1 and 10 nM insulin, respectively. The effect of PPP was identical with both concentrations used (0.05 μM and 0.5 μM). 2-DG uptake in OA chondrocytes was increased to 112±2.1% and 123±2.5% relative to the respective control cells, by treatment with 1 or 10 nM insulin, respectively, but pretreatment with either concentration of PPP had no effect on insulin-induced 2-DG uptake. Similarly, treatment with PPP decreased insulin-induced Akt phosphorylation in normal but not in OA chondrocytes Total GLUT-1 protein content was increased by treatment with insulin 10 nM in normal, but not in OA chondrocytes, whereas 1 nM had no effect either in normal or OA chondrocytes.

**Conclusions:** Adult human chondrocytes express functional IR. Insulin increases glucose transport in normal and OA chondrocytes, but in the latter its effects seem to be mediated by the IR alone, as indicated by the inability of the specific IGFR inhibitor, PPP, to decrease insulin-induced 2-DG uptake and Akt phosphorylation, a signaling event common to IR and IGFR. Insulin responses mediated by its specific receptor may overcome the previously reported defective IGFR signaling in OA chondrocytes.

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**DIFFERENT AREAS OF THE HUMAN OSTEOARTHRITIC KNEE CARTILAGE RESPOND DIFFERENTLY TO CATABOLIC AND ANABOLIC STIMULATION WHEN CULTURED**

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**Purpose:** There is a vast need for developing models for investigation of the pathobiology of cartilage turnover. A model system that have proven worthy in investigation of cartilage turnover is the bovine explants model; it show distinct patterns in response to defined catabolic and anabolic stimuli. It is desirable to extend this model to human articular cartilage, however, obtainable human cartilage will most likely be affected by OA. We therefore investigated how different areas of human knee OA cartilage would respond to catabolic and anabolic stimuli, when cultured for 3 weeks.

**Methods:** Human articular cartilage was isolated from patients undergoing knee replacement. Explants were taken from either the center (slightly macroscopically damaged) or the rim of the tibia plateau (macroscopically undamaged), or the femur condyle (no macroscopic damage). Explants were cultured at optimized conditions with and without cytokines and growth factors: 1) without stimulation (w/o), 2) TNFα and oncostatin M (O+T), 3) IGF-1, and 4) Parathyroid hormone (PTH). The explants were cultured for 21 days and medium was renewed every 2-3 days. At day 21, the explants were formalin-fixed and paraffin embedded. Explants were analyzed by safranin O/fast green. Supernatants were analyzed by formation and degradation biomarkers (ELISA assays): PIINP, CTX-II, 342-G2, 374-G2 and CIIM (new Col II marker).

**Results:** All stimulated samples were compared with w/o. Aggrecanase fragments (374-G2) were continuously released from all explants throughout the culturing period, and there was only little effect of the different stimuli. MMP fragments of aggrecan (342-G2) and type II collagen (CTX-II and CIIM) were significantly released as explants from both damaged and undamaged tibial cartilage when treated with O+T, but not by femur explants (all time points). Neither IGF-1 nor PTH could significantly alter the release of aggrecanase- or MMP-derived fragments. Type II collagen formation (PIINP) was suppressed when treated with O+T in explants from the femur and the undamaged tibial cartilage, but only slightly in the late stage of the damaged cartilage explants. Both IGF-1 and PTH could induce collagen type II formation, but again the effect was only significant in the femur and the tibial undamaged explants. These data was supported by histological assessments.

**Conclusion:** We found that different areas of the OA cartilage display different biological potential. We found that the aggrecanase activity could not be induced further by either anabolic or catabolic stimulation. In contrast, MMP activity could be induced by cytokines in both slightly damaged and undamaged cartilage explants. Furthermore, anabolic stimulation had greater effect on femur condyle and undamaged tibial cartilage, than on slightly damaged tibial cartilage. This means that different areas of the cartilage display different biological profile and this information should be incorporated when designing OA models (e.g. cartilage explants). Moreover this could with advantage be used for investigation of particular OA situation.

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**AN IKKα ASSOCIATED GENE EXPRESSION PROFILE IN DIFFERENTIATING CHONDOCYTES**

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**Purpose:** We previously reported that IKKα, one of the two NF-κB activating kinases, has an unexpected role in ECM remodelling and progression to hypertrophy and terminal differentiation of primary osteoarthritic chondrocytes grown in 3D mineralizing cultures. As an unbiased approach to deciphering IKKα’s mechanisms of action in this context we have begun to evaluate the effects of IKKα ablation on the gene expression profile of primary OA chondrocytes. In addition we have also begun to investigate if the expression of genes affected by IKKα in this in vitro context reflects similar or different alterations in gene expression associated with OA disease.

**Methods:** IKKα expression was evaluated by immunohistochemistry in full thickness explants of cartilage and subchondral bone derived from OA patients, and graded by Safranin-O staining. To investigate IKKα dependent gene expression, primary OA chondrocytes, derived from several independent patients undergoing joint arthroplasty, were seeded into differentiating microcultures. Endogenous IKKα expression was ablated just prior to micromass seeding by stable retrotransduction with IKKα shRNAs (Oliotto et al A&R 2008) with a firefly lucerase shRNA retrovector as a control. After ~1 week of micromass differentiation, Affymetrix arrays were employed to reveal the gene expression profile linked to IKKα in this context and the latter gene expression profile was subsequently evaluated in the context of normal and OA cartilage.

**Results:** IHC analysis showed that the degree of IKKα protein expression correlated with OA severity, and was more upregulated in the vicinity of the middle to deep zones of late stage OA cartilage compared to normal cartilage. The expression profile of ninety-two genes in differentiating microcultures were linked to IKKα. Pathway analysis (Kegg mapp) indicated that the pathways significantly affected by IKKα specify gene products associated with ECM-receptor interaction, focal adhesion, cell communication, T cell receptor signaling pathway and the cell cycle. Noteworthy, most of the genes encoding ECM proteins were up-
regulated in IKK\(\alpha\) KD micromass samples, suggesting a common or concerted regulatory mechanism linked to IKK\(\alpha\). Conversely, MMP-10, recently shown to function as a collagenase activator in OA cartilage, was downregulated in IKK\(\alpha\) KD samples. Overall, these findings suggest that IKK\(\alpha\) can coordinately affect a number of different processes, some of which are linked to ECM remodeling, whose deregulated control is associated with OA disease. A subset of the genes affected by IKK\(\alpha\) were also differentially expressed in normal vs. OA cartilage.

Conclusions: IKK\(\alpha\) protein is upregulated in late OA cartilage. In the context of differentiating chondrocytes in in vitro micromasses and on the basis of DNA microarray analysis, the expression of IKK\(\alpha\) was linked to a functional profile specifying effectors of ECM remodeling, cell cycle and cellular communication with some of the same genes exhibiting differentially expression in normal vs. OA cartilage. Subsequent experiments will examine the expression profile of these and other IKK\(\alpha\) linked genes in OA progression to better understand IKK\(\alpha\)’s potential role in OA disease onset and and/or development.

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IN Volvement of P38 MAP KINASE PHOSPHORYLATION IN CHONDROCYTE APOPTOSIS INDUCED BY MECHANICAL STRESS AND HEAT STRESS

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Purpose: There are some reports that osteoarthritis (OA) cartilage has a higher number of apoptotic chondrocytes than does normal cartilage. Like a JNK, p38 MAP kinase participates in a signaling cascade controlling cellular responses to cytokines and stress. p38 MAP kinase plays an important role in the regulation of the immune response and has been shown to regulate proliferation or differentiation. In addition, activation of p38 MAP kinase has traditionally been associated with the stress response and some apoptotic processes. However, it is still clearly unknown the function of the p38 MAP kinase in chondrocytes. In this study, we analyzed the expression of p38 and phosphorylated p38 in chondrocytes of OA cartilage and normal cartilage. We induced chondrocytes apoptosis by various stress, and then investigated the relationship between chondrocytes apoptosis and phosphorylated p38.

Methods: Normal cartilage samples were obtained from femoral head undergoing joint replacement surgery for the neck fracture of the femur. OA cartilage samples were collected from patients at the time of total knee joint replacement surgery. The expression of p38 and phosphorylated p38 in OA cartilage and normal cartilage were analyzed by Western blotting.

Next, chondrocytes apoptosis were induced by various stress, mechanical stress and heat stress. To apply mechanical stress or heat stress to NHAC-kn cells (human normal chondrocytes), both stress were induced apoptosis and also increased phosphorylated p38. However, in control, we could not detect chondrocytes apoptosis and phosphorylated p38.

Conclusions: We revealed that chondrocytes with mechanical stress and heat stress increased phosphorylated p38 the same as OA chondrocytes. From these results, to reveal the relationship between phosphorylated p38 and chondrocytes apoptosis could be one of the key to elucidate OA etiology.

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THE RESPONSE OF BOVINE ARTICULAR CARTILAGE TO CYCLIC LOADING IN VITRO

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Purpose: Articular cartilage protects the joint by distributing applied loads and providing a low-friction bearing surface to enable free movement. Physiological loading is repetitive and it has been previously shown that both human and animal chondrocytes are sensitive to idealised cyclic loading in vitro. Details of the mechanisms remain unclear but it has been suggested that members of the mitogen activated protein kinase (MAPK) family and fibroblast growth factor (FGF) family of proteins play a role. Our goal was to explore the effect of cyclic loading on bovine articular cartilage in vitro, in order to explore the mechanisms by which mechanical loading influences chondrocyte behaviour.

Methods: Full-depth articular cartilage explants (5 mm diameter) were freshly removed from bovine carpometacarpal joints (n=3). The explants were collected in PBS and cultured for 48 hours in DMEM. Following this, explants in 1 ml of culture medium were subjected to 1 hour or 4 hours of cyclic loading (1 MPa; 2 s on/2 s off) using a custom built, pneumatically driven device. Immediately after loading, the medium was collected and the explants were frozen in liquid nitrogen; both were stored at -20 °C until required. Frozen cartilage explants from each treatment group were combined (65-140 mg), freezer milled into a fine powder and lysed in RIPA buffer for protein extraction. In the culture medium, glycosaminoglycan (GAG) release was measured using the DMMB assay and levels of nitric oxide (NO) assayed using the Greiss reaction. In addition, vascular endothelial growth factor (VEGF) expression was analysed using an ELISA kit. Total protein in the lysate was measured using the BCA assay and western blotting used to identify levels of pERK, ERK, FGF2, FGF18, FGFR3, MMP13, WNT16 and actin in the pooled explants.