

## Effect of Limited Nutrition on Intervertebral Disc Cells Under “Physiological” Loading – A 21 Day Culture

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**INTRODUCTION:** The underlying causes of disc degeneration are multifactorial. Besides genetic factors and aging, limited nutrition is generally believed to be an etiological factor [1]. Although this effect has been demonstrated in cell culture, no investigations have been reported in actual discs. Recently; a novel in vitro system was developed for culturing whole intervertebral disc (IVD) explants with natural controlled loading through intact endplates (EPs). In this study, the mid term (21 days) response of disc cells, in their native extra cellular matrix (ECM), to nutritional challenge and “physiological” load was investigated.

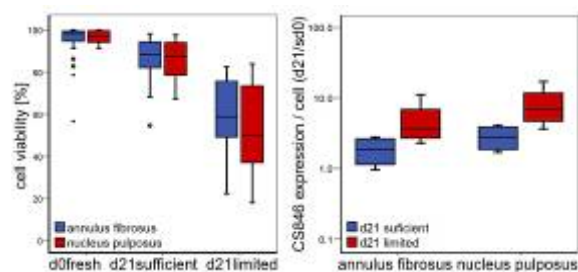
**METHODS:** Discs were cultured for 21 days, under “physiological” loading i.e. diurnal axial load (0.2/0.6 MPa, 8/16 h) with cyclic load during the 0.6 MPa-phase (0.2 Hz ± 0.2 MPa, 2x4 h), and cultured in media with limited (lim=2 g/l) or sufficient (suf=4.5 g/l) glucose concentration. Cell viability was determined with LIVE/DEAD stain and cLSM and quantified using NIH/ImageJ software.

Relative gene expression of selected anabolic and catabolic genes was quantified by relative real-time RT-PCR ( $\Delta\Delta C_t$ ). Synthesis rate of aggrecan chondroitin sulfate was measured with CS846 epitope ELISA (IBEX) and normalized to number of living cell (from cell viability quantification). For statistical analysis all data were normalized to d0 and a Wilcoxon signed rank test blocked for sheep was used for cell viability, RT-PCR and GAG synthesis rate. For all statistical analyses, a  $p < 0.05$  was considered significant.

**RESULTS:** Cell viability in fresh discs was ~90% and could be maintained for 21 days cultured under sufficient nutrition, cell viability dropped to ~50% when cultured under limited nutrition (Fig. 1A). Although CS846 content did increase with limited vs. sufficient glucose in the, this was not statistically significant (AF: suf=0.023  $\mu\text{g}/\text{cell}$  (0.009-0.028) lim=0.041  $\mu\text{g}/\text{cell}$  (0.019-0.105), NP: suf= 0.041  $\mu\text{g}/\text{cell}$

(0.066-0.454), lim=0.128  $\mu\text{g}/\text{cell}$  (0.1177-0.3087) values are medians,  $\pm$  range; Fig.1B). In gene expression, no significant differences between culture conditions for AF or NP were detected (Data not shown).

Fig. 1: Boxplots of A) cell viability and B)



CS846 content in the annulus fibrosus (AF) and nucleus pulposus (NP) of discs cultured for 7 day.

**DISCUSSION & CONCLUSIONS:** Only cell viability and GAG synthesis rate followed the expected patterns of early degeneration. To induce severe signs of matrix breakdown 21 days of culture seems not long enough. To our knowledge, our study is the first to confirm Horner & Urban’s (2001) cell work *in situ*. They found that cell viability stabilized to a new equilibrium after 2-4 days of culture. Here, the new equilibrium was reached within days and kept stable up to three weeks. In a next step we will investigate the synergistic effects of limited nutrition crossed with low (0.2Hz) and extreme high (10Hz) frequency loading in a seven day culture.

**REFERENCES:** <sup>1</sup>Urban, J.P.; Smith, S.; Fairbank, J.C. (2004) *Spine* 29:2700-2709, <sup>2</sup> Gantenbein, B.; Gruenhagen, T.; Lee, C.R.; Van Donkelaar, C.C.; Alini, M.; Ito, K. (2006), *Spine* 31:2665-2673

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