Introduction: Previous studies have independently shown intervertebral disc (IVD) cells respond to applied mechanical load (1) and to normal aging processes (2) with changes in the relative catabolic/anabolic gene expression profile, raising the question: Does loading affect young and mature IVD cells differently? Variations in ages and species of models used to study the biological response to loading, combined with differences in methodology used to apply dynamic loads (compression, hydrostatic forces) has left this important question unanswered.

This study addressed two aims. Aim 1 studied changes in mechanosensitivity of IVD cells with increasing age, hypothesizing that cells from older animals will be less sensitive to mechanical stimulation. Aim 2 determined the effects of varying frequencies on NP and AF cell behavior in 3D alginate culture, with the hypothesis that GAG content and nitric oxide production will increase with load frequency.

Materials and Methods: Intervertebral discs were removed from mature (18-24 mo.) and young (4-6 weeks) bovine tails. Nucleus pulposus (NP) and annulus fibrosus (AF) tissue were separated, enzymatically digested, and expanded at high cell density. Alginate gel constructs were created in 96 well plates using a slow set technique with cells seeded at a density of 4 million cells/ml. After curing, gels were placed into mechanical stimulation test dishes with culture medium (hg DMEM, 100 U/ml penicillin and streptomycin, 0.25 μg/ml fungizone, 10% FBS, 50 μg/ml ascorbic acid and 0.5% v/v 5M NaCl and 0.4M KCl to adjust medium osmolarity) and allowed to equilibrate overnight.

Mechanical stimulation consisted of 2 hours daily compressive strain from 2-12% for 7 days at one of three frequencies (0.1, 1 or 3 Hz) or unstimulated control (Table 1). Table 1: Experimental set-up (repeated on days 7, 14 and 21). Annulus fibrosus (AF) and nucleus pulposus (NP) cells were seeded into alginate and tested at two ages (rows) and four loading conditions (columns).

After 7 days of loading, gels were harvested for analysis or further cultured and harvested on days 14 and 21 of culture with media changes every 3-4 days.

Cell viability was checked using 1μM calcine-AM and 1μM ethidium homodimer-1. The remaining dissociated gel was centrifuged and the supernatant was carefully removed from the separated pellet and stored. Pellets and supernatants were digested using papain incubated at 60°C overnight and analyzed for DNA content (Picogreen) and GAG content (DMMB). Real time RT-PCR was performed on dissociated gels (n = 5 per group). After RNA isolation and cDNA transcription, primers for bovine aggrecan, collagen types I and II, MMP -3, -13 and TIMP -1 and -2 were analyzed using SYBR green. Resulting gene expression levels were normalized to the housekeeping gene GAPDH and the gene expression of IVD cells with increasing age, hypothesizing that cells from older animals will be less sensitive to mechanical stimulation. Aim 2 determined the effects of varying frequencies on NP and AF cell behavior in 3D alginate culture, with the hypothesis that GAG content and nitric oxide production will increase with load frequency.

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Results: Viability was maintained throughout the experiment. Total GAG tended to increase with time, however no effect was observed with increased frequencies of mechanical loading. In general, GAG per DNA was higher per cell for NP versus AF constructs.

Mechanical stimulation upregulated gene expression for aggrecan, collagen I and II in the mature NP and for MMP-3 in the young NP (Figure 1).

Discussion: Effects of animal age and loading frequency on extracellular matrix production of isolated IVD cells in 3D gel culture were examined. Young and mature IVD cells remained viable and mechanically responsive in 3D alginate culture. Aging affected the response to mechanical loading, with load-induced upregulation of anabolic genes in mature NP cells, and load-induced downregulation of catabolic gene expression in young NP cells. Therefore, contrary to hypothesis 1, increasing age did not diminish the mechanosensory response of IVD cells, but rather it did change the relative anabolic and catabolic response to loading. Overall, age effects dominated the effects of loading, with few effects of load frequency noted, contrasting hypothesis 2.

Results suggest aging plays an important role in IVD homeostasis and also influences the cell response to externally applied stimulation by mechanical loading.


Acknowledgements: NIH Grant (R01AR051146) and the Intramural Research Program of the NIH, National Institute of Arthritis and Musculoskeletal and Skin Diseases.