Nitric oxide (NO) is a multifunctional cellular messenger molecule that coordinates diverse physiological processes and is also an intraarticular free radical associated with the development of inflammatory or degenerative arthritis. Articular chondrocytes release NO in response to a variety of stimuli such as IL-1, TNF-α, INF-γ, endotoxin and shear stress. The effects of NO on chondrocyte metabolism down-regulates the synthesis of proteoglycan and enhances the catabolism of cartilage matrix proteins through matrix metalloprotease activities. In addition, NO can also increase the synthesis of prostaglandin E₂ which in turn can mediate local inflammatory responses and influence skeletal responses to mechanical loading. Mechanical stimuli provide cues for the appropriate differentiation and maintenance of cartilage homeostasis throughout cartilage development and after maturation. In tissue loading models and in vitro studies, intermittent hydrostatic pressure inhibits NO synthesis and degeneration of articular cartilage and enhances cartilage matrix synthesis. This study tested the hypothesis that intermittent hydrostatic pressure counteracts inhibitory effects of NO on chondrocyte matrix protein mRNA expression.

**Methods** Human osteoarthritic chondrocytes from patients receiving total knee replacement for osteoarthritis were plated at high density. Monolayer cultures of cells were subjected to the induction of NO-mediated responses by a NO donor (sodium nitroprusside 20 μM or 2000 μM), bacterial E. coli lipopolysaccharide (LPS, 1 μg/ml), and fluid-induced shear stress (1.64 Pa) in serum free Dulbecco's modified Eagle medium containing 25 μg/ml gentamicin, 50 μg/ml ascorbic acid, 1 mM selenium, and liposomes. Intermittent hydrostatic pressure (10 MPa) was applied at the frequency of 1 Hz as a sinusoidal wave-form was applied to the cells by a servohydraulic MTS machine for 4 hours. The cells were incubated under the identical culture conditions for 24 hours for the assessment of post-loading cellular responses. NO levels were monitored by analyzing nitrite, the stable end product of NO synthesis, in culture medium using Griess reagents. Cartilage matrix mRNA levels were analyzed by reverse transcription polymerase chain reaction (RT-PCR).

**Results** Exposure to intermittent hydrostatic pressure decreased the NO levels from 12 to 8 μM and from 46 to 33 μM, in 20 μM and 2000 μM NO donor-treated chondrocytes, respectively (ANOVA p<0.01). Intermittent hydrostatic pressure also inhibited LPS-induced NO levels to 53% of the unloaded controls (ANOVA p<0.001). In shear stress-preconditioned chondrocytes, NO release was decreased to 64% of the original by intermittent hydrostatic pressure (ANOVA p<0.05) (Fig. 1). The effect of intermittent hydrostatic pressure on signal levels of cartilage matrix mRNA in the NO donor-treated chondrocytes is illustrated. (Fig. 2) The NO donor at 20 μM inhibited type II collagen and aggrecan mRNA by an average of 14% and at 2000 μM by an average of 24%. Application of intermittent hydrostatic pressure to the 20 μM NO donor-treated cells resulted in the upregulation of type II collagen and aggrecan mRNA (ANOVA p<0.005). However, in the 2000 μM NO donor groups, application of intermittent hydrostatic pressure counteracted the downregulating effect on aggrecan but not type II collagen mRNA expression (Fig. 3 & 4). In LPS-activated cells, type II collagen expression was downregulated by 67% and aggrecan expression was downregulated by 56% (Fig. 5) (ANOVA p<0.001). Application of intermittent hydrostatic pressure to LPS-activated cells resulted in an average of 1.7 fold increase in both type II collagen and aggrecan mRNA expression (Fig. 3 & 4) (ANOVA p<0.001). Shear stress (1.64 Pa) for 2 hours decreased signal levels of type II collagen mRNA by 27% and aggrecan mRNA by 30% (Fig. 3 & 4). However, the inhibition of matrix macromolecule gene expression in shear stress-preconditioned chondrocytes was not affected by the subsequent application of intermittent hydrostatic pressure.

**Discussion** In cartilage, NO may act as a localized or a widespread signaling messenger. It is unclear whether chondrocytes at different areas of a joint exposed to the same amount of synovial NO will respond differently. The data presented here shows that mechanical loading influences chondrocyte matrix gene expression by modulating NO levels. Intermittent hydrostatic pressure effectively counteracted the NO donor- and LPS-mediated down-regulation of cartilage matrix mRNA in chondrocytes treated with sodium nitroprusside or LPS. However, intermittent hydrostatic pressure failed to reestablish the shear stress-mediated down-regulation of cartilage matrix protein gene expression. An explanation for this behavior may be due to complex effects of shear stress on chondrocytes or that chondrocytes in monolayer cultures without extracellular matrix support require a longer time for recovery. These data suggest that intermittent hydrostatic pressure may function in vivo to down-regulate effects of nitric oxide on cartilage matrix metabolism.

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