INTRODUCTION
Matrix remodeling in articular cartilage occurs by the elevation and activation of aggrecanases (ADAMTS-4 and ADAMTS-5) and matrix metalloproteinases (MMPs) [2–4, 7–9, 10]. Recent studies from our and other groups have shown that mechanical loading can counteract interleukin 1 (IL-1) induced pro-inflammatory and catabolic events by down-regulating aggrecanases, MMPs, and pro-inflammatory genes [1, 3, 5, 6], but the molecular mechanism is not clear. Potential mediators that counteract the pro-inflammatory effects of IL-1 include anti-inflammatory cytokines (TGF-β, IL-10, IL-6 and interferon γ), IL-1 receptor related proteins (IL-1Rα, IL-1R1, IL-1R2), and the intracellular inhibitory proteins called Suppressor Of Cytokine Signaling (SOCS) SOCS1 and SOCS3 are especially important, since they can inhibit both MAPK and NF-κB pathways induced by IL-1 [12]. The objective of this study was to determine whether mechanical load affected anti-inflammatory mediators along with anti-catabolic events.

MATERIALS AND METHODS
Cartilage explants, harvested from the trochlear groove of mature (>18 month) bovine knees using a 7-mm diameter biopsy punch, were cultured in serum-free DMEM for 72 hours. The explants were divided into four groups: Control, IL-1 (10ng/ml IL-1), Load and IL-1+Load. Explants in the Load and IL-1+Load groups received cyclic confined compression at 1.0MPa for 24 hours, as previously described [4,11]. Culture medium was collected at day 0 and day 1 after treatments for analyzing PG release. Explants were immediately snap-frozen in liquid nitrogen and stored at -80°C prior to RNA isolation. Briefly, cartilage explants were homogenized and RNA was extracted using tri-reagent (Sigma) and RNeasy mini kit (Qiagen) [4]. Equal amounts of total RNA were reverse transcribed into cDNA (iScript, BioRad). Real time PCR was performed using a MyiQ Real time PCR machine (BioRad), SYBR Green supermix (BioRad), as well as the primers for GAPDH, COL2A1 (type II collagen) AGG (aggrecan), anti-inflammatory cytokines (TGF-β, IL-10, IL-6, and IFN-γ), IL-1 receptor related (IL1R1, IL1R2, IL1Ra), and intracellular inhibitory proteins (SOCS1 and SOCS3). Specific primers were designed according to previously published sequences of each target gene. The relative mRNA expression of each gene was normalized to the expression of the housekeeping gene GAPDH. Proteoglycan content in the culture medium was determined using DMMB assay. The effects of the treatment were evaluated by one-way ANOVA or Student’s t-test using Excel (Microsoft) or Systat (10.2, SPSS). P-value < 0.05 was considered significant.

RESULTS
A significant increase of PG content was found in the IL-1 group while no changes in PG content were found in the Load and IL-1+Load groups (Fig. 1). Similar to our previous finding at 0.5 MPa [4], the overexpression of ADAMTS4 and ADAMTS5 induced by IL-1 was abrogated by mechanical load at 1.0 MPa where TIMP-3 was upregulated (Fig. 1). There was a downregulation of aggrecan gene (AGG) by IL-1, which was overcome by mechanical load (Fig. 3.) Although loading has no effect on IL-1R2, it significantly downregulated the expression of IL-1R1. Since IL-1 receptor 1 is the major receptor for IL-1, this suggested that the downregulation of IL-1 receptor 1 may play a role in the inhibitory effect of load. Load, however, downregulated IL-1 receptor antagonist (Fig. 2) and had no effect on TGF-β and IL-10 (Fig. 3). This suggests that IL-1Rα, TGF-β and IL-10 may not be involved in the anti-catabolic mechanism of load. We also found that load inhibited IL-1 induced IL-6 overexpression (Fig. 3). Of particular interest, we found that IL-1 had no effect on IFN-γ and SOCS3 expression while load highly upregulated the expression of IFN-γ (5.4-7.1 folds) and SOCS3 (10-12.6 fold) in the absence and presence of IL-1 (Fig. 3). This contrasted to the upregulation of SOCS1 by IL-1 in the absence or presence of load.

DISCUSSION
Our findings suggest that mechanical load at 1.0MPa can abrogate IL-1 induce degradative events similar to 0.5MPa. Among all inhibitory cytokines, we found that IFN-γ was the only one highly upregulated by mechanical load in the presence or absence of IL-1 along with an upregulation of SOCS3. Since IFN-γ can induce SOCS3 and SOCS1, both of which are the major intracellular proteins for both MAPK and NF-κB pathways, our findings suggest that IFN-γ is the main extracellular mediator for the inhibitory effect of load while IL-1R1 may play a minor role.

REFERENCES

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