Changes in proteoglycan content of articular cartilage during avian degenerative joint disease

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Abstract

Objective

To determine the biochemical changes in articular cartilage composition associated with the development of avian degenerative joint disease (DJD) in ad libitum fed broiler fowl, in comparison to feed-restricted broilers and J-line fowl (non-susceptible to DJD).

Methods

Articular cartilage from the distal tibiotarsus (DTT) was characterised up to age 180 days. Proteoglycan content was determined by uronic acid and sulphated glycosaminoglycan analysis, cellularity by assay for DNA content, and collagen content and crosslinking by hydroxyproline and pyridinoline analysis, respectively.

Results

Disease development was accompanied by increased hydration and proteoglycan content (particularly sulphated proteoglycans) and decreased cellularity, with no significant differences in either total collagen content or in mature collagen cross-linking.

Conclusion

The biochemical features of avian DJD are similar to those observed in other animal models. This bipedal model is exceptional however since cartilage alterations occur spontaneously and in a load-dependent manner.

Key words

Articular cartilage, biochemistry, osteoarthritis, poultry

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Introduction

Avian degenerative joint disease (DJD) causes lameness in broiler strain fowl. The histopathology of avian DJD, which typically shows areas of articular cartilage thinning, surface fibrillation and necrosis (1) is similar to that seen in human DJD [osteoarthrosis (2)]. In order to understand the relative contributions of genetic and other factors in the development of avian DJD, we have recently carried out a long-term study of three groups of fowl, including broiler strain fowl fed either *ad libitum* or on a feed-restricted diet, and J-line (light, layer strain) fowl (3). Morphological and histopathological analysis showed that broiler fowl fed *ad libitum* developed DJD earlier and more severely than their lighter, feed-restricted counterparts, while no gross pathology was seen in the J-line group. Of the four joint surfaces examined (distal tibiotarsus (DTT)), proximal tarsometatarsus (PTM), antitrochanter (AT) and proximal humerus (PH)), articular cartilage from the DTT was the worst affected.

Within articular cartilage, chondrocytes are enmeshed in a network of collagens [usually types II, VI, IX and XI but also type I in avian tissue; (4-6)] which are stabilised by a variety of lysine or hydroxylysine derived cross-links, mainly pyridinoline in mature tissue (7, 8). Proteoglycans such as aggrecan, entrapped within the fibrillar collagen network, draw water into the cartilage due to the osmotic swelling pressure provided by their fixed negative charge. This swelling pressure is resisted by the collagen network, thereby controlling cartilage hydration, so that normally proteoglycans are constrained to occupy 20% of their volume in free solution (9). Increased hydration has been widely associated with DJD (10).

Here we describe the biochemical composition of the DTT at different stages of development in the three experimental groups, in terms of hydration and uronic acid, sulphated glycosaminoglycan (SGAG), DNA, collagen and pyridinoline content. We show that the onset of DJD in the DTT of *ad libitum* fed broilers is associated with increased proteoglycan content (per tissue dry weight) compared to the other experimental groups. The results are consistent with a role for increased body mass in the development of DJD in avian articular cartilage.

Materials and methods

Experimental groups

As part of a larger study (3, 11), three groups of fowl were investigated: *ad libitum* fed broiler strain fowl (n = 140), feed-restricted broiler strain fowl (n = 110) and J-line fowl (n = 70). *Ad libitum* fed broilers show greater susceptibility to DJD than feed-restricted broilers (3), while J-line (laying strain) fowl (12) do not develop DJD. Rearing conditions are fully described in reference 3.

Sample collection and biochemical analysis

Five birds from each of the three groups were killed at 1, 19, 61, 79, 113 and 180 days of age. The mass of each bird was recorded and samples were collected (both right and left legs) from the distal tibiotarsus (DTT), proximal tarsometatarsus (PTM), antitrochanter (AT) and proximal humerus (PH). The joint was dissected sagitally and one half was preserved for histological examination in buffered neutral formalin (3). Cartilage samples for biochemical analysis were removed from the remaining part of the articular surface. Tissue hydration was calculated from the mass of each sample before and after drying overnight at 60°C. DNA and uronic acid contents were determined after digestion of samples with SDS/proteinase K (13), where carbazole assays (for uronic acid) were carried out using a method adapted for microtitre plates (14). Additional samples were taken at day 180 for papain digestion (0.1 M phosphate buffer pH 7.0,10 mM cysteine, 2 mM EDTA with 125 g/ml papain for 18 hours at 60°C, followed by maceration and additional papain, another 125 g/ml, for a further 18 hours). Papain digested samples were assayed for sulphated glycosaminoglycans (15) using a method adapted for a microplate reader.

Pyridinoline content (per mole total collagen, as assayed by hydroxyproline analysis) was assayed at day 180. Hydroxyproline assays were carried out using the method of Firschein and Shill (16) an adaptation of the original method of Woessner (17). For each sample a portion of the acid hydrolysate used for hydroxyproline analysis was dried under vacuum, redissolved in water and analysed using ion-pair reversed phase HPLC directly linked to an automated sample preparation system for solid-phase extraction on disposable columns (18).

Statistical evaluation

The results were analysed by one-way analysis of variance (ANOVA) with Tukey's method to compare means, using Instat Version 3.0 for Windows (GraphPad Software Inc., San Diego USA) corrected for multiple comparisons. Significance was set at $P < 0.05$. Analysis of covariance was performed using Genstat 5 - Version 3.2 for Windows (Lawes Agricultural Trust -IACR - Rothamsted, UK).

Results

In general, in all experimental groups (*ad libitum*, feed -restricted and J-line) and in all joint surfaces examined (DTT, PTM, PH, AT) hydration of articular cartilage decreased with age, as shown for the DTT in Figure 1. Hydration of samples from both the *ad libitum* fed and feed-restricted groups was generally higher than in the corresponding J-line samples, consistent with the absence of observed gross pathology in the J-line group up to day 180 (3, 11). ANOVA analysis for the entire DTT hydration data set showed the inter-group variation to be significant ($p < 0.0001$). During the period of disease development and progression (days 113-180), the broiler strain consistently showed significantly higher hydration levels in DTT cartilage than samples from disease resistant J-line fowl (Tukey tests $p < 0.001$). There were no differences in hydration however between the *ad libitum* fed birds, which develop severe DJD pathology, and the feed-restricted birds, which develop only mild pathology.

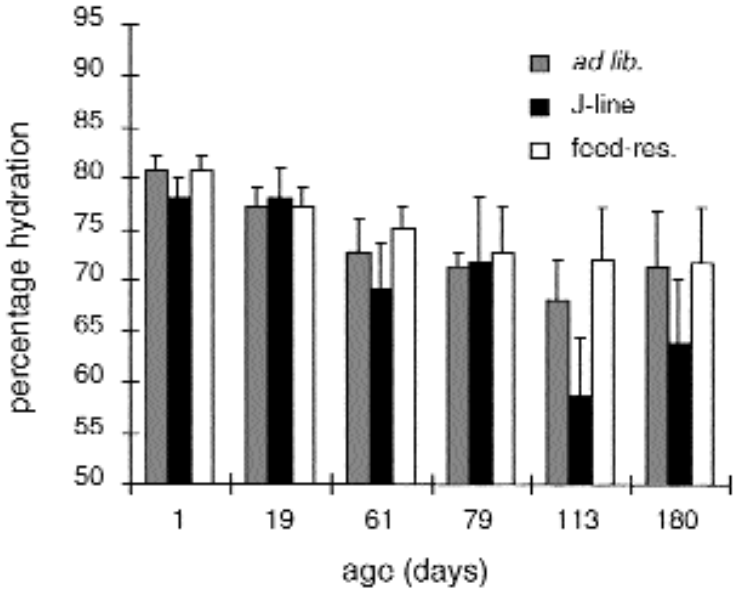

Fig. 1. Hydration of DTT articular cartilage from the three experimental groups. Error bars are ± SD.

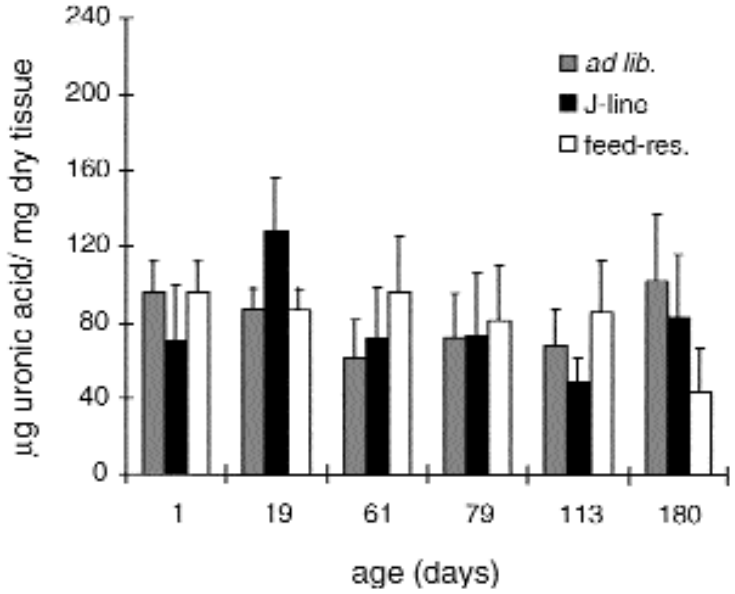

Fig. 2. Uronic acid content of DTT articular cartilage from the three experimental groups. Error bars are ± SD.

Data for uronic acid content showed a pattern similar to that for hydration, with significant inter-group variation for the DTT (Figure 2; ANOVA result for the entire data set, $p < 0.0001$). At day 180 in particular, uronic acid content in articular cartilage from diseased (13) DTT of the *ad libitum* fed group was significantly greater than in healthy DTT from the feed-restricted group ($p < 0.001$). Consistent with the uronic acid data, assay of sulphated glycosaminoglycans revealed significantly higher sulphated glycosamino-

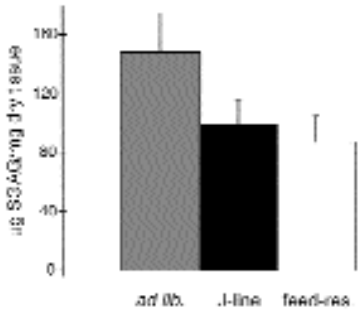

Fig. 3. SGAG content of the DTT for the three experimental groups at day 180. Error bars are ± SD.

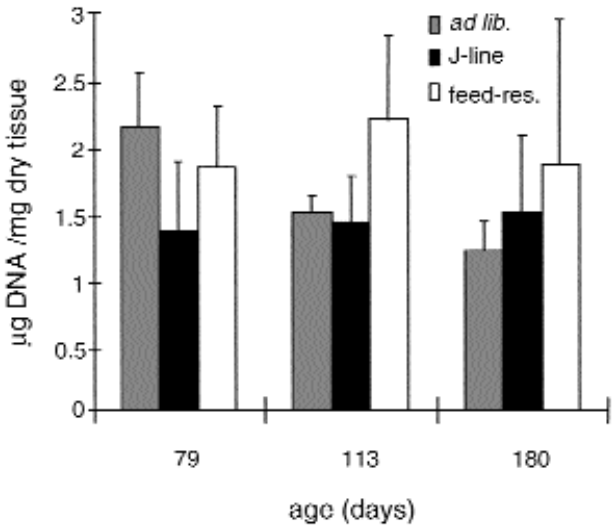

Fig. 4. DNA content of the DTT for the three experimental groups for days 79-180. Error bars are ± SD.

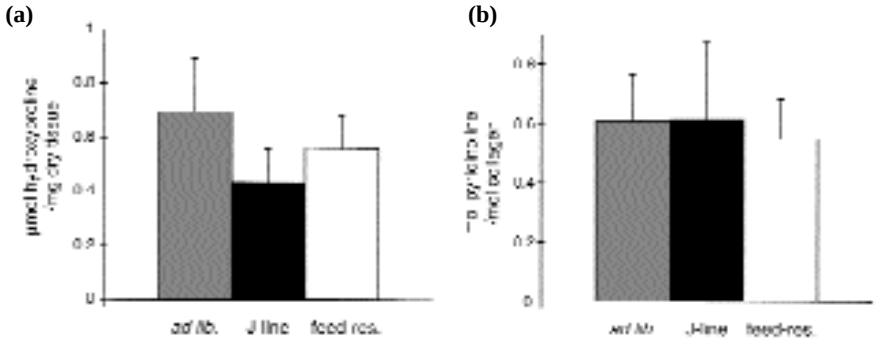

Fig. 5. (**a**) Hydroxyproline and (**b**) pyridinoline content of DTT articular cartilage for the three experimental groups, at day 180. Error bars are ± SD.

glycan content (Fig. 3) in the DTT from *ad libitum* fed fowl, compared to both J-line ($p < 0.01$) and feed-restricted broilers ($p < 0.01$), at day 180 (ANOVA result $p < 0.0001$). The higher uronic acid and sulphated glycosaminoglycan contents are coincident with the appearance of DJD pathology (3) and increased proteoglycan degradation (11).

Differences between *ad libitum* fed broilers and the other experimental groups were also apparent in the DNA content (Fig. 4). During disease development (days 79 to 180) in the DTT of the *ad libitum* fed broilers, DNA content continued to fall, whilst being relatively constant in the other samples. Analysis of covariance showed that the change in DNA content with age from days 79 to 180 was significantly different between disease susceptible *ad libitum* fed broiler strain fowl and disease resistant J-line fowl ($p = 0.02$).

To determine whether the observed increase in DTT tissue hydration in *ad libitum* fed broilers might be due to changes in collagen content or in the extent of collagen cross-linking, total hydroxyproline and pyridinoline content were measured. At day 180, however, there were no significant differences in either hydroxyproline content (Fig. 5a) or pyridinoline content (Fig. 5b) between all three experimental groups.

Discussion

The general decrease in hydration with time is in agreement with the age related changes seen in other species (19, 20). One of the most consistent features of the biochemistry of DJD cartilage is the higher water content when compared to normal cartilage (21). This was also true for avian DJD. The hydration of the DTT cartilage of the DJD susceptible broiler strain was consistently higher than that of the resistant J-line strain, during the period when DJD pathology was observed at days 113 and 180 (3, 11). There were however no differences in hydration between *ad libitum* fed and feed-restricted broilers. It should be noted that, unlike J-line birds, the DTT of feed-restricted broilers is also susceptible to mild DJD in older birds (3).

The development of DJD in the DTT of *ad libitum* fed broilers was associated with increased levels of proteoglycans, as shown by determination of both uronic acid and sulphated glycosaminoglycans, when compared to both feed restricted and J-line birds. These changes are similar to those seen in other animal models of disease. In canine models of OA, before the phase of massive cartilage loss, the cartilage first exhibits a hypertrophic response (22, 23). The increase in uronic acid content of the *ad libitum* fed broiler DTT at day 180 is in agreement with these observations. The high SGAG content of the DTT compared to the other groups at this time is supported by observations in rabbit and canine models both *in vivo* (24) and *in vitro* (25, 26) that proteoglycan synthesis is increased in early experimental OA.

In a parallel study of proteoglycan metabolism in articular cartilage explants taken from the avian model (11), we found increased degradation but no increase in synthesis during the development of DJD pathology in the DTT. It is possible that the absence of serum factors in these *in vitro* experiments may have masked the increase in proteoglycan synthesis that would be required to account for the increased total proteoglycan content reported here.

During the period of disease development (days 79-180) there was a continuing decrease in DNA content in *ad libitum* fed DTT when the DNA content of the other samples was constant. Decreased DNA content is consistent with the decline in numerical cell density observed in similar early stage experimental OA, when cartilage fibrillation is minimal or absent and chondrocyte clustering is modest (27).

Table I. Biochemical composition of DTT articular cartilage in the three experimental groups, in relation to strain DJD susceptibility and pathology.

Experimental group	DJD susceptibility	DJD pathology	Hydration	Uronic acid content	SGAG
J-line	No	No	Low	High	Low
Feed-restricted	Yes	No	High	Low	Low
Ad libitum fed	Yes	Yes	High	High	High

Direct DNA measurements of similar cartilage also show a decline (28).

Articular cartilage from broiler strain fowl contains similar levels of collagen cross-linking as measured by pyridinoline analysis, to that of J-line fowl. These results, however, provide no information of the extent of collagen network breakdown. The lack of association of pyridinoline content with disease is in agreement with the observation that total pyridinoline content did not change with development of OA in partially menisectomised rabbits (29). Also in humans there is no difference in pyridinoline content between OA and normal articular cartilage samples (30).

The characteristics of the DTT cartilage from the three groups at day 180 are summarised in Table I. This shows certain characteristics which are associated with disease and loading. Strain DJD susceptibility appears to be associated with high cartilage hydration, since both feed restricted and *ad libitum* fed broilers are relatively highly hydrated. Uronic acid content however is high in the J-line and *ad libitum* fed groups and low in the feed restricted group. This can be related to the differences in loading of articular cartilage *in vivo*, which is a function of both body mass and articular surface area. While articular surface area is similar for both feed-restricted and *ad libitum* fed broilers, differences in body mass result in relatively low loading in the feed restricted group. Body mass is also low in J-line birds, but so is articular surface area, resulting in relatively high loading. Both highly loaded groups (*ad libitum* fed broilers and J-line) are associated with high uronic acid contents, resulting in the increased swelling pressure required to counteract the increased load. In contrast, SGAG content is related to disease only; it is high in the *ad libitum* fed group and low in the other two groups. Hence the development of DJD pathology in avian articular cartilage may be a consequence of load induced changes in proteoglycan sulphation. The extent to which these changes are the direct result of increased loading, rather than associated metabolic changes, remains unknown.

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