effects of short and long-term exposure of leptin on both normal and osteoarthritic chondrocyte metabolism.

Methods: Human normal and OA cartilage tissues were obtained following autopsy or joint surgery respectively. Chondrocytes were isolated from cartilage by enzymatic digestion and resuspended in alginate beads at 2 × 10^6/ml. Chondrocytes were permitted to stabilize in 5% FBS for 72 hrs, and transferred to serum free media supplemented with "mini-ITS" for 3 days prior to experimental treatments. [35S] was added for the final 18 hr of culture and sulphate incorporation was measured by liquid scintillation. Cytokine release in culture supernatants was measured using a custom multiplex bead immunoassay.

Results: Short-term leptin exposure resulted in an increase in proteoglycan synthesis in normal cartilage, and no change in response from OA cartilage. Continued treatment with leptin induced a decrease in proteoglycan synthesis from normal and OA cartilage. Only modest increases in cytokine production were detected from normal chondrocytes following leptin treatment and varied among donors.

Conclusions: Prolonged exposure to leptin induces a catabolic response in both normal and OA cartilage, independent of cytokine production. These observations indicate that the chronic increased levels of leptin present in obesity may contribute to the development and progression of OA. Future studies are planned to determine if inhibition of leptin signaling provides a protective effect on cartilage.

![Graph showing the effect of leptin on normal chondrocyte proteoglycan synthesis and cytokine production.](image)

Normal chondrocytes, 2 day treatment.

Normal chondrocytes, 4 day treatment.

**RECIROCAL REGULATION OF ADAMTS BY IL-1 AND TGF-β IN CHONDROCYTES: MODULATION BY SELECTIVE PPAR AGONISTS**

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Purpose: Recent findings suggest that ADAMTS (A Desintegrin And Metalloprotease with Thrombospondin motifs) play a key role in cartilage destruction in osteoarthritis (OA) and many ADAMTS are able to cleave aggrecan. IL-1 and TGF-β, which contribute to OA by promoting cartilage degradation and osteophytosis respectively, are key modulators of some ADAMTS. Peroxisome Proliferators-Activated Receptors (PPAR) are nuclear transcription factors able to suppress IL-1-induced inflammatory responses as well as TGF-β-induced synthesis of extracellular matrix components in joint cells. To investigate the regulation of several ADAMTS by IL-1 and TGF-β, we used alone or in combination, in rat or human OA chondrocytes and to determine the modulating potency of selective PPAR agonists.

Methods: Rat and human OA chondrocytes cultured as monolayers were stimulated with 10 ng/ml of recombinant homolog IL-1 or 10 ng/ml of recombinant TGF-β used alone or as costimulators. Levels of mRNA for ADAMTS-1, -4, -5, -8, -9, and -15 were assessed by real-time quantitative PCR. Aggrecanase activity was measured by a commercial enzyme-linked immunosorbent ELISA-based kit (InvLISA®, Invitrogen). In some experiments, selective PPAR agonists, Wy14643 (100 μM) or GW7647 (250 μM) for PPARγ, GW501516 (100 μM) for PPARα, pioglitazone (Pio, 30 μM) or rosiglitazone (Rosi, 10 μM) for PPARβ/δ, were added to culture medium 2 hours before cytokine stimulation. These concentrations were shown previously to activate PPAR-target genes in a subtype selective manner in rat chondrocytes.

Results: A preliminary experiment showed that the inducing effect of IL-1 on ADAMTS-4 expression was higher in human chondrocytes obtained from monolayers than from alginate beads. In human chondrocytes, IL-1 reduced ADAMTS-1 and -5 mRNA levels by 3.8- & 3.3-fold respectively, increased ADAMTS-4 and -9 mRNA level by 175- & 1.9-fold, without affecting ADAMTS-5. In contrast, IL-1 increased ADAMTS-1, -5 and -8 mRNA levels by 12.4-, 5.9- & 90-fold respectively, without modifying ADAMTS-4, -9 and -15 expression in rat chondrocytes. In human chondrocytes TGF-β increased ADAMTS-4 mRNA level by 20-fold but decreased ADAMTS-1, -5, -9 and -15 mRNAs by 15-, 1.9-, 4.5- and 3.8-fold respectively. In contrast, TGF-β increased ADAMTS-4 mRNA level by 16.4-fold and decreased ADAMTS-9 and -15 mRNAs by 3- and 3.5-fold respectively without affecting ADAMTS-1, -4 and -9 expression in rat chondrocytes. When added together, TGF-β potentiated the inhibitory effect of IL-1 on ADAMTS-1 expression and its stimulating effect on ADAMTS-4 expression in human chondrocytes. In contrast, TGF-β counteracted the stimulating effect of IL-1 on ADAMTS-9 and its inhibitory potency on ADAMTS-15. In rat chondrocytes, TGF-β counteracted the stimulating effect of IL-1 on ADAMTS-1, -5 and -8 mRNA levels. In human chondrocytes, the global aggrecanase activity was increased by IL-1, decreased by TGF-β which was also able to antagonize IL-1 effect. When added before IL-1 or TGF-β stimulation, PPAR agonists failed to modulate ADAMTS mRNA levels in human chondrocytes as well as IL-1-induced changes in ADAMTS expression in rat chondrocytes. However, Pio and Rosi decreased the basal expression of ADAMTS-5 in human chondrocytes but this did not translate into significant reduction of aggrecanase activity.

Conclusions: Our data show that: (i) the regulation of ADAMTS expression by IL-1 and TGF-β and IL-1 differed between rat and human chondrocytes; (ii) the effect of IL-1 depended on the ADAMTS considered whereas TGF-β was globally inhibitory; (iii) TGF-β counteracted the stimulating effect of IL-1 on ADAMTS and aggrecanase activity; (iv) PPAR agonists were inactive on IL-1 or TGF-β-induced changes in ADAMTS.