

Intervertebral disc mechanobiology and the kinetics of gene expression

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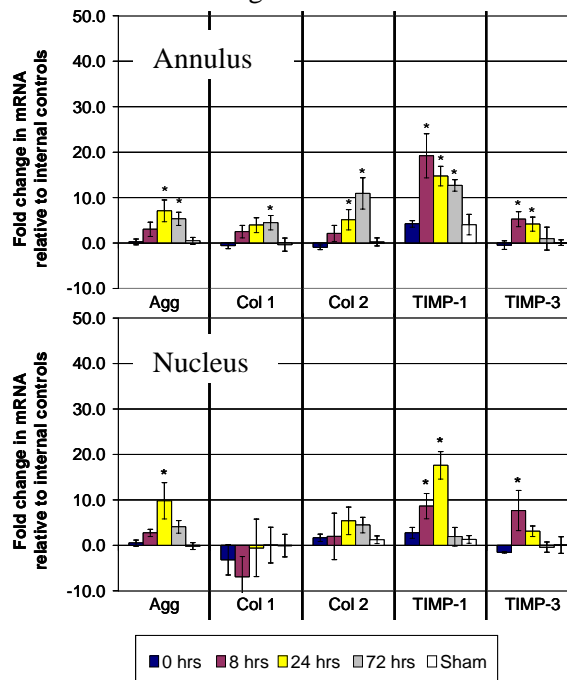
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Introduction: Disc degeneration may be accelerated through mechanical loading. In vivo studies on rodent models defined a clear relationship between mRNA expression and compression loading magnitude, frequency, and duration. It is unknown when changes in gene expression peak and for how long they remain altered following loading. Defining the kinetics of gene expression in response to mechanical loading is a research priority, represents a large gap in the spine literature, and has clinical implications related to how frequently strenuous activity may be repeated. The purpose of this study was to investigate kinetics of gene expression by recording mRNA levels 0, 8, 24 and 72 hours after a single loading event.

Methods: This study was approved by the University of Vermont IACUC. Forty-nine skeletally mature Wistar rats (>12 weeks) were instrumented with an Ilizarov-type device spanning caudal disc 8-9. 72 hours after surgery rats were anesthetized and the c8-9 discs were subjected to 1.5 hours of loading at 1MPa and 1Hz. Shams (n=9) were subjected to 1.5 hours of anesthesia without loading. Animals in the loading groups were euthanized either immediately (0 hrs) or awakened and allowed to recover for 8, 24, or 72 hours (n=10 per group). The instrumented disc along with the proximal and distal control levels from each rat were harvested, and annulus (AF) and nucleus (NP) tissue separated. Control and loaded disc levels from all animals were dissected and annulus and nucleus tissue were separately analyzed by real-time RT-PCR for levels of rat-specific collagen 1A1, collagen 2A1, aggrecan, stromelysin (MMP3), collagenase-3 (MMP13), gelatinase (MMP2) aggrecanase-4 (ADAMTS-4), TIMP-1, and TIMP-3 mRNA. Each gene was normalized to 18S rRNA levels. ANOVA with post-hoc testing was performed.

Results: In the annulus, peak gene expression occurred after 8hrs in TIMP-1 and TIMP-3; after 24 hrs in agg, ADAMTS-4, and MMP-3; and after 72 hrs in Col-I, Col-II, and MMP-13. No change was observed in MMP-2 (Figure, catabolic results not shown). In the nucleus, maximum changes in gene expression occurred after 8hrs in Col-1 and TIMP-3; and after 24 hrs in aggrecan, TIMP-1,

ADAMTS-4, MMP-13, and MMP-3. No changes were observed in collagen-II or MMP-2.



Discussion: In the nucleus genes were generally up-regulated within 1.5 hours after initiation of compression, were significantly modified after 24 hours and were returning to sham levels by 72 hours. Annulus displayed similar patterns but message persisted longer than in the nucleus with several genes significantly up-regulated 72 hours following load cessation. The increased levels in the annulus at later time points could reflect a prolonged time of elevated expression or a slower rate of message degradation. TIMP-1 and TIMP-3 genes displayed the most rapid response to loading with peak levels occurring within 8 hours, indicating mechano-sensitivity of these regulatory genes. This in vivo study on the mRNA kinetics response to loading defined recovery times required for gene expression to return to baseline levels and may more precisely characterize healthy and risky loading conditions. This information is also useful for optimizing tissue harvest time and for determining the frequency of repeated loading episodes in chronic mechanobiology studies.

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