Extracellular matrix changes in early osteochondrotic defects in foals: a key role for collagen?

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Abstract

Osteochondrosis (OC) is the most important developmental orthopaedic disease in the horse. Despite some decades of research, much of the pathogenesis of the disorder remains obscure. Increasing knowledge of articular cartilage development in juvenile animals led to the presumption that the role of collagen in OC might be more important than previously thought. To study collagen characteristics of both cartilage and subchondral bone in young (5 and 11 months of age) horses, samples were taken of subchondral bone and articular cartilage from a group of 43 Dutch Warmblood foals and yearlings that suffered from varying degrees of OC. Based on a histological classification, lesions were graded as early, middle and end stage. Collagen content and some posttranslational modifications (lysyl hydroxylation, hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) cross-links) were determined, as was proteoglycan content. Data were compensated for site effects and analysed for differences due to the stage of the lesion.

In early lesions total collagen was significantly decreased in both cartilage and subchondral bone of 5- and 11-month-old foals. Also in cartilage, HP cross-linking was reduced in the early lesions of 5- and 11-month-old foals, while LP cross-linking was decreased in subchondral bone of the end-stage lesions of both 5- and 11-month-old foals. Hydroxylysine content was unaffected. Collagen content remained reduced in cartilage from middle- and end-stage lesions, but returned to normal in subchondral bone. In cartilage there was a decrease in proteoglycan content in the end-stage lesions of both age groups.

Thus, alterations of the collagen component, but not of the proteoglycan component, of the extracellular matrix might play a role in early OC. More severe lesions show a more general picture of an unspecific repair reaction. Biomarkers of collagen metabolism can be expected to be good candidates for early detection of OC.

Keywords: Osteochondrosis; Dyschondroplasia; Joint development; Horse; Cartilage; Mineralisation; Growth; Collagen; Glycosaminoglycan

1. Introduction

Osteochondrosis (OC) can be defined as a disturbance of the process of endochondral ossification, i.e. as an aberration of the physiological process that occurs during growth of the young individual in which the cartilaginous predecessors of the osseous components of the skeleton turn to bone [1]. The last parts of the long bones that undergo this process are the physis or growth plates that account for virtually all growth in longitudinal direction of the bone, and the epiphyses. Where the physes of long bones close and mineralise completely when growth ceases, the epiphyses retain a thin layer of cartilage that serves as articular cartilage throughout the life of the individual. Disturbances in the process of endochondral ossification occur in many species and may have different clinical manifestations, depending on where and when problems arise. In man, osteochondrosis juvenilis Scheuermann is a relatively rare, but well-known clinical entity [2,3], in chicken so-called dyschondroplasia is common in the (tibial) growth plate [4], and in dogs, pigs and horses osteochondrosis (OC) or osteochondritis dissecans (OCD) is common in the epiphy-
seal cartilage of a number of joints [5]. Of these species, OC in the horse is of special importance. It has been estimated that 20,000–25,000 foals are born annually in Northwestern Europe alone that will suffer from some degree of OC [6]. Although most of these animals will eventually be able to perform, surgery is required in many cases, which means that the disease causes a vast loss to the equine industry, both in economic terms and in terms of animal welfare.

The principal event in the process of endochondral ossification is the transformation of the cartilage extracellular matrix (ECM) into an ECM that is typical for bone. Cartilage ECM is characterised by a collagen type II network as the structural component that is interspersed with strongly hydrated proteoglycan aggregates. Bone has a heavily mineralised organic matrix that itself has collagen type I molecules as structural units with again proteoglycans as the major non-collagenous organic component. In the process of endochondral ossification, most if not all ECM components will have to be replaced or remodelled. It seems, based on recent literature, that the role of collagen in both cartilage and bone is very prominent in processes of remodelling of the ECM, making it likely that collagen may be a key factor in certain pathological conditions as well. In callus formation in bone, Wassen et al. [7] demonstrated that it is the collagen matrix that largely directs the process of mineralisation. Obviously, mineralisation is a key element in the process of endochondral ossification and it can be speculated that collagen may have a role here as well. In cartilage, Bramà et al. [8] showed that there is a very active turnover of the collagen matrix in the young foal that results in a distinct topographical heterogeneity in the mature animal. This biochemical heterogeneity has been shown to match with site differences in biomechanical loading [9] and is thought to be an adaptation of the tissue to the biomechanical challenges it is exposed to once the joints are started to be loaded after birth. This process of remodelling and modification seems to be a general phenomenon in at least a number of mammalian species [10]. In the horse, it has been shown to be most active in the first months after birth and to decline rapidly after this period [8]. It is in this same period that endochondral ossification is at its height and that osteochondrotic lesions are known to develop [11,12]. The process of endochondral ossification and the formation of topographical heterogeneity over the articular surface are therefore concurrent processes, both implying a very active collagen turnover. Although the exact pathogenesis of OC has not been elucidated so far [13], there is increasing evidence that a disturbance in collagen metabolism may be at least one of the key factors. Various studies have focussed on the distribution of proteinases that may be implicated in collagen metabolism in normal articular cartilage and in samples from osteochondrotic lesions [14–17]. Laverty et al. [18] found alterations in cartilage type II-procollagen in synovial fluids from horses with OC and in another study showed evidence for a significant increase in type II collagen cleavage, but no evidence for an increase in proteoglycan degradation [19].

A major drawback of most of the studies mentioned is that they are either based on very limited numbers of cases, or make use of samples from clinical cases that were presented for surgical removal of loose osteochondrotic fragments. In the latter case this means that it is the end stage of the disease that is studied. Osteochondrosis has been shown to be a very dynamic disorder in which lesions may become visible during the first months of life and then either develop further or regress, partially or even completely [11,20,21]. These observations have led to the idea that clinical OC is determined by two separate processes: the occurrence of lesions, i.e. the real pathogenesis, and the ensuing repair process of the lesion after it has formed [22]. Although both processes have effect on the ultimate incidence of clinical OC at the age when the horse is put into use, they are not necessarily influenced by the same factors. Whereas pathogenetical factors can be specific for OC, repair can be presumed to be a fairly general process that is influenced by the metabolic status of the cartilage, but is largely independent from the actual cause of the damage. In this line, it has recently been shown that a good copper status at birth in foals positively influenced the repair process of OC lesions, but had no effect on the pathogenesis of these lesions [23]. When studying established OC lesions, the relatively unspecific repair process will be focussed at, making it difficult to discriminate between various causes of cartilage damage [24,25]. It may be expected that the study of less mature lesions will yield more relevant information that might be useful for gaining a better insight in the early events of OC.

In order to test the hypothesis that disturbances in collagen metabolism are major events in early equine OC, which will be reflected in the make-up of the collagen network of the ECM, the present study focuses on the biochemical composition of the extracellular matrix in a large number of young horses in various, well-defined stages of osteochondrosis, including very early stages. The analysis includes the proteoglycan component of the ECM, but emphasises collagen and its posttranslational modifications. As osteochondrosis is a disturbance of the process in which cartilage changes into bone, subchondral bone samples were included in this analysis. We investigated total collagen content, hydroxylsine content, collagen cross-linking, and glycosaminoglycan (GAG) content in early-, middle-, and end-stage osteochondrotic lesions in foals and yearlings.

2. Materials and methods

2.1. Origin of the foals and experimental design

In this study, 43 Dutch Warmblood foals were used. These foals took part in a large research project on the development of osteochondrosis and the influence of exer-
exercise on the development of the equine musculoskeletal system. This project is extensively discussed elsewhere, including animal welfare and ethical considerations [6]. Briefly, the foals were the offspring of eight stallions with radiographically proven osteochondrosis in either the femoropatellar \((n = 4)\) or the tibial tarsal joint \((n = 4)\) and the mare population of the Institute for Horse Husbandry in Lelystad, the Netherlands. Four out of 43 of these mares had radiological osteochondrosis in the tibial tarsal joint and seven in the femoropatellar joint.

At birth, the foals were randomly assigned to three exercise regimens that included free pasture exercise, box-rest and box-rest together with a specific training programme. At the age of 5 months all foals were weaned. Twenty-four were randomly selected and euthanased by an overdose of pentobarbital directly after weaning. The remaining 19 foals were housed together in a loose house with access to a small paddock for an additional 6 months before being sacrificed at the age of 11 months. As the exercise regimens did not significantly affect the manifestation of OC [12], this factor was not taken into account in the present study.

2.2. Sample collection

Directly after euthanasia, the right femoropatellar and tibial tarsal joints were dissected. Samples were taken from two different joints: the femoropatellar joint and the tibial tarsal joint. In the femoropatellar joint two sites were sampled, which were located at the lateral and the medial trochlear ridge of the femur, respectively. In the tibial tarsal joint samples were taken from four sites. These were: the medial and lateral trochlear ridge of the talus and the medial malleolus and the cranial part of the sagittal ridge of the tibia. Samples were taken from these six sites in all animals, irrespective whether there were signs of osteochondrosis or not. Any osteochondrosis present was scored on a scale of 0 to 4 as described by Van Weeren and Barneveld [12] and based on the histological description of Henson et al. [26].

In this scale, grade 0 represents normal tissue, grade 1 features an indentation of the ossification front without histological signs of OC, in grade 2 there is loss of the normal, regular columnar arrangement of the chondrocytes and chondrone formation, grade 3 shows additionally fissures and necrosis of the deep layers of the cartilage, in grade 4 there is an overt lesion with a semi-loose fragment, and in grade 5 there is a loose fragment in the joint. Sites with scores of 2 or more were considered osteochondrotic. Sites with scores 0 were used as controls. Scores of 1 were considered inconclusive and hence not classified. In this classification, grade 2 lesions can be considered early stage OC, grade 3 middle stage and grades 4–5 end stage. In the present paper they are indicated as such.

Care was taken that the cartilage sample included the full thickness of the articular cartilage down to, but not including, the subchondral bone. Samples were cut in half, one for GAG analysis and one for determination of the collagen parameters. After recording wet weight, samples were lyophilised and dry weight was recorded.

Subchondral bone samples of approximately 2 mm in thickness were harvested after removal of the cartilage layer by use of a woodcraft knife. Dry weight was recorded after lyophilization of the sample. Finally, all samples were sealed, frozen, and stored at \(-20\,^\circ\text{C}\) until analysis as a single batch.

2.3. Determination of total collagen, and posttranslational modifications of collagen

Before hydrolysis, subchondral bone samples were demineralised three times with 1-ml 0.5 M EDTA (pH 7.4) during 24 h for each step. To remove excessive EDTA, the samples were washed with water (three times during 6 h each) and dried in vacuo. Further processing was similar for cartilage and subchondral bone samples. After hydrolysis in 6 N HCl, amounts of hydroxyproline (Hyp), hydroxylysine (Hyl), and the cross-link hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) were quantified by high performance liquid chromatography (HPLC) [27]. Collagen content was calculated from the Hyp content assuming that a collagen triple helix contains 300 Hyp residues, and choosing an average molecular weight of 300 kDa for collagen [27]. Collagen is expressed as mg collagen/mg dw. Levels of Hyl and the cross-links HP and LP were expressed as moles per mole of triple helical collagen (mol/mol collagen).

2.4. Determination of the sulfated GAG content

Prior to GAG analysis, cartilage samples were digested with 1 U/ml papain (Sigma, St. Louis, USA) in 400 μl of 50 mM phosphate buffer (pH 6.5), containing 2 mM Na2EDTA and 2 mM cysteine, for 4 h at 65 C. Sulfated GAGs were analysed by a modified 1,9-dimethylmethylen blue (DMMB) assay described by Farndale et al. [28]. To 40-μl sample, 40-μl 3% (w/v) bovine serum albumin (Sigma) and 1 ml of reagent (46 μM DMMB (Sigma), 40 mM glycine and 42 mM NaCl (adjusted to pH 3.0 with HCl) were added and after 30 min the absorbance at 525 nm was measured. The assay was standardised with shark chondroitin sulfate (Sigma).

2.5. Data analysis

Anatomical site and age differences were analysed with a two-way analysis of variance (two-way ANOVA); \(P\) values below 0.05 were considered significant. Interactions between anatomical site and OC-severity were analysed using three-way ANOVA (parameters: anatomical site, age and OC-severity). Variation due to differences in anatomical site was compensated for in order to analyse statistically the difference between normal and osteochondrotic samples in itself. To achieve this, each value was compensated for the average site effect of the corresponding age. The values of
all sites with a specified degree of OC per animal were averaged and considered as one data point, restricting the number of values per foal to one normal value, and none to three osteochondrotic values, dependent on the occurrence and grade of osteochondrotic lesions. For statistical analysis of multiple groups (i.e. osteochondrotic grades and age), a two-way ANOVA was used, in combination with Dunnett’s-T3 post hoc (equal variance is not assumed) test to evaluate the level of significance. Values were expressed as mean ± S.D., unless otherwise stated.

3. Results

3.1. Anatomical site and age differences in normal tissue

Considerable anatomical site and age differences were observed in most of the parameters in normal tissue of both cartilage and subchondral bone. The most prominent site and age differences were observed in the collagen content of normal cartilage tissue (Fig. 1A). Age appeared to have only an effect on the collagen content of cartilage samples that were taken from the tibial tarsal joint, whereas age had no effect on samples that were taken from the femoropatellar joint (Fig. 1A). The average grade of osteochondrotic lesions found in 26 preselected joints of each of the foals (for calculations early-, middle- and end-stage lesions were ranked as described in Section 2) indicates that there was a gradual shift in the distribution of lesions from the less severe early lesions in the 5-month-old foals (2.85 ± 0.10, n = 23) to the more severe end-stage lesions in the 11-month-old foals (3.20 ± 0.13, n = 17) (P < 0.05, Mann–Whitney test) (Fig. 1B).

Three-way ANOVA (parameters: anatomical site, age and OC-severity) revealed that there was no interaction between anatomical site and OC-severity. Because of this,

Fig. 1. Age differences on total collagen content and the average lesion severity in juvenile horses. (A) Influence of age and the anatomical site where the sample was taken on the total collagen content of non-osteochondrotic cartilage in 5- (open bars) (n = 24) and 11-month-old (solid bars) (n = 19) foals. Collagen content was deduced from hydroxyproline content as described in Section 2. Cartilage samples were taken from the lateral (IT) and medial (mT) trochlear ridge of the femur (Fem), and the talus (Tal), and the medial malleolus (Timm) and cranial part of the sagittal ridge (Tis) of the tibia. (B) Average severity of osteochondrotic lesions that were found within 26 preselected joints of 40 individual horses (with osteochondrotic lesions) of ages 5 (n = 23) or 11 (n = 17) months. Lines represent geometric mean ± 95% confidence interval. *P < 0.05 (Mann Whitney test).

Fig. 2. Total collagen content (mean ± S.E.) of cartilage (A) and subchondral bone (B) samples in increasing pathological stages of osteochondrotic lesions in 5- (open bars) and 11-month-old (solid bars) foals. Collagen content was deduced from hydroxyproline content as described in Section 2. All values were corrected anatomical site variations. n = 43 (24 + 19), 13 (10 + 3), 5 (3 + 2) and 8 (5 + 3) for control, early, middle and end, respectively; numbers between brackets represent n for 5 and 11 months, respectively. Asterisks indicate statistical differences between control and the indicated OC-severity group, where *, ** and *** represent P < 0.05, P < 0.01 and P < 0.001, respectively.
the statistical model was simplified by compensating each value for the average site effect of the corresponding age. Obviously, after correction of the samples for site effects, differences within this parameter were completely eliminated ($P > 0.96$). Also no statistical interactions were observed between age and OC-severity (two- and three-way ANOVA). For this reason, we do not go into detail about age effects, since these are extensively discussed earlier by Brama et al. [29].

3.2. Biochemical parameters after compensation for site effects

3.2.1. Collagen content

A highly significant ($P < 0.001$) decrease in collagen content of cartilage in osteochondrotic lesions was observed (0.56 ± 0.04 ($n = 43$), 0.48 ± 0.04 ($n = 13$), 0.49 ± 0.07 ($n = 5$) and 0.47 ± 0.14 ($n = 8$) mg/mg dw for control, early-, middle- and end-stage lesions, respectively). The lower collagen content was clearly present in the mildest form of osteochondrosis, and was stable in the more severe grades (Fig. 2A). In subchondral bone there was a significant ($P < 0.01$) decrease in collagen content in early lesions (0.15 ± 0.02 ($n = 13$) mg/mg dw) compared to the control group (0.21 ± 0.11 ($n = 43$) mg/mg dw) (Fig. 2B). However, collagen content tended to increase in the middle- and end-stage forms of osteochondrosis (0.23 ± 0.03 ($n = 5$) and 0.25 ± 0.08 ($n = 8$) mg/mg dw, respectively), although this increase was not significant.

3.2.2. Posttranslational modifications of collagen

The hydroxylysine level was very constant in both control and osteochondrotic samples from cartilage (50.7 ± 3.3 ($n = 43$) mol/mol triple helix) as well as from subchondral bone (19.1 ± 2.1 ($n = 43$) mol/triple helix) (Fig. 3).

Collagen HP cross-links were significantly decreased in cartilage from early lesions (0.86 ± 0.08 ($n = 43$) vs. 0.72 ± 0.15 ($n = 13$) mol HP/mol triple helix, for control
and early lesions, respectively, \( P < 0.01 \). However, this effect disappeared when the lesions worsened (Fig. 4). No differences were found in the HP cross-links of subchondral bone samples.

The level of LP cross-links in cartilage was found to be very low, and just above the detection limit. For this reason, no reliable data could be provided. In subchondral bone the level of LP cross-links in the control group was about seven times lower compared to the level of HP cross-links. LP cross-links in subchondral bone tended to decrease slowly with worsening of the osteochondrotic lesions. In severe lesions, this decrease became significant (0.040 ± 0.009 \( n = 43 \) vs. 0.027 ± 0.008 \( n = 8 \) mol LP/mol triple helix, for control and severe lesions, respectively, \( P < 0.01 \) (Fig. 5).

3.2.3. **Sulfated GAG content**

Sulfated GAG content was only altered in cartilage in the most severe form of osteochondrosis. In these lesions, GAG content decreased from 32.3 ± 10.3 \( n = 43 \) \( \mu g/mg \) ww for control cartilage to 20.3 ± 4.5 \( n = 8 \) \( \mu g/mg \) ww (Fig. 6). In subchondral bone the variation in GAG content appeared to be extremely high; for this reason, no GAG data for this tissue is provided.

4. Discussion

In this study we had the unique opportunity to study the biochemical changes of early osteochondrotic lesions in a relatively large number of young animals. Juvenile articular cartilage is a metabolically very active tissue in which rapid changes occur, with various processes going on at the same time. In the process of endochondral ossification, the cartilaginous precursors of the skeletal long bones gradually ossify, except for a thin layer of cartilage at the extreme ends of the epiphyses that become the articular cartilage. At the same time, this articular cartilage gradually assumes a typical topographical heterogeneity in biochemical make-up, a process initiated by the onset of articulation and weightbearing. The biochemical heterogeneity causes distinct differences in biomechanical characteristics across the joint surface and allows the joint to counteract successfully the biomechanical challenges it is exposed to. In the horse, this process of functional adaptation takes place principally in the first months of life [8]. Collagen is the major structural element of the tissues involved and the processes of remodelling and adaptation can proceed, thanks to the high collagen metabolism in the young animal. In articular cartilage this metabolic activity is known to decline exponentially after birth and to become extremely low in mature individuals. In man, collagen type II turnover time has been estimated to be between 200 and 400 years [30,31]. Against this background, the conclusion seems justified that developmental disorders such as OC can only be studied in young animals, at least if it is the pathogenesis of the disease that is subject of study and not the repair process of existing lesions.

There were interesting age-related site differences in collagen content between the femoropatellar and the tibial tarsal joint. In the latter, collagen content increased significantly from age 5 to age 11 months. This pattern was found also in the metacarpophalangeal joints of the same animals [29]. However, in the femoropatellar joint collagen, levels were already at a higher level at age 5 months and stayed at that level. There is no easy explanation for this phenomenon, but it stresses the earlier observation that age-related changes in equine joints may not progress at the same rate in all joints [22]. This study focussed on the analysis of the biochemical composition of cartilage and subchondral bone in various classes of osteochondrotic lesions. Since the occurrence and severity of lesions among sites of provenance were rather diverse, all samples were compensated for site differences before further processing. Two-way ANOVA revealed no interaction between age and the level of OC in none of the analysed parameter; meaning that age had the same effect on all groups of osteochondrotic severity in all analysed biochemical parameters.

There were distinct differences in collagen characteristics in the early-, middle- and end-stage lesions. In early lesions a highly significant decrease in total collagen content was found, remarkably both in cartilage and in subchondral bone. It should be realised in this respect that biochemical analysis of (osteochondrotic) subchondral bone samples has a potential pitfall. Since in osteochondrosis the border between cartilage and bone is, by definition, not sharp and distinct, contamination of subchondral bone samples with cartilaginous tissue may occur. This was almost certainly the reason why we failed to establish reliable GAG-values for subchondral bone as GAGs are much more abundant in cartilage than in bone. However, for collagen measurement, cartilage contaminations are less critical as the levels in both
tissue types do not differ much. The decrease in collagen content persisted in cartilage when the lesions grew older, but not in subchondral bone. The explanation may be that the declining metabolic level in cartilage (middle- and end-stage lesions were more prominent in 11-month-old animals than in 5-month-olds, Fig. 1B) affects the ability of this tissue to regain normal collagen levels. Collagen metabolism in bone remains high throughout life, thus enabling a quick recuperation. It therefore seems that a disturbance in collagen metabolism is an early event in OC in both cartilage and subchondral bone.

In cartilage, but not in subchondral bone, the degree of HP-cross-linking was decreased as well. Reduced cross-linking in cartilage from osteochondrotic lesions was earlier found in studies in which these lesions were provoked by means of inducing copper-deficiency [32,33]. It is known that the HP/LP ratio has a strong relationship with mineralisation in callus tissue [7]. This ratio is a result of a specific packing of intra-fibrillar collagen molecules [34], and thus of the spatial arrangement of these fibrils. Although we failed to analyse LP in cartilage, we too found a relation between low HP levels and lack of mineralisation in early OC lesions. It is therefore tempting to speculate that a similar influence of the collagen network might exist in the process of endochondral ossification and that spatial arrangements of the collagen fibrils may have a pivotal role herein.

The decrease in collagen levels may be caused by various mechanisms. First, the rate of collagen synthesis can be affected. The process of collagen synthesis is extremely active in the neonate and in the early postnatal period when mineralisation is most active, reflected by high levels of type X collagen and chondrocalcin (the C-propeptide of type II collagen) [35–37]. The highly active processes are more vulnerable to disturbances than low-rate processes. The change for such a disturbance to happen is greater when the growth rate is high, which is a well-known risk factor for the development of OC [1]. Second, the incorporation of newly formed collagen may be disturbed, which can lead to a too early release of these molecules from the matrix. Third, collagen breakdown may increase due to, for example, increased proteolytic activity. Based on the present study, no discrimination can be made between these possible mechanisms. Further, two or more mechanisms may be of importance at the same moment or sequentially in time. However, taking into account information from other recent studies, some speculations can be made.

Billinghurst et al. [38] showed alterations of biomarkers of collagen metabolism in serum samples from the same group of animals as used in this study. In particular, it appears that elevations in the serum levels of a marker of type II collagen synthesis, CPII, during the first year of life, is a consistent indicator of foals that have or will develop OC. They also showed a decrease in the serum levels of the 234CEQ-epitope, which is a marker specific for type II collagen degradation, and an increase in serum COL2-3/4Cshort, a less specific biomarker for type I and type II collagen degradation, in 5-month-olds with high OC scores compared to low OC scores. At the age of 5 months, most lesions are in an early phase (Fig. 1B). Brama et al. [39] found a sharp age-related decrease in MMP-1 (tissue collagenase) levels in synovial fluid from horses, reflecting the decline in collagen metabolism, but in another study found a relatively higher level of MMP-1 activity in foals affected by OC compared to age-matched controls without OC (unpublished data). MMPs are known to play an active role in cartilage repair and remodelling [40–42]. Van den Hoogen et al. [43] demonstrated a higher metabolic activity of chondrocytes originating from osteochondrotic lesions in young foals compared to chondrocytes from more mature lesions in yearlings. Together, these findings seem to indicate that in early osteochondrosis overall metabolic activity is increased, including collagen turnover, but specific collagen type II breakdown seems to be impaired. This leads to a lagging behind in development of the ECM of the affected area, giving it a more juvenile phenotype and hence a lower collagen level.

The importance of collagen metabolism during endochondral ossification and the aberration of collagen metabolism as an important feature of early OC are supported by recent reports on enzymatic activity in equine articular cartilage and on collagen degradation products in explant cultures. Hernandez-Vidal et al. [14,15] showed differences in distribution patterns of the cathepsins B and D in osteochondrotic cartilage compared to normal using immunolocalisation. Gläser et al. [17] have provided evidence for differences and site-specific roles of cathepsins B and L in articular cartilage during the process of endochondral ossification. The cysteine proteinases cathepsin B and L have been shown to have collagenolytic activity as well [44–46]. Laverty et al. [19] found an increase in the COL2-3/4Cshort-epitope, but not in proteoglycan degradation products in explants of osteochondrotic cartilage that were taken from young (<12 months) horses. Finally, in a recent study, Squires et al. [47] found that in human osteoarthritic cartilage a reduced collagen content is also associated with an increased collagenase activity, suggesting that a drop in collagen content is more commonly associated with an increased cleavage of collagen by collagenase.

It should be pointed out that, although data from this and other studies point to an important mechanistic role of collagen metabolism in the early phase of OC, there is no proof that changes in collagen metabolism are primary events. There are many putative factors involved in the process of endochondral ossification, which is a multi-step process regulated by a complex network of signalling systems [48]. The process of chondrocytic hypertrophy, apoptosis and mineralisation is governed by regulatory mechanisms in which parathyroid hormone-related protein (PTHrP) and Indian hedgehog (Ihh) play prominent roles [49]. Other important systemic factors include TGF-, IGF-1, thyroid hormones and basic FGF [13,50,51]. It is possible and even likely that the changes in collagen
metabolism are secondary to events in the upstream regulatory mechanisms.

Proteoglycan depletion was present in end-stage lesions, but not in earlier lesions. This finding is in line with the results of Lillich et al. [52], who found significantly lower quantities of uronic acid, GAGs and chondroitin sulfate in samples from OC cartilage compared to normal cartilage in a group of 16–24-month-old horses, which, given their age, must have featured end-stage lesions. Laverty et al. [19] found no evidence for an increase in proteoglycan degradation from horses aged 7–12 months. These results indicate that disturbances of proteoglycan turnover most likely are no specific sign of early OC, but are a feature of general joint pathology (as in OA), giving further support for the discrimination between a pathogenetic phase and a repair phase within the syndrome of OC.

It is concluded that this study, together with other recent publications, gives strong support for an important mechanistic role of changes in collagen metabolism during the early phases of osteochondrosis. The insufficient maturation and remodelling of the juvenile cartilage will lead to a thickening of the cartilage layer, but also to a delay in the normal age-related increase in collagen content and cross-linking, making the tissue more vulnerable for external influences. In older lesions, a more general repair process seems to take place that results in a less specific biochemical profile of the tissues. However, it should be realised that, although it seems that changes in collagen metabolism are of great importance in the pathogenesis of OC (and hence may be used for diagnostic purposes or even targeted for therapeutic intervention), it is not likely that these changes are primary causes of osteochondrosis.

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