# Chondroitin sulphation in human joint tissues varies with age, zone and topography

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The joint is an organ consisting of diverse connective tissues, each with its own specific extracellular matrix composition, whose metabolism is controlled by an equally diverse range of cell types. Nevertheless, the physiological function of the joint depends upon the integrated activity of all tissue structures.

Although analyses of matrix macromolecules in synovial fluid can provide unique insights into the metabolism of the joint, many of these molecular fragments, particularly chondroitin sulphate, are ubiquitous connective tissue components. The effectiveness of 'markers' must lie in the specificity with which they define a biological process (tissue, cell, matrix component, repair, catabolism, etc.,). Thus, it is imperative that the potential of individual tissues to contribute a component to the synovial fluid pool should be known so that changes which occur in pathology can be correctly ascribed to a particular metabolic process and a meaningful assessment of them as temporal markers of joint disease can be made.

The need for a better understanding of the sulphation pattern of proteoglycans in human joint tissues was prompted by two published observations. The first of these concerns the generally accepted view that osteoarthritic chondrocytes synthesise proteoglycans with an 'immature' composition, which is in keeping with the hypermetabolic activity of the tissue (Mankin and Lippiello 1971). This conclusion was based on analysis of the cartilage remaining on resected hip joints which had an increased content of 4-sulphated disaccharide. However, the study took no account of the anatomical, morphological or ageing patterns that are known to exist and made no attempt to differentiate between anabolic and catabolic events. The concept of a chondroblastic phenotype in osteoarthritis may be appealing in relation to tissue repair, but there is little direct evidence to support it. Clarification of these experimental findings is urgently required, not just to determine their place in the pathogenesis of osteoarthritis, but also because measurements of 4- and 6-sulphated disaccharides in joint fluids are now being published as 'markers' of cartilage proteoglycan metabolism (Shinmei et al. 1992) without reference to the normal, macro- or micro-heterogeneity of these structures and without a clear appreciation of which tissue's turnover they reflect. The second of these observations concerns the identification, using monoclonal antibodies, of atypical structures in the chondroitin sulphate chains of animal and human osteoarthritic cartilage (Caterson et al. 1990, Slater et al. 1995). In osteoarthritic cartilage there is increasing evidence that they represent an attempt at matrix remodelling and repair.

The present study is a preliminary report of an extensive investigation designed to provide a detailed data-base of normal joint tissue composition and metabolic activity, which it is hoped will be of use for subsequent studies of diseased tissues.

## Materials and methods

Tissues were obtained from normal intact knee joints at surgery for bone tumours not involving the joint space. Tissues sampled included, a mid-slice of the lateral and medial meniscus, a mid-slice of the anterior cruciate ligament and a sample of the synovium from the supra-patellar pouch area. Full-thickness cartilage was taken from the complete femoral condylar surface of over 100 specimens of different ages and a representative sample was used for analysis. Topographical variations were assessed by taking 4 mm diameter cores of cartilage from twelve sites on the femoral condyle and tibial plateau at five different

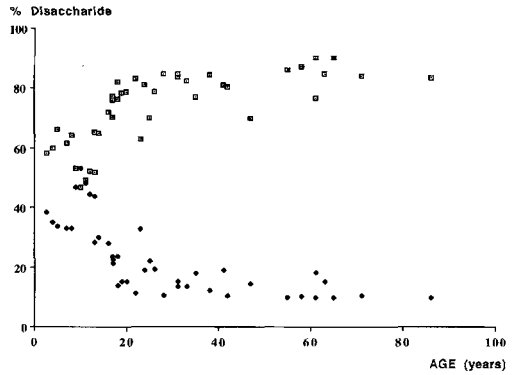


Figure 1. The Δdi-6S (■) and Δdi-4S (◆) content of human femoral condylar cartilage measured by capillary electrophoresis.

Table 1. Comparison of the chondroitin sulphate and hyaluronan composition of normal human knee joint tissues aged 10 years and 35 years

| Tissue | %Δdi-HA | %Δdi-0S | %Δdi-6S | %Δdi-4S |
|---|---|---|---|---|
| *Aged 10 years* | | | | |
| Tibial plateau | 1.59 | 3.38 | 63.28 | 31.74 |
| Femoral condyle | 0.94 | 3.38 | 53.41 | 42.27 |
| Meniscus | 12.46 | 15.06 | 27.30 | 45.18 |
| Ligament | 16.18 | 9.40 | 12.98 | 61.40 |
| Synovium | 32.10 | 9.60 | 16.60 | 41.70 |
| *Aged 35 years* | | | | |
| Tibial plateau | 0.62 | 3.13 | 89.81 | 6.44 |
| Femoral condyle | 1.14 | 2.37 | 88.62 | 7.87 |
| Meniscus | 5.70 | 4.80 | 43.40 | 45.90 |
| Ligament | 14.30 | 8.80 | 23.90 | 52.90 |
| Synovium | 40.00 | 23.81 | 11.85 | 24.20 |

ages. Each core was sectioned at 50 μm from the articular surface, pooling every four sections through the tissue depth, in order to assess zonal variations. Glycosaminoglycans were prepared by papain digestion of the tissues. These were treated with chondroitinase ABC to generate chondroitin sulphate (Δdi-0S, Δdi-4S, Δdi-6S) and hyaluronan (Δdi-HA) disaccharides which were separated on a 270 A-HT capillary electrophoresis system as described by Carney and Osborne (1991). For biosynthetic studies, cartilage cores were cultured for 8 hrs in HAM's F12 containing 50 μCi/ml $^{35}SO_4$. Labelled disaccharides were prepared as described above and separated by HPLC on a Partisil 5PAC column with an on-line radioactivity monitor.

## Results

Analysis of full depth pieces of articular cartilage confirmed earlier studies that there is an increase in the 6-sulphation and a concomitant decrease in 4-sulphation of chondroitin sulphate, with increasing age. However, analysis of over 100 specimens showed that this change in composition was confined to the period of tissue development and maturation and was largely complete by 20–25 years of age; thereafter, the Δdi-6S:Δdi-4S ratio remained relatively constant (Figure 1). In contrast, the proportion of Δdi-0S remained unchanged from birth to 90 years of age. Although evidence was also found for other di- and tri-sulphated structures, these never accounted for more than 1% of the total disaccharide content.

When the chondroitin sulphate composition of different tissues from the same joint were compared, each tissue had a different content of Δdi-6S and Δdi-4S. Although articular cartilage always had the highest Δdi-6S:Δdi-4S ratio, all tissues except synovium showed an age-related increase in the proportion of the 6-sulphated moeity and the extent of this increase was also tissue specific (Table 1). In the synovium the proportions of Δdi-HA and Δdi-0S increased with age, which resulted in a lower concentration of both Δdi-6S and Δdi-4S.

In order to determine what the relative contribution of anabolism and catabolism was to the age-related changes in sulphation, full-depth cores of articular cartilage were biosynthetically labelled with $^{35}S$-sulphate. A comparison of newly synthesised and endogenous chondroitin sulphate chains, indicated that at all ages the disaccharide composition of both pools was very similar. Moreover, the same experiment also demonstrated that there were considerable zonal variations in disaccharide composition, however, at each age and within each zone, both pools had similar Δdi-6S:Δdi-4S ratios (Table 2).

The influence of topography on chondroitin sulphation was investigated by sampling articular cartilage from a number of defined sites on the femoral condyles and tibial plateaus of knee joints of different ages. Although it is not possible to present the data for all of the specimens examined so far, the results illustrated in Figure 2 are an example of the variable composition that has been measured at different sites on the articular surface.

## Discussion

Proteoglycans are major components of the connective tissues which make up the synovial joint. They have key roles in the normal physiology of these tis-

Table 2. A comparison of the sulphation pattern of endogenous ($UV_{232}$) and newly synthesised ($^{35}S$) chondroitin sulphate in different zones of articular cartilage. Articular surface = Slice 1

| | 14 years (Δdi-6S:Δdi-4S) | | 60 years | |
|---|---|---|---|---|
| | $^{35}S$ | $UV_{232}$ | $^{35}S$ | $UV_{232}$ |
| 1. | 1.16 | 1.67 | 1.62 | 2.33 |
| 2. | 1.47 | 2.07 | 2.17 | 4.07 |
| 3. | 1.80 | 2.51 | 3.31 | 6.80 |
| 4. | 2.10 | 2.40 | 4.82 | 6.34 |
| 5. | 2.23 | 2.00 | 4.80 | 5.22 |
| 6. | 1.65 | 1.60 | 4.33 | 4.70 |
| 7. | 1.62 | 1.44 | 3.76 | 4.10 |
| 8. | 1.24 | 1.35 | 3.70 | 4.20 |
| 9. | 1.25 | 1.18 | 3.52 | 3.90 |
| 10. | 1.06 | 1.06 | — | — |
| 11. | 0.66 | 0.87 | — | — |
| 12. | 0.69 | 0.76 | — | — |
| 13. | 0.70 | 0.77 | — | — |
| 14. | 0.89 | 0.78 | — | — |

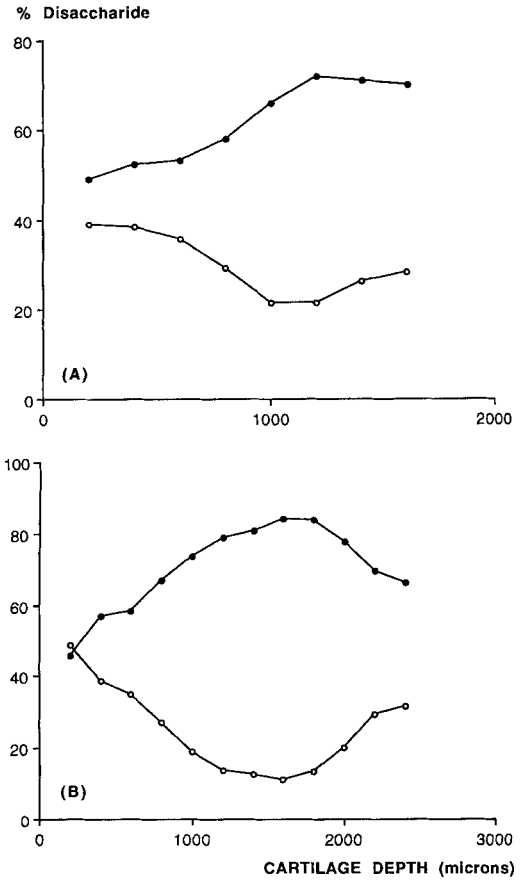


Figure 2. The distribution of Δdi-6S (●) and Δdi-4S (○) through the depth of human articular cartilage (30 years). Medial femoral condyle (A); Medial tibial plateau (B).

sues and can influence fundamental biological processes such as cell proliferation, migration and recognition, cytodifferentiation, extracellular matrix deposition and morphogenesis. The control of glycosaminoglycan structure and in particular the pattern of sulphation appears to be used to change the properties of proteoglycans to suit biological needs. Thus there have been a number of reports in the literature describing changes in the Δdi-6S:Δdi-4S ratio of proteoglycans in human osteoarthritic cartilage, and more recently the expression of novel sulphation patterns on chondroitin sulphate chains have been identified. It has also been proposed that the levels of chondroitin sulphate isomers and the Δdi-6S:Δdi-4S ratio in joint fluids reflect cartilage proteoglycan metabolism and can be used to diagnose joint diseases and predict articular cartilage destruction.

The present study has revealed some potentially important observations which may affect the interpretation of previous studies and which should also be considered in future investigations of joint disease. In particular, age is a major determinant of chondroitin sulphate composition. For articular cartilage this may not seem particularly surprising given the well documented reports of age-related changes in aggrecan structure. However, the effect of age on the composition of other chondroitin sulphate-containing proteoglycans such as decorin, biglycan and versican is unknown. It is imperative that we have this information for tissues such as meniscus, ligament and synovium where these proteoglycans are known to be concentrated. The different disaccharide composition of chondroitin sulphate in normal joint tissues is partly a reflection of the variable proteoglycan populations that are present and it is not unreasonable therefore to expect that the turnover of each of them would also be tissue dependent. Consider then the difficulties which arise in interpreting synovial fluid analyses of chondroitin sulphate; not only are the molecular and tissue sources unknown, but the clinical stage of joint disease will also undoubtedly have a differential effect on each tissue.

The same arguments apply to zonal and topographical variations in the composition of chondroitin sulphate. The results obtained for articular cartilage illustrate the futility of comparing the composition of full-thickness normal with partial-thickness diseased cartilage, or of comparing different sites on the articular surface. It is also interesting to note that the results shown in Table 2. suggest that changes in sulphation are mainly biosynthetic in origin and that for diseased, adult cartilage to attain an 'immature' composition there must be a change in the activity of

intracellular sulphotransferases. This is in marked contrast to the increased heterogeneity in size of aggrecan which is thought to be largely due to proteolytic modification in the extracellular matrix. Recent studies (Bayliss and Hickery, unpublished results) have provided further evidence for the independent control of sulphation (sulphotransferase activity) in human cartilage, by showing that IL-1α, TNF-α and TGF-ß have the same effects on the incorporation of $^{35}$S-sulphate and the Δdi-6S:Δdi-4S ratio of chondroitin sulphate chains uncoupled from protein core by xylosides, as when the chains are covalently attached to the protein. Needless to say, the considerations discussed above are equally applicable to the proteoglycans in other joint tissues and to other matrix macromolecules.

Thus, analysis of chondroitin sulphate contents in joint fluids may represent a new way to study joint diseases but, as for other 'markers', it is worth sounding a cautionary note: we need to improve our knowledge about the structure and metabolism of matrix macromolecules of all the joint tissues if we are to take full advantage of the information they can provide about disease mechanisms.

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