expression in response to IL-1β was similar to free-swelling culture, with a peak in expression at 6h. However, the magnitude of expression was greater than under free swelling conditions, with 145 fold increase in levels. Dynamic compression or the presence of SB203580 in unstrained constructs reduced but did not abolish the IL-1β induced iNOS expression at 6h (59 fold and 40 fold, respectively). However, the addition of SB203580 in combination with dynamic compression resulted in a further reduction in the IL-1β induced iNOS expression (8.9 fold). IL-1β increased COX-2 expression which peaked at 12h in unstrained constructs (44 fold). The application of dynamic compression or presence of SB203580 completely abolished the IL-1β induced COX-2 expression (2.4 fold and 1.3 fold, respectively). Co-stimulation with both dynamic compression and the inhibitor had no further effect when compared to each in isolation. In the absence of the cytokine, dynamic compression enhanced aggrecan levels at 12h (2.4 fold). This effect was reduced by IL-1β and restored with both dynamic compression and the inhibitor. By contrast, the expression levels of collagen type II were not influenced by IL-1β and/or SB203580 in unstrained or strained constructs.
Conclusions: These results suggest that dynamic compression directly influences the expression levels of iNOS and COX-2 and are primarily mediated by a p38 MAPK dependent pathway. These enzymes are current targets for pharmacological intervention, raising the possibility for integrated pharmacological and biophysical therapies for the treatment of cartilage joint disorders.

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BIOMECHANICAL SIGNALS BLOCK IKK ACTIVATION TO INHIBIT NF-κB-MEDIATED PROINFLAMMATORY GENE TRANSCRIPTION IN ARTICULAR CHONDROCYTES

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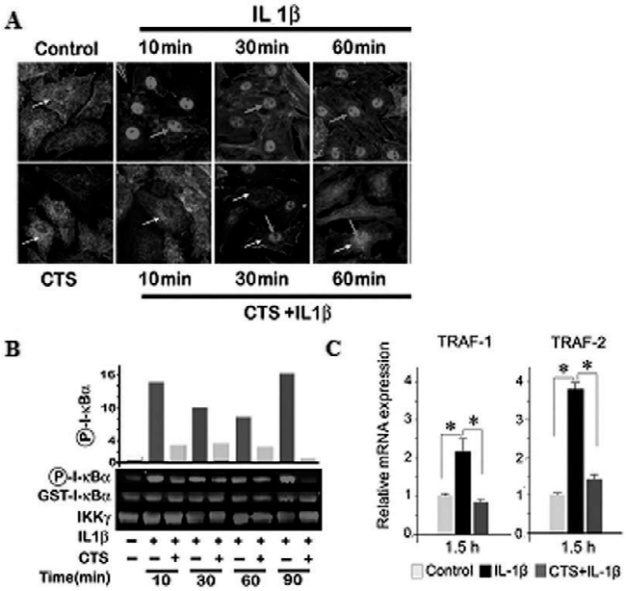
Purpose: Therapeutic exercise is beneficial for the arthritic joints. But how this mobilization helps the joints at molecular level has been an enigma for many years. Recently, signals generated by mechanical strain are shown to inhibit proinflammatory gene induction in chondrocytes and inflammation of joints afflicted with antigen-induced arthritis. In this study to understand the molecular basis of exercise mediated effects, we dissected the NF-kB pathway to identify molecules that are regulated by biomechanical signals in chondrocytes.
Methods: *Cell culture:* Articular chondrocytes from the knees of 10-12-week-old Sprague-Dawley rats, were cultured in Ham/F12, 10% FBS, pen/strep and 2mM L-glutamine, and used during the first 3 passages. *Application of CTS:* Cells (7 X 10^4/well) grown on collagen I-coated BioFlex-I culture plates to 80% confluence were subjected to cyclic tensile strain (CTS; 3% strain at 0.25 Hz) in a Flexcell loading station. Four different treatment regimens were: i) untreated controls, ii) cells treated with IL-1β, iii) cells treated with CTS, iv) cells treated with CTS and IL-1β. *Real time PCR*: PCR was performed using Taqman primers and probes. *Western Blot analysis and Immunofluorescence:* The blots were probed with anti-IκB-α, anti-IκB-β (Santa Cruz Biotech, CA), and phospho-IκBα Ser32, and Ser 36, phospho-NF-κB p65 Ser536, and Ser276 antibodies and their respective secondary antibodies. Alternatively, activation of NF-κB, IκB-α and IκB-β was assessed by immunofluorescence using above antibodies and visualized under an epifluorescence microscope (Zeiss Axioimage). *Statistical analysis:* One-Way ANOVA and the post hoc multiple comparison Dunnett's test were applied to determine the significance.
Results: 1) IL-1β induced a rapid nuclear translocation of NF-κB at all the time points tested (Fig. A), whereas CTS inhibited this IL-1β induced nuclear translocation at 30, 60 and 90 minutes. 2) Inhibition of NF-κB nuclear translocation by CTS was paralleled by a significant suppression of iNOS mRNA expression. 3) CTS blocked mRNA expression for I-κBα that is upregulated by IL-1β at 10, 30, 60 and 90 minutes. Western blots and immunofluoresence revealed that IL-1β induced rapid degradation of IκB-α at 10 and 30 minutes, and upregulation of its mRNA expression. CTS inhibited IκB-α degradation at 10 and 30 minutes and an inhibition of resynthesis at 60 and 90 minutes. 4) IL-1β significantly induced the degradation of I-κBβ protein synthesis at all the time points tested and this degradation was inhibited by CTS. 4) Event upstream of I-κBα showed that CTS inhibited IL-1β-induced TAK-1 activation and resulting IKK-beta activation (Fig. B). 5) Furthermore, CTS downregulated NF-κB dependent genes IL-R1, IL-R2, TRAF-1 and TRAF-2 (Fig. C).



A. CTS mediated inhibition of IL-1β-induced NF-κB nuclear translocation. B. CTS mediated inhibition of IL-1β-induced IKKβ activation and I-κBα phosphorylation. C. CTS mediated inhibition of IL-1β-induced TRAF-1 and TRAF-2 mRNA expression.

Conclusions: The results demonstrate that mechanical signals inhibit IL-1β induced proinflammatory gene transcription by regulating activation of IKK-β to inhibit NF-κB transactivation. Thus, the beneficial effects of physiological levels of biomechanical signals or exercise may be explained by their ability to suppress the signal transduction pathways of proinflammatory/catabolic mediators. Whether these signals are also anabolic is yet to be elucidated. Regardless, biomechanical forces generate complex magnitude- and frequency-dependent signals within the inflammatory microenvironment of the cartilage that ultimately allow the remarkable and beneficial effects that are realized at tissue level.
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CHANGE OF HUMAN MENISCI IN OSTEOARTHRITIS

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Purpose: Menisci in knee joints undergo obvious degenerative changes in osteoarthritis (OA), but the details of the changes are still not known well. This study was performed to investigate the change of menisci in OA by histological and molecular biological evaluations.
Methods: This study was performed under the approval of in-