Third Carpal Bone Sclerosis

Radiographic evaluation and clinical implications
in Standardbred trotters

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Abstract

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The aims of this thesis were to evaluate the DPr-DDiO projection for the assessment of sclerosis and to study the relationships between altered third carpal bone (C3) radiopacity and previous exercise, previous carpal lameness, clinical appearance, and prognosis for future performance.
Effects of positioning on radiographic appearance and depth of evaluated C3 were investigated in frozen specimens under beam-cassette angles of 15° to 45°. Beam-cassette angles near 40° produced maximal depth of evaluated C3 but grading of sclerosis appeared insensitive to variations in beam-cassette angle in the range 25°-40°. A comparison between grading of sclerosis and histomorphometrical bone density estimations in specimens from Standardbred trotters showed a higher bone volume density in subchondral compared to central cancellous bone. Mild sclerosis indicated that the subchondral cancellous bone had reached its maximal density while increasing sclerosis only reflected a further density-increase in central cancellous bone.
The association between altered C3 radiopacity and degree of lameness and prognosis for racing was evaluated in a retrospective study of 89 Standardbred trotters diagnosed with traumatic carpitis. No significant relationships between degree of sclerosis and lameness or prognosis for racing within 30 months were found. However, radiolucencies ≥ 2 mm were found to significantly influence degree of lameness at presentation and time to start but did not affect chances of racing within 30 months.
Factors affecting development of increased C3 radiopacity were evaluated in a longitudinal study of 14 Standardbred trotters in professional training, between the mean ages of 20 and 42 months. Carpal lameness was significantly associated with progression of sclerosis although in most cases, sclerosis developed irrespective of carpal lameness. No significant associations were found between grade of sclerosis and previous carpal lameness or between grade of sclerosis and level of performance, classified as training, qualified for racing and actively racing.
Sclerosis appears to be of limited value as an indicator of clinical carpal disease or level of performance in Standardbred trotters while radiolucencies are clinically significant findings warranting further research.

Key words: equine, Standardbred, carpal, sclerosis, osteolysis, subchondral bone, lameness, radiography, histomorphometry

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Radiologisk utvärdering och klinisk betydelse av skleros i tredje karpalbenet hos travhästar

Skleros, eller ökad röntgentäthet, i tredje karpalbenet är ett välbekant fynd hos kapplöpningshästar. Den ökade röntgentätheten orsakas av att ben nybildas inne i det porösa tredje karpalbenet. Eftersom benbildningen är ett svar på ökad belastning är det tänkbart att den ökade röntgentätheten skulle kunna fungera som en indikator för hästens träningsnivå eller för överbelastningsskador. Tidigare studier utförda på fullblodsgaloppörer har visat att skleros i tredje karpalbenet skulle kunna vara ett tecken på en ledskada och bidra till uppkomsten av frakturer på tredje karpalbenet. I denna studie av varmblodiga travhästar har radiologisk bedömning av skleros i DPr-DDiO projektion utvärderats och sambanden mellan förändrad röntgentäthet och träningsnivå, tidigare karpalledsproblem, aktuell klinisk status och framtida prestation har undersökts.

Projektionsvinkelns (vinkeln mellan röntgenstråle och filmkassett) betydelse för det radiologiska utseendet och för hur stor del av tredje karpalbenet som kunde bedömas studerades på frysta frambenspreparat. Vinklar mellan 15° och 45° undersöktes och det visade sig att vinklar nära 40° resulterade i maximalt undersökningsdjup. Graderingen av skleros påverkades dock inte av att projektionsvinkeln varierade mellan 25°-40°. Jämförelser mellan radiologiskt bedömd skleros och histomorfometriskt bedömd bentäthet visade att den porösa benvävnaden närmast ledbrosket var mer kompakt än den centrala benvävnaden. I ben som bedömts ha lindrig skleros hade den porösa benvävnaden närmast ledbrosket nått maximal täthet medan tilltagande skleros enbart avspeglade en alltmer ökande täthet i den centrala benvävnaden.

Sambanden mellan täthetsförändringar i tredje karpalbenet och grad av hälta respektive framtida prestation undersöktes i en retrospektiv studie. Radiologiskt utseende jämfördes med patientdata från 89 varmblodiga travhästar som fått diagnosen icke-infektiös karpalledsinflammation. Inga signifikanta samband sågs mellan grad av skleros och grad av hälta respektive prestation under de följande 30 månaderna. Däremot sågs signifikanta samband mellan fynd av områden med nedsatt röntgentäthet $\geq$ 2 mm och ökad hälta respektive förlängd tid till start. Andelen hästar som tävlade inom 30 månader påverkades dock inte av fynd av områden med nedsatt röntgentäthet.

Faktorer som påverkade uppkomsten och utvecklingen av ökad röntgentäthet utvärderades i en longitudinell studie av 14 professionellt tränade varmblodiga travhästar från 20 till 42 månaders ålder. Ett signifikant samband sågs mellan karpalhälta och tilltagande skleros men i de flesta fall utvecklades skleros i tredje karpalbenet oberoende av hälta. Inga signifikanta samband sågs mellan grad av skleros och tidigare karpalhälta, eller mellan grad av skleros och träningsnivå, graderad som: tränande, startkvalificerad och tävlande.

Radiologiskt utvärderad skleros tycks ha ett begränsat värde som indikator både för kliniska karpalproblem och för träningsnivå. Lokalt nedsatt röntgentäthet i tredje karpalbenet är emellertid ett klinisk betydelsefullt fynd vilket motiverar vidare studier kring dess uppkomst och avläkning.

"All trotters and pacers that go fast enough and compete long enough to be useful racehorses become lame at some time in their career..."

<div align="right">Scott E. Palmer, 1990</div>

Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I.   Uhlhorn H, Eksell P. The dorsoproximal-dorsodistal projection of the distal carpal bones in horses: an evaluation of different beam-cassette angles. Vet Radiol Ultrasound. 1999;40:480-485.

II.   Uhlhorn H, Ekman S, Haglund A, Carlsten J. The accuracy of the dorsoproximal-dorsodistal projection in assessing third carpal bone sclerosis in Standardbred trotters. Vet Radiol Ultrasound. 1998;39:412-417.

III. Uhlhorn H, Carlsten J. Retrospective study of subchondral sclerosis and lucency in the third carpal bone of Standardbred trotters. Equine Vet J. 1999;31:500-505.

IV.   Uhlhorn H, Eksell P Sandgren B, Carlsten J. Sclerosis of the Third Carpal Bone. A Prospective Study of its Significance in a Group of Young Standardbred Trotters. Acta Vet Scand. 2000;41:51-61.

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Abbreviations

ADR – areas of decreased radiopacity (or radiolucency)
BGR – continous blue-green-red colour scale
BMU – basic multicellular unit
b.w. – body weight
C3 – third carpal bone (os carpale III)
CT – computed tomography
DL-PaMO - dorsolateral-palmaromedial oblique
DM-PaLO - dorsomedial-palmarolateral oblique
DPa – dorsopalmar
DPr-DDiO - dorsoproximal-dorsodistal oblique
DXA – dual-energy x-ray absorptiometry
HDP – hydroxymethylenediphosphonate
IRU – increased radiopharmaceutical uptake
i.v. – intra venous
LM – lateromedial
MC3 – third metacarpal bone (os metacarpale III)
MRI – magnetic resonance imaging
ROI – region of interest

Introduction

The term sclerosis, from the Greek "σκληροξ" meaning hard, is used for conditions when tissues become harder than normal due to excessive connective tissue formation and/or mineralisation. In porous cancellous bone, sclerosis implies new bone formation on the surface of existing bone trabeculae and reduced intertrabecular bone marrow space. In radiology, sclerosis denotes an increased radiographic opacity of bone, generally in connection with subchondral cancellous bone. In Thoroughbred and Standardbred racehorses, increased radiographic opacity of the third carpal bone (C3) is a common finding. In this thesis, the term sclerosis is used to denote an increased cancellous bone volume density that has been detected using radiography.

Bone

Characteristics of bone

Bone can be regarded as a specialised connective tissue composed of cells (10% by weight) and calcified intercellular matrix (Schiller 1994). Three main types of cells, responsible for the synthesis, maintenance and resorption of bone matrix are found. The osteoblast is a bone-forming cell found along the surface of newly formed, non-calcified bone matrix, or osteoid. Osteoblasts derive from progenitor cells emanating from mesenchymal stem cells associated with bone marrow (Owen 1980). The osteoblast has a cuboidal shape and basophilic cytoplasm indicative of abundant rough endoplasmic reticulum and active protein synthesis. Osteoblasts synthesise organic bone matrix; collagen fibres and ground substance that later mineralises. The tightly appositioned flattened osteoblasts, or bone-lining cells, covering the bone matrix are also thought to play a role as regulators of matrix resorption by osteoclasts both through physical exposure of mineralised matrix (Chambers and Fuller 1985) and by mediating the hormonal/cytokine signal for resorption (Thomson et al. 1986). The osteocyte is an endstage differentiated osteoblast that has been surrounded by newly formed bone during matrix formation. Osteocytes are singly situated in lacunae, being in contact with each other via cytoplasmic processes and gap junctions through thin cylindrical canaliculi. The osteocyte has a flattened shape, condensed nuclear chromatin, reduced rough endoplasmic reticulum but a relatively large Golgi complex (Jande 1971). Osteocytes are necessary for matrix maintenance and their death is followed by matrix resorption (Van Sickle et al. 1993). It has also been suggested that osteocytes play a key role in regulating the modelling and remodelling processes of bone (Cowin et al. 1991, Burger et al. 1999). The osteoclast is a large, multinuclear, branched, motile cell thought to form from the fusion of mononuclear cells of the bone marrow (Roodman et al. 1985). Osteoclasts attach to the matrix surface where they form resorption cavities. The cells secrete collagenase and other lytic enzymes together with hydrogen ions into an enclosed space between cell and bone matrix, digesting collagen and dissolving bone

mineral. Dry bone matrix in mature bone contains inorganic and organic matter in a ratio of 2:1 (Getty 1975). The organic matter or osteoid, laid down by the osteoblasts, consists of collagen, non-collagenous proteins and proteoglycans. The major part of the organic component consists of type 1 collagen forming a cohesive scaffold upon which the inorganic matter, primarily hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2)$, is deposited. Mineralisation takes place 1-10 days after matrix deposition (Gehron-Robey 1989). The initial hydroxyapatite crystals are deposited in the gap regions of the collagen fibrils and in mature bone the mineral is located within and between the fibrils (Weiner and Traub 1986). Bone specific non-collagenous proteins may be mediators of hydroxyapatite precipitation (Gehron-Robey 1989).

On a gross level, mature bone exists in two structural forms: cortical (dense) bone and cancellous (trabecular) bone. Dense cortical bone forms the external shell of the bone within which is the meshwork arrangement of cancellous bone. The architecture of interconnected trabeculae has been shown to adapt to functional loads (Radin et al. 1982) and the direction of trabeculae has been shown to reflect the principal strain directions on the surface of bone (Lanyon 1974). Typical long bones have a hollow shaft forming a medullary cavity thus confining the cancellous bone to the metaphyses and epiphyses while the short cuboidal bones of the carpus and the tarsus consist of a thin shell of dense cortical bone surrounding cancellous bone. The medullary cavity and the intertrabecular space are filled with bone marrow. The bone cortex accounts for a large part of the mechanical strength of the bone while the cancellous bone has a greater capacity for deformation and shock absorption. Cancellous bone distributes forces applied at the articular surface to surrounding bone (Hayes et al. 1978, Pugh et al. 1973). Its density and intrinsic architecture (Pugh et al. 1973) determine the compliance of cancellous bone. Stiffness of C3 cancellous bone of horses has been shown to be proportional to bone volume density (Young et al. 1991). The soft tissues of the cavities of bone contribute very little to the mechanical properties of the bone (Currey 1984). A proportionally higher surface area and number of bone cells to the volume of bone is found in the cancellous compared to the cortical bone reflecting a higher metabolic bone activity (Weiss 1988).

Remodelling and modelling of bone

Bone is a viable, dynamic tissue. In horses 50% of the primary bone formed during the foetal period and first years of life has been removed through osteoclastic activity and has been replaced with mature, highly organised lamellar bone by three years of age (Riggs and Evans 1990). This process, termed remodelling, continues, although with declining intensity, throughout adult life thus facilitating both the repair of damaged tissue and adaptation of bone tissue to mechanical demands. Remodelling takes place within specific cell populations, or basic multicellular units (BMUs) (Frost 1964). Osteoclasts and small mononuclear cells resorbing bone are followed by osteoblasts laying down osteoid. Resorption and formation of bone are coupled, ensuring that the amount of bone remains approximately constant (Frost 1964). In cortical bone, the BMUs
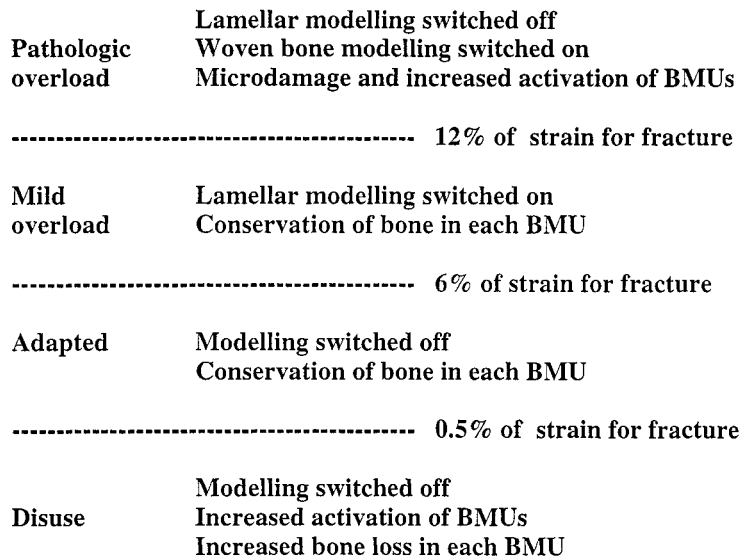
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may cut canals longitudinally through the bone creating so called Haversian systems. In cancellous bone, the osteoclasts of a remodelling BMUs are wrapped over the trabecular surfaces (Lucht 1972) but may also perforate trabeculae (Parfitt 1992). The activation of remodelling BMUs may be regulated via a systemic influence of hormones, local factors released from microfractures or signals from osteocytes or other bone lining cells (Eriksen and Kassem 1992). In humans, the duration of an entire remodelling cycle exceeds 4 months (Agerbaek et al. 1991) and an increased recruitment of BMUs will cause a temporary reduction in bone mass (Marcus 1987). An irreversible reduction in bone mass due to reduced bone formation during remodelling is seen in humans with increasing age (Sambrook et al. 1993). A similar age related loss of bone has not been described in horses although a decline in breaking strength of the third metacarpal bone after 6.3 years of age has been reported (Lawrence et al. 1994). Growth and shaping of bones are performed through modelling when formation and resorption of bone take place in different locations (Jee and Frost 1992). In the process of growth, new bone formation takes place on the periosteal (external) surface of cortical bones while resorption takes place on the endosteal (internal) surface resulting in larger and stronger bones (Parfitt 1994). While remodelling essentially conserves bone mass, the primary objective of modelling is to adjust the strength of bones to present needs (Kimmel 1993). In Thoroughbred racehorses, subchondral sclerosis due to formation of bone on trabecular surfaces, is a frequent finding (Young 1987) representing modelling in response to increased loading.

It needs to be recognised that the balance between bone formation and resorption is not only affected by direct influence of mechanical usage. A number of hormones, growth factors, nutrition, drugs, genetic factors and age influence bone homeostasis either directly or by offsetting the setpoints for the mechanical thresholds (Jee and Frost 1992).

Bone and exercise

To cope with body growth, microdamage, and changing mechanical demands as well as to serve as a reservoir of calcium, bone has a need for continuous adaptation through modelling and remodelling. The exact mechanisms whereby mechanical stimuli regulate the adaptive response of bone are not known but several theories relating the two have been presented and osteocytes have been indicated as the principal mechanosensor cells of bone (Burger et al. 1999). From the different models some generally accepted principles governing bone adaptation have been derived (Burr and Martin 1992). Firstly, adaptation of bone occurs in response to deformation or strain, i.e. a fractional change in length in response to stress, or force exerted on a certain area. Secondly, to elicit signals for an adaptive response, strains must be above or below certain perceived thresholds. The range may be exceeded either because of changes in the outer environment or because of alterations in the mechanical properties of the bone itself.

Figure 1: The mechanical usage windows suggested by Frost (Bone Miner. 1992;19:257-271). Approximate strain thresholds indicate typical peak bone strains in relation to fracture strain. Threshold levels might change with age, animal species and bone and the model does not account for effects of strain rates and strain gradients and non-mechanical factors.

Pathologic overload	**Lamellar modelling switched off** **Woven bone modelling switched on** **Microdamage and increased activation of BMUs**

--- **12% of strain for fracture**

Mild overload	**Lamellar modelling switched on** **Conservation of bone in each BMU**

--- **6% of strain for fracture**

Adapted	**Modelling switched off** **Conservation of bone in each BMU**

--- **0.5% of strain for fracture**

Disuse	**Modelling switched off** **Increased activation of BMUs** **Increased bone loss in each BMU**

In the Mechanostate model, Frost (1992) suggested the existence of three thresholds and four mechanical usage windows (Fig. 1). If the mechanical stimuli affecting the skeleton produce strain within a "normal" range, bone tends to be conserved, no modelling takes place and remodelling is balanced resulting in no net formation or resorption of bone. If strains fall below the lower threshold of the adapted window the mechanical stimulus will be insufficient to prevent loss of bone. No modelling of the bone surface takes place and an increased number of BMUs are recruited together with decreased bone formation during remodelling. When mechanical stimuli are increased until strains exceed the upper threshold of the adapted window, net bone formation is initiated. When strains are in the mild overload window lamellar bone is formed on periosteal and endosteal surfaces. Remodelling is balanced in each BMU tending to conserve existing bone. In rats, overload resulted in increased bone mass due to increased appositional bone formation and due to a lower number of BMUs being recruited (Jee and Li 1990). If strains increase, exceeding the threshold for pathologic overload, the slow formation of highly organised lamellar bone ceases and rapidly formed, poorly organised woven bone is produced in a reparative attempt. Mechanical overload during rapid exercise in racehorses has been shown result in trabecular microfracture (Norrdin et al. 1998). Pathological overload and microfracturing initiate a reparative response involving increased remodelling. This reparative process may result in a paradoxical loss of bone during the initial resorptive phase of bone remodelling, even contributing to bone failure. In humans, long-term

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exhaustive exercise has been reported to induce inhibition of bone formation as well as increased bone resorption (Brahm et al. 1996). In cancellous bone, trabeculae are thought to become thicker, more contiguous, and closer together in regions that carry high loads. The opposite occurs if load bearing is reduced, resulting in trabecular thinning or loss of entire trabeculae (Lanyon 1982).

Studies of avian bone have shown that repetitive loading is necessary to initiate an adaptive response (Lanyon and Rubin 1984), but that the number of cycles needed is low and that a further increased number of cycles does not result in increased bone formation (Rubin and Lanyon 1984). Experimental studies have also demonstrated the existence of strain related thresholds for modelling and remodelling in turkeys (Rubin and Lanyon 1985) and rats (Turner et al. 1991). *In vivo* studies have indicated that irregular strain distribution (Burr and Martin 1992) has a greater effect on the adaptive response of bone. This implies that an adaptive response is more readily generated by a variety of different loading conditions than by increased repetitions and that bone may be prone to respond to accidental loading (Burr and Martin 1992).

The equine carpus

Anatomy and biomechanics

The skeleton of the horse is a complex framework consisting of some 205 bones (Getty 1975) of various sizes, shape and function. Long bones of the limbs act as supporting columns and as levers while short bones are believed to play a role in attenuating load (Getty 1975, Bramlage 1988). In the equine carpus, positioned between the two columns of the antebrachium and the metacarpus, the short carpal bones are arranged in two rows, proximal and distal (Fig. 2). The proximal row has three axially weight bearing bones and one lateral accessory bone that may be regarded as a sesamoid bone interposed in the tendons of the flexor carpi ulnaris and ulnaris lateralis muscles. The distal row of carpal bones has three axially weight bearing bones and one small inconstant bone embedded in the medial collateral ligament of the carpus. The latter generally lacks articular facets and is present in about 50% of the horses (Getty 1975). Occasional findings of a palmarolateral fifth carpal bone (Butler et al. 2000) and separate ossicles, palmar to the distal row of carpal bones (Martens 1999) have been reported. Each carpal bone develops from a separate centre of ossification except for the accessory carpal bone that may have additional centres of ossification (Auer 1982). Ossification begins in the 9th month of pregnancy (Getty 1975, Soana et al. 1998) and the bones have a mature radiographic silhouette at 2-4 months of age (Auer 1982). The cuboidal third carpal bone is the largest of the carpal bones of the distal row. It is composed of cancellous bone sandwiched between two subchondral bone plates. The proximal surface consists of two articular facets and both the proximal and distal surfaces are covered by hyaline articular cartilage. Bone trabeculae of the third carpal bone have a predominantly proximo-distal arrangement (Young 1987).

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Figure 2: Dorsal view of the carpus. The carpal bones are arranged in two rows between the radius (R) and the metacarpal bones (MC). The radial- (Cr), intermediate- (Ci), ulnar- (Cu) and accessory (indicated with a broken line) carpal bones form the proximal row and the second- (C2), third- (C3) and fourth (C4) carpal bones form the distal row.
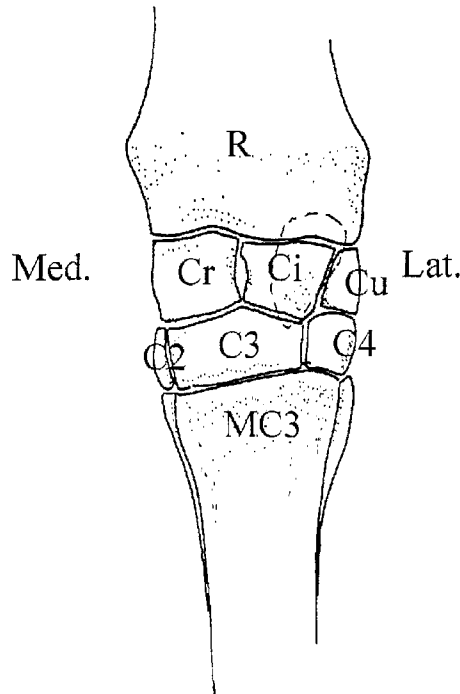
Two transverse dorsal ligaments and two interosseus ligaments connect the weight bearing bones within each row. Palmar ligaments and the thick, dense palmar part of the fibrous joint capsule connects the proximal row to the radius, the proximal to the distal rows, and the distal row to the metacarpus. Dorsal and interosseus ligaments help to attach the distal row of carpal bones firmly to the metacarpus (Getty 1975). The fibrous part of the carpal joint capsule is common to all joints but the synovial membrane forms three sacs corresponding to the three transversal joints separated at the levels of the interosseous ligaments (Getty 1975). The radiocarpal sac includes the joints of the accessory carpal bone. The intercarpal and the carpometacarpal sacs communicate between the third and fourth carpal bones and sometimes on the palmar aspect of the joint (Ford et al. 1988). Finally, the carpal joint has two wide collateral ligaments connecting the radius and the metacarpus.

During the non weight-bearing phase of a stride, the carpus will flex, thus reducing the moment of inertia when the limb is brought forward (Johnston et al.

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1997). Joint movement is almost entirely confined to the radiocarpal and intercarpal joints. Shortly before impact the limb is extended (van Weeren et al. 1993). Most of the sagittal articulations between the carpal bones have an overlapping arrangement so that axial load may be partially attenuated through medial-to-lateral displacement of the carpal bones, dissipating stress to the intercarpal ligaments. However, the medial aspect of the intercarpal joint is unable to attenuate axial load through interosseous displacement. The distal articular surface of the radial carpal bone is centred over the concave radial fossa of the third carpal bone transmitting its full load on to the dorsoproximal articular surface of the third carpal bone (Bramlage et al. 1988). When trotting at higher speeds the carpus is overextended during most of the stance phase (van Weeren 1993, Johnston et al. 1997). Overextension consists of two modes, an early rapid mode associated with the breaking of the hoof and a less rapid mode mediated by the opposing tension of the palmar supporting structures (Johnston et al. 1997). Under experimental conditions an increase in pressure and a shift of weight bearing area towards the dorsal aspect of the C3 with increasing load has been shown (Palmer et al. 1994). Due to the rotating action of the radiocarpal joint overextension and dissipation of load to the palmar soft tissue may take place but this is not possible in the ginglymus intercarpal joint. This renders the dorsomedial aspect of the carpus, particularly the interface between the radial and third carpal bones, liable to chronic overload (Bramlage et al. 1988).

Carpal lameness

In clinical practice, carpal injury, and lameness, is a common occurrence in horses performing at maximum speed over short distances (Bramlage et al. 1988). In Thoroughbred racehorses, carpal lameness has been shown to constitute a major cause of days lost from training (Rossdale et al. 1985). Reports generally agree that carpal fracture involves the intercarpal joint twice as frequently as the radiocarpal joint (Stashak 1987). In Standardbred trotters, most carpal fractures have been found to occur in the C3 (Schneider et al. 1988, Palmer 1986), primarily in the radial fossa (Schneider et al. 1988). The intercarpal joint is, second to the fetlock joint, the joint most frequently treated for traumatic aseptic arthritis in Standardbred trotters in Sweden (Kallings 1997). Early signs of carpal disease in Standardbred trotters include bearing in or bearing out during exercise (Ross 1991). Usually the horse is reported to be on the ipsilateral line and lameness is more obvious around turns (Ross 1991). Standardbreds with carpal soreness may abduce the affected limb in advancement and may have reduced carpal flexion (Palmer 1990, Ross 1991). Carpal soreness may be present in absence of synovial distension or pain at flexion and the diagnosis of carpitis should be confirmed by itra-articular anaesthesia (Palmer 1990).

Degenerative changes associated with intercarpal joint disease may involve one or more of the tissues forming the joint such as ligaments, joint capsule, synovial fluid, articular cartilage and subchondral bone. Due to the complex interaction between different tissues, degenerative changes in one of the joint components

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may eventually affect other tissues and the function of the joint. Damage to the articular cartilage as a result of concussion may lead to progressive depletion of matrix components and the release of cartilage degradation products that may induce synovial inflammation. Cartilage proteoglycans injected intra-articularly into rabbit knees has been shown to provoke synovitis and synovial hypertrophy as well as erosion of articulating surfaces (Boniface et al. 1988). Primary or secondary synovitis and capsulitis may cause alterations in the production of synovial fluid and result in the release of proteolytic enzymes, inflammatory mediators and cytokines impairing cartilage matrix production and leading to cartilage degradation (McIlwraith 1996). Equine synoviocytes stimulated with the inflammatory mediator interleukin 1 show collagenase and caseinase activity (Spiers et al. 1994). Tearing of the ligamentous apparatus may lead to joint instability and detrimental loading of articular cartilage and subchondral bone. Primary damage to the subchondral bone may lead to cartilage damage due to lack of support or due to the release of cytokines.

Mechanisms involving altered subchondral bone density, pertinent to the development of third carpal bone disease in horses have primarily been investigated in other species. Repeated longitudinal impact loading has been shown to cause increased subchondral bone stiffness prior to cartilage degeneration with fibrillation and glucosaminoglycan depletion in guinea pig knees (Simon et al. 1972). This supports the concept that early subchondral bone sclerosis causes a reduction in the shock-absorbing capacity of the subchondral bone thereby increasing the risk of shear induced damage to the articular cartilage. Experimentally induced subchondral bone stiffening in sheep (Burr and Schaffler 1997) as well as repeated longitudinal impact loading in horses (Murray et al. 1999) have also been shown to result in increasing depth of calcified cartilage, thereby decreasing the thickness of non-calcified cartilage. Decreased non-calcified cartilage thickness may alter the distribution of stress in articular cartilage contributing to cartilage overload and subsequent osteoarthrosis (Burr and Schaffler 1997).

All supportive tissues of the joint are capable of responding to alterations in the mechanical demands through remodelling, but not necessarily at the same rate and joint lesions develop when tissues fail to adapt to the biomechanical demands (Palmer and Bertone 1996).

Radiography of the C3

The C3 can be evaluated in all of the five standard radiographic projections of the carpus dorsopalmar (DPa), lateromedial (LM), flexed LM, dorsolateral-palmaromedial oblique (DL-PaMO) and dorsomedial-palmarolateral oblique (DM-PaLO) (Boring 1998, Butler et al. 2000). The direction of the x-ray beam is tangential to different areas of the carpus in the different projections, evaluating the C3 with varying skeletal superimposition. However, the dorsoproximal-dorsodistal oblique (DPr-DDiO) projection of the distal row of carpal bones is the only projection permitting the assessment of C3 cancellous bone structure and density, free of superimposed bone (O'Brien 1977). The DPr-DDiO (or "skyline")

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projection was introduced in equine radiology (O'Brien 1977) with the primary objective to improve the diagnosis and assessment of minimally displaced fractures of the third carpal bone. It has become one of the principal projections of the radiographic investigation in cases of carpal lameness and suspected carpal fracture (Morgan and Silverman 1984, Boring 1998, Butler et al. 2000).

Figure 3: Positioning for the DPr-DDiO projection of the distal row of carpal bones.

35°
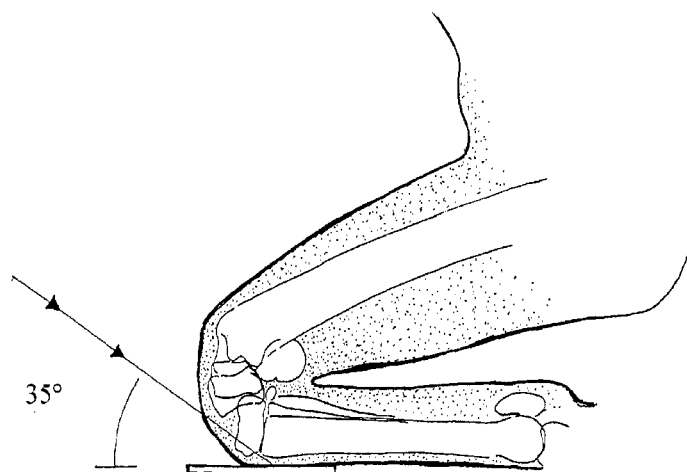
Positioning for the DPr-DDiO projection is illustrated in Fig. 3. The carpus should be flexed to a maximum and the cassette held firmly against the proximal metacarpal region, nearly parallel to the floor (Morgan and Silverman 1984). The x-ray beam should be directed proximally to distally, projecting the dorsal part of the C3 free of adjacent bone (O'Brien 1977). A specific beam-cassette angle was not given but subsequent authors describing the projection have recommended angles of 20-25° (Blevins and Widmer 1990), 30° (Douglas et al. 1987; Morgan et al. 1991; Morgan and Silverman 1993, Wright and Butler 1997, Boring 1998) or 35° (Butler et al. 2000). High-definition film/screen combinations are preferred. In a properly positioned DPr-DDiO radiograph of the distal row of carpal bones (Butler et al. 2000), the dorsal borders of the second, third and fourth carpal bones should be clearly outlined. The third carpal bone is central, with the second and fourth carpal bones at the medial and lateral aspects respectively. Each carpal bone should have a smooth outline, with an even trabecular pattern and sharply defined corticomedullary border.

Studies on altered radiopacity of the equine third carpal bone

Prevalence

Foci of increased radiopacity and loss of subchondral bone structure in the C3 was first reported in association with carpal fractures in Thoroughbred racehorses (O'Brien 1977). Retrospective investigations indicated that C3 sclerosis was common in Thoroughbred racehorses diagnosed with carpal lameness. Sclerosis was present in 48 of 50 limbs, either in combination with fracture or as a single finding (O'Brien et al. 1985). Similar investigations, in non-lame, racing Thoroughbreds (O'Brien et al. 1985) and in cadaver specimens from racing Thoroughbreds presumed free of lameness (Young et al. 1988), revealed sclerosis in approximately half of the investigated carpi.

Foci of decreased subchondral bone radiopacity (or subchondral radiolucency, or cystic lesions (O'Brien 1977)), of dorsoproximal third carpal bones have been described as an infrequent finding that should not be confused with vascular channels (O'Brien 1977). Ross et al. (1989) described 13 cases of subchondral radiolucency and sclerosis of the third carpal bone in Standardbred trotters from a total caseload of 27,000 horses.

To the author's knowledge no other studies describing the prevalence of sclerosis or radiolucency of the third carpal bone in other breeds have been published. But it has been stated that sclerosis is a frequent finding in Thoroughbred and Standardbred racehorses (Butler et al. 2000) and in high-performance horses (Boring 1998).

Development

Using histomorphometrical and microradiographic methods it has been shown that localised hypertrophy of subchondral bone takes place in the third carpal bone of Thoroughbreds during training and racing (Young 1987, Young et al. 1991) as well as during controlled, high intensity treadmill exercise of Thoroughbred horses (Firth et al. 1999). The subchondral bone plate has been shown to thicken (Young 1987) and to become more compact (Young 1987, Firth et al. 1999), and the thickness of underlying cancellous bone trabeculae has been shown to increase (Young 1987). In untrained Thoroughbred horses, 2- to 3-year-olds have been shown to have a higher cancellous bone density than yearlings (Young et al. 1991) but no age-related differences in bone density have been found in racing and training horses (Young 1987, Young et al. 1991).

Increased subchondral bone radiopacity of the dorsoproximal third carpal bone has been reported in Thoroughbreds that have trained and/or raced (O'Brien et al. 1985, Young 1987) and in Standardbreds that have raced (Young 1987). Severe sclerosis (loss of trabecular structure in the radial and/or intermediate fossa, O'Brien et al. 1985) has only been reported from racing Thoroughbreds (O'Brien et al. 1985, Young 1987) primarily in connection with carpal fracture and lameness (DeHaan et al. 1987). Cancellous bone volume density measurements have also been reported to be higher in third carpal bones from intercarpal joints diagnosed with fractures and severe arthrosis compared to third carpal bones from

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non-lame trained Thoroughbreds (Young et al. 1991). Sclerosis of the C3 is generally seen as a persistent radiographic finding but regression of sclerosis after resting has been described in a Thoroughbred racehorse diagnosed with acute carpal lameness and mild radiographic sclerosis of the dorsoproximal third carpal bone. A follow-up investigation of the same horse after four months revealed a cancellous bone pattern of both third carpal bones of almost normal radiopacity but with a coarse and fuzzy appearance. Histologic examination revealed large areas of cancellous bone that had undergone resorption (Young 1987).

Foci of decreased radiopacity of the C3 have been ascribed to excessive compressive forces acting on the radial fossa but the pathogenesis is unclear (Ross et al. 1989). One study (Ross et al. 1989) has reported the mean age of affected horses to be 4.1 years which indicates that subchondral lucency will appear after prolonged demanding physical exercise and is not a finding of horses in early training or racing.

Clinical significance

Evidence of clinical importance of findings of abnormal C3 radiopacity is largely circumstantial. The very high proportion of lame, racing Thoroughbreds reported to have C3 sclerosis, particularly in combination with C3 fracture led to suggestions that C3 sclerosis was indicative of microfracture and cartilage damage (DeHaan et al. 1987) and that sclerosis may be a lesion preceding C3 fracture (O'Brien et al. 1985). Subchondral lucency in Standardbred racehorses has been claimed to cause acute, severe forelimb lameness (Ross et al. 1989). This was based on a study where an acute moderate to severe lameness referable to the middle carpal joint was observed in 13 horses diagnosed with foci of decreased subchondral bone density commonly in combination with sclerosis.

Histological examination of third carpal bones with marked sclerosis from Thoroughbred and Standardbred racehorses has revealed extensive appositional growth of woven bone on trabecular surfaces (Young 1987). Foci of dead bone were found in subchondral areas of particularly sclerotic bones where new bone formation had obliterated marrow spaces possibly cutting off areas of bone from its blood supply. It was suggested that necrotic bone might induce resorption and weakening of subchondral bone predisposing it to fracture (Young 1987). Biomechanical investigations of third carpal bones of lame Thoroughbreds (Young et al. 1991) have shown a dorsomedial front of increased cancellous bone density and a sharp gradient in stiffness, 5-10 mm from the dorsal edge of the bone. These correspond to the most common plane of fracture of the C3 in Thoroughbreds (Stephens et al. 1988). It has been hypothesised that this steep gradient in stiffness may result in a concentration of shear stress leading to carpal fracture (Young et al. 1991). Areas of subchondral bone necrosis and resorption in combination with sclerosis have been described in the C3 radial fossa of racing Thoroughbreds (Young 1987, Pool 1996). These lesions, evident as radiolucent areas at radiographic examination have been implicated in the development of C3 slab fracture. Histologic investigations of fracture fragments from C3 slab

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fractures showing evidence of chronic ischemia and necrosis corroborate this theory (Pool 1996).

Background to this thesis

Including foals, older horses and imports, Standardbred trotters represent slightly less than one third of the Swedish horse population of 200,000. Registered Swedish Standardbred trotters in competing age (2-12 years) number approximately 45,000 (Anon 1999). The Standardbred trotter is almost exclusively used for racing and is the predominant racing breed in Sweden.

The typical Standardbred trotter is broken in the autumn the year after it was born. Training progresses with increasing speed, loading and duration until the horse qualifies for racing. Approximately 40% of Swedish Standardbred trotters race as 3-year-olds (Anon 1997). However, only 24% of horses at competing age actually race in a year (Anon 1997) due to lameness, disease, lack of talent or breeding activities. Carpal lameness is stated to be frequent in racing horses and to be rare in other breeds (Bramlage et al. 1988). Likewise the presence of C3 sclerosis is primarily reported in racehorses (Butler et al. 2000). We may assume that radiographic findings of C3 sclerosis in racehorses result from loading during racing and training, whether this loading is physiological, pathological, or both has not been determined. To determine this and the potential impact of sclerosis on future performance is quintessencial to the development of training programs and for our understanding of clinical carpal problems. Assuming that increased radiopacity results from increased loading, grade of sclerosis might indicate attained level of performance and/or previous carpal trauma. Previous studies in Thoroughbred racehorses have also indicated sclerosis as a risk factor for clinical carpal disease and carpal fracture. While a number of techniques are available for the evaluation of bone density, radiography remains the only clinically available method for evaluation of C3 density in horses (Firth et al. 1999). In spite of this, current literature describes C3 sclerosis in racehorses both as a sign of normal adaptation to exercise (Pool 1996) and as a sign of degenerative joint disease warranting removal of a horse from training (Ferraro 1990). The lack of clinical evaluation severely restricts the usefulness of the technique and allows for arbitrary assessment of clinical status and prognosis.

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Aims

The purpose of this thesis was to evaluate the radiographic DPr-DDiO projection of the distal row of carpal bones for the assessment of C3 sclerosis and to investigate the relationships between altered C3 radiopacity and previous exercise, previous carpal lameness, clinical appearance and prognosis for future performance.

With this in view the following questions were asked:

- What extent of the C3 is possible to evaluate in the DPr-DDiO or "skyline" projection of the distal carpal bones, and how does variation in beam-cassette angle influence the result?

- How does grade of sclerosis relate to histomorphometric bone volume density?

- Do findings of sclerosis or radiolucency of the C3 relate to degree of lameness and length of convalescence in lame Standardbred trotters?

- At what age, or stage of training, does sclerosis develop in the C3 and is the development of sclerosis of the C3 indicative of previous carpal trauma and lameness or of specific levels of performance?

Comments on materials and methods

Details of the methods used in the thesis are presented in the separate papers. This section presents an overview and comments on the methods employed.

Study design

Methodological evaluation was performed in two studies. The influence of beam-cassette angle on radiographic appearance and depth of evaluated C3 was investigated in one experimental study (I). Radiopacity estimations and histomorphometric bone volume density measurements were compared in one observational study (II). Clinical significance of radiographic signs of altered bone density was evaluated in two observational studies. One retrospective, cross-sectional study (III) was performed to investigate the associations between altered radiopacity and degree of lameness and length of convalescence in lame clinical cases and one prospective, longitudinal study (IV) was performed to investigate development of increased radiopacity in relation to previous carpal lameness and performance. In the prospective study examinations were performed at the mean ages of 20, 23, 26, 30, 34 and 42 months.

Horses

For the evaluation of different beam-cassette angles for the DPr-DDiO projection (I), limbs from mature horses of various sizes and breeds were examined to ensure general applicability of the results. The other investigations concerning sclerosis and decreased radiopacity, were carried out on Standardbred trotters or on specimens taken from the same breed.

This thesis is based on four materials.

Study I
Ten limbs from 5 horses with clinically normal carpi and three limbs from 2 Standardbred trotters diagnosed with C3 sclerosis, were collected at post mortem in 1998 from adult horses (6-19 years) euthanised for reasons unrelated to the carpus. The limbs were severed at the cubital joint immediately after death. They were flexed at the carpus, the antebrachium resting on the metacarpus from its own weight, and frozen in that position for radiographic examination. When compared, carpal flexion in the experimental and clinical situation was similar.

Study II
Thirty-five carpal joints from 20 Standardbred trotters, euthanised for non-orthopaedic reasons during 1991 were examined radiographically and the distal rows of carpal bones were collected at post mortem. The horses were 1-7 years of age and all horses older than 1 year (n =16) were either training or racing.

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Study III
Radiographs and case records from 89 Standardbred trotters, 2-7 years of age, diagnosed with traumatic carpitis in absence of fractures and severe degenerative joint disease at the University Veterinary Hospital SLU, Uppsala during 1991-1994 were reviewed. Lameness of the fore limb had been completely blocked after intra-articular anaesthesia of the middle carpal joint. In 38 of the cases a unilateral carpitis was the only source of lameness.

Study IV
All one-year-old Standardbred trotters (11 colts and 11 fillies) which started training at a professional training camp in 1994-95 were examined. The horses were kept under the same management conditions and were trained according to the same protocol. Eight horses (3 colts and 5 fillies) were lost from the study, 1 horse died and 7 were sold or transferred to other trainers. At the end of the study, the 14 remaining horses were either training or racing. Six of the horses raced before the end of the study as 3-year-olds and 5 horses began racing as 4-year-olds. Three horses were regarded as poor performers and their training was discontinued soon after the final examination.

Recordings of demographic, performance and clinical parameters

Data on age, breed and gender were obtained from case records or from the register of the Swedish Trotting Association (STC). Racing records and total earnings were obtained from the register of the STC. Lameness was evaluated by trotting the horse in hand on a hard surface 25-30 m straight away and towards the examiner. Lameness at presentation was graded subjectively on a scale from 0 to 5 where grade 0 was not lame and grade 5 was not weight-bearing. Lameness after flexion test was evaluated after one minute of flexion of the carpus before trotting using the same scale as for lameness at presentation. In the retrospective study (III), lameness and treatment data were obtained from case records, in the prospective study (IV), all lameness examinations and treatments were performed by the same clinician.

Radiographic examination

An ORBIX turnable X-ray tube arm (Siemens-Elema AB, Stockholm, Sweden, maximum focus-film distance 100 cm) with a Siemens Biangulix 125/20/40-100 tube was used for the evaluation of beam-cassette angles for the DPr-DDiO projection (I) and a ceiling mounted Siemens Biangulix 150/30/101R tube for all other radiographic examinations. Both tubes were used in combination with a Siemens Pandoros Optimatic, 200 kV, 3-phase generator (Siemens-Elema AB, Stockholm, Sweden).
Examinations of the distal row of carpal bones in the DPr-DDiO projection and in proximodistal projection were done using high detail intensifying screens (Fuji HR Mammo fine) and mammography film (Fuji UM-MA HC). The screen and

film combination used for all other projections were regular screens (Fuji FG-8) and high-resolution film (Fuji HR-L 30), (Fuji Photo Co., Ltd., Tokyo, Japan).

All third carpal bones were evaluated in the D35°Pr-DDiO projection of the distal carpal bones. In study I beam-cassette angles from 15° to 45° were compared and in study II, D35°Pr-DDiO radiographs were compared to specimen radiographs in proximodistal projection. Radiographic examinations of lame carpi examined in the D35°Pr-DDiO projection of the distal row of carpal bones (III), included a minimum of 3 standard projections (LM, D60°L-PaMO, Pa60°L-DMO). In the prospective study (IV) an initial radiographic examination including 3 standard projections (DPa, LM, D60°L-PaMO) was performed. For repeated examinations only the D60°L-PaMO and D35°Pr-DDiO projections were used unless there were clinical or scintigraphic findings associated with the carpal area, when an extended radiographic examination was performed. The dorsal and medial aspects of the radiocarpal and middle carpal joints have been indicated as areas of high loading and as predilection sites for stress related trauma (Bramlage et al. 1988, Palmer et al. 1994). These aspects of the carpus are also regarded as the primary location for radiographic signs of remodelling and degenerative joint disease (Butler et al. 2000). Based on this and for reasons of cost and radiation safety, most of the repeated examinations in the study of Standardbred trotters in training (IV) were performed only in two projections.

Radiographic evaluation

Radiographs in DPa, LM, D60°L-PaMO and Pa60°L-DMO projections were evaluated for signs of carpal degenerative joint disease (joint capsule distension, periarticular swelling, periarticular osteophytes, rounding of articular margins and subchondral sclerosis or lucent zones (Butler et al. 2000)). DPr-DDiO and proximodistal radiographs were evaluated for depth of proximal articular surface examined (I), radiopacity, trabecular thickening and clarity of trabeculation of the radial fossa of the C3. Sclerosis was graded in four classes: none, mild, moderate and severe sclerosis, according to criteria modified from O'Brien et al. (1985) (Table 1). Radiolucency was graded as presence of radiolucent zones $\geq$ 2 mm (lateromedially) (II) or according to lateromedial width (III).

Scintigraphic examination

Bone phase skeletal scintigraphy (IV) was performed 2 hours after i.v. administration of 8-10 MBq/kg b.w. 99Tc-HDP (HDP, Mallickrodt Medical B.V. Petten, Holland). Static 150k (lateral) and 200k (dorsal) count images of the carpal area were acquired in a 128x128x16 matrix, using a gamma camera with a low energy, all purpose collimator (Picker SX 300, 37x37 cm, Picker International Inc., Cleveland, Ohio 44143, USA). Dorsal images included both carpal areas.

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Table 1: The four grades of sclerosis
0 - No sclerosis: trabecular bone of uniform opacity, no loss of trabecular structure or definition between radioopaque margin and trabecular bone.
1 - Mild sclerosis: distinct focal increase in trabecular opacity with focal thickening of trabeculation but no loss of trabecular structure or definition between radioopaque margin and trabecular bone.
2 - Moderate sclerosis: focal loss of trabecular structure, indistinct delineation between radioopaque margin and trabecular bone.
3 - Severe sclerosis: loss of trabecular structure and definition between radioopaque margin and trabecular bone.

Scintigraphic evaluation

Scintigrams were evaluated using a commercial software package (Hermes, NUD, Nuclear Diagnostics, Hägersten, Stockholm, Sweden). Assessment of radiopharmaceutical uptake in the carpus were made in dorsal images. Subjective grading and ranking of increased radiopharmaceutical uptake in the C3 area was performed in a blue-green-red (BGR) colour scale after filtering the scintigrams with a medium resolution Metz filter. Objective evaluation of increased radiopharmaceutical uptake in the C3 area was performed using region-of-interest (ROI) analysis. ROI's were drawn in filtered images and pixel counts calculated in reset images. Filtering has been shown to significantly increase sensitivity with almost constant specificity in visual detection of increased radiopharmaceutical uptake in equine skeletal scintigraphy using the BGR colour scale (Eksell 2000).

Third carpal bone specimen collection and preparation

Third carpal bones were removed immediately after death (II). The bones were macroscopically examined, radiographed and the proximal articular surface was photographed. The bones were cut in oblique sagittal slabs perpendicular to the dorsomedial surface through the radial and intermediate fossas and the slabs were immersed in buffered formalin. The fixed bone slabs were radiographed and then decalcified in formic acid.

Histomorphometry

Histomorphometric studies were performed on 6-μm thick paraffin sections taken from the C3 slab section with the highest radiographic opacity in the radial fossa and from the slab section in the middle of the intermediate fossa. The volume densities of trabecular bone tissue and bone marrow was determined by point counting (Weibel 1979, Elias and Hyde 1980) at a final magnification of 100x. The point counting procedure was performed by superimposing a grid of 11x11 intersecting lines over the screen image of every 6-mm diameter microscopic field studied. The number of intersections on the grid (test points, n $\approx$ 100) overlying bone and marrow tissue was then counted, and the ratio of the number of these points to the total number was considered to be the volume density of that component. Three fields were examined from each of three proximodistal

levels of the oblique sagittal sections. Volume densities were calculated for each field, for each level and as a mean for the whole section.

Statistical methods

Data were treated using the JMP program (JMP 3.0.2 (II, III), JMP 3.2 (I, IV), SAS Institute Inc., Cary, North Carolina, USA) and analysed statistically using the same program or using manual methods (Kappa (II), Friedman analysis of variance (IV)). Grouped continuous data having a normal distribution (I, II) were compared using ANOVA and further evaluated using a Tukey Kramer HSD test while symmetrically distributed continuous data lacking a normal distribution were analysed using Wilcoxon signed rank tests (IV). Predictors influencing clinical and prognostic data in lame horses (III) were evaluated using multivariate ordinal or nominal logistic regression or multiple linear regression. Repeated measurements (IV) were compared over time using a Friedman two-way analysis of variance (Lehman and D'Abrera 1975) and further evaluated using a multiple comparison procedure suggested by Sprent (1993). Dichotomized clinical data (IV) were compared for each time of examination using contingency table analysis. Gradings of sclerosis in specimen and clinical radiographs (II) were compared using weighted Kappa (Altman 1991).

Results and discussion

Beam-cassette angle and the DPr-DDiO projection (I)

The depth of the dorsal C3 free of superimposing structures measured in DPr-DDiO radiographs, increased with in average 37% when beam-cassette angles increased from 15° to 40° but variation between horses was large and this increase was not significant. The true dorsopalmar depth of the C3 that was evaluated, compared to measurements in the DPr-DDiO projection, ranged from 27% at 15° to 84% at 40°. The evaluated depth showed a significant ($p \leq 0.05$), linear increase with increasing beam-cassette angle from 15° to 40°. In 9/10 cases, the evaluated dorsopalmar depth continued to increase up to 45° but the degree of increase varied considerably between horses. It has previously been claimed that the DPr-DDiO projection evaluates the dorsal half to one third of the proximal surface of the C3 (Young 1987) which seems to be an optimistic estimate and certainly requires the use of large beam-cassette angles. It must also be emphasised that the present measurements were made on dissected limbs from sound horses, with the extensor muscles relaxed. In a clinical situation with a horse in pain and unwilling to comply with carpal flexion, the maximal beam-cassette angle and hence the evaluated depth of C3 is likely to be smaller (O'Brien 1977).

The majority of DPr-DDiO radiographs made at beam-cassette angles of 15° or 20° had a blurry appearance and lacked distinct trabecular patterning. This was probably due to increasing distortion at smaller angles and to the increasing influence of dorsal surface irregularities as the evaluated portion of the articular surface decreased. Overlapping by the proximal carpal bones was evident in all radiographs made at 45° and to a slight degree in 6/10 radiographs made at 40°. A dorsal radiopaque margin was identified in 8/10 limbs but the angles under which the radiopaque margin was visible varied considerably among the limbs. In spite of the variation in size and breed between the examined horses the radiographic appearance of the C3 was quite consistent which confirmed previous statements (O'Brien 1977). However, the inconsistent finding of a dorsal radiopaque margin indicates that it is not a reliable criterion for a properly positioned DPr-DDiO radiograph as has previously been stated (Butler et al. 2000).

Radiographic examinations of three sclerotic C3 under different beam-cassette angles (I) indicated that the subjective grading of sclerosis did not seem to be affected by variations in beam-cassette angle between 25° and 40°. For angles < 25° and > 40°, distortion and superimposition interfered with the evaluation. A limited influence of variation in beam-cassette angle on grading of sclerosis is also supported by the good agreement found between subjective assessment of sclerosis in the D35°Pr-DDiO projection and in proximo-distal specimen radiographs (II). Although bone density estimations appeared relatively insensitive to variations in beam cassette angle, it must be emphasised that small

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beam-cassette angles of 20°-25° (Blevins and Widmer 1990) substantially reduces the investigated portion of the C3 and increases the risk of incorrect assessments due to distortion.

## Comparison of sclerosis and bone volume density (II)

Histomorphometric bone volume density of the radial fossa appeared to be uniformly distributed in a transversal plane in the proximal, central and distal levels of the third carpal bone. However, considerable differences were seen between the different levels. Subchondral cancellous bone was more compact compared to central cancellous bone. Previous investigations in Thoroughbred racehorses have shown a dorsomedial front of increased bone volume density 5-10 mm from the dorsal edge of the radial fossa, in the central level of C3 from lame horses (Young et al. 1991) and for pooled proximo-distal measurements from training, non-lame horses (Young 1987). A similar gradient in bone volume density was not seen in Standardbred racehorses in the present study, which might indicate an influence of breed or gait on the intrinsic C3 anatomy. Bone volume density estimations in study II were based on one oblique sagittal cross section through the radial fossa while the previous two studies investigated larger areas. This may have limited the ability of the present study to discern dorsal to palmar bone volume density variation but a DM-PaL cross section, transecting the previously indicated area of maximum density was chosen to minimize this risk. In all three proximo-distal levels of the C3, radial fossae graded as sclerotic in the D35°Pr-DDiO projection had significantly higher bone volume density compared to non-sclerotic radial fossae. In the central level, bone volume density continued to increase significantly with increased sclerosis whereas the volume density of the more compact proximal and distal levels appeared to have reached a maximum at the onset of sclerosis and did not increase any further (Fig. 4). Consequently, it appears that the examined parts of all three proximo-distal levels contributed to distinguish sclerotic from non-sclerotic C3. On the other hand, perceived differences in radiographic opacity between sclerosis graded 1-3 were explained by volume density variations only at the central level. Accordingly, beam-cassette angle influenced the decision of sclerosis vs. no sclerosis less than the grading of sclerosis as 1, 2 or 3 (II).

Clinical importance of altered radiopacity in lame Standardbred trotters (III)

In lame Standardbred trotters, no significant relationships between radiographic findings of C3 sclerosis and degree of lameness, or prognosis for racing, were found. The multifactorial origin of carpal lameness and the limited numbers of horses, particularly horses with severe sclerosis, resulted in low power of analysis but no significant relationships were found even at the $p = 0.25$ level. However, horses with areas of decreased radiopacity (ADR) ≥ 2 mm had significantly higher lameness scores at presentation, and findings of submarginal ADR ≥ 2 mm

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entailed significantly longer time to start (3 months) although chances of racing within 30 months were not affected.

Figure 4: Mean bone volume density ($\pm$1SD) in central cancellous bone (filled circle) and in proximal and distal subchondral bone (filled square) versus grade of sclerosis in the D35°Pr-DDiO projection in 35 C3 (II).
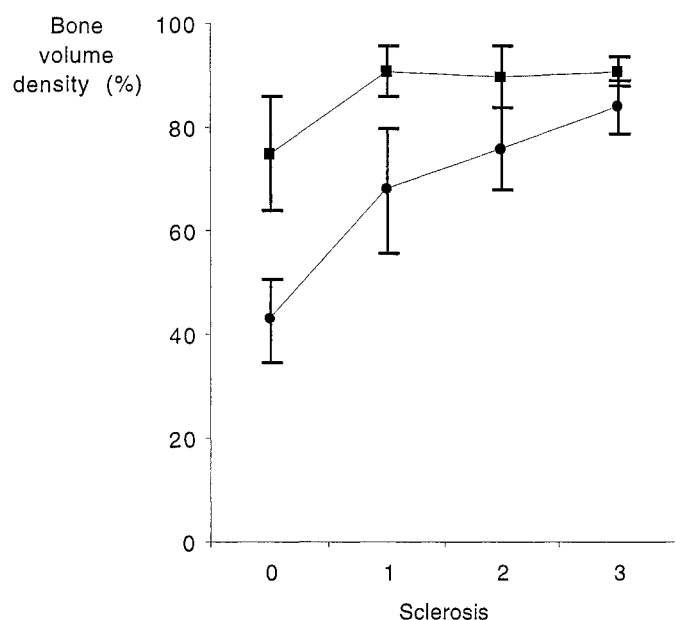
Both sclerosis and areas of decreased radiopacity were prevalent findings in the present material. There was a strong association between patent sclerosis and ADR $\geq$ 2 mm. In moderately to severely sclerotic C3, 59% had ADR $\geq$ 2 mm. Areas of decreased radiopacity $\geq$ 2 mm were only found in combination with subchondral sclerosis while ADR < 2 mm were found in both sclerotic and non sclerotic C3. Dorsal vascular channels have been found in 90% of C3 from racing and training Thoroughbreds (Young et al. 1988). These are usually identified approximately 1 cm from the dorsal margin of the C3 in DPr-DDiO radiographs (O'Brien et al. 1985). In the present material, no association between ADR < 2 mm and lameness scores was found and most ADR < 2 mm were considered to represent vascular channels. The results of the present study, showed that ADR $\geq$ 2 mm significantly related to lameness scores at presentation but not to lameness after flexion test. This concurred with a previous report where 13 Standardbred racehorses with radiolucency of the C3 had chronic mild lameness that acutely exacerbated after training or racing, and minimal or inconsistent response to carpal flexion (Ross et al. 1989).

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In young Thoroughbred racehorses, moderate to severe C3 sclerosis has been suggested to represent a clinical problem and to precede carpal fracture while none or mild sclerosis has been considered normal (O'Brien et al. 1985, DeHaan et al. 1987). However, the low prevalence of patent sclerosis and osteolysis have resulted in small study materials and the ensuing low power of analysis has led to weak inferences, particularly from negative findings. Small numbers of severely sclerotic C3 precluded definite conclusions about this condition in a previous study (Young 1987). In this study of lame clinical cases, a selection for increased numbers of severely sclerotic C3 would have been expected had severe sclerosis been a risk factor for carpal lameness. In the present study, the prevalence of severe sclerosis was identical to the prevalence (6%) found in the previous study of Thoroughbreds presumed free of carpal lameness (Young 1987). Consequently, the results in this study do not support the idea that patent sclerosis indicates a clinical problem in Standardbred trotters even though a significantly larger mean area of cartilage erosion was seen with sclerosis in study II. However, the association between moderate and severe sclerosis and ADR ≥ 2 mm could account for a presumed association between sclerosis and clinical carpal problems particularly since cartilage erosions have been reported always to accompany C3 radiolucencies (Ross et al. 1989, study II).

Factors affecting development of sclerosis (IV)

For economical and management reasons, the longitudinal investigation of factors affecting development of C3 sclerosis (IV) was based on 22 horses from one training camp. The camp was of medium size, subjectively judged as typical of training facilities in Sweden and having a proportion of horses racing as 3-year-olds, similar to the population mean. The use of privately owned horses resulted in a loss of horses from the study. However, the recorded radiographic, scintigraphic and performance data indicated no significant differences between horses lost from the study and the remaining horses and there was no indication that the material was biased through the loss of horses.

In the investigated group of horses, the development of C3 sclerosis was associated primarily with the period of intense training and racing. The majority of horses (18/22) were free from C3 sclerosis until the beginning of speed training (18-22 months of age). During the subsequent training period, level of sclerosis in the investigated group increased continuously over time (Fig. 5). As three-year-olds, the majority of the investigated horses (12/14) had developed unilateral or bilateral C3 sclerosis. Radiolucencies ≥ 2 mm were not present. In post mortem material (II), radiographic signs of sclerosis were only found in C3 from racing or training horses, whereas the majority of non-sclerotic C3 originated from untrained horses ≤ 2 years of age. These findings in Standardbred trotters are supported by histomorphometrical studies in Thoroughbreds indicating that low intensity exercise is an insufficient stimulus for increased C3 bone density (Firth

et al. 1999), and that localised hypertrophy of subchondral bone follows training and racing (Young 1987, Young et al. 1991).

Figure 5: Level of C3 sclerosis over time in 14 Standardbred trotters (IV), illustrated as the summed individual rankings of sclerosis for each examination, for left (Scl-L) and right (Scl-R) limbs.
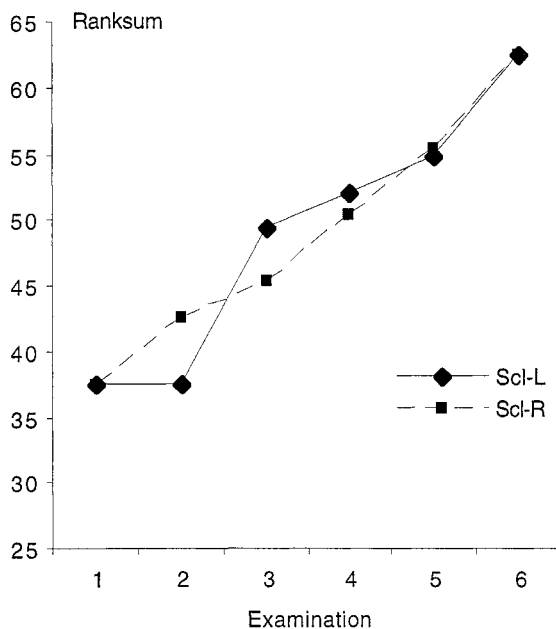
In most cases radiographic signs of C3 sclerosis developed in absence of carpal lameness even when increasing from none to moderate in 3 months (IV). Horses diagnosed with C3 sclerosis included both horses qualified for racing and unqualified horses. Prevalence of sclerosis was similar in the two groups (6/8 compared to 4/6). At the final examination, grade of sclerosis or increased grade of sclerosis compared to previous examination was not significantly associated with having qualified for racing, having raced, or number of starts but increased grade of sclerosis related significantly to carpal lameness since previous examination (p = 0.045). Mean scintigraphic ratios were significantly (p = 0.03) higher for limbs where C3 sclerosis had increased since previous examination. At the first examination, presence of sclerosis was significantly associated (p = 0.005) with increased radiopharmaceutical uptake (IRU) in the C3 region. The first examination was performed in the first month of speed training and some of the horses had 1-2 weeks of intense training prior to the first examination. The strong association between presence of sclerosis and IRU indicated that most of the sclerosis represented active processes and may have developed with the

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beginning of speed training. At the examinations following the first, no significant associations were found between presence of sclerosis and IRU.

Mean objective scintigraphic ratios and prevalence of subjective IRU was higher in right limbs at every examination although differences were only significant ($p < 0.03$) for objective scintigraphic ratios at examinations 1-3. This consistent difference between right and left limbs may have been related to predominantly unidirectional training and racing similar to what has been reported for the incidence of C3 fracture in Thoroughbred racehorses (Schneider et al. 1988; Stephens et al. 1988; Martin et al. 1988). In study IV, the higher scintigraphic activity of right carpi was not associated with higher frequency of sclerosis or frequency of lameness. Similarly, performance appeared to influence scintigraphic activity. Horses that had qualified for racing had significantly higher scintigraphic ratios at examination 6.

General discussion

This study was focused on radiographic evaluation of the C3 because it is the commonly available, *in vivo* method for detection of bone changes in horses. In the horse, the use of computed tomography (CT), dual-energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) to evaluate cancellous bone has been reported but high costs and equipment designs unsuited to the needs of horses have so far limited the use mainly to cadaver and specimen investigations.

Radiography is often stated to be imprecise in the assessment of bone mass or mineral content, requiring changes of approximately 30% in bone mineral content (Butler et al. 2000) or a 60% reduction of cancellous bone thickness (Edelstyn et al. 1967) for visual evaluation. This would imply that considerable changes in bone volume density could have taken place in the third carpal bone without this being evident in the DPr-DDiO radiograph. This is particularly relevant since skeletal anatomy limits radiographic evaluation of C3 subchondral bone density to the dorsoproximal part of the bone. Furthermore radiography will underestimate the volume fraction of newly formed bone due to its lower mineral content (Riggs and Boyde 1999). Under certain conditions, the accuracy of density estimations from radiographs can be increased considerably using photodensitometry when the optical densities of a bone and of a known thickness of a standard material are compared in a radiograph (Meakim et al. 1981). But for the DPr-DDiO projection, oblique angles of evaluation and resulting image distortion in combination with inadvertent variability in beam-cassette angle make photodensitometry unsuitable. The present results indicate that the DPr-DDiO projection may perform better than radiography in general and that bone volume density changes of 10-15% may be evaluated (II). The evaluation of bone volume density in the C3 is made on small quantities of cancellous bone without skeletal superimposition and the assessment is based on comparison of regional variation in opacity within the C3 and between the C3 and the second and fourth carpal bones. This comparative estimation appears more precise and relatively insensitive to variations in beam-cassette angle.

Third carpal bone sclerosis is described in racehorses (Boring 1998, Butler et al. 2000). In this study, increased C3 radiopacity primarily developed during speed training and racing. This agrees with previous studies in Thoroughbreds showing increased bone volume density or bone mineral density following training, racing, and intense treadmill exercise (Young 1987, Young et al. 1991, Firth et al. 1999). Although an age related increase in bone volume density has been reported in untrained Thoroughbreds (Young et al. 1991), this increase was apparently insignificant compared to exercise induced changes. This indicates that increased C3 radiopacity is not an inherent breed characteristic of Standardbred and Thoroughbred racehorses, but a result of conditions imposed during training and

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racing. In this study, no association between C3 sclerosis and degree of lameness or prognosis for racing was found in lame horses. The association between carpal lameness and increase in C3 radiopacity (IV) shows that to describe C3 sclerosis exclusively as a sign of normal adaptation (Pool 1996) is incorrect. Findings of appositional growth of woven bone in sclerotic areas of C3 from training and racing Thoroughbreds (Young 1987) also indicate that increased C3 radiopacity may represent a reparative response rather than physiological adaptation. It has previously been found that an osteogenic response is more readily generated by a variety of different loading conditions than by increased repetitions and that bone may be prone to respond to accidental loading (Burr and Martin 1992). This, in combination with the finding that increased C3 radiopacity often developed unilaterally (IV), suggests that increased C3 radiopacity may result from random events during exercise rather than representing adaptation of the C3, necessary for the bone to withstand loading at high speeds. Furthermore, the observation that mild and moderate sclerosis primarily reflects bone volume density changes in the central cancellous bone (II) raises questions as to the relevance of grade of sclerosis as an indicator of C3 mechanical properties with respect to articular cartilage. Experimental studies in dogs indicate that only bony change within 3 mm of the osteochondral junction affects overlying cartilage (Burr and Schaffler 1997). These observations, together with the lack of trends between specific levels of performance and sclerosis seen in study IV, indicate that radiographic examination of the C3 will be of little use in the development of training protocols. This also means that in spite of over twenty years of controversy over the clinical significance of equine C3 sclerosis still no evidence of a detrimental influence on clinical status or performance has been demonstrated. As a consequence, until more extensive studies have proven otherwise, ascribing clinical significance to sclerosis of the third carpal bone should be avoided.

In the present study, radiolucencies ≥ 2 mm, were significantly associated both with higher degree of lameness and longer convalescence. Previous studies have indicated associations between C3 radiolucencies and lameness (Ross et al. 1989) and development of C3 fracture (Bramlage et al. 1988, Pool and Meagher 1990, Pool 1996). No studies investigating the development of C3 radiolucencies have been published although underlying mechanisms have been suggested (Young 1987, Bramlage et al. 1988, Ross et al. 1989, Pool 1996). These authors assumed that chronic cyclic loading of the C3 may cause fatigue damage, microfracturing and necrosis of the subchondral bone. Osteolysis ensues in a situation when continuous damage from cyclic loading exceeds the reparative response of the bone. Ross et al. (1989) also suggested that radiolucencies might result from acute osteochondral collapse or acute fracture of the proximal articular surface. Development of radiolucencies was not documented in the prospective study (IV). This was probably due to the size of the study in combination with low incidence and to the supposedly late appearance of the condition (mean age 4.1 years, Ross et al. 1989), (range 4-7 years, study II). The low incidence and late appearance make longitudinal studies of radiolucencies cumbersome and costly,

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limiting research to retrospective investigations. The small portion of the C3 accessible for radiographic investigation and the image distortion resulting from oblique beam-cassette angles have precluded detailed non-invasive investigations of the appearance of radiolucencies. Radiolucencies or subchondral lucencies of the C3 have generally been described as focal areas of necrotic bone and bone debris (Ross et al. 1989, Pool 1996) associated with sclerosis and cartilage erosion (Ross et al. 1989, study II). Findings of punctate (<1 mm diameter) cartilage depressions associated with collapsed subchondral bone in absence of documented bone necrosis has also been suggested to represent subchondral lucencies (Little et al. 1997). Our clinical records show that Standardbred trotters that develop C3 radiolucency sometimes continue to race successfully after convalescence, in spite of remaining lucencies. At present little has been published about the repair process of C3 lucencies and the radiographic appearance of lucencies undergoing repair.

For practical and economical reasons, radiography is likely to remain the principal imaging method for clinical examinations of lame horses with suspected carpal fractures or degenerative joint disease. In clinical practice, scintigraphy provides a valuable complement to the radiographic evaluation of the carpus. Scintigraphy is a sensitive technique for early detection of increased bone metabolism (Devous and Twardock 1984, Chambers et al. 1995). Unfortunately, scintigraphy has poor spatial resolution and is unable to differentiate between bone formation and bone resorption (Francis and Fogelman 1987). However, to further improve our diagnostic abilities by refining upon assessments of carpal bone density and three-dimensional structure, there is a need for high resolution, tomographic techniques. The use of CT and MRI modified to the needs of equine clinical practice, preferably in combination with scintigraphy to assess bone metabolism, could greatly improve our ability to monitor e.g. the processes of development and repair of C3 subchondral lucencies.

Diseases of the equine skeleton due to injury during training and racing are a major welfare problem both to the horses and to the racing industry. Successful preventive measures depend on a reliable assessment of skeletal adaptation relative to the level of exercise. The present results showing that increased C3 radiopacity may represent both adaptive and reparative skeletal responses suggest that evaluation of the C3 based on X-ray techniques is insufficient for this purpose. To differentiate between adaptive and reparative bone responses *in vivo*, markers reflecting the balance between bone formation and resorption must be identified. Biochemical markers of bone turnover measured in equine blood or urine may provide rapid, sensitive and non-invasive montoring of bone formation and resorption but a specific equine serum marker for bone resorption has not yet been identified (Price 1998). Current investigations also show that serum and urine concentrations of bone markers in horses depend on seasonal- (Price 1998) and diurnal (Black et al. 1999) variation as well as age (Lepage et al. 1998, Black et al. 1999) and breed (Lepage et al. 1998). However, in combination with

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techniques providing information about skeletal mass and architecture, biochemical markers of bone metabolism could provide a tool for identifying horses that are at risk of developing carpal injuries. The optimal mass and architecture of the carpal bones to withstand loading at certain levels of exercise have not been determined. The previously discussed shortcomings of the radiographic DPr-DDiO projection in this context means that techniques providing more exact estimations of bone mass and three-dimensional structure will be necessary for this purpose.

There are three important fields of application for the results of this thesis. In the clinical situation, positioning for, and interpretation of C3 radiographs can be improved. In the clinical and legal situations better assessments of clinical importance of radiographic findings can be made for prognostic, treatment and insurance purposes. In exercise physiology, limitations to radiographic assessment of training effects on the subchondral C3 have been outlined and main paths for future research have been suggested.

Conclusions

- Beam-cassette angles near 40° are recommended for maximum depth of evaluated C3 but assessment of sclerosis in the DPr-DDiO projection appears unaffected by moderate angular variation.

- In a group of Standardbred trotters in professional training, the majority of horses developed sclerosis of the C3, primarily during speed training and racing.

- Grading of sclerosis primarily reflects the volume density of central cancellous bone while the more compact subchondral bone has reached its maximum density with mild sclerosis.

- Sclerosis appears to be of limited value as indicator of attained level of performance, clinical carpal disease or future performance in Standardbred trotters.

- Radiolucencies ≥ 2 mm of the C3 are clinically significant findings associated with increased lameness and longer time to start in Standardbred trotters.

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