ever. Se addition reduced this expression. In contrast, exposure to DON, NIV, and T-2 toxin caused depressed TIMP-1 and TIMP-3 levels compared with the Control; this reduction was reversed with Se addition. Se addition also reversed the expression. α2 macroglobulin that was lowered by the three Toxins.

**Conclusions:** This study indicates that three toxins (DON, NIV and T-2 toxin) all depress type II collagen and aggregan expression in tissue engineered cartilage grafts cultured in vitro. The levels of MMP-1 and MMP-3 were increased but the levels of TIMP-1, TIMP-3 and α2 macroglobulin were all decreased in the presence of Toxins. Addition of Se reversed all of the expression effects produced by these Toxins. These in vitro results provide evidence for the potential biological mechanisms underlying cartilage degradation in the pathogenesis of KBD and how Selenium addition reverses these pathological effects.

### 263

**REGULATION OF MICROSOMAL PROSTAGLANDIN E2 SYNTHASE-1 AND 5-LIPOXYGENASE-ACTIVATING PROTEIN/5-LIPOXYGENASE BY 4-HYDROXYNONENAL IN OSTEOARTHRITIC CHONDROCYTES**

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**Purpose:** Recently, we reported that the induction of cyclooxygenase-2 (COX-2) decreased dramatically after 24 hrs of incubation with 4-hydroxynonenal (4-HNE), a product of lipid peroxidation. This study aimed to investigate whether HNE is responsible for the shunt from COX-2 to 5-lipoxygenase-activating protein (FLAP)/5-lipoxygenase (5-LOX) in human osteoarthritic (OA) chondrocytes.

**Methods:** OA chondrocytes were treated with 10 μM of 4-HNE at different times of incubation (0 to 72 hrs). The protein level of mPGES-1 was evaluated by Western blot and that of prostaglandin E2 (PGE2) and leukotriene 4 (LTB4) was determined by EIA using commercial kits. The level of mPGES-1 and FLAP/5-LOX mRNA was measured by real-time RT-PCR.

**Results:** HNE induced the production of both PGE2 and LTB4 by chondrocytes, but in opposite fashion. The level of PGE2 increased during the short period of stimulation (0-24 hrs), whereas that of LTB4 increased after a longer period of stimulation (48 and 72 hrs), where the level of PGE2 decreased. The Western blot data showed that protein expression of mPGES-1 increased gradually in treated cells with 4-HNE, to reach a maximum level after 16 hrs of incubation. At mRNA level, 4-HNE induced mPGES-1 and FLAP/5-LOX expression after 4 and 48 hr of incubation respectively.

**Conclusions:** Our results showed the shunt from the COX-2 to FLAP/5-LOX pathway in HNE-induced human OA chondrocytes. The increase in LTB4 level may be due to the decrease in that of PGE2. Further experiments are in progress in other to determine the molecular mechanism underlying this switch in OA chondrocytes.

### 264

**EFFECT OF THREE DIFFERENT CHONDROITIN SULFATES IN HUMAN OSTEOARTHRITIS CARTILAGE**

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**Purpose:** During the osteoarthritis (OA) process, the altered metabolism of the chondrocytes is responsible for the extensive breakdown of the cartilage in which pro-inflammatory cytokines and matrix metalloproteases (MMPs) are highly implicated. Previous studies have demonstrated that chondroitin sulfate (CS) exerts a protective effect on the cartilage. However, due to differences in CS in terms of origin, purity and the production/purification process, we compared the effects of three different types of CS on human OA cartilage.

**Methods:** Three types of CS were tested: namely CS#1 (porcine, purity: 90.4%), CS#2 (bovine, purity: 93.0%), and CS#3 (Bioibérica S.A.; bovine, purity: 99.9%). Treatment with each CS at 200 and 1000 μg/ml were performed in human OA cartilage explants in the presence/absence of IL-1β, and the protein modulations of factors, including PGE2, IL-6, and MMP-1, were investigated by specific ELISA. The CS effect on the expression of the pro-anabolic factor, collagen type II, was also investigated on OA chondrocytes using quantitative PCR.

**Results:** In the presence of IL-1β, CS#3 at 200 μg/ml, but not at 1000 μg/ml, reduced the level of MMP-1, PGE2 and IL-6. CS#2 followed the same trend as CS#3, however, at a much higher concentration, 1000 μg/ml. On the other hand, CS#1 was much less efficient in reducing the same catabolic markers and very surprisingly, in the absence of IL-1β, it increased the three catabolic factors tested, PGE2, IL-6, and MMP-1. As expected IL-1β inhibits the gene expression level of the collagen type II; only CS#3 was able to limit this inhibition. CS#1, in the presence or absence of IL-1β, further decreased collagen type II expression.

**Conclusions:** This study provides data on the effect of different CS on the cartilage metabolism. In this context, CS prescribed for alleviating OA symptoms should be taken with care as the origin, purity and/or production/purification of the CS compound could orientate the current OA disease process toward increased catabolic pathways.

### 265

**PATHOLOGY OF THE OUTERBRIDGE IV LESION; THERAPEUTIC IMPLICATIONS**

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**Purpose:** The purpose of this study is to examine the gross and microscopic characteristics of the Outerbridge IV lesion that may serve as the foundation for cartilage repair. Preclinical studies have demonstrated that low-intensity pulsed ultrasound (LIPUS) may improve the healing of cartilage lesions.

**Methods:** Human osteochondral specimens having Outerbridge IV lesions were harvested following total knee surgery. They were subject to visual examination before and after Safranin O staining. Correlative histology was examined.

**Results:** The stained gross specimens showed cartilaginous aggregates on the surface as well as multiple small depressions. The microscopy showed cartilaginous aggregates on the surface...