Introduction:
The collagen fibril network maintains the volume, shape and tensile strength of the extracellular matrix of articular cartilage. One of the features of osteoarthritis (OA) is the disruption of this fibrillar network by collagenases. The Hartley guinea pig develops a spontaneous and progressive degenerative of knee joints that closely resembles OA in humans (1,2). This animal model of primary OA is especially attractive for studies of OA pathobiology due to the unique biology of guinea pig cartilage. The interstitial collagenase-1 and -3 are found in humans (3), and whose expression has been associated with cartilage damage both in humans (4) and guinea pigs (3). In our previous work, we identified collagenase-3 expression and collagen cleavage in the guinea pig knee joint cartilage as early as two months of age (3). We hypothesized that a reduction in normal cartilage birefringence of knee joints would be a sensitive indicator of OA onset and the relative loss of birefringence would correlate with histological progression of OA in this model system. We have utilized a novel technique to examine the progressive loss of normal collagen birefringence in the Hartley guinea pig model longitudinally.

Methods: Forty male Hartley guinea pigs were obtained from Charles River Laboratories at 2 months of age and raised until sacrificed at 2 (n=6), 4 (n=6), 7 (n=6), 10 (n=6), 12 (n=6), and 18 (n=10) months of age. The Institutional Animal Care and Use Committee approved all procedures. The right knee joint from each animal was fixed for 24 hours in 10% buffered formalin, followed by decalcification in 10% EDTA in 0.1M phosphate buffer, pH 7.6-7.8 for approximately 3 months. Paraffin sections (5 μM) of the central region of the joint were stained with either picrosirius red and examined under polarized light to evaluate the collagen fibril network or toluidine blue for histological analyses. Slides containing histologic sections for collagen network orientation were exhaustively deparaffinized after which they were treated at 37°C for 18 hours in 2.0 mg bovine testicular hyaluronidase in 1.0 ml 0.1M phosphate buffer at pH 6.0 to remove chondroitin sulfate molecules, which might mask the cationic binding sites of the collagen for the saturated picric acid and then washed, dehydrated and mounted with cover slips. Sections were analyzed with a Nikon microscope equipped with polarizing filters. The relative sign of induced birefringence was determined by turning the analyzer in two opposite directions. The optical properties of the extracellular matrix (i.e. the presence or absence of birefringence indicating orientation of the collagen fibers (6,7)) of the articular cartilage were examined. Normal patterns of collagen network birefringence were observed and then the areas of normal birefringence were expressed as a percentage of the area of articular cartilage observed. These assessments were made blinded to animal age and histological score. A semi-quantitative histological grading system described previously (2) was used to evaluate OA severity in each tibial plateau and femoral condyle. This included a blinded evaluation of articular cartilage structure (extent and severity of surface irregularities including fibrillation and clefts [0-8]), and proteoglycan loss (as determined by loss of toluidine blue staining [0-6]). These two features of chondropathy were summed to obtain a total histological score ranging from 0-14 for each compartment of the joint. Differences in birefringence with respect to age and 4 joint surfaces (medial and lateral femoral condyles; medial and lateral tibial plateaus) were analyzed by ANOVA, non-parametric, followed by the appropriate post-hoc tests using Instat. Linear regressions were performed using Statistica. P-values of ≤ 0.05 were considered statistically significant.

Results: There was a progressive loss of normal collagen birefringence with age (Figure 1A). Overall, the birefringent area of cartilage declined 41% from 2 to 18 months of age. The area of normal collagen network birefringence was reduced to 75% at 4 months, from a baseline value of 86% at 2 months. Declines in birefringence were similar among the 4 joint surfaces at these early ages. From 7 months on, significant differences were noted among the 4 joint surfaces studied (p<0.0001).

Reductions in normal birefringence were consistently greater on the tibial side of the joint when compared with the corresponding femoral surface. Similarly, reductions in the medial side of the joint were greater than changes in the corresponding lateral side of the joint. The development of histological knee OA was apparent at 4 months of age and steadily progressed in severity through 12 months of age. Appreciable further worsening of histological OA severity was not discernible for animals 18 months of age (data not shown). A highly significant negative correlation was observed between percent area of normal birefringence and histologic OA score. This was apparent for the total joint (Figure 1B) as well as for both the medial and lateral compartments (p<0.0001, r=-0.67 and p<0.0001, r=-0.64, respectively).

Discussion: In the present study, we have shown that reductions in normal collagen network birefringence corresponded with histological progression of OA in the Hartley guinea pig knee. Interestingly, the timecourse of decline in collagen integrity was similar between the medial and lateral compartments up to 4 months of age, even though the medial tibial plateau is the site at which histologic damage is first discerned. Thereafter, changes were more severe in the medial tibial plateau. This finding is consistent with our previous work showing positive immunostaining of young guinea pig knee joint cartilage with 9A4, a monoclonal antibody detecting a collagenase generated neoepitope site within collagen (3). These results show that disruption in the collagen fibril network is a feature of guinea pig OA and suggest that collagen network disruption is discernible prior to evidence of histological OA.