INTRODUCTION: Compressive loading of the intervertebral disc is associated with physical stimuli, such as altered osmotic pressure, strain and stress, that have been shown to regulate disc cell biosynthesis. In particular, static compressive loads have been shown to inhibit post-transcriptional measures of proteoglycan synthesis in vitro (1,2), and aggrecan gene expression in vivo (3). In general, cells of the anulus fibrosus (AF) do not respond to compression with the same magnitude of change in proteoglycan biosynthesis as observed in cells of the nucleus pulposus (NP), suggesting a zonal-specific cellular response to the altered physical stimuli. In this study, we examined compression-induced changes in gene expression for proteoglycans of the intervertebral disc (aggrecan, decorin, biglycan and lumican) in cells of the AF and NP loaded in alginate culture in vitro.

METHODS: Cell Culture. Cells were isolated from the AF and NP regions of intervertebral discs harvested from skeletally-immature porcine lumbar spines (4-5 months) (4). After one subculture, cells were suspended at a density of 2x10^7 cells/ml in cylindrical constructs of 2% crosslinked sodium alginate (d=8 mm, h=2 mm). Cell-alginate discs were incubated in OPTI-MEM I (GIBCO reduced serum medium) for 24 hours prior to loading. Static Compression. A tare strain of 1% compression was applied to all cell-alginate discs in a custom-built apparatus. In the experimental samples, an additional displacement corresponding to 25% compressive strain was applied for a period of 2, 18 or 30 hours. “Unloaded control” samples were cultured for the same time periods under the tare strain only (AF: n=4 each unloaded and compressed samples at each timepoint; NP: n=2). All discs were cultured in OPTI-MEM I media throughout the duration. Immediately after compression, cells were released from alginate and cell viability was tested by trypan blue dye exclusion. The cell pellet was then used for RNA isolation with RNeasy mini kit plus DNase I digestion (Qiagen). Cells were released from one set of discs at the start of the experiment, prior to any loading, for use as a “0 hour control.” Real-time Quantitative RT-PCR. mRNA of aggrecan, decorin, biglycan and lumican were quantified by real-time quantitative RT-PCR using comparative Ct method as described previously (5). mRNA levels (relative 18s rRNA as an internal control) under unloaded and compressed conditions at each time were normalized to that of the “0 hour control.” Statistical Analysis. Duplicate or 4 replicates of PCRs were performed for each target gene and 18s rRNA. Statistical analyses were performed to detect a difference in Ct values between compressed and unloaded samples at each timepoint using a one-factor ANOVA. For each time point, a particular gene was considered to be significantly regulated by compression if the fold-difference was greater than or equal to 2, and if ANOVA detected a statistically significant difference at p<0.05. RESULTS: There was no difference in cell viability or the yield of total RNA between compressed and unloaded samples for either NP or AF cells. In AF cells, mRNA levels for aggrecan, biglycan, decorin and lumican appeared to increase at 2 and 18 hours in both unloaded and compressed samples, compared to the 0 hour control (Figure A). The magnitude of this increase varied among genes, but was generally between 2 to 5-fold. This trend suggests an attempt of the AF cells to regenerate matrix for the first ~48 hours after being placed in the alginate gel. This trend reversed in AF cells at the 30 hour timepoint, although few differences in gene expression were noted at the earlier times between compressed and uncompressed samples. In contrast, cells of the NP were not responsive to culture conditions nor to compressive stimuli. Previous studies suggest that AF cells may not respond to compression with changes in proteoglycan gene expression (3) and post-translational biosynthesis (1,2) to the same extent as NP cells. The differences between results of the previous and current studies may reflect the presence of a large notochordal cell population in the immature NP of the porcine intervertebral disc studied here (6). Previous studies have demonstrated that NP cells from this tissue do not respond to either conditioned medium (7) or altered osmolarity (5) with changes in gene expression for relevant extracellular matrix proteins. The biological function of the notochordal cells in this population is unclear, although they remain biosynthetically active in the post-natal tissue (7). Together with these prior studies, the current study suggests that the biological response of NP cells to environmental stimuli and soluble mediators is severely restricted, as compared to neighboring, age-matched cells of the intervertebral disc. Furthermore, the results for compression-induced stimulation of proteoglycan gene expression in the AF cells suggest that compression may act to sustain biosynthesis in the fibrochondrocytes, but at longer times after loading.

DISCUSSION: The results of this study demonstrate that cells of the AF and NP have dramatically different responses to compressive stimuli in vitro. AF cells expressed high mRNA levels for all proteoglycans between 2 to 30 hours in the uncompressed constructs, compared to values at the 0 hour control. Compression served to sustain mRNA levels for all proteoglycans at high levels in the AF cells at the 30 hour timepoint, although few differences in gene expression were noted at the earlier times between compressed and uncompressed samples. In contrast, cells of the NP were not responsive to culture conditions nor to compressive stimuli. Previous studies suggest that AF cells may not respond to compression with changes in proteoglycan gene expression (3) and post-translational biosynthesis (1,2) to the same extent as NP cells. The differences between results of the previous and current studies may reflect the presence of a large notochordal cell population in the immature NP of the porcine intervertebral disc studied here (6). Previous studies have demonstrated that NP cells from this tissue do not respond to either conditioned medium (7) or altered osmolarity (5) with changes in gene expression for relevant extracellular matrix proteins. The biological function of the notochordal cells in this population is unclear, although they remain biosynthetically active in the post-natal tissue (7). Together with these prior studies, the current study suggests that the biological response of NP cells to environmental stimuli and soluble mediators is severely restricted, as compared to neighboring, age-matched cells of the intervertebral disc. Furthermore, the results for compression-induced stimulation of proteoglycan gene expression in the AF cells suggest that compression may act to sustain biosynthesis in the fibrochondrocytes, but at longer times after loading.


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FIGURES: Relative mRNA levels (fold-difference = 2^{ΔΔCt} ± SE) for AF and NP cells following static compression. mRNA levels normalized to 0 hour control values. (* >2-fold difference between compressed and control samples and p<0.05, ANOVA).