Regarding galectin mRNA levels in human chondrocytes (n=8), we found expression in the order: LGALS1 (0.36±0.13) > LGALS3 (0.18±0.06) > LGALS8 (0.09±0.03) > LGALS9 (LGALS7 < 0.00004).

**Conclusions:** Here, we have portrayed for the first time the complete glycome of human chondrocytes out of minute amounts of cultured cells (100,000 cells). IL-1β and TNF-α induce a range of specific alterations in chondrocyte glycopolymers supporting the hypothesis that the chondrocyte glyobiology might be of relevance for malfunctioning cell-cell interactions or altered galectin function under disease conditions.

### Role of K(ATP) Channels in Modulating GLUT-1 Content in Normal and Osteoarthritic Human Chondrocytes

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**Purpose:** Our previous studies showed that human chondrocytes adjust their glucose transport capacity and facilitative glucose transporter (GLUT)-1 content in response to high and low extracellular glucose concentrations. OA chondrocytes, however, fail to downregulate GLUT-1 content and glucose transport when exposed to high extracellular glucose concentrations. The consequent intracellular glucose accumulation causes prolonged oxidative stress which can compromise chondrocyte functions. The mechanisms by which chondrocytes sense the extracellular glucose concentration and subsequently adjust GLUT-1 content are unknown. Functional ATP-sensitive potassium [K(ATP)] channels were demonstrated in equine chondrocytes and in other cells were shown to play an important role as metabolic sensors. In pancreatic β cells, high extracellular glucose causes these channels to close, increasing the intracellular calcium concentration and insulin secretion. Thus, we hypothesized that K(ATP) channels may function as metabolic sensors in chondrocytes, generating the signals that lead to adjustment of GLUT-1 content. To test this hypothesis, we evaluated the ability of specific blockers (glibenclamide) and openers (levcromakalin) of the K(ATP) channels to close, increasing the intracellular calcium concentration and insulin secretion.

**Methods:** Normal (N=5, 31–59 years old, mean=44) and OA (N=6, 60–72 years old, mean=65) human chondrocytes were obtained from multi-organ donors or patients undergoing total knee replacement surgery, respectively, at the University Hospitals of Coimbra. Non-proliferating chondrocyte cultures were treated with glibenclamide or levcromakalin for 18h. GLUT-1 content and actin levels were evaluated in total cell extracts by western blot.

**Results:** No significant difference was found between basal GLUT-1 content in normal and OA chondrocytes. Treatment of normal chondrocytes with 10, 20 and 100 nM glibenclamide significantly decreased GLUT-1 content (61.8±10.1%, 57.9±11.3% and 61.2±7.9%, respectively) while 20 μM (112.2±7.3%) had no effect, relatively to untreated cells. In the OA group, however, none of the glibenclamide concentrations tested significantly affected GLUT-1 content. Treatment with 0.5 μM levcromakalin had no significant effect on GLUT-1 content either in normal (96±24.8%) or OA (108±20.4%) chondrocytes.

**Conclusions:** These results suggest that closure of K(ATP) channels is involved in GLUT-1 downregulation in human chondrocytes, consequently modulating glucose transport. In OA chondrocytes, however, this process seems to be impaired and thus may compromise their ability to adjust glucose transport to metabolic needs.

### Human Osteoarthritic Chondrocytes Express the Calcitonin Receptor

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**Purpose:** A growing body of evidence suggests that calcitonin (CT) has a direct chondroprotective effect against degenerative joint disease in articular cartilage. The expression of the calcitonin receptor by chondrocytes is currently a matter of debate. In this study, we sought to test the hypothesis that human chondrocytes do express the calcitonin receptor.

**Methods:** We worked with fresh primary chondrocyte samples isolated from arthroplasty cartilage specimens dissected from osteoarthritic patients. We established a dedicated reverse transcription polymerase chain reaction (RT-PCR) protocol followed by a nested PCR assay aiming at amplifying the full coding region of the calcitonin receptor mRNA (CALCR). We optimized a western blotting protocol for characterizing calcitonin receptor protein (CTR) immunoreactivity in chondrocyte samples using human osteoestlasts as positive controls. We validated an immunochemical procedure for localizing CTR in both primary and in situ chondrocytes.

**Results:** We amplified and sequenced four different allelic variants of CALCR in four patients. We detected a protein reacting against different CTR antibodies in four other patients. The molecular weight of the chondrocyte protein corresponds to that expressed by human osteoestlasts. The plasma membrane of primary chondrocytes was intensely stained with one of the CTR antibodies validated by western blotting.

**Conclusions:** Human articular cartilage chondrocytes express both the mRNA and protein molecules of the calcitonin receptor. These findings signal the calcitonin receptor as a new pharmacological target in osteoarthritis treatment.

### Leptin Produced by Joint White Adipose Tissue Induces Cartilage Degradation by Up-Regulation and Activation of Matrix Metalloproteinases

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**Purpose:** investigate the effect of leptin on cartilage destruction in osteoarthritis.

**Methods:** The release of proteoglycan and collagen was assessed in bovine cartilage explant cultures, while collagenolytic activities and gelatinolytic activity in culture supernates were determined by bioassay and gelatin zymography. The expression and production of MMPs was analyzed by real-time reverse transcription polymerase chain reaction (RT-PCR). Signalling pathway activation was studied by immunoblotting. Levels of leptin in the supernatants from cultured osteoarthritic (OA) joint infrapatellar fat pad was measured by ELISA.

**Results:** Leptin significantly induced collagen release from bovine cartilage, by up-regulation of collagenolytic and gelatinolytic activity. When added in combination with IL-1, a marked synergistic and a dose-dependent increase in matrix collagen release and enzyme activity was observed. In cultured chondrocytes, leptin induced MMP-1 and MMP-13 expression with a concomitant activation STATs – I.3, -5, MAPK (JNK, Erk, p38), Akt and NFκB pathways. Blockade of PI3K, P38, Erk and AKT pathway with selective inhibitors significantly reduced MMP-1 and MMP-13 expression in chondrocytes, and reduced collagen release induced by leptin or leptin + IL-1 in bovine cartilage. Furthermore, we found that supernatants from cultured OA joint infrapatellar fat pad contained leptin and can significant induce cartilage proteoglycan and collagen release from cartilage, and increase MMP-1 and MMP-13 expression chondrocytes. An anti-leptin antibody was able to partially suppress the effects of the fat conditioned media with respect to the induction of the collagenases by human chondrocytes.

**Conclusions:** This study demonstrates that leptin acts as a pro-inflammatory cytokine with a catabolic role on cartilage metabolism via the up-regulation and activation of proteolytic enzymes via PI3K, Akt, Erk and STATs signalling pathways. Moreover, our data suggests that the infrapatellar fat pad in arthritic joints is a local producer of leptin which may contribute to inflammatory and degenerative processes in cartilage metabolism, providing a mechanistic link between obesity and OA.