**Introduction:** Loading of cartilage (such as in walking) is accompanied by a displacement of proteoglycans—a movement that is resisted by the collagen network. By doing so, the collagen network experiences increased tensile stresses. One of the earliest changes seen in osteoarthritis (OA) is an increase in water content, due to a damaged collagen network. Freeman [1] suggested that the primary event in the pathogenesis of primary OA is a collagen fatigue failure. Fatigue refers to the failure of a material because of repetitive stressing at a level below the ultimate strength of the material. An important feature of the collagen network in adult human articular cartilage is that it shows hardly any turnover from age 20 years onwards [2]. Thus, in adults, the collagen network experiences a large number of stress cycles during life. We hypothesized that, to withstand fatigue, the physical condition of the collagen network laid down at adolescence is of crucial importance towards the age of onset of OA. Indeed, two recurrent point mutations and a reduced expression of collagen type II have been found in early onset OA [3], highlighting the importance of collagen fibril quality in the pathogenesis of OA. We have investigated whether more subtle changes of the collagen network could be involved in the development of OA at older age. Therefore, we have measured cross-link and lysyl hydroxylating levels in normal (N) and macroscopically fibrillated cartilage obtained from the knee of donors without a clinical history of OA. This degenerated (DG) cartilage contains increased levels of damaged collagen molecules [4] and is considered an early, preclinical phase of OA [5].

**Material and methods:** N and DG cartilage was obtained from the weight-bearing areas of 6 human femoral knee condyles (age 49, 60, 72, 83, 86 and 92 years). DG cartilage (defined by macroscopic focal fibrillation of the articular surface) was taken from the lateral or medial condyle; N cartilage (with a glossy, white, smooth surface) was obtained from a comparable position of the contralateral knee of the same donor. All cartilage samples were divided in an upper half (the region near the surface) and a lower half (the region adjacent to the bone). The samples were hydrolyzed in 6 M HCl for 20 h at 108 °C and subsequently subjected to amino acid and cross-link analysis as described previously [2]. The quantities of hydroxylysyl (Hyl), and hydroxylysylpyridinoline (HP) were expressed as the number of residues per collagen molecule, assuming 300 Hyp residues per triple helix. In addition, the non-enzymatic crosslink pentosidine was measured. Pentosidine accumulates in articular cartilage over several decades, and was used as a measure of the molecular age of the collagen network. Furthermore, the relative amount of proteoglycans (PGs) was estimated by the Hyp/Pro ratio.

**Results:** Within each donor, N and DG cartilage contained identical pentosidine levels, indicating that the collagen network in these samples are of the same age (i.e., no significant remodelling of the collagen network in DG cartilage occurred). Thus, comparison of N and DG cartilage is not confounded by de novo synthesis of collagen: the non-remodelled collagen network in DG cartilage represents the network laid down at adolescence (age 20 years). Significantly higher levels of Hyl were found in the upper and lower part of DG cartilage compared to normal cartilage (paired Student’s t-test; P<0.005 and <0.001, respectively; Fig. 1). The Hyl levels in the upper and lower part of DG cartilage are 17 ± 7% and 8 ± 3% (mean ± S.D.) higher than in normal cartilage, respectively. Furthermore, a significantly higher (14 ± 11%) HP level is found in the upper part of DG cartilage compared to normal cartilage (P<0.05; Fig. 2A). The HP level of the collagen molecules in DG cartilage from the lower part is the same as in normal cartilage (Fig. 2B). There was no significant difference between the Hyp/Pro ratio of N and DG cartilage (Fig. 3), indicating that in N and DG cartilage the relative amount of PGs and collagen is the same.

**Discussion:** It is well known that PGs induce tensile stresses in the collagen network: the higher the PG concentration, the higher the tensile stress. It seems unlikely that PGs play a role in the impairment of the collagen network in the studied DG samples, since we did not find differences in PG content between N and DG cartilage. As we found differences in lysyl hydroxylation and HP cross-linking, it is reasonable to assume that the problem is located in the collagen network itself, the major structural component of cartilage. Since the observed changes in DG cartilage must have been present several decades before cartilage became degenerated, it seems that high levels of lysyl hydroxylation and pyridinoline cross-linking result in a collagen network that fails mechanically in long term loading. Areas containing collagen with low Hyl and HP levels are less prone to degeneration. As such, this study indicates that post-translational modifications of collagen molecules synthesized at adolescence are causally involved in the pathogenesis of OA.

![Figures 1-3](image_url)