TOWARDS DETECTION OF FUNCTIONAL FAILURE OF EQUINE ARTICULAR CARTILAGE

THE METACARPOPHALANGEAL JOINT UNDER SCRUTINY

HAROLD BROMMER

UTRECHT – 2005

TOWARDS DETECTION OF FUNCTIONAL FAILURE OF EQUINE ARTICULAR CARTILAGE

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HARMEN BROMMER

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PROMOTORES: Barneveld A, Prof, DVM, PhD
Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Jurvelin JS, Prof, PhD
Department of Clinical Physiology and Nuclear Medicine and Department of Applied Physics,
Kuopio University, Finland

CO-PROMOTORES: Brama PAJ, DVM, PhD
Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Van Weeren PR, DVM, PhD
Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

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***Aan* Mirjam en Wiemer**

***Voor* mijn vader en moeder**

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PRINCIPLES RELATED TO FUNCTION AND FAILURE OF ARTICULAR CARTILAGE

- GENERAL INTRODUCTION -

INTRODUCTION

Lameness is the most common cause of impaired athletic performance in the horse (Olivier *et al.* 1997, Rossdale *et al.* 1985, Todhunter and Lust 1990). Among all disorders of the musculoskeletal system, joint injuries and joint diseases are most frequently encountered and represent a major part of the caseload for equine clinicians (Pool 1996, Todhunter and Lust 1992). Within the joint, damage to the articular cartilage is most critical as this highly specialized tissue has a very limited repair capability. The limited potential of articular cartilage for regeneration and healing has been appreciated for over 2 centuries. In 1743, the British surgeon and anatomist William Hunter stated: "To the present age, it is universally allowed that ulcerated cartilage is a troublesome thing and that, when once destroyed, it is not repaired". Therefore, maintenance of healthy cartilage is of major concern.

Given the fact that articular cartilage is a functional tissue *par excellence*, cartilage is considered to be healthy when it is not impaired in the execution of its key function: transmission and absorption of applied forces and, together with the synovial fluid, provision of low-friction articulation during locomotion and performance (Buckwalter and Martin 1995, Hasler *et al.* 1999, Hayes *et al.* 2001, Mow *et al.* 1990, Todhunter 1996). Therefore, management of joint injuries and joint diseases is basically focussed on the maintenance and possible restoration of the specific biomechanical properties of the articular cartilage (Palmer and Bertone 1996, Todhunter 1996). In recent years much effort has been dedicated to developing methods for early detection of joint disorders, such as identification of biomarkers (McIlwraith 2004, Ray *et al.* 1996, Van den Boom 2004) and development and validation of magnetic resonance imaging techniques (Burstein *et al.* 2000, Nieminen 2002). However, assessment of the biomechanical characteristics of articular cartilage is the most direct, and potentially the most powerful diagnostic modality for evaluation of the functional status of the tissue, and hence for prognostication of joint disease (Laasanen 2003, Lyyra 1997). In this introductory chapter, functionality of articular cartilage is the central theme and loading and biomechanical characteristics (*i.e.* the capacity to sustain load) are the key words. First, the constituents and structure of articular cartilage in the context of their relationships with cartilage biomechanics under physiological and pathophysiological conditions are discussed. Then, a synopsis is given of the role of joint loading in the physiological maintenance and deterioration of articular cartilage. Finally, the techniques how to measure cartilage biomechanical properties are reviewed, and some information on the equine metacarpophalangeal joint and the susceptibility of this joint to injury is given in

order to provide a basis for understanding of the experimental work that is reported in the following chapters of this thesis, and of which a brief outline is given at the end of this introduction.

CONSTITUENTS AND STRUCTURE OF ARTICULAR CARTILAGE

Articular cartilage is a thin layer of avascular, aneural, and alymphatic tissue that forms the smooth gliding surface at the end of the articulating bones in synovial joints (Hayes *et al*. 2001, Todhunter 1996) (figure 1). The tissue consists of 3 main components: a limited number of chondrocytes (< 5%), the extracellular matrix (20 - 30%), made up mainly of proteoglycans (5 - 10% on dry weight basis) and collagens (50 - 80% on dry weight basis), and interstitial water (60 - 80%) (Brama *et al*. 2000a and 2000b, Hayes *et al*. 2001, Johnson *et al*. 1980, Mow *et al*. 1990, Todhunter 1996, Vachon *et al*. 1990). These constituents have a zonal variation in contents and structure throughout the cartilage layer (Hasler *et al*. 1999, Mow *et al*. 1990) (figure 2).

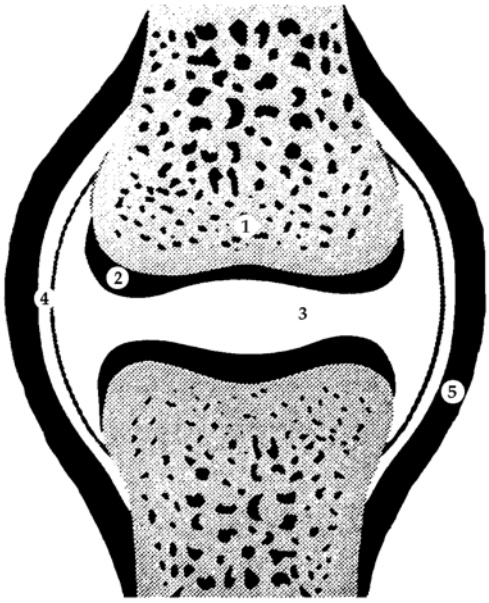

FIGURE 1: *Schematic representation of a synovial joint: 1) bone, 2) articular cartilage, 3) synovial fluid, 4) joint capsule with synovial membrane, 5) collateral ligament.*

CHONDROCYTES

Chondrocytes, although relatively low in number, are specialized cells and their homeostatic activity coordinates and controls the rate of synthesis and deposition of matrix components with the rate of degradation and removal (Maroudas 1975, Muir 1995, Stockwell and Meachim 1973). The cells are most numerous in the superficial zone, where they are small and flattened in shape. In the intermediate zone chondrocytes are moderate in size with an oval or rounded shape. The deep zone is occupied by large rounded cells arranged in vertical columns (Collins and McElliot 1960, Stockwell and Meachim 1973).

PROTEOGLYCANS

Proteoglycans are made up of monomers, which are linked to a hyaluronan backbone by a link protein, forming large aggregates (Muir 1972, Todhunter 1996). A proteoglycan monomer consists of numerous repeating units of glycosaminoglycans, of which chondroitin sulphate is most common, linked to a protein core (Franzen *et al*. 1981, Paulsson *et al*. 1987, Perin *et al*. 1987, Todhunter 1996). The main proteoglycan is aggrecan, but there exist various other proteoglycans in small amounts (Fife and Brandt 1993, Heinegard and Oldberg 1989, Paulsson *et al.* 1987). The concentration is lowest at the articular surface and increases towards deeper parts of the tissue (Kiviranta *et al*. 1985, Maroudas *et al*. 1980). The negatively charged glycosaminoglycans attract water and cations into the tissue, creating an osmotic pressure gradient in the cartilage, which causes cartilage to swell (Maroudas and Bannon 1981, Maroudas and Urban 1980, Maroudas *et al.* 1985 and 1991, Urban *et al*. 1979).

COLLAGENS

The collagens are mostly of type II and are organized in a fibrillar network (Eyre and Wu 1995, Hayes *et al*. 2001, Mow *et al*. 1984, Todhunter 1996). The basic element in collagen is a coiled structure composed of 3 polypeptide α-chains, forming a triple helix. These 'building blocks' are crosslinked in order to form fibrils together with other macromolecules (Eyre and Wu 1995, Todhunter 1996). The collagen fibres restrict the swelling tendency of the proteoglycans and, hence, induce tensile strains in the collagen network (Basser *et al*. 1998, Maroudas 1976, Maroudas *et al*. 1992a, Mizrahi *et al*. 1986). At different depths of cartilage, the collagen fibrils show a varying spatial arrangement (Benninghoff 1925, Clark 1990, Redler 1974). In the superficial zone, the collagen fibrils are arranged parallel to the articular surface and begin to arcade towards the intermediate zone (5 - 15% of the

total thickness). In the intermediate zone, the fibrils have turned to form a randomly organized meshwork (1 - 15% of the total thickness). In the deep zone, the fibrils become radially arranged and are anchored to the calcified cartilage at the cartilage-bone interface (70 - 90% of the total thickness) (Benninghoff 1925, Clark 1990, Redler 1974). The relative contribution of collagen to total tissue dry weight decreases from superficial to deep (Ven and Maroudas 1977).

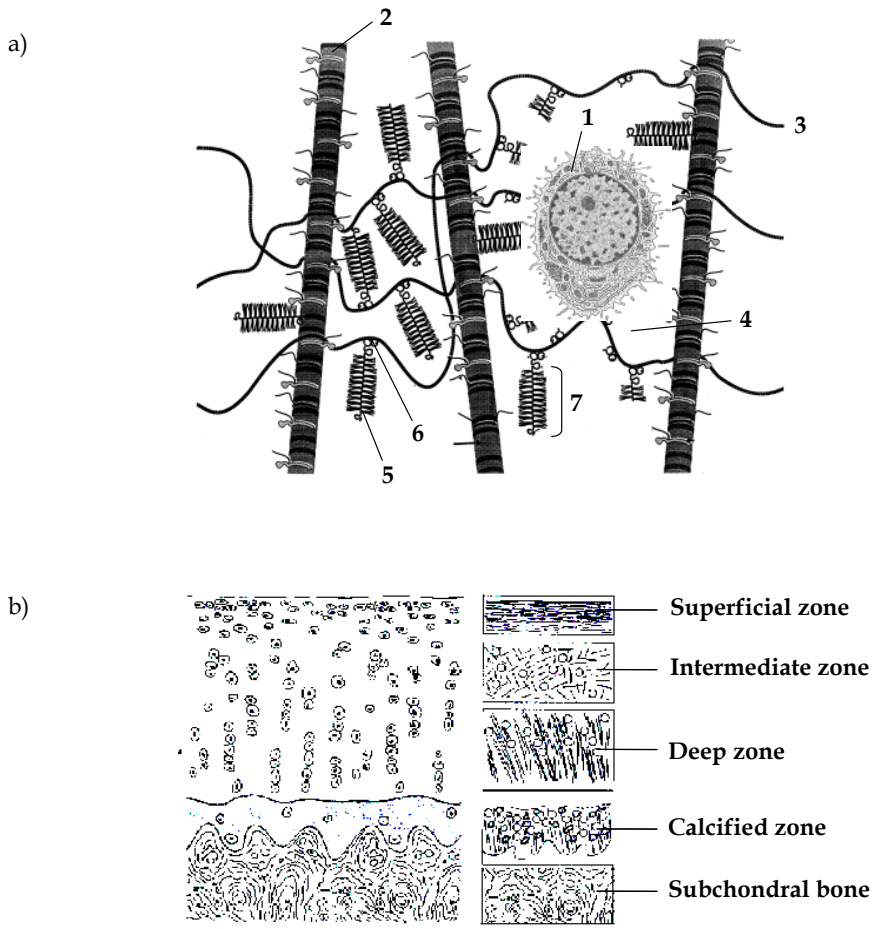

FIGURE 2: *a) Schematic representation of the 3-dimensional arrangement of the constituents of articular cartilage. 1) chondrocyte, 2) collagen fibril, 3) hyaluronan, 4) interstitial fluid, 5) glycosaminoglycan unit, 6) link protein, 7) proteoglycan monomer. b) Cartilage tissue consists of different zones in which structure, composition, and mechanical properties differ.*

INTERSTITIAL WATER

The space surrounding the macromolecules and chondrocytes is filled with water, in which electrolytes, anabolites and catabolites are dissolved (figure 2). About 30% of water is trapped in the intrafibrillar space within the collagen fibrils, while the remainder exists in the solution formed by water and proteoglycans (Mow *et al*. 1991). The interstitial fluid plays an important role in chondrocyte metabolism, as it is a transport medium for new matrix components and waste products and, thus, it has a function in maintaining the integrity of the articular cartilage (Maroudas 1985, Mow *et al.* 1984). The water content is highest in the superficial zone and decreases towards the deep tissue (Lemperg *et al*. 1971).

INTERACTION BETWEEN CONSTITUENTS

The collagen network and proteoglycans in articular cartilage are functionally interdependent. Molecular interactions occur via collagen-collagen, proteoglycan-collagen and proteoglycan-proteoglycan interactions (Broom and Poole 1983, Mow *et al*. 1991). Covalent collagen-collagen crosslinking is essential in providing a strong and stiff collagen network (Mow *et al*. 1992). The physical interaction between collagen and proteoglycans arises from electrostatic forces, in which negatively charged groups of glycosaminoglycans interact with positively charged groups in the collagen, or is due to the strong frictional interaction between proteoglycans and the collagen network when the swelling pressure of the proteoglycans is counterbalanced by the collagen framework (Mow *et al.* 1992, Schmidt *et al*. 1990). The proteoglycan-proteoglycan interaction arises from the repulsive forces between the negatively charged glycosaminoglycans (Mow *et al*. 1992).

PHYSIOLOGY OF CARTILAGE BIOMECHANICS: STRUCTURE - FUNCTION RELATIONSHIPS

The ability of articular cartilage to perform its physiological function under different types of loading, such as low-level constant loading during weightbearing, intermittent loading during locomotion, and high-impact loading during strenuous training and performance, depends critically on the delicate interaction of the cartilage constituents and the flow of interstitial fluid through the permeable matrix (Hasler *et al*. 1999, Kempson 1980, Mow *et al*. 1984). In unloaded cartilage, the tensile forces in the collagen fibrils are in balance with the osmotic swelling forces of the proteoglycans (Bader *et al*. 1992, Maroudas 1979). During physiological joint loading cartilage deforms, resulting in an increase of contact

areas and in improvement of local joint congruence, and generating compressive, tensile and shear stresses (Mow *et al*. 1990).
The effect of a suddenly applied compressive force can be considered to occur in 2 stages (Bader and Kempson 1994, Comper and Lyons 1993, Mow *et al*. 1990, Myers and Mow 1983). In the first stage, the cartilage responds rapidly by a change of shape at a constant volume. Lateral expansion occurs, which causes a sideways movement of the proteoglycans and water away from the region of pressure. In fact, the loaded area is increased, thereby reducing the applied stress. Lateral expansion is restricted by the network of the collagen fibrils, which experiences an increase in tensile strain. Following the initial response, and continuous with it, water efflux occurs from the region that is under high pressure to the surrounding regions that experience less pressure. The volume of the cartilage decreases and the deformation of the articular surface increases gradually but at a decreasing rate until finally, if sufficient time is allowed, a new equilibrium is reached when the applied force is balanced by the new swelling pressure and the new tensile strains in the collagen fibrils (figure 3).

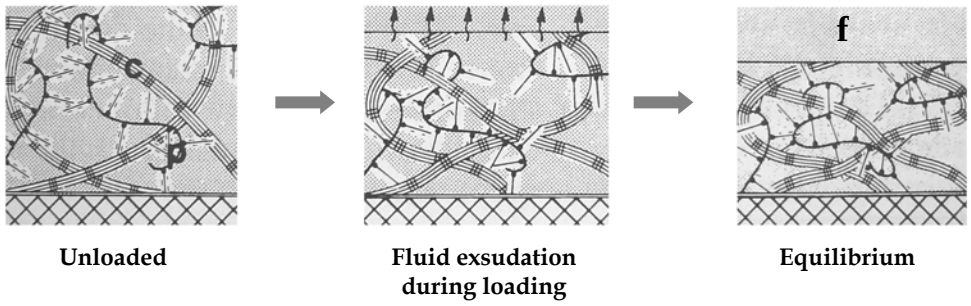

FIGURE 3: *Deformation and fluid exsudation of articular cartilage during compressive loading. c = collagen, p = proteoglycan, f = exsudated fluid.*
(Modified from Myers and Mow 1983)

After the release of loading, relaxation of the strain in the collagen network causes initially a change in shape at a constant volume again. This induces a negative swelling pressure, resulting in an influx of water. The volume of cartilage increases gradually until the original equilibrium between the swelling pressure and the tensile stress of the collagen fibrils is restored.
During normal locomotion, the articular cartilage is dynamically loaded and the compressive forces applied to the cartilage increase and decrease rapidly and

repetitively in a cyclical manner. The response of cartilage to dynamic loading will depend on its short-term viscoelastic properties (Bader and Kempson 1994) and is related with the frequency of loading (Mak *et al.* 1987, Wong *et al.* 2000). At high frequencies of loading, cartilage is assumed to be instantaneously incompressible (Mak *et al.* 1987, Wong *et al.* 2000), which means that in these circumstances there is no outflow of fluid from the cartilage (Maroudas 1973).

The deformation at a constant volume represents the elastic properties of the cartilage and is largely controlled by the characteristics of the collagen network (Bader and Kempson 1994, Bader *et al.* 1992). The collagen network is mainly responsible for the tensile stiffness of articular cartilage (Kempson *et al.* 1968, Mow *et al.* 1990). The deformation that occurs as a result of fluid flow indicates the viscous behaviour and is mainly influenced by the proteoglycans (Mow *et al.* 1980 and 1984). The proteoglycans control the compressive stiffness of articular cartilage (Bader and Kempson 1994, Bader *et al.* 1992, Korhonen *et al.* 2003a, Mow *et al.* 1990). It is difficult to uncouple the elastic and viscous phases in the mechanical response as the elastic response occurs so rapidly and, therefore, the point at which the fluid flow starts is difficult, if not impossible, to define.

The biomechanical properties of articular cartilage are not unidirectional. Mechanical anisotropy is related with the nonhomogeneous distribution and structure of the constituents in the articular cartilage (Bank *et al.* 2000, Laasanen *et al.* 2003, Zheng *et al.* 2001). Associated with the arcade model of the collagen fibrils (Benninghoff 1925), the superficial layer is the stiffest zone of cartilage in tension (Kempson *et al.* 1968). Generally, in an axial direction tensile stiffness decreases and compressive stiffness increases with increasing distance from the surface (Akizuki *et al.* 1986, Guilak *et al.* 1995, Schinagl *et al.* 1997, Wang *et al.* 2002). Apart for the axial anisotropy, there is also anisotropy parallel to the articular surface. This anisotropic conformation is responsible for the fact that cartilage is more strain limiting in tension along the split-line direction, *i.e.* cartilage exhibits greatly increased stiffness with increasing deformation or strain parallel to the fibrils (Kempson 1980, Woo *et al.* 1976).

Collagen integrity is more critical for the maintenance of articular cartilage stiffness than the proteoglycan content (Oakley *et al.* 2004). This is undoubtedly related with large differences in turnover rate of these constituents. Collagen turnover times have been estimated to be 120 - 350 years (Maroudas 1980, Maroudas *et al.* 1992b, Todhunter 1996). This means that collagen is a very static tissue and, once damaged, can not be repaired during the lifespan of the individual. On the contrary, turnover times of the proteoglycans range from 3 - 1800 days and,

therefore, allow a steady renewal of tissue components without disruption of the functional characteristics of the extracellular matrix (Maroudas 1980, Maroudas *et al*. 1992b, Palmer and Bertone 1994, Platt 1996, Todhunter 1996).

PATHOPHYSIOLOGY OF CARTILAGE BIOMECHANICS: DEGENERATION - DYSFUNCTION RELATIONSHIPS

Damage of the collagen meshwork is the decisive initiating factor in the pathophysiology of cartilage biomechanics. Subsequently, a decrease in proteoglycan aggregation will occur as a result of disturbance of the trapping mechanism by the damaged collagen network. Together with an increase in the water content, these changes, which are initiated in the superficial layer of the cartilage, are the earliest regressive symptoms prior to the appearance of macroscopically visible cartilage degeneration (Altman *et al.* 1984, Arokoski *et al.* 2000, Bank *et al*. 2000, Guilak *et al*. 1994, Mankin and Thrasher 1975). However, there are 2 opposing views about the initial phase of cartilage damage in degenerative joint disorders. Several reports claim that, indeed, the disease begins in the articular cartilage (Dedrick *et al.* 1993, Donohue *et al*. 1983, Yamada *et al*. 2002), but there are also studies in which alterations of the subchondral bone precede changes in the articular cartilage (Burr 1998, Carlson *et al*. 1994, Dequeker *et al*. 1995, Radin and Rose 1986, Radin *et al*. 1984 and 1991). The question whether subchondral bone changes occur before articular cartilage deterioration or subsequent to it, has not been resolved (Adolphson *et al*. 1994, Bailey and Mansell 1997, Burr and Schaffler 1997, Muehleman *et al*. 2002, Oegema *et al.* 1997). In fact, these changes are closely intermingled because of the tight functional relationship, as has recently been investigated in the horse (Van der Harst 2005).

Changes in the superficial layer of articular cartilage are associated with a compensatory increase in proteoglycan synthesis in the deeper zones such that there is initial no net loss of proteoglycans from the total cartilage layer (Mankin 1974, Poole *et al*. 1992). Collagen synthesis also appears to be increased in this phase, reflecting an attempt to repair the damage by the chondrocytes in the deeper zones (Fukui *et al*. 2001, Guilak *et al*. 1994, Mankin 1974). The total concentration of collagen remains unchanged, but the composition changes as degenerated cartilage contains elevated levels of degraded collagen molecules (Bank *et al.* 1997, Dodge and Poole 1989, Hollander *et al.* 1994). Degraded collagen molecules remain incorporated in the fibrils because of the crosslinked structure of the collagen network. However, because of the fact that the collagen molecule has suffered cleavage, some of these crosslinks are no longer functional, since the

crosslinks between both ends of the collagen molecule have lost their connection. As a consequence, the depolymerised collagen molecules will slide along each other during tensile loading, which explains why the fibrils stretch more easily under a given load (Bank *et al.* 2000). Therefore, the amount of degraded collagen molecules is strongly related with the decrease in stiffness of the cartilage (Bank *et al*. 2000). As a result of damage to the collagen network and the decline of proteoglycan aggregation, both the tensile and compressive stiffness of the superficial cartilage layer are reduced (Armstrong and Mow 1982, Kempson *et al*. 1971). As the superficial zone is the stiffest zone of cartilage in tension, it plays an important role in the compressive and tensile behaviour of articular cartilage, and substantial changes of the properties in this zone will have a significant effect on the overall mechanics of the tissue (Akizuki *et al.* 1986, Guilak *et al.* 1994, Kempson *et al.* 1968, Setton *et al*. 1993). In this stage, axial compression of the articular cartilage surface will lead to a lateral expansion that is larger than normal in the first stage of the cartilage response (the elastic phase), as there is less tensile restriction from the damaged collagen network. As a consequence, the loaded area will be further increased compared to the normal situation. Adjacent areas will experience abnormally high loads, resulting in larger tensile strains in the collagen meshwork. This elevation of tensile strains could conceivably be sufficiently large to cause mechanical damage to collagen fibres in these adjacent areas (Kempson *et al*. 1971). This will lead to a further reduction of the proteoglycan aggregation and a further increase in swelling of the solid matrix. As a functional consequence, a further reduction in the compressive and tensile stiffness of the cartilage layer will occur (Akizuki *et al.* 1986, Guilak *et al.* 1994, Setton *et al*. 1993) and a vicious circle is initiated. Hence, once any disruption in the collagen framework occurs, the continuation of normal loading can lead to progressive damage and to further loss of mechanical strength with progressive negative consequences for the functionality of the cartilage (Kempson *et al*. 1973).

THE ROLE OF JOINT LOADING IN CARTILAGE BIOMECHANICAL PHYSIOLOGY AND PATHOPHYSIOLOGY

The biomechanical environment of articular cartilage is very critical for both the physiological maintenance and pathophysiological deterioration of the functionality of the tissue (Buckwalter and Mankin 1997). In bone, Wolff's law postulates a direct relationship between the primary stress fields and the corresponding orientation of trabeculae (Wolff 1892). Articular cartilage is also a dynamic tissue, responding actively to changes in loading conditions (Adams and

Brandt 1991, Jurvelin *et al*. 1989, Palmoski *et al*. 1979, Sah *et al*. 1989, Torzilli *et al*. 1997). Accordingly, articular cartilage also obeys Wolff's law to a certain extent (Helminen *et al.* 2000). The stress-strain fields of the extracellular matrix are transmitted across the cell to the genome either by a direct mechanical signal transmission, or by the force-dependent release of chemical second messengers that activate gene transcription, which, in turn, influence the biosynthetic response of the chondrocyte (Guilak *et al*. 1997, Hasler *et al*. 1999, Hering 1999).

Proteoglycan synthesis and metabolism respond much more actively than the collagen metabolism to alterations of joint loading (Arokoski *et al*. 1993, Jurvelin *et al*. 1986, Kiviranta *et al*. 1988, 1992 and 1997, Säämänen *et al*. 1989 and 1994, Vasan 1983). However, the capacity of the adaptive response of articular cartilage depends significantly on the age of the individual as the remodelling and turnover rate of the tissue decrease sharply with increasing age (Bank *et al*. 1998, Maroudas 1980).

At birth, articular cartilage is immature and the extracellular matrix is to a large extent homogeneous with respect to its constituents (Brama *et al*. 2000a). During growth and development, the metabolism of matrix components is still very high, and during this period a process of functional adaptation takes place, in which the biochemical characteristics of cartilage are changed according to the loading to which it is subjected to, leading to topographical heterogeneity of the components (Brama *et al*. 2000a and 2000b). Even the collagen fibril network appears to be prone to considerable alterations at young age (Brama *et al*. 2000a, Helminen *et al*. 2000). In fact, optimal joint loading at young age seems to be crucial for the establishment and strengthening of the articular cartilage collagen network and is, therefore, pivotal for the ultimate quality and, hence, for injury resistance of the tissue (Brama 1999, Helminen *et al*. 2000). Once mature, especially the collagen metabolism is at such a low rate that substantial modifications are not possible anymore (Bank *et al*. 1998, Brama *et al*. 1999, Maroudas 1980), and the integrity of the tissue remains unaffected as long as the adaptive capacity is not exceeded (Brama 1999, Cornwall 1984).

It is well accepted that regular loading of articular cartilage within physiological limits throughout life is necessary to maintain normal function (Burton-Wurster *et al*. 1993, Sah *et al*. 1989 and 1992, Torzilli *et al*. 1997). Static compressive loading in the absence of motion decreases biosynthetic rates in a dose-dependent manner, whereas subjection of the tissue to intermittent cyclic stimuli at specific frequencies increases biosynthesis of the chondrocytes (Buckwalter and Martin 1995, Burton-Wurster *et al*. 1993, Sah *et al*. 1989 and 1992, Torzilli *et al*. 1997). Regular moderate,

and possibly even strenuous activity does not appear to cause or accelerate cartilage damage in normal joints (Buckwalter and Martin 1995, Kiviranta *et al*. 1988, Säämänen *et al*. 1989).

In the development of cartilage pathology, mechanical influences have been reported to be a major initiating factor as well (Freeman 1980, Hayes *et al*. 2001, Radin 1983, Radin *et al*. 1972). Damage of cartilage will occur when the applied load exceeds the load absorbing capacity of the tissue (Cornwall 1984). Various mechanisms can be involved. First, mechanical insult can occur acutely when a joint is forced beyond its usual range of motion, causing stress on softer areas of cartilage that are unable to support the load adequately, and thus may cause damage to the collagen network. Articular cartilage was found to be physically stiffer in areas of increased mechanical stress and softer in areas that bore less weight (Cameron *et al*. 1975). Second, a more chronic way of mechanical damage may occur during different types of sporting exercise, in which joints are repetitively exposed to high levels of impact loading, and which are associated with fatigue failure (Adams 1976, Andersson *et al.* 1989, Kujala *et al*. 1994 and 1995, Lane 1995, Radin and Paul 1971, Vingard *et al*. 1991). Third, deviations from normal loading patterns and aberrant repetitive joint use, in particular following joint injury, have been shown to lead to cartilage damage and degeneration in a variety of animal models (Adams and Brandt 1991, Brandt *et al.* 1991, Herzog *et al*. 1998, McDevitt *et al.* 1977, Pond and Nuki 1973, Sah *et al.* 1997, Setton *et al.* 1994 and 1995, Simmons *et al*. 1999). However, the pathobiology of the joint and the response to injury is complex and considerably more factors are involved than simple physical stress injury. A variety of matrix degrading enzymes, cytokines, and growth factors, originating from different cell types are activated, which mediate further degradation of the extracellular matrix (Hayes *et al.* 2001, Kempson *et al*. 1970, Lane *et al*. 1979, McDevitt *et al.* 1977, McIlwraith 1996, Meachim *et al*. 1965, Mow *et al*. 1990).

MEASUREMENT OF BIOMECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

MEASUREMENT TECHNIQUES

Mechanical properties can be determined using loading geometries, in which an external force disturbs the balance between the swelling pressure of the proteoglycans and the restrictive forces attributable to the collagen network. Loading geometries are classified according to the way of confinement of the sample and application of the load (Hasler *et al*. 1999, Laasanen 2003, Nieminen 2002) (figure 4). Indentation is a measurement technique, in which intact or

isolated cartilage is pressed with a perpendicularly-aligned rigid indenter that may be impervious or permeable. In unconfined compression, the cartilage sample is detached from the subchondral bone, and is subsequently deformed between 2 smooth impervious metallic plates, allowing fluid flow in lateral direction. In confined compression, the sample is inserted in a confining chamber to prevent lateral expansion, and loaded with a permeable piston to allow an axial flow of fluid in and out of the sample.

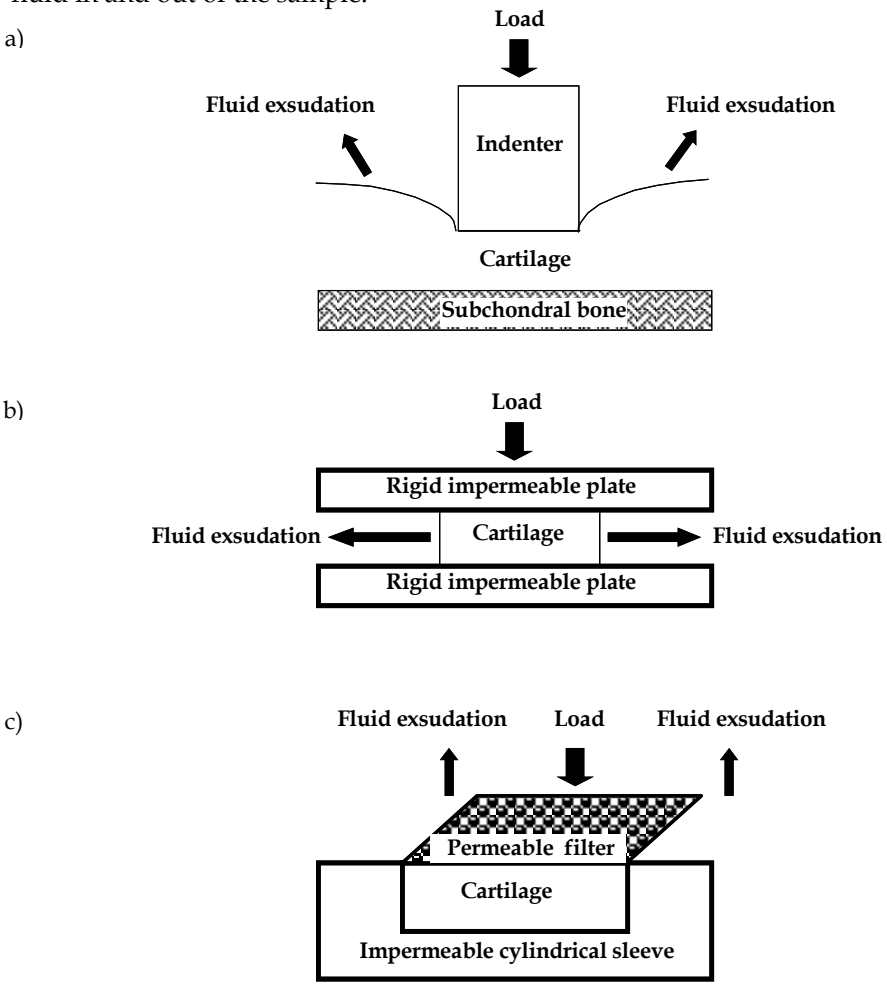

FIGURE 4: *Commonly used experimental configurations for determination of biomechanical properties of articular cartilage: a) indentation, b) unconfined compression, c) confined compression.*
(From Hasler et al. 1999, reprinted by kind permission of Begell House Inc., New York, USA)

Four compressive loading schemes can be applied (Hasler *et al*. 1999, Laasanen 2003, Nieminen 2002): stress-relaxation, creep, dynamic testing, and destructive testing (figure 5).

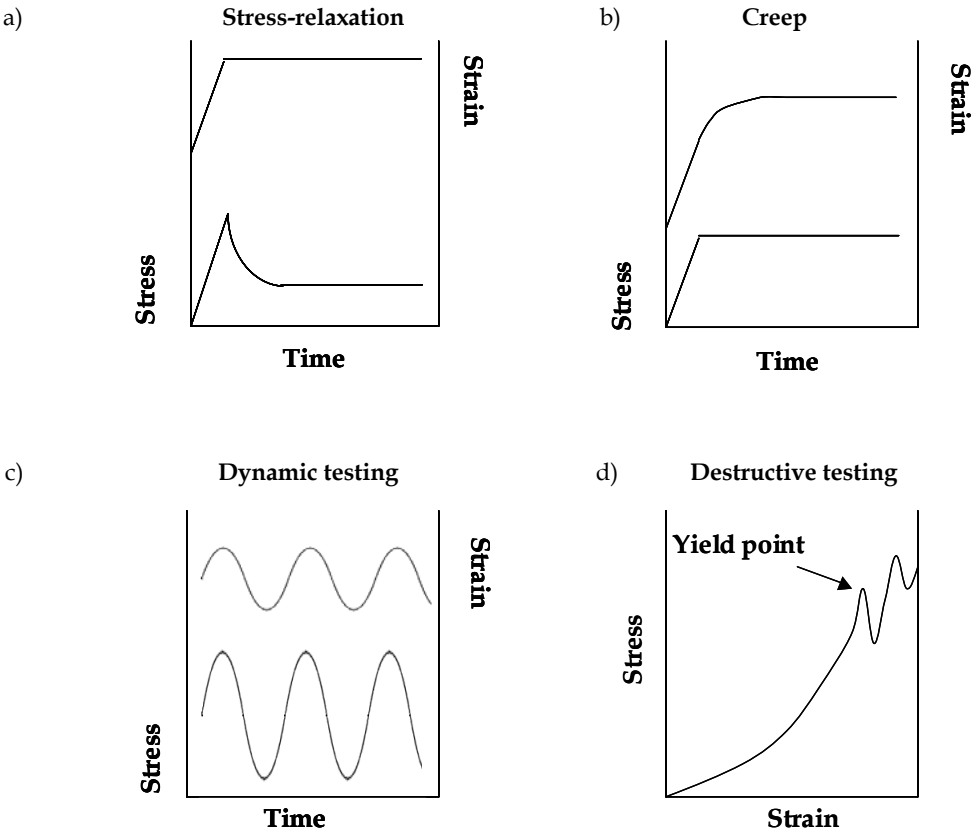

FIGURE 5: Different compressive loading schemes for determination of cartilage biomechanical properties: a) stress-relaxation, b) creep, c) dynamic testing, d) destructive testing.

In stress-relaxation, a constant deformation is applied and the relaxation of the reaction force is measured as a function of time. The creep test involves the application of a step force, while the time-dependent deformation of the tissue is recorded. In dynamic loading, several sinusoidal cycles are applied at a certain

strain (or stress) and the corresponding stress (or strain) is recorded as a function of time. Destructive testing can be used to determine the ultimate strength of the tissue from the point of failure in the stress-strain curve. Besides these compressive loading configurations, also other tests in different loading modes are possible, such as shear tests and tensile tests.

BIOMECHANICAL PARAMETERS

Various models have been developed to characterize the loading response of articular cartilage. Most of these consider cartilage to be homogeneous and isotropic for the sake of simplicity. They include the single-phase elastic model (Hayes *et al.* 1972), the viscoelastic model (Parsons and Black 1977), the biphasic model (Mow *et al*. 1980), and the triphasic model (Lai *et al.* 1991).

Typical biomechanical parameters are (Laasanen 2003, Nieminen 2002, Palmer and Bertone 1996): 1) Young's modulus at equilibrium, basically a ratio of axial stress and strain in unconfined compression; 2) Aggregate modulus, based on the assumption of material isotropy and describing the equilibrium modulus in confined compression; 3) Dynamic modulus, proportional to the elastically stored energy and viscous energy dissipated in the loading process and basically a ratio of dynamic stress and strain; 4) Shear modulus, basically the ratio of the shear stress and the angular shear distortion; 5) Poisson's ratio, describing the lateral expansion during axial compression and is basically defined as the ratio of lateral and axial strains; 6) Permeability, describing the movement of fluid through the extracellular matrix and dependent on the pore size and the impedance (Comper and Lyons 1993).

Recently developed models, such as the transversely isotropic biphasic (Cohen *et al*. 1998) and fibril-reinforced models (Korhonen *et al*. 2002, Li *et al.* 2000, Soulhat *et al*. 1999), attempt to account for the more complicated cartilage structure.

MEASUREMENT OF CARTILAGE BIOMECHANICAL PROPERTIES IN VIVO

A number of instruments have been introduced for arthroscopic evaluation of stiffness of articular cartilage (Appleyard *et al.* 2001, Dashefsky 1987, Lyyra *et al*. 1995, Niederauer *et al.* 1998). Use of these instruments is based on mechanical indentation and they employ strain technology or miniature load cells to measure the resisting force of cartilage to an applied deformation. The instantaneous or the equilibrium load-deformation behaviour is typically recorded. The results are affected by the thickness of the cartilage, especially when the cartilage layer is relatively thin (Hayes *et al.* 1972, Mak *et al*. 1987, Zhang *et al*. 1997). The newer

generations of these instruments have been adapted by replacing plane-ended indenters for spherical-ended instruments, and by using smaller reference plates and pressure forces to make the measured indentation stiffness less sensitive to the surface irregularities and thickness of the cartilage (Korhonen *et al*. 2003b, Lyyra-Laitinen *et al*. 1999). Recently, new instruments have been developed in which a high frequency ultrasound probe is integrated in the indenter tip (Kawchuk and Elliott 1998, Laasanen *et al*. 2002, Suh *et al*. 2001, Zheng and Mak 1996). The ultrasound transducer is used as an indenter and the thickness and deformation of the tissue can be calculated from the ultrasound signal during compression (Kawchuk and Elliott 1998, Laasanen *et al*. 2002, Suh *et al*. 2001, Zheng and Mak 1996). This technique enables an objective calculation of biomechanical parameters, provided that the speed of sound in the cartilage is known and a valid model of cartilage mechanics is used.

THE EQUINE METACARPOPHALANGEAL JOINT: HIGH SUSCEPTIBILITY TO INJURY

The equine metacarpophalangeal (MCP) joint consists of the distal extremity of the third metacarpal bone (MCIII), the proximal extremity of the proximal phalanx (P1), and the 2 proximal sesamoid bones (PSBs) (figure 6).

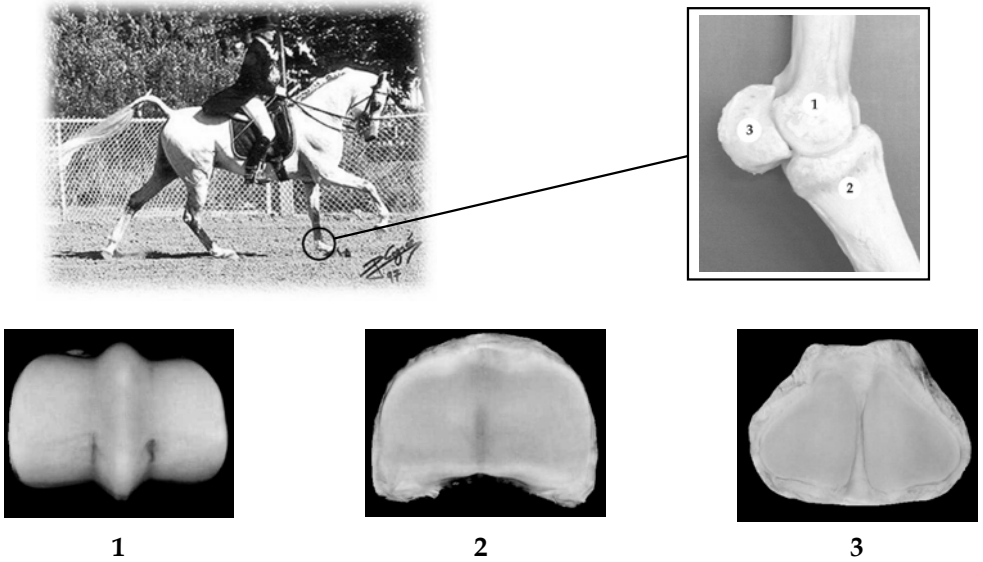

FIGURE 6: *The equine metacarpophalangeal (MCP) joint: 1) third metacarpal bone (MCIII), 2) proximal phalanx (P1), 3) proximal sesamoid bones (PSBs).*

The equine MCP joint is of great clinical importance as the joint has a high susceptibility to injury, probably related to its high-motion character, its distal position in the appendicular skeleton, and its relatively small cartilage surface area in relation to the large body weight of the horse (MacDonald *et al.* 2002, Pool 1996, Pool and Meagher 1990). Most of the lesions commonly encountered in the equine MCP joint are osteoarthritic in nature (Pool 1996). Although trauma is the central aetiology of osteoarthritis (OA), several interrelating pathways have been discerned in the pathogenesis of the disease (McIlwraith 2002). In the first pathway, trauma is associated with synovitis and capsulitis, also known as traumatic arthritis, in which a primary release of matrix degrading enzymes and cytokines from different cell types may secondarily affect the articular cartilage (McIlwraith 1996 and 2002). The second pathway is related with direct damage to the cartilage in regions of concussion, leading to physical cell injury, which also results in the release of matrix degrading enzymes and, subsequently, to further degeneration of articular cartilage. The third pathway may reflect intrinsic cartilage degeneration associated with age, in which trauma is induced by superimposition of normal continuous joint use on the intrinsically degenerated, and thus inferior cartilage, which again leads to progressive cartilage damage by the enzymatic degradative cascade (McIlwraith 1996 and 2002). In the fourth pathway, OA may develop secondary in the course of other primary joint diseases, such as intra-articular fractures, dislocations, ligamentous ruptures, wounds, infectious arthritis, and osteochondrosis (McIlwraith 1996 and 2002). The common end-stage of OA is characterised by progressive degradation of articular cartilage, accompanied by changes in the bone (sclerosis and osteophyte formation) and soft tissues (fibrosis) (McIlwraith 1996 and 2002). OA involving the equine MCP joint leads to a spectrum of specific pathologic lesions. Degenerative changes and chip fractures are frequently found at the dorsal articular margin of P1 and MCIII, probably related with impaction of the proximodorsal articular margin of P1 upon the dorso-distal end of MCIII due to repeated overextension of the joint (Hardy *et al*. 1987, Kawcak and McIlwraith 1994, McIlwraith 1996, Palmer and Bertone 1996, Pool 1996). Small solitary ovoid lesions on either the medial or lateral condylar surface of MCIII, wear lines aligned in the sagittal plane on the articular surfaces of P1, PSBs, and MCIII, and OA changes on the transverse ridge of MCIII, ranging from mild fibrillation to cavitary ulcerations extending into the subchondral bone, are other pathologic entities which are frequently encountered in the equine MCP joint (Pool 1996).

AIM AND SCOPE OF THE STUDY

The ultimate objective of this thesis was to quantitatively assess cartilage functionality, *i.e.* cartilage biomechanical properties, in the equine MCP joint in relation to OA-associated cartilage degeneration in order to improve the diagnosis and prognostication of this chronic degenerative joint disorder. The study particularly focused on the articular surface of the proximal phalanx (P1) as, certainly in Warmblood horses, the articular surface of P1 is considered to be representative for the overall condition of cartilage in the MCP joint. To reach this goal, several steps, that were partly very basic but indispensable, had to be taken. The first step was to study the normal physiological development of articular cartilage biomechanical properties after birth (chapter II). Knowledge of functional adaptation of articular cartilage after birth has resulted from earlier biochemical research (Brama 1999) and, as there is a strong relationship between cartilage tissue constitution and function (Hayes *et al*. 2001, Mow *et al*. 1990), it could be assumed that the biomechanical properties would adapt accordingly. Insight in this normal physiological adaptation process after birth is essential before pathological conditions of cartilage biomechanics can be highlighted.

In the study of structure-function and degeneration-dysfunction relationships of articular cartilage, measurements of biomechanical properties would have to be interpreted against the background of a quantitative measure of cartilage damage. Chapter III presents a new technique for such a quantitative assessment of articular cartilage degeneration. This method takes into account that OA is heterogeneous in nature, featuring severely affected and seemingly unaffected sites within the same joint (Chang *et al*. 1997, Pool 1996). The technique described in this chapter is capable of detecting degenerated cartilage across the entire joint surface, but also of areas of special interest.

Once any disruption of the collagen network with a subsequent decrease in cartilage stiffness has arisen in a particular region within the joint, the damage may be progressive when normal joint loading is continued (Kempson *et al*. 1973). In order to better understand the dynamic nature and progression of OA, it is therefore essential to know the spreading pattern of cartilage degeneration within the joint. The topographical distribution of cartilage degeneration across the articular cartilage surface in the MCP joint is investigated in chapter IV and compared with the metatarsophalangeal (MTP) joint in chapter V. The equine MCP and MTP joints have virtually the same geometric anatomy, but are subject to different biomechanical loading environments as the kinematics of the forelimb are different in comparison to the hindlimb (Back 1994, Back *et al*. 1995, Merkens 1987,

Merkens and Schamhardt 1994). In chapter V the hypothesis was tested that these differences would be associated with differences in the development and spread of cartilage degeneration over the respective surfaces.
In chapter VI the functional consequences of cartilage degeneration in the MCP joint are investigated and discussed. Biomechanical properties are determined and related to the amount of cartilage damage at 2 sites with different loading patterns (Brama *et al.* 2001), and with differences in the relative contribution to overall cartilage degeneration in the joint, as described in chapter IV.
For the *in vivo* evaluation and direct visualisation of macroscopic damage of articular cartilage, arthroscopy is the only minimally invasive diagnostic modality, which provides a direct, magnified view of the cartilage surface (McIlwraith 1990, Trotter and McIlwraith 1996). The accuracy of this technique for the assessment of the severity of cartilage surface damage in the MCP joint is evaluated in chapter VII.
In chapter VIII, a spherical-ended arthroscopic indentation instrument is tested as a potentially useful tool for the estimation of cartilage stiffness and level of cartilage degeneration.
Determination of the speed of sound (SOS) in equine articular cartilage, a parameter that is needed for the correct interpretation of the recently developed ultrasound-based indentation technique, is described in chapter IX. The possible influences of age, site within the joint, and cartilage degeneration are also investigated, as interaction of these parameters with the SOS may affect the clinical applicability of this technique (Töyräs *et al.* 2003).
Finally, the main results are summarised and discussed in the concluding chapter of this thesis (chapter X), in which clinical implications and directions of future research are also outlined.

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- CHAPTER II -

FUNCTIONAL ADAPTATION OF ARTICULAR CARTILAGE FROM BIRTH TO MATURITY UNDER THE INFLUENCE OF LOADING: A BIOMECHANICAL ANALYSIS

Brommer H[a] – Brama PAJ[a] – Laasanen MS[b] – Helminen HJ[c] - Van Weeren PR[a] – Jurvelin JS[b,d]

[a] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[b] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Finland
[c] Department of Anatomy, Kuopio University, Finland
[d] Department of Applied Physics, Kuopio University, Finland

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SUMMARY

Reasons for performing study: **The concept of functional adapatation of articular cartilage during maturation has emerged from earlier biochemical research. However, articular cartilage has principally a biomechanical function governed by joint loading.**

Objectives: **To verify whether the concept of functional adaptation can be confirmed by direct measurement of biomechanical properties of cartilage.**

Hypothesis: **Fetuses have homogeneous (*i.e.* site-independent) cartilage with regard to biomechanical properties. During growth and development to maturity, the biomechanical characteristics adapt according to functional (loading) demands, leading to distinct, site-dependent biomechanical heterogeneity of articular cartilage.**

Methods: **Osteochondral plugs were drilled out of the surface at 2 differently loaded sites (site 1: intermittent impact-loading during locomotion, site 2: low-level constant loading during weightbearing) of the proximal articular cartilage surface of the proximal phalanx in the forelimb from stillborn foals (n = 8), horses of age 5 (n = 9) and 18 months (n = 9), and mature horses (n = 13). Cartilage thickness was measured using ultrasonic, optical and needle-probe techniques. The osteochondral samples were biomechanically tested in indentation geometry. Young's modulus at equilibrium, dynamic modulus at 1.0 Hz, and the ratios of these moduli values between sites 1 and 2 were calculated. Age and site effects were statistically evaluated using ANOVA tests. The level of significance was set at $p < 0.05$.**

Results: **Fetal cartilage was significantly thicker compared to the other ages with no further age-dependent differences in the cartilage thickness from age 5 months onwards. Young's modulus stayed constant at site 1, whereas at site 2 there was a gradual, statistically significant increase in modulus during maturation. Values of dynamic modulus both at sites 1 and 2 were significantly higher in the fetus and decreased after birth. Values for both moduli were significantly different between sites 1 and 2 from age 18 months onwards. The ratio of values between sites 1 and 2 for Young's modulus and dynamic modulus showed a gradual decrease from ~ 1.0 at birth to 0.5 - 0.6 in the mature horse. At age 18 months, all values were comparable to those in the mature horse.**

Conclusions: **In line with the concept of functional adaptation, the neonate is born with biomechanically 'blank' or homogeneous cartilage. Functional adaptation of biomechanical properties takes place early in life resulting in cartilage with a distinct heterogeneity in functional characteristics. At age 18 months, functional adaptation, as assessed by the biomechanical characteristics,**

has progressed to a level comparable to the mature horse, and after this age no major adaptations seem to occur.
Potential relevance: **Throughout life, different areas of articular cartilage are subjected to different types of loading. Differences in loading can adequately be met only when the tissue is biomechanically adapted to withstand these different loading conditions without injury. This process of functional adaptation starts immediately after birth and is completed well before maturity. This makes the factor loading at young age a crucial variable, and emphasises the necessity to optimise joint loading during early life in order to create an optimal biomechanical quality of articular cartilage, which may well turn out to be the best prevention for joint injury later in life.**

INTRODUCTION

Articular cartilage has principally a biomechanical function and its response to physiological loading is governed by the interactions between 3 main components, *i.e.* proteoglycans, collagen and water (Hasler *et al*. 1999, Hayes *et al*. 2001, Mow *et al*. 1990). The proteoglycans are trapped by the collagen molecules, which are organised in a fibrillar network. Water is drawn into the tissue as a result of osmotic forces created by the negatively charged glycosaminoglycans (GAG's). The balance between the tension in the collagen network and the osmotic swelling capacity of the proteoglycans is responsible for the biomechanical characteristics of the articular cartilage (Hasler *et al*. 1999, Hayes *et al*. 2001, Mow *et al*. 1990).
The relationship between biomechanical characteristics of articular cartilage with the biochemical constitution of the tissue has been a subject of many studies (Armstrong *et al*. 1995, Jurvelin *et al.* 2000, Kiviranta *et al*. 1987b, Lewis *et al*. 1998, Maroudas *et al.* 1973). The static or long-term time-dependent properties of cartilage are essentially dependent on the proteoglycans (Armstrong and Mow 1982, Mow *et al*. 1990), while the collagen network strongly affects the dynamic properties of cartilage (Bader and Kempson 1994, Bader *et al*. 1992, Korhonen *et al*. 2003).
Within a joint, different areas are subject to different types of loading, such as low-level constant loading during weightbearing, intermittent loading during locomotion, and high-impact loading during training and athletic performance. As a result, each area within a joint is subjected to different biomechanical demands, which can only be met adequately by articular cartilage with topographically varying biochemical and biomechanical characteristics (Armstong *et al*. 1995,

Jurvelin *et al.* 2000, Kiviranta *et al*. 1987b, Maroudas *et al*. 1973, Murray *et al*. 1995 and 1999, Palmer *et al*. 1995).

In horses, it has also been shown that, in the metacarpophalangeal (MCP) joint, a marked topographical heterogeneity exists with respect to the biochemical composition that is matched with local differences in loading during normal locomotion and activity (Brama *et al*. 2000b and 2001). This heterogeneity appears not to be present in the newborn foal (Brama *et al*. 2000a and 2002), but is formed in the early juvenile period, presumably under the influence of joint loading (Brama *et al*. 2000a and 2002, Carter *et al*. 1987, Wong and Carter 2003). These observations have led to the development of the concept of functional adaptation, in which it is hypothesised that the factor joint loading (*i.e.* exercise) in young and growing animals is assumed to play a crucial role in the development of the biochemical and biomechanical constitution of articular cartilage, resulting in cartilage of the mature animal that undergoes no more substantial changes during maturity (Brama *et al.* 2000a and 2002). A similar hypothesis was introduced by Helminen *et al*. (2000), who stated that regular joint loading in youth assists in establishing and strengthening the collagen fibril network of articular cartilage, which contributes to prevention of osteoarthritis (OA) later in life.

Research regarding the biomechanical characteristics of equine articular cartilage has been limited. In the mature horse, the biomechanical properties of carpal cartilage and the relationship between biochemical composition and biomechanical challenges associated with exercise, training and cartilage deterioration has been determined (Murray *et al*. 1995, 1999, 2000 and 2001, Palmer *et al.* 1995). However, in the equine MCP joint, which has been shown to have the largest number of traumatic and degenerative lesions (Pool 1996), no research in this area has been performed. Neither has work been performed on the developmental aspect of the biomechanical characteristics of articular cartilage in the horse.

The aim of the present study was to investigate topographically the development of the biomechanical properties of articular cartilage from birth to maturity under the influence of joint loading. To achieve this, cartilage thickness, Young's modulus at equilibrium as a measure for static stiffness during weightbearing, and dynamic modulus as a measure for dynamic stiffness during locomotion were determined at 2 differently loaded sites of the proximal articular surface of the proximal phalanx (P1) in a number of MCP joints from horses of different ages, ranging from the fetal stage to the mature horse. The first site of interest was located at the dorsal articular margin, being intermittently and impact-loaded during locomotion (Brama *et al*. 2001). The second site of interest was located at the central fovea,

being constantly and low-level loaded during weightbearing (Brama *et al*. 2001). It is hypothesised that fetuses have homogeneous cartilage with regard to biomechanical properties and that, during development to maturity, these properties adapt according to different loading conditions to which the cartilage is subjected, leading to a distinct biomechanical heterogeneity of articular cartilage.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

MCP joints were harvested from stillborn foals (Warmbloods, n = 8); foals kept on pasture, age 5 months (Warmbloods, n = 9); foals kept on pasture, age 18 months (Thoroughbreds, n = 9); and mature horses age 3 – 17 years (mean $\pm$ s.d. 9.0 $\pm$ 4.7 years, Warmbloods, n = 13). The P1s were extracted and the proximal articular cartilage surfaces, including approximately 5 cm of bone, were cut off using a band saw. The cartilage surfaces were checked macroscopically for the absence of OA. The samples were stored at -20°C until processed. The results of a study by Kiefer *et al*. (1989) indicated that cryopreservation did not have an effect that could be measured on specific mechanical behaviours of articular cartilage.

Before performing the measurements, the samples were thawed at 7°C in wrapped gauzes soaked in phosphate-buffered saline (PBS). Two predefined test sites (diameter 8 mm) were marked on the proximal articular cartilage surface of P1. Site 1 was located at the lateral dorsal margin, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin; site 2 was located at the lateral central fovea, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins (figure 1). Subsequently, *in situ* articular cartilage thickness of sites 1 and 2 was measured using an ultrasound method as described below. After these ultrasound measurements, osteochondral plugs (diameter 8 mm) at sites 1 and 2 were drilled out of the cartilage surfaces. The subchondral bone was cut off beyond the cartilage-subchondral bone interface, leaving a 3 mm long subchondral bone plug attached to the articular cartilage layer.

MEASUREMENTS OF CARTILAGE THICKNESS

In order to enable stress-relaxation measurements in indentation geometry, *in situ* cartilage thickness was determined nondestructively at the centre of sites 1 and 2 using a recently developed ultrasound indentation instrument (Laasanen *et al.* 2002). Briefly, an unfocused, 10.2 MHz broadband ultrasound transducer (diameter

3.0 mm, Panametrics XMS-310)[1], mounted on the tip of an arthroscopic indentation instrument (Artscan 200)[2], was used to determine the ultrasound flight time between the cartilage surface and subchondral bone (Laasanen *et al.* 2002). Subsequently, cartilage thickness was calculated by multiplying the flight time with a sound speed of 1650 m/s, which was based on earlier findings in bovine cartilage (Töyräs *et al.* 2003) and on pilot measurements using equine articular cartilage.

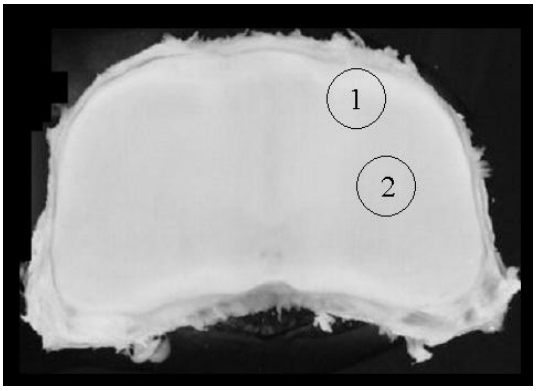

FIGURE 1: *Proximal articular cartilage surface of the equine first phalanx (P1) showing the sites of interest. Site 1 (halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin) is subjected to intermittent and impact loading. Site 2 (halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins) is subjected to low-level constant loading.*

After biomechanical testing, articular cartilage thickness at the site of indentation was measured using a well-validated, slightly destructive needle-probe technique (Jurvelin *et al*. 1995). The penetration velocity of the needle was 10 μm/s.
In fetal cartilage, however, ultrasound and needle-probe measurements were not feasible due to lack of strong echo or change in needle force from the cartilage-subchondral bone interface. Therefore, in the fetal samples optical measurement of cartilage thickness was conducted instead (Jurvelin *et al.* 1987 and 1995). The thickness of the cartilage layer was determined from the osteochondral plugs using

a stereomicroscope[3] equipped with a calibrated measuring ocular (Jurvelin *et al*. 1987). A magnification of x 25 was used. The thickness of cartilage was calculated as the mean of 4 values, measured from 4 evenly spaced sites along the plug edge. Optical determination of cartilage thickness was also performed in a number of samples of the 5-month-old (site 1: n = 4, site 2: n = 4) and 18-month-old horses (site 1: n = 5, site 2: n = 5).

The accuracy of different techniques was studied by comparing thickness values obtained by these 3 techniques. The ultrasound and needle-probe thickness values were similar in the tested samples (mean difference –2%, Pearson's linear correlation coefficient $r = 0.94$, $p < 0.001$, $n = 62$). Thickness values from optical measurements were slightly higher (mean difference to needle-probe thickness 10%, Pearson's linear correlation coefficient $r = 0.90$, $p < 0.001$, $n = 18$).

BIOMECHANICAL TESTING

The osteochondral samples were glued onto the bottom of a sample holder with cyanoacrylate. The sample was submerged in PBS and biomechanically tested in indentation geometry using a custom-made, computer-controlled, high-resolution material testing instrument (resolution 5 mN for force and 0.1 μm for position) as described by Töyräs *et al.* (1999) (figure 2a). A cylindrical, porous, plane-ended indenter (diameter 1.041 mm) was used (figure 2b). Samples were tested using a stress-relaxation protocol (two 7.5% strain steps with a velocity of 1.0 μm/s and a relaxation time of 1200 s), followed by dynamic testing (1.0% amplitude of the compressed thickness at a frequency of 1.0 Hz) (figure 2c). Biomechanical testing was performed using the ultrasound thickness values. For the fetal samples, values obtained by the optical technique were used. Software for data acquisition and analysis was developed with LabVIEW (version 6.1)[4].

DETERMINATION OF YOUNG'S MODULUS

Young's modulus at equilibrium was derived from the equilibrium stress-strain response during the second step (7.5 - 15%) of compression. For a linearly elastic, isotropic material, the expression for Young's modulus (E) is given as (Hayes *et al.* 1972):

$$E = (\sigma/\varepsilon) \times (\pi\alpha/2h\kappa) \times (1 - \upsilon^2)$$

where σ = stress, ε = strain, α = indenter radius, h = thickness of the cartilage, υ = Poisson's ratio, and κ = theoretical scale factor that accounts for the variable cartilage thickness (Hayes *et al.* 1972, Jurvelin *et al.* 1990).

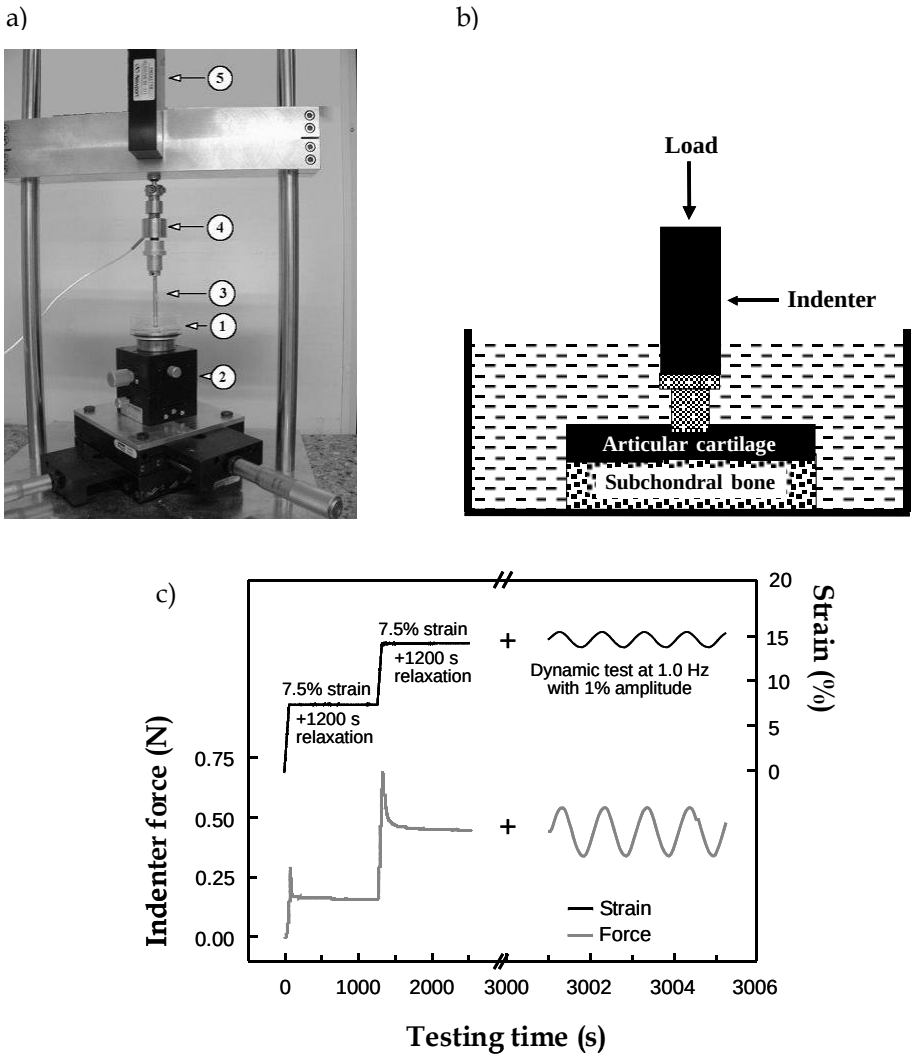

FIGURE 2: *a) Setup of the biomechanical testing device: 1) sample and sample holder, 2) controller for alignment of the cartilage surface, 3) indenter, 4) load cell, 5) computer-assisted motion controller. b) During biomechanical testing, the cartilage sample is compressed with a cylindrical, porous, plane-ended indenter. The sample, glued onto bottom of the sample holder with cyanoacrylate, is submerged in phosphate buffered saline.*
c) Loading response of the stress-relaxation and subsequent dynamic loading tests. Young's modulus was calculated from the equilibrium response of the second 7.5% step and dynamic modulus from the last 2 cycles.

When computing values of the Young's modulus, the thickness values measured by the needle-probe technique were used for all except fetal samples. For fetal samples, the optical thickness values were used. The Poisson's ratio was set to 0.1 for calculation of Young's modulus, based on previous results (Jurvelin *et al*. 1997).

DETERMINATION OF DYNAMIC MODULUS

For calculation of the dynamic modulus, the same indentation model was used. However, cartilage was assumed to be incompressible during 1.0 Hz cyclic compression and, therefore, Poisson's ratio was set to 0.5 (Mak *et al*. 1987, Wong *et al*. 2000). Dynamic modulus was calculated from the last 2 cycles of the dynamic test.

STATISTICAL ANALYSIS

All measured parameters are expressed as mean $\pm$ s.d. Age and site effects on cartilage thickness, Young's modulus, dynamic modulus, and modulus ratios between sites 1 and 2 were statistically evaluated using ANOVA tests with Bonferroni *post hoc* comparisons. All tests were run using SPSS software (version 10.0) [5]. The level of significance was set at $p < 0.05$.

RESULTS

CARTILAGE THICKNESS

Fetal cartilage appeared to be significantly thicker both at sites 1 (4181 $\pm$ 1586 μm) and 2 (3421 $\pm$ 1476 μm), compared to the cartilage from other age groups (figure 3). There was a large variation in thickness values in the fetuses and differences between sites 1 and 2 were not significant. At age 5 months, cartilage thickness at site 1 (1268 $\pm$ 104 μm, needle-probe values) was significantly higher than at site 2 (885 $\pm$ 81 μm, needle-probe values). From age 5 months to maturity, there were no significant age-dependent differences in cartilage thickness. In addition, significant differences in cartilage thickness between sites 1 and 2 disappeared at age 18 months.

YOUNG'S MODULUS

Young's modulus at equilibrium at site 1 was virtually constant and there were no significant differences between horses of different ages (figure 4). At site 2, there was a gradual increase in the modulus, ranging from 1.35 $\pm$ 0.39 MPa in the fetus to 3.19 $\pm$ 1.39 MPa in the mature horse. Values in the mature horse were significantly higher compared to the fetal and 5-month-old foals. In the fetus and at

age 5 months there were no differences between sites 1 and 2, whereas in 18-month-old and mature horses, differences between sites 1 and 2 were statistically significant. The modulus ratio between sites 1 and 2 gradually decreased from 1.04 ± 0.30 in the fetus to 0.51 ± 0.17 in the mature horse (figure 6). The ratio between sites 1 and 2 in the mature horse was significantly lower than in the fetal stage and 5-month-old foals.

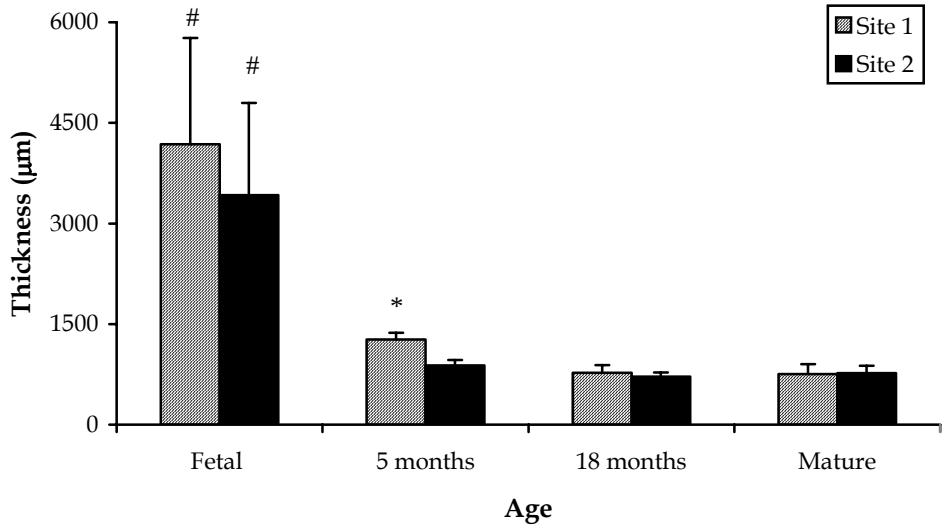

FIGURE 3: *Thickness (mean ± s.d., µm) of articular cartilage at sites 1 and 2 in fetal specimens (optical values), at 5 age months (needle-probe values), at age 18 months (needle-probe values), and in the mature horse (needle-probe values).*
** Significant site effects ($p < 0.05$)*
 # Significant age effects ($p < 0.05$) vs. age 5 months, age 18 months, and mature horses

DYNAMIC MODULUS

During maturation, dynamic modulus decreased at both sites 1 and 2 (figure 5). Values in the fetus (6.63 ± 0.73 MPa and 7.47 ± 1.68 MPa for sites 1 and 2, respectively) were significantly higher compared with those at the other ages. At age 5 months, dynamic modulus at site 1 (4.94 ± 1.41 MPa) was still significantly higher than in the 18-month-old and mature horses. At age 18 months modulus values did not differ significantly from those of mature horses. At site 2, dynamic modulus at age 5 months (5.02 ± 1.24 MPa) was similar to that of 18-month-old and

mature horses and the differences were not significant. The ratio between sites 1 and 2 showed the same pattern as for the Young's modulus (figure 6). There was a gradual decrease of the ratio value from the fetal stage (0.93 ± 0.22) to maturity (0.61 ± 0.25). Again, the ratio between sites 1 and 2 in the mature horse was significantly lower than in the fetal stage and in 5-month-old horses.

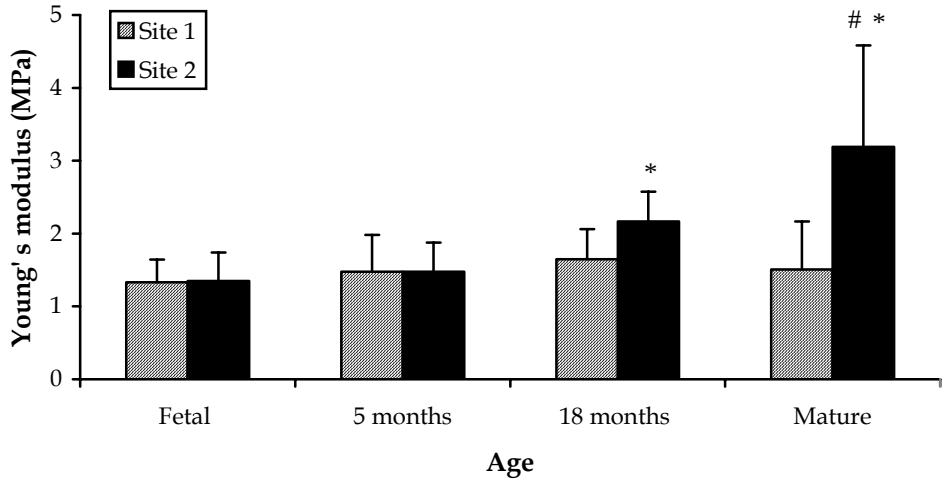

FIGURE 4: *Young's modulus at equilibrium (mean ± s.d., MPa) at sites 1 and 2 in the fetal stage, at age 5 months, at age 18 months, and in the mature horse.*
** Significant site effects ($p < 0.05$)*
 # Significant age effects ($p < 0.05$) vs. fetal and age 5 months

DISCUSSION

In this study, changes in thickness and biomechanical characteristics of equine cartilage on the articular surface of P1 were investigated at 2 different loaded sites from birth until maturity. Cartilage at site 1 is subjected to intermittent impact-loading whereas cartilage at site 2 is subjected to more constant low-level loading (Brama *et al.* 2001). The relatively thin cartilage on the equine P1 combined with a rather pronounced curvature of the articular surface made confined or unconfined compression techniques unsuitable in this study. For this reason, the biomechanical tests were performed using the indentation geometry. The values of Young's modulus from indentation tests have been found to be higher than those

obtained directly from unconfined compression tests, however, modulus values between these techniques were highly linearly correlated (r ~ 0.9, Korhonen *et al*. 2002). Indentation tests were conducted under displacement control to enable tests with small strains. The results indicate that, biomechanically, fetal tissue is homogeneous, showing no site-dependent differences. As the horse gets older and tissue maturates, cartilage becomes gradually heterogeneous, showing topographical variations in both thickness and compressive stiffness. Our results suggest that these biomechanical alterations are controlled by joint loading, representing further evidence for the concept of functional adaptation of articular cartilage (Brama *et al.* 2000a and 2002, Helminen *et al.* 2000).

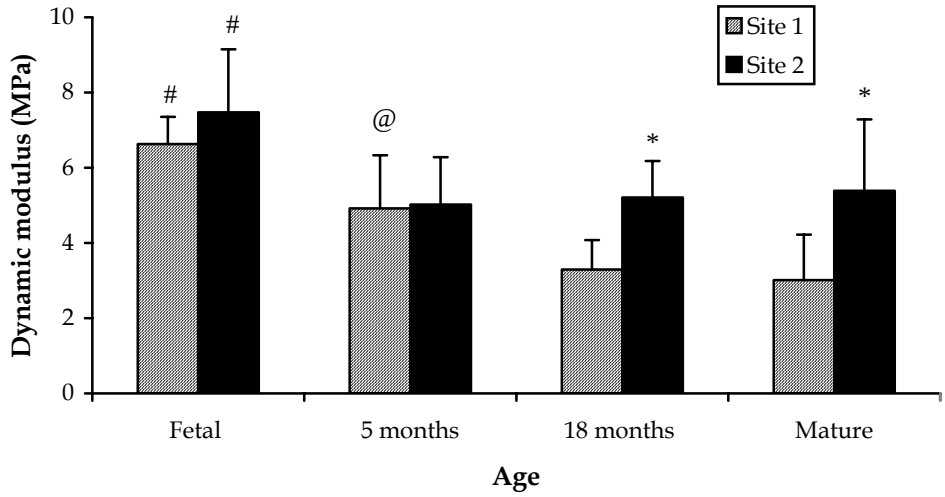

FIGURE 5: *Dynamic modulus (mean ± s.d., MPa) at sites 1 and 2 in the fetal stage, at age 5 months, at age 18 months, and in the mature horse.*
** Significant site effects ($p < 0.05$)*
 # Significant age effects ($p < 0.05$) vs. age 5 months, age 18 months, and mature horses
@ Significant age effects ($p < 0.05$) vs. age 18 months and mature horses

Fetal cartilage was significantly thicker compared to samples from the other ages at both sites 1 and 2. In the fetal period, ossification of the cartilaginous models of the long bones begins (Baltadjiev 1986, Glorieux *et al*. 1991, Salle *et al.* 2002). The speed of this ossification is fast during the last part of gestation, but the process is not

completed at the time of delivery and it continues after birth (Firth 1996, Firth and Greydanus 1987). Cartilage is thick at most articular surfaces in newborn foals, but thickness decreases rapidly with increasing age (Firth 1996, Firth and Greydanus 1987). Therefore, small differences in the ages of foals may result in large differences in thickness values. This may explain the large range of cartilage thickness in fetuses. At age 5 months, differences in cartilage thickness were no longer significantly different compared to 18-month-old and mature horses. This indicates that during the first 5 months of life there is high activity in the bone ends, turning cartilage vigorously into bone. At age 5 months, cartilage thickness at site 1 is still higher than at site 2. This suggests that the remodelling rate at site 2 is higher than at site 1 during the first months after birth. This indirectly also implies that at site 1 remodeling goes on for a longer period and at a higher rate than at site 2 from age 5 - 18 months.

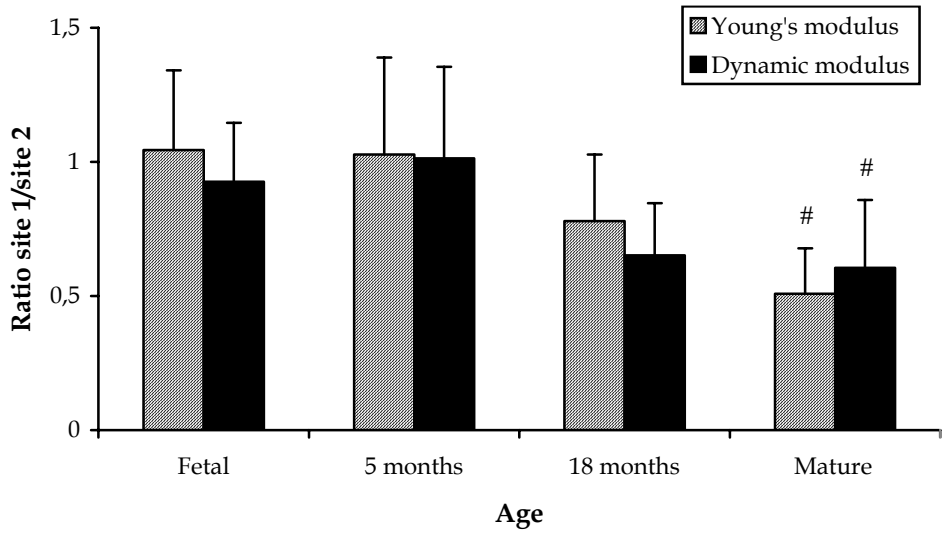

FIGURE 6: *Ratio site 1/site 2 (mean ± s.d.) for Young's modulus at equilibrium and dynamic modulus in the fetal stage, at age 5 months, at age 18 months, and in the mature horse.*
 # Significant age effects ($p < 0.05$) vs. fetal and age 5 months

Young's modulus is related to the static (or equilibrium) properties of cartilage and shows positive correlation with the concentration of proteoglycans (Armstrong

and Mow 1982, Mow *et al*. 1990). Young's modulus at site 1 remained constant and no age effects were detected. This may relate to the fact that site 1 is not a constantly loaded cartilage area (Brama *et al.* 2001) which would require increase in compressive stiffness during age-related weight gain. At site 2, however, which is continuously loaded during weightbearing (Brama *et al.* 2001), there was a gradual increase in the values of Young's modulus, generating mature values that were significantly different from values determined at other ages. Possibly, during growth, development and aging, the increase of body weight augments static loading of the cartilage at P1, *i.e.* in the central area of the joint. The ratio between sites 1 and 2 decreased from approximately 1.0 in the fetus to about 0.5 in the mature horse, a consequence of the unchanged modulus at site 1 and the higher modulus at site 2. The fetal value 1.0 indicates a topographical homogeneity of cartilage properties with regard to the static stiffness. These findings are in line with GAG levels in articular cartilage in the equine MCP joint found previously. In the fetal stage there is homogeneity with respect to GAG levels. In the mature animal, GAG levels are higher in the central area (*i.e.* the constant load-bearing part) of the joint, compared to the more peripheral area (*i.e.* the intermittently high-impact loaded part) of the joint (Brama *et al*. 2000a and 2000b).

Dynamic modulus has been related to impact loads and shear during exercise and is strongly determined by the characteristics of the collagen network (Bader and Kempson 1994, Bader *et al*. 1992, Korhonen *et al.* 2003). In the fetus, dynamic modulus at both sites 1 and 2 was significantly higher compared to the other ages. The finding of high dynamic modulus values (and low equilibrium modulus values) in the fetus compared to adult tissue, is consistent with results from an earlier study revealing high peak loads and strong stress relaxation of fetal bovine cartilage in unconfined compression (Wong *et al*. 2000). Numerical analyses using the fibril re-inforced biphasic model can relate the dynamic stiffness of cartilage to the architectural characteristics of the collagen network (Korhonen *et al*. 2004). The relatively high dynamic modulus of fetal tissue with a complex collagen network may be related to the recently found differences in the structure and orientation of collagen fibrils in the extracellular matrix, as visualised by polarised light microscopy (Rieppo *et al*. 2003). In the fetus, the collagen fibrils appear to be aligned parallel to the joint surface across the major part of the depth of cartilage layer, resulting in dynamically stiff cartilage (unpublished data). Under the influence of loading, the fibrils gradually take their characteristic position in arcades, which was determined 80 years ago (Benninghoff 1925): parallel to the surface in the superficial zone and perpendicular to the surface in the deep zones.

This reconfiguration might contribute significantly to the changes in dynamic stiffness during maturation. Here again, the dynamic modulus was not significantly different at sites 1 and 2 in the fetal stage, but this changed during aging. This process corresponds with earlier findings in the amount of collagen and levels of hydroxylysylpyridinoline (HP) crosslinks. In fetuses, levels of collagen were almost equal at sites 1 and 2, but in the mature horse, larger collagen content was found at site 1 (Brama *et al.* 2000a and 2000b). The amount of HP crosslinks increases significantly during maturation (Brama *et al*. 1999). The differences between sites 1 and 2 were not present until age 1 year, but in mature horses, higher levels of HP crosslinks were found at the joint edge (Brama *et al.* 1999, 2000a and 2000b). At age 5 months, values of dynamic modulus were already comparable with the mature values at site 2. At site 1 this was not the case before age 18 months. Here too, development of functional characteristics was therefore faster at site 2 than at site 1. This difference in pace of the maturation probably depends on the fact that site 2 is constantly loaded while site 1 is not. Dynamic modulus was significantly higher at site 2 than at site 1 in 18-month-old and mature horses. The dynamic modulus therefore indicates that a less stiff cartilage resides at the joint margins, which is possibly functionally important for optimal absorption of the impact forces.

Most of the cartilage samples used in this study originated from Warmblood horses. Samples of the 18-month-old horses were from Thoroughbred horses. Breed differences with regard to cartilage constitution have not been reported in the horse, but they might exist. However, it can be reasoned, based on the present results, that such differences appear to be of minor importance for the biomechanical characterisation of cartilage. Possible breed differences in cartilage thickness seem not to be relevant here, since the effect of cartilage thickness on the Young's modulus and dynamic modulus was minimised by the use of a theoretical model for indentation (Hayes *et al.* 1972). In this model, the dynamic behaviour of biphasic cartilage was idealized to be equivalent to that of incompressible ($\upsilon = 0.5$) elastic material (Mak *et al.* 1987), whereas for the equilibrium analysis the value of Poisson´s ratio was adopted from earlier studies indicating small values (~ 0.1) of Poisson´s ratio for both immature and mature tissue (Jurvelin *et al.* 1997, Wong *et al.* 2000). Nevertheless, possible variations in the values of Poisson's ratio may contribute to age- and site-dependent differences found in the values of Young's modulus.

While the theoretical analysis includes aspect ratio (α/h) correction, the thickness measurement can be critical for indentation analysis (Hayes *et al.* 1972, Jurvelin *et*

al. 1990). In this study, we measured articular cartilage thickness nondestructively prior to biomechanical testing by using a new ultrasound instrument (Laasanen *et al*. 2002) and also using an optical (or microscopic) technique (Jurvelin *et al*. 1987). Both techniques were validated against a destructive needle-probe measurement, conducted after biomechanical testing. Although the optically derived thickness was slightly (~ 10%) higher than the thickness obtained by the ultrasound or needle-probe techniques, it was adopted for the measurement of fetal samples, which showed only weak ultrasound echo or needle force change at the cartilage-bone interface, thereby invalidating the use of other techniques. Due to high thickness of fetal cartilage (and small aspect ratios, α/h), these differences in thickness induce only minor changes in the modulus values of fetal cartilage (Jurvelin *et al*. 1990). After age 5 months the cartilage thickness at site 2 was highly constant. This results confirm that the statistically significant increase in Young's modulus arises from true changes in material properties of articular cartilage.

In conclusion, this study supports the concept of functional adaptation of articular cartilage suggested by earlier studies (Helminen *et al.* 2000, Brama *et al*. 2000a and 2002). Simultaneously with the development of topographical biochemical heterogeneity starting after birth, local differences in biomechanical behaviour emerge. This functional adaptation of the biomechanical properties to loading takes place early in life. The first 5 months of the horse's life in particular witness a tremendous development of cartilage with regard to the biomechanical properties. At age 18 months, biomechanical adaptation has progressed to a level comparable to the mature horse. After this age, only minor adaptations appear to be possible in mature cartilage.

It appears that the functional adaptation of dynamic biomechanical characteristics proceeds at a faster rate than the static biomechanical properties. This is probably related to the fact that the dynamic properties are mainly determined by the collagen network whereas the static characteristics are principally influenced by the proteoglycan content (Armstrong and Mow 1982, Bader and Kempson 1994, Bader *et al*. 1992, Korhonen *et al.* 2003, Mow *et al*. 1990). According to the prevailing view, the collagen characteristics do not change essentially once the network has been formed, development of nonenzymatic crosslinks excluded, while proteoglycan concentration can be altered on account of joint loading or disuse (Jurvelin *et al.* 1988 and 1990, Kiviranta *et al.* 1987a and 1988, Maroudas 1980).

It should be realised that loading plays a crucial role in the process of functional adaptation in early life that is so essential for future cartilage properties. This emphasises the need to optimise rearing conditions and determine the optimal

exercise regimen during early life in order to create the most optimal biomechanical quality of articular cartilage, which may well turn out to be the best prevention for joint injury later in life (Brama *et al*. 2002, Helminen *et al*. 2000).

MANUFACTURERS' ADDRESSES

[1] Panametrics Inc., Waltham, USA
[2] Artscan Oy, Helsinki, Finland
[3] Olympus Optical Co. Ltd, Tokyo, Japan
[4] LabVIEW, National Instruments, Austin, USA
[5] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER III -

NEW APPROACH FOR QUANTITATIVE ASSESSMENT OF ARTICULAR CARTILAGE DEGENERATION IN HORSES WITH OSTEOARTHRITIS

Brommer H - Van Weeren PR – Brama PAJ

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Am J Vet Res 2003, 64: 83-87

SUMMARY

***Objective*: To evaluate a modified digital imaging technique for quantitative assessment of the grade of osteoarthritis (OA) across the proximal articular surface of the first phalanx (P1) in horses.**

***Sample population*: Six metacarpophalangeal (fetlock) joint specimens from 6 horses with various stages of OA.**

***Procedure*: P1 specimens, together with 4 gray scale reference calibration targets, were positioned in a bath with the proximal articular cartilage surface submerged in saline (0.9% NaCl) solution. Digital images were obtained from the articular surface before and after staining with Indian ink. Computer-controlled gray level analysis of the nonstained and Indian ink stained cartilage surfaces and gray scale reference calibration targets was performed by use of the mean pixel value (based on 255-gray scale). An increase in the mean pixel value after staining was used to calculate the Cartilage Degeneration Index (CDI).**

***Results*: CDI values of the proximal articular cartilage surface of the P1 specimens ranged from 9.2 ± 5.7% (early stage OA) to 41.5 ± 3.6% (late stage OA). The effect of repeating the measurement 6 times in nonstained (including repositioning) and stained specimens (including repositioning and restaining) was not significant. Up to 10 measurements of nonstained specimens could be made without refreshing the bath solution. In stained specimens, mean gray level increased significantly after the 6^{th} measurement.**

***Conclusions and clinical relevance*: The modified digital imaging technique allowed quantitative assessment of cartilage degeneration across the articular cartilage surface. The CDI is the first quantitative measure for OA-induced cartilage degeneration over an entire joint surface in horses.**

INTRODUCTION

Osteoarthritis (OA) and associated lameness is the most common cause of impaired athletic performance in horses (Pool 1996). It is defined as a disease of diarthrodial joints characterised by variable degrees of articular cartilage degeneration, subchondral bone sclerosis, and marginal osteophyte formation (Kidd *et al*. 2002, Meachim 1969, Poole 1999).

OA is usually diagnosed by use of radiology. However, OA is known to be an insidious disease in which substantial damage to the articular cartilage may occur before radiographic changes become visible. It is known that in mature individuals articular cartilage damage will not be fully repaired, making all treatments primarily palliative (Clegg and Booth 2000, Clegg *et al*. 1997). Therefore, research presently is focused on prevention and therapeutic intervention in the initial stages of cartilage degeneration (Clegg and Booth 2000, Clegg *et al.* 1997, Platt 2001, Wright 2001). These approaches require early detection of the disorder. Recent developments in molecular biology have opened the way for the identification of a large number of potential molecular markers in either synovial fluid or serum that may indicate early cartilage degeneration (Myers 1999). However, the assessment of the usefulness of these potential markers and their eventual validation are severely hampered by the fact that there is no measure for the quantitative assessment of the severity of OA over an entire joint surface.

To date, subjective qualitative and semiquantitative grading scales at macroscopic and microscopic levels are used to assess the extent of cartilage degeneration (Cantley *et al.* 1999, Mankin *et al.* 1971, Meachim 1972, Meachim and Emery 1974, Yoshioka *et al.* 1996). Grading at the histopathologic level is dependent on the selected sample sites as OA is known to be heterogeneous in nature with severely affected and seemingly unaffected sites within the same joint (Cantley *et al.* 1999, Pool 1996). A technique that is capable of detecting OA-induced cartilage changes over the entire joint surface is staining with Indian ink (Cantley *et al*. 1999). Indian ink particles are prevented from entering an intact cartilage surface with an unaffected proteoglycan-rich matrix, but have a high affinity for articular cartilage with surface fibrillation and proteoglycan depletion (Chang *et al*. 1997, Madsen *et al*. 1992). The intensity of Indian ink uptake is related to the reduction in the proteoglycan content of the cartilage (Ficat and Maroudas 1975, Maroudas *et al*. 1973). As Indian ink particles absorb and scatter incident light, the reflection of incident light from articular cartilage is relative to the degree of Indian ink staining (Madsen *et al*. 1992). The technique has been used in horses in a semiquantitative way (Cantley *et al*. 1999). In the rabbit, the same principle was used to develop a

quantitative analysis method based on computer-assisted gray level determination (Chang *et al*. 1997).
The purpose of the study presented here was to develop a fully quantitative analysis technique for evaluation of OA at the level of the entire joint surface on the basis of Indian ink staining and digital imaging techniques, and to evaluate the reproducibility of this method. Because of the high incidence of OA in the equine metacarpophalangeal (MCP) joint, the proximal articular surface of the first phalanx (P1) was chosen as the subject of study.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF SPECIMENS

Six MCP joints from 6 horses (age range 1 - 22 years) were harvested immediately after normal slaughter and stored at –20°C until processing. No clinical data about the horses were available. Two days before the measurements were performed, the joints were thawed at 7°C, and the joints were opened by a circumferential incision of the joint capsule. The proximal two-thirds of P1 were cut off using a band saw and freed from the surrounding tissues. Keeping the cartilage surface moist with isotonic saline (0.9% NaCl) solution prevented drying out of the cartilage.

EXPERIMENTAL SETUP AND IMAGE ACQUISITION

The P1 specimens were mounted in a clamp with the proximal articular cartilage surface facing up. Care was taken that the lines connecting the medial and lateral, and the dorsal and palmar articular margins were exactly in a horizontal plane. To achieve this, a rectangular piece of synthetic material (Perspex)[1] was laid over the articular margins and a leveler was used to ensure this position. Specimens were then submerged in a bath containing isotonic saline solution at room temperature (approx. 20°C) with the fluid level 1 to 2 millimeters above the most proximal parts of the articular surface.
A mold was positioned around the P1 in the bath in such a fashion that the proximal articular cartilage surface was projected through the hole in the center of the mold. Professional gray scale reference calibration targets (Q13)[2] in the shades white (#A), light gray (#3), dark gray (#M), and black (#19) were placed at random on the 4 sides of the mold. Also, 4 rulers were mounted on the 4 sides of the mold. The mold was submerged in the bath solution to the same level as the P1 specimen (figure 1).
The system was set up under darkroom conditions. Illumination was provided by 4 100W lamps[3]. Each light bulb was positioned at the corners of a square frame

(80 × 80 cm) with the center of the bulb at 55 cm from the center of the sulcus of P1 and 25 cm above the fluid level.

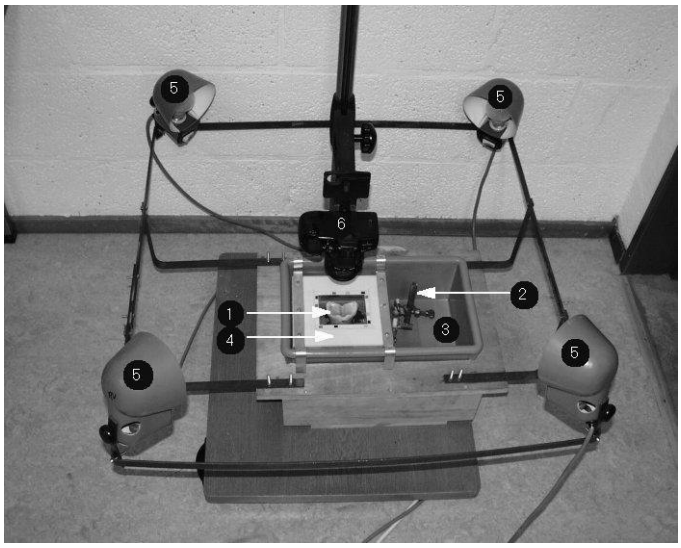

FIGURE 1: *Photograph of the experimental setup: 1) first phalanx (P1) specimen, 2) clamp, 3) bath, 4) mold with the reference calibration targets and rulers, 5) light bulbs, 6) digital camera. Measurements were performed under darkroom conditions.*

Above the P1 specimen, a digital camera (Camedia C-2500L)[4] was mounted with the lens position exactly perpendicular to the joint surface and above the centre of the sulcus of P1. The distance between the lens of the camera and the fluid level was 12 cm, the film speed setting was 100 ISO (as determined by the International Standards Organisation), the lens was maximally zoomed in, and the supermacro close-up photographic mode with high quality preference was chosen. With this configuration, an area of 11 × 14 cm was imaged at a resolution of 1024 × 1280 pixels. The diaphragm was fully opened, shutter speed was 1/50 s, and the camera was set on autofocus. The flashlight function was turned off. The white balance of the camera was adjusted to the special illumination design. Images were stored on computer using commercially available software (Olympus Media Suite Professional, version 1.02.0504)[4].

IMAGE PROCESSING AND ANALYSIS

Computer-assisted gray level analysis of the digitally imaged cartilage surfaces and the reference calibration targets was performed. The mean pixel value was determined as a measure for gray level, by use of image analysis software (Scion Image Beta 4.0.2)[5]. Digital images were processed so that high reflectance of illumination gave low values, and low reflectance gave relatively high mean pixel values. Sizes of the 4 rulers were measured and divided by the original size to get the related ruler size (RRS) on the top (RRS_t), at the bottom (RRS_b), on the right (RRS_r), and on the left side (RRS_l) of the acquired digital image.

INDIAN INK STAINING

The P1 specimens were turned upside down in a bath containing Indian ink[6] for 5 minutes. Excess ink solution was removed by gentle rinsing with 0.5 L of isotonic saline solution. The imaging procedure after staining was identical to the nonstained specimen.

CALCULATION OF THE CARTILAGE DEGENERATION INDEX

The amount of cartilage stained by Indian ink was calculated as follows:

$$[(GL_{iis} - GL_{ns})/(255 - GL_{ns})] \times 100\%$$

in which GL_{iis} is the mean pixel value of the articular cartilage surface after staining with Indian ink, GL_{ns} stands for the mean pixel value of the nonstained articular cartilage surface, and 255 represents the maximum pixel value.

For calculation of the amount of degenerated cartilage, the value for GL_{iis} was corrected, as nondegenerated articular cartilage takes up a small amount of Indian ink (Chang *et al*. 1997). Therefore,

$$GL_{iis,cor} = GL_{iis} - GL_{ndc}$$

in which $GL_{iis,cor}$ is the corrected mean pixel value of the Indian ink stained articular cartilage surface and GL_{ndc} represents the mean pixel value caused by Indian ink uptake of nondegenerated cartilage. The value of GL_{ndc} is variable as the amount of nondegenerated cartilage is inversely proportional to the amount of degenerated cartilage. Thus,

$$GL_{ndc} = C \times (255 - GL_{iis})/(255 - GL_{ns})$$

in which C stands for the increase in the mean pixel value of the articular cartilage surface caused by Indian ink uptake in cartilage without degeneration. A piece of cartilage with a normal macroscopic appearance from each P1 specimen was used for calculation of the mean value for C.

The relative amount of degenerated articular cartilage, called the Cartilage Degeneration Index (CDI), was then calculated as follows:

$$CDI = \{[GL_{iis} - C \times (255 - GL_{iis})/(255 - GL_{ns}) - GL_{ns}]/(255 - GL_{ns})\} \times 100\%$$

DETERMINATION OF REPRODUCIBILITY AND ERROR ANALYSIS

The experimental setup was evaluated for the reproducibility of the articular cartilage alignment, the influence of the isotonic saline solution in the bath, magnification and distortion effects, steadiness of the illumination, and reproducibility of the gray level measurements of the articular cartilage surface and the Indian ink staining procedure. To achieve this, 10 digital images were obtained of each nonstained P1 specimen. For all images, the P1 specimens were remounted in the clamp and repositioned in the bath. The bath solution was refreshed after every 10^{th} measurement. The same procedure was performed for the Indian ink stained P1 specimens. In those instances, the articular cartilage surface was cleaned of Indian ink after each measurement by gently rubbing and rinsing with isotonic saline solution.

STATISTICAL ANALYSIS

Differences between repetitive measurements were analysed by use of a repeated measurement and multiple comparison test (SPSS, version 10.0[7]). The level of significance was set at $p < 0.05$.

RESULTS

Large variations in the amount of Indian ink uptake between the 6 specimens and across the articular surface of each specimen were found. In 2 specimens, small amounts of Indian ink uptake were found at the medial and lateral dorsal joint margins only. In 3 specimens, Indian ink uptake extended to the medial central fovea. In 1 specimen, Indian ink uptake was by wear lines across the entire cartilage surface.

The mean ± s.e.m. C value (increase in pixel value caused by Indian ink uptake in articular cartilage without degeneration) was 25 ± 3. This resulted in CDI values that ranged from 9.2 ± 5.7% (minor OA characteristics at the medial and lateral dorsal margins) to 41.5 ± 3.6% (severe OA changes with extension to the medial central fovea).

REPRODUCIBILITY OF THE PROCEDURE

Six repetitive measurements of the GL_{ns} (mean pixel value of the nonstained articular cartilage surface) after repositioning of the specimens yielded no significant differences among measurements. The GL_{ns} values of the 6 specimens varied between 43 ± 2 and 58 ± 1. Six repetitive measurements of the GL_{iis} (mean pixel value of the stained articular cartilage surface) yielded no significant differences among measurements. The GL_{iis} values of the 6 specimens varied between 89 ± 10 and 149 ± 7 (figure 2).

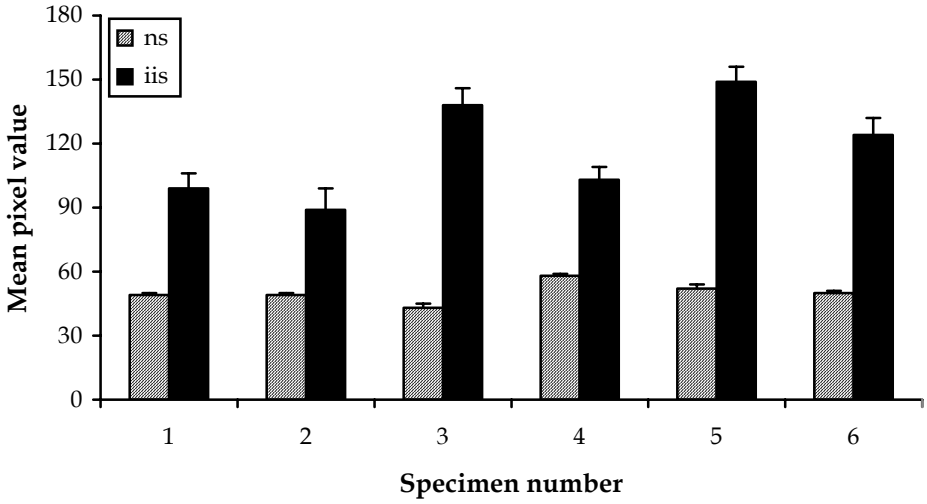

FIGURE 2: *Mean (± s.e.m.) pixel values (n = 6 measurements) of nonstained (ns) and Indian ink stained (iis) proximal articular cartilage surfaces of first phalanx (P1) specimens from 6 horses. Specimens were repositioned and restained (iis only) after each measurement. Measurements were made on the basis of maximum pixel values of 255. No significant ($p < 0.05$) differences in mean pixel values between the measurements were found for any specimens.*

MAGNIFICATION OR DISTORTION ERRORS

No significant differences were observed between mean (± s.e.m.) values of RRS_t (1.01 ± 0.01), RRS_b (1.03 ± 0.01), RRS_r (1.00 ± 0.01), and RRS_l (1.01 ± 0.01).

STEADINESS OF ILLUMINATION

The mean (± s.e.m.) pixel value of each gray scale reference calibration target did not differ significantly within images. Mean (± s.e.m.) values were 17 ± 2, 44 ± 2, 103 ± 2, and 238 ± 1 for the white, light gray, dark gray, and black targets, respectively.

INFLUENCE OF THE ISOTONIC SALINE SOLUTION IN THE BATH

Ten successive measurements of nonstained specimens resulted in no significant differences in the mean pixel values of the reference calibration standards within all images and between all successive images (figure 3). For the Indian ink stained specimens, no significant differences were found in the mean pixel values of the reference calibration targets within each image, but a significant increase in the mean pixel values of the white, light gray, and dark gray reference calibration targets was found after the 6th measurement. No significant increase in the mean pixel value of the black reference calibration standard was found.

DISCUSSION

The digital imaging technique for Indian ink stained cartilage specimens described in our study proved to be a reliable and highly reproducible method for the quantitative assessment of degenerated cartilage across the entire proximal articular surface of P1. Although the technique is not particularly difficult, precision and accuracy are needed, and a standardised setup with regard to articular cartilage surface alignment, bath solution, calibration targets, lighting conditions, camera position, and staining procedure is essential. Further, some specific requirements should be taken into account.

As the proximal articular surface of the equine P1 is not flat, specimens had to be submerged in fluid to eliminate illumination reflection at the sites of cartilage curvature (Chang *et al.* 1997). The dependence of the reflected light intensity on the layer of fluid overlying the cartilage surface was neglected, as it has been shown by spectrophotometric measurements that the transmittance of light is decreased only slightly (3%) by a 4 mm path length of phosphate-buffered saline solution (Chang *et al.* 1997). The volume of fluid above the articular cartilage surface should theoretically be kept minimal, as the inevitable contamination of the bath solution

by tissue fluid or Indian ink will result in absorption of light and overrating of the mean pixel value of the articular cartilage surface.

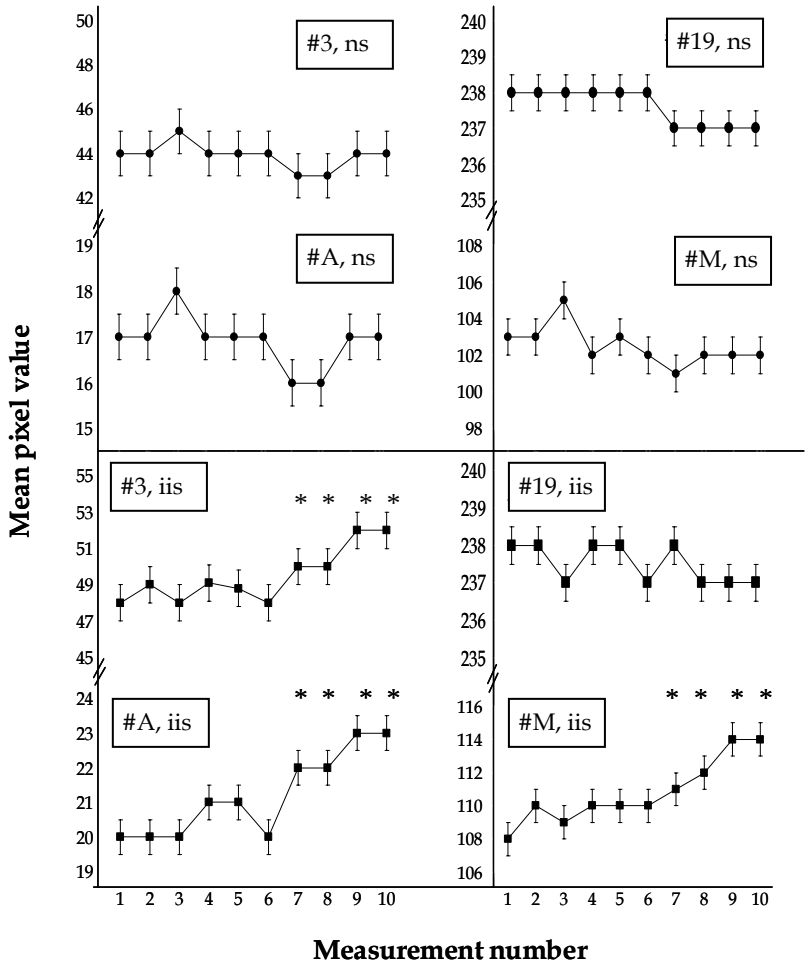

FIGURE 3: Mean (± s.e.m.) pixel values of the 4 reference calibration targets (white [#A], light gray [#3], dark gray [#M], and black [#19]) after 10 successive measurements of the nonstained (ns) and Indian ink stained (iis) proximal articular cartilage surfaces of 6 equine first phalanx (P1) specimens. Measurements were made on the basis of maximum pixel values of 255.
** Significantly different ($p < 0.05$) from preceding measurements*

However, contamination does not seem to be a major problem, and it is not necessary to refresh the bath solution after every measurement. No significant differences were found in the mean pixel values of the 4 reference calibration standards after 10 successive measurements of nonstained specimens, so refreshment of the isotonic saline solution after every 10th measurement is acceptable. For Indian ink stained specimens, refreshment after every 6th measurement is necessary, as significant differences in the mean pixel values of the white, light gray, and dark gray reference calibration standards were noticed after the 6th measurement. Significant differences in mean pixel values were not found for the black reference calibration standard, even after the 10th measurement, which most likely is a result of the fact that this standard has a mean pixel (238) value that approximates the maximum mean pixel value (255). These findings are in line with the study by Chang *et al*. (1997), who found only a slight (4%) reduction of light transmission after consecutive staining and submersion of 4 joints. As in our study, they also found a negligible (1.7%) distortion and magnification error. Whereas Chang *et al.* (1997) used a ring light source, we used 4 separate bulbs under darkroom conditions. The equal mean pixel values of the calibration targets along each of the 4 sides indicated that light conditions were identical at all sides.

The technique of Indian ink staining requires particular attention. Because Indian ink particles are either absorbed to the articular surface or trapped in fissures extending from the articular surface into the cartilage, particles can be removed by vigorous washing or wiping (Chang *et al.* 1997). Therefore, extreme care was taken to remove excess ink gently by only rinsing with 0.5 L of isotonic saline solution. The articular surface was constantly kept moist as drying out influences Indian ink uptake (Madsen *et al.* 1992). As no significant differences in the mean pixel value of the stained articular cartilage surfaces were found after repeated staining, it was concluded that the staining procedure was adequate and reproducible.

The degree of cartilage degeneration across the entire joint surface was expressed as the CDI. Theoretically, this index can range from 0 - 100% and represents the amount of articular cartilage across the entire joint surface that is stained with Indian ink. It is known that the intensity of ink staining is related to a reduction in the proteoglycan content (Ficat and Maroudas 1975; Maroudas *et al.* 1973). However, some Indian ink staining of articular cartilage occurs in the absence of degeneration (Chang *et al.* 1997). In an earlier study, 2 - 4% staining of nondegenerated cartilage by Indian ink was found, which was assessed as insignificant for correction (Chang *et al.* 1997). In our study we found an error of

approximately 10%, making correction necessary. The difference may be related to the different species that were studied, rabbits versus horses. The amount of nondegenerated cartilage is inversely proportional to the amount of degenerated cartilage, which indicates that the relative importance of Indian ink uptake by nondegenerated cartilage decreases with increasing stages of OA. Correction of the mean pixel value of the Indian ink stained articular cartilage surface was made on the basis of this assumption.

The CDI seems a valuable tool to quantify cartilage damage, but some considerations should be kept in mind. If cartilage ulceration has extended to the level of the underlying subchondral bone, the method of quantification of cartilage degeneration described here will be inadequate, as Indian ink does not stain subchondral bone. This makes the method particularly useful for the initial stages of cartilage degeneration, in which articular cartilage ulceration to the subchondral bone is absent, but less for advanced stages. Further, translating the 3-dimensional architecture of the joint surface into a 2-dimensional digital image has consequences for the ultimate assessment of articular cartilage degeneration, because articular cartilage geometry will influence the reflected light intensity. This problem is of minor importance for the proximal articular cartilage surface of the equine P1, but the larger the joint curvature, the more important this factor becomes. In an earlier experiment (Chang *et al.* 1997), the reflected light intensity was within 5% of the maximum in a central region of a 6.4-mm-diameter spherical target, defined by surface angles of 36° or less with the horizontal. Near the edges of the spherical target, the intensity of the reflected light fell, but remained within 10% of the maximum at surface angles of 50° with the horizontal (Chang *et al.* 1997). For the equine fetlock joint, this indicates that this factor has to be taken into account if cartilage damage at the metacarpal or metatarsal side of the joint is going to be quantitated with this technique.

The method described in our study provides a highly accurate and reproducible method for the quantification of Indian ink uptake by degenerated cartilage of the proximal articular surface of P1 and, hence, for the overall assessment of damage to the articular cartilage. Quantification of articular cartilage degeneration, enabling overall joint OA scoring, is of great relevance to OA research in horses (May *et al.* 1996). Quantification techniques for early OA such as the one described in our study are expected to be of great help for the screening of potential molecular markers for this crippling disorder (Myers 1999). Once markers have been identified, OA quantification techniques may serve as a standard to assess the relative importance of these markers and to establish the guidelines for the

interpretation of specific marker levels. The same applies to other techniques that are being used to detect early cartilage damage in the live animal such as magnetic resonance imaging (Adams *et al.* 1991) and arthroscopic inspection (Ayral *et al.* 1996). It should be realised, however, that variables such as the CDI reflect cartilage damage only. No other features of OA, such as changes in the subchondral bone (Kawcak *et al.* 2001) or joint capsule (Palmer and Bertone 1994), are taken into account. This is a strength and weakness of the method. An advantage is that the so-far obscure early stages of OA can now be quantified, a disadvantage is that the CDI refers to only a part of the problem. The relationship of the CDI with clinical assessments, such as lameness scores, therefore seems worthwhile to investigate.

MANUFACTURERS' ADDRESSES

[1] Vink Co., Didam, The Netherlands
[2] Eastman Kodak Co., Rochester, NY, USA
[3] Philips Co., Eindhoven, The Netherlands
[4] Olympus Co., Hamburg, Germany
[5] Scion Corp., Frederick, Maryland, USA
[6] Royal Talens Inc., Apeldoorn, The Netherlands
[7] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER IV -

QUANTIFICATION AND AGE-RELATED DISTRIBUTION OF ARTICULAR CARTILAGE DEGENERATION IN THE EQUINE FETLOCK JOINT

Brommer H - Van Weeren PR - Brama PAJ - Barneveld A

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Equine Vet J 2003, 35: 697-701

SUMMARY

Reasons for performing study: **The equine fetlock joint has the largest number of traumatic and degenerative lesions of all joints of the appendicular skeleton.**

Objective: **To gain insight into the distribution of cartilage degeneration across the articular surface in relation to age in order better to understand the dynamic nature and progression of osteoarthritis (OA)**

Hypothesis: **That there would be a specific age-related distribution pattern of cartilage degeneration in the equine metacarpophalangeal (MCP) joint.**

Methods: **The proximal articular cartilage surfaces of the first phalanges (P1) of 73 slaughter horses (age range 0.4 - 23 years) with different stages of OA were scored semiquantitatively on a 0 - 5 scale and also assessed quantitatively using the Cartilage Degeneration Index (CDI_{P1}), which ranges from 0 - 100%. Furthermore, CDI values were determined for special areas of interest: medial dorsal surface (CDI_{mds}), lateral dorsal surface (CDI_{lds}), medial central fovea (CDI_{mcf}), and lateral central fovea (CDI_{lcf}). Correlations were calculated between CDI_{P1} values and macroscopic scores and between CDI_{P1} values and age. For each macroscopic score, CDI values (mean $\pm$ s.d.) at the specific areas of interest were determined and statistically analysed ($p < 0.05$).**

Results: **There was a high correlation between the semiquantitative macroscopic score and the quantitative CDI_{P1} values ($r = 0.92$, $p < 0.001$). A macroscopic score of 0 (*i.e.* no obvious cartilage degeneration) corresponded with a CDI_{P1} value of 2.5 $\pm$ 2.8% and a macroscopic score of 5 (*i.e.* severe cartilage degeneration in localised areas) with a value of 38.1 $\pm$ 7.9%. There was a moderate but highly significant correlation between the CDI_{P1} value and the age of the horses ($r = 0.41$, $p < 0.001$). Highest CDI values were calculated for the medial dorsal surface (from 10.6 $\pm$ 2.8% at macroscopic grade 0 to 63.1 $\pm$ 8.4% at grade 5). At the lateral dorsal surface these values were 5.9 $\pm$ 1.4% and 47.2 $\pm$ 10.4%, respectively. The CDI_{mcf} and CDI_{lcf} were significantly lower ($p < 0.05$) than the CDI_{mds} and CDI_{lds} at all grades. The CDI_{mcf} ranged from 1.0 $\pm$ 2.9% at grade 0 to 43.7 $\pm$ 9.1% at grade 5. Laterally these values were 1.5 $\pm$ 2.6% and 15.2 $\pm$ 6.2%, respectively.**

Conclusions: **CDI grading increased from lateral to medial and from central to dorsal. This specific distribution pattern confirms the heterogeneous nature of the OA process and strongly supports an important role for biomechanical loading, superimposed on age-related changes, in the spread of the disorder over the joint.**

Potential relevance: **Knowledge of the development of OA across the articular surface is essential for understanding the dynamic nature and progression of the**

disease and can form a basis for improvements in diagnostic and therapeutic approaches to degenerative joint disease.

INTRODUCTION

The equine fetlock joint has the largest number of traumatic and degenerative lesions of all joints of the appendicular skeleton (Pool 1996). Of these, most are osteoarthritic in nature. Osteoarthritis (OA) is defined as a disease of diarthrodial joints characterised by variable degrees of articular cartilage destruction, subchondral bone sclerosis, and marginal osteophyte formation (Raker 1966).

In the equine fetlock joint, degenerative cartilage changes can be found at various sites. Most of the pathologic signs are encountered at the articular cartilage surface of the first phalanx (P1). Osteochondral fragmentation and OA changes are seen frequently at the proximodorsal articular margin and, less frequently, at the proximopalmar/-plantar articular margins. At both the dorsal and proximopalmar/-plantar margins, the medial side has been reported to be involved more frequently than the lateral side (Birkeland 1970, Hardy *et al*. 1987, Kawcak and McIlwraith 1994, Petterson and Ryden 1982, Whitton and Kannegieter 1994). Another common feature is the so-called 'wear lines' (also referred to as linear grooving) across the cartilage surface of P1, the third metacarpal bone (MCIII) and the proximal sesamoid bones (PSBs). These run in the direction of the articulation and may be accompanied by other joint lesions, such as chip fractures, but may be found independently as well (Pool and Meagher 1990). They have been incriminated as the earliest macroscopic sign of degenerative joint disease (Kennedy and Palmer 1985).

With respect to pathogenesis, biomechanical factors are commonly believed to be of great importance (Freeman 1980, Radin 1983, Radin *et al*. 1972). The high susceptibility to injury of the fetlock joint has been related to the relatively small surface area, large range of motion, and impact of full bodyweight during the stance phase (Pool 1991 and 1996). It has been proposed that repeated overextension of the joint may result in impaction of the proximodorsal articular margin of P1 upon the distal end of MCIII and may therefore play a prominent role in the high incidence of joint pathologies found at these sites (McIllwraith 1996, Palmer and Bertone 1996, Pool 1991, Schryver *et al*. 1978). Chondropathies at the palmar/plantar articular surface of P1 have been associated with the attachment of several ligaments, such as the short and cruciate sesamoidean ligaments, that extend from the distal parts of the PSBs to the proximopalmar/-plantar aspects of

P1 (Weaver *et al*. 1992). These attachments are challenged by repetitive peak loading too.

So far, no major cross-sectional study has been performed that describes the distribution of OA lesions across the proximal articular surface of P1. Insight into the distribution of these lesions in relation to age should be helpful in understanding the dynamic nature and progression of OA. In the present study, a recently developed method to objectively quantify the degree of cartilage degeneration across the entire joint surface (Brommer *et al*. 2003) was used to study the presence and distribution of OA lesions in a large number of fetlock joints from horses of varying ages. The objective of the present study was to quantify age-related articular cartilage degeneration in the entire equine fetlock joint with different grades of OA, and characterise the expansion of cartilage degeneration in this joint by quantifying articular cartilage degeneration in special areas of interest.

MATERIALS AND METHODS

SAMPLE COLLECTION

Seventy-three right front fetlock joints from slaughter horses (age range 5 months - 23 years) with different grades of OA were harvested immediately after death and stored at –20°C until processing. No clinical data of these horses were available. One day before the measurements, the fetlock joints were thawed and opened. The proximal two-thirds of P1 were isolated from the rest of the limb.

QUANTITATIVE ASSESSMENT OF CARTILAGE DEGENERATION

Articular cartilage degeneration was quantified using the recently described Cartilage Degeneration Index (CDI) (Brommer *et al*. 2003). The CDI is based on the fact that degenerated articular cartilage, which is depleted of proteoglycans and shows surface fibrillation, takes up Indian ink particles, whereas intact cartilage is hardly stained (Chang *et al.* 1997, Madsen *et al*. 1992). Briefly, the amount of Indian ink uptake across the entire cartilage surface is quantified by digital imaging of the native and Indian ink stained articular cartilage surfaces under standardised conditions. First, the native P1 specimens were mounted in a clamp with the proximal articular cartilage surface facing up. The specimens were submerged in a bath containing isotonic saline solution at room temperature with the fluid level 1 - 2 mm above the most proximal parts of the articular surface. The system was set up under darkroom conditions. Illumination was provided by 4 100W lamps[1]. Above the P1 specimen a digital camera (Camedia C-2500L)[2] was mounted and centred on the sulcus of P1. The distance between the lens of the camera and the

fluid level was 12 cm, the lens was maximally zoomed in and the supermacro close-up photographic mode with high quality preference was chosen. The diaphragm was fully opened, shutter speed was 1/50 s and the camera was set on autofocus. The flashlight function was turned off. Professional grey scale reference calibration targets (Q13)[3] in the shades white, light grey, dark grey, and black were positioned on a mold around P1 and were also digitised, serving as internal control of the acquired digital images. Images were stored on computer using commercially available software (Olympus Media Suite Professional, version 1.02.0504)[2].

After imaging the native cartilage surfaces, the P1 specimens were turned upside down in a bath, containing Indian ink[4], for 5 minutes. Excess ink solution was removed by gentle rinsing with 0.5 L of isotonic saline solution. The imaging procedure after staining was identical to that for the nonstained specimen. A typical example of a digitised native and Indian ink stained cartilage surface with the greyscale reference calibration targets is illustrated in figure 1.

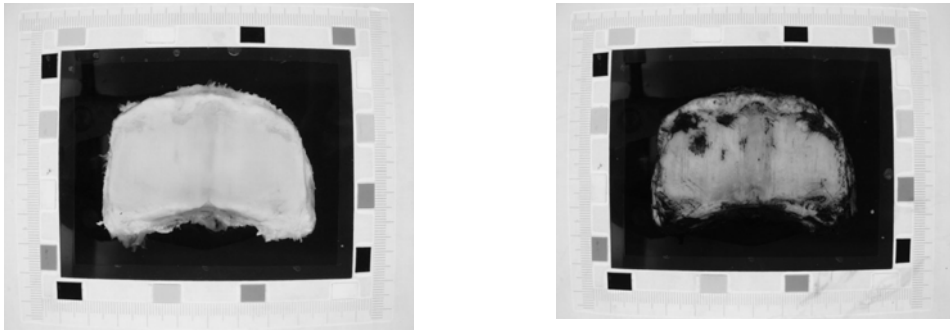

FIGURE 1: *Typical illustration of a digital picture of the native (left) and the Indian ink stained (right) surface of the proximal articular cartilage of the first phalanx (P1). The articular surfaces are surrounded by the grey scale reference calibration targets.*

Computer-assisted grey level analysis of the digitally imaged cartilage surfaces were performed. The mean pixel value was determined as a measure for grey level, using image analysis software (Scion Image Beta 4.0.2)[5]. Digital images were processed only when the grey levels of the professional calibration targets were within the reference value ranges. The increase in mean grey level of the articular

surface is the basis for calculation of the CDI (range 0 - 100%) using a specially developed formula that corrects for the fact that intact cartilage shows some uptake (Brommer *et al*. 2003). The Indian ink stained proximal articular cartilage surface was also macroscopically scored semiquantitatively using a 0 - 5 scale as proposed by Cantley *et al.* (1999).

DISTRIBUTION OF ARTICULAR CARTILAGE DEGENERATION

Apart from the overall CDI value (CDI_{P1}), CDI values of 4 circular areas of interest (1 cm diameter) were determined. These were the medial dorsal surface (CDI_{mds}), halfway between the medial edge of the sulcus articularis and the medial border of the articular surface, adjacent to the dorsal articular margin; the lateral dorsal surface (CDI_{lds}) at the same location, but laterally; the medial central fovea (CDI_{mcf}), halfway between the medial edge of the sulcus articularis and the medial border of the articular surface, and halfway between the dorsal and palmar articular margins; and the lateral central fovea (CDI_{lcf}), located at the same level but laterally (figure 2).

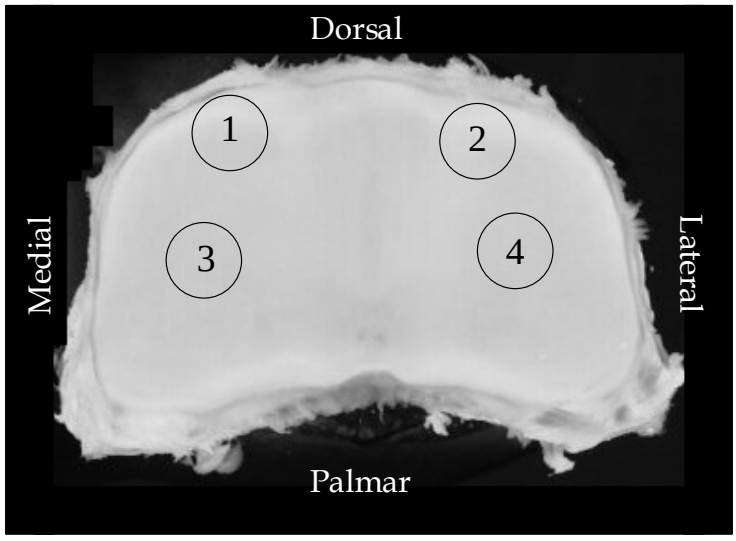

FIGURE 2: *Proximal articular cartilage surface of the first phalanx (P1). Cartilage Degeneration Index (CDI) values were determined for the total surface and for the 4 areas of interest: medial dorsal surface (1), lateral dorsal surface (2), medial central fovea (3), and lateral central fovea (4).*

DATA ANALYSIS

Pearson correlation coeffients (r) with matching p values were determined for the correlation between CDI_{P1} and the macroscopic scoring grade, and for correlation between CDI_{P1} and the age of the horses.

Differences between the values of CDI_{P1}, CDI_{mds}, CDI_{lds}, CDI_{mcf}, and CDI_{lcf} were statistically evaluated in ANOVA multiple comparison test. All tests were run using SPSS software (version 10.0)[6]. The level of significance was set at $p < 0.05$.

RESULTS

MACROSCOPIC GRADING AND QUANTIFICATION OF CARTILAGE DEGENERATION

Following macroscopic scoring 2, 15, 26, 13, 13, and 4 specimens were classified as grades 0, 1, 2, 3, 4, and 5, respectively (figure 3). A macroscopic score of 0 (*i.e.* no obvious cartilage degeneration) corresponded with a CDI_{P1} value of mean $\pm$ s.d. 2.5 $\pm$ 2.8% and a score of 5 (*i.e.* severe cartilage degeneration) with a CDI_{P1} value of 38.1 $\pm$ 7.9%. From grade 1 onwards, the CDI_{P1} values were significantly different between each grade ($p < 0.05$). There was a high level of correlation ($r = 0.92$, $p < 0.001$) between the semiquantitative macroscopic score and the quantitative CDI_{P1} value.

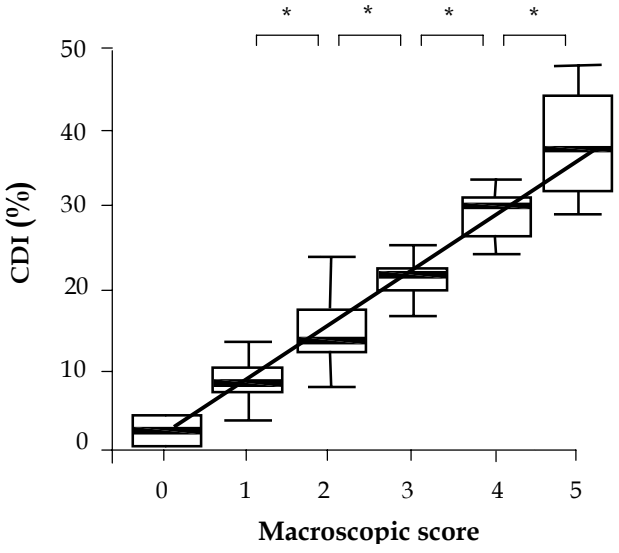

FIGURE 3: *Box plot showing the correlation between semiquantitative macroscopic grading according to Cantley et al. (1999) and the quantitative Cartilage Degeneration Index (CDI, %) ($r = 0.92$, $p < 0.001$, $n = 73$). Regression line indicated.*
** $p < 0.05$*

RELATION WITH AGE

There was a moderate, but significant correlation between CDI_{P1} and the age of the horses ($r = 0.41$, $p < 0.001$) (figure 4).

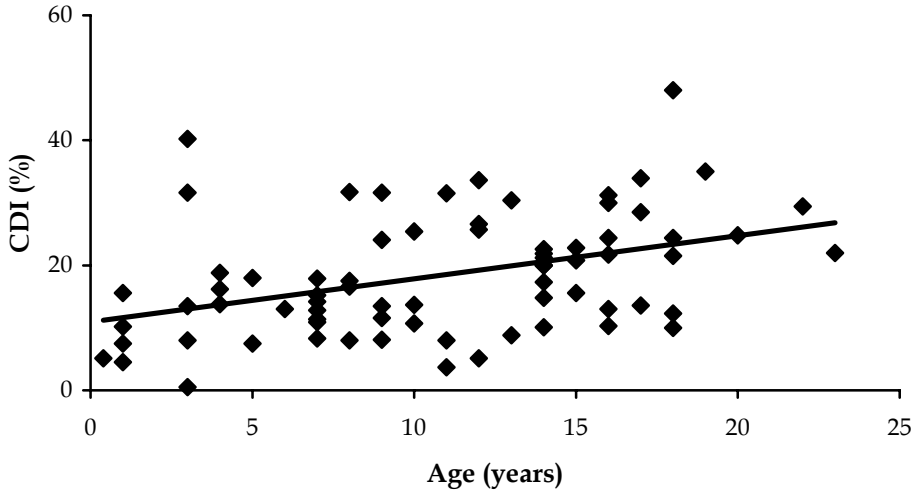

FIGURE 4: *Scatter plot showing the correlation between the Cartilage Degeneration Index (CDI, %) of the proximal articular cartilage surface of the first phalanx (P1) and the age of the horses (years) ($r = 0.41$, $p < 0.001$, $n = 73$). Regression line indicated.*

DISTRIBUTION OF CARTILAGE DEGENERATION IN RELATION TO SEVERITY OF OA

Highest CDI values were calculated for the medial dorsal surface of the joint, ranging from mean $\pm$ s.d. 10.6 $\pm$ 2.8% at macroscopic grade 0 to 63.1 $\pm$ 8.4% at macroscopic grade 5 (figure 5). For the lateral dorsal surface, CDI values varied from 5.9 $\pm$ 1.4% to 47.2 $\pm$ 10.4% at grades 0 and 5, respectively (figure 5). Only at grade 5, the differences between CDI_{mds} and CDI_{lds} were statistically significant ($p<0.05$).

CDI_{mcf} and CDI_{lcf} were significantly lower ($p<0.05$) than CDI_{mds} and CDI_{lds} at all grades except grade 0 (figure 5). In fact, no cartilage degeneration was found at these sites in joints that were macroscopically graded 0. From grades 1 – 5, the CDI_{mcf} increased from 1.0 $\pm$ 2.9% to 43.7 $\pm$ 9.1% (figure 5). For the lateral central fovea, these values were 1.5 $\pm$ 2.6% and 15.2 $\pm$ 6.2%, respectively (figure 5). Again, only at grade 5, the differences between CDI_{mcf} and CDI_{lcf} were statistically significant ($p < 0.05$).

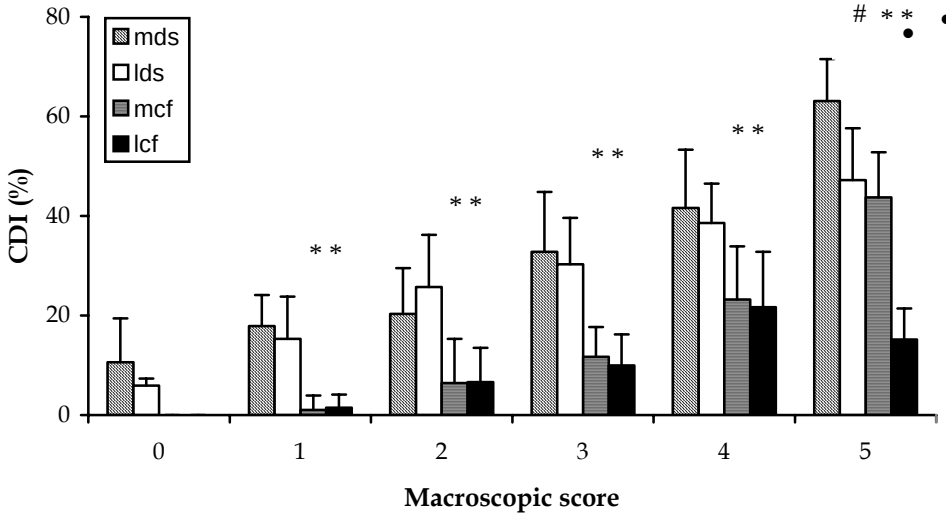

FIGURE 5: *Cartilage Degeneration Index (CDI, mean ± s.d, %) for the medial dorsal surface (mds), lateral dorsal surface (lds), medial central fovea (mcf), and lateral central fovea (lcf) of the proximal articular cartilage surface of the first phalanx (P1) classified according to macroscopic scoring.*
** $p < 0.05$ when compared to mds and lds*
 # $p < 0.05$ when compared to mds
• $p < 0.05$ when compared to mcf

DISCUSSION

The Cartilage Degeneration Index (CDI) has previously been shown to be a reliable tool for the quantification of articular cartilage damage across a joint surface (Brommer *et al*. 2003). In the present study this was confirmed, as in this relatively large series of specimens there was an excellent correlation with the semiquantitative scoring technique described previously by Cantley *et al*. (1999). Determination of articular cartilage degeneration was restricted to the articular cartilage surface of P1, as in this part of the joint lesions are reported most frequently (Cantley *et al*. 1999, Pool 1996) and because, so far, the CDI has been validated for this articular surface only.

No obvious cartilage degeneration corresponded with a CDI_{P1} of 2.5 ± 2.8% and in joints with severe cartilage degeneration the CDI_{P1} increased up to 38.1 ± 7.9%. Therefore, loss of normal articular cartilage in cases with severe OA may amount to somewhat less than 40% of the entire joint surface. Unfortunately, no clinical

data were available for these horses and no inferences can therefore be made about the relationship of the area affected and the clinical presentation.

In man, there is a strong relationship between articular cartilage degeneration and increasing age. This process has been related to high levels of nonenzymatic glycation (NEG) products, such as pentosidine crosslinks, which are known to accumulate with age, resulting in a stiffer and more brittle collagen network of the extracellular cartilage matrix (Verzijl *et al*. 2000). In equine articular cartilage, there is an age-related increase in pentosidine crosslinks too, although absolute numbers are much lower than reported in man (Brama *et al*. 2000). Age-related changes consistent with OA have been shown in the fetlock joints of wild horses (Cantley *et al*. 1999). In that study, it was postulated that there was an age-related OA process naturally present in the equine species, with stress of racing and training accelerating this ageing process in performance horses. The moderate, but highly significant correlation of cartilage degeneration found in the present study seems to support the conclusion that OA may, in principle, be a naturally occurring age-related process in the horse.

Degenerated cartilage was not evenly distributed over the joint. The spread of affected areas follows a typical pattern with increasing severity of OA. Initial cartilage degeneration started invariably at the medial dorsal margin of P1, extending to the lateral dorsal margin. With a further increase in OA severity, cartilage degeneration appeared at the medial and ultimately at the lateral central fovea. The difference in frequency of OA between the dorsal and central areas can most probably be explained by differences in loading. Brama *et al.* (2001) showed that the central area of the proximal articular surface of the equine P1 is subjected to constant, but relatively moderate loading. The dorsal articular area is a noncontact area when the horse moves at slow gaits, but becomes heavily loaded during galloping and jumping. It is probable that this intermittent loading during locomotion, with very high and sudden loading during strenuous exercise and/or athletic performance, causes repetitive episodes of trauma, making the area pivotal in the initiation and progression of the disease (McIlwraith 1996, Palmer and Bertone 1996). Crucial to the fact that OA may start at the dorsal margins rather than in the central area of the joint may be the heterogeneous nature of the biochemical composition of the tissue. Cartilage at the joint margins has the highest levels of collagen content and hydroxylysylpyridinoline crosslinks and the lowest levels of glycosaminoglycans (Brama *et al.* 2000). The more centrally located sites have an opposite pattern: higher contents of glycosaminoglycans with variable but faster turnover rates, and lower contents of collagen and

hydroxylysylpyridinoline crosslinks (Brama *et al.* 2000, Maroudas 1980, Maroudas *et al*. 1992, Todhunter 1996). Collagen is known to have an extremely low turnover rate (Maroudas 1980, Maroudas *et al.* 1992, Todhunter 1996). Therefore, although the dorsal areas are better prepared for heavy loading by their higher collagen and crosslink content, once damage has occurred this will be repaired much more slowly, offering an insidious disease such as OA a chance to develop. The medial to lateral difference is less obvious, but consistent. It is in line with clinical reports that mention a higher incidence medially than laterally (Birkeland 1970, Hardy *et al*. 1987, Kawcak and McIlwraith 1994, Petterson and Ryden 1982, Whitton and Kannegieter 1994). It is probable that biomechanical factors again play a role, as loading of the proximal articular surface of P1 is asymmetrical and higher in the medial area due to the eccentric position of the centre of gravity of the horse in relation to the axis of the limb. This difference was not demonstrated in the study by Brama *et al.* (2001), but they loaded the limbs along the limb axis in an *in vitro* setting. The medial to lateral distribution pattern may have important clinical implications, as it is a clear indication that asymmetrical weightbearing, whether natural in the form of conformational faults or induced by the application of unilateral wedges, can strongly influence the 3-dimensional behaviour of the fetlock joint and therefore the development of cartilage degeneration (Chateau *et al*. 2001).

In conclusion, quantification of articular cartilage degeneration and description of its distribution is important in the understanding of the development and progression of OA. This study presents a comprehensive and quantitative description of OA-related articular cartilage degeneration in the equine fetlock joint in a range of horses of various ages. Increases in severity of OA from lateral to medial and from central to dorsal over the P1 joint surface confirm the heterogeneous nature of the OA process and strongly support the role of biomechanical loading, superimposed on age-related changes, in the spread of the disorder over the joint.

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MANUFACTURERS' ADDRESSES

[1] Philips Co., Eindhoven, The Netherlands
[2] Olympus Co., Hamburg, Germany
[3] Eastman Kodak Co., Rochester, NY, USA

[4] Royal Talens, Apeldoorn, The Netherlands
[5] Scion Corp., Frederick, Maryland, USA
[6] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER V -

DIFFERENCES IN THE TOPOGRAPHICAL DISTRIBUTION OF ARTICULAR CARTILAGE DEGENERATION BETWEEN THE EQUINE METACARPO- AND METATARSOPHALANGEAL JOINTS

Brommer H - Brama PAJ - Barneveld A - Van Weeren PR

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Equine Vet J 2004, 36: 506-510

SUMMARY

Reasons for performing study: **The equine metacarpophalangeal (MCP) joint and metatarsophalangeal (MTP) joint, although having virtually the same geometrical appearance, differ in the prevalence of joint pathologies such as osteochondral fragmentation, and in biomechanical behaviour. The recently developed Cartilage Degeneration Index (CDI) technique offers a possibility to quantitatively assess differences in cartilage degeneration between these joints and to compare these with known differences in biomechanics and clinical observations.**

Objectives: **To compare the topographical distribution of articular cartilage degeneration across the proximal articular surface of the proximal phalanx (P1) in the equine fore- and hindlimb.**

Methods: **In 24 distal hindlimbs from 24 horses, articular cartilage degeneration of the proximal articular surface of P1 was quantified, using the CDI. The overall CDI value (CDI_{P1}) and CDI values of 6 areas of interest were determined: the medial dorsal surface (mds), lateral dorsal surface (lds), medial central fovea (mcf), lateral central fovea (lcf), medial plantar surface (mps), and lateral plantar surface (lps). The joints were divided into 4 equally sized groups of increasing CDI_{P1} values. From an existing CDI database of MCP joints, 24 joints were selected with matching CDI_{P1} values to the MTP joints and CDI values for the same areas of interest were determined.**

Results: **In both the MCP and MTP joint, highest CDI values were determined at the dorsal articular surfaces. Values were not significantly different between fore- and hindlimbs. In contrast to the MCP joint, CDI values at the plantar joint margin were significantly higher compared to CDI values in the central sites in the MTP joint. CDI values for the plantar surfaces of P1 were significantly higher than those for the palmar surfaces in the forelimb in joints with advanced stages of OA. CDI values for the central regions of P1 were significantly lower in the hindlimb compared with the forelimb in joints with severe OA.**

Conclusions: **In both fore- and hindlimbs, initial cartilage degeneration started at the dorsal articular margin of P1. There was a major difference in the spread of cartilage degeneration: in the forelimb both the central and palmar parts are about equally involved, whereas in the hindlimb the plantar parts were significantly more and the central parts significantly less involved. These differences can be linked to differences in biomechanical loading reported elsewhere.**

Potential relevance: **This study supports the hypothesis that differences in biokinematics between fore- and hindlimbs are associated with differences in the development of cartilage degeneration and other joint pathologies such as osteochondral fragmentation in the MCP and MTP joints. This information is indispensable for a better understanding of the dynamic nature and progression of these joint disorders and may be of help when monitoring the effects of therapeutic interventions and preventive measures.**

INTRODUCTION

Joint injury and joint disease are consistently diagnosed as principal causes of lameness in the equine athlete (Pool 1996, Rossdale *et al*. 1985). Of all joint disorders, osteoarthritis (OA) is the most important (Pool 1996). It is generally thought that biomechanical stress is an important initiating factor in the development of OA, as it may result in the release of matrix-degrading enzymes, shifting the delicate balance between anabolic and katabolic processes in the extracellular matrix (Freeman 1980, Helminen *et al*. 2000, McIlwraith 1996, Radin 1983, Radin *et al*. 1972).

Of all joints, the metacarpophalangeal (MCP) joint is most commonly affected (Pool 1996). Advanced OA in the MCP joint is characterised, as in any other joint, by cartilage degeneration, subchondral bone sclerosis and osteophyte formation (Pool 1996), but osteochondral fragmentation is also often seen (Kawcak and McIlwraith 1994, Yovich and McIlwraith 1986).

The metatarsophalangeal (MTP) joint has a virtually identical geometry but is less frequently affected with respect to osteochondral fragmentation. There are also differences in localisation. In the forelimb, the proximodorsal articular margin of the proximal phalanx (P1) is more frequently involved than the proximopalmar articular margin, whereas in the hindlimb osteochondral fragmentation is more frequently seen at the proximoplantar aspects of P1 (Birkeland 1970, Fortier *et al.* 1995, Hardy *et al*. 1987, Nixon and Pool 1995, Sandgren 1988, Whitton and Kannegieter 1994). Fore- and hindlimbs have in common that dorsal and palmar/plantar osteochondral fragments (POFs) are more commonly encountered in the medial than in the lateral part of the joint (Fortier *et al.* 1995, Kawcak and McIlwraith 1994, Nixon and Pool 1995, Roneus *et al.* 1998, Sandgren 1988, Whitton and Kannegieter 1994, Yovich and McIlwraith 1986).

Differences in clinical manifestation of joint disease between these similar articulations may be related to differences in biomechanical behaviour of the fore- and hindfetlocks, as reported by various kinematical studies (Back *et al*. 1995,

Holmström *et al.* 1994, Martinez-delCampo *et al*. 1991) and ground reaction force (GRF) experiments (Merkens *et al*. 1985 and 1993, Pratt and O'Connor 1976, Ueda *et al*. 1981). The outcome of these kinematic and GRF studies can be summarised in the concept that the forelimb acts as a propulsive strut, making the forelimb 'bouncing' at impact. The hindlimb contributes more to propulsion, which results in a more sliding impact at the beginning of the stance phase (Back *et al.* 1995, Merkens *et al.* 1993). Biomechanical stress has been reported to have a detrimental effect on joints only when impulsive loading is combined with the evocation of oscillations (Radin *et al.* 1991). It has, therefore, been hypothesised that, as the combination of impulsive loading with rapid oscillations is higher in the forelimb than in the hindlimb due to the different biokinetic behaviour (Back *et al*. 1995, Merkens and Schamhardt 1994), this could be the basis of differences in the clinical manifestation of joint disease.
In a recent study, the topographical distribution pattern of articular cartilage degeneration across the proximal articular surface of P1 in the MCP joint has been described (Brommer *et al*. 2003b). It is the aim of the present study to compare the development of OA in the MCP joint with development of OA in the MTP joint, in order to test the hypothesis that the spread of OA-affected areas on the joint surface is distinctly different between the MCP and MTP joints and is in line with the differences in clinical manifestation of osteochondral fragmentation and differences in biomechanical behaviour.

MATERIALS AND METHODS

SAMPLE COLLECTION

Twenty-four distal hindlimbs from 24 horses (age 1 - 33 years) were collected in a slaughterhouse immediately after death of the animals. The limbs were stored at -20°C until processing. No clinical data of these horses available. One day before the measurements, the limbs were thawed and opened. The proximal two-thirds of P1 were isolated from the rest of the limb.

QUANTITATIVE ASSESSMENT OF CARTILAGE DEGENERATION

Articular cartilage degeneration of the entire proximal articular surface of P1 was quantified using the recently described Cartilage Degeneration Index (CDI) technique (Brommer *et al*. 2003a), in which degenerated articular cartilage, depleted of proteoglycans and showing surface fibrillation, takes up Indian ink particles whereas intact cartilage is hardly stained (Chang *et al.* 1997, Madsen *et al.* 1992). The amount of Indian ink uptake across the entire cartilage surface is

quantified by digital imaging of the native and the Indian ink stained articular cartilage surfaces under standardised conditions. The mean pixel value is then determined as a measure for grey level and the increase in mean grey level of the articular surface is the basis for calculation of the CDI (range 0 - 100%), using a specially developed formula that corrects for the fact that intact cartilage will show some uptake (Brommer *et al*. 2003a).

TOPOGRAPHICAL DISTRIBUTION OF ARTICULAR CARTILAGE DEGENERATION

Apart from the overall CDI value for the entire proximal articular surface of P1 (CDI_{P1}), CDI values of 6 circular areas of interest (diameter 1 cm) were determined. These were the medial dorsal surface (CDI_{mds}), halfway between the medial edge of the sulcus articularis and the medial border of the articular surface, adjacent to the dorsal articular margin; the lateral dorsal surface (CDI_{lds}), at the same location but laterally; the medial central fovea (CDI_{mcf}), halfway between the medial edge of the sulcus articularis and the medial border of the articular surface, and halfway between the dorsal and palmar articular margins; the lateral central fovea (CDI_{lcf}), located at the same level but laterally; the medial plantar surface (CDI_{mps}), halfway between the medial edge of the sulcus articularis and the medial border of the articular surface, adjacent to the plantar articular margin; and the lateral plantar surface (CDI_{lps}) at the same location, but laterally (figure 1).

DATA ANALYSIS

The joints were divided into 4 groups of 6 joints, based on the CDI_{P1} values. Group 1 consisted of 6 joints with the lowest CDI_{P1} values, and group 4 contained 6 joints with the highest CDI_{P1} values. Groups 2 and 3 formed 2 sets of 6 joints with intermediate CDI_{P1} values.

From an existing database that contains digital images of the proximal articular surface of 147 P1s of forelimbs (produced under the same standardised conditions as described by Brommer *et al*. 2003a), 24 MCP joints of 24 horses were selected for which the CDI_{P1} value matched with that determined for the MTP joints in this study. These MCP joints all originated from horses other than those from which the MTP joints were used. In this group of MCP specimens, the CDI values for the same areas of interest of P1 were determined in an identical way.

Differences between the values of CDI_{mds}, CDI_{lds}, CDI_{mcf}, CDI_{lcf}, CDI_{mps}, and CDI_{lps} in both fore- and hindlimbs were statistically evaluated using an ANOVA multiple comparison test and differences between fore- and hindlimbs were evaluated using

an independent samples t-test. All tests were run using SPSS software (version 10.0)[1]. The level of significance was set at $p < 0.05$.

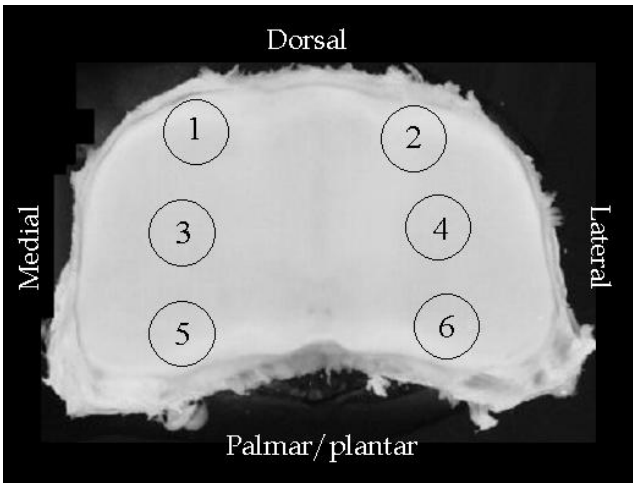

FIGURE 1: *Proximal articular cartilage surface of the proximal phalanx (P1). Cartilage Degeneration Index (CDI) values were determined for the total surface and for the 6 areas of interest: medial dorsal surface (1), lateral dorsal surface (2), medial central fovea (3), lateral central fovea (4), medial palmar/ plantar surface (5), and lateral palmar/ plantar surface (6).*

RESULTS

The CDI_{P1} value ranges for the MTP joints were 7.2% - 14.6% for group 1, 14.6% - 18.1% for group 2, 18.9% - 22.9% for group 3, and 23.9% - 29.8% for group 4, respectively. For the matching group of MCP joints these values were 7.5% - 14.2%, 15.2% - 17.9%, 18.8% - 24.1 % and 24.4 – 30.0% for groups 1, 2, 3, and 4, respectively.

DISTRIBUTION OF CARTILAGE DEGENERATION IN THE MCP JOINT

Highest CDI values (mean $\pm$ s.d.) were measured for the medial dorsal surface, ranging from 19.5 $\pm$ 5.1% in group 1 to 42.7 $\pm$ 15.9% in group 4 (figure 2). Values for CDI_{lds} were comparable and not significantly different from CDI_{mds} values, ranging from 19.3 $\pm$ 9.9% in group 1 to 34.4 $\pm$ 7.6% in group 4. In group 1 the CDI value for the medial dorsal surface was significantly higher than for all other areas.

The CDI value for the lateral dorsal surface in group 1 was significantly higher than the values for the medial and lateral central fovea. In group 4 the CDI values for the medial and lateral dorsal surface were significantly higher than for all other areas. In groups 2 and 3, this was only true with respect to the medial and lateral central fovea.

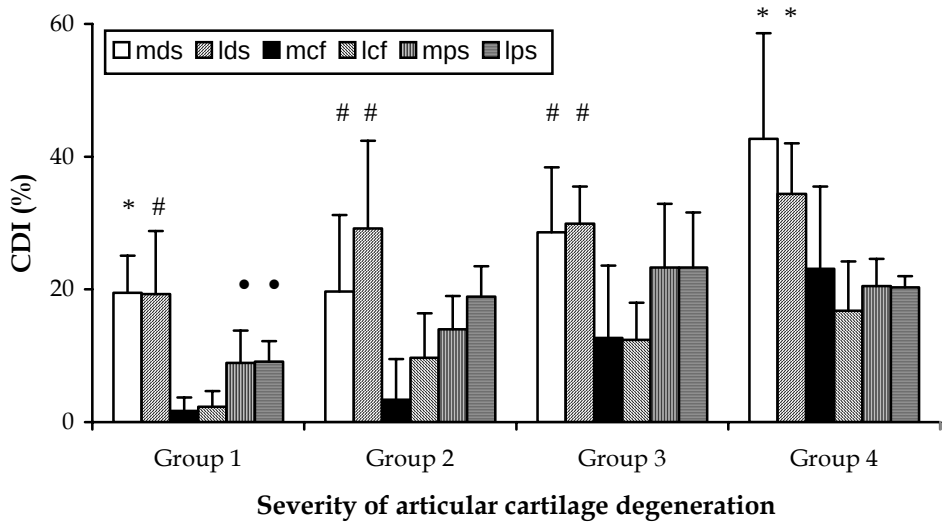

FIGURE 2: *Cartilage Degeneration Index (CDI, mean ± s.d., %) for the medial dorsal surface (mds), lateral dorsal surface (lds), medial central fovea (mcf), lateral central fovea (lcf), medial palmar surface (mps), and lateral palmar surface (lps) of the proximal articular cartilage surface of the proximal phalanx (P1) in relation to severity of osteoarthritis (OA) in the equine metacarpophalangeal (MCP) joint.*

** $p < 0.05$ when compared to mcf, lcf, mps and lps*

 # $p < 0.05$ when compared to mcf and lcf

• $p < 0.05$ when compared to mcf and lcf

Significant differences between CDI values for the palmar and central sites were present only in group 1. In all other groups, there were no significant differences between the palmar and central sites. There were no significant differences between the medial and lateral compartments at either the dorsal, central, or palmar sites of P1.

DISTRIBUTION OF CARTILAGE DEGENERATION IN THE MTP JOINT

Highest CDI values (mean ± s.d.) were measured for the lateral dorsal surface, ranging from 31.2% ± 9.4% in group 1 to 61.2 ± 15.0% in group 4 (figure 3). Values for CDI_{mds} came close to CDI_{lds} values, ranging from 26.8 ± 12.4% in group 1 to 53.2 ± 16.9% in group 4 and were not significantly different. CDI_{mds} and CDI_{lds} values were not significantly different from those in the forelimb.

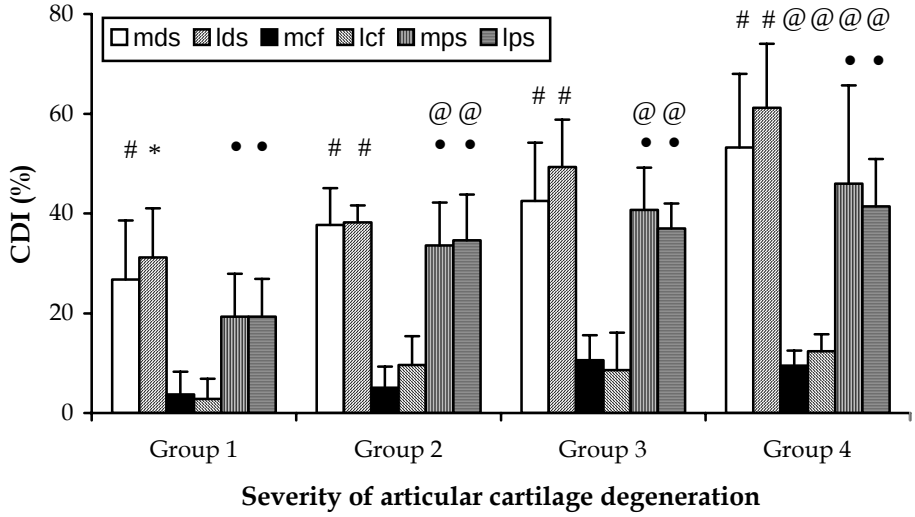

FIGURE 3: *Cartilage Degeneration Index (CDI, mean ± s.d., %) for the medial dorsal surface (mds), lateral dorsal surface (lds), medial central fovea (mcf), lateral central fovea (lcf), medial plantar surface (mps), and lateral plantar surface (lps) of the proximal articular cartilage surface of the proximal phalanx (P1) in relation to severity of osteoarthritis (OA) in the equine metatarsophalangeal (MTP) joint.*

** $p < 0.05$ when compared to mcf, lcf, mps and lps*

 # $p < 0.05$ when compared to mcf and lcf

• $p < 0.05$ when compared to mcf and lcf

@ $p < 0.05$ when compared to the CDI value of the identical area in the MCP joint

The CDI values for the medial and lateral plantar surfaces were lower than those for the dorsal areas, but the difference was not significant in any of the groups. Values ranged from 19.3 ± 9.2% in group 1 to 46.0 ± 17.7% in group 4 for CDI_{mps}. For CDI_{lps} these values ranged from 19.2 ± 9.1% to 41.4 ± 9.7%. CDI_{mps} and CDI_{lps}

values were significantly higher than those on the palmar surfaces in the forelimb in groups 2 - 4. A consistent finding in all groups was that CDI_{mcf} and CDI_{lcf} were significantly lower ($p < 0.05$) compared to CDI_{mds}, CDI_{lds}, CDI_{mps}, and CDI_{lps}. Mean CDI values for these central areas never became more than 13%, even in the most severely affected group. This was different from the forelimbs where central CDI values were significantly lower in comparison to the palmar sites in group 1 only. In group 4, CDI_{mcf} and CDI_{lcf} values were significantly lower than their counterparts in the forelimb. There were no significant differences between the medial and lateral compartments at either the dorsal, central, or plantar sites of P1.

DISCUSSION

The CDI technique was originally developed and validated for use on the proximal articular surface of P1 of the equine forelimb (Brommer *et al*. 2003a). However, as the geometry of the proximal articular surface of P1 in the equine hindlimb is virtually identical in the forelimb, use of this technique for studying the process of cartilage degeneration across the proximal articular surface of P1 in the hindlimb was deemed justifiable.

There appeared to be major differences in the distribution of cartilage degeneration across the articular surface of P1 between fore- and hindlimbs. In the forelimb, both the central and the palmar parts of the articular surface of P1 were about equally involved in the progress of cartilage degeneration, whereas in the hindlimb the plantar parts were significantly more involved than the palmar aspects in the forelimb and there was a huge difference in degree of cartilage degeneration in the central area between the fore- and hindlimb. In fact, the central fovea of P1 hardly showed any cartilage degeneration in the hindlimb, but in the forelimb high CDI values were measured in more advanced stages of OA. These differences may be related to differences in biomechanical loading during impact and in the early part of the stance phase. It has been stated, based on the relationship between kinematic and GRF data in the walking and trotting horse, that the forelimb 'bounces' at impact, while the hindlimb 'slides' (Back *et al.* 1995, Merkens *et al.* 1993). From the moment of initial ground contact until approximately 14% of total stride duration, both fore- and hindlimb fetlock joint oscillate (Back *et al*. 1995, Merkens and Schamhardt 1994). These oscillations have been reported to have a detrimental effect on joints only when combined with impulsive loading (Radin *et al.* 1991) and the term 'concussion' has been used to indicate the combination of rapid oscillations and impulsive loading at the beginning of the stance phase. Concussion has been shown to be higher in the forelimb than in the hindlimb (Back

et al. 1995, Merkens and Schamhardt 1994), which explains the difference in severity of degenerative lesions in the central parts of the articular surface of P1 in the fore- and hindlimbs. It should be recognised in this respect that this study was performed in joints from fore- and hindlimbs that had a comparable overall status of cartilage degeneration (CDI_{P1} value). Hence, this study provides insight in the relative contribution of different joint areas in a given stage of disease, but cannot be used to compare fore- and hindfetlocks within the same individual. Therefore, the study does not provide data on the time-relation of the development of OA in the MCP and MTP joint within one horse. Unfortunately, no clinical data were available for the animals used in this study and, therefore, no inferences can be made about the relationship of the areas affected and the clinical presentation.

The other major difference between front- and hindlimbs, the much larger involvement of the plantar region than the palmar region, can possibly be explained from differences in biomechanical loading as well. During the swing phase, the fetlock in the hindlimb shows a higher maximal flexion at an earlier timepoint than in the forelimb (Back *et al*. 1995). In this phenomenon, the reciprocal apparatus in the hindlimb, which couples flexion and extension of the stifle joint, tarsal joint, and the MTP joint, and which is absent in the forelimb, may play an important role. Where the reciprocal apparatus in the hindlimb forces the MTP joint to flex maximally until the plantar joint margin is stopped by the sesamoid bones, the MCP joint is basically flexed by inertiae forces during the swing phase of the stride (Back *et al.* 1995), and will normally not flex to its physical maximum. Further, the hoof in the forelimb has been found to land most commonly toe-first, whereas in the hindlimb the hoof lands heel-first (Back *et al*. 1995). Although the total amount of action force during the retardation and propulsion phases is about the same in fore- and hindlimbs, the retardatory peak amplitudes and impulses are larger and the duration of the retardatory phase is longer in forelimbs than in hindlimbs. The opposite holds for the propulsory forces and impulses in the hindlimb, where the peak amplitudes and impulses exceed those of the forelimb (Merkens *et al.* 1993, Ueda *et al*. 1981). From these kinematic and GRF data, it can be concluded that during the total stride the plantar joint margins of P1 in the hindlimb are subjected to a heavier biomechanical load than the palmar aspects of P1 in the forelimb. This may explain the differences in spread of cartilage degeneration and is also in line with the clinical findings that POFs are more frequently encountered in the hindlimb than in the forelimb (Fortier *et al*. 1995, Hardy *et al*. 1987, Roneus *et al.* 1998, Sandgren 1988, Whitton and Kannegieter 1994).

In both the MCP and MTP joints, initial cartilage degeneration started at the dorsal articular margin of P1 and differences in CDI values were not significant. Degenerative lesions on the dorsal margin of P1 in both the fore- and hindlimb have been associated with overextension of the joint and subsequent impaction of the dorsal margin during the stance phase. Overextension angles at midstance can be presumed to be most important in this respect, as this is the moment in the stride cycle when weightbearing is maximal. At midstance, fetlock angles are comparable in the fore- and hindlimb (Back *et al*. 1995, Holmström *et al.* 1994, Martinez-delCampo *et al*. 1991), which may explain the similarity in relative contribution of this area to overall cartilage damage as observed in this study. However, there certainly are differences in biomechanical behaviour. The fetlock has been shown to more rapidly extend in the forelimb than in the hindlimb, and also the maximal and minimal accelerations of the fetlock were found to be significantly higher in the forelimb than in the hindlimb (Back *et al*. 1995). Moreover, from GRF studies it appeared that the vertical reaction force in the forelimb is about 1.3 times as high as the vertical reaction force in the hindlimb (Merkens *et al.* 1985 and 1993, Ueda *et al*. 1981), partly caused by the fact that the forelimbs carry 60% of the bodyweight and the hindlimbs 40% (Stashak 1987). These differences may possibly explain the clinical observation that osteochondral fragmentation at the dorsal aspects of P1 is more frequently encountered in the forelimb than in the hindlimb (Kawcak and McIlwraith 1994, Yovich and McIlwraith 1986).

CDI values on the medial aspects of P1 were not significantly different from those on the lateral parts in both fore- and hindlimbs. Only in the most severely affected group (4), CDI values at the medial aspects of the articular surface of P1 had a tendency to be higher than laterally in MCP joints. This is in contrast to our previous study (Brommer *et al*. 2003b), where significantly higher CDI values were found at the medial than at the lateral side of the articular surface of P1 in forelimbs. However, in that study, far more joints were investigated (n = 73). Clinically, osteochondral fragmentation is found more at the medial side of the joint, but this difference is rather marginal as such fragments are also found laterally in both the MCP and MTP joints (Fortier *et al.* 1995, Kawcak and McIlwraith 1994, Nixon and Pool 1995, Roneus *et al*. 1998, Sandgren 1988, Whitton and Kannegieter 1994, Yovich and McIlwraith 1986). The difference is thought to be caused by differences in loading of the medial part of the articular surface compared to the lateral side due to the eccentric position of the centre of gravity of the horse in relation to the axis of the limb.

It is recognised that the equine MCP and MTP joints, although having the same macroscopic appearance, may differ in the 3-dimensional geometry, which in turn may be related with the 3-dimensional biomechanics at joint level. Using the principle of a joint coordinate system, the normal 3-dimensional behaviour of the MCP joint has been described in great detail in terms of small amplitudes of collateromotion and axial rotation superimposed on large amplitudes of flexion and extension (Chateau *et al*. 2001). Unfortunately, similar studies on the MTP joint are still lacking in veterinary literature. It would be of great interest to investigate possible differences in 3-dimensional anatomy and 3-dimensional biomechanics between the MCP and MTP joints given the growing insight in the relationship between articular cartilage function and structure as recently reviewed by Van Weeren and Brama (2003). Along these principles, differences in the 3-dimensional behaviour of the MCP versus the MTP joint might have a significant impact on the site-specific biochemical constitution of articular cartilage and subchondral bone.
In conclusion, differences in the topographical distribution of cartilage degeneration across the articular surface of P1 between fore- and hindlimbs as found in this study appear to support the hypothesis that differences in biomechanical loading between the distal fore- and hindlimb lead to differences in the development of cartilage degeneration in the MCP versus MTP joint. There is a clear association between the prevalence of osteochondral fragmentation at the palmar and plantar margins of the MCP and MTP joints, respectively, and the degree to which these areas contribute to the overall degree of cartilage degeneration in a given joint. This information is of indispensable value for a better understanding of the dynamic process of the development of cartilage degeneration in the equine MCP and MTP joints and may be of use when monitoring the effect of therapeutic interventions and/or preventive measures.

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MANUFACTURERS' ADDRESSES
[1] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER VI -

FUNCTIONAL CONSEQUENCES OF CARTILAGE DEGENERATION IN THE EQUINE METACARPOPHALANGEAL JOINT: QUANTITATIVE ASSESSMENT OF CARTILAGE STIFFNESS

Brommer H[a] - Laasanen MS[b] – Brama PAJ[a] - Van Weeren PR[a] – Helminen HJ[c] – Jurvelin JS[b,d]

[a] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[b] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Finland
[c] Department of Anatomy, Kuopio University, Finland
[d] Department of Applied Physics, Kuopio University, Finland

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SUMMARY

Reasons for performing study: **A functional consequence of cartilage degeneration, as seen during the development of osteoarthritis (OA), is the impairment of absorption and transmission of applied forces. In the horse, the metacarpophalangeal (MCP) joint is most commonly affected, but no quantitative data exist on the relationship of the occurrence of cartilage degeneration and changes in site-specific biomechanical properties in this joint.**

Objectives: **To gain insight into the biomechanical consequences of cartilage deterioration at 2 differently loaded sites on the proximal articular surface of the proximal phalanx (P1).**

Hypothesis: **Static and dynamic stiffness of articular cartilage decreases significantly in degenerated cartilage.**

Methods: **Cartilage Degeneration Index (CDI) values were measured at the lateral dorsal margin (site 1), at the lateral central fovea (site 2), and of the entire joint surface of P1 (CDI_{P1}) in 30 horses. The samples were divided into 2 groups, based on CDI_{P1} values. Group 1 contained joints without cartilage degeneration (CDI_{P1} values < 25%, n = 22). Joints with signs of cartilage degeneration (CDI_{P1} values > 25%) were assigned to group 2 (n = 8). Cartilage thickness at sites 1 and 2 was measured using ultrasonic and needle-probe techniques. Osteochondral plugs were drilled out from sites 1 and 2 and, subsequently, biomechanically tested in indentation geometry. Young's modulus at equilibrium and dynamic modulus were determined. Statistical analyses were performed using ANOVA tests ($p < 0.05$). Values are expressed as mean $\pm$ s.d.**

Results: **Mean CDI value at site 1 was 30.0 $\pm$ 14.1% in joints without cartilage degeneration and 59.7 $\pm$ 12.3% in joints with degenerated cartilage. At site 2, these values were 3.7 $\pm$ 3.2% and 16.3 $\pm$ 5.3%, respectively. Cartilage thickness values were not significantly different between the 2 groups and sites. Young's modulus at site 1 was significantly lower in joints with cartilage degeneration than in joints without cartilage degeneration (1.0 $\pm$ 0.4 MPa and 1.6 $\pm$ 0.6 MPa, respectively). At site 2, the difference was not significant (2.2 $\pm$ 1.1 MPa and 2.8 $\pm$ 1.2 MPa). Dynamic modulus values were significantly lower in joints with cartilage degeneration than in joints without degenerated cartilage at both sites (site 1: 2.1 $\pm$ 1.2 MPa and 3.1 $\pm$ 1.0 MPa; site 2: 3.6 $\pm$ 1.9 MPa and 5.3 $\pm$ 1.6 MPa, respectively).**

Conclusions: **The occurrence of degenerative cartilage changes is clearly related to loss of stiffness of the tissue. Absolute changes in cartilage integrity in terms of CDI are largest at the joint margin, but concomitant changes are present at the joint centre as well with a comparable decrease of the biomechanical moduli at**

the 2 sites. Therefore, significant cartilage degradation at the joint margin not only reflects local deterioration of biomechanical properties, but is also indicative of the functional quality in the centre.
Potential relevance: **Knowledge of site-related changes in cartilage biomechanics during OA-like cartilage degeneration is required for the assessment of tissue function. These findings may be important for improving prognostication and for the development of preventive measures.**

INTRODUCTION

The specific biomechanical properties of articular cartilage are determined by the delicate interactions of the main constituents of the extracellular matrix (ECM), *i.e.* proteoglycans, collagen, and water (Mow *et al*. 1990, Todhunter 1996). In healthy cartilage, these properties are matched with the functional demands imposed on the tissue (Helminen *et al*. 2000, Palmer and Bertone 1996). During early life, articular cartilage maturates under the influence of joint loading, both in biochemical and in biomechanical terms (Brama *et al*. 2000a, Brommer *et al*. 2005). The maturation enables the tissue to withstand different types of stresses, such as low-level constant loading during weightbearing, intermittent loading during locomotion, and high impact loading during training and racing (Palmer and Bertone 1996). Damage of cartilage will occur when the applied load exceeds the biomechanical load absorbing capacity of the tissue. Mechanical injury has been reported to be the major mechanism which leads to cartilage degeneration in the process of osteoarthritis (OA) (Freeman 1980, Hayes *et al*. 2001, Radin 1983, Radin *et al*. 1972). Cartilage degeneration results in depletion of proteoglycans and degradation of the collagen network (Arokoski *et al.* 2000, Buckwalter and Mankin 1997, Mow *et al*. 1990). In biomechanical terms, structural and compositional changes in cartilage during the development of degeneration lead to an increase of cartilage permeability and a decrease of cartilage stiffness (Armstrong and Mow 1982, Kempson *et al.* 1971 and 1973, Palmoski and Brandt 1981, Wirth *et al*. 1980). Once a particular threshold level has been passed, most often following substantial damage to the collagen network, the cartilage will no longer withstand normal biomechanical forces acting upon it, which makes the tissue more vulnerable to further degeneration and, in the end, leads to complete loss of biomechanical function (Palmoski and Brandt 1981).
Knowledge of the predisposing factors and understanding of the dynamic nature and progression of OA-like cartilage degeneration is of importance for attempts to prevent or diagnose the disorder at an early stage (Helminen *et al*. 2000, Ray *et al*.

1996). The athletic demands imposed on performance horses may result in overloading of cartilage, especially in the metacarpophalangeal (MCP) joint, the joint in which cartilage degeneration in the process of OA has been encountered most frequently (Pool 1996). This joint is characterised by a relatively small cartilage surface area and a large range of motion. These factors, together with its distal position in the appendicular skeleton, makes the MCP joint prone to the development of degenerative disorders (Pool 1996). Recently, the progress of cartilage degeneration across the articular surface of the proximal phalanx (P1) has been described (Brommer *et al.* 2003b). Cartilage degeneration invariably starts at the joint margin, which is subjected to intermittent and high impact loading, and then progresses to the centre of the joint, an area which is continuously loaded, but at a lower level. These 2 differently loaded sites have different biomechanical characteristics in healthy joints (Brommer *et al*. 2005), but the consequences of the development of cartilage degeneration on the load absorption capacity at these particular sites are not known.

The aim of the present study was to investigate the biomechanical properties of cartilage at the joint margin and at the joint centre on the articular surface of P1 in joints without cartilage degeneration and in joints with degenerated cartilage. Young's modulus, primarily dependent on the proteoglycan component of the ECM (Armstrong and Mow 1982, Mow *et al*. 1990), was determined as a measure for the static or long-term, time-dependent capacity to withstand loading of articular cartilage. Dynamic modulus, reflecting the properties of the collagen network (Bader and Kempson 1994, Bader *et al*. 1992, Korhonen *et al.* 2003), was used as a parameter for the dynamic characteristics of cartilage tissue, or the instantaneous response to loading. Both moduli were determined in indentation geometry. We anticipated that cartilage degeneration would adversely affect the biomechanical properties of the tissue as reflected by these parameters, but that peripheral and central cartilage areas would respond differently. By improving our insight into the functional changes that take place in the equine MCP joint during development of degenerative joint disorders, we may expect progress in the quest for the improvement of early diagnostics and the development of preventive measures.

MATERIALS AND METHODS

SAMPLE COLLECTION

Thirty MCP joints from 30 horses (mixed population of Warmbloods and Thoroughbreds, age range 1.5 - 22 years) were selected from a sample bank of

equine MCP joints, collected from horses in the slaughter house or from horses used for experimental studies. The P1s were isolated and the proximal articular cartilage surfaces, including approximately 5 cm of bone, were cut off using a bandsaw. The samples were stored at -20°C until processing. The samples were thawed at 7°C in wrapped gauzes soaked in phosphate buffered saline (PBS) during 14 - 18 hours before performing the measurements.

SITES OF INTEREST AND MEASUREMENT OF DEGENERATIVE STATE

Two predefined sites of interest (diameter 8 - 10 mm) were marked on the proximal articular cartilage surface of P1. Site 1, which has been shown to become affected in the initial stage of OA-like cartilage degeneration (Brommer *et al*. 2003b), was located at the lateral dorsal margin, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin. Site 2, which becomes affected in a later phase (Brommer *et al*. 2003b), was located at the lateral central fovea, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins (figure 1). The Cartilage Degeneration Index (CDI) technique (Brommer *et al*. 2003a) was used to determine the degenerative state of the 2 sites of interest. Moreover, the CDI of the entire cartilage surface of P1 (CDI_{P1}) was determined.

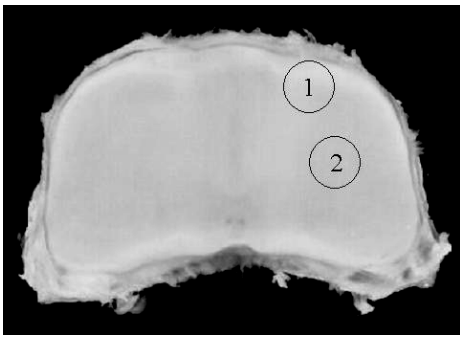

***FIGURE 1**: Proximal articular cartilage surface of the equine first phalanx (P1) with the sites of interest. Site 1, which becomes affected in the initial stage of osteoarthritis (OA), is located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin. Site 2, which becomes affected during further progression of the disease, is located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins.*

MEASUREMENT OF CARTILAGE THICKNESS

In order to enable stress-relaxation measurements in indentation geometry, *in situ* cartilage thickness was determined nondestructively at the centre of sites 1 and 2, using a recently developed ultrasound indentation instrument (Laasanen *et al.* 2002). Briefly, an unfocused, 10 MHz broadband ultrasound transducer (diameter 3.0 mm, Panametrics XMS-310)[1], mounted on the tip of an arthroscopic indentation instrument (Artscan 200)[2], was used to determine the ultrasound time of flight (TOF) between the cartilage surface and subchondral bone (Laasanen *et al.* 2002). Subsequently, sample thickness was calculated by multiplying the predefined speed of sound (SOS, 1650 m/s) with the term TOF/2. The predefined SOS was obtained from earlier findings of bovine cartilage (Töyräs *et al.* 2003), and from pilot measurements using equine articular cartilage.

After biomechanical testing, articular cartilage thickness at the site of indentation was measured again, now using a validated, slightly destructive needle-probe technique (Jurvelin *et al.* 1995). The penetration velocity of the needle was 10 μm/s. LabVIEW software (version 5.1)[3] was developed and used for data acquisition and analysis.

The accuracy of thickness measurements was studied by calculating Pearson's linear correlation coefficient and the relative mean difference of the thickness values obtained by the 2 techniques.

PREPARATION OF SAMPLES AND BIOMECHANICAL TESTING

The same protocol for sample preparation and biomechanical testing was used as described previously (Brommer *et al.* 2005). Briefly, osteochondral plugs (diameter 8 mm) of sites 1 and 2 were drilled out from the cartilage surface. The subchondral bone was smoothly cut off distal to the cartilage-subchondral bone interface, leaving 3 mm of subchondral bone attached to the articular cartilage layer. The osteochondral samples were glued in a sample holder with cyanoacrylate. The sample was submerged in PBS and biomechanically tested in indentation geometry, using a custom-made, computer-controlled, high-resolution material testing instrument (resolution 5 mN and 0.1 μm for the force and position, respectively). A cylindrical, porous, plane-ended indenter was used with a diameter of 1.041 mm. Samples were tested in a stress-relaxation protocol (2 steps of each 7.5% strain with a velocity of 1.0 μm/s and a relaxation time of 1200 s), followed by dynamic testing (1.0% amplitude of the compressed thickness at a frequency of 1.0 Hz). Biomechanical testing was performed using the thickness values determined by ultrasound. Care was taken that the contact point of the

cartilage surface was aligned perpendicular to the indenter. LabVIEW software (version 6.1)[3] was developed and used for data acquisition and analysis.

DETERMINATION OF YOUNG'S MODULUS

Young's modulus at equilibrium was derived from the equilibrium stress-strain response during the second step (7.5 - 15%) of compression. For a linearly elastic, isotropic material, the expression for Young's modulus (E) is given as follows (Hayes *et al.* 1972):

$$E = (\sigma/\varepsilon) \times (\pi\alpha/2h\kappa) \times (1 - \upsilon^2)$$

where σ = stress, ε = strain, α = indenter radius, h = thickness of the cartilage, υ = Poisson's ratio, and κ = theoretical scale factor that accounts for the variable cartilage thickness (Hayes *et al.* 1972, Jurvelin *et al.* 1990). When computing values of Young's modulus, the thickness values measured by the needle-probe technique were used. The Poisson's ratio was set at 0.1 for calculation of Young's modulus, based on previous results (Jurvelin *et al*. 1997).

DETERMINATION OF DYNAMIC MODULUS

For calculation of the dynamic modulus, the same model (Hayes *et al*. 1972) was used. However, cartilage was assumed to be incompressible during 1.0 Hz cyclic compression and, therefore, Poisson's ratio was set to 0.5 (Mak *et al*. 1987, Wong *et al*. 2000). The dynamic modulus was calculated from the last 2 cycles of the dynamical test.

STATISTICAL ANALYSIS

The samples were divided into 2 groups, based on CDI_{P1} values. The first group contained the joints with no cartilage degeneration, having CDI_{P1} values < 25% (n = 22). The second group was made up of joints that showed cartilage degeneration, having CDI_{P1} values > 25% (n = 8). For each group and site, values (mean $\pm$ s.d.) for CDI, thickness, Young's modulus, and dynamic modulus were calculated.

Differences between the groups and sites were statistically evaluated using ANOVA tests. SPSS software (version 10.0)[4] was used and the level of significance was set at $p < 0.05$.

RESULTS

DEGENERATIVE STATE OF THE CARTILAGE

The division of the samples based on the CDI for the entire joint surface (CDI_{P1}) resulted in marked and significant differences in local CDI for sites 1 and 2 (figure 2). CDI value at site 1 was 30.0 ± 14.1% in joints without cartilage degeneration and 59.7 ± 12.3% in joints with cartilage degeneration ($p < 0.001$). At site 2, the mean CDI value was also significantly higher in joints with cartilage degeneration (16.3 ± 5.3%) than in joints without cartilage degeneration (3.7 ± 3.2%, $p < 0.001$). Differences between sites 1 and 2 were significant in both groups of joints ($p < 0.001$).

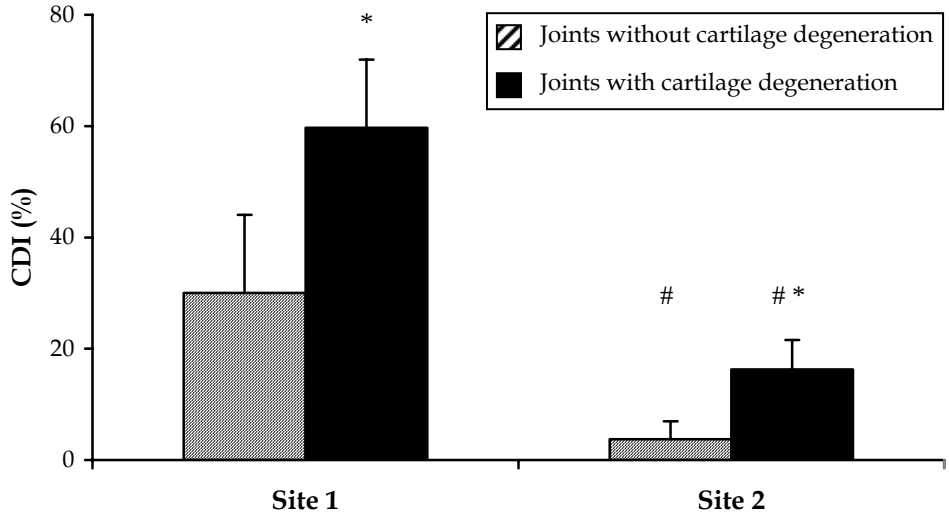

***FIGURE 2**: CDI (mean ± s.d., %) at sites 1 and 2 in metacarpophalangeal (MCP) joints without cartilage degeneration and in MCP joints with cartilage degeneration.*
** Significant difference between the 2 groups of joints ($p < 0.05$)*
 # Significant difference between sites 1 and 2 ($p < 0.05$)

THICKNESS

The mean cartilage thicknesses (needle-probe values) were not significantly different between the 2 groups and 2 sites ($p > 0.05$). At site 1, the mean values were 762 ± 131 μm for joints without cartilage degeneration and 788 ± 51 μm for

joints with cartilage degeneration. At site 2, these values were 747 ± 97 μm and 784 ± 112 μm, respectively (figure 3). The thickness values obtained by the ultrasound technique and those obtained by the needle-probe method were highly similar (Pearson's linear correlation coefficient $r = 0.94$, $p < 0.001$, $n = 60$, mean difference 2.3%), justifying the use of the ultrasound-generated values for biomechanical testing.

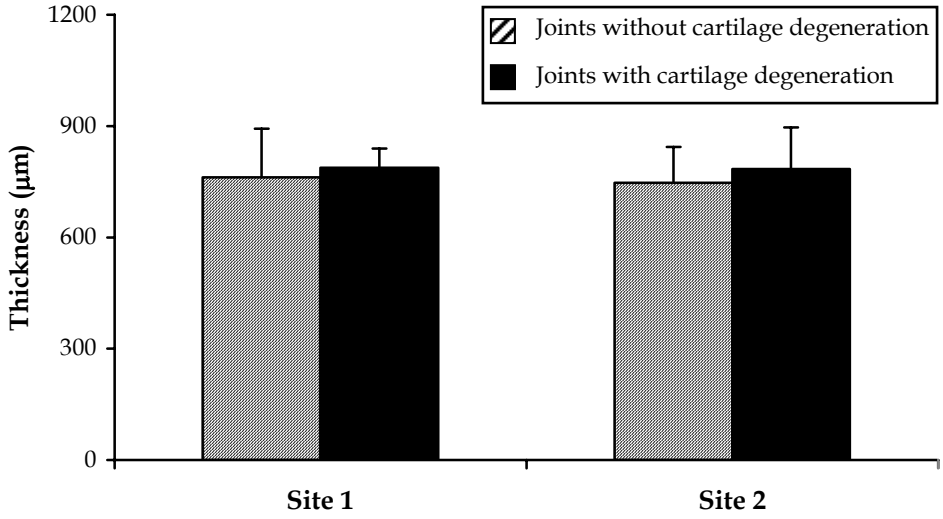

FIGURE 3: *Cartilage thickness (mean ± s.d., μm, needle-probe values) at sites 1 and 2 in metacarpophalangeal (MCP) joints without cartilage degeneration and in MCP joints with cartilage degeneration.*

YOUNG'S MODULUS

Young's modulus at equilibrium at site 1 was significantly lower (1.0 ± 0.4 MPa) in joints with cartilage degeneration compared to joints without cartilage degeneration (1.6 ± 0.6 MPa, $p = 0.01$). At site 2, there was a difference between the moduli of both groups, but the difference was not significant (2.2 ± 1.1 MPa and 2.8 ± 1.2 MPa, respectively, $p = 0.25$). Differences between sites 1 and 2 were significant in both groups ($p < 0.01$), with site 2 having the highest modulus values (figure 4).

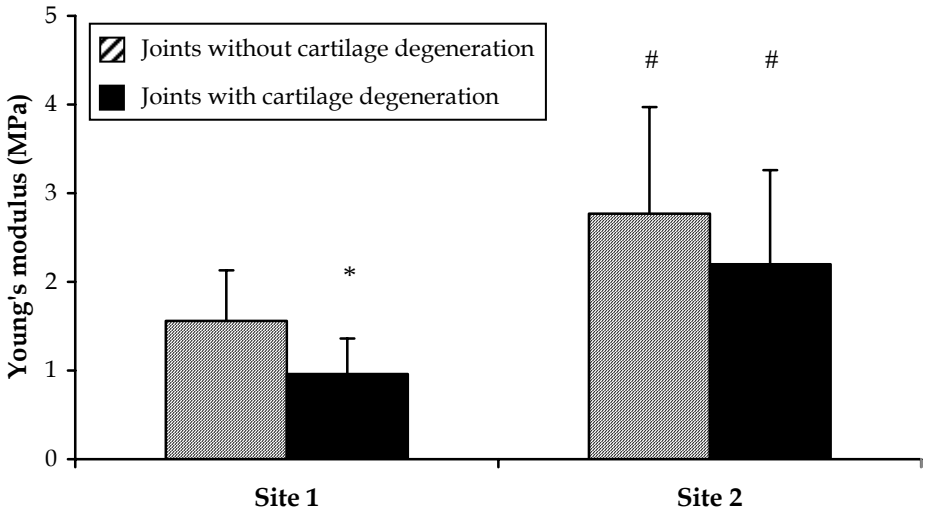

FIGURE 4: *Young's modulus (mean ± s.d., MPa) at sites 1 and 2 in metacarpophalangeal (MCP) joints without cartilage degeneration and in MCP joints with cartilage degeneration.*
** Significant difference between the 2 groups of joints ($p < 0.05$)*
 # Significant difference between sites 1 and 2 ($p < 0.05$)

DYNAMIC MODULUS

Dynamic modulus values at site 1 were significantly lower (2.1 ± 1.2 MPa) in joints with cartilage degeneration than in joints without cartilage degeneration (3.1 ± 1.0 MPa, $p = 0.02$). At site 2, there were also significant differences between both groups of joints (3.6 ± 1.9 MPa and 5.3 ± 1.6 MPa, respectively, $p = 0.02$). Differences between sites 1 and 2 were significant in joints without degeneration and in joints with cartilage degeneration ($p < 0.001$). The highest modulus values were found at site 2 (figure 5).

DISCUSSION

Biomechanical tests were analysed using the analytical single-phase elastic model of cartilage indentation (Hayes *et al.* 1972). In this model, the dynamic behaviour (at 1.0 Hz) of biphasic cartilage is idealised to be equivalent to that of incompressible ($\upsilon = 0.5$) elastic material (Mak *et al.* 1987), whereas, for the equilibrium analysis, smaller, true values of Poisson´s ratio (~ 0.1) must be assumed (Jurvelin *et al.* 1997, Wong *et al.* 2000). However, the value of cartilage

Poisson's ratio may vary and consequently contribute to differences found in the values of Young's modulus. Previously, the values of Young's modulus from indentation tests were found to be consistent with the modulus values obtained directly from unconfined compression tests (Korhonen *et al*. 2002). Unconfined compression tests were unsuitable in this study due to the relatively thin cartilage on the equine P1 combined with a rather pronounced curvature of the articular surface. While the theoretical model (Hayes *et al*. 1972) includes aspect ratio (α/h) correction, the thickness measurement can be critical for indentation analysis (Hayes *et al.* 1972, Jurvelin *et al.* 1990). In this study, we measured articular cartilage thickness nondestructively prior to biomechanical testing by using a new ultrasound instrument (Laasanen *et al*. 2002). The accuracy of this technique was validated against a destructive needle-probe measurement, conducted after biomechanical testing. As thickness values were highly similar between specimens with and without cartilage degeneration, the decrease in Young's modulus and dynamic modulus in cartilage from joints with cartilage degeneration can be attributed to true changes in tissue properties.

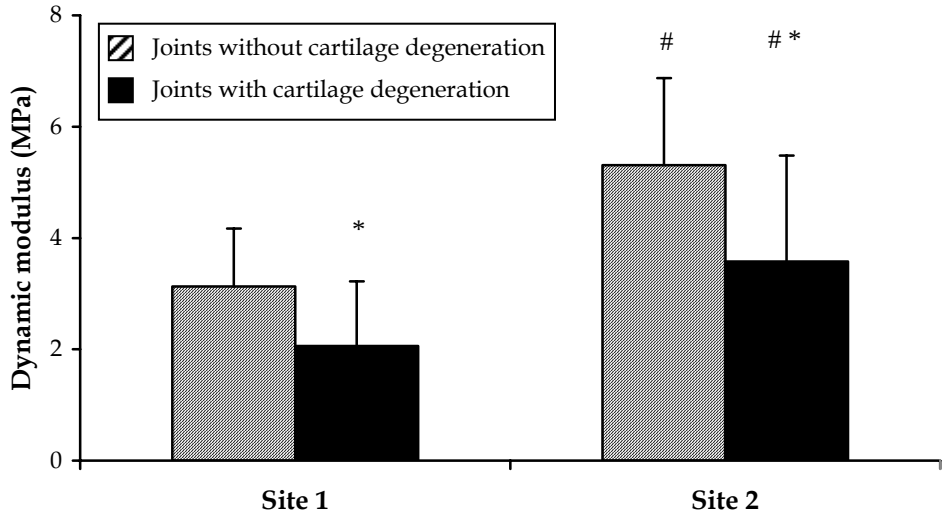

***FIGURE 5**: Dynamic modulus (mean ± s.d., MPa) at sites 1 and 2 in metacarpophalangeal (MCP) joints without cartilage degeneration and in MCP joints with cartilage degeneration.*
** Significant difference between the 2 groups ($p < 0.05$)*
 # Significant difference between sites 1 and 2 ($p < 0.05$)

There were significant differences in biomechanical characteristics between the joint edge and joint centre of the proximal articular surface of P1 in MCP joints without cartilage degeneration, which is a confirmation of earlier findings (Brommer *et al*. 2005). It is likely that these differences are associated with the different loading patterns of these sites, since the dorsal joint margin is subject to intermittent high impact loading and the joint centre is continuously loaded at a lower level (Brama *et al*. 2001). The present study revealed significant changes in biomechanical properties at both sites in joints featuring cartilage degeneration. The 2 sites play a different role in the progression of the disease as the cartilage at the joint margin is affected in the initial stages and the joint centre becomes only involved in the more advanced stages of the process (Brommer *et al*. 2003b). This is illustrated by the significantly different mean CDI values of both sites in joints with an overall CDI of < 25%, *i.e.* without appreciable cartilage degeneration, and the different ratios of increase of the local site-specific values in joints with cartilage degeneration. The increase in the mean CDI value at site 1 (~ x 2) was less than at site 2 (~ x 3.4), but the absolute CDI value was still substantially and significantly higher at site 1 than at site 2 in joints with cartilage degeneration. This difference in rate of increase of CDI at these sites was not reflected by equivalent changes in the static and dynamic stiffness of cartilage at sites 1 (~ 30 - 40%) and 2 (~ 20 - 30%). This observation suggests that there is no linear relationship between changes in CDI and changes in both stiffness indexes and may reflect differences in the sensitivity of both parameters for characterisation of cartilage degeneration.

It has been suggested that the thickness of the cartilage layer decreases during cartilage degeneration due to fibrillation as a result of disruption of the collagen network and depletion of proteoglycans (Buckwalter and Mankin 1997, Buckwalter and Martin 1995, McIlwraith 1996). In this study, no significant differences in cartilage thickness between normal and relatively mildly diseased joints were found. However, a significant decrease was found in the static and dynamic biomechanical properties of articular cartilage, indicating a softening of the tissue. This softening prior to changes in cartilage thickness is in agreement with other studies that suggest that softening is one of first detectable signs of cartilage degeneration (Armstrong and Mow 1982, Kempson *et al*. 1971, Palmoski and Brandt 1981).

Young's modulus at equilibrium has been related with the static (long-term) properties of cartilage and it has a correlation with the concentration of proteoglycans in the tissue (Armstrong and Mow 1982, Mow *et al*. 1990). In healthy cartilage, glycosaminoglycan levels were found to be higher in the central area of

the joint (*i.e.* the area that is constantly loaded), compared to the more peripheral area (*i.e.* the area that is exposed intermittently to high impact loads) (Brama *et al*. 2000a and 2000b), which explains the higher value for Young's modulus at site 2. A decrease of Young's modulus has been related with loss of proteoglycans (Armstrong and Mow 1982, Kempson *et al*. 1971). Significant proteoglycan loss was recently demonstrated in comparable samples from the articular cartilage surface of P1 of the equine MCP joint that showed cartilage degeneration (Van der Harst *et al*. accepted for publication).

The dynamic modulus has been related with the impact loads during exercise and is strongly determined by the characteristics of the collagen network (Bader and Kempson 1994, Bader *et al*. 1992). In normal joints, the dynamic modulus is lower at site 1, indicating the presence of less stiff cartilage at the joint margins, which may be of importance to cope with the strongly variable loading pattern in this region. Cartilage at this site is less stiff despite the presence of more collagen and higher levels of hydroxylysylpyridinoline crosslinks compared to site 2 (Brama *et al*. 1999, 2000a and 2000b). Probably, characteristics other than biochemical parameters of the collagen network, such as structure and orientation of the collagen fibrils, also have influence on the dynamic biomechanics of the tissue. In human cartilage, compressive stiffness of the tissue was found to be primarily dependent on the integrity of the ECM and less on the levels of the major cartilage constituents (Franz *et al*. 2001). The integrity of the collagen network is essential for trapping of proteoglycans, which attract water into the tissue as a result of osmotic forces created by the negatively charged glycosaminoglycans. It is the balance between the tension in the collagen network and the osmotic swelling capacity of the proteoglycans that is responsible for the ultimate biomechanical characteristics of the articular cartilage (Hasler *et al*. 1999, Hayes *et al*. 2001, Mow *et al*. 1990). Decrease of the dynamic modulus at sites 1 and 2 in the process of cartilage degeneration is primarily related with damage to type II collagen (Buckwalter and Mankin 1997, Poole 1999). Damage of the collagen network results also in a loss of proteoglycans as the trapping mechanism is disturbed (Armstrong and Mow 1982, Buckwalter and Mankin 1997, Kempson *et al*. 1971, Poole 1999). This explains why in the process of cartilage degeneration both the Young's modulus and the dynamic modulus values decrease simultaneously. In a recent study, no significant differences between joints with and without cartilage degeneration were found in the levels of denatured collagen and hydroxylysylpyridinoline crosslinks at sites 1 and 2 of the equine P1 (Van der Harst *et al*. accepted for publication). This is a further indication that structure and orientation of collagen fibrils may be more

important than the collagen content and the amount of crosslinks for the dynamic characteristics of articular cartilage.
The dorsal aspect of P1 is the most common site of clinical lesions in the MCP joint (Hardy *et al.* 1987, Kawcak and McIlwraith 1994, Yovich and McIlwraith 1986). The softening of the articular cartilage at this location, as shown in this study, results in impaired biomechanical function and a decrease of load absorbing capacity of the tissue. This in turn will increase loading of the underlying subchondral bone. Once a particular threshold level has been passed, the situation may lead to osteochondral fragmentation. It is likely therefore, that the clinical problem of osteochondral fragmentation in the MCP joint has a similar biomechanical background as the osteochondral fragmentation of the small carpal bones (Murray *et al*. 1995 and 1998).
In conclusion, articular cartilage stiffness decreases during the progress of cartilage degeneration. This results in a decrease of both Young's modulus, which is a measure of static stiffness, and of the dynamic modulus, which is a measure of dynamic stiffness. Changes in the integrity of the articular cartilage are largest at the dorsal margin of the joint, but there are concomitant changes at the joint centre and there is a simultaneous decrease of the biomechanical moduli at the 2 sites. Significant cartilage degradation at the joint margin therefore not only reflects a local deterioration of the biomechanical properties, but is indicative of the functional quality in central area as well. This finding has implications for the interpretation of the arthroscopic examination of the articular cartilage using indentation instruments. The dorsal margin of P1 is arthroscopically accessable while the joint centre is not. Thus, indentation data from the joint margin indirectly provide information about the biomechanical status of the cartilage in the central area of the joint. The data provided by this study give a better understanding of the biomechanical function of cartilage at P1 in healthy MCP joints and in joints affected by cartilage degeneration. This knowledge may be of use in the ongoing quest for improvements in diagnostics and prognostication of degenerative joint disease. It may also contribute to the development and design of adequate preventive measures.

MANUFACTURERS' ADDRESSES

[1] Panametrics Inc., Waltham, USA
[2] Artscan Oy, Helsinki, Finland
[3] LabVIEW, National Instruments, Austin, USA
[4] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER VII -

ACCURACY OF DIAGNOSTIC ARTHROSCOPY FOR THE ASSESSMENT OF CARTILAGE DAMAGE IN THE EQUINE METACARPOPHALANGEAL JOINT

Brommer H - Rijkenhuizen ABM - Brama PAJ - Barneveld A - Van Weeren PR

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Equine Vet J 2004, 36: 331-335

SUMMARY

Reasons for performing study: **There are many non-invasive diagnostic methods used for evaluating chronic progressive joint disease, but each has severe limitations in the detection of early articular cartilage damage.**

Objectives: **To evaluate the accuracy of arthroscopy as a diagnostic method for the assessment of the severity of cartilage surface damage on the proximal articular margin of the equine first phalanx (P1).**

Hypothesis: **That arthroscopic assessment of the visible cartilage provides 1) a good indication of the integrity of the cartilage surface and 2) a good estimation of the status of the cartilage surface of the entire articular area of P1.**

Materials and methods: **Arthroscopic examination of the dorsal pouch of the metacarpophalangeal (MCP) joint was performed in the left front limbs of 74 slaughter horses (age 5 months - 23 years). The appearance of the visible cartilage of P1 was scored by 2 independent arthroscopists, using the Société Française d'Arthroscopie (SFA) arthroscopic grading system. The joints were dissected after completion of the arthroscopic procedure. The Cartilage Degeneration Index (CDI_{P1}) was determined and used as a quantitative measure for the overall degree of cartilage surface deterioration on the articular area of P1. Further, the CDI value was determined for the dorsal articular margin of P1 (CDI_{dam}), *i.e.* the area that can be visualised with arthroscopy. The CDI_{dam} values were classified in 3 groups (CDI_{dam} < 25%, minor lesions; CDI_{dam} between 25 - 45%, moderate lesions; and CDI_{dam} > 45%, severe lesions). Differences between the 2 arthroscopists were statistically evaluated in a nonparametric test and Pearson correlation coefficients (r) with matching p values were determined for the correlations between SFA and CDI_{dam} and between CDI_{P1} and CDI_{dam}. The level of significance was set at $p < 0.05$.**

Results: **Differences between SFA scores of the 2 arthroscopists were not significant ($p = 0.22$). In the group of joints with minor cartilage changes, there was no correlation between SFA and CDI_{dam} ($r = 0.12$, $p = 0.71$), but there was a significant correlation between CDI_{P1} and CDI_{dam} ($r = 0.95$, $p < 0.01$). In the group with moderate cartilage damage, there was an increase in correlation between SFA and CDI_{dam} ($r = 0.27$, $p = 0.09$) and a decrease in correlation between CDI_{P1} and CDI_{dam} ($r = 0.48$, $p < 0.01$). In the group with severe cartilage changes, there was a significant correlation between SFA and CDI_{dam} ($r = 0.58$, $p < 0.01$), but no significant correlation between CDI_{P1} and CDI_{dam} ($r = 0.43$, $p = 0.06$).**

Conclusions: **Arthroscopic assessment of cartilage lesions on the proximal articular surface of P1 in joints with minor cartilage damage leads to an underestimation of the actual damage because proteoglycan depletion and light**

cartilage fibrillation can not be detected arthroscopically. In cases with mild cartilage damage, the status of the cartilage surface of the visible area of P1 is a good representation of the status of the entire articular surface. In cases with severe cartilage lesions, there is an overestimation of the real damage. In such joints, the arthroscopic scoring system provides reliable information, but the visible area is not representative of the entire articular surface.
Potential relevance: **From a practical viewpoint, it can be stated that the arthroscopic grading of visible lesions on the equine P1 gives the best impression of overall cartilage damage in joints with moderately severe cartilage lesions. It should be realised, however, that this is the result of an underestimation due to the shortcomings of the grading system, which is neutralised by an overestimation due to the fact that the severity of lesions on the visible area of P1 is not representative for the entire articular surface.**

INTRODUCTION

There are many diagnostic noninvasive or minimally invasive methods used for the evaluation of chronic progressive joint diseases, such as osteoarthritis (OA). Radiography, scintigraphy, computed tomography (CT), and synovial fluid analysis have long been available (Park *et al*. 1996, Ray *et al*. 1996, Trotter and McIlwraith 1996). Although all techniques may yield useful information, there are severe limitations as well, particularly in the detection of early articular cartilage damage.
The state of articular cartilage can be more directly assessed using techniques such as ultrasonography and magnetic resonance imaging (MRI) (Denoix 1996, Park *et al*. 1996). However, accurate assessment of articular cartilage integrity is still relatively difficult.
As an invasive method, arthroscopy provides a direct, magnified view of the cartilage surface and, therefore, this technique has been considered to be the golden standard for evaluating cartilage damage (McIlwraith and Fessler 1978, Trotter and McIlwraith 1996). However, an in-depth validation of the use of diagnostic arthroscopy in equine practice has not been reported.
In human orthopaedic surgery, various systems for the semiquantitative assessment of arthroscopically visible cartilage lesions have been developed. The system adopted by the Société Française d'Arthroscopie (French Arthroscopic Society, SFA) uses a grading based on both depth and extension of the visible lesions (Ayral *et al.* 1996, Dougados *et al.* 1994). However, the question remains to what extent this or similar systems give a truthful indication of the integrity of the

cartilage surface and of the status of the entire joint, as a complete arthroscopic examination of the entire articular cartilage surface is not possible in most joints.
Recently, we developed an *in vitro* technique to quantify objectively the degree of cartilage degeneration across the entire joint surface: the Cartilage Degeneration Index (CDI) (Brommer *et al.* 2003a). The technique has been validated first for the proximal articular cartilage surface of the proximal phalanx (P1) because the metacarpophalangeal (MCP) joint has the largest number of traumatic and degenerative lesions of all joints of the appendicular skeleton in the horse (Pool 1996). It was shown that, in degenerative joint disease, the spread of affected cartilage areas follows a typical pattern with increasing severity. Initially, cartilage degeneration starts at the medial dorsal margin of P1 and then extends to the lateral dorsal margin. With a further increase in severity, cartilage degeneration appears at the medial and ultimately at the lateral central fovea (Brommer *et al.* 2003b).
The aim of the present study was to investigate the accuracy of diagnostic arthroscopy for assessment of cartilage damage in the MCP joint. The cartilage was scored arthroscopically under clinical conditions, using the SFA grading system (Ayral *et al.* 1996, Dougados *et al.* 1994), and the CDI (Brommer *et al.* 2003a) was subsequently determined. The hypothesis was tested that arthroscopic assessment of the visible cartilage provides 1) a good indication of the integrity of the cartilage surface and 2) a good representation of the status of the cartilage surface of the entire joint.

MATERIALS AND METHODS

SAMPLE COLLECTION

Seventy-four left front distal limbs from slaughter horses (age range 5 months – 23 years) with different grades of cartilage surface lesions in the MCP joint were harvested immediately after death and stored at –20°C until processing. No clinical data of these horses were available. One day before the measurements, the limbs were thawed at room temperature.

ARTHROSCOPIC SCORING OF ARTICULAR CARTILAGE

Arthroscopy of the MCP joints was performed using the dorsal approach as described by McIlwraith (1990). The joint was effused with 60 millilitres of isotonic saline and the arthroscope (4.5 mm, 30° oblique lens)[1] was inserted medial to the common digital extensor tendon, using a blunt trocar. The arthroscopic findings were recorded on a U-Matic video cassette (KCA-60BRS)[2].

The degree of chondropathy on the dorsal articular margin of P1 was assessed (HB) using the SFA grading system (Ayral *et al*. 1996, Dougados *et al*. 1994). Briefly, the depth of cartilage lesions was classified on a 0 - 4 scale. Grade 0 indicates normal cartilage, grade 1 swelling and softening of the cartilage, grade 2 superficial fibrillation, grade 3 deep fibrillation down to subchondral bone, and grade 4 exposure of subchondral bone. The size of the lesions was estimated as a percentage of the whole articular cartilage that could be seen with the arthroscope. The SFA score was calculated using a specially developed formula resulting in a continuous variable with a range from 0 - 100% (Ayral *et al*. 1996). After the arthroscopic procedure was finished, the joints were dissected and the sizes of the lesions that had been estimated with help of the arthroscope were verified by direct macroscopic inspection. The videotapes were also judged independently by a second experienced orthopaedic surgeon (ABMR).

QUANTIFICATION OF ARTICULAR CARTILAGE DEGENERATION

After macroscopic inspection of the joints, the proximal two-thirds of P1 were isolated from the rest of the limb. Articular cartilage degeneration was quantified using the recently described CDI (Brommer *et al*. 2003a). This technique is based on the fact that degenerated articular cartilage, which is depleted of proteoglycans and shows surface fibrillation, takes up Indian ink particles, whereas intact cartilage is hardly stained (Chang *et al.* 1997, Madsen *et al.* 1992). Briefly, the amount of Indian ink uptake across the entire cartilage surface is quantified by digitally imaging the native and the Indian ink stained articular cartilage surfaces under standardised conditions. The increase in mean grey level of the articular surface (measured as the mean pixel value) is the basis for calculation of the CDI (range from 0 - 100%), using a specially developed formula (Brommer *et al*. 2003a). Apart from the overall CDI value of the entire cartilage surface of P1 (CDI_{P1}), the CDI value of the dorsal articular margin of P1 (*i.e.* that part of the articular surface that can be visualised with arthroscopy) was also determined (CDI_{dam}) (figure 1).

DATA ANALYSIS

Differences in the determination of sizes of the cartilage lesions using arthroscopy and direct macroscopic inspection, and differences between the SFA values obtained from the 2 arthroscopists were statistically evaluated in a nonparametric ANOVA repeated measurement and multiple comparison test. CDI_{dam} values were categorised into three groups: CDI_{dam} < 25% (minor lesions), CDI_{dam} between 25 - 45% (moderate lesions), and CDI_{dam} > 45% (severe lesions). Data were checked

for normal distribution and Pearson correlation coefficients (r) with matching p values were determined for correlations between SFA and CDI_{dam} and between CDI_{P1} and CDI_{dam} for each group. SPSS software (version 10.0)[3] was used and the level of significance was set at $p < 0.05$.

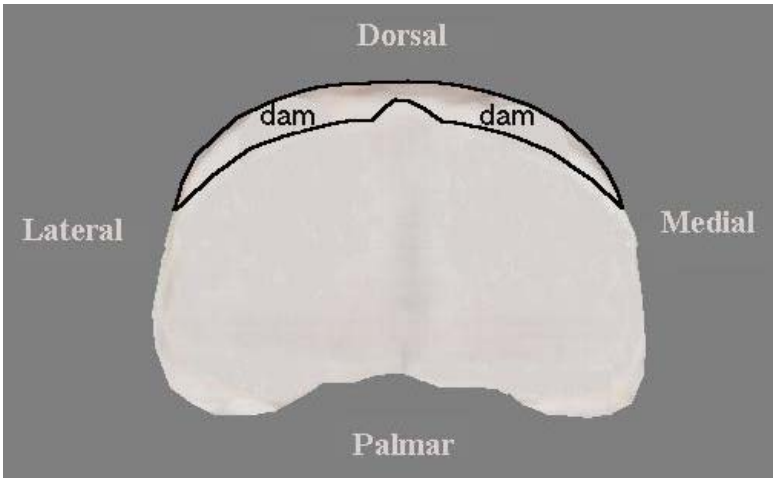

FIGURE 1: *Proximal articular cartilage surface of the first phalanx (P1). Cartilage Degeneration Index (CDI) values were determined for the total surface (CDI_{P1}) and for the dorsal articular margin (CDI_{dam}), i.e. the area that can be visualised arthroscopically.*

RESULTS

There were no differences in lesion sizes as determined by arthroscopy or by direct macroscopic inspection ($p = 0.31$).

The SFA values of arthroscopist 2 (ABMR) were consistently higher than for arthroscopist 1 (HB) (figure 2), but these differences were not significant ($p = 0.22$). This enabled averaging of the SFA scores of both arthroscopists. The mean $\pm$ s.d. SFA values were 8.9 $\pm$ 5.8% in the group with minor cartilage lesions ($CDI_{dam} < 25\%$, 12 joints), 26.4 $\pm$ 16.3% in the group with moderate cartilage lesions (CDI_{dam} between 25 - 45%, 41 joints) and 35.4 $\pm$ 16.3% in the group with severe cartilage lesions ($CDI_{dam} > 45\%$, 21 joints).

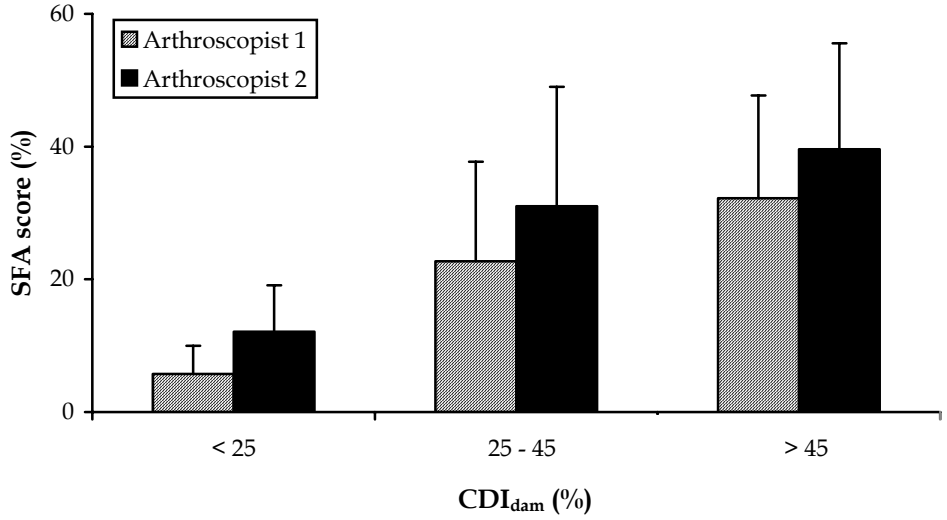

FIGURE 2: *Chondropathy assessment by arthroscopy: SFA scores (mean ± s.d., %) for the 3 CDI_{dam} groups and the 2 arthroscopists.*

CORRELATION BETWEEN SFA AND CDI_{DAM}

In joints with minor lesions (CDI_{dam} < 25%), there was no correlation between SFA scores and CDI_{dam} values (r = 0.12; p = 0.71). In those with moderate cartilage damage (CDI_{dam} between 25 - 45%), there was an increase in the correlation between SFA values and CDI_{dam} scores (r = 0.27), but significance was not reached (p = 0.09). In the group with severe lesions (CDI_{dam} > 45%), there was a further increase of the correlation coefficient between SFA scores and CDI_{dam} values (r = 0.58), which was now significant (p < 0.01) (figure 3).

CORRELATION BETWEEN CDI_{P1} AND CDI_{DAM}

There was a significant correlation between CDI_{P1} and CDI_{dam} values in joints with CDI_{dam} < 25% (r = 0.95, p < 0.01). The correlation coefficient decreased in the group with moderate damage (r = 0.48), but the correlation was still significant (p < 0.01). In the joints with CDI_{dam} > 45%, the correlation coefficient between CDI_{P1} and CDI_{dam} further decreased (r = 0.43) and became nonsignificant (p = 0.06) (figure 3).

DISCUSSION

The arthroscopic assessment of cartilage damage in this study was performed using the SFA grading system. This method was developed and validated for

human patients, who suffered mainly from meniscal tears and had generally mild chondropathies (Ayral *et al.* 1996). The system seems to be a fully quantitative measure as it calculates with a continuous variable between 0 - 100%, but it has to be realised that it is in fact a semiquantitative technique, because the method is based on classification of cartilage lesions into 5 categories (Ayral *et al.* 1996).

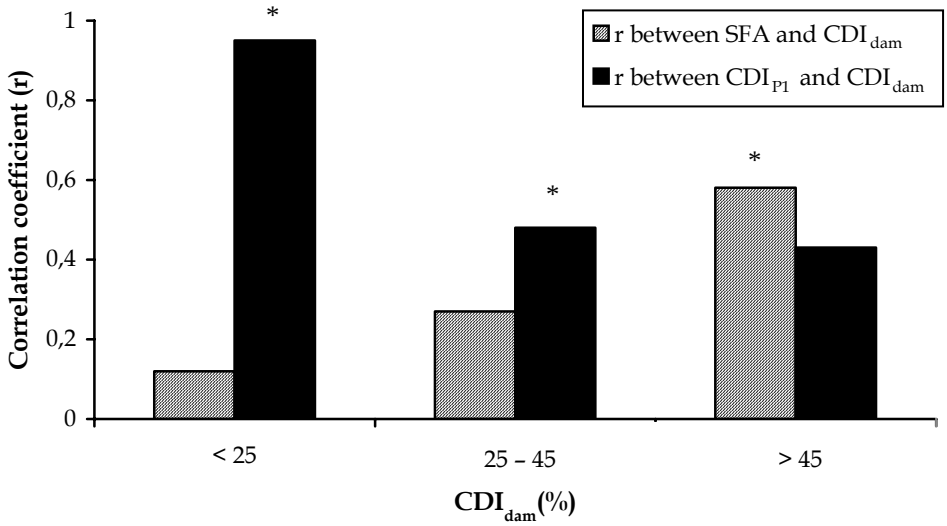

FIGURE 3: *Correlation coefficients between SFA and CDI_{dam} and between CDI_{P1} and CDI_{dam} for the mean SFA score of the 2 arthroscopists and for the 3 groups of CDI_{dam}.*
** Correlation is significant ($p < 0.05$)*

Extrapolation of the SFA grading system was assessed to be appropriate for this study because it incorporates depth and size of cartilage lesions which are also the main features that are used to judge the severity of articular cartilage damage in the horse (McIlwraith and Fessler 1978, Trotter and McIlwraith 1996). As most of the articular cartilage lesions were irregularly shaped, it was unfeasible to give the exact dimensions of these lesions. The arthroscopic estimation of the size of the lesions was compared with direct macroscopic inspection and appeared to give the same results. This indicates that the magnifying effect of the arthroscope was convenient in the assessment of the estimation of the extent of cartilage lesions. As in any scoring system, interobserver differences exist. In our study, SFA values were consistently higher for 1 of the arthroscopists, a feature which was also found

in human studies concerning arthroscopic evaluation of chondropathies (Ayral *et al.* 1996, Dougados *et al.* 1994). However, because it was a systematic error, this had no consequences for the determination of correlation coefficients and scores of the 2 observers could be averaged. With a more differentiated classification of cartilage lesions, for example based on the use of a probe or cartilage indenter (Brama *et al*. 2001) and combined with intra-articular size measuring equipment, scores as the SFA would come closer to fully quantitative and, therefore, more objective variables.

Only the dorsal approach of the MCP joint was used and assessment of the articular cartilage of the proximal sesamoid bones and the third metacarpal bone was not included in this study. This was because the CDI method has been validated only for the quantification of articular cartilage degeneration across the proximal articular surface of P1 (Brommer *et al*. 2003a). The CDI is, so far, the only fully quantitative and objective parameter for the assessment of cartilage surface integrity that has been described, and was seen as the most appropriate reference standard.

The SFA score appeared to be a good measure for cartilage damage in joints with advanced cartilage lesions only. An explanation may be that proteoglycan depletion and light cartilage fibrillation, which are the characteristics of chondropathy in early stage osteoarthritis (OA), can not be seen at arthroscopy. However, these mild forms of chondropathy will cause an increased uptake of Indian Ink (Chang *et al*. 1997, Madsen *et al*. 1992) and hence lead to a rise in CDI. Therefore, SFA scores of the dorsal articular margin of P1 give an underestimation of the real damage of the entire cartilage surface in all but severe cases. With respect to the usefulness of cartilage damage at the arthroscopically visible dorsal articular margin (CDI_{dam}) as an estimate for overall cartilage damage (CDI_{P1}) of the entire surface, the situation is reverse. Light cartilage damage at the dorsal articular margin appears to be a good representation of overall light cartilage damage of the entire surface of P1. However, severe lesions are not representative due to the irregular spread of this class of lesions over the entire joint surface (Brommer *et al*. 2003b). Therefore, in cases with severe chondropathy at the dorsal margin, CDI_{dam} gives an overestimation of CDI_{P1}.

Unfortunately, no clinical data were available for these horses. Therefore, no inferences can be made about the relationship with the clinical presentation. From previously published work, it has become clear that the correlation between severity of clinical signs and arthroscopic presentation of the articular cartilage is limited. In fact, a range of articular cartilage lesions, varying from small focal

lesions to extensive osteochondral fragmentation, can be found in both lame horses with acute clinical signs and in those presented for necropsy for conditions unrelated to the musculoskeletal system (Houttu 1991, Pool 1996, Roneus *et al*. 1997, Yovich and McIlwraith 1986). This is probably caused by the fact that the arthroscopic aspect of articular cartilage (and also the CDI) reflects cumulative damage more than the acute stage of the disease. The pathogenesis of articular chondropathy is rather complex: acute trauma and chronic repetitive (micro)trauma can induce an inflammatory process that affects the chondrocytes and the synoviocytes which, in turn, respond by producing proteolytic enzymes and cytokines that enzymatically and chemically affect the extracellular matrix. It is the intensity of this inflammatory process that determines the severity of lameness and other clinical signs (McIlwraith 1996, Pool 1996).
In equine practice, arthroscopy has long been considered the golden standard for assessing cartilage lesions (McIlwraith and Fessler 1978, Trotter and McIlwraith 1996). Recently, MRI has been advocated as the imaging modality of the future in both human and equine medicine (Eckstein *et al*. 2001, Martinelli *et al*. 1996, Recht *et al*. 2001). Therefore, it would be interesting to investigate the correlation between MRI parameters and CDI grading to gain more insight into the power of MRI for the detection of articular cartilage lesions.
It can be conluded that the cartilage characteristics in the equine MCP joint with mild signs of chondropathy can not be visualised effectively with arthroscopy, leading to an underestimation of the real damage. On the other hand, there will be an overestimation of the actual damage in cases with severe cartilage lesions if the visible area of P1 is taken as representative of the entire cartilage surface of P1. From a practical viewpoint, it can be stated that the arthroscopical grading of visible lesions on the equine P1 gives the best impression of overall cartilage damage in moderate cases of articular chondropathy. It should be realised, however, that this is achieved by the neutralisation of an error, related to arthroscopic shortcomings (underestimation of actual cartilage damage), by an unrelated other error (overestimation of severity of damage on the entire joint surface based on cartilage damage of the visible area). It therefore seems that arthroscopy may at present be the best, but not ideal, method for the clinical assessment of cartilage damage.

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MANUFACTURERS' ADDRESSES

[1] Karl Storz GmbH, Tuttlingen, Germany
[2] Sony Corp., Tokyo, Japan
[3] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER VIII -

EVALUATION OF AN ARTHROSCOPIC INDENTATION INSTRUMENT TO ESTIMATE CARTILAGE STIFFNESS AND LEVEL OF CARTILAGE DEGENERATION IN THE EQUINE METACARPOPHALANGEAL JOINT

Brommer H[a] - Laasanen MS[b] – Brama PAJ[a] - Van Weeren PR[a] – Helminen HJ[c] – Jurvelin JS[b,d]

[a] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[b] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Finland
[c] Department of Anatomy, Kuopio University, Finland
[d] Department of Applied Physics, Kuopio University, Finland

Submitted

SUMMARY

Reasons for performing study: **Early diagnosis of osteoarthritis (OA) is of great importance for appropriate therapeutic interventions and for prevention of further degeneration of articular cartilage.**

Objective: **Evaluation of a commercially available arthroscopic indentation instrument to estimate cartilage stiffness and the level of cartilage degeneration in the equine metacarpophalangeal (MCP) joint.**

Hypothesis: **The device is efficacious in the detection of changes in cartilage stiffness and in the estimation of the grade of cartilage degeneration.**

Methods: **The coefficient of variation (CV) was determined from repetitive measurements of the indenter force with the arthroscopic instrument at 2 predefined sites (site 1: joint margin, site 2: joint centre) on the articular surface of isolated proximal phalanges (P1) in 39 MCP joints. Dynamic modulus values of the samples were measured and used as reference standard for cartilage stiffness. The thickness and Cartilage Degeneration Index (CDI) value of each sample was determined as both thickness and cartilage degeneration may influence the results of indentation measurements. The relationship between indenter force measured at site 1, the only site that is arthroscopically accessible *in vivo*, and CDI values at sites 1 and 2 was established. Results were statistically evaluated ($p < 0.05$) using Pearson's linear correlation analysis and ANOVA between 4 groups of cartilage samples with increasing indenter force values (soft, moderately soft, moderately stiff, and stiff cartilage, respectively).**

Results: **The CV value was 9.0%. Moderate but significant correlations were found between indenter force and dynamic modulus ($r = 0.48$, $p < 0.001$), indenter force at site 1 and CDI at site 1 ($r = -0.46$, $p = 0.01$), and indenter force at site 1 and CDI at site 2 ($r = -0.57$, $p < 0.001$). Cartilage thickness values were not significantly different between the 4 groups of different cartilage stiffnesses ($p = 0.49$). Mean dynamic modulus of samples with low indenter force values (soft cartilage) was significantly lower ($p = 0.003$) than of the other 3 groups, which among them were not significantly different. At site 1, soft cartilage had significantly higher CDI values (48 $\pm$ 14%) than stiff cartilage (24 $\pm$ 13%) ($p = 0.04$). Soft cartilage at site 1 was also related with significantly higher CDI values at site 2 (11 $\pm$ 6%) compared to moderately stiff (3 $\pm$ 4%) and stiff cartilage (1 $\pm$ 2%) ($p = 0.005$). No significant differences in indenter force and CDI values between moderately soft, moderately stiff, and stiff cartilage could be measured ($p > 0.05$).**

Interpretation of results: **The reproducibility of the measurements with the arthroscopic indentation instrument was adequate. Sensitivity was limited, as**

the device could reveal a decrease in cartilage stiffness only when the mechanical properties of the cartilage were considerably changed. In cases of substantial cartilage damage at the arthroscopically accessible joint margin, there was also an increase in the level of cartilage degeneration at the inaccessible centre of the joint. The instrument was insensitive to reveal early functional changes of articular cartilage.
Potential relevance: **Usefulness of this indentation instrument during arthroscopic surgery is limited in the initial phase of OA-like cartilage degeneration, but the device may yield valuable additional information in more advanced stages of the disease.**

INTRODUCTION

Osteoarthritis (OA) and associated lameness is the most common cause of impaired athletic performance in horses (Pool 1996). Early diagnosis of this joint disorder is essential for appropriate therapeutic interventions and for prevention of further degeneration of articular cartilage (Price 2002, Ray *et al.* 1996). One of the first signs of incipient OA, prior to the appearance of macroscopically visible cartilage degradation, is softening of the tissue, which is mainly attributable to deterioration of the superficial collagen network and depletion of proteoglycans (Altman *et al.* 1984, Arokoski *et al.* 2000, Lane *et al.* 1979, Mow *et al.* 1990).
Arthroscopy is the diagnostic modality of choice for a direct assessment of the cartilage of the joint surface (McIlwraith 1990a, O'Connor 1977). Indentation instruments have been developed and applied to judge the stiffness of cartilage at arthroscopic examination in order to evaluate cartilage functional integrity (Appleyard *et al.* 2001, Dashefsky 1987, Lyyra 1997, Lyyra *et al.* 1995, Niederauer *et al.* 1998). The main criteria for a clinically applicable instrument are reproducibility, accuracy, and usefulness in relation to the geometric configuration of the joint surface.
In the last decade, there has been extensive research on arthroscopic indentation instruments that aimed to optimize the fulfillment of these demands (Lyyra 1997). Indentation stiffness is dependent on size and shape of the indenter (Lyyra-Laitinen *et al.* 1999, Töyräs *et al.* 2001) and on the thickness of the cartilage, a variable which will not be known during arthroscopy and which may be subject to changes during the progress of osteoarthritic disease (Töyräs *et al.* 2001). Especially in relatively thin cartilage, variations in cartilage thickness affect the outcome of indentation measurements (Hayes *et al.* 1972, Jurvelin *et al.* 1990, Mak *et al.* 1987, Töyräs *et al.* 2001). A change from plane-ended to spherical-ended indenters has

made the measured indentation stiffness less sensitive to alterations in cartilage thickness (Lyyra-Laitinen *et al.* 1999). Moreover, optimal dimensions for diameter and height of the indenter were designed for relatively thin cartilage layers, *i.e.* thickness in the range from 0.7-1.8 mm (Lyyra-Laitinen *et al.* 1999). Further, experimental and numerical tests indicated that use of a smaller reference plate and a lower pressure force additionally improves the ease of use and reproducibility of the arthroscopic indentation instrument compared to the original instrument configuration (Korhonen *et al.* 2003). Finally, high frequency ultrasound (US) was integrated in the indentation measurements to measure cartilage thickness, which further improved the sensitivity of the calculation of cartilage stiffness (Laasanen *et al.* 2002, Saarakkala *et al.* 2003, Suh *et al.* 2001, Töyräs *et al.* 2001, Zheng and Mak 1996). However, the technique of US indentation is still in a developmental phase and instruments of this type are not commercially available at the moment.

In the horse, indenter systems have been applied to predict biochemical properties (Brama *et al.* 2001), but no data exist with regard to the relation of cartilage stiffness, as measured by an indenter system, with the functional quality and, therefore, with the health status of the tissue. The aim of the present study was to test the hypothesis that use of a commercially available spherical-ended arthroscopic indentation device during arthroscopic surgery yields valuable additional information that will improve the assessment of the status of the cartilage. To this end, first the reproducibility of the indentation procedure was determined. As the results of measurement with the hand-held arthroscopic indentation instrument are a measure of dynamic stiffness of the tissue (Hayes *et al.* 1972, Lyyra *et al.* 1995, Mak *et al.* 1987), the dynamic modulus of the cartilage specimens were determined by use of a custom-made material testing device, and used as reference values. Further, the influence of cartilage thickness in specimens featuring different degrees of cartilage degeneration was investigated, as both factors are known to affect the outcome of the indentation procedure. Finally, results of indentation measurements were compared to Cartilage Degeneration Index (CDI) values as a measure for cartilage surface integrity (Brommer *et al.* 2003a).

MATERIALS AND METHODS

SAMPLE COLLECTION

Thirty-nine MCP joints were harvested from 39 horses (mixed population of Warmbloods and Thoroughbreds, age range 5 months - 22 years). The proximal

phalanx (P1) was isolated and the proximal articular cartilage surface, including approximately 5 cm of the underlying bone, was cut off using a band saw. The samples were stored at –20°C until processed. The samples were thawed at 7°C in phosphate buffered saline (PBS) soaked gauzes, 14 - 22 hours before the measurements were performed. Freezing and thawing is believed to have no effect on the tissue's intrinsic material properties (Athanasiou *et al.* 1991, Kiefer *et al.* 1989).

AREAS OF INTEREST

Two predefined locations (diameter 8 - 10 mm) were marked on the proximal articular cartilage surface of P1. Site 1 was located at the lateral dorsal margin, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin, and site 2 was located at the lateral central fovea, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins (figure 1). The 2 sites of interest have been shown to differ in their contribution to the process of cartilage degeneration across the joint surface of P1 (Brommer *et al.* 2003b) and have different biomechanical characteristics (Brommer *et al.* 2005). Moreover, site 1 is an arthroscopically accessible part of the joint, whereas site 2 is arthroscopically inaccessible (McIlwraith 1990b).

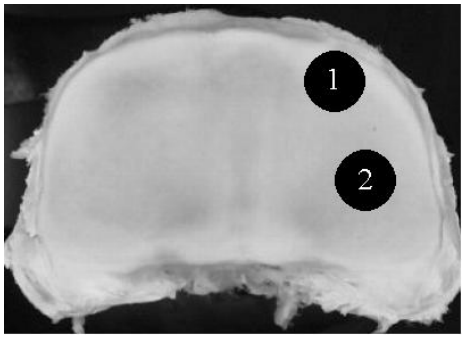

FIGURE 1: *Proximal articular cartilage surface of the equine first phalanx (P1) with the sites of interest. Site 1 was located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin. Site 2 was located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins.*

MEASUREMENT OF CARTILAGE STIFFNESS WITH THE ARTHROSCOPIC INDENTATION INSTRUMENT

Measurement of articular cartilage stiffness *in situ* at the 2 sites of interest was performed with a commercially available arthroscopic indentation instrument (Artscan 200)[1] (figure 2). Basically, the instrument produces a standard deformation of the articular cartilage and measures the force by which the cartilage resists the compression by the indenter. The instrument consists of a handle, connected to a measurement rod (diameter 5 mm). The distal end of the measurement rod contains a reference plate with an inclination of 20° to the axis of the rod. The reference plate (diameter 3 mm, height 0.48 mm) has a small, spherical-ended, cylindrical indenter in the centre (figure 2, set-in). Indenter diameter (0.6 mm) and height (0.14 mm) are small to minimize the effect of cartilage thickness on the indentation response. Both the reference plate and the indenter are connected to strain gauge transducers inside the rod, by which the exerted force and the indenter force can be measured. Labview software (version 6.1)[2] was used for data storage and analysis.

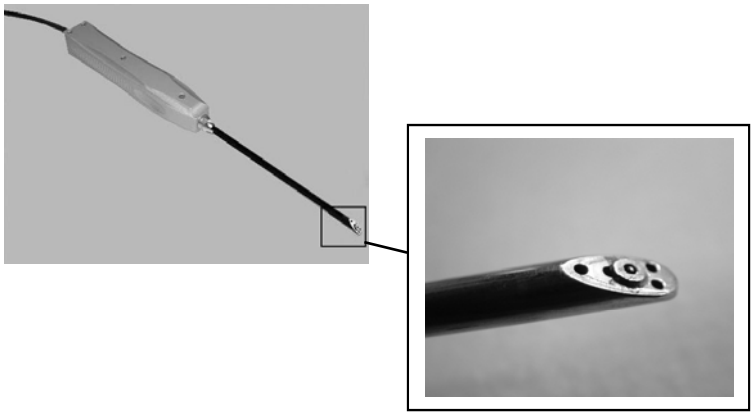

FIGURE 2: *The arthroscopic indentation instrument. Set-in: the reference plate with the spherical-ended indenter in the centre.*

The transducers were calibrated by loading the indenter with masses up to 300 grams. Calibration measurements were performed with a pressure force of 3.0 N, which was shown to be the optimal force in earlier validation experiments and

which had produced a highly linear response for both transducers (Korhonen *et al.* 2003). Care was taken that the reference plate was positioned as parallel as possible to the articular surface. In 1 run, several brief measurements were performed and the maximum indenter force for each loading with a clear stress-relaxation response was determined. Three comparable indenter forces were used for analysis. For each site 3 runs were performed. In this way, 9 indenter force measurement values were obtained for each sample site, of which mean $\pm$ s.d. was determined.

MEASUREMENT OF DYNAMIC MODULUS WITH THE MATERIAL TESTING DEVICE

Osteochondral plugs (diameter 8 mm) at sites 1 and 2 were drilled out of the cartilage surface. The subchondral bone was cut off beyond the cartilage-subchondral bone interface, leaving a 3-mm-thick subchondral bone plug attached to the articular cartilage layer. The osteochondral samples were glued onto the bottom of a sample holder with cyanoacrylate. The sample was submerged in PBS and biomechanically tested in indentation geometry using a custom made computer-controlled, high-resolution material testing device (resolution 5 mN for force and 0.1 μm for position, respectively) as described by Töyräs *et al.* (1999). A cylindrical, porous, plane-ended indenter (diameter 1.041 mm) was used. Samples were tested using a stress-relaxation protocol (two 7.5% strain steps with a velocity of 1.0 μm/s and a relaxation time of 1200 s), followed by dynamic testing (1.0% amplitude of the compressed thickness) at a frequency of 1.0 Hz. Biomechanical testing was performed using cartilage thickness values determined by ultrasound, as decribed below. Software for data acquisition and analysis, developed with LabVIEW (version 6.1)[2], was used. Dynamic modulus, serving as reference standard for the measurements with the arthroscopic instrument, was calculated from the last 2 cycles of the dynamic test, using an analytical model for cartilage indentation (Hayes *et al.* 1972). When computing values of dynamic modulus, the thickness values measured by the needle-probe technique as described below, were used. Cartilage was assumed to be incompressible during 1.0 Hz cyclic compression and, therefore, Poisson's ratio was set at 0.5 (Wong *et al.* 2000, Mak *et al.* 1987).

MEASUREMENT OF CARTILAGE THICKNESS

Before the biomechanical tests were performed, cartilage thickness at the centre of sites 1 and 2 *in situ* was determined ultrasonographically as described by Laasanen *et al.* (2002). Cartilage thickness was calculated by multiplying the flight time with

a speed of sound of 1650 m/s, which was based on findings in bovine cartilage (Töyräs *et al.* 2003), and on pilot measurements using equine articular cartilage. Software for data acquisition and analysis was developed, using LabVIEW (version 6.1)[2]. After biomechanical testing of the samples in the material testing device, the thickness was measured again, now using the well-validated needle-probe technique (Jurvelin *et al.* 1995). The penetration velocity of the needle was 10 μm/s. LabVIEW software (version 5.1)[2] was used for data acquisition and analysis.

MEASUREMENT OF CARTILAGE DEGENERATIVE STATE

The degenerative state of the 2 sites of interest was determined *in situ* in 30 of 39 joints, using the CDI technique (Brommer *et al.* 2003a). The CDI is based on the observation that degenerated articular cartilage, which is depleted of proteoglycans and shows surface fibrillation, takes up Indian ink particles whereas intact cartilage hardly stains. The amount of Indian ink uptake is quantified by digital imaging of the native and the Indian ink stained articular cartilage surfaces under standardized conditions. The mean pixel value is then determined as a measure for the grey level and the increase in the mean grey level of the articular surface is the basis for calculation of the CDI, which has been shown to have excellent correlation with a macroscopic grading system for cartilage degeneration (Brommer *et al.* 2003a).

DATA ANALYSIS

Prior to data analysis, it was tested that all values for indenter force, dynamic modulus, cartilage thickness, and CDI were normally distributed.

REPRODUCIBILITY OF INDENTER FORCE MEASUREMENTS

Coefficient of variation (CV) was calculated as the ratio of the standard deviation (σ) and the mean (x) of the repeated measurements of the indenter force. For a number (n) of samples, the CV is the root mean square averaged value (CV_{RMS}) (Blake *et al.* 1999, Mead *et al.* 1993):

$$CV_{RMS} = \sqrt{\left(\sum_{i=1}^{n} (\sigma_{x_i} / \bar{x}_i)^2\right)/n}$$

RELATIONSHIP BETWEEN INDENTER FORCE AND DYNAMIC MODULUS

In order to study the sensitivity of indenter force measurements with the arthroscopic device, Pearson's linear correlation coefficient was calculated between

indenter force and the reference dynamic modulus values of the tested samples (n = 78). The samples were ranked to increasing values of the indenter force, and classified in 4 categories of equal numbers: soft cartilage (n = 20), moderately soft cartilage (n = 19), moderately stiff cartilage (n = 19), and stiff cartilage (n = 20). Mean $\pm$ s.d. of dynamic modulus values was calculated for each group.

RELATIONSHIP BETWEEN INDENTER FORCE AND CARTILAGE THICKNESS

To evaluate possible influences of cartilage thickness on indenter force measurements, the accuracy of thickness measurements was studied first by calculating Pearson's linear correlation coefficient and the relative mean difference of the thickness values obtained by the ultrasonographic and needle-probe technique (n = 78). Then, Pearson's linear correlation coefficient was determined between indenter force and needle probe thickness values (n = 78). Finally, mean $\pm$ s.d. of needle-probe thickness values was calculated for the 4 indenter force classes.

RELATIONSHIP BETWEEN INDENTER FORCE AND LEVEL OF CARTILAGE DEGENERATION

To characterise a possible relationship between indenter force and cartilage surface integrity (or cartilage degeneration), Pearson's linear correlation coefficient was calculated between indenter force values measured on site 1 (which is, in contrast to site 2, arthroscopically accessible *in vivo*), and CDI values on sites 1 and 2, respectively (n = 30 for both sites, 9 joints were excluded as CDI data were not determined for these joints). Of the indenter force values measured at site 1 in these 30 joints, 13 fell in the category of soft cartilage, and 5, 6, and 6 were classified as moderately soft cartilage, moderately stiff cartilage, and stiff cartilage, respectively. For each group, mean $\pm$ s.d. of the CDI values on sites 1 and 2 was calculated.

STATISTICAL ANALYSIS

Differences in dynamic modulus, cartilage thickness (needle-probe values), and CDI values between the 4 groups of cartilage stiffness were statistically analysed, using ANOVA with Bonferroni post hoc comparisons. The level of significance was set at $p < 0.05$. All tests were run using SPSS software (version 10.0)[3].

RESULTS

REPRODUCIBILITY OF INDENTER FORCE MEASUREMENTS

Reproducibility of the indenter force measurements by single user, as indicated by CV_{RMS}, was 9.0% (n = 78).

RELATIONSHIP BETWEEN INDENTER FORCE AND DYNAMIC MODULUS

There was a moderate but significant linear correlation between indenter force and dynamic modulus values ($r = 0.48$, $p < 0.001$, $n = 78$). The indenter force ranges in the groups of soft cartilage, moderately soft cartilage, moderately stiff cartilage, and stiffcartilage were 0.45 - 0.65 N, 0.65 - 0.75 N, 0.75 - 0.86 N, and 0.89 - 1.20 N, respectively. The mean value of dynamic modulus of soft cartilage (2.7 $\pm$ 1.3 MPa, $n = 20$) was significantly lower ($p = 0.003$) than that of moderately soft (4.5 $\pm$ 1.6 MPa, $n = 19$), moderately stiff (4.5 $\pm$ 1.6 MPa, $n = 19$), and stiff cartilage (4.8 $\pm$ 1.7 MPa, $n = 20$). Differences between moderately soft, moderately stiff, and stiff cartilage were not significant ($p = 0.9$) (figure 3).

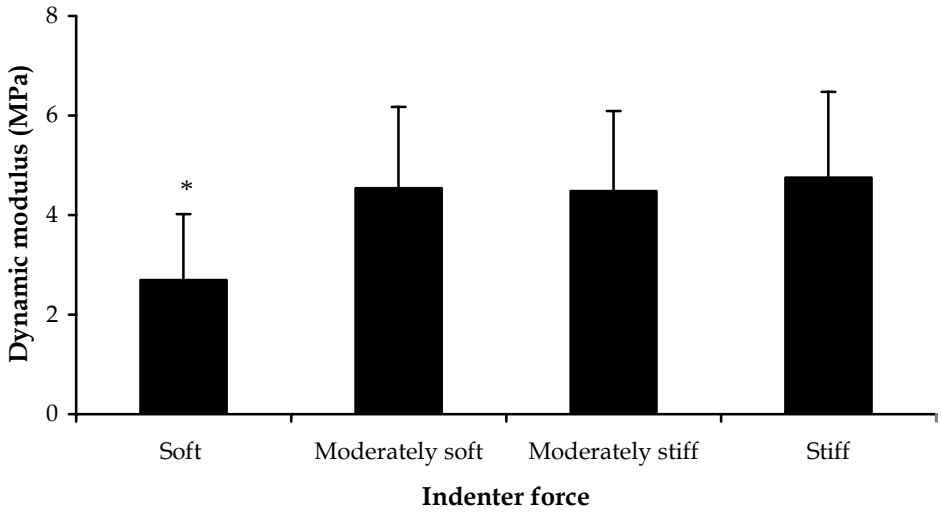

FIGURE 3: *Dynamic modulus (mean $\pm$ s.d., MPa) in soft cartilage, moderately soft cartilage, moderately stiff cartilage, and stiff cartilage, as identified by indenter force.*
** $p < 0.05$ vs. moderately soft, moderately stiff, and stiff cartilage*

RELATIONSHIP BETWEEN INDENTER FORCE AND CARTILAGE THICKNESS

Pearson's linear correlation coefficient between ultrasonographic and needle-probe thickness values was 0.94 ($p < 0.001$, $n = 78$). The relative mean difference of the thickness values obtained by both techniques was – 1.8%. There was a rather weak but significant inverse linear correlation between indenter force and cartilage needle-probe thickness values ($r = - 0.27$, $p = 0.02$, $n = 78$). The mean needle-probe

thickness values for soft cartilage (896 ± 194 μm, n = 20), moderately soft cartilage (846 ± 198 μm, n = 19), moderately stiff cartilage (887 ± 210 μm, n = 19), and stiff cartilage (805 ± 224 μm, n = 20) were not significantly different (p = 0.49).

RELATIONSHIP BETWEEN INDENTER FORCE AT SITE 1 AND THE LEVEL OF CARTILAGE DEGENERATION

There were moderate but significant inverse linear correlations between indenter force at site 1 and CDI values at site 1 ($r = -0.46$, $p = 0.011$, $n = 30$) and between indenter force at site 1 and CDI values at site 2 ($r = -0.57$, $p < 0.001$, $n = 30$). At site 1, soft cartilage had significantly higher CDI values (48 ± 14%, n = 13) than stiff cartilage (24 ± 13%, n = 6) (p = 0.04). Soft cartilage at site 1 was also related with significantly higher CDI values at site 2 (11 ± 6%, n = 13) compared to moderately stiff (3 ± 4%, n = 6) and stiff cartilage (1 ± 2%, n = 6) (p = 0.005). Differences in CDI values between moderately soft, moderately stiff, and stiff cartilage were not significant at both sites 1 and 2 (p = 0.49 and p = 0.12, respectively) (figure 4).

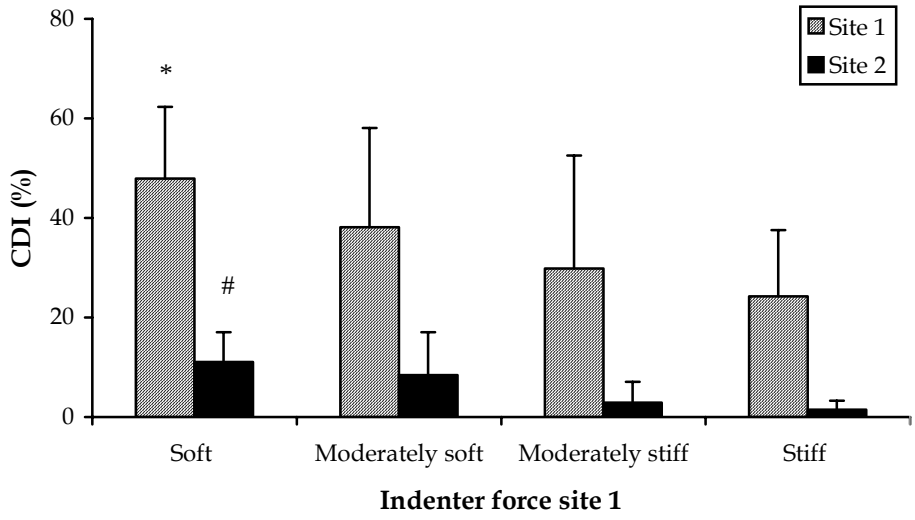

FIGURE 4: *Cartilage Degeneration Index (CDI) values (mean ± s.d., %) at site 1 and 2 in joints with soft, moderately soft, moderately stiff, and stiff cartilage at site 1, as identified by indenter force.*
* *p < 0.05 vs. stiff cartilage*
# *p < 0.05 vs. moderately stiff and stiff cartilage*

DISCUSSION

Reproducibility of indentation measurements with the hand-held arthroscopic indentation device was adequate. The CV of 9.0% is in accordance with findings of other studies (Franz *et al.* 2001, Lyyra-Laitinen *et al.* 1999). The analytical single-phase elastic model of cartilage indentation (Hayes *et al.* 1972) was used to analyse the outcome of the reference biomechanical tests in the material testing device. In this theoretical model, measurement of cartilage thickness can be critical (Hayes *et al.* 1972, Jurvelin *et al.* 1990). In the present study, we measured articular cartilage thickness nondestructively prior to biomechanical testing, using ultrasonography (Laasanen *et al.* 2002). The accuracy of this technique was validated against a slightly destructive needle-probe measurement, conducted after biomechanical testing (Jurvelin *et al.* 1995). The ultrasound and needle-probe thickness values were highly similar in the tested samples. As the mean cartilage thickness of the groups with soft, moderately soft, moderately stiff, and stiff cartilage was similar, a decrease in dynamic modulus can be attributed to true changes in the mechanical properties of the cartilage.

Soft cartilage, as measured with the arthroscopic indentation device, showed significantly lower dynamic modulus values compared to moderately soft, moderately stiff, and stiff cartilage. Furthermore, soft cartilage at site 1, which is the arthroscopically accessible part of the MCP joint, corresponded not only with significantly elevated CDI values at this site, but was also related with a significant increase of the CDI value at site 2, which is an arthroscopically inaccessible area of the MCP joint. This finding implies that in these cases, the process of cartilage degeneration across the articular surface of P1 has progressed to a more advanced stage, as cartilage degeneration invariably starts at the dorsal margin of P1 and extends to the central fovea in more advanced stages (Brommer *et al.* 2003b). In a previous study, it was shown that a significant increase in the level of cartilage degradation at the joint centre was related with a significant decrease of dynamic stiffness at this location (Brommer *et al.* accepted for publication).

Moderately soft, moderately stiff, and stiff cartilage could not be distinguished from each other with the arthroscopic indentation instrument. In these groups, the CDI values of the samples gradually increased with decreasing indenter force values, but the differences were not significant. This suggests that the instrument was not sensitive enough to detect early functional changes in cartilage stiffness before severe changes in CDI values occur. However, some critical notes regarding this apparent lack of sensitivity, as found in this study, should be noticed. The tested instrument has been primarily developed for use in humans, and, given the

variation of mechanical properties of articular cartilage between different species (Athanasiou *et al.* 1991, Simon 1970), the measurement capability range of the instrument may not have been ideal for the stiffness characteristics of articular cartilage in horses. Further, the reference plate has to make full contact with the articular surface and has to be positioned as parallel to the cartilage surface as possible. In the case of the equine P1, which has a rather concave surface, optimal positioning may not always be possible due anatomical constraints. This may explain the lower linear correlation coefficient between indenter force and dynamic modulus value as compared to previously performed validation studies of the indentation instrument, in which correlation coefficients of 0.8-0.9 have been reported (Korhonen *et al.* 2003, Lyyra *et al.* 1995, Lyyra-Laitinen *et al.* 1999). In this context, it should be stressed that the arthroscopic instrument was tested on isolated P1 bones in this study. In the intact MCP joint, the problem of correct positioning of the device on P1 will probably even be greater as the presence of the third metacarpal bone precludes optimal positioning of the indenter.

Another factor that may have contributed to the difficulty of distinguishing between the higher stiffness ranges, is the relatively thin cartilage of the equine P1. Cartilage thickness of this joint surface ranges between 598-1405 μm. Especially in cases of thin cartilage, which is bonded to the rigid subchondral bone, a small variation in cartilage thickness may have a significant impact on the results of individual indentation measurements (Hayes *et al.* 1972, Jurvelin *et al.* 1990, Mak *et al.* 1987, Töyräs *et al.* 2001). In numerical analysis studies using Hayes' elastic model (Hayes *et al.* 1972), variation of the cartilage thickness in the range of 0.5 – 1.2 mm affected the indenter force by about 25%, and evaluation of the effect using a finite element model revealed an effect of thickness variation on indenter force of 42% (Lyyra-Laitinen *et al.* 1999). The indenter force is virtually independent on cartilage thickness when cartilage is thicker than 2 mm, as determined for plane-ended indenters with a diameter of 1.3 mm and height of 300 μm (Lyyra *et al.* 1995). Even though the introduction of a spherical-ended indenter has made the indentation stiffness less sensitive to alterations in cartilage thickness (Lyyra-Laitinen *et al.* 1999, Töyräs *et al.* 2001), this geometrical adaptation may be less effective in the case of the thin cartilage layer of the equine MCP joint.

Despite these limitations, the instrument could well identify the soft cartilage and may, therefore, enhance the value of diagnostic arthroscopy in more advanced cases of OA-like cartilage degeneration.

Arthroscopic indentation measurements are not only suitable for evaluation of the biomechanical functionality of cartilage, but are potentially also useful for

detecting degradation of the superficial collagen network of the extracellular matrix (ECM) of cartilage as the collagen fibrils provide high tensile stiffness and effectively control the instantaneous elastic response in compression (Bader and Kempson 1994, Bader *et al.* 1992). In a previous study on the application of an indenter system in the MCP joint of horses, no correlation was found between the measured indenter force and collagen content of the cartilage (Brama *et al.* 2001). In a biochemical study of joints with and without early OA changes, no significant differences were found in the levels of hydroxylysylpyridinoline crosslinks at the joint margin and at the joint centre of the equine P1 (Van der Harst *et al.* accepted for publication). Both observations suggest that the indentation force, as determined with the arthroscopic indentation device, is primarily related to the structural integrity of the collagen matrix and not to content or biochemical make-up of collagen. This is in line with earlier findings in human cartilage (Bank *et al.* 2000, Franz *et al.* 2001). The proteoglycan component of the ECM has been shown to play a less important role in the reduction of the tensile stiffness of degenerated cartilage (Bank *et al.* 2000). However, high maximum indenter forces correlated positively with high glycosaminoglycan (GAG) levels (Brama *et al.* 2001) and levels of GAG have been shown to decrease in the articular surface of P1 in MCP joints affected by OA (Van der Harst *et al.* accepted for publication). This suggests that a decrease of indentation forces reflect a decrease in GAG levels of the cartilage.

It is concluded that application of the commercially available spherical-ended arthroscopic indentation instrument may have the potential to increase the diagnostic information about the cartilage status that can be obtained during arthroscopy of equine MCP joints. Direct information can be obtained about cartilage stiffness at the joint margin, which in turn provides indirect information about the cartilage status at the joint centre, which cannot be visualised during arthroscopy. However, the instrument in its present configuration does not detect the initial functional changes in cartilage stiffness and is, therefore, insensitive to detect cartilage degeneration associated with OA at an early timepoint. This lack of sensitivity is probably related to the surface geometry of P1 and the relatively thin and stiff cartilage in the equine MCP joint. Integration of ultrasound in indentation measurements can overcome the problem of unknown cartilage thickness, enabling improvements in the calculation of material stiffness (Suh *et al.* 2001, Zheng and Mak 1996). Results of validation studies are promising (Laasanen *et al.* 2002 and 2003, Saarakkala *et al.* 2003 and 2004, Töyräs *et al.* 2001), but further technical development and research are needed before instruments of this type can be recommended for routine clinical use in equine orthopaedics.

MANUFACTURERS' ADDRESSES

[1] Artscan Oy, Helsinki, Finland

[2] LabVIEW, National Instruments, Austin, USA

[3] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER IX -

DETERMINATION OF THE SPEED OF SOUND IN EQUINE ARTICULAR CARTILAGE AND THE INFLUENCE OF AGE, SITE, AND DEGENERATIVE STATE

Brommer H[a] – Laasanen MS[b] – Brama PAJ[a] - Van Weeren PR[a] – Barneveld A[a] – Helminen HJ[c] – Jurvelin JS[b,d]

[a] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[b] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Finland
[c] Department of Anatomy, Kuopio University, Finland
[d] Department of Applied Physics, Kuopio University, Finland

Am J Vet Res, accepted for publication

SUMMARY

Objective: **To determine the speed of sound (SOS) in equine articular cartilage and to investigate the influence of age, site in the joint, and cartilage degeneration on the SOS.**

Sample population: **Cartilage samples from metacarpophalangeal joints of 38 horses (age 0.5 - 22 years).**

Procedure: **Osteochondral plugs were collected from 2 articular sites of the proximal phalanx after the degenerative state was characterized by use of the Cartilage Degeneration Index (CDI) technique. The SOS was calculated (ratio of needle-probe cartilage thickness to time of flight of the ultrasound pulse) and relationships between SOS and age, site, and cartilage degeneration were evaluated. An analytical model of cartilage indentation was used to evaluate the effect of variation in true SOS on the determination of cartilage thickness and dynamic modulus with the US indentation technique.**

Results: **The mean SOS for all samples was 1696 $\pm$ 126 m/s. Age, site, and cartilage degeneration had no significant influence on the SOS ($p > 0.05$). The analytical model revealed that use of the mean speed of sound of 1696 m/s was associated with maximum errors of 17.5% on cartilage thickness and 7.0% on dynamic modulus in a SOS range that covered 95% of the individual measurements.**

Conclusions and clinical relevance: **In equine articular cartilage, use of a mean SOS of 1696 m/s in US indentation measurements introduces some inaccuracy on cartilage thickness determinations, but the dynamic modulus of cartilage can be estimated with acceptable accuracy in horses regardless of age, site in the joint, or stage of cartilage degeneration.**

INTRODUCTION

Osteoarthritis (OA) is one of the main causes of impaired performance in equine athletes (Pool 1996). Early diagnosis of this joint disorder is essential for successful prevention of further degeneration of articular cartilage through appropriate therapeutic interventions (Price 2002). The first signs of early cartilage damage are a decreased proteoglycan (PG) concentration in the superficial layer and a disorganization of the collagen network (Arokoski *et al.* 2000, Mow *et al.* 1990). These lead to changes in the biomechanical and structural properties of the cartilage, such as softening and surface roughness or fibrillation (Arokoski *et al.* 2000, McIlwraith 1996). Modern diagnostic imaging modalities, such as quantitative magnetic resonance imaging (MRI), have improved the evaluation of articular cartilage structural integrity (Anastasiou *et al.* 2003, Burstein *et al.* 2000). For evaluation of cartilage functional quality, direct mechanical indentation during arthroscopy has been applied (Lyyra *et al.* 1995, Niederauer *et al.* 1998). However, indentation stiffness is dependent on the thickness of the cartilage and an unknown thickness induces uncertainty in the results (Hayes *et al.* 1972, Mak *et al.* 1987, Töyräs *et al.* 2001). For this reason, high frequency ultrasound (US) was integrated in indentation measurements. The combined use of US and mechanical indentation may provide a sensitive tool to diagnose changes in thickness and biomechanical properties of cartilage in the very early stage of osteoarthritic disease (Laasanen *et al.* 2002, Saarakkala *et al.* 2003, Suh *et al.* 2001). In the equine metacarpophalangeal (MCP) joint with degenerative joint disease, values of cartilage biomechanical parameters, *i.e.* Young's modulus and dynamic modulus, may decrease as much as 40%, depending on the severity of the disease, without significant changes in cartilage thickness (Brommer *et al.* accepted for publication).

Accurate application of US indentation requires the appropriate value for the speed of sound (SOS) in articular cartilage. The SOS in equine cartilage is unknown, but values have been determined in human and bovine articular cartilage (Agemura *et al.* 1990, Kolmonen *et al.* 1995, Myers *et al.* 1995, Suh *et al.* 2001, Töyräs *et al.* 1999 and 2003, Yao and Seedhom 1999, Youn *et al.* 1999). Topographical variations in SOS at different sites of the articular cartilage surface have been reported, and these may jeopardize the accuracy of US indentation measurements (Töyräs *et al.* 2003, Yao and Seedhom 1999). Moreover, the SOS appears to be related to cartilage composition, structure, and degenerative state (Agemura *et al.* 1990, Joiner *et al.* 2001, Myers *et al.* 1995, Nieminen *et al.* 2002 and 2004, Suh *et al.* 2001, Töyräs *et al.* 1999 and 2003). Although significant correlations have been found between the quality of cartilage and the SOS, differences in the

SOS between normal and degenerated cartilage were rather small, and studies in bovine cartilage indicated that use of a constant SOS was deemed justifiable (Nieminen *et al.* 2002, Töyräs *et al.* 2003).
The purpose of the study reported here was to determine the SOS in equine articular cartilage. Cartilage of the proximal articular surface of the proximal phalanx (P1) was chosen as object of study because of its clinical importance: the MCP joint is the joint that sustains the largest number of traumatic and degenerative lesions (Pool 1996). Further, the influence of 3 main clinical variables, *i.e.* age, site, and cartilage degeneration, on the SOS was investigated. In addition, the effect of variation in the SOS on the uncertainty associated with determination of cartilage thickness and dynamic modulus with an US indentation instrument was simulated by use of an analytic model of cartilage indentation (Hayes *et al.* 1972, Mak *et al.* 1987).

MATERIALS AND METHODS

SAMPLE COLLECTION

Thirty-eight MCP joints from 38 horses (mixed population of Warmblood horses and Thoroughbred horses) were selected from a sample bank of equine MCP joints that had been collected from horses at the slaughter house or from horses used in experimental studies. The series of samples consisted of left MCP joints collected from 8 horses of age 5 months with no joint disease, right MCP joints collected from 9 horses of age 1.5 years with no joint disease, and left MCP joints collected from 21 mature horses (age 3 - 22 years) with different stages of OA. From each joint, P1 was isolated and the proximal articular cartilage surface, including approximately 5 cm of the underlying bone, was cut off by use of a band saw. The samples were stored at –20°C until processed. On the previous afternoon before the measurements were performed, the samples were wrapped in gauzes, soaked with phosphate buffered saline (PBS), and thawed at 7°C. It has been shown that freezing and thawing have no or only minor effect on the biomechanical and acoustic properties of soft tissues (D'Astous *et al.* 1986, Geleskie *et al.* 1982, Kiefer *et al.* 1989).

AREAS OF INTEREST AND MEASUREMENT OF DEGENERATIVE STATE

Two predefined locations (diameter 8 - 10 mm) were marked on the proximal articular cartilage surface of P1. Site 1 was located at the lateral dorsal margin, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin. Site 2 was located at

the lateral central fovea, halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins (figure 1). The biochemical and biomechanical properties of these 2 sites differ markedly and significantly (Brama *et al.* 2000a, Brommer *et al.* 2005), and *in vivo*, these sites are subject to different loading conditions (Brama *et al.* 2001). Except for the samples from the 5-month-old horses, the degenerative state of the 2 sites of interest was measured postmortem, using the Cartilage Degeneration Index (CDI) technique (Brommer *et al.* 2003). Briefly, the CDI is based on the fact that degenerated articular cartilage, which is depleted of proteoglycans and shows surface fibrillation, takes up Indian ink particles whereas intact cartilage is hardly stained. In this method, the amount of Indian ink uptake is quantified under standardized conditions via digital imaging of the native (*i.e.* unstained) and the Indian ink stained articular cartilage surfaces. The mean pixel value is then determined as a measure for grey level and the increase in mean grey level of the articular surface after staining is the basis for calculation of the CDI (range 0 - 100%).

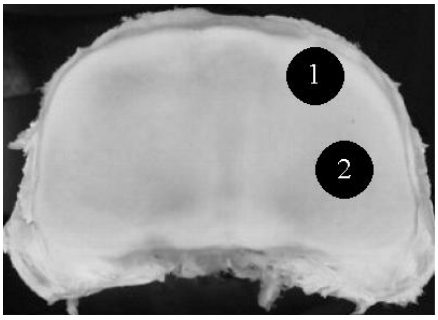

FIGURE 1: *Proximal articular cartilage surface of the equine first phalanx (P1) with the sites of interest. Site 1 was located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface, adjacent to the dorsal articular margin. Site 2 was located halfway between the lateral edge of the sulcus articularis and the lateral border of the articular surface and halfway between the dorsal and palmar articular margins.*

ACOUSTIC MEASUREMENT OF CARTILAGE THICKNESS

Cartilage thickness at the centre of the marked sites of interest was measured by use of a recently developed US indentation instrument (Laasanen *et al.* 2002,

Saarakkala *et al.* 2003). Briefly, an unfocused 10 MHz broadband ultrasound transducer (diameter 3 mm, Panametrics XMS-310)[1] was mounted on the tip of an arthroscopic indentation instrument (Artscan 200)[2]. The ultrasound transducer was controlled with UltraPAC-system[3] containing a 500 MHz AD-board and a 0.5 - 100 MHz pulse receiver board. During ultrasound measurements, bandpass filtering (2.0 - 18 MHz, rolloff 24 dB/oct) was utilized to enhance the signal to noise ratio. Software for data acquisition and analysis was developed, using LabVIEW (version 6.1)[4]. The predefined SOS was set to 1650 m/s, based on findings in bovine cartilage (Töyräs *et al.* 2003) and pilot measurements.
The acoustic measurements were conducted with a pressure of 1.0 N and the cartilage surface was kept moist with PBS solution. For each cartilage sample, the thickness measured at the maximum ultrasound echo amplitude was determined 3 times per sample and mean $\pm$ s.d. was determined.

MEAN VARIABILITY AND REPEATABILITY OF US THICKNESS MEASUREMENTS

For each sample, the relative variability of the 3 US thickness measurements was determined as the ratio of 4 x s.d. (equivalent to the 95% range) to the mean value, multiplied by 100%. Then, the mean $\pm$ s.d. of the relative variability of all samples was calculated for sites 1 and 2. Repeatability of the US thickness measurements was determined in terms of coefficient of variation (CV) (Blake *et al.* 1999, Mead *et al.* 1993).

NEEDLE-PROBE MEASUREMENT OF CARTILAGE THICKNESS

After the CDI and acoustic measurements were completed, osteochondral plugs (diameter 8 mm) were drilled out from the predefined sites 1 and 2. The subchondral bone was smoothly cut off distal to the cartilage-subchondral bone interface, so that 3 mm of subchondral bone tissue remained on the articular cartilage layer. Subsequently, the samples were glued onto a sample holder with cyanoacrylate and submerged in PBS for 1 hour. The sample was aligned in such a way that the cartilage in the centre of the plug was in horizontal position. Articular cartilage thickness at the centre of the plug was measured, using the needle-probe technique (Jurvelin *et al.* 1995). With this technique, a load cell is used to sense the movements when a sharp-pointed needle presses the articular surface and when it contacts the subchondral bone. The penetration velocity of the needle was 10 μm/s. LabVIEW software (version 5.1)[4] was developed and used for data acquisition and analysis.

DETERMINATION OF SPEED OF SOUND (SOS) IN CARTILAGE

While measuring cartilage thickness with the US indentation instrument, the ultrasound time of flight (TOF) was determined from the maximum value of the Hamming windowed Hilbert's envelope calculated for the signal. Details of this technique have been presented in a previous study (Saarakkala *et al.* 2003). The SOS was calculated as the ratio of needle-probe thickness x 2 (equivalent to the length of US travel) to the TOF. The mean US thickness value of each sample was used for the TOF calculations.

MEAN SOS AND CORRELATION BETWEEN NEEDLE-PROBE AND US THICKNESS VALUES

All samples were pooled and the mean $\pm$ s.d. of the SOS value was calculated. The US thickness values, based on the hypothesized SOS of 1650 m/s, were recalculated, using the mean SOS for all the samples pooled. Pearson's linear correlation coefficient was calculated between the needle-probe thickness values and these recalculated US thickness values. Because the correlation coefficient indicates the strength of a relation between 2 variables, but not the real agreement between them, the relative difference in thickness values between the methods was determined for each sample, calculated as the ratio of the measurement difference to the mean value, multiplied by 100%. Then, the relative differences in thickness values were plotted against the mean thickness values (Bland and Altman 1986), and the mean $\pm$ s.d. of all samples was calculated.

SOS IN RELATION TO AGE, SITE, AND CARTILAGE DEGENERATION

Multivariate statistical analysis was performed to screen the effects of age, site, and cartilage degeneration on the SOS value. To characterize the relationship between age and SOS, Pearson's linear correlation coefficient was determined. Subsequently, the samples were categorized into 3 groups on the basis of age of the horses: 5 months (n = 16), 1.5 years (n = 18), and > 3 years (mature horses, n = 42). To address the possible influence of site differences on SOS, the samples were separated into 2 groups: samples harvested from site 1 (n = 38) and from site 2 (n = 38). When characterizing the relationship between cartilage degeneration and SOS, samples from the 5-month-old horses were excluded because CDI values were not determined. From all other samples, Pearson's linear correlation coefficient was determined between SOS and CDI values. Moreover, the samples were categorized into 3 groups, based on CDI values: group 1 contained samples with CDI values < 20% (n = 34), group 2 contained samples with CDI values between 20 - 40% (n = 14), and group 3 contained samples with CDI values > 40%

(n = 12). The mean ± s.d. of the SOS value was calculated for each group. Differences in SOS among the age groups, between the 2 sites, and among the groups with different degenerative states of the cartilage were statistically evaluated using the Kruskal-Wallis test. For all statistics, SPSS software (version 10.0)[5] was used and the level of significance was set at $p < 0.05$.

SIMULATION OF THE EFFECT OF VARIATION IN SOS

The effect of variation in the true SOS on the error (%) of cartilage thickness and dynamic modulus, as determined with the US indentation instrument, by use of a constant predefined SOS (*i.e.* the mean SOS for all samples pooled) was simulated by modeling cartilage as if it was isotropic and instantaneously incompressible (Hayes *et al.* 1972, Mak *et al.* 1987). An indenter diameter of 3 mm was used. The simulation was conducted for a SOS range that covered 95% of the individual measurements (*i.e.* mean ± 2 x s.d.) and for the cartilage thickness range of the tested samples. Subsequently, errors on cartilage thickness and dynamic modulus were calculated for thickness of so called 'typical cartilage' (*i.e.* the mean cartilage thickness of the samples used in this study).

RESULTS

MEAN VARIABILITY AND REPEATABILITY OF US THICKNESS MEASUREMENTS

The variability of US thickness measurements was 6.7 ± 7.6% for site 1 and 7.5 ± 5.6% for site 2, respectively. The coefficient of variation (CV) was 2.4%.

MEAN SOS AND CORRELATION BETWEEN NEEDLE-PROBE AND US THICKNESS VALUES

The mean SOS for all samples was 1696 ± 126 m/s. There was a strong linear correlation between needle-probe thickness values and US thickness values using the mean SOS ($r = 0.96$, $p < 0.001$, $n = 76$, figure 2). The mean relative difference between the US and needle-probe thickness values was 0.3 ± 7.4% (figure 3).

SOS IN RELATION TO AGE, SITE, AND CARTILAGE DEGENERATION

Multivariate analysis revealed no significant influence of age ($p = 0.48$), site ($p = 0.79$), and CDI ($p = 0.78$) on the SOS.

There was no significant correlation between SOS and age ($r = -0.19$, $p = 0.10$). Values for SOS were 1695 ± 160 m/s , 1748 ± 127 m/s, and 1675 ± 107 m/s for age 5 months, 1.5 years, and mature horses, respectively. The differences between these values were not significant ($p = 0.12$).

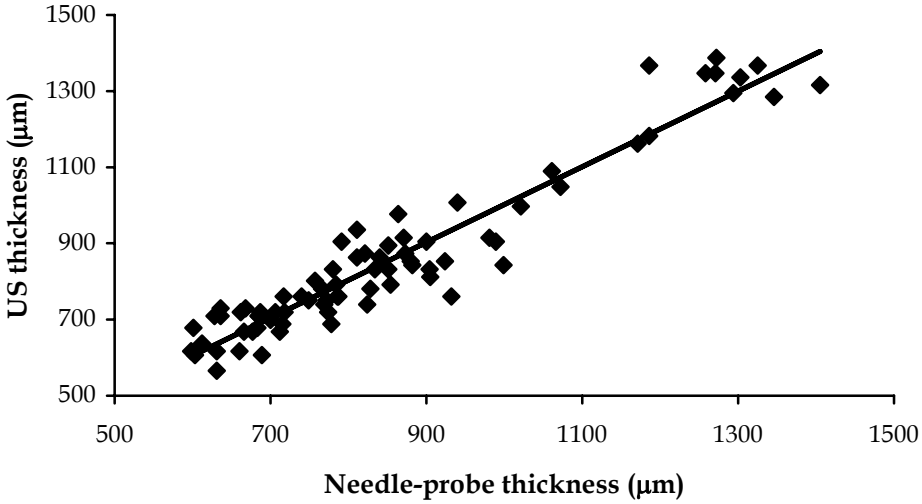

FIGURE 2: *Pearson's linear correlation between values of cartilage thickness (µm) obtained with the ultrasound (US) and needle-probe technique (r = 0.96, p < 0.001, n = 76). US detected thickness was calculated using a predefined speed of sound (SOS) of 1696 m/s.*

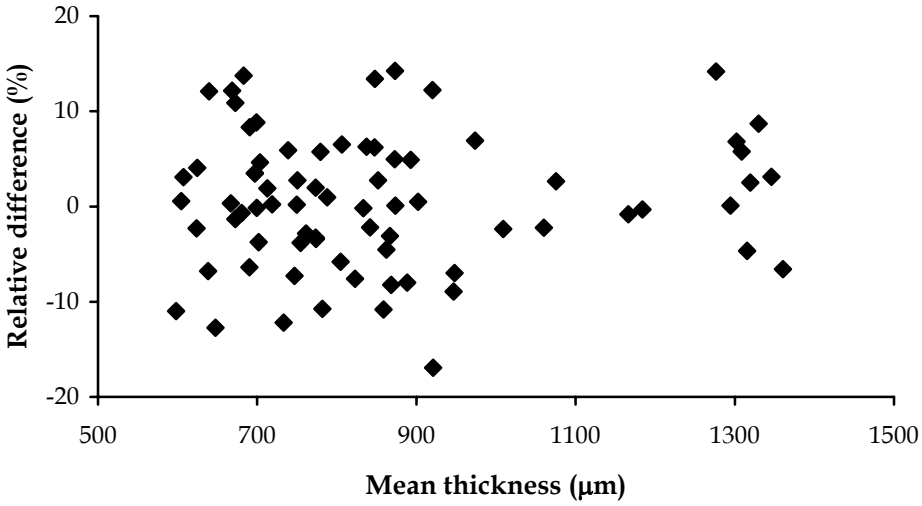

FIGURE 3: *Scatter plot showing the relative difference (%) between the cartilage thickness (µm) obtained with the ultrasound (US), using the mean speed of sound (SOS) of 1696 m/s, and needle-probe technique.*

There were no significant differences between the 2 sites of interest (p = 0.82). The SOS values were 1705 ± 123 m/s and 1688 ± 130 m/s for sites 1 and 2, respectively. There was no significant correlation between SOS and CDI values (r = 0.17, p = 0.19). The SOS was 1681 ± 109 m/s, 1706 ± 145 m/s, and 1731 ± 104 m/s for cartilage with CDI < 20%, CDI between 20 - 40%, and CDI > 40%, respectively. Among the 3 CDI groups, there were no significant differences (p = 0.56).

SIMULATION OF THE EFFECT OF VARIATION IN SOS ON CARTILAGE THICKNESS AND DYNAMIC MODULUS

Simulation of the error induced by the variable SOS on the determination of cartilage properties with the US indentation technique revealed that use of the mean SOS for all samples (*i.e.* 1696 m/s), instead of the true speed, induced a maximum error of 17.5% on cartilage thickness and a maximum error of 7.0% on dynamic modulus for the mean cartilage thickness of the tested samples (855 μm) in the SOS range that covered 95% of the individual measurements for SOS (1444 – 1948 m/s) (figure 4).

DISCUSSION

The present study revealed no significant differences in the SOS of equine cartilage samples with different age, site, and degenerative state characteristics. Variability of US thickness measurements was comparable between the 2 sites of interest and the repeatability of this technique was fairly good (CV 2.4%), which indicates that the insignificant SOS differences of the present study were true and not a result from use of a technique with low repeatability. This is in line with the results of a recently published US indentation study in bovine cartilage, in which a CV of 5.0% was reported for the measurement of cartilage thickness (Saarakkala *et al.* 2004). Use of a mean value of 1696 m/s resulted in some uncertainty in the measurement of articular cartilage thickness in horses. However, the error was typically small, within few percents, and the higher errors were related to extreme cases (*i.e.* SOS values at the edges of the 95% range). Results of the numerical simulation indicated that use of this mean SOS value was associated with a maximum error of 7.0% on dynamic modulus values. In our opinion, this error is acceptable, because values of biomechanical parameters can change as much as 40% from baseline values during the development of degenerative joint disease (Brommer *et al.* accepted for publication). Further, dynamic stiffness, measured with the US indentation instrument, appears to be consistent with the dynamic modulus, measured as a reference in the unconfined compression geometry (Saarakkala *et al.*

2004). It has been concluded that, even if there is a significant variation in clinical variables such as age of the horse, site in the joint, and cartilage degeneration, the use of the mean predefined value of SOS is valid during clinical US indentation measurements. The mean SOS value determined in equine cartilage specimens was in agreement with mean values determined for bovine and human cartilage, 1627 – 1765 m/s (Agemura *et al.* 1990, Kolmonen *et al.* 1995, Myers *et al.* 1995, Töyräs *et al.* 1999 and 2003, Youn *et al.* 1999).

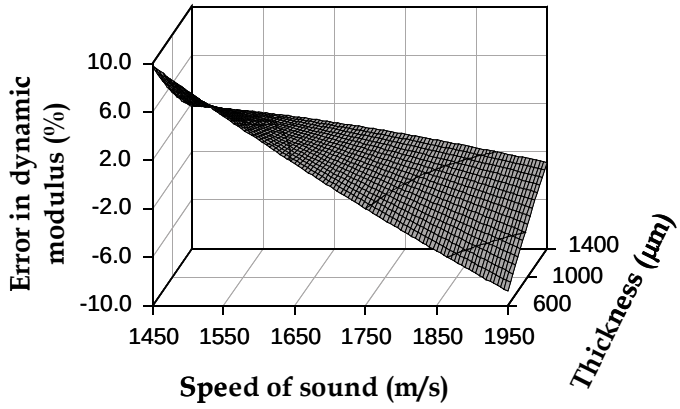

FIGURE 4: *Simulated error (%) in dynamic modulus, as obtained with the ultrasound (US) indentation instrument by assuming a constant speed of sound (SOS) of 1696 m/s. The simulation was conducted for a SOS range that covered 95% of the individual measurements (1444 - 1948 m/s) and for the cartilage thickness range of the tested samples (601 - 1405 μm).*

The composition, structure, and degree of degradation of cartilage have been reported to be main determinants for the SOS (Agemura *et al.* 1990, Joiner *et al.* 2001, Myers *et al.* 1995, Nieminen *et al.* 2004, Suh *et al.* 2001, Töyräs *et al.* 1999). In particular, the collagen characteristics and water content may influence the SOS (Agemura *et al.* 1990, Töyräs *et al.* 2003). Because there are age-related changes in cartilage composition in horses (Brama *et al.* 2000b and 2002), the SOS in cartilage may vary with age. In our study, values for SOS in the 1.5-year-old horses were (not significantly) higher than values determined for the 5-month-old and mature horses. In a study by Joiner *et al.* (2001), the SOS values in cartilage of young people (29 - 34 years of age) were similar to values in older people (mean age 71 years).

Compositional differences have been determined between the joint edge and the joint centre in the MCP joint of adult horses (Brama *et al.* 1999 and 2000a), but highly similar values for the SOS were found at these 2 sites. Differences in biochemical and biomechanical properties are marked between these 2 sites (Brama *et al.* 2000a, Brommer *et al.* 2005). As there were no significant differences in SOS between the joint margin and joint centre, it is probable that only minor site-dependent differences will exist at the other sites of the articular surface of P1. Substantial variation in the SOS in cartilage at different articular surfaces within a given joint has been reported in humans (Yao and Seedhom 1999). However, the absolute differences in SOS among sites in bovine cartilage were rather small (Töyräs *et al.* 2003). The differences in site-dependence of SOS between the species may be related to more or less prominent differences in basic constituents of cartilage, but may also be related to methodological differences in those studies. It should be emphasized that the findings of the present study were derived from MCP joints of horses and may not necessarily be applicable to other joints. Theoretically, values for the SOS may vary among different joints as cartilage in those joints may differ in composition and structure.

The SOS in cartilage has been reported to decrease with degeneration (Agemura *et al.* 1990, Joiner *et al.* 2001, Myers *et al.* 1995, Nieminen *et al.* 2002, Suh *et al.* 2001, Töyräs *et al.* 1999 and 2003), but this could not be confirmed in the present study. The differences in the absolute values of the SOS in human and bovine cartilage with various degenerative states were rather small (Joiner *et al.* 2001, Myers *et al.* 1995, Nieminen *et al.* 2002, Töyräs *et al.* 2003). In our study, the CDI technique, which assesses the extent of degradation of the superficial layer of the cartilage surface (Brommer *et al.* 2003), was used for the grading of the degenerative state of the cartilage specimens. Our data suggest that the SOS is not significantly influenced when cartilage degeneration is restricted to the top layer of the surface.

Variations in the SOS values may have arisen, at least partially, from the uncertainty in the reference measurement of cartilage thickness, or from other potential methodological inaccuracies of the present study. First, the TOF (*i.e.* the pulse transit time) was determined from the maximum of the signal envelope. Depending on the degree of frequency-dependent attenuation, this may induce a measurement error, albeit a small one compared to the biological variation of the SOS in cartilage (Ragozzino 1981). Second, the SOS was calculated from the thickness values measured by use of the needle-probe technique (Jurvelin *et al.* 1995). The sound waveform represents energy reflected from a considerable area of the cartilage-bone interface, which is larger by orders of magnitude than the area

of the cartilage-bone interface detected by the sharp-tipped needle (Mann 2001). Whereas the cartilage surface is relatively smooth, the cartilage-bone interface is highly irregular on a small scale. Thus, the US will determine the mean cartilage thickness over a large area, whereas the needle may contact the bone at a small prominence or enter a cavity and thereby introduce variance to the measurements (Mann 2001). In this context, the type, geometrical size, and frequency range of the transducer also play a role. Despite these uncertainties, the numerical simulation indicated an acceptable accuracy for determination of the dynamic modulus of the cartilage.

Clinical application of the arthroscope-guided US indentation technique in the horse will be challenging. The US transducer has to make a full, perpendicular contact to the cartilage surface. In the intact MCP joint, such positioning may be limited, because the articular cartilage surface of P1 is rather concave, and a large part of the surface articulates with the third metacarpal bone. As each joint has its own anatomical geometry, the problem of correct positioning is probably joint specific.

Our data have indicated that use of a constant value for SOS of 1696 m/s is acceptable in US indentation measurements of articular cartilage in horses regardless of clinical variables, such as age, site in the joint, and degenerative state of the cartilage. These results suggest that US indentation measurements may have a potential value for clinical use in the horse to detect changes in the functional properties of cartilage in the very early stage of osteoarthritic disease. Further studies are warranted to test the *in vivo* applicability of this technique.

MANUFACTURERS' ADDRESSES

[1] Panametrics Inc., Waltham, MA, USA
[2] Artscan Oy, Helsinki, Finland
[3] Physical Acoustics Corp., Princeton, USA
[4] LabVIEW, National Instruments, Austin, USA
[5] SPSS Inc., Chicago, Illinois, USA

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- CHAPTER X -

ON THE EDGE OF FUNCTION AND FAILURE

- GENERAL DISCUSSION -

FUNCTIONAL ARTICULAR CARTILAGE IS PIVOTAL FOR PERFORMANCE

The horse is nowadays used as a sports animal and, as in human athletes, high-level performance activities often lead to a high incidence of injuries. Slight injuries are of great importance as they may preclude top performance. In the horse, the musculoskeletal system is known to be loaded close to the limit, and it is no surprise that joint disorders count among the most common injuries (Rossdale *et al*. 1985). Among these, articular cartilage injuries are notorious for their poor quality of repair, and there is growing awareness that prevention and early diagnosis of this type of injury are of utmost importance.

In July 2004, the cutting-edge technology and the latest ideas with respect to equine joint health care were reviewed at the AAEP's annual focus symposium. The current understanding and state of pathogenetic factors (Riggs 2004), biomarker profiles (McIlwraith 2004), imaging modalities (Park 2004), treatment options (Frisbie 2004), and preventive strategies of joint diseases in the horse (Firth 2004) were the main topics of this meeting. There are many procedures, including synovial fluid analysis and diagnostic imaging techniques such as radiography, ultrasonography, arthroscopy, computer tomography (CT), and magnetic resonance imaging (MRI), that are used nowadays to generate indirect information about joint function, but the direct assessment of articular cartilage functionality in terms of biomechanical characteristics has received surprisingly little attention so far. Given the well-defined function of articular cartilage, *i.e.* absorption and transmission of loading forces during locomotion and performance (Hasler *et al.* 1999, Hayes *et al*. 2001, Mow *et al.* 1990, Todhunter 1996), the maintenance of its specific biomechanical properties is paramount to guarantee functionality, and thus pivotal in the management of joint disorders. Therefore, the biomechanical condition is in fact the best criterion to assess the status of articular cartilage (Palmer and Bertone 1996, Todhunter 1996). Some work in the field of characterisation of cartilage biomechanical properties has been performed on equine carpal cartilage (Murray *et al*. 1995, 1998 and 1999, Palmer *et al*. 1995, 1996 and 1998), incited by the fact that carpal osteochondral injury is a typical problem in racehorses (Palmer 1986, Pool 1996, Pool and Meagher 1990) and is, therefore, of economic interest in the Thoroughbred industry. In other horses, joint diseases are more frequently encountered in the metacarpophalangeal (MCP) joint, and especially osteoarthritis (OA) is very prominent (Pool 1996, Pool and Meagher 1990). However, notwithstanding the clinical importance, knowledge with respect to the functional characteristics of articular cartilage in the equine MCP joint is lacking. This gap is to a certain extent filled by this thesis, in which the first efforts

are made to come to a quantitative assessment of OA-associated degeneration-dysfunction relationships of articular cartilage in this joint in order to evaluate the diagnostic potential of measurement of biomechanical properties for early detection of cartilage damage.

CARTILAGE DEGENERATION: BIOMECHANICAL PROOF OF FUNCTIONAL FAILURE

The first signs of cartilage degeneration, *i.e.* deterioration of the collagen network and depletion of proteoglycans, emerge in the superficial layer of articular cartilage, leading to softening of the tissue (Altman *et al*. 1984, Arokoski *et al*. 2000, Lane *et al*. 1979, Mow *et al*. 1990). Therefore, this layer plays an important role in cartilage functionality and, hence, in the detection of functional failure. The Cartilage Degeneration Index (CDI) technique (chapter III) and the indentation geometry of biomechanical testing (Korhonen *et al*. 2002) are both principally influenced by the biochemical, structural, and degenerative characteristics of this superficial layer of articular cartilage, which make these methods optimal for the assessment of degeneration-dysfunction relationships.

The occurrence of degenerative cartilage changes in the equine MCP joint clearly entails loss of stiffness of the tissue (chapter VI). Degenerative changes were larger at the joint margin compared to the joint centre (chapter IV and VI), but loss of biomechanical stiffness was comparable at these 2 sites (chapter VI). This indicates that functional changes of articular cartilage can be detected at an earlier time-point than macroscopic structural changes, which is in line with results of other reports (Lane *et al*. 1979, Mow *et al*. 1990), and underlines the importance of evaluation of cartilage functionality as a suitable diagnostic modality for early detection of cartilage deterioration. This observation implies that significant loss of biomechanical stiffness of articular cartilage at the joint margin not only reflects local cartilage damage, but is also indicative of the functional quality at the centre of the joint (chapter VI), a feature that is of prognostic importance.

LOADING-RELATED SPREAD OF CARTILAGE DEGENERATION WHEN FUNCTION FALTERS

Loss of integrity of the collagen network of the extracellular matrix (ECM) is the critical feature that ultimately may result in even physiological joint loading becoming harmful (Kempson *et al*. 1973). In response to regional differences in the physiological loading pattern of articular surface in the equine MCP joint during normal joint use (Brama *et al*. 2001), regional differences in 'wear and tear' of articular cartilage develop when cartilage function falters during progressive OA (chapter IV). The specific loading-related spreading pattern of cartilage

degeneration from medial to lateral and from peripheral to central over the articular surface in the equine MCP joint differs from the pattern in the anatomically closely resembling equine metatarsophalangeal (MTP) joint (chapters IV and V). This can be related to the differences in biokinematic behaviour between these 2 joints (Back *et al.* 1995, Merkens and Schamhardt 1994), and the observation emphasizes the strong relationship between physical stress and damage of articular cartilage and the significant influence of joint biomechanics on the progression of degenerative joint disorders (Freeman 1980, Hayes *et al*. 2001, Radin 1983, Radin *et al*. 1972).

CLINICAL ASSESSMENT OF ARTICULAR CARTILAGE FUNCTIONALITY AT PRESENT: EARLY DIAGNOSIS OF CARTILAGE DAMAGE ?

At present, arthroscopy is the only, minimally invasive technique with a direct assessment of articular cartilage *in vivo*. Therefore, this technique has been considered the gold standard for evaluation of cartilage damage (McIlwraith 1990, Trotter and McIlwraith 1996). However, only a limited part of the joint can be visualized and only the surface integrity of the cartilage layer can be assessed. As the CDI technique measures surface damage, and is therefore also related with the surface integrity of the cartilage (chapter III), the technique is very suitable to evaluate an arthroscopic visual scoring system of cartilage damage. In the equine MCP joint, the value of arthroscopic inspection of cartilage damage appears to be very limited (chapter VII). The presence of light cartilage fibrillation at the joint margin leads to an underestimation of cartilage damage of the entire joint surface. On the other hand, severe cartilage lesions at the joint margin lead to an overestimation of the cartilage damage over the entire joint surface (chapter VII). These limitations of visual inspection have been encountered also in some human arthroscopy studies (Lubowitz *et al*. 2004, Schafer *et al*. 2003), and there has been an extensive search for additional techniques for a proper assessment of the health status of the articular cartilage. Among these, studies on the efficacy of arthroscope-guided indentation instruments take a prominent place (Laasanen 2003, Lyyra 1997).

The reproducibility of the arthroscopic indentation instrument, that was used in this thesis to estimate cartilage stiffness and the level of cartilage degeneration, was found to be adequate (chapter VIII). However, it should be mentioned that the instrument was tested on isolated bones. Problems may be encountered during application of these arthroscope-guided instruments *in vivo,* because of the 3-dimensional anatomy of the joint and the limited space for manoeuvring. The

instrument was able to detect substantial cartilage damage at the joint margin and to predict cartilage degeneration at the inaccessible centre of the joint based on the measurements at the joint margin (chapter VIII). Therefore, the instrument was judged as potentially useful for the evaluation of cartilage functionality in more advanced stages of OA-associated cartilage degeneration in the equine MCP joint. Nevertheless, further modification of the instrument is indicated to increase the sensitivity, as in its present configuration the device was not able to detect early functional changes in the initial phase of cartilage degeneration (chapter VIII).

EARLY DIAGNOSIS OF CARTILAGE DAMAGE IN THE FUTURE: WISHFUL THINKING OR A REALITY THAT IS WITHIN REACH?

Synovial fluid analysis and diagnostic imaging techniques are the 2 main approaches that are used to obtain clinical information about the integrity of the articular cartilage. Synovial fluid has been recognised as an important mirror of joint (patho)physiology (McIlwraith 2004, Van den Boom 2004). Although promising, recent studies have shown that many interpretative uncertainties have still to be overcome before synovial biomarkers may become fully operational as a diagnostic modality (Van den Boom 2004). The main disadvantage of synovial biomarkers is that no information is obtained with regard to the functional status of articular cartilage. It would thus be very worthwhile to direct future research efforts at the relationship between concentration and activity of biomarkers and the biomechanical functionality of the cartilage.

Imaging techniques are the other group of diagnostic modalities that are used for the assessment of damage of articular cartilage. Direct assessment of the specific biomechanical properties of articular cartilage by arthroscope-guided instruments is promising, although the technique has to be further adapted for accurate clinical application in the horse (chapter VIII). The low sensitivity of the commercially available instruments may be related with the relatively thin and stiff cartilage in the equine MCP joint (chapter II and VI). Instruments have been developed for use in humans, and, given the variation in mechanical properties of articular cartilage from different species (Athanasiou *et al.* 1991, Simon 1970), the stiffness characteristics of articular cartilage of horses may be at the far end of the range of these instruments. Thickness of articular cartilage is an important factor in biomechanical analysis of the tissue and cartilage thickness in a particular joint is related with the anatomical configuration and the specific joint biomechanics. Joints with a relatively small surface area and a large range of motion, such as the equine MCP joint, permit a wide range of movement with great inherent stability

when there is a relatively high level of cartilage congruency in the joint (Palmer and Bertone 1996). Congruent joints contain thinner cartilage to decrease the diffusion pathway for nutrient delivery and removal of waste products while simultaneously dissipating the stress applied to the joint without excessive cartilage deformation (Afoke *et al*. 1984, Palmer and Bertone 1996). In less congruent joints, a thicker cartilage layer allows efficient transport of nutrients and waste products via mechanical pumping, as loading applied to the joint is dissipated through a greater extent of cartilage deformation (Afoke *et al*. 1984, Palmer and Bertone 1996). As the error in indentation testing is inversely related to the thickness of the cartilage (Hayes *et al.* 1972, Mak *et al*. 1987), the sensitivity of the indentation instruments can be improved by the concurrent measurement of cartilage thickness with help of ultrasonography during the indentation procedure (Laasanen *et al*. 2002, Suh *et al*. 2001). Knowledge of cartilage thickness at the site of indentation will lead to a more accurate calculation of cartilage biomechanical properties (Laasanen *et al*. 2002, Suh *et al*. 2001). Ultrasound (US) indentation instruments have been developed and tested in the laboratory and the first results are promising (Laasanen 2003, Saarakkala *et al*. 2003 and 2004). In chapter IX, preparatory work was performed for the application of the US indentation technique in the horse by determination of the speed of sound (SOS) in equine articular cartilage. The results indicate that use of a predefined SOS (1696 m/s) leads to estimations of dynamic modulus with an acceptable accuracy in horses with varying ages, at different sites in the joint, and with varying stages of cartilage degeneration (chapter IX). Comparable results were found in bovine cartilage (Töyräs *et al*. 2003). This suggests that US indentation is a promising technique for clinical use in the horse, as the sensitivity in the determination of biomechanical properties can be improved and, therefore, the detection of functional failure of articular cartilage will be more accurate. Further *in vivo* research under clinical conditions is indicated. Nevertheless, it remains an invasive approach and requires general anaesthesia. These disadvantages make integration of this technique in a standard protocol of joint evaluation not feasible.

MRI is an imaging technique that provides exceptionally good anatomic, pathologic, and pathophysiologic information of articular and periarticular structures (Burstein *et al*. 2000, Park 2004, Werpy *et al*. 2004). Quantitative MRI strongly correlates with the integrity and organisation of the collagen network (Fragonas *et al*. 1998, Mlynarik *et al*. 1999, Nieminen 2002), and in the presence of contrast agent there was a high correlation with the proteoglycan content of the cartilage (Bashir *et al.* 1997, Nieminen 2002). Interestingly, MRI findings were also

highly correlated with the biomechanical properties of articular cartilage (Nieminen 2002, Nieminen *et al.* 2004). Therefore, quantitative MRI techniques appear to be very promising, not only for the compositional and structural characterization, but also for determination of the biomechanical properties of articular cartilage. As MRI is a non-invasive technique and general anaesthesia is not required in the MRI configuration for standing horses, it is a diagnostic modality that could potentially be integrated in a standard protocol to image the functional characteristics of articular cartilage. There is in fact a striking similarity with recent research on another biomechanically important and lesion prone tissue in the horse, the equine flexor tendon. Noninvasive computerised ultrasonography has been proven to be useful for the assessment of biomechanical properties of injured tendons through its ability to discriminate between lesion types and quality of repair (Van Schie 2004). Similarly, MRI imaging could be the way to topographically map the functional characteristics of articular cartilage within a given joint in order to assess the capacity of the cartilage to sustain the typical loads as they can be expected during various types of exercise. Such a noninvasive assessment of the cartilage status would be invaluable for the rehabilitation of sport horses, as a good functional quality of articular cartilage is an absolute prerequisite for superior performance. A point of concern at this moment is the inferior resolution of clinical MRI systems (Nieminen *et al.* 2004), but technical improvements are being made almost on a day by day basis and likely this problem will be solved with time.

OTHER APPLICATIONS OF FUNCTIONAL ASSESSMENT OF ARTICULAR CARTILAGE

Good, quantitative, and reliable techniques for the determination of cartilage functionality would have more applications than only the improvement of diagnoses and increasing the accuracy of prognostication in clinical cases. These applications cover the entire spectrum from the determination of adequate exercise protocols for juvenile horses to obtain optimal conditioning of the articular cartilage to the hopelessly damaged joint in which artificial repair is the only treatment option.

CONDITIONING OF ARTICULAR CARTILAGE: THE YOUNGSTERS MAKE THE DIFFERENCE

The rapidly increasing insight in joint physiology and pathophysiology has made clear that the basis for of a good functional quality of articular cartilage in the performance horse is laid in the immediate postnatal period. In fact, the prevailing principle of prevention of degenerative joint disorders later in life is the

recognition of the importance of optimal joint loading in the early juvenile period (Brama 1999, Helminen *et al.* 2000). The biomechanical characteristics (chapter II) and biochemical composition (Brama *et al.* 2000a) of articular cartilage in a newborn foal are homogeneous, *i.e.* site-independent, over the entire joint surface. Immediately after birth, loading forces will be applied to the articular cartilage with the foal's first steps, and the initially 'blank' cartilage layer develops under these influences in the juvenile animal into the specific load-adapted topographical heterogeneity, *i.e.* distinct site differences with respect to biomechanical (chapter II) and biochemical characteristics (Brama *et al.* 2000b), which are typical for the mature joint. This developmental process has been called 'functional adaptation' (Brama 1999, Helminen *et al.* 2000). Functional adaptation of the biomechanical properties of articular cartilage takes place under the influence of joint loading until an age of approximately 18 months is reached after which no major adaptations seem to occur anymore (chapter II). The factor loading, *i.e.* exercise, at young age is, therefore, a crucial variable that shapes the final functional make-up of articular cartilage. There is thus a need to determine the optimal exercise regimen during early life for the development of the best functional quality of articular cartilage. *In vivo* evaluation of the biomechanical properties of articular cartilage may aid in controlling and optimising this physiological adaptation process because such an evaluation will yield the main criteria to which the effects of training programmes can be judged.

EVALUATION OF CARTILAGE REPAIR

With respect to therapy of extensive cartilage damage, extrinsic cartilage repair techniques become more and more popular in human orthopaedics (Galois *et al.* 2004, Resinger *et al.* 2004). Postoperative assessment of the biomechanical properties of extrinsically repaired cartilage is of great importance during the healing process in order to evaluate the mechanical carrying capacity of the tissue, and thus the degree to which loading can be sustained (Laasanen *et al*. 2003). Arthroscope-guided US indentation instruments have been reported to provide a means for the monitoring of functional healing after repair surgery (Laasanen *et al.* 2003). Recently, there have also been encouraging reports on the value of MRI for the evaluation of articular cartilage repair tissue (Marlovits *et al*. 2004, Verstraete *et al*. 2004). Cartilage repair techniques are in their infancy in veterinary medicine. However, it can be expected that there may be developments in this field in the coming years, and assessment of the functional characteristics of repair tissue will then become an important item.

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- MAIN CONCLUSIONS -

I

The neonate is born with biomechanically 'blank' or homogeneous cartilage. Functional adaptation of the biomechanical properties to different loading conditions takes mainly place during the first 18 months of life, resulting in cartilage with a distinct heterogeneity in functional characteristics (chapter II). This early period of life is, therefore, of vital importance for the capacity to sustain loading without damage, which may contribute to the prevention of degenerative joint disorders later in life.

II

The Cartilage Degeneration Index (CDI) technique is a reliable and highly reproducible method for the quantitative assessment of cartilage surface damage on the articular surface of the equine first phalanx (P1) (chapter III). This technique provides a value for the overall integrity status of the articular suface and/or for special areas of interest, and is a useful tool to study osteoarthritis (OA)-associated degenerative characteristics of articular cartilage.

III

In the progression of OA, the spreading of cartilage degeneration over the articular surface of the equine P1 follows a typical pattern (chapter IV). The spread from medial to lateral and from the joint margin to the joint centre can be explained by the inhomogeneous loading of the cartilage surface.

IV

Differences in kinematics between the anatomically closely resembling metacarpophalangeal (MCP) and metatarsophalangeal (MTP) joints in the horse are associated with differences in the distribution pattern of cartilage degeneration over the articular surface of P1. This finding strongly supports earlier observations that joint biomechanics play an important role during the progression of degenerative joint disorders (chapter V).

V

Loss of stiffness is a hallmark of cartilage degeneration. Significant cartilage degradation at the joint margin of P1 not only reflects local deterioration of the biomechanical properties, but is also indicative of loss of functional quality in the joint centre (chapter VI). This is of clinical importance, as the joint margin is arthroscopically accessible while the joint centre is not.

VI

Evaluation of cartilage damage by arthroscopic visualisation leads in the equine MCP joint to an underestimation of actual damage in joints with minor lesions at the visible surface area, and to an overestimation in joints featuring severe lesions at the joint margin (chapter VII). Therefore, with respect to the cartilage surface integrity, arthroscopic inspection of the accessible areas of the joint should be interpreted with care and cannot be straightforward extrapolated.

VII

A spherical-ended indentation instrument, that can be employed during arthroscopic surgery, is a useful tool to detect and quantify substantial cartilage damage at the joint margin and to predict cartilage degeneration at the inaccessible centre of the MCP joint in more advanced stages of OA-like degenerative joint disorders (chapter VIII). However, the instrument was not sensitive enough to detect functional changes in the earliest stages of cartilage degeneration.

VIII

Use of a predefined speed of sound (SOS) of 1696 m/s during ultrasound (US) indentation measurements leads to acceptable accuracy in the prediction of dynamic modulus of articular cartilage in horses of different ages, at different sites in the joint, and with varying stages of cartilage degeneration (chapter IX). The US indentation technique has promising value to determine the biomechanical properties of articular cartilage more accurately and, therefore, may increase the sensitivity of assessment of articular cartilage functionality *in vivo*.

- SUMMARY -

TOWARDS DETECTION OF FUNCTIONAL FAILURE OF EQUINE ARTICULAR CARTILAGE

Joint disease is a serious problem in the horse that often leads to early retirement from performance activities. Of all joints, the metacarpophalangeal (MCP) joint is most frequently affected, and the most frequently diagnosed joint disorder is osteoarthritis (OA). One of the hallmarks of OA is damage and degeneration of articular cartilage, which have severe implications as this highly specialized tissue has a limited capacity for repair. Therefore, prevention and early diagnosis are of great importance.

The most important functions of articular cartilage are absorption and transmission of the forces that are generated during locomotion and athletic activity. Proper fulfilment of these functions requires an intact cartilage layer that features adequate biomechanical characteristics, such as degree of stiffness and elasticity. One of the first signs of cartilage degeneration is a decrease of stiffness, and thus a partial loss of biomechanical function. Therefore, management of joint disease should be principally focused on the maintenance of the functional characteristics of the articular cartilage, and the evaluation of the functional health status of articular cartilage is important for the prognostication of joint injuries.

In this thesis, the principles related to function and failure of articular cartilage are summarised in chapter I that serves as a basis for the following chapters where several aspects associated to degeneration - dysfunction relationships of articular cartilage in the equine MCP joint are studied within the framework of the development of tools and techniques that may help in the early detection of functional failure of articular cartilage.

FUNCTIONAL ADAPTATION OF BIOMECHANICAL PROPERTIES

The first step was to study the normal physiological development of biomechanical properties of articular cartilage (chapter II). Earlier biochemical research had shown that in the juvenile animal there is a process called 'functional adaptation', in which the biochemical composition of the tissue changes from homogeneous at birth to heterogeneous in the mature animal. In the latter, there is a match between biochemical make-up and the biomechanical loading pattern. Given the strong relationship between biochemical composition and biomechanical function, a similar process of functional adaptation of the biomechanical properties was expected. Young's modulus, a measure for static stiffness and related to the proteoglycan concentration, and dynamic modulus, a measure for dynamic stiffness and related to the collagen characteristics, were determined at 2 differently loaded sites (joint margin and joint centre) of the articular surface of

the proximal phalanx (P1) in a number of horses of different ages, varying from the fetal stage to the mature horse. It was found that, also in biomechanical sense, the foal is born with biomechanically 'blank' (*i.e.* site-independent) cartilage, and that during the first 18 months of life both the static and dynamic stiffnesses adapt under the influence of joint loading. At age 18 months, functional adaptation of the biomechanical characteristics has progressed to a level that is similar to that in the mature horse, and after this age no major adaptations seem to occur anymore.

QUANTIFICATION OF CARTILAGE DEGENERATION

To study degeneration - dysfunction relationships of articular cartilage, a quantitative measure of cartilage degeneration is needed. To create such a measure, a new technique was developed that takes into account that OA is heterogeneous in nature with affected and unaffected areas of cartilage deterioration in the same joint (chapter III). The technique is based on the fact that degenerated cartilage, that is characterised by surface fibrillation and proteoglycan depletion, takes up Indian ink particles, whereas sound cartilage does not. The technique then uses digital images that are made of the articular surface of P1 under standardised conditions before and after staining with Indian ink. The increase in the mean grey level, measured as mean pixel value, is a measure for ink uptake, and hence for the amount of cartilage degeneration, and serves as the basis for the calculation of the Cartilage Degeneration Index (CDI). The reproducibility of the technique appeared to be adequate, and the CDI values showed an excellent correlation with a macroscopic grading system for OA (chapter IV). The CDI technique is used throughout the thesis to quantify degenerated cartilage over the entire cartilage surface of P1 and also of special areas of interest.

SPREAD OF CARTILAGE DEGENERATION DURING PROGRESSION OF OA

Once the process of OA is initiated, damage to articular cartilage will often be progressive. In order to better understand the dynamic nature of this disease, it is essential to know the distribution pattern of cartilage degeneration within a joint. In chapter IV, the distribution pattern of cartilage degeneration in the equine MCP joint is investigated. In a large number of MCP joints, overall cartilage degeneration was scored and CDI values of special areas of interest (medial and lateral dorsal margin and medial and lateral central fovea of P1) were also determined. It was found that OA-associated cartilage degeneration follows a typical pattern over the joint surface of P1: from medial to lateral and from peripheral to central. These results nicely illustrates the heterogeneous nature of

OA and support an important role for biomechanical loading in the progression of the disease.

THE ROLE OF JOINT BIOMECHANICS ON THE SPREAD OF OA-ASSOCIATED CARTILAGE DEGENERATION

To further study the influence of joint loading on the progression of OA-associated cartilage degeneration, and as a logical sequence to chapter IV, the spread of cartilage degeneration in the MCP joint was compared with that of the equine metatarsophalangeal (MTP) joint (chapter V). The MCP and MTP joints show great similarity in anatomy, but are subject to a different biomechanical environment during locomotion and performance. The frequency and location of joint pathologies also differ between the MCP and MTP joints. It was hypothesised that, in line with these differences, the spread of cartilage degeneration would be distinctly different between these 2 joints. CDI values of special areas of interest, now also including the medial and lateral palmar/plantar margins of P1, were determined in a number of MCP and MTP joints, categorised into groups of increasing severity of OA, based on CDI values of the total articular surface of P1. In both the MCP and MTP joints, highest CDI values were measured on the dorsal articular surfaces. There was, however, a major difference in the spread of cartilage degeneration between the MCP and MTP joints. In the MCP joint, both the central and palmar parts are equally involved, which was thought to be related with the fact that the forelimb 'bounces' at impact and acts as a propulsive strut. In the MTP joint, the plantar parts are more and the central areas less involved in the process of OA-associated cartilage degeneration, which can be related with the fact that the hindlimb 'slides' at impact and contributes more to propulsion. The results of both chapters IV and V support earlier findings that mechanical factors (*i.e.* joint biomechanics) are very important in the progression of OA and underline the strong relationship between physical stress and cartilage damage.

FUNCTIONAL CONSEQUENCES OF CARTILAGE DEGENERATION

The functional consequences of cartilage degeneration in the equine MCP joint are described in chapter VI. In a similar approach as in chapter II, Young's modulus at equilibrium as a measure for static stiffness, and dynamic modulus as a measure for dynamic stiffness, were determined at the joint margin and the joint centre, but now in equine MCP joints with and without cartilage degeneration. The degree of cartilage degeneration of these particular sites was quantified using the CDI technique. Joints with OA-associated cartilage degeneration had significantly

higher CDI values, lower values for Young's modulus, and lower values for dynamic modulus at the joint margin. At the joint centre, the results were comparable. There was no significant change in the thickness of the cartilage layer of these 2 sites, indicating that a decrease in biomechanical properties occurs at an earlier time-point than thinning of the cartilage layer. It was concluded that the occurrence of degenerative cartilage changes is clearly related to loss of both static and dynamic stiffness. Absolute changes in cartilage integrity (CDI) were largest at the joint margin, but the decrease in the biomechanical parameters was similar at the joint margin compared to the joint centre. Therefore, substantial cartilage degradation at the joint margin not only reflects local loss of biomechanical function, but also reflects impairment of biomechanical function in the centre of the MCP joint, a finding that is of prognostic importance.

ARTHROSCOPIC INSPECTION OF CARTILAGE DAMAGE

At present, arthroscopy is the only, minimally invasive, technique that provides a direct assessment of articular cartilage *in vivo,* and, therefore, this technique is considered the gold standard for the evaluation of the cartilage integrity. The accuracy of diagnostic arthroscopy for the assessment of cartilage damage in the equine MCP joint was investigated in chapter VII. Arthroscopic inspection of the dorsal pouch of the joint was performed in a number of MCP joints and the hypothesis was tested that arthroscopic assessment of the visible cartilage, quantified using the 'Société Française d'Arthroscopie' (SFA) score, would provide a good indication of the integrity of the visible cartilage surface and would also serve as a good estimation of the status of the cartilage of the entire surface area of P1, thus including the arthroscopically invisible parts. CDI values were used as reference. It resulted that, in cases of minor cartilage damage on the visible part of the equine P1, arthroscopic grading leads to an underestimation of actual damage because light fibrillation and proteoglycan depletion cannot be detected arthroscopically. On the other hand, severe damage on the visible margin of P1 leads to an overestimation of the actual damage in the joint as damage on the visible area is not representative for the overall damage in the joint. Therefore, arthroscopic inspection of articular cartilage has a very limited value for the assessment of the health status of the tissue.

APPLICABILITY OF ARTHROSCOPE-GUIDED INDENTATION INSTRUMENTS

A commercially available arthroscopic indentation instrument was tested for its reproducibility and sensitivity in the estimation of cartilage stiffness and the level

of cartilage degeneration at the same 2 predefined sites on the articular surface (joint margin and joint centre) of isolated P1 bones (Chapter VIII). Dynamic modulus values of the tested samples were determined and used as a reference for the indenter force measurements of the arthroscopic indentation instrument. The degenerative state of the samples was quantified by use of the CDI technique. Based on the indenter force measurements, the samples were categorised as soft, moderately soft, moderately stiff, or stiff cartilage. The reproducibility of the measurements was adequate, but the sensitivity of the instrument appeared to be insufficient. The arthroscopic indentation device could reveal a decrease in cartilage stiffness only when the change was substantial, *i.e.* when the cartilage was classified as 'soft'. Moderately soft, moderately stiff, and stiff cartilage could not be distinguished from each other. Soft cartilage at the joint margin was related with a significantly higher CDI value at this site, but also with an increase of the CDI value at the centre of the joint. It was concluded that the usefulness of this instrument during arthroscopic surgery is limited in the initial phase of OA-associated cartilage degeneration, but that the device may yield additional information in more advanced stages of the disease.

DETERMINATION OF THE SPEED OF SOUND IN CARTILAGE FOR USE IN ULTRASOUND INDENTATION TECHNIQUES

Indentation stiffness, as measured by arthroscope-guided indentation instruments, is dependent on the thickness of the cartilage, a variable which is not known during arthroscopy and which may be subject to changes during the process of OA. Cartilage of the equine MCP joint is relatively thin and especially in this kind of cartilage, variation in cartilage thickness affects the outcome of indentation measurements. For this reason, indentation instruments have been adapted by the integration of a high-frequency ultrasound (US) transducer in the indenter tip that measures cartilage thickness at the site of indentation, allowing a more accurate calculation of the biomechanical properties of the cartilage. In chapter IX, the determination of the speed of sound (SOS) in equine articular cartilage is described and the influence of 3 main clinical variables (age, site in the joint, and cartilage degeneration) on the SOS is determined. The SOS was calculated as the ratio between cartilage thickness, measured by needle-probe, and the time of flight of the US pulse in a number of cartilage samples with different age, site (joint margin and joint centre of P1), and degenerative characteristics (quantified using the CDI method). These variables had no significant influence on the SOS, and simulation of the error induced by the variable SOS with the US indentation technique

revealed that use of the mean SOS induced a maximum error of 7.0% on dynamic modulus values in a SOS range that covered 95% of the individual measurements. Use of a mean SOS of 1696 m/s was found to be acceptable in US indentation measurements in the horse without taking into account clinical variables, such as age, site in the joint, and degenerative state of the cartilage. Therefore, US indentation techniques may have potential value for clinical use in the horse for early detection of functional changes of cartilage in the initial phase of degenerative joint disorders.

ASSESSMENT OF CARTILAGE FUNCTIONALITY IN PERSPECTIVE

The current state of techniques for the clinical assessment of the biomechanical characteristics of equine articular cartilage and the potentially interesting novel methods are discussed in the last chapter of this thesis (chapter X). At present, functional failure of articular cartilage can be detected, using arthroscopically-guided indentation instruments, in cases of more advanced stages of cartilage degeneration. However, early detection of functional changes in the initial phase of cartilage deterioration is not yet possible. It is expected that US indentation instruments and quantitative magnetic resonance imaging (MRI) techniques will become available in the near future as more sensitive methods for detection of functional changes of articular cartilage in the horse. Evaluation of the biomechanical properties of articular cartilage is not only important for diagnosis and prognostication, but is also of great value with respect to the prevention of degenerative joint disorders. Assessment of cartilage functionality may contribute in the determination of the optimal level of biomechanical loading for young and growing individuals in order to optimise the functional adaptation process. Conditioning through well-balanced exercise programmes may be the best way to obtain the best functional quality of articular cartilage that can sustain loading without suffering injuries throughout life. Finally, evaluation of the biomechanical properties of articular cartilage may be important in relation to the development of therapeutic strategies, for example in the monitoring of functional healing after extrinsic cartilage repair surgery.

- NEDERLANDSE SAMENVATTING -

(SUMMARY IN DUTCH)

OP WEG NAAR DETECTIE VAN FUNCTIONEEL FALEN VAN GEWRICHTSKRAAKBEEN BIJ HET PAARD

Gewrichtsziekten vormen een groot probleem bij het paard, hetgeen nogal eens kan leiden tot een vroegtijdige beëindiging van de sportcarrière. Het metacarpophalangeale gewricht, ook wel het kootgewricht genoemd, is een gewricht dat bijzonder gevoelig is met betrekking tot het ontwikkelen van gewrichtsziekten. De belangrijkste gewrichtsziekte is osteoarthrose (OA), in de volksmond 'gewrichtsslijtage' genoemd. Eén van de kenmerken van OA is beschadiging en degeneratie van het kraakbeen in het gewricht. Schade aan gewrichtskraakbeen kan ernstige gevolgen hebben omdat dit weefsel maar een beperkte mogelijkheid heeft om de schade te herstellen. Daarom zijn maatregelen ter preventie en vroegtijdige diagnostiek uitermate belangrijk om de hiermee gepaard gaande kreupelheid te voorkomen.

De belangrijkste functies van het gewrichtskraakbeen zijn absorptie en transmissie van krachten die opgewekt worden tijdens het normale voortbewegen van het individu. Voor het goed kunnen uitoefenen van deze functies is een gezonde en intacte kraakbeenlaag van belang, waarbij het kraakbeen specifieke biomechanische eigenschappen moet hebben, zoals een bepaalde mate van stijfheid en elasticiteit. Gezien het enorme belang van deze biomechanische functie moet het management rondom gewrichtsziekten uiteindelijk gericht zijn op het behoud van de specifieke biomechanische eigenschappen van het kraakbeen. Evaluatie van de functionele gezondheidstoestand van het kraakbeen is dan ook belangrijk voor de prognosestelling van gewrichtsziekten.

In dit proefschrift worden de elementen die belangrijk zijn voor de functie en het functionele falen van gewrichtskraakbeen samengevat in hoofdstuk I. In de volgende hoofdstukken worden vervolgens studies beschreven waarin diverse aspecten van kraakbeenschade en de daarmee gerelateerde stoornis in de biomechanische functie aan de orde komen. De resultaten van deze studies moeten gezien worden in het perspectief van ontwikkeling van methoden en technieken die uiteindelijk vroege opsporing van functioneel falen van gewrichtskraakbeen mogelijk moeten maken. De aandacht in dit proefschrift is gericht op het kootgewricht omdat dit gewricht klinisch het meest van belang is.

FUNCTIONELE AANPASSING VAN DE BIOMECHANISCHE EIGENSCHAPPEN

Als eerste is de normale fysiologische ontwikkeling van de biomechanische eigenschappen van gewrichtskraakbeen bestudeerd (hoofdstuk II). Uit eerder biochemisch onderzoek is gebleken dat er na de geboorte een proces in gang gezet

wordt, genoemd 'functionele aanpassing', waarbij de biochemische samenstelling van kraakbeen verandert vanuit een homogene toestand naar een heterogene situatie. Biochemische homogeniteit, zoals dat bij het jonge dier na de geboorte aanwezig is, wil zeggen dat de biochemische samenstelling over het gehele kraakbeenoppervlak min of meer hetzelfde is. Biochemische heterogeniteit van kraakbeen is karakteristiek voor het volwassen dier en betekent dat de biochemische eigenschappen van het kraakbeen van plaats tot plaats verschillen, waarbij er een duidelijke relatie is tussen de biochemische samenstelling en de belasting van het kraakbeenoppervlak, die tevens van plaats tot plaats verschillend is. De verwachting was, gegeven het feit dat de biochemische samenstelling bepalend is voor de biomechanische eigenschappen van het kraakbeen, dat er een soortgelijk proces van functionele aanpassing van de biomechanische eigenschappen plaatsvindt na de geboorte. Voor de studie naar deze biomechanische aanpassing van het kraakbeen zijn kootgewrichten verzameld van doodgeboren veulens, slachtpaarden, en paarden die om andere redenen doodgegaan zijn. Het kraakbeen van de doodgeboren veulens is nooit belast geweest, en door de biomechanische eigenschappen van dit kraakbeen te vergelijken met (gezond) kraakbeen van paarden van diverse leeftijden, is het mogelijk de normale aanpassing van de biomechanische eigenschappen van het kraakbeen te bestuderen. Op 2 plaatsen van het kraakbeenoppervlak van het kootbeen die in het levende paard verschillend belast worden, namelijk de rand en het centrum van het gewricht, zijn de Young's modulus en de dynamische modulus bepaald. De Young's modulus is een maat voor de statische stijfheid en is gerelateerd aan de concentratie van de proteoglycanen in het kraakbeen. De dynamische modulus is een maat voor de dynamische stijfheid en is gerelateerd aan de karakteristieken van het collagene netwerk in het kraakbeen. Het bleek dat het kraakbeen van het veulen biomechanisch 'blank' is, dat wil zeggen dat de biomechanische eigenschappen van het kraakbeen over het oppervlak overal hetzelfde, en dus homogeen zijn. Na de geboorte, als het veulen gaat staan, gaat het kraakbeen belast worden en onder invloed van belasting vindt er een aanpassing plaats van zowel de statische als de dynamische stijfheid van het kraakbeen, waardoor het kraakbeen die functionele eigenschappen krijgt die het mogelijk maken dat de opgelegde belasting ook verdragen kan worden zonder dat er schade optreedt. Op een leeftijd van 18 maanden bleek deze functionele aanpassing van de biomechanische eigenschappen voortgeschreden te zijn tot een niveau dat vergelijkbaar is met dat van het volwassen paard. Daarna treden er nauwelijks meer veranderingen op.

KWANTIFICERING VAN KRAAKBEENDEGENERATIE

Om de veranderingen van de biomechanische eigenschappen in relatie tot kraakbeendegeneratie, zoals dat gezien wordt bij OA, te bestuderen, is kwantificering van kraakbeendegeneratie noodzakelijk. In hoofdstuk III is de ontwikkeling van een nieuwe techniek beschreven die een dergelijke kwantificering mogelijk maakt. Met behulp van deze methode wordt rekening gehouden met het feit dat er op het gewrichtsoppervlak zowel aangetaste als niet aangetaste gebieden naast elkaar voorkomen, hetgeen veroorzaakt wordt door het heterogene verloop van kraakbeendegeneratie in het proces van OA. De techniek is gebaseerd op het gegeven dat gedegenereerd kraakbeen Oostindische inkt bindt door de fibrillering van het oppervlak en de depletie van proteoglycanen. Gezond kraakbeen heeft deze kenmerken niet en bindt dan ook geen Oostindische inkt. Binding van Oostindische inkt geeft een zwarte kleur van het kraakbeenoppervlak op die plaatsen waar het kraakbeen gedegenereerd is. De toename van de zwarting is dan een maat voor de hoeveelheid gebonden Oostindische inkt, die op zijn beurt weer een maat is voor de hoeveelheid gedegenereerd kraakbeen. De toename van de zwartkleuring van het kraakbeenoppervlak kan bepaald worden door van het kraakbeenoppervlak digitale opnames te maken zowel voor als na kleuring met Oostindische inkt, en hieruit computermatig de toename van de gemiddelde grijswaarde van de pixels te meten. Belangrijke voorwaarden zijn een gestandaardiseerde belichting en positionering van het kraakbeenoppervlak. Vervolgens kan met deze gegevens de kraakbeen degeneratie index (Cartilage Degeneration Index, CDI) berekend worden. De techniek is gevalideerd voor het kraakbeenoppervlak van het kootbeen. De reproduceerbaarheid bleek redelijk goed te zijn en er was een goede en significante correlatie met een reeds eerder beschreven macroscopisch scoringssysteem voor OA (hoofdstuk IV). De CDI techniek is in dit proefschrift in de diverse hoofdstukken gebruikt om zowel van het gehele gewrichtsoppervlak, als ook van bepaalde geselecteerde gebieden, de hoeveelheid gedegenereerd kraakbeen te kwantificeren.

VERSPREIDINGSPATROON VAN KRAAKBEENDEGENERATIE IN HET KOOTGEWRICHT GEDURENDE HET PROCES VAN OA

Degeneratie van het gewrichtskraakbeen is vaak progressief gedurende het proces van OA, waarbij de snelheid van progressie afhangt van de mate van belasting van het gewricht. Voor een beter begrip van OA is het noodzakelijk om inzicht te krijgen in het verspreidingspatroon van kraakbeendegeneratie over het gewrichtsoppervlak. In hoofdstuk IV is het verspreidingspatroon van

kraakbeendegeneratie in het kootgewricht van het paard beschreven. In 73 gewrichten van slachtpaarden is de OA-status van het totale gewrichtsoppervlak van het kootbeen bepaald, alsmede de CDI waarden van 4 omschreven gebieden: de mediale en laterale dorsale rand, en de mediale en laterale centrale fovea. OA-gerelateerde kraakbeendegeneratie heeft een specifiek verspreidingspatroon over het gewrichtsoppervlak: van mediaal naar lateraal en van perifeer naar centraal. De resultaten bevestigen het heterogene verloop van OA met betrekking tot de degeneratie van het kraakbeen. Dit specifieke verspreidingspatroon hangt zeer waarschijnlijk nauw samen met verschillen in biomechanische belasting van de verschillende plaatsen van het kraakbeenoppervlak.

DE INVLOED VAN BELASTING OP HET VERSPREIDINGSPATROON VAN OA-GERELATEERDE KRAAKBEENDEGENERATIE

Als min of meer logisch vervolg op hoofdstuk IV zijn in hoofdstuk V de resultaten van een vervolgstudie beschreven aangaande het belang van de factor belasting op de topografische ontwikkeling van OA-gerelateerde kraakbeendegeneratie. In dit hoofdstuk is het verspreidingspatroon van kraakbeendegeneratie, zoals gevonden in het kootgewricht van het voorbeen, vergeleken met dat in het achterbeen. Het kootgewricht van het voorbeen is anatomisch vergelijkbaar met dat van het achterbeen, echter, de belasting van deze 2 gewrichten is verschillend gedurende de normale voortbeweging van het paard. Bovendien zijn er in de literatuur verschillen beschreven met betrekking tot de frequentie en lokalisatie van bot-kraakbeen fragmenten in het kootgewricht van het voor- respectievelijk achterbeen. Gezien deze verschillen werd de hypothese gelanceerd dat de topografische ontwikkeling van kraakbeendegeneratie in het kootgewricht van het voorbeen verschillend is van die in het achterbeen. In kootgewrichten van het voor- en achterbeen zijn de CDI waarden bepaald zoals beschreven in hoofdstuk IV, maar nu uitgebreid met de mediale en laterale palmaire/plantaire gebieden van het kootbeen. In zowel voor- als achterbeen werden de hoogste CDI waarden gemeten op de dorsale rand van het gewrichtsoppervlak. Echter, gedurende de verdere ontwikkeling van OA werd er een verschil geconstateerd in het verspreidingspatroon van kraakbeendegeneratie: in het kootgewricht van het voorbeen verscheen er gedegenereerd kraakbeen ongeveer gelijktijdig zowel op de centrale als op de palmaire delen van het gewrichtsoppervlak, terwijl in het achterbeen significant meer kraakbeendegeneratie op de plantaire gebieden gevonden werd in vergelijking met de centrale regio's. Dit verschil wordt

toegeschreven aan het feit dat het voorbeen meer met een klap wordt neergezet en meer betrokken is bij het dragen van het lichaamsgewicht van het paard, terwijl het achterbeen meer glijdend wordt neergezet en een belangrijke functie vervult bij de voortbeweging van het paard. De resultaten van hoofdstuk IV en V bevestigen de bevindingen uit de literatuur dat belasting een belangrijke factor is in de progressie van OA en dat er een duidelijke relatie is tussen fysische stress en kraakbeenschade.

FUNCTIONELE CONSEQUENTIES VAN KRAAKBEENDEGENERATIE

De gevolgen van kraakbeendegeneratie voor de biomechanische eigenschappen zijn beschreven in hoofdstuk VI. Voor de uitvoering van deze studie is een vergelijkbare benadering gekozen zoals die gebruikt is voor het experiment beschreven in hoofdstuk II. De Young's modulus als maat voor de statische stijfheid en de dynamische modulus als maat voor de dynamische stijfheid van het kraakbeen zijn bepaald in gezonde kootgewrichten en in kootgewrichten met OA-gerelateerde kraakbeendegeneratie. Deze biomechanische parameters zijn gemeten op zowel de rand als in het centrum van het gewricht. De mate van kraakbeenschade op deze 2 plaatsen was weer gemeten met behulp van de CDI techniek. Gewrichten met OA hadden op de gewrichtsrand significant hogere CDI waarden, lagere waarden voor Young's modulus, en lagere waarden voor de dynamische modulus. In het centrum van het gewricht werden vergelijkbare resultaten gevonden. Tevens zijn de kraakbeendiktes gemeten. Er bleken geen verschillen te bestaan in de kraakbeendiktes tussen gezonde gewrichten en gewrichten met OA. Dit betekent dus dat veranderingen in de biomechanische eigenschappen van het kraakbeen eerder optreden dan veranderingen in de dikte van de kraakbeenlaag. De conclusie was dat kraakbeendegeneratie gevolgen heeft voor zowel de statische als de dynamische stijfheid van het kraakbeen en daarmee voor de functionaliteit van het weefsel. Beide parameters dalen in waarde gedurende het proces van OA. De absolute hoeveelheid kraakbeendegeneratie was groter op de dorsale rand van het gewrichtsoppervlak, maar de veranderingen in de biomechanische eigenschappen waren aan de rand vergelijkbaar met die in het centrum van het gewricht. Substantiële kraakbeendegeneratie op de dorsale rand van het kootbeen heeft dus niet alleen gevolgen voor de lokale kraakbeenstijfheid, maar is tevens gerelateerd met een vermindering van de elasticiteit in het centrum van het gewricht, een bevinding die van belang kan zijn in het kader van de prognosestelling van degeneratieve gewrichtsziekten.

INSPECTIE VAN KRAAKBEENSCHADE MIDDELS ARTHROSCOPIE

Arthroscopie, ook wel 'kijkoperatie' genoemd, is momenteel de enige minimaal invasieve techniek die een directe benadering van het gewrichtskraakbeen mogelijk maakt. Deze techniek is dan ook 'de gouden standaard' met betrekking tot het inventariseren van kraakbeenschade. De diagnostische arthroscopie van het kootgewricht is geëvalueerd met betrekking tot het vaststellen van OA-gerelateerde kraakbeenletsels (hoofdstuk VII). Arthroscopie van de dorsale gewrichtszak van het kootgewricht is uitgevoerd in 74 onderbenen van slachtpaarden. De hypothese werd getoetst dat het bekijken van het arthroscopisch zichtbare deel van het kraakbeenoppervlak een goede indicatie geeft van de toestand van zichtbare kraakbeen en dat hiermee tevens een goede inschatting gemaakt kan worden van de toestand van het kraakbeen van het totale kraakbeenoppervlak van het kootbeen, dus inclusief die delen die normaliter met een arthroscopie niet zichtbaar zijn. De 'Société Française d'Arthroscopie' (SFA) techniek (een scoringssysteem ontwikkeld door de Franse vereniging voor arthroscopie) werd gebruikt om het kraakbeen (semi)kwantitatief te beoordelen. Opnieuw werd de CDI techniek als referentie gebruikt. De resultaten gaven aan dat er een onderschatting van de totale schade in het gewricht gemaakt wordt in het geval er weinig letsels op het zichtbare kraakbeenoppervlak van het kootbeen aanwezig zijn. De oorzaak is wellicht het onzichtbaar zijn van geringe fibrillering en proteoglycaandepletie. Aan de andere kant wordt er een overschatting van de totale hoeveelheid kraakbeenschade in het gewricht gemaakt als er ernstige letsels op het zichtbare deel gediagnostiseerd worden, omdat schade op het arthroscopisch zichtbare deel niet representatief is voor het totale gewrichtsoppervlak. De uiteindelijke conclusie is dat inspectie van kraakbeenschade middels arthroscopie maar een beperkte waarde heeft voor het bepalen van de gezondheidstoestand van het kraakbeenweefsel in het kootgewricht.

TOEPASBAARHEID VAN KRAAKBEENINDENTERS

Kraakbeenindenters zijn instrumenten die onder begeleiding van de arthroscoop gebruikt kunnen worden om de mechanische eigenschappen van het kraakbeen te meten. Hiermee kan dan een indruk verkregen worden van de functionele toestand van het kraakbeen. Een commercieel verkrijgbare kraakbeenindenter is getest op reproduceerbaarheid en gevoeligheid met betrekking tot het meten van de kraakbeenstijfheid en de inschatting van het niveau van kraakbeendegeneratie (hoofdstuk VIII). In de studie zijn de metingen verricht op vrijgeprepareerde

kootbenen en weer is gekozen voor 2 plaatsen op het gewrichtsoppervlak: de rand en het centrum. Van elke testplaats is tevens de dynamische modulus bepaald die gebruikt is als referentiewaarde voor de metingen met de kraakbeenindenter. CDI waarden zijn bepaald als referentie voor de graad van kraakbeendegeneratie. De geteste kraakbeenmonsters zijn onderverdeeld in 4 groepen, gebaseerd op de uitkomsten van de metingen met de kraakbeenindenter: zacht, matig zacht, matig stijf, en stijf kraakbeen. De reproduceerbaarheid van het apparaat was redelijk goed, echter de gevoeligheid van het apparaat bleek onvoldoende. De kraakbeenindenter kon alleen een verschil in stijfheid aantonen wanneer er reeds aanzienlijke kraakbeendegeneratie opgetreden was en het kraakbeen als 'zacht' geclassificeerd werd. Matig zacht, matig stijf, en stijf kraakbeen kon met dit apparaat niet van elkaar onderscheiden worden. De aanwezigheid van zacht kraakbeen op de rand van het gewrichtsoppervlak was gerelateerd met een significant hogere CDI waarde op zowel de rand als in het centrum van het gewricht. Samen met de bevindingen uit hoofdstuk IV werd geconcludeerd dat deze kraakbeenindenter niet geschikt is om de beginfase van OA-gerelateerde kraakbeendegeneratie bij het paard te detecteren. In meer gevorderde gevallen van OA is het apparaat te gebruiken om gegevens omtrent de functionele en degeneratieve toestand van het kraakbeen te kwantificeren.

BEPALING VAN DE GELUIDSSNELHEID IN GEWRICHTSKRAAKBEEN VAN HET PAARD

Het resultaat van het meten van de kraakbeenstijfheid met behulp van een kraakbeenindenter is afhankelijk van de dikte van het kraakbeen. De dikte van de kraakbeenlaag is niet bekend tijdens een routine arthroscopie. Bovendien kan de dikte van het kraakbeen veranderen gedurende het proces van OA. Het kraakbeen in het kootgewricht van het paard is relatief dun en speciaal in dunne kraakbeenlagen heeft een kleine verandering van de kraakbeendikte aanzienlijke invloed op de resultaten van de metingen van de kraakbeenstijfheid. Om dit probleem te ondervangen kan in de indenter een echotransducer geïmplanteerd worden, waarmee op de plaats van meting de kraakbeendikte bepaald kan worden. Kennis omtrent de juiste kraakbeendikte op de plaats van meting maakt het dan mogelijk om de biomechanische eigenschappen van het kraakbeen nauwkeuriger te berekenen. Voorwaarde is wel dat de geluidssnelheid in het gewrichtskraakbeen bekend is. In hoofdstuk IX is de geluidssnelheid in kraakbeen van het paard bepaald en is onderzocht wat de invloed hierop is van 3 belangrijke klinische variabelen, namelijk de leeftijd van het paard, de plaats in het gewricht, en degeneratie van het kraakbeen. De geluidssnelheid is berekend uit de

kraakbeendikte, zoals gemeten met de naaldmethode, en de tijdsduur van de echogolf. Uit de resultaten bleek dat er geen significante invloed was van leeftijd, plaats in het gewricht, en kraakbeendegeneratie op de geluidssnelheid in het kraakbeen. De gemiddelde geluidssnelheid bleek 1696 $\pm$ 126 m/s te zijn. Tevens is er een simulatie uitgevoerd waaruit bleek dat er een maximale fout van 7.0% geïnduceerd wordt in de berekening van de dynamische modulus wanneer deze gemiddelde geluidssnelheid gebruikt wordt in de indentermetingen in de range voor geluidssnelheden die 95% van de waarnemingen uit de studie bevatte. De conclusie is dat het gebruik van de gemiddelde geluidssnelheid van 1696 m/s acceptabel is voor kraakbeenindenter metingen gecombineerd met echografische meting van de kraakbeendikte zonder dat er rekening gehouden hoeft te worden met klinische variabelen als leeftijd, plaats in het gewricht, en eventueel degeneratie van het gewrichtskraakbeen. Deze techniek heeft dan ook potentiële waarde voor klinisch gebruik bij het paard om vroege veranderingen in de biomechanische eigenschappen van het kraakbeen in het kader van degeneratieve gewrichtsziekten op te sporen.

BEPALING VAN KRAAKBEENFUNCTIONALITEIT IN PERSPECTIEF

In het laatste deel van dit proefschrift (hoofdstuk X) wordt ingegaan op de huidige stand van zaken met betrekking tot de waarde en het bepalen van de biomechanische eigenschappen van gewrichtskraakbeen en wat de verwachting is van eventuele ontwikkelingen op dit terrein. Op dit moment kan met behulp van de kraakbeenindenter tijdens een arthroscopie pas een verandering in de biomechanische eigenschappen van het kraakbeen vastgesteld worden wanneer het proces van kraakbeendegeneratie reeds in een gevorderd stadium is. Vroege detectie van functionele veranderingen in de beginfase van kraakbeendegeneratie is echter nog niet mogelijk. Het is te verwachten dat de ontwikkeling van kraakbeenindenters doorgaat en dat er in de nabije toekomst indenters met geïntegreerde echotransducers op de markt komen, waardoor de gevoeligheid van dit soort instrumenten verbeterd zal worden. Tevens zal in de toekomst met de verdere ontwikkeling van kwantitatieve 'magnetic resonance imaging' (MRI) technieken de functionele eigenschappen, en daarmee de gezondheidstoestand van het gewrichtskraakbeen, bepaald kunnen worden. Evaluatie van de biomechanische eigenschappen van het gewrichtskraakbeen is niet alleen van belang voor vroege diagnostiek en daarmee voor de prognose, het is tevens een belangrijke parameter met betrekking tot preventie van degeneratieve gewrichtsziekten. Het bepalen van de biomechanische karakteristieken van het

gewrichtskraakbeen kan bijdragen bij het zoeken naar het optimale bewegingsregime voor jonge opgroeiende paarden om daarmee de functionele aanpassing van het kraakbeen zo gunstig mogelijk te laten verlopen. Het op deze manier conditioneren van kraakbeen is de beste manier om een zo goed mogelijke functionele kwaliteit te krijgen, waardoor dit weefsel gedurende het hele leven de opgelegde belasting op kan vangen zonder beschadigd te worden. Tenslotte kan het bepalen van de biomechanische eigenschappen van het kraakbeen een belangrijke waarde hebben bij bepaalde therapeutische behandelingen, bijvoorbeeld in het monitoren van functioneel herstel na een chirurgisch uitgevoerde kraakbeentransplantatie.

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Harold Brommer
Utrecht, april 2005

- CURRICULUM VITAE -

A GOOD FUNCTIONAL QUALITY OF ARTICULAR CARTILAGE IS A PREREQUISITE FOR TOP PERFORMANCE

Winner of the open class hackney ponies in harness, UTV competition 1993, Utrecht, The Netherlands.
(By courtesy of Paard en Foto, Amersfoort, The Netherlands)

Harold Brommer was born on September 13th, 1971 in Zwolle. He grew up in Wezep, and in 1990 he passed his final exams at the grammar school Lambert Franckens College in Elburg. In that year, he started the study veterinary medicine at Utrecht University. He passed his first year examination *with honours*. During his study, he participated in an 'Excellent Tracé' at the Department of General and Large Animal Surgery, presently the Department of Equine Sciences. He investigated the biomechanical effects of the walking cast, a research project in the field of fracture treatment and fracture healing. He graduated to Master of Veterinary Research (MVR) in 1995. He finished his study *cum laude* in 1998. Since September 1998, he has been working at the Department of Equine Sciences as 'ADIKO', in which he combines a PhD tract with a residency. For the ADIKO programme, a stipendium was offered by the Dutch Organisation for Health Research and Development (ZON MW). The results of his PhD study are described in this thesis. In 2002, he was registered as Diplomate in Equine Surgery by the Royal Dutch Veterinary Association.

Harold Brommer werd geboren op 13 september 1971 in Zwolle. Hij groeide op in Wezep en behaalde in 1990 het VWO diploma aan het Lambert Franckens College te Elburg. In datzelfde jaar begon hij met de studie diergeneeskunde aan de Universiteit van Utrecht. Een jaar later werd het propedeutisch examen *met genoegen* gehaald. Tijdens zijn opleiding volgde hij het 'Excellent Tracé' bij de vakgroep Algemene Heelkunde en Heelkunde der Grote Huisdieren, later de Hoofdafdeling Gezondheidszorg Paard. Hij bestudeerde het biomechanisch effect van de loopbeugel, een onderzoek op het gebied van de fractuurbehandeling en fractuurgenezing, hetgeen in 1995 resulteerde in de onderscheiding Master of Veterinary Research (MVR). Hij studeerde *cum laude* af in 1998. Sinds september 1998 is hij in dienst bij de reeds genoemde Hoofdafdeling Gezondheidszorg Paard (HGP). In de functie van Assistent Diergeneeskundige In opleiding tot Klinisch Onderzoeker (ADIKO) deed hij wetenschappelijk onderzoek en volgde hij een klinische opleiding tot specialist. Voor het ADIKO programma werd een stipendium toegekend door Zorg Onderzoek Nederland en Medische Wetenschappen (ZON MW). De resultaten van zijn promotieonderzoek zijn beschreven in dit proefschrift. In 2002 werd hij door de Koninklijke Nederlandse Maatschappij voor Diergeneeskunde geregistreerd als Specialist Chirurgie van het Paard.

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ABSTRACTS

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Who is not fascinated by the elegance of the top-level dressage horse or impressed by the power and agility of the show jumper? The horse appeals to everybody's imagination as an athlete *pur sang*, thanks to its powerful musculoskeletal system. However, there are several conditions that threat the proper functioning of the equine musculoskeletal system. Among these, osteoarthritis (OA) takes, as in man, the most prominent place.

Driven by his eagerness to improve the diagnosis and prognostication of joint disease, Harold Brommer focuses in this thesis on the function and failure of articular cartilage, a tissue in which adequate functioning is equal to proper biomechanical behaviour. After setting the standard by determining the biomechanical characteristics of developing joints from birth to maturity, he develops the Cartilage Degeneration Index (CDI), an objective and quantitative measure of cartilage damage. The CDI is then used to trace how OA insidiously spreads over the joint surfaces of the equine metacarpophalangeal and metatarsophalangeal joints during the natural course of the disease. In the quest for an adequate and early diagnosis of OA, the index is also used to test the validity of the arthroscopic evaluation of a joint for cartilage damage and the value of a special indenter device that is meant to give information about material properties, *i.e.* biomechanical tissue characteristics. Results are promising, but further R&D is necessary before clinical use can be recommended.

'Towards detection of functional failure of equine articular cartilage' puts articular cartilage research in the perspective of function and performance. It gives an overview of present-day state of the art assessment of cartilage functionality in the horse, and, through its general concept, the conclusions are of equal relevance to man. In the end, it presents a clear vision where to go when aiming at further improvement of prevention, diagnosis, prognostication, and treatment strategies for degenerative joint disorders.