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MORPHOLOGICAL AND MOLECULAR CHANGES IN DEVELOPING GUINEA PIG OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis (OA)—an unspecific term for late joint destruction—is one of the most common causes of pain and disability in elderly. OA is characterized by destruction of articular cartilage and insufficient tissue repair. The tissue tensile strength and integrity mainly rely on a collagen network retaining highly negatively charged proteoglycan (PG) molecules, responsible for the cartilage compressive stiffness. In OA development molecular changes are likely to precede structural changes. At present only symptomatic therapy is available, due to a lack of knowledge regarding etiology and mechanisms. Such knowledge is a prerequisite for development of novel therapeutic strategies and requires studies on OA models with predictable onset and low variability.

Hartley guinea pigs develop a spontaneous knee arthropathy similar to primary human knee OA. The lesions progress slowly with age and increasing body weight, first occurring in the non-meniscus covered part of the medial tibial condyle. Load redistribution by valgus femoral osteotomy and amputation retards development on surfaces with decreased load, while surfaces with increased load show a more progressive OA pattern.

Biochemical changes are unevenly distributed depending on distance from the articular surface as well as distance from the cells. In **study I** we used gold-labeled antibodies directed towards chondroitin4-sulfate, a PG constituent in cartilage, to analyze variations in PG concentration at different sites. The PG concentration decreased in the upper zones of cartilage developing overt OA, while it increased in deeper zones. Cartilage with advanced OA had lower PG concentration in the upper zones than non-OA cartilage of the same age.

In **study II** we analyzed the effect of load redistribution, e.g. below knee amputation and valgus femoral osteotomy, on PG and collagen concentrations. Increased load was followed by decreased cartilage PG concentration whereas decreased load resulted in increased concentration, indicating a cell-mediated process with parallel biochemical and morphological patterns.

The importance of load was further investigated in **study III** by analyzing the effects of body weight and exercise. Animals were allocated to three groups: controls living under standard laboratory conditions with food ad libitum; mobilized animals with unrestricted motion in a large room with food ad libitum; and a diet group weight-matched with the mobilized group. The diet and mobilized animals had a 30–40% lower bodyweight than controls. The mobilized animals tended to have the least severe lesions at 9 months. At 12 months, lesions had progressed in mobilized and control animals, being most severe in mobilized animals. Lesions were stationary in diet animals. Thus, body mass reduction appears to retard OA progression in animals mainly subjected to a static load, but not sufficiently in animals with a more dynamic load. Dynamic load is perhaps chondroprotective in the early phase but harmful in later stages.

To identify early events in guinea pig OA we sequentially studied cartilage morphology, hydration and PG concentration in young guinea pigs **(study IV)**. We found an age-related decreased hydration and PG concentration in OA as well as non-OA cartilage, but higher PG concentration and hydration in the medial condyle (OA) than in the lateral condyle (non-OA). The overall decreased hydration and PG concentration probably represent maturation while the difference between condyles may be a compensatory reaction to higher load.

Interactions between the collagen II fibers are considered to involve cartilage matrix proteins such as fibronectin and cartilage oligomeric matrix protein (COMP). In **study V** we analyzed the incorporation of labeled SO_4 and leucine in newly synthesized cartilage matrix proteins and the cartilage concentration of COMP and fibronectin. We found increased synthesis and concentration of fibronectin and a presumed increased turnover of COMP in OA cartilage, hypothetically reflecting repair mechanisms to maintain cartilage integrity.

The integrity of the superficial layer seems to be crucial to withstand further destruction of articular cartilage. A constant morphological pattern in the studies presented in this thesis was early cell depletion in the superficial zone parallel with fibrillation associated with metabolic changes, e.g. altered concentrations of proteoglycans and matrix proteins, indicating a cell-mediated process.

LIST OF PUBLICATIONS

I. Wei L, Brismar BH, Hultenby K, Hjerpe A, Svensson O. Distribution of Chondroitin 4-Sulfate Epitopes (2/B/6) in Different Zones and Compartments of Articular Cartilage in Guinea Pig Osteoarthrosis. *Acta Orthop Scand.* Accepted for publication

II. Wei L, Hjerpe A, Brismar BH, Svensson O. Effect of load on articular cartilage matrix and the development of guinea-pig osteoarthritis. *Osteoarthritis Cartilage* 2001;9(5):447-53

III. Brismar BH, Wei L, Hjerpe A, Svensson O. The Effect of Body Mass and Physical Activity on the Development of Guinea Pig Osteoarthrosis. *Acta Orthop Scand.* Accepted for publication

IV. Brismar BH, Vassiliou D, Hjerpe A, Heinegård D, Svensson O. Morphological and biochemical changes in early guinea pig osteoarthritis. Manuscript

V. Brismar BH, Lorenzo P, Zunino L, Svensson O, Heinegård D. Distinct alterations in synthesis of ECM-proteins in spontaneous guinea pig osteoarthritis of the knee. Manuscript.

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LIST OF ABBREVIATIONS

OA	Osteoarthritis
PG	Proteoglycan
GAG	Glycosaminoglycan
CS	Chondroitin Sulfate
FN	Fibronectin
COMP	Cartilage Oligomeric Matrix Protein
CILP	Cartilage Intermediate Layer Protein
HA	Hyaluronan
HPLC	High Performance Liquid Chromatography
PBS	Phosphate Buffered Saline
SDS	Sodium dodecylsulfate
GuHCL	Guanidine hydrochloride
ELISA	Enzyme Linked Immunosorbent Assay
MRI	Magnetic Resonance Imaging
ACR	American College of Rheumatology

INTRODUCTION

JOINTS

The synovial joint can be regarded as an organ, which simultaneously ensures stability and motion. The millimeter-thin articular cartilage minimizes friction, but is too thin to have any substantial shock absorbing function. Rather, the deformable cartilage distributes load to a larger tissue volume, thereby minimizing peak load [1]. From the cartilage the load is transferred to the less deformable subchondral bone, which further distributes load. Muscles and ligaments ensure joint stability. Neuromuscular reflexes contribute to the shock absorbing capacity and protect the joint from injury. Conditions with reduced sensibility, e.g. diabetes, may result in a rapid joint destruction. The joint is thus a sophisticated transformer of mechanical load between different skeletal segments.

A capsule encloses the articular cavity. Its fibrous outer layer is in continuum with the periosteum. Its inner surface, the highly vascular and innervated synovial membrane, is the metabolic interface of the joint and serves as a filter for diffusion of compounds between plasma, synovial fluid and the avascular cartilage. The synovial membrane contains specialized cells, which can be divided into type A, macrophages, and type B, synovial intimal cells, which regulate synovial fluid volume and composition. Under physiological conditions the “joint cavity” is obliterated, since the synovial membrane adheres to the joint surfaces due to a negative intraarticular pressure. The unique properties of the thin film of synovial fluid that covers the surfaces, aid in minimizing friction between cartilage and the synovial surfaces [2].

Cartilage

Cartilage is a specialized form of connective tissue found in many locations in the body. Three kinds of cartilage are distinguished on the basis of their relative amount of extracellular matrix and the abundance of collagen and elastic fibers: elastic, fibrous, and hyaline cartilage. The viscous nucleus pulposus of the intervertebral disc is sometimes referred to as a fourth type of cartilage, pseudocartilage [3]. Elastic cartilage, e.g. in the external ear and nose, contains collagen II fibrils and elastic fibers enabling it to deform and regain shape. Fibrous cartilage contains both collagen I and II, and is present in areas where cartilage must withstand tensile forces, e.g. at attachments of tendons to bones, the symphysis, intervertebral discs and the menisci. Hyaline cartilage is found in the growth plates, on the ventral ends of ribs, in the tracheal rings and larynx, and on the articular surfaces of joints [4]. The structure and composition of hyaline cartilage covering the articular surfaces will be described in detail below.

Articular cartilage

The articular cartilage has a typical structural organization that has been highly preserved during evolution For descriptive purposes it can be subdivided into four zones aligned parallel to the articular surface (Figure 1):

1. The superficial or tangential zone is characterized by discoid cells, oriented with their long axis parallel to the surface. The cells are surrounded by densely packed fine collagen fibrils aligned parallel to the surface. This zone in considerably thinner than the following zones.
2. The intermediate or transitional zone has spheroid cells and thicker collagen fibrils with a predominant oblique direction, reflecting an arcade like organization.
3. The deep or radial zone, where the chondrocytes typically arrange in columns with their axis more or less perpendicular to the articular surface, is usually the thickest zone. Here collagen fibrils are thickest, with a predominant vertical orientation.
4. The calcified zone, which is anchored to the subchondral bone.

The transitions between zones 1–3 are somewhat merging, while that between zones 3 and 4 is more distinctly separated by a basophilic stained line, “the tidemark”. The cell density is highest in the superficial zone and decreases progressively through zones 2–4 [5].

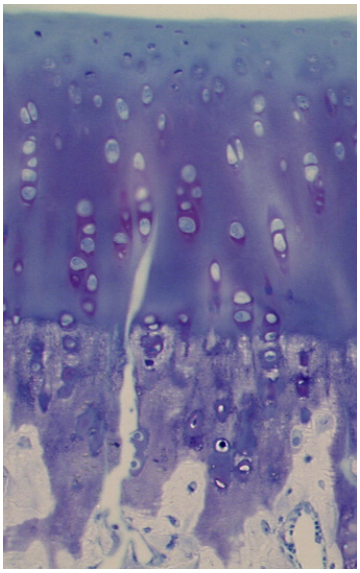

Figure 1. Structural organization of articular cartilage. Section from the central part of the lateral tibial condyle in a 6-months-old guinea pig.

The chondrocyte

Chondrocytes, cartilage cells, constitute a few percent of the total articular cartilage volume in man. In small animals the cell fraction may be considerably larger. However, the cell densities may not be species-dependant but rather inversely correlated to the thickness of cartilage, which is related to body size and the degree of incongruity of the joint surfaces. Hypothetically, this may reflect the amount of mechanical loading of the cartilage. Chondrocytes put under a high load are probably dependant on the maintenance of a more protective matrix [6]. Each chondrocyte could be regarded as an individual metabolic unit, which initially produce and then continuously maintain their surrounding matrix.

The extracellular matrix

The cartilage extracellular matrix is composed of three compartments. Closest to the cell is the thin pericellular compartment, characterized by an absence of cross-banded fibrillar collagen and richness in proteoglycans. The next compartment, the territorial compartment, surrounds and encapsulates the pericellular area. Here the matrix forms a basket-like network of fibrillar collagen fibrils. This organization probably serves as a mechanical support for the cells. The remaining matrix furthest away from the chondrocyte, the interterritorial compartment, constitutes the largest tissue volume. It is characterized by cross-banded, parallel collagen fibrils [7]. The interterritorial matrix forms a rather dense fibrous tissue, quite unlike the other compartments. The metabolic turnover is high pericellularly but appears to occur at a considerably slower pace interterritorially [8, 9]. Thus, the zones and compartments differ considerably, and this reflects differences in function.

Water constitutes 65–85 % of the total tissue weight. Becides water, the two quantitatively most important extracellular constituents are macromolecular: collagen II fibrils (50% of the dry weight), responsible for cartilage's tensile strength, and aggrecan (25% of the dry weight), the large aggregating proteoglycan of essence in maintaining tissue hydration and thereby cartilage compressive stiffness [10]. The remainder is essentially accounted for by other collagens, hyaluronan, link protein and a number of matrix proteins.

Collagens

Collagens are a large group of structurally related proteins, which have been highly conserved during evolution, and form the fibrous elements found in the extracellular spaces of most connective tissues. Collagens are the quantitatively most important proteins in animals. Common to all collagens is a triple helical structure, formed by three polypeptide α-chains with extensive repeats of a three amino acid sequence, glycine-X-Y, where X and Y often are proline and hydroxyproline. There are three residues per turn of the helix and the only residue that can fit in an interior position is glycine, the smallest amino acid. The ring structure of proline sterically stabilizes the helical conformation in each α-

chain. Collagens can roughly be divided into fibrillar, fibril-associated and network-forming collagens (Table I). Fibrillar collagens are synthesized and secreted in a triple helical proform. Specific proteolytic enzymes outside the cell remove the N- and C-terminal ends and subsequently the collagen molecules self-assemble into fibrils. The fibrils often aggregate into cable like bundles, called collagen fibers. Fibril-associated collagens decorate the surface of collagen fibrils and are thought to link fibrils to one another or to other matrix components. In this way they play a part in determining the organization of the fibrils in the matrix. Network-forming collagens assemble into meshwork [11].

Table I. Some collagens found in articular cartilage [12]

Group	Type	Characteristics
Fibril-forming (fibrillar)	II	About 90% of collagens in mature cartilage. Provides the tensile strength to cartilage.
	III	Copolymerize with collagen II
	XI	About 3% of collagens in mature cartilage. Copolymerize with collagen II. Controls lateral fibril growth?
Fibril-associated	IX	About 1% of collagens in mature cartilage. Covalently bound to the surface of collagen II. Enhances the mechanical stability of the fibrillar three-dimensional meshwork?
	XII	Non-covalently bound to the surface of collagen II. Unknown function.
	XIV	Non-covalently bound to the surface of collagen II. Unknown function
Network-forming	VI	Self assembles into a filamentous network, most concentrated pericellularly.

Proteoglycans

Proteoglycans (PG) are defined as proteins to which at least one glycosaminoglycan (GAG) is covalently bound. (A GAG is a chain of unbranched disaccharide repeats of which one sugar is glucosamine or galactosamine and the other one is hexuronic acid or galactose.) PGs are found in all tissues: intracellularly, on cell surfaces, and in the extracellular matrix. The composition and length of the protein core as well as the number of attached GAGs varies considerably.

Aggrecan is the predominant PG in articular cartilage. Each aggrecan molecule consists of a protein core of some 2000 amino acids, with three globular regions

(G1–G3) and an extended region of attached negatively charged GAGs—i.e. chondroitin sulfate (~100 chains) and keratan sulfate (~30 chains). The chondroitin sulfate chain is an unbranched polymer of 40–50 repeating disaccharide units, formed by one N-acetylgalactosamine and one glucuronic acid (Figure 2). The glucuronic acid carries a negatively charged carboxyl group and the galactosamine a negatively charged sulfate group in one of two isomeric positions, C4 or C6. A few percent of the chondroitin sulfates are unsulfated. The keratan sulfate chain is considerably shorter and consists of 5–10 disaccharides (Figure 3) [13].

Aggrecan binds to hyaluronan, a linear, unsulfated, high-molecular weight GAG. A link protein stabilizes the binding. As many as 200 aggrecan molecules can bind to a single hyaluronan molecule forming a highly negatively charged giant macromolecule complex creating a high osmotic pressure, that draws water into the tissue and expands the collagen network. The compressive properties characteristic for articular cartilage are based on the hydraulic mechanism balancing the osmotic pressure and the tension in the collagen fibers [14].

Biglycan, decorin, fibromodulin and lumican are some smaller proteoglycans with one or two attached GAG chains. They can all interact with collagen II and contain a leucine-rich repeat protein region exposing a so-called β-sheet structure, which is classically known to participate in protein-protein interactions [13].

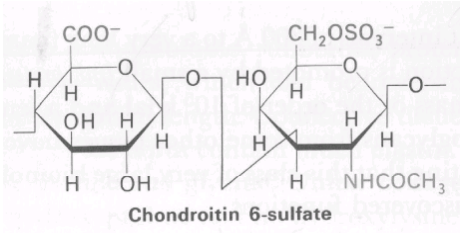

Figure 2. Structure of the disaccharide repeating unit of Chondroitin-6-sulfate (From Stryer 1981) [15]

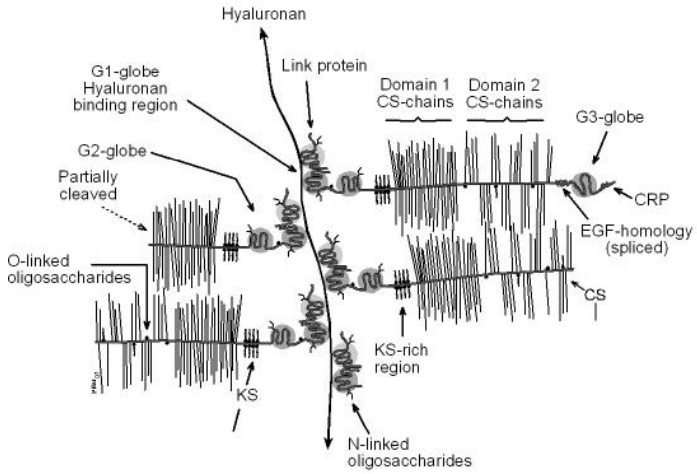

Figure 3. Illustration of the proteoglycan aggregate (D. Heinegård)

Other matrix proteins

There are also several small matrix proteins, which are neither collagens nor PGs. Some of them, e.g. chondroadherin and fibronectin (FN), interact with integrins on the chondrocyte surface. (Integrins are proteins spanning the plasma membrane, differing from cell-surface receptors in that they bind their ligand with relatively low affinity and are usually present at about 10- to 100-fold higher concentration [11].)

FN is often assigned a role in cell migration [16]: It binds to collagen, and cell surfaces and may also be involved in tissue repair by its ability to form own fibrillar structures [17]. It is found in most tissues and body fluids.

Cartilage intermediate layer protein (CILP) and cartilage oligomeric matrix protein (COMP) are examples of more cartilage specific proteins [18, 19]. COMP is composed of five identical subunits and has a bouquet of tulip like molecular structure [18]. It binds to collagens I/II, procollagen I/II and collagen IX, and its structure may explain its roles in collagen fibril assembly [20, 21]. Another matrix protein present in cartilage is the recently identified asporin, with yet unknown functions [22].

OSTEOARTHRITIS

Osteoarthritis (OA) is a common name for a group of chronic, non-inflammatory joint disorders, leading to cartilage destruction. It is thus not a single entity, but may rather be described as a common end stage—joint failure—of several disorders of different etiologies [23]. Although there is continuous debate on the precise definition, most authors agree that it involves cartilage destruction and loss of function. Most definitions of OA will entail radiological, clinical or pathologic components, or combinations thereof (Table II).

Table II. Definitions of OA

Author	Definition of OA
Altman et al, 1986 [24]	"A heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone and at the joint margins."
Keuttner & Goldberg, 1995 [25]	"A group of overlapping distinct diseases, which may have different etiologies but with similar biologic, morphologic, and clinical outcomes. The disease processes not only affect the articular cartilage, but involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration, and full thickness loss of the joint surface"
Radin, 1995 [26]	"Symptomatic loss of significant articular cartilage in a habitual load-bearing area of a joint, associated with subchondral sclerosis and osteophyte formation"

Traditionally, clinical diagnostic criteria are radiological [27, 28] (Table III). As is often the case in gradation of diseases and disorders, a 5-grade scale is used that eventually boils down to a dichotomy, i.e. normal or abnormal. This is especially true in OA where there is a poor correlation between pathological anatomy and radiology and symptoms—predominantly pain (Figure 4). Thus, a grade ≥ 2 on the Kellgren-Lawrence scale [27] is often used as a definition of radiographic OA. Furthermore, radiographic OA classification is uncertain, especially in the early stages [29, 30]. Conventional radiographs only show indirect signs of cartilage destruction and reactive bone changes—not unmineralized cartilage. Magnetic resonance imaging (MRI) and arthroscopy allow detection of more subtle changes [31, 32], although their clinical relevance is uncertain, as is discussed later. Several classification systems are used, some are purely descriptive, e.g. Collins (Table IV) [33], other systems, e.g. the French

Society of Arthroscopy classification system (SFA), involve calculations of scores based on depth, size and location of lesions [34]. However, arthroscopic grading of early OA lesions seems to be rather inexact [35].

Table III. Radiographic classification of knee OA

Kellgren and Lawrence grade	Kellgren and Lawrence definition [27]	Ahlbäck grade	Ahlbäck definition (width of articular space) [28, 36]
0	Normal	0	Normal
1	Minute osteophyte, doubtful narrowing		
2	Definite osteophyte, absent or questionable narrowing		
3	Moderate osteophyte, definite narrowing, some sclerosis, possible deformity	1	Narrowing <3 mm
4	Large osteophytes, marked narrowing, severe sclerosis, definite deformity	2	Obliteration
		3	Bone attrition <5 mm
		4	Bone attrition 5-10 mm
		5	Bone attrition >10 mm

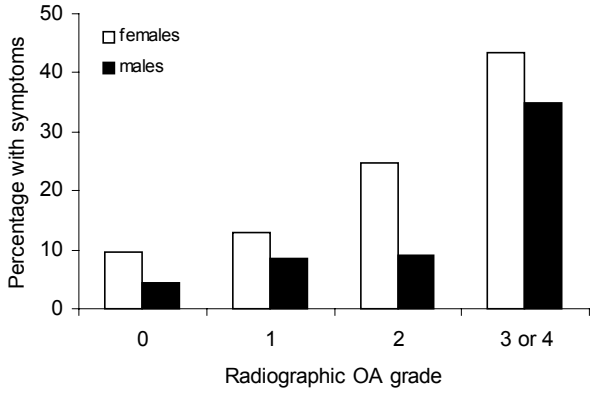

Figure 4. Radiographic OA classified with the Kellgren-Lawrence scale and percentage of subjects with symptoms. (Data from Felson et al. 1987) [37]

Table IV. Morphological classification of OA (modified Collins classification) [33]

Grade	Definition
0	Normal cartilage
1	Destruction of superficial cartilage, confined to areas of greatest pressure and movement - tangential flaking, early fibrillation, shallow pits or grooves
2	More extensive destruction of cartilage, but still confined to pressure and movement areas and not denuding bone. Deep fibrillation and notable loss of cartilage substance
3	Total loss of cartilage in one or more pressure areas with exposure and usually eburnation, of bone. Widespread fibrillation and flaking of remaining cartilage. Regions of unaffected cartilage in parts least subjected to pressure and movements
4	Complete loss of cartilage from large areas of joint surface. Eburnation of exposed bone.

Pain, as previously mentioned, is the predominant clincal problem in OA. Therefore criteria have been developed focusing on clinical symptoms e.g. the American College of Rheumatology (ACR) criteria for classification and reporting of OA (Table V) [24]. Pain is an inherently subjective symptom and is difficult to quantify, not least as it varies with individual susceptibility and activity grade, since patients often adapt their life style in order to reduce pain.

Table V. ACR criteria for classification of OA of the knee [24]

Clinical and radiographic criteria	OA is present if the items present are:
1. Knee pain for most days of prior month	1, 2, 3
2. Osteophytes at joint margins (radiograph)	or 1, 2, 4
3. Age>50	or 1, 2, 5
4. Morning stiffness<30 minutes in duration	
5. Crepitus on active joint motion	

In experimental studies diagnostic criteria will be purely morphological or biochemical, since pain is even more difficult to assess in animals. These criteria will be described in detail later.

OA can be classified dependent upon its cause as either idiopathic (primary) OA developing without known etiology or secondary OA caused by an underlying disease e.g. following septic arthritis, osteonecrosis or fractures leading to incongruity of the joint surface. In fact almost any condition that causes

weakness or injury to cartilage, or disturbs the normal biomechanics or the neuromusculoskeletal protection reflexes, may cause OA.

Several risk factors for OA development/progression have been reported (Table VI). However, data from epidemiological studies are often difficult to interpret due to confounding factors and selection bias. The odds ratios representing increased risk of OA are usually not high and generally associated with wide confidence intervals.

Table VI. Risk factors for developing OA

Generalized susceptibility		
Obesity	Knee OA	Felson et al, 1997 [38]
Heredity	Knee and hand OA	Spector et al, 1996 [39]
Mechanical factors		
Joint injury	Cruciate ligament injuries	Kannus et al, 1989 [40]
	Meniscal tears	Roos et al, 1998 [41]
	Intraarticular fractures	Rasmussen, 1972 [42]
Joint shape	Legg-Calvé-Perthes	Lecuire, 2002 [43]
	Congenital hip dislocation	Hasegawa et al, 2000 [44]
	Slipped epiphysis	Goodman et al, 1997 [45]
Occupation	Jobs with prolonged squatting, kneeling, climbing stairs	Cooper et al, 1994 [46]
Exercise	Running (hip OA)	Marti et al, 1989 [47]
	Soccer (hip OA)	Lindberg et al, 1993 [48]

OA is the most common form of joint disorder and also the most frequent cause of disability in elderly [49, 50]. It is most prevalent in the spine, hip, knee, and finger joints, while the elbow, and curiously enough the ankle joint—a highly loaded joint—seldom develops OA. The prevalence is higher in women and this difference increases with age (Figure 5) [51]. Lesions normally develop for decades. The incidence is low in youth, rising steeply with age (Figure 6) [52].

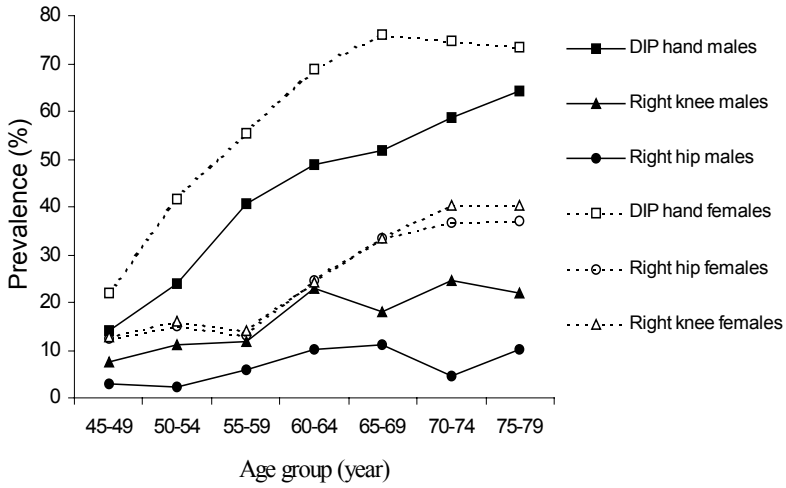

Figure 5. Prevalence of mild to severe OA in men and women (Modified from van Saase et al. 1989) [51]

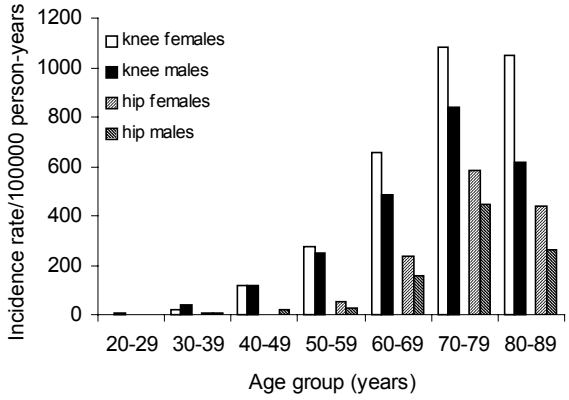

Figure 6. Incidence of OA of the knee and hip, in members of the Fallon Community Health plan, 1991-1992, by age and sex (Modified from Olivera et al. 1995) [52]

Clincal findings are pain related, i.e. reduced range of motion and loss of function in the affected joint. The only treatments available today are symptomatic with the aim of reducing pain and inflammation. Most patients with OA can manage with conservative treatment, i.e. pain control, restriction of physical activity and physical therapy. Surgery is generally only used in the elderly with late stage OA. The development of chondroprotective drugs and perhaps even means to regenerate injured cartilage awaits better understanding of cartilage biology.

Osteoarthritis theories

Since OA is not one distinct entity it certainly has different etiologies. Hypothetically OA may be due to excessive demands on normal cartilage, normal demands on susceptible cartilage or a combination thereof. The excessive demand could be related to age, such as a decline in muscle function and impairment of joint proprioception, or to more mechanical factors like mal-alignment, obesity, excessive load (occupational/recreational), trauma and altered function/properties of the cruciate ligaments and the subchondral bone. A reduction of vascular supply and nutritional support to the joint, heredity, hormonal balance, proteolytic enzymes and apoptosis could influence the susceptibility.

Morphological changes in osteoarthritis

On gross examination normal articular cartilage is firm, smooth and shining in young individuals. In normal ageing, cartilage becomes more opaque and later yellowish. In OA, the cartilage turns softer, irregular and lusterless. Age-associated fibrillations, often localized to the periphery of joints or areas with low load, are a frequent phenomenon in middle aged and elderly [53]. However, age-related fibrillations are normally benign and non-progressive. In contrast, OA lesions are progressive and located to areas of high load. Typically there are lesions of varying degrees in the same joint. Initially lesions are parallel to the surface (superficial fibrillation). Gradually, lesions progress into vertical clefts (deep fibrillation). Later, horizontal splitting between the unmineralized and mineralized cartilage occurs and also shedding of mineralized cartilage. In advanced OA the subchondral bone is exposed. In areas close to lesions there is a reduced cell density, while adjacent unaffected sites show chondrocyte clustering and proliferation [54, 55]. There are also parallel changes in the subchondral bone such as thickening (subchondral sclerosis), osteonecrosis (cyst formation) and fractures through the bone plate. Bone and cartilage formation at the joint margins (osteophytosis) is another prominent feature.

Molecular events in osteoarthritic cartilage

When cartilage lesions give symptoms they are generally of rather severe degree and have usually developed during a longer period of time. However, even the

earliest cartilage lesions seen at arthroscopy or MRI are likely to be preceded by molecular events.

A key factor controlling matrix integrity is the regulation of synthesis and degradation of matrix components. Degradation of aggrecan appears to be orchestrated by proteolytic enzymes, e.g. aggrecanses and metalloproteinases [56]. In OA cartilage there may be a loss of control of the proteolytic turnover resulting in a relative increase in degradation. Studies on femoral head cartilage, retrieved from patients undergoing hip replacement, show lower GAG levels in OA cartilage than non-OA cartilage and a negative correlation of GAG concentration to OA grade, despite an increased GAG synthesis [57, 58]. More recent studies have shown higher GAG concentrations diffusing from OA cartilage explants than from normal tissue. These fragments were mainly the result of aggrecanase cleavage, and an aggrecanase inhibitor blocked this degradation [59]. The in vivo concentration of aggrecan fragments in OA synovial fluid is also increased [60]. The results from experimental studies are somewhat diverging. Some show decreased GAG levels [61], while in others an increased GAG content and GAG synthesis [62], but also an increased GAG turnover [63] have been shown. These metabolic differences may be explained by OA being studied at different stages. In early OA there may be a hypertrophic state, which later turns into a more degradative phase.

An early event in the OA process appears to be an extracellular matrix edema, which has been attributed to a defective collagen network [64]. This may be due to fatigue related degeneration of the collagen molecule or enzymatic cleavage, as implied by the finding of enhanced cleavage of collagen II and increased amounts of the denaturated collagen II in OA cartilage [65, 66]. This cleavage appear to be secondary to proteoglycan loss [67]. It has been hypothesized that aggrecan protects the collagen network from proteolytic attack by collagenases due to steric and charge hindrance [59]. The overall collagen levels appear to be unchanged [64, 68]. An altered expression of small collagens and non-collagenous matrix proteins, involved in collagen fibril assembly, may also affect the strength of the network.

Animal studies

Clinical studies on early OA are hampered by difficulties in defining the disease state and monitoring its onset and progress. Furthermore, there are ethical difficulties involved in taking cartilage biopsies of loaded cartilage surfaces from patients in view of the poor healing capacity of articular cartilage. Animal models are therefore necessary for studies on initiating events and mechanisms of progression. Moreover, studies of whole joints are possible, thus incorporating the concept of the joint being viewed as an organ. However, there are limitations involved in animal studies. First, it can not be taken for granted that results in an animal model are applicable to humans, especially considering differences in size of joints, loading patterns and gait. Also, the main clinical symptom, pain, is not readily registered in animals.

Induced osteoarthritis models

Most animal models involve a graded injury to the joint, e.g. transection of the cruciate ligament [69, 70] and/or meniscectomy [61, 71]. A drawback of such models is that the surgical trauma may induce acute unspecific effects. Thus, surgical models are more relevant to secondary than primary OA. Alternatively, OA can be triggered by altered load over the joint e.g. by osteotomy [72-74] or strenuous exercise [75]. The relevance of these models for primary OA is questionable since the cartilage changes do not always progress to full depth lesions. Finally, a repetitive impulse loading model has been described, resulting in thickening and stiffening of subchondral bone and consequent cartilage changes affecting the intermediate and deep zones [76], but the OA progression in that model appears to be unlike the one of primary human OA. An alternative model of progressively developing OA is the transgene mice, harboring a deletion mutation of the type II collagen gene [77].

Spontaneous osteoarthritis models

There are also several progressive spontaneous animal OA models, e.g. in mice [78], monkeys (rhesus macaques) [79] and guinea pigs [80, 81].

Guinea pigs originate from South America and belong to the order rodentia, like squirrels, rats and mice. Their life span is 4–5 years and they reach puberty at about 2–3 months. The rate of weight gain decreases at 4–6 months (Figure 7) and the femur length does not increase substantially after 6 months (Table VII), even though the physis is still open in 12-months-animals.

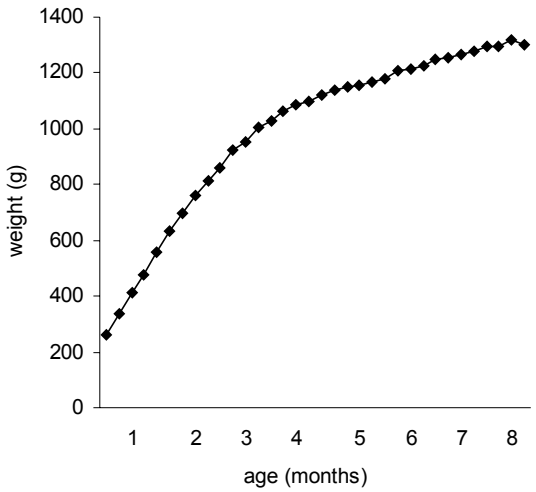

Figure 7. Weight curve of ageing guinea pigs (mean values for animals in study IV)

Table VII. Femur lengths of growing Hartley guinea pigs (mean values for a subgroup animals in study V, n=4)

Age (months)	Right (mm)	Left (mm)
3	43	43
6	48	47
9	47	47
12	48	48

In Hartley guinea pigs cartilage lesions typically develop in the non-meniscus covered part of the medial tibial condyle at 3–6 months [81] in some studies somewhat later [82], and increase in size and depth with increasing age and bodyweight. At one year lesions are severe with extensive cartilage fibrillation and exposed mineralized cartilage. Biochemically, the proteoglycan and collagen content increase before overt OA has developed [83], but in cartilage with advanced morphological changes the concentrations are diminished, partly due to a decreased synthesis [84]. It is possible to modify the natural history of guinea pig OA by changing load, by diet restriction [85], by femur valgus osteotomy (transferring load from the medial to the lateral side) and by below knee amputation [86].

PRESENT INVESTIGATION

AIMS

Biochemical and morphological aspects of early OA are difficult to study in humans because the earliest changes occur before patients develop symptoms and it is not ethical to take tissue biopsies from loaded joint areas. Therefore animal models are necessary. Hartley guinea pigs spontaneously develop a knee disorder, similar to human knee OA [81]. This thesis examines morphological and molecular patterns in developing guinea pig OA with emphasis on articular cartilage.

The specific aims were:

I. To examine changes in PG distribution in relation to zones and matrix compartments during the development of guinea pig OA

II. To evaluate the effect of altered load on cartilage biochemistry in developing guinea pig OA

III. To quantitatively evaluate the morphological and biochemical effects of body mass and physical activity on the development of guinea pig OA

IV. To characterize early guinea pig OA with regard to morphology, hydration, and PG and collagen contents

V. To define alterations in the metabolism of cartilage matrix proteins at different stages of spontaneously developing OA.

MATERIAL AND METHODS

Study design

We used 163 male Hartley guinea pigs supplied from Møllegaard, Copenhagen, Denmark (study I–II) and Sahlins, Malmö, Sweden (study III–V). In study II, two groups underwent surgical intervention at 9 months, while one group was left untreated as a control. All animals were sampled at 12 months. In study I, IV and V animals were allocated to groups by age. In study III, one group was allowed free mobilization in a larger area, one group had restricted diet and a third group served as a control living in an ordinary cage with food ad libitum. Each group was further subdivided into age groups, 9 and 12 months. Table VIII provides a schematic overview of the study design used in the separate studies.

Cartilage from the medial (study I–V) and lateral tibial condyles (study I–IV) and the humeral trochlea (study V) was studied, as was serum (study V).

Table VIII. A schematic overview of the study design in studies I-V

Study	Groups	Intervention	Age at sampling (months)	N	Studied parameters
I	I	---	6	5	Morphology and PG distribution/concentration
	II	---	12	5	
II	I	---	12	8	PG and collagen concentration
	II	Valgus femoral osteotomy at 9 months	12	8	
	III	Amputation at 9 months	12	8	
III	I	---	9	8	Morphology and GAG concentration
	II	---	12	6	
	III	Mobilized in shared big cage	9	5	
	IV	Mobilized in shared big cage	12	10	
	V	Diet restriction	9	8	
	VI	Diet restriction	12	8	
IV	I	---	3	5	Morphology, GAG and collagen concentrations and hydration
	II	---	4	5	
	III	---	5	5	
	IV	---	6	5	
	V	---	8	8	
V	I	---	3	14	Morphology, synthesis and concentration of extracellular matrix proteins
	II	---	6	14	
	III	---	9	14	
	IV	---	12	14	

Morphology

Electron microscopy

Study I

Cartilage was fixed by vascular perfusion, using an even mixture of 0.3% phosphate-buffered glutaraldehyde and 0.3% paraformaldehyde [87]. Thin blocks, including cartilage and a part of the subchondral bone, were cut from the non-meniscus-covered parts, dehydrated at low temperature and embedded in a polar resin [88].

Two central 35–40 nm-thin sections from two blocks of each condyle were cut and placed on formvar-coated nickel grids. Sections were digested with chondroitinase ABC, blocked and incubated with the monoclonal 2/B/6 antibody, reacting with unsaturated, terminal 4-sulfated chondroitin disaccharides. Bound antibodies were detected by secondary gold-conjugated antibodies. Subsequently, the sections were contrasted with 4% uranyl acetate and lead citrate. Electron micrographs (final magnification x 66000) were used to monitor the pericellular and interterritorial chondroitin-4-sulfate distribution in the superficial, intermediate, deep and calcified zones.

Light microscopy

Study I

1 μm-thick toluidine blue stained sections from 2–4 resin embedded blocks of each condyle were used for light microcopy. One micrograph covering the four zones was taken from each section. The different zones were defined according to the description by Meachim and Stockwell [5].Volume densities of matrix and chondrocytes in the various zones were estimated by point counting [89].

Studies III–IV

Cartilage was fixed in neutral buffered 4 % paraformaldehyde, decalcified, paraffin embedded and serially sectioned at 250 μm-intervals (section thickness 5 μm). The sections were stained by haematoxylin and eosin, coded, mixed and analyzed blindly using a light microscope (x150) with a computerized image analyzer (CAST-Grid, Olympus, Aarhus, Denmark). The central third of each condyle, corresponding to a surface area of 3–4 mm^2, was analyzed. The proportion of intact cartilage and exposed mineralized cartilage was measured with a digital ruler. Volume density of subchondral bone, including the subchondral bone plate and the subchondral trabecular bone down to the physis, was measured by point counting. Average cartilage thickness was estimated by dividing the cartilage volume by the projection of the corresponding surface area.

Study V

Cartilage was freeze sectioned (6 μm), stained with toluidine blue and analyzed qualitatively.

Biochemistry

GAGs and collagens

Study II

PGs were extracted with 4 M guanidine hydrochloride (GuHCl), containing EDTA and protease inhibitors [90]. Residues not extracted were digested with papain and aliquots of these digests were hydrolyzed in 6 M HCl and analyzed for hydroxyproline contents [91].

GAG sulfation pattern was monitored by high performance liquid chromatography (HPLC) after incubation with chondroitinase AC and ABC. Contents of chondroitin sulfate (CS) and hyaluronan (HA) were obtained by further digestion with chondroitinase-4 and chondroitinase-6 sulfatases followed by ion suppression HPLC [92].

Large and small PGs were separated electrophoretically [93]. The aggregability of PG monomers was monitored by incubating aliquots with HA before electrophoresis and comparing the mobility with other aliquots, in which the HA binding capacity had been blocked by reduction [93]. The gels were stained with toluidine blue and the distribution was scanned and evaluated using densitometry.

Study III and IV

Cartilage was solubilized with a papain digestion buffer containing EDTA. Following inactivation of remaining enzyme, one aliquot was retained for analysis of hydroxyproline (collagen) [91]. GAGs were precipitated from the remaining digest, by adding 4 volumes of ethanol. The precipitates were digested using a mixture of chondroitinase AC and ABC. HPLC was used to characterize the sulfation pattern and uronic acid content (GAG).

Hydration

Study IV

Cartilage biopsies were incubated in [^{3}H]-urea and subsequently equilibrated in PBS. The [^{3}H]-urea content in the incubation medium and the admixture of [^{3}H]-urea in the PBS was determined by scintillation counting. The biopsy water content was calculated assuming equilibration between the original incubation medium and the cartilage. The dry weight was determined after dehydration in acetone. Hydration was estimated as the amount of tissue water divided by the wet weight (tissue water + dry weight).

Matrix protein metabolism

Study V

Cartilage was incubated in culture medium supplemented with [^{3}H]leucine and [^{35}S]sulfate for 4 hours at 37°C. Proteins were extracted in 4 M GuHCl with EDTA. Aliquots of each extract were applied on a 1 ml Mono Q anion exchange HPLC column and eluted with a gradient urea buffer. Fractions collected were analyzed for protein patterns by SDS-polyacrylamide gel electrophoresis and fluorography. Developed films were scanned and the band intensity was estimated. The relative single band intensity, corresponding to specific proteins, was determined by densitometry and related to total matrix protein amount or label in the gel.

Cartilage COMP and fibronectin concentration

Study V

Cartilage proteins were extracted in 4 M GuHCl with EDTA, precipitated with ethanol and dissolved in 0.8% SDS. Proteins were measured by inhibition enzyme linked immunosorbent assays (ELISA) using rabbit anti guinea pig COMP and rabbit anti guinea pig FN. Microtiter plates were coated with purified bovine COMP and guinea pig FN antibodies. The protein content was related to the hydroxyproline content, which was determined using a colorimetric assay after acid hydrolysis [91].

RESULTS

Effects of interventions

Study II

After osteotomy and wound healing the animals moved about unhindered. Bone healing was confirmed radiologically. The amputated animals initially had restricted movement, but were soon able to walk tripedally. At 12 months of age, all animals appeared to be unimpaired with a normal knee passive range of motion.

Study III

Animals mobilized in a shared big cage were physically more active than single caged animals. Mobilized and diet animals weighed about 0.8–0.9 kg and controls 1.3–1.4 kg.

Morphology

Study I

Fibrillation developed between 6 and 12 months in the central part of the medial tibial condyle, parallel with a decreasing superficial zone cell volume density. Cell densities in this zone were higher laterally than medially at both time points. No lesions were seen laterally at 12 months.

Study III

Most cartilage was intact in the mobilized group at 9 months, whereas fibrillations had developed in large areas in the other two groups. Lesions progressed between 9 and 12 months in the control and mobilized group, but were stationary in the diet group. At 12 months, lesions down to the mineralized cartilage affected 60% of the central cartilage surface in the mobilized group, while the corresponding figure was 25% in the control group and 2% in the diet group. The subchondral bone density was higher medially than laterally.

Study IV

Superficial fibrillations occurred already at 4 months medially, parallel with cell depletion in the superficial zone and hypertrophy of chondrocytes in the intermediate zone and upper part of the deep zone. The most extensive changes were seen at 8 months. No lateral condyle lesions were seen. Cartilage was thicker and the subchondral bone density higher medially than laterally at all ages studied. Subchondral bone density did not change between 3 and 6 months.

Study V

Fibrillations developed between 3 and 6 months in the medial tibial condyle while the humeral trochlea cartilage was still intact at 12 months.

Biochemistry

GAGs and Collagen

Study I

Medial tibial condyle PG concentration decreased in the interterritorial compartments of the superficial and intermediate zones between 6 and 12 months and increased in the deep zone. Laterally, the concentration increased in all zones. At 6 months, the PG concentration was equal or slightly higher medially than laterally. At 12 months it was almost 100% higher in the two upper zones laterally.

Study II

Decreased load—on the ipsilateral medial condyle after osteotomy, and on the ipsilateral medial and lateral condyles following tibia amputation—was associated with an increase in PG concentration compared with corresponding condyles in sham-operated animals. Condyles with increased load had a decreased PG concentration. Collagen concentration followed a similar pattern in the osteotomy group. In the amputated animals collagen concentration went down irrespective of changes in load.

Study III

The GAG concentration (uronic acid/hydroxyproline) in the medial tibial condyle varied considerably between animals, similar to the morphometric pattern. It decreased between 9 and 12 months in mobilized animals but was unchanged in controls and animals with diet restriction. The lowest levels were seen in mobilized animals at 12 months, i.e. the group with the most extensive cartilage lesions.

Study IV

The GAG concentration and hydration was higher medially than laterally and decreased between 3 and 6 months in both condyles. There was a correlation between GAG concentration and hydration, $r^2=0.44$ ($p<0.001$).

The collagen concentration did not differ between the medial and lateral condyles, but tended to decrease medially with increasing age.

Matrix proteins

Study V

Fibronectin synthesis and concentration increased in cartilage developing OA, where it was higher than in non-OA cartilage. This change was most prominent early in the OA process. Also COMP synthesis appeared to be higher in OA cartilage, while there was no marked difference in COMP concentration. A high intensity 39-kDa band, corresponding to asporin and GP39/YKL-40 was seen in tibia cartilage before OA lesions had developed.

Guinea pig knee OA development follows a distinct and predictable pattern, starting in the non-meniscus covered part of the medial condyle and later also appearing in the lateral condyle. The two condyles may thus represent two phases in the natural history of the disorder. This temporal pattern is constant and the inter-individual variation small when compared with clinical material. Within a year the animals have developed a severe arthropathy. Some studies indicate an onset at about 4 months [81], similar to the findings in studies III–V, while others have reported a somewhat later onset [82] as seen in studies I–II. There is no obvious explanation to this difference, except that different breeders supplied the animals in studies I–II and III–V. It is possible that the animals from the two breeders have a slightly different genetic background and/or have been bred under different conditions. Recent findings of differential susceptibility to OA in guinea pigs from one inbred strain, Strain 13 and Hartley guinea pigs supports the possibility of genetic differences [94].

Guinea pig OA displays several similarities to human primary knee OA, e.g. the structural and biochemical changes are similar, the lesions occur naturally, the progression is fairly slow and development is affected by load redistribution. However there are also some differences, e.g. the 100% incidence, which indicates a strong genetic predisposition, the considerably smaller joint size and the quadripedal load pattern with a substantial load on the forelegs. In addition the growth plates in guinea pigs never completely close. They appear to grow until 6 months of age, after which growth is negligible.

A drawback with the model is of course the lack of controls, although the lateral condyle in some sense could serve as an internal control, since it develops OA at a later time than the medial one. The elbow joint is an alternative control, but its different kinetics and configuration are important aspects, which must be taken into consideration when comparing results.

Evaluation of cartilage lesions may be either qualitative, e.g. using different scales [57], or quantitative. Qualitative scales involve a great portion of subjectivity and it is questionable to summarize ordinal values into scores [95]. In studies III-IV quantitatively measurable variables, were therefore chosen, such as proportion of intact and exposed mineralized cartilage. These are rather crude estimates, and there may be a wide variety of intermediate changes. However, considering the rather variable depth and extent of cartilage lesions often found on affected surfaces, there are advantages with simple and unbiased estimates.

Cartilage and subchondral bone plate thickness were not directly measured for similar reasons. Cartilage thickness is difficult to assess in fibrillated cartilage due to the varying topography of the surface. This is also true for subchondral bone, which has a complicated structure. The subchondral plate is visible on radiographs, but under the microscope the lower part of the “plate” is continuous with the underlying bone trabeculae making distinctions of plate demarcations unsure. Furthermore, direct measures of thickness require subjective decisions

on directions. In order to further reduce the risk of bias all sections were blinded and randomized.

As pointed out earlier, OA cartilage displays variable responses with destruction and cell death in some areas but regeneration quite close by. This heterogeneity in tissue response is evident both in vertical and horizontal directions. We therefore analyzed cartilage from a well-defined area of the condyles using a standardized biopsy instrument. However, diverging zonal and/or compartmental biochemical changes may have been missed, since we used full thickness cartilage samples (study II-V). There are several obstacles analyzing different zones individually due to the small amount of tissue.

This was taken into account in study I. Distribution and amount of gold labeled antibodies, directed towards the 2/B/6 epitope of 4-sulfated 4,5 unsaturated chondroitin, were stereologically quantified using transmission electron microscopy. With the tissue embedded in a plastic resin, the reaction only takes place on the section surface with no dependence on penetration of enzyme or antibodies, nor will there be any rearrangement of protein cores within the tissue. The developed epitopes may be located anywhere in the GAG chains depending on the cut surface and the extent of the limited chondroitinase digestion preceding the labeling with antibody. Therefore, the observed distribution of gold grains within a section reflects that of the total 4-sulfated chondroitin disaccharides in the tissue, regardless of possible variations in sulfation along the chain. Furthermore, the fairly constant sulfation pattern in corresponding guinea pigs [83], suggests that the 2/B/6 labeling would also mirror the overall distribution of chondroitin sulfate.

Due to the limited amount of material available samples were pooled in study II (all biochemical analyses) and study V (analysis of matrix protein synthesis). These results have therefore not been tested statistically. However, the pattern of load dependant biochemical changes was consistent in study II, and protein concentration alterations (FN) in study V were in concordance with synthesis patterns.

DISCUSSION

One of the earliest and most striking findings was the cell depletion in the superficial layer and the reactive hypertrophic cell changes just some 50μm further down, parallel with fibrillation development (study I, III–IV). The qualitative changes suggest that the cellular changes occur first, but the nature of the cell-matrix interactions that ensue and the mechanism of matrix destruction remain to be elucidated. Apoptosis in OA cartilage is still a moot question [96, 97], but has not been studied in the guinea pig.

A reduced cell number has an obvious impact on cartilage integrity, since the chondrocytes continuously govern the turnover of matrix components to adapt to altered demands, such as altered load. This was evident in study I, where the GAG concentration decreased together with a reduced cell volume density in the superficial zone. An opposite pattern was seen in the deep zone, with chondrocyte hypertrophy and increased GAG concentration. In the lateral condyle, concentrations increased in all compartments, probably reflecting a compensatory state, which is also seen medially before OA lesions develop [83]. A similar hypertrophic pattern has been reported in dogs following anterior cruciate ligament transection [62], where cartilage in areas of greatest load bearing showed the greatest increase in PG synthesis [98]. Hypothetically, this compensatory state may eventually become insufficient to withstand a sustained excessive load and is followed by cell death, defective tissue repair and cartilage destruction, associated with decreased PG levels [57, 83]. However, the reduced PG concentration may also be the result of an increased enzymatic degradation.

The PGs are crucial for cartilage compressive stiffness [10]. Indeed, OA cartilage is more compliant than intact cartilage in compression tests [99]. The polyanionic GAG chains create a high osmotic pressure that draws water into the tissue. Paradoxically, hydration is increased in human OA cartilage although GAG content is decreased [100]. This has been attributed to a defective collagen network [101]. It was therfore somewhat surprising to find that hydration decreased in early guinea pig OA (study IV), which however represents a much earlier state than that investigated in clinical studies. Considering the different structure of zones and compartments it is likely that the response varies within the tissue, and that the overall result may not represent the true picture.

The integrity of the superficial zone seems to be crucial in guinea pig articular cartilage. This thin surface cover has a high collagen content with fibrils running in a strict and constant meshwork pattern parallel to the surface [102]. The fibrils are supposed to oppose tensile forces on the superficial cartilage [103]. The integrity of the superficial zone was broken already at 4 months in study IV, but there was no marked change in total collagen concentration. Similarly the collagen content has been reported to be unchanged in human OA cartilage [68]. This is not unexpected considering the almost non-existing turnover of collagen in normal cartilage [104]. Furthermore, it is possible that defective collagen molecules are trapped in the tissue and thus do not negatively affect the total concentration. Interactions between collagen fibrils are considered to involve

cartilage matrix proteins [20, 105, 106]. It is possible that an altered expression or loss of function of these proteins result in a weaker network more prone to disintegration with resulting cartilage fibrillation. New techniques, e.g. tissue processing techniques and immunolocalization at the ultrastructural level, may reveal such molecular interactions. Cleavage of collagens is another possibility. Collagenase 1 is expressed in higher levels in the medial condyle cartilage and is also focally localized to the extracellular matrix of guinea pig OA lesion sites [107].

As stated before, chondrocytes continuously regulate matrix turnover to adapt to altered demands. There are no cell-cell contacts in cartilage, no nerve fibers and no vascular supply. Thus, the chondrocyte is dependent on diffusion of molecules or conformational changes of surrounding matrix to interact with its environment. Some of these interactions are believed to be mediated by matrix proteins. The increased synthesis and concentration of one of these, FN, coincided with the initial tibial cartilage OA development. Levels were considerably lower in the non-OA humerus trochlea cartilage (study V). This is consistent with findings of increased FN synthesis and concentration in spontaneous dog hip OA [108]. Bearing in mind the role of FN in cell-to-matrix interactions, one may speculate that its increased synthesis in OA cartilage is an attempt to tissue repair.

COMP synthesis was higher in tibia (OA cartilage) than in humerus (non OA cartilage), but with equal or even higher concentrations in the humerus cartilage (study V). This implies an increased turnover of COMP in the OA cartilage. COMP appears to have roles in collagen fibril assembly [20] and an altered metabolism of the molecule may thus have implications for cartilage tissue integrity.

Another interesting finding was the high synthesis of asporin or GP-39, at 3 months in tibial cartilage—the time for OA initiation at this site (study V). Moreover, high asporin as well as GP-39 mRNA levels have been found in human OA articular cartilage. Thus, even before gross structural changes develop, the synthesis pattern of a number of matrix proteins is altered. These early changes may assist in the understanding of the subsequent tissue breakdown in guinea pig OA.

Load appears to be a key factor in guinea pig OA. Load redistribution by femoral valgus osteotomy or below knee amputation retard OA development in cartilage with reduced load and accelerate development in cartilage with increased load [86]. PG concentrations were also affected, being higher in condyles with decreased load and lower in condyles with increased load. Chondrocytes therefore seem to be directly affected by the load redistribution (study II).

The effect of load was further evaluated in study III. Animals in the mobilized group were more active than controls, which is supported by the difference in body mass. Although difficult to measure, it is reasonable to believe that the mobilized animals place more dynamic loads on their joints than the sedentary control and diet animals. It is also probable that leaner diet animals' joints were loaded less than those of the controls. The result at 9 months suggests that dynamic loading in guinea pigs, at least initially, is chondroprotective since

mobilized animals had a smaller proportion of the surface affected by fibrillation than diet animals. However, when fibrillation eventually develops, dynamic load appears to be more harmful than static, which is illustrated by the extensive lesions in the mobilized group at twelve months. The supposed chondroprotective effect of exercise corroborates with results on hamster spontaneous OA [109] and may in part be explained by findings of higher collagen fibril assembly in the superficial cartilage in exercising young guinea pigs [110]. A similar protective effect of exercise has been reported in a transgenic mouse line [111], while the opposite pattern was seen in another line [112]. The previously reported protective effect of weight reduction in guinea pig OA [85] was evident comparing the result of diet and control animals at twelve months. In accordance with previous results, the biochemical changes followed the morphology, i.e. GAG levels decreased in mobilized animals (with progressive OA) and were stationary in diet animals (with a more stationary condition).

Load, though, is not the only pathogenic factor in guinea pig OA in view of the absence of OA in elbow cartilage (study V). Guinea pigs carry a higher load on the fore than the hind extremities (unpublished results Svensson O, Lundberg A). It is likely that factors specific to the knee, e.g. a complex movement pattern and/or ligaments and menisci are involved in the OA development. The elbow, a hinge joint, has simpler kinetics. There are a number of clinical phenomena that corroborate such a notion, e.g. that primary OA is very rare in the talocrural joint in contrast to the knee and hip, two joints with a more complicated motion pattern.

An increased subchondral bone density, leading to stiffening of subchondral bone and increased cartilage stresses with horizontal splitting and tearing of deep and intermediate layers of cartilage has been suggested as an initiating mechanism in OA [76, 113]. That mechanism is unlikely in guinea pig OA since the first structural changes were confined to the cartilage. Notably, the subchondral bone density did not differ between animals with intact or deranged cartilage surfaces at 9 months in study III and between 3 and 6 months in study IV. Furthermore, below knee amputation did not entirely stop OA progression, in spite of a striking decrease of subchondral bone thickness [86]. Also, a recent comparative analysis of two strains of guinea pigs with differential susceptibility to OA showed less severe OA in the strain with the thickest subchondral bone [94]. This indicates that guinea pig OA and increased subchondral bone density are not interconnected, but rather represent parallel responses to altered load. Whether this is also true for large and heavy-duty human joints remains to be shown.

CONCLUSIONS

I. The tissue response in guinea pig OA is heterogeneous with varying biochemical changes in different zones and compartments. This indicates a cell-mediated process

II. The striking morphological difference between the medial (OA) and lateral (non-OA) condyle in early guinea pig OA, is reflected by higher hydration and GAG concentration in the medial condyle, probably representing an adaptive response to higher load. The concomitant cell depletion in the disrupted superficial layer and hypertrophy of cells in the deep zone further corroborate the notion of a cell-mediated process

III. Cartilage matrix protein synthesis is affected early in the OA process, as reflected by the altered metabolism of FN, COMP, asporin and/or GP-39 in developing guinea pig OA. Altered metabolic patterns may reflect repair attempts to maintain cartilage integrity

IV. Load appears to be a key external factor in guinea pig OA. A reduced static load, i.e. lower bodyweight, slow down OA progression, while dynamic load, i.e. exercise appear to increase the rate of cartilage destruction. However, in the early phase—before lesions have developed—dynamic load may perhaps be chondroprotective

V. Biochemical events in guinea pig OA are affected by load redistribution and correlate to morphological changes

VI. The window of opportunity for studying molecular pathogenic mechanisms in guinea pig OA appears to be between 3 and 6 months before gross changes have developed. This stage seems to be of special relevance in the development of chondroprotective agents

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