INTRODUCTION

Age-dependent changes in articular cartilage have been documented extensively and include a decrease in the mechanical performance of the tissue, decreases in the total proteoglycan content and in the size of the proteoglycans in the tissue [1]. While a significant age dependency exists, it is still unclear whether these changes are specific for degenerative and osteoarthritic cartilage or whether they are involved in the normal aging process. The causes of these changes are unknown, but are thought to involve both mechanical loading [2] as well as local and systemic biochemical factors, which are difficult to decouple. The nasal septum is composed of hyaline cartilage similar to that of articular cartilage in an unloaded environment. As such it provides a model system by which cartilage aging can be studied in the absence of mechanical factors. The objectives of this study are to quantify the biochemical and biomechanical changes that occur in nasal septum cartilage with age.

METHODS

Human septal cartilage was obtained from reconstructive septorhinoplasty in accordance with the guidelines of the University of Massachusetts Medical School and Englewood Hospital. A total of 45 patients ranged in age from 15 to 60 years with a mean age of 31.7 ± 12.3. Nasal cartilage specimens were placed in DMEM with 100 U/ml penicillin G, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B immediately after retrieval from patients until processing. Specimens were freed of surrounding perichondrium, bone or connective tissue and weighed. Then a 6 mm punch biopsy was obtained and frozen at -20°C until biomechanical testing.

Biomechanical Analysis

Cartilage samples were placed in a cylindrical confining chamber, mounted in a servo-controlled Dynastat mechanical spectrometer [3]. Samples were equilibrated at pH 7.4 at room temperature in a solution of phosphate-buffered saline containing protease inhibitors (10 mM EDTA, 10 mM benzamidine, 1 mM PMSF and 1mM NEM). The thickness of 6 mm biopsies were recorded with a caliper and samples were placed in uniaxial confined compression between a porous polyethylene platen and the base of the chamber. Disks were compressed by sequential increments of 2% strain, up to a maximum of 20% total strain with load recorded every 0.5 seconds for 200 sec/step. Imposed displacements and measured loads were normalized to sample thicknesses and areas to yield strains and stresses, respectively. Stress relaxation data was fit to a poroelastic model of tissue behavior [3,4] to calculate equilibrium modulus and hydraulic permeability.

Biochemical Analysis

Cartilage disks were digested in 2 ml of 10mM cysteine 125 µg/ml papain solution at 60°C for 16 hours. Aliquots (10 µl) of the digests were assayed spectrophotometrically for the glycosaminoglycan content with dimethylmethine blue dye [5] using shark chondroitin sulfate as the standard. The hydroxyproline content of 100 µl aliquots was measured spectrophotometrically at 560 nm after hydrolysis in 6N HCl for 16 hours at 125°C and reaction with 50 mM chloramine T and pDAB [6]. Cartilage samples not digested for analysis were placed in 2 ml of 4M guanidine HCl containing protease inhibitors described above to extract proteoglycans. Samples of extracts were eluted on a 105 cm Sepharose CL-4B column to determine the size profile of nasal cartilage proteoglycans.

Statistical Analysis

Trends in equilibrium modulus, hydraulic permeability, GAG content, and hydroxyproline content with age were determined by calculating a coefficient of linear correlation, R, and comparing to critical values of R to determine significance levels.

RESULTS

The equilibrium modulus decreased significantly with age (p<0.01) from a high value of 723 kPa in a 16 year old to a low of 34.1 kPa in a 54 year old. The average permeability was 5.99 × 10⁻¹⁴ ± 8.22 × 10⁻¹⁵ m²Pa⁻¹s⁻¹, 10-20 higher than the reported hydraulic permeability of human articular cartilage (Armstrong and Mow, 1982).

The total GAG content decreased significantly with increasing age (p<0.05) (Fig. 2A). The magnitude of age-related changes in GAG content was much less than that of mechanical properties, with ~25% average decrease from age 15 to 60. The average total GAG content of human nasal cartilage was 43.01 ± 1.29 µg/mg, similar to that reported for human articular cartilage [8]. The hydroxyproline content of human nasal cartilage showed a slight, but not statistically significant increase with age. Mean hydroxyproline content of was 19.90 ± 1.40 µg/mg, higher than that of articular cartilage [8].

Average proteoglycan (PG) size in human nasal cartilage also decreased with age. Three distinct sizes were noted on CL-4B chromatography. PGs from youngest tissues eluted early with a sharp profile. Samples from middle-aged patients eluted as a broad peak with the largest portion in the middle peak. Proteoglycans from the oldest specimens also eluted broadly with the greatest portion in the last (lowest MW) peak (Fig 2B).

DISCUSSION

Compressive equilibrium modulus, hydraulic permeability, and GAG content of human nasal cartilage varied significantly with donor age, while hydroxyproline content was found to change only slightly. The decrease in size of proteoglycans in nasal cartilage with age suggests that the tissue is enzymatically degraded with age. This general pattern of age-related changes is consistent with studies of the aging process in human articular cartilage [7]. The fact that these changes occur in a nonloaded cartilage suggests that there is a biochemical component of age-induced cartilage degradation that is independent of mechanical factors.

REFERENCES


ACKNOWLEDGMENTS

This study was supported by a grant from the German Academic Exchange Society (N.R.) and the University of Massachusetts Medical School.

**Englewood Hospital, Englewood, NJ.**