2	6	2	2	1	4	D	0.158	5	95	300	8	3	3	4	2	0.6	0.6
2	6	2	2	1	4	E	0.159	5	95	300	8	3	3	4	2	0.6	0.6
2	6	2	2	2	2	A	0.143	2	200	500	1	2	1	5	3	0.9	0.8
2	6	2	2	2	2	B	0.136	2	200	500	1	2	1	5	3	0.9	0.8
2	6	2	2	2	2	C	0.141	2	200	500	1	2	1	5	3	0.9	0.8
2	6	2	2	2	2	D	0.148	2	200	500	1	2	1	5	3	0.9	0.8
2	6	2	2	2	2	E	0.142	2	200	500	1	2	1	5	3	0.9	0.8
2	8	3	1	1	4	A	0.167	2	145	400	2	2	2	5	2	0.7	0.6

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
2	8	3	1	1	4	B	0.182	2	145	400	2	2	2	5	2	0.7	0.6
2	8	3	1	1	4	C	0.182	2	145	400	2	2	2	5	2	0.7	0.6
2	8	3	1	1	4	D	0.155	2	145	400	2	2	2	5	2	0.7	0.6
2	8	3	1	1	4	E	0.152	2	145	400	2	2	2	5	2	0.7	0.6
2	8	3	1	2	3	A	0.148	1	170	500	0	0	1	5	2	0.7	0.6
2	8	3	1	2	3	B	0.149	1	170	500	0	0	1	5	2	0.7	0.6
2	8	3	1	2	3	C	0.132	1	170	500	0	0	1	5	2	0.7	0.6
2	8	3	1	2	3	D	0.151	1	170	500	0	0	1	5	2	0.7	0.6
2	8	3	1	2	3	E	0.154	1	170	500	0	0	1	5	2	0.7	0.6
2	8	3	2	1	3	A	0.174	2	160	400	3	2	1	5	1	0.8	0.7
2	8	3	2	1	3	B	0.171	2	160	400	3	2	1	5	1	0.8	0.7
2	8	3	2	1	3	C	0.167	2	160	400	3	2	1	5	1	0.8	0.7
2	8	3	2	1	3	D	0.163	2	160	400	3	2	1	5	1	0.8	0.7
2	8	3	2	1	3	E	0.163	2	160	400	3	2	1	5	1	0.8	0.7
2	8	3	2	2	2	A	0.153	1	200	500	0	1	1	5	3	0.9	0.8
2	8	3	2	2	2	B	0.146	1	200	500	0	1	1	5	3	0.9	0.8
2	8	3	2	2	2	C	0.155	1	200	500	0	1	1	5	3	0.9	0.8
2	8	3	2	2	2	D	0.133	1	200	500	0	1	1	5	3	0.9	0.8
2	8	3	2	2	2	E	0.146	1	200	500	0	1	1	5	3	0.9	0.8
2	8	4	1	2	2	A	0.182	2	170	300	3	1	1	5	2	0.8	0.7
2	8	4	1	2	2	B	0.179	2	170	300	3	1	1	5	2	0.8	0.7
2	8	4	1	2	2	C	0.189	2	170	300	3	1	1	5	2	0.8	0.7
2	8	4	1	2	2	D	0.159	2	170	300	3	1	1	5	2	0.8	0.7
2	8	4	1	2	2	E	0.154	2	170	300	3	1	1	5	2	0.8	0.7
2	8	4	1	1	3	A	0.188	2	180	300	2	1	1	5	1	0.9	0.7
2	8	4	1	1	3	B	0.154	2	180	300	2	1	1	5	1	0.9	0.7
2	8	4	1	1	3	C	0.146	2	180	300	2	1	1	5	1	0.9	0.7
2	8	4	1	1	3	D	0.173	2	180	300	2	1	1	5	1	0.9	0.7
2	8	4	1	1	3	E	0.16	2	180	300	2	1	1	5	1	0.9	0.7
2	8	4	2	2	3	A	0.17	2	160	250	2	1	2	4	1	0.7	0.65
2	8	4	2	2	3	B	0.169	2	160	250	2	1	2	4	1	0.7	0.65
2	8	4	2	2	3	C	0.162	2	160	250	2	1	2	4	1	0.7	0.65
2	8	4	2	2	3	D	0.152	2	160	250	2	1	2	4	1	0.7	0.65
2	8	4	2	2	3	E	0.169	2	160	250	2	1	2	4	1	0.7	0.65
2	8	4	2	1	4	A	0.184	3	150	200	3	2	2	4	1	0.9	0.7
2	8	4	2	1	4	B	0.173	3	150	200	3	2	2	4	1	0.9	0.7
2	8	4	2	1	4	C	0.174	3	150	200	3	2	2	4	1	0.9	0.7
2	8	4	2	1	4	D	0.173	3	150	200	3	2	2	4	1	0.9	0.7
2	8	4	2	1	4	E	0.161	3	150	200	3	2	2	4	1	0.9	0.7
2	9	5	1	2	5	A	0.194	4	90	100	6	3	4	4	0	0.4	0.8
2	9	5	1	2	5	B	0.169	4	90	100	6	3	4	4	0	0.4	0.8
2	9	5	1	2	5	C	0.166	4	90	100	6	3	4	4	0	0.4	0.8
2	9	5	1	2	5	D	0.157	4	90	100	6	3	4	4	0	0.4	0.8
2	9	5	1	2	5	E	0.154	4	90	100	6	3	4	4	0	0.4	0.8
2	9	5	1	1	3	A	0.19	3	130	200	4	2	3	3	0	0.5	0.6
2	9	5	1	1	3	B	0.179	3	130	200	4	2	3	3	0	0.5	0.6
2	9	5	1	1	3	C	0.179	3	130	200	4	2	3	3	0	0.5	0.6

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
2	9	5	1	1	3	D	0.177	3	130	200	4	2	3	3	0	0.5	0.6
2	9	5	1	1	3	E	0.169	3	130	200	4	2	3	3	0	0.5	0.6
2	9	5	2	2	5	A	0.199	5	70	100	4	3	3	3	0	0.5	0.6
2	9	5	2	2	5	B	0.165	5	70	100	4	3	3	3	0	0.5	0.6
2	9	5	2	2	5	C	0.158	5	70	100	4	3	3	3	0	0.5	0.6
2	9	5	2	2	5	D	0.183	5	70	100	4	3	3	3	0	0.5	0.6
2	9	5	2	2	5	E	0.186	5	70	100	4	3	3	3	0	0.5	0.6
2	9	5	2	2	4	A	0.189	4	90	150	3	2	3	4	0	0.65	0.75
2	9	5	2	2	4	B	0.169	4	90	150	3	2	3	4	0	0.65	0.75
2	9	5	2	2	4	C	0.173	4	90	150	3	2	3	4	0	0.65	0.75
2	9	5	2	2	4	D	0.17	4	90	150	3	2	3	4	0	0.65	0.75
2	9	5	2	2	4	E	0.186	4	90	150	3	2	3	4	0	0.65	0.75
2	5	6	1	1	1	A	0.162	2	150	250	2	1	3	3	1	0.65	0.5
2	5	6	1	1	1	B	0.148	2	150	250	2	1	3	3	1	0.65	0.5
2	5	6	1	1	1	C	0.15	2	150	250	2	1	3	3	1	0.65	0.5
2	5	6	1	1	1	D	0.148	2	150	250	2	1	3	3	1	0.65	0.5
2	5	6	1	1	1	E	0.15	2	150	250	2	1	3	3	1	0.65	0.5
2	5	6	1	2	0	A	0.143	1	200	300	1	1	2	4	2	0.75	0.6
2	5	6	1	2	0	B	0.141	1	200	300	1	1	2	4	2	0.75	0.6
2	5	6	1	2	0	C	0.143	1	200	300	1	1	2	4	2	0.75	0.6
2	5	6	1	2	0	D	0.141	1	200	300	1	1	2	4	2	0.75	0.6
2	5	6	1	2	0	E	0.154	1	200	300	1	1	2	4	2	0.75	0.6
2	5	6	2	1	1	A	0.154	2	160	250	2	1	2	3	2	0.7	0.6
2	5	6	2	1	1	B	0.147	2	160	250	2	1	2	3	2	0.7	0.6
2	5	6	2	1	1	C	0.153	2	160	250	2	1	2	3	2	0.7	0.6
2	5	6	2	1	1	D	0.167	2	160	250	2	1	2	3	2	0.7	0.6
2	5	6	2	1	1	E	0.17	2	160	250	2	1	2	3	2	0.7	0.6
2	5	6	2	2	1	A	0.166	1	180	300	0	0	1	4	3	0.9	0.8
2	5	6	2	2	1	B	0.169	1	180	300	0	0	1	4	3	0.9	0.8
2	5	6	2	2	1	C	0.162	1	180	300	0	0	1	4	3	0.9	0.8
2	5	6	2	2	1	D	0.167	1	180	300	0	0	1	4	3	0.9	0.8
2	5	6	2	2	1	E	0.178	1	180	300	0	0	1	4	3	0.9	0.8
2	7	7	1	1	4	A	0.182	4	90	120	3	3	3	3	1	0.6	0.5
2	7	7	1	1	4	B	0.186	4	90	120	3	3	3	3	1	0.6	0.5
2	7	7	1	1	4	C	0.181	4	90	120	3	3	3	3	1	0.6	0.5
2	7	7	1	1	4	D	0.174	4	90	120	3	3	3	3	1	0.6	0.5
2	7	7	1	1	4	E	0.155	4	90	120	3	3	3	3	1	0.6	0.5
2	7	7	1	2	1	A	0.162	1	150	200	0	1	2	4	2	0.8	0.6
2	7	7	1	2	1	B	0.169	1	150	200	0	1	2	4	2	0.8	0.6
2	7	7	1	2	1	C	0.171	1	150	200	0	1	2	4	2	0.8	0.6
2	7	7	1	2	1	D	0.163	1	150	200	0	1	2	4	2	0.8	0.6
2	7	7	1	2	1	E	0.133	1	150	200	0	1	2	4	2	0.8	0.6
2	7	7	2	1	5	A	0.199	5	60	100	3	3	3	3	1	0.7	0.5
2	7	7	2	1	5	B	0.151	5	60	100	3	3	3	3	1	0.7	0.5
2	7	7	2	1	5	C	0.158	5	60	100	3	3	3	3	1	0.7	0.5
2	7	7	2	1	5	D	0.199	5	60	100	3	3	3	3	1	0.7	0.5
2	7	7	2	1	5	E	0.165	5	60	100	3	3	3	3	1	0.7	0.5

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
2	7	7	2	2	4	A	0.188	2	100	150	1	1	1	3	2	0.85	0.6
2	7	7	2	2	4	B	0.179	2	100	150	1	1	1	3	2	0.85	0.6
2	7	7	2	2	4	C	0.162	2	100	150	1	1	1	3	2	0.85	0.6
2	7	7	2	2	4	D	0.185	2	100	150	1	1	1	3	2	0.85	0.6
2	7	7	2	2	4	E	0.177	2	100	150	1	1	1	3	2	0.85	0.6
2	7	8	1	1	4	A	0.175	4	90	150	5	3	3	3	1	0.65	0.5
2	7	8	1	1	4	B	0.164	4	90	150	5	3	3	3	1	0.65	0.5
2	7	8	1	1	4	C	0.154	4	90	150	5	3	3	3	1	0.65	0.5
2	7	8	1	1	4	D	0.161	4	90	150	5	3	3	3	1	0.65	0.5
2	7	8	1	1	4	E	0.172	4	90	150	5	3	3	3	1	0.65	0.5
2	7	8	1	2	4	A	0.172	5	60	90	7	3	5	2	0	0.6	0.45
2	7	8	1	2	4	B	0.177	5	60	90	7	3	5	2	0	0.6	0.45
2	7	8	1	2	4	C	0.189	5	60	90	7	3	5	2	0	0.6	0.45
2	7	8	1	2	4	D	0.193	5	60	90	7	3	5	2	0	0.6	0.46
2	7	8	1	2	4	E	0.174	5	60	90	7	3	5	2	0	0.6	0.45
2	7	8	2	1	4	A	0.188	4	90	100	3	3	4	2	0	0.75	0.6
2	7	8	2	1	4	B	0.145	4	90	100	3	3	4	2	0	0.75	0.6
2	7	8	2	1	4	C	0.14	4	90	100	3	3	4	2	0	0.75	0.6
2	7	8	2	1	4	D	0.164	4	90	100	3	3	4	2	0	0.75	0.6
2	7	8	2	1	4	E	0.172	4	90	100	3	3	4	2	0	0.75	0.6
2	7	8	2	2	3	A	0.165	3	80	100	1	2	3	3	1	0.65	0.5
2	7	8	2	2	3	B	0.136	3	80	100	1	2	3	3	1	0.65	0.5
2	7	8	2	2	3	C	0.157	3	80	100	1	2	3	3	1	0.65	0.5
2	7	8	2	2	3	D	0.142	3	80	100	1	2	3	3	1	0.65	0.5
2	7	8	2	2	3	E	0.163	3	80	100	1	2	3	3	1	0.65	0.5
2	6	9	1	1	1	A	0.138	1	200	300	0	1	2	4	2	0.8	0.7
2	6	9	1	1	1	B	0.136	1	200	300	0	1	2	4	2	0.8	0.7
2	6	9	1	1	1	C	0.12	1	200	300	0	1	2	4	2	0.8	0.7
2	6	9	1	1	1	D	0.131	1	200	300	0	1	2	4	2	0.8	0.7
2	6	9	1	1	1	E	0.123	1	200	300	0	1	2	4	2	0.8	0.7
2	6	9	1	2	1	A	0.137	1	180	250	0	0	1	4	2	0.85	0.7
2	6	9	1	2	1	B	0.142	1	180	250	0	0	1	4	2	0.85	0.7
2	6	9	1	2	1	C	0.136	1	180	250	0	0	1	4	2	0.85	0.7
2	6	9	1	2	1	D	0.125	1	180	250	0	0	1	4	2	0.85	0.7
2	6	9	1	2	1	E	0.136	1	180	250	0	0	1	4	2	0.85	0.7
2	6	9	2	1	2	A	0.143	3	150	200	2	2	3	3	1	0.65	0.6
2	6	9	2	1	2	B	0.144	3	150	200	2	2	3	3	1	0.65	0.6
2	6	9	2	1	2	C	0.147	3	150	200	2	2	3	3	1	0.65	0.6
2	6	9	2	1	2	D	0.144	3	150	200	2	2	3	3	1	0.65	0.6
2	6	9	2	1	2	E	0.14	3	150	200	2	2	3	3	1	0.65	0.6
2	6	9	2	2	2	A	0.134	2	160	220	1	2	2	3	2	0.7	0.65
2	6	9	2	2	2	B	0.136	2	160	220	1	2	2	3	2	0.7	0.65
2	6	9	2	2	2	C	0.139	2	160	220	1	2	2	3	2	0.7	0.65
2	6	9	2	2	2	D	0.139	2	160	220	1	2	2	3	2	0.7	0.65
2	6	9	2	2	2	E	0.143	2	160	220	1	2	2	3	2	0.7	0.65
2	8	10	1	1	4	A	0.174	4	60	100	3	2	3	3	1	0.65	0.5
2	8	10	1	1	4	B	0.169	4	60	100	3	2	3	3	1	0.65	0.5

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
2	8	10	1	1	4	C	0.167	4	60	100	3	2	3	3	1	0.65	0.5
2	8	10	1	1	4	D	0.168	4	60	100	3	2	3	3	1	0.65	0.5
2	8	10	1	1	4	E	0.154	4	60	100	3	2	3	3	1	0.65	0.5
2	8	10	1	2	3	A	0.169	3	90	100	2	3	3	4	1	0.65	0.5
2	8	10	1	2	3	B	0.148	3	90	100	2	3	3	4	1	0.65	0.5
2	8	10	1	2	3	C	0.159	3	90	100	2	3	3	4	1	0.65	0.5
2	8	10	1	2	3	D	0.164	3	90	100	2	3	3	4	1	0.65	0.5
2	8	10	1	2	3	E	0.156	3	90	100	2	3	3	4	1	0.65	0.5
2	8	10	2	1	3	A	0.161	2	100	150	1	2	3	4	1	0.6	0.5
2	8	10	2	1	3	B	0.158	2	100	150	1	2	3	4	1	0.6	0.5
2	8	10	2	1	3	C	0.164	2	100	150	1	2	3	4	1	0.6	0.5
2	8	10	2	1	3	D	0.163	2	100	150	1	2	3	4	1	0.6	0.5
2	8	10	2	1	3	E	0.159	2	100	150	1	2	3	4	1	0.6	0.5
2	8	10	2	2	4	A	0.174	4	60	120	6	3	4	4	0	0.45	0.4
2	8	10	2	2	4	B	0.174	4	60	120	6	3	4	4	0	0.45	0.4
2	8	10	2	2	4	C	0.175	4	60	120	6	3	4	4	0	0.45	0.4
2	8	10	2	2	4	D	0.162	4	60	120	6	3	4	4	0	0.45	0.4
2	8	10	2	2	4	E	0.159	4	60	120	6	3	4	4	0	0.45	0.4
2	9	11	1	1	4	A	0.187	4	50	100	7	3	4	4	2	0.65	0.6
2	9	11	1	1	4	B	0.18	4	50	100	7	3	4	4	2	0.65	0.6
2	9	11	1	1	4	C	0.186	4	50	100	7	3	4	4	2	0.65	0.6
2	9	11	1	1	4	D	0.171	4	50	100	7	3	4	4	2	0.65	0.6
2	9	11	1	1	4	E	0.167	4	50	100	7	3	4	4	2	0.65	0.6
2	9	11	1	2	3	A	0.174	3	90	100	3	2	3	4	2	0.8	0.7
2	9	11	1	2	3	B	0.164	3	90	100	3	2	3	4	2	0.8	0.7
2	9	11	1	2	3	C	0.172	3	90	100	3	2	3	4	2	0.8	0.7
2	9	11	1	2	3	D	0.165	3	90	100	3	2	3	4	2	0.8	0.7
2	9	11	1	2	3	E	0.166	3	90	100	3	3	3	4	2	0.8	0.7
2	9	11	2	1	4	A	0.173	4	60	90	5	3	5	4	2	0.7	0.65
2	9	11	2	1	4	B	0.169	4	60	90	5	3	5	4	2	0.7	0.65
2	9	11	2	1	4	C	0.166	4	60	90	5	3	5	4	2	0.7	0.65
2	9	11	2	1	4	D	0.163	4	60	90	5	3	5	4	2	0.7	0.65
2	9	11	2	1	4	E	0.162	4	60	90	5	3	5	4	2	0.7	0.65
2	9	11	2	2	3	A	0.164	3	85	150	2	1	4	4	2	0.7	0.55
2	9	11	2	2	3	B	0.167	3	85	150	2	1	4	4	2	0.7	0.55
2	9	11	2	2	3	C	0.169	3	85	150	2	1	4	4	2	0.7	0.55
2	9	11	2	2	3	D	0.164	3	85	150	2	1	4	4	2	0.7	0.55
2	9	11	2	2	3	E	0.16	3	85	150	2	1	4	4	2	0.7	0.55
3	11	1	1	1	5	A	0.207	5	65	100	10	4	3	2	0	0.45	0.65
3	11	1	1	1	5	B	0.186	5	65	100	10	4	3	2	0	0.45	0.65
3	11	1	1	1	5	C	0.183	5	65	100	10	4	3	2	0	0.45	0.65
3	11	1	1	1	5	D	0.192	5	65	100	10	4	3	2	0	0.45	0.65
3	11	1	1	1	5	E	0.178	5	65	100	10	4	3	2	0	0.45	0.65
3	11	1	1	2	3	A	0.168	3	70	150	5	3	3	3	0	0.6	0.65
3	11	1	1	2	3	B	0.155	3	70	150	5	3	3	3	0	0.6	0.65
3	11	1	1	2	3	C	0.168	3	70	150	5	3	3	3	0	0.6	0.65
3	11	1	1	2	3	D	0.151	3	70	150	5	3	3	3	0	0.6	0.65

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
3	11	1	1	2	3	E	0.157	3	70	150	5	3	3	3	0	0.6	0.65
3	11	1	2	1	3	A	0.169	4	70	120	6	3	3	2	0	0.5	0.5
3	11	1	2	1	3	B	0.166	4	70	120	6	3	3	2	0	0.5	0.5
3	11	1	2	1	3	C	0.16	4	70	120	6	3	3	2	0	0.5	0.5
3	11	1	2	1	3	D	0.169	4	70	120	6	3	3	2	0	0.5	0.5
3	11	1	2	1	3	E	0.168	4	70	120	6	3	3	2	0	0.5	0.5
3	11	1	2	2	3	A	0.164	3	85	150	4	2	3	3	1	0.7	0.6
3	11	1	2	2	3	B	0.157	3	85	150	4	2	3	3	1	0.7	0.6
3	11	1	2	2	3	C	0.153	3	85	150	4	2	3	3	1	0.7	0.6
3	11	1	2	2	3	D	0.148	3	85	150	4	2	3	3	1	0.7	0.6
3	11	1	2	2	3	E	0.155	3	85	150	4	2	3	3	1	0.7	0.6
3	10	2	1	1	3	A	0.182	3	50	100	4	3	2	3	0	0.6	0.65
3	10	2	1	1	3	B	0.177	3	50	100	4	3	2	3	0	0.6	0.65
3	10	2	1	1	3	C	0.178	3	50	100	4	3	2	3	0	0.6	0.65
3	10	2	1	1	3	D	0.177	3	50	100	4	3	2	3	0	0.6	0.65
3	10	2	1	1	3	E	0.172	3	50	100	4	3	2	3	0	0.6	0.65
3	10	2	1	2	3	A	0.187	2	65	100	3	3	2	3	0	0.65	0.6
3	10	2	1	2	3	B	0.161	2	65	100	3	3	2	3	0	0.65	0.6
3	10	2	1	2	3	C	0.165	2	65	100	3	3	2	3	0	0.65	0.6
3	10	2	1	2	3	D	0.163	2	65	100	3	3	2	3	0	0.65	0.6
3	10	2	1	2	3	E	0.169	2	65	100	3	3	2	3	0	0.65	0.6
3	10	2	2	1	3	A	0.172	3	60	90	5	3	3	2	0	0.5	0.5
3	10	2	2	1	3	B	0.168	3	60	90	5	3	3	2	0	0.5	0.5
3	10	2	2	1	3	C	0.164	3	60	90	5	3	3	2	0	0.5	0.5
3	10	2	2	1	3	D	0.16	3	60	90	5	3	3	2	0	0.5	0.5
3	10	2	2	1	3	E	0.165	3	60	90	5	3	3	2	0	0.5	0.5
3	10	2	2	2	3	A	0.178	4	65	90	8	4	3	2	0	0.45	0.5
3	10	2	2	2	3	B	0.153	4	65	90	8	4	3	2	0	0.45	0.5
3	10	2	2	2	3	C	0.159	4	65	90	8	4	3	2	0	0.45	0.5
3	10	2	2	2	3	D	0.167	4	65	90	8	4	3	2	0	0.45	0.5
3	10	2	2	2	3	E	0.163	4	65	90	8	4	3	2	0	0.45	0.5
3	14	3	1	1	4	A	0.184	4	70	90	7	3	2	2	1	0.7	0.5
3	14	3	1	1	4	B	0.175	4	70	90	7	3	2	2	1	0.7	0.5
3	14	3	1	1	4	C	0.167	4	70	90	7	3	2	2	1	0.7	0.5
3	14	3	1	1	4	D	0.177	4	70	90	7	3	2	2	1	0.7	0.5
3	14	3	1	1	4	E	0.178	4	70	90	7	3	3	2	1	0.7	0.5
3	14	3	1	2	5	A	0.189	4	85	100	4	3	3	3	2	0.75	0.45
3	14	3	1	2	5	B	0.185	4	85	100	4	3	3	3	2	0.75	0.45
3	14	3	1	2	5	C	0.189	4	85	100	4	3	3	3	2	0.75	0.45
3	14	3	1	2	5	D	0.18	4	85	100	4	3	3	3	2	0.75	0.45
3	14	3	1	2	5	E	0.181	4	85	100	4	3	3	3	2	0.75	0.45
3	14	3	2	1	5	A	0.186	5	50	85	5	5	4	1	1	0.6	0.5
3	14	3	2	1	5	B	0.182	5	50	85	5	5	4	1	1	0.6	0.5
3	14	3	2	1	5	C	0.183	5	50	85	5	5	4	1	1	0.6	0.5
3	14	3	2	1	5	D	0.183	5	50	85	5	5	4	1	1	0.6	0.5
3	14	3	2	1	5	E	0.182	5	50	85	5	5	4	1	1	0.6	0.5
3	14	3	2	2	4	A	0.177	3	70	90	3	3	3	2	2	0.6	0.5

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
3	14	3	2	2	4	B	0.16	3	70	90	3	3	3	2	2	0.6	0.5
3	14	3	2	2	4	C	0.169	3	70	90	3	3	3	2	2	0.6	0.5
3	14	3	2	2	4	D	0.171	3	70	90	3	3	3	2	2	0.6	0.5
3	14	3	2	2	4	E	0.174	3	70	90	3	3	3	2	2	0.6	0.5
3	10	4	1	1	2	A	0.16	3	80	100	6	3	3	3	1	0.8	0.7
3	10	4	1	1	2	B	0.159	3	80	100	6	3	3	3	1	0.8	0.7
3	10	4	1	1	2	C	0.154	3	80	100	6	3	3	3	1	0.8	0.7
3	10	4	1	1	2	D	0.16	3	80	100	6	3	3	3	1	0.8	0.7
3	10	4	1	1	2	E	0.159	3	80	100	6	3	3	3	1	0.8	0.7
3	10	4	1	2	3	A	0.177	3	70	120	3	3	2	4	2	0.8	0.75
3	10	4	1	2	3	B	0.174	3	70	120	3	3	2	4	2	0.8	0.75
3	10	4	1	2	3	C	0.172	3	70	120	3	3	2	4	2	0.8	0.75
3	10	4	1	2	3	D	0.169	3	70	120	3	3	2	4	2	0.8	0.75
3	10	4	1	2	3	E	0.168	3	70	120	3	3	2	4	2	0.8	0.75
3	10	4	2	1	3	A	0.18	3	60	100	6	2	2	3	1	0.65	0.6
3	10	4	2	1	3	B	0.172	3	60	100	6	2	2	3	1	0.65	0.6
3	10	4	2	1	3	C	0.176	3	60	100	6	2	2	3	1	0.65	0.6
3	10	4	2	1	3	D	0.178	3	60	100	6	2	2	3	1	0.65	0.6
3	10	4	2	1	3	E	0.172	3	60	100	6	2	2	3	1	0.65	0.6
3	10	4	2	2	2	A	0.169	2	70	100	3	2	2	3	2	0.9	0.7
3	10	4	2	2	2	B	0.163	2	70	100	3	2	2	3	2	0.9	0.7
3	10	4	2	2	2	C	0.163	2	70	100	3	2	2	3	2	0.9	0.7
3	10	4	2	2	2	D	0.167	2	70	100	3	2	2	3	2	0.9	0.7
3	10	4	2	2	2	E	0.159	2	70	100	3	2	2	3	2	0.9	0.7
3	12	5	1	1	4	A	0.183	4	50	90	7	4	3	3	2	0.65	0.6
3	12	5	1	1	4	B	0.182	4	50	90	7	4	3	3	2	0.65	0.6
3	12	5	1	1	4	C	0.173	4	50	90	7	4	3	3	2	0.65	0.6
3	12	5	1	1	4	D	0.179	4	50	90	7	4	3	3	2	0.65	0.6
3	12	5	1	1	4	E	0.18	4	50	90	7	4	3	3	2	0.65	0.6
3	12	5	1	2	2	A	0.17	3	90	100	3	3	3	3	2	0.75	0.7
3	12	5	1	2	2	B	0.168	3	90	100	3	3	3	3	2	0.75	0.7
3	12	5	1	2	2	C	0.166	3	90	100	3	3	3	3	2	0.75	0.7
3	12	5	1	2	2	D	0.17	3	90	100	3	3	3	3	2	0.75	0.7
3	12	5	1	2	2	E	0.169	3	90	100	3	3	3	3	2	0.75	0.7
3	12	5	2	1	4	A	0.188	4	60	90	5	3	3	3	2	0.65	0.6
3	12	5	2	1	4	B	0.178	4	60	90	5	3	3	3	2	0.65	0.6
3	12	5	2	1	4	C	0.175	4	60	90	5	3	3	3	2	0.65	0.6
3	12	5	2	1	4	D	0.182	4	60	90	5	3	3	3	2	0.65	0.6
3	12	5	2	1	4	E	0.179	4	60	90	5	3	3	3	2	0.65	0.6
3	12	5	2	2	3	A	0.169	3	65	90	3	2	3	3	2	0.7	0.55
3	12	5	2	2	3	B	0.172	3	65	90	3	2	3	3	2	0.7	0.55
3	12	5	2	2	3	C	0.167	3	65	90	3	2	3	3	2	0.7	0.55
3	12	5	2	2	3	D	0.171	3	65	90	3	2	3	3	2	0.7	0.55
3	12	5	2	2	3	E	0.169	3	65	90	3	2	3	3	2	0.7	0.55
3	12	6	1	1	5	A	0.191	5	60	90	5	3	3	3	1	0.55	0.5
3	12	6	1	1	5	B	0.187	5	60	90	5	3	3	3	1	0.55	0.5
3	12	6	1	1	5	C	0.179	5	60	90	5	3	3	3	1	0.55	0.5

Group	Age	ID	Leg	Aspect	MLS	Area	BMD	SHS	LCN (Z1)	LCN (Z3)	CH	EL	TLG	TRN (Z3)	TRN (Z1)	CT (Z1)	CT (Z3)
3	12	6	1	1	5	D	0.185	5	60	90	5	3	3	3	1	0.55	0.5
3	12	6	1	1	5	E	0.181	5	60	90	5	3	3	3	1	0.55	0.5
3	12	6	1	2	3	A	0.174	3	70	120	3	3	2	3	2	0.65	0.55
3	12	6	1	2	3	B	0.173	3	70	120	3	3	2	3	2	0.65	0.55
3	12	6	1	2	3	C	0.167	3	70	120	3	3	2	3	2	0.65	0.55
3	12	6	1	2	3	D	0.168	3	70	120	3	3	2	3	2	0.65	0.55
3	12	6	1	2	3	E	0.169	3	70	120	3	3	2	3	2	0.65	0.55
3	12	6	2	1	4	A	0.179	4	80	100	4	3	3	3	2	0.75	0.7
3	12	6	2	1	4	B	0.176	4	80	100	4	3	3	3	2	0.75	0.7
3	12	6	2	1	4	C	0.169	4	80	100	4	3	3	3	2	0.75	0.7
3	12	6	2	1	4	D	0.175	4	80	100	4	3	3	3	2	0.75	0.7
3	12	6	2	1	4	E	0.174	4	80	100	4	3	3	3	2	0.75	0.7
3	12	6	2	2	4	A	0.178	3	85	120	3	3	3	3	1	0.75	0.65
3	12	6	2	2	4	B	0.177	3	85	120	3	3	3	3	1	0.75	0.65
3	12	6	2	2	4	C	0.174	3	85	120	3	3	3	3	1	0.75	0.65
3	12	6	2	2	4	D	0.169	3	85	120	3	3	3	3	1	0.75	0.65
3	12	6	2	2	4	E	0.171	3	85	120	3	3	3	3	1	0.75	0.65