INTRODUCTION
The beneficial effects of continuous passive motion on arthritic knees are well recognized, and signals generated by mechanical strain are shown to inhibit inflammation of joints afflicted with antigen-induced arthritis (1). The anti-inflammatory effects of biomechanical signals are mediated by the inhibition of the nuclear translocation of NF-κB in vivo (2), which subsequently leads to a significant inhibition of the transcription and translation of pro-inflammatory genes. In this study we examined the target molecules in the NF-κB pathway that are responsible for the inhibition of NF-κB nuclear translocation in a pro-inflammatory environment.

METHODS
Cell culture: Chondrocytes were obtained from the articular cartilage from the knees of 10-12-week-old Sprague-Dawley rats. Cells were released from the cartilage by enzymatic digestion, cultured in TC [Ham/F12, 10% FBS, 10 μg/ml penicillin and 100 U/ml streptomycin, and 1% L-glutamine], and used during the first 3 passages. During these passages the chondrocytic phenotype (aggrecan, biglycan, versican and collagen type II) was found to be stable.

Application of CTS: Cells (7 X 10^4) were grown for 5-6 days on collagen I-coated BioFlex-I culture plates to 80% confluence and subjected to cyclic tensile strain (CTS) in a Flexcell loading station (Flexcell Int, NC) at a magnitude of 3% and frequency of 0.25 Hz. Four different treatment regimens were assigned: i) untreated controls, ii) cells treated with recombinant human IL-1β, iii) cells treated with CTS, iv) cells treated with CTS and with rhIL-1β. The cells were harvested following a 10, 30, 60 and 90 minutes exposure to all four regimens.

Real time PCR: PCR was performed on a Biorad iCycler iQ (Biorad, CA). Taqman primers and probes were used and the data was analyzed using the comparative threshold cycle (CT) method.

Western Blot analysis: The blots were probed with anti-IκBα, anti-IκBβ (Santa Cruz Biotech, CA), and phospho-IκBα Ser32, and Ser36, phospho-NF-κB p65 Ser536, and Ser276 antibodies. The binding of primary antibodies was revealed with a horseradish peroxidase (HRP)–conjugated secondary antibodies. The bands were visualized with Western Lightening chemiluminescence reagent used as a substrate for HRP. The blots were exposed to Blue Lite Autorad Film and assessed by densitometric analysis using Kodak Image Station 1000, and image J software.

Immunofluorescence: Activation of NF-κB, IκBα and IκBβ was assessed by immunofluorescence using rabbit anti-NF-κB p65, anti-IκBα, or anti-IκBβ IgG as primary antibodies and Cy3-conjugated goat anti-rabbit IgG as secondary antibody. Phalloidin-FITC was used as a counter-stain to visualize F-actin. The stained cells were visualized under an epifluorescence microscope (Zeiss Axiosimage), and densitometrically analyzed by Zeiss Axiovision software (Carl Zeiss, Germany).

Statistical analysis: All experiments were done three times, and means ± S.E.M. were calculated. One-Way ANOVA and the post hoc multiple comparison Dunnett’s test were applied to determine whether significant differences exist between the groups. Values of p ≤ 0.05 were considered to be significant.

RESULTS
1) IL-1β induced a rapid nuclear translocation of NF-κB at all the time points tested, whereas CTS inhibited this IL-1β induced nuclear translocation at 30, 60 and 90 minutes (Fig.1). 2) Inhibition of NF-κB nuclear translocation by CTS was paralleled by a significant suppression of more than 90% of the IL-1β-induced iNOS mRNA expression during the initial 90 minutes. 3) CTS blocked mRNA expression for IκBα that is upregulated by the treatment of chondrocytes with IL-1β at 10, 30, 60 and 90 minutes. Examination of the IκBα protein by Western blots and immunofluorescence revealed that IL-1β induced rapid degradation of IκBα at 10 and 30 minutes, accompanied by its resynthesis at 60 and 90 minutes. Exposure to CTS resulted in an inhibition of IκBα degradation at 10 and 30 minutes and an inhibition of resynthesis at 60 and 90 minutes. 4) IL-1β significantly induced the degradation of IκBβ protein synthesis at all the time points tested and this degradation was inhibited by CTS at 30, 60 and 90 minutes. 4) Examination of the NF-κB dependent genes IL-R1, IL-R2, TRAF-1 and TRAF-2 revealed that IL-1β markedly increased the expression of these genes, while CTS significantly downregulated their expression.

DISCUSSION
The results demonstrate that mechanical signals inhibit IL-1β induced inflammatory gene transcription by regulating the early events upstream of NF-κB nuclear translocation. Mechanical signals inhibit IL-1-induced inflammatory responses by both, blocking the activation of inhibitors of NF-κB, IκBα, IκBβ, and thus NF-κB. Furthermore, inhibition of nuclear translocation of NF-κB results in the Inhibition of NF-κB, IκBα, and IκBβ mRNA expression and synthesis. Thus, mechanical signals regulate IκB degradation at earlier time points and failure to inhibit resynthesis at later time points. Thus, by blocking NF-κB pathway at the site of IκB activation, mechanical signals inhibit proinflammatory effects of IL-1β and help in avoiding tissue degradation induced by proinflammatory genes. [This study was supported by grants from NIH AT00646, AR04878, NIDCR DE15399]

References: