**Introduction:** In osteoarthritis, articular cartilage is eroded by an imbalance between catabolic and anabolic cellular processes. This results in a net loss of extra-cellular matrix molecules, such as chondroitin sulfate (CS) and hyaluronic acid (HA), which are critical to the ability of cartilage to withstand physical activity. Previous studies have attempted to characterize the metabolic differences between “normal” and “osteoarthritic” human cartilage primarily by assessing donor tissue in various *in vitro* models. However, *in vitro* results can be problematic since the recovery of human cartilage biopsies often injures chondrocytes. Moreover, articular cartilage interacts with surrounding tissues *in vivo*, such as bone, joint capsule, and synovial fluid, all of which influence the tissue phenotype. An *in vivo* assay has been developed for animal models in which the stable isotope deuterium from heavy water (\(\text{D}_2\text{O}\)) is incorporated into newly-synthesized glycosaminoglycan extracted either from articular cartilage or synovial fluid. The objective of this study was to modify this *in vivo* assay for use in people to determine the physiological synthesis rates of CS and HA in human articular cartilage after obtaining either cartilage biopsies or synovial fluid (SF) aspirates.

**Materials and Methods:** Patients scheduled for ACL reconstruction surgery were recruited for this IRB-approved study. Patients took 2-3 oral doses of heavy water (40-50 ml 70% \(\text{D}_2\text{O}\)) daily until their scheduled surgery (3-6 weeks). On the day of surgery, small biopsies of articular cartilage (1-2 mg) were harvested during routine notchplasty. A sample of SF also was obtained from all subjects. Body water enrichment was monitored from saliva samples, obtained every week during the labeling period. Following surgery, HA and CS disaccharides were isolated from cartilage and SF samples as chondroitinase ABC cleavage products and analyzed by gas chromatography / mass spectrometry. Specifically, fractional synthesis was determined from the \(\text{D}_2\text{O}\)-enrichment of N-acetyl glucosamine and N-acetyl galactosamine, respectively. Data are presented as mean ± SD.

**Results:** Five patients were given heavy water for 15-42 days prior to surgery, resulting in \(\text{D}_2\text{O}\) enrichment in body water of 1.5±0.3% and \(\text{D}_2\text{O}\)-enrichments in CS that ranged from 0.3-1.3% and increased 0.04% per day (\(p=0.01, r^2=1\)). These \(\text{D}_2\text{O}\)-enrichments were above the lower limit of sensitivity of the GC / MS (>0.2% EM1).

Fractional synthesis of CS in the articular cartilage was 0.8±0.3% per day (Fig. 1; n=5 patients), a rate of turnover corresponding to a half-life of 92 days. This represented an interpatient coefficient of variation (CV) of 40%. For patients from which multiple cartilage specimens were obtained, intra-patient CV averaged 48% (n=4).

Fractional HA synthesis in the cartilage was 0.4±0.2% per day (Fig. 1), about 50% lower than the CS turnover rate (\(p=0.02, n=5\)). Inter- and intra-patient variability in HA synthesis was 48% (n=5) and 19% (n=3), respectively.

The CS from SF exhibited a fractional synthesis rate of 1.9±0.6% per day (n=5), 2.5X greater than that in the articular cartilage (p=0.03), suggesting that the CS in the SF was preferentially derived from newly-synthesized CS, rather than from degraded matrix. Regression analysis did not indicate a relationship between synthesis rates, suggesting that the breakdown products in the SF may provide a representative biopsy of the underlying cartilage. In humans, however, the data from this study did not support the notion that the CS in SF aspirates can be used to quantitatively infer CS synthesis of the joint cartilage. In the same fashion, this method of directly measuring cartilage matrix synthesis would be useful in validating other SF or circulating biomarkers.

**Discussion:** We present the first *in vivo* measurements of the physiological synthesis rates of CS and HA by human articular cartilage. Half-lives were 92 and 181 days, respectively. These half-lives were markedly lower than those previously measured when using *in vitro* models for assessment (e.g., 243-481 days for sulfated GAGs), which may reflect some of the limitations of *in vitro* approaches, such as cell viability. While previous studies have shown good correlation between *in vivo* and *in vitro* methods for quantifying radiosulfate incorporation into the articular cartilage from normal rabbits and dogs, it is unclear whether this would also hold in pathogenic joint conditions, where the abnormal joint environment may play a critical role in regulating cartilage metabolism. Indeed, regulatory factors associated with traumatic knee injury may have stimulated the cartilage studied here, since previous animal studies, using both this method and *in vitro* approaches, have demonstrated that mechanical instability can induce a hypertrophic repair response in articular cartilage. Additional work including a proper “normal” control group of patients may elucidate the stimulatory effects of ACL tear as well as provide a framework for evaluating any metabolic alterations that occur during OA progression and therapeutic intervention.

Evaluation of “normal” human cartilage would be facilitated by an ability to measure cartilage GAG synthesis from SF aspirates. The majority of CS in SF is typically bound to aggrecan, indicating that it originates from articular cartilage. In previous rat studies, we have observed that increased fractional CS synthesis due to mechanical instability is also reflected in the CS collected in synovial fluid aspirates, suggesting that the breakdown products in the SF may provide a representative biopsy of the underlying cartilage. In humans, however, the data from this study did not support the notion that the CS in SF aspirates can be used to quantitatively infer CS synthesis of the joint cartilage. In the same fashion, this method of directly measuring cartilage matrix synthesis would be useful in validating other SF or circulating biomarkers.

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**CLINICAL MEASUREMENTS OF GAG SYNTHESIS IN HUMAN ARTICULAR CARTILAGE IN VIVO**
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**Figure 1.** Fractional synthesis rates of CS and HA in individual cartilage biopsies taken from five ACL reconstruction patients.