# Betamethasone Chondrotoxicity in Hip Cartilage Explants from Symptomatic Femoral Acetabular Impingement

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INTRODUCTION: Femoral acetabular impingement (FAI) is characterized by the mechanical destruction of articular cartilage secondary to irregularities in hip morphology, such as at the junction of the femoral head and neck in the cam subtype (CFAI). Although initially asymptomatic, patients typically present at young ages and may be predisposed to the development of osteoarthritis (OA).[1] Treatment for FAI begins with non-operative interventions including intraarticular corticosteroid injections. Current formulations of betamethasone have been shown to have chondrotoxic effects postulated to be secondary to preservatives such as benzalkonium chloride.[2] The purpose of this study was to assess the chondrotoxic effects of commercial betamethasone sodium phosphate and betamethasone acetate solution on cartilage explants from patients with CFAI, and compare the impact of differing betamethasone solution concentrations on expression of chondrogenic markers and catabolic enzymes as well as cartilage architecture.

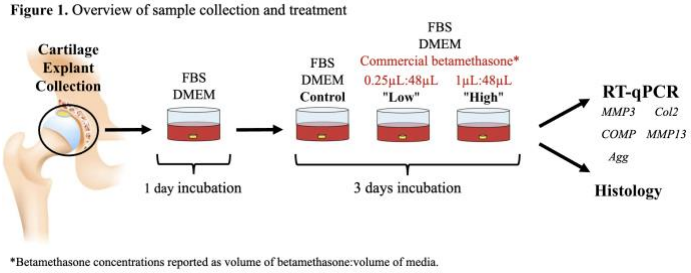
METHODS: Articular cartilage samples were obtained from 6 patients (3 male, 3 female) who underwent hip arthroscopy for cam lesion removal in symptomatic FAI. Radiographs and visual inspection were used to ensure that all cartilage samples were harvested from the apex of the cam lesion for each patient. Samples were deidentified and partitioned into three separate cartilage explants of equal size (2cm height x 2cm width) that were incubated individually at 37ºC in Dulbecco's Modified Eagle Medium (DMEM) buffered with 10% phosphate buffered saline (PBS) and 0.1% penicillin-streptomycin (PS). After 24 hours of incubation, the media was replaced with DMEM with 10% PBS and 0.1% PS solution with one of three concentrations of betamethasone sodium phosphate and betamethasone acetate solution to media: no betamethasone, "control" group; 0.25µL betamethasone:48uL media, "low steroid" group; or 1µL betamethasone:48µL media, "high steroid" group. Cartilage explants incubated in the appropriate media for 3 days, and the media was exchanged for fresh media every 24 hours. Cartilage explants were then removed. Samples were fixed and stained with Safranin-O following standard technique, and gene expression analyses were performed via real-time quantitative PCR. Chondrogenic (*Agg, Col2, COMP*) and catabolic enzyme (*MMP3*, *MMP13*) gene expression was assessed, and expression levels were compared within each patient relative to the control sample. Gene expression was presented as fold-change relative to the control. Significance was $p<0.05$.

RESULTS: Explants from 6 patients (3 male, 3 female) were collected and separately treated with each experimental condition (Figure 1). Both *Agg* and *COMP* expression were greater in the low steroid group than in the high steroid group, although this did not reach significance (Figure 2). Expression of *Col2* and *MMP3* was comparable between the betamethasone-treated groups, whereas *MMP13* expression was notably higher in cartilage explants incubated in the high concentration of betamethasone. Safranin-O staining revealed marked differences in cartilage organization and matrix architecture across conditions (Figure 3). The distribution and uniformity of Safranin-O staining, along with overall tissue integrity, decreased progressively with increasing steroid concentration. The control group showed the most intense Safranin-O staining and well-preserved chondrocyte morphology, whereas the low-steroid and high-steroid groups exhibited reduced proteoglycan content and matrix preservation in a dose-dependent manner. Evidence of cartilage degeneration, reflected by prominent Fast Green counterstaining, was present in all groups.

DISCUSSION: In this study, CFAI-derived cartilage explants were exposed to differing concentrations of commercial betamethasone solution, demonstrating a dose-dependent chondrotoxic effect. Explants cultured in higher steroid concentrations demonstrated decreased expression of chondrogenic markers (*Col2*, *AGG*, *COMP*) and increased expression of catabolic enzymes (*MMP3*, *MMP13*), although this did not reach significance. These trends may indicate a shift toward extracellular matrix (ECM) degradation. At the molecular level, high doses of corticosteroids can impair cartilage homeostasis by suppressing anabolic gene transcription while promoting catabolic enzyme activity. Reduced *Col2* and *Agg* expression may reflect diminished synthesis of structural components responsible for cartilage tensile strength and compressive resilience. Concurrently, elevated *MMP3* and *MMP13* levels suggest increased collagenase activity, which may accelerate ECM breakdown via high-dose steroid-induced chondrocyte stress. This imbalance between matrix synthesis and degradation may explain the diminished proteoglycan staining observed in the high-steroid condition. Commercial corticosteroid formulations contain constituents known to exhibit chondrotoxic effects, potentially amplifying the effects of the steroid.[2]

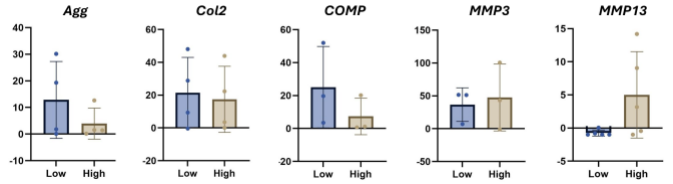
SIGNIFICANCE: These findings suggest that incubation of hip cartilage explants from patients with symptomatic CFAI in commercial betamethasone solution results in dose-dependent chondrotoxicity. This work underscores the need for careful evaluation of intra-articular corticosteroid use and formulation-specific safety in the management of joint disease such as FAI. Future studies exploring alternative dosing regimens may help identify thresholds that preserve therapeutic benefit while minimizing cartilage injury and further examine excipients in corticosteroid solutions like benzalkonium chloride that may contribute to oxidative stress and chondrocyte toxicity.

**Figure 1.** Overview of sample collection and treatment




*Betamethasone concentrations reported as volume of betamethasone:volume of media.

**Figure 2.** Fold-change in expression relative to control group.



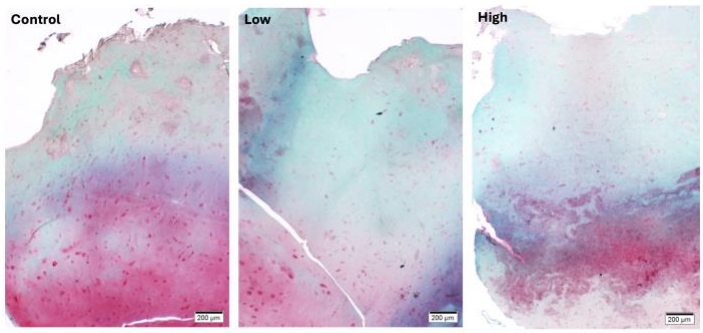




**Figure 3.** Safranin-O staining of articular cartilage obtained from patients who underwent hip arthroscopy for symptomatic CFAI and exposed to betamethasone (control, low, or high concentration).

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