The combined effects of high Frequency Loading and Limited Nutrition on Intervertebral Discs

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INTRODUCTION:

The underlying causes of disc degeneration are multifactorial. Besides genetic factors and aging, limited nutrition and certain mechanical stimuli are generally believed to be etiological factors [1-2]. Although these effects and their interactions have been demonstrated in cell culture, no investigations have been reported in actual discs. An in vitro system was developed for culturing whole intervertebral disc (IVD) explants with "simulated physiological" loading through intact endplates (EPs) and the response of disc cells to nutritional challenge was investigated [3]. In this study, the synergistic effects of limited nutrition combined with low (0.2 Hz) and high (10 Hz) frequency loading in a seven-day culture were investigated.

MATERIALS AND METHODS:

Discs were harvested from 7 skeletally mature (3-5 year-old) Swiss Alpine sheep. The 5-6 caudal IVDs were prepared from each sheep (vertebrae cut just proximal and distal to EPs with a histological band saw and cleaned) and loaded in bioreactors [4]. Discs were cultured for 7 days either under static (0.2 MPa), simulated-physiological (low) or high-frequency (high) loading, i.e. diurnal axial load (0.2/0.6 MPa, 8/16 h) with 2 x 4 h cyclic load during the 0.6 MPa-phase with simulated-physiological (0.2 Hz, ±0.2 MPa) or higher-frequency (10 Hz; ±0.2 MPa) loading. Discs were cultured either in media with limited (lim = 2 g/l) or sufficient (suf = 4.5 g/l) glucose concentration. Cell viability was determined by calcine AM and ethidium homodimer. Imaging and quantification is described elsewhere [5]. Relative expression of selected anabolic and catabolic genes was quantified by real-time RT-PCR [4]. Synthesis rate of aggrecan chondroitin sulphate 846 (CS846) was measured with CS846 epitope ELISA (IBEX, Inc.). Disc height (after dissection and after culture), total water (wet – dry weight of discs) and proteoglycan content (DMMB dye assay of disc tissue and media) were determined. For statistical analyses univariate GLM with subsequent pairwise post hoc testing was performed (Fisher’s LSD and Games-Howell). For all statistical analyses a value less than 0.05 was considered significant.

RESULTS:

In fresh discs cell viability was 86.02% (84.36-89.89) in annulus fibrosus (AF) and 92.24% (90.75-95.97) in nucleus pulposus (NP), values are median (IQR).

For discs cultured in sufficient media under static (suf/stat) or simulated-physiological (suf/low) conditions, the viability could be maintained [suf/stat: AF = 79.54% (77.72-87.58) NP = 84.93% (82.59-87.40); suf/low: AF = 88.54% (81.82-89.47) NP = 87.87% (85.57-90.48)]. Whereas culturing under limited condition or high frequency load resulted in decreased cell viability of 50-65% in AF and 60-68% in NP. Synergistic expression of limited condition and high frequency load in NP (lim/high) yielded in a drop of viable cells down to 38% in AF and 39% in NP (Fig. 1). Relative gene expression analysis revealed significant changes in the pattern of the anabolic gene COL1A1 and the catabolic gene MMP13. Compared to d0, in the AF, COL1A1 was ~55 times up regulated when cultured under lim/low condition (p = 0.04, Fig. 2). In the NP it dropped ~40 times in the suf/stat group and was maintained in the suf/low group, whereas suf/high cultured discs expressed COL1A1 ~400 times more (compared to: suf/stat p = 0.003; suf/low p = 0.009).

In the AF, the expression of MMP13 dropped ~150 times relative to d0 when discs where cultured under suf/low conditions, whereas its expression pattern did not change when cultured with suf/high loading (p = 0.073). In the NP the expression profile did not change when cultured under suf/stat or suf/low loading, but culture with suf/high, lim/low or lim/high frequency intensely increased the expression of MMP13 [suf/high = 1,890 (1’345-12’323; p = 0.02) lim/low = 800 (519-6’739; p = 0.032) lim/high = 627 (322-1’327; p = 0.068)]. No significant changes of ACAN (= AGC1, aggrecan), COL2A1, ADAMTS4, MMP7 and HSPA4 (= heat shock 70K protein 4) were observed.

A reduction in disc height was observed in all discs, fewest in the suf/low culture [39% (36.83-45.19)] followed by suf/stat [43% (39.38-54.14)]. It decreased further when cultured under suf/high [49.21% (46.20-51.65)] lim/low [59.30% (47.22-61.22)] or lim/high [54.55% (53.27-57.87)] conditions. For the limited cultured discs this was significant (rel. to suf/low: lim/low p = 0.02 lim/high p = 0.027). No significant differences between culture conditions were detected for the CS846 synthesis rate, water yield, GAG content in tissue or GAG release in media.

DISCUSSION:

In this culture system, with media containing sufficient concentration of glucose and static or simulated-physiological loading cell viability and matrix synthesis at the gene expression level could be maintained. In agreement with previous studies [1,3] limited nutrition caused increased cell death. It is known that high body vibration can cause low back pain [2]. For example, a frequency of ~10Hz is experienced when flying in a helicopter. Pope et al [2] simulated this environment and measured a loss of comfort of the volunteers already after a 2-hours exposure. Here we confirm in a whole organ culture that high frequency load has a negative effect on cell viability; the synergistic culture of limiting nutrition and high frequency increased the cell death even further. With the partial up regulation of COL1A1 and MMP13 the stressed discs (suf/high, lim/low or lim/high) also responded on mRNA level. Another sign for the negative effect of high frequency load and limited nutrition is the lost in disc height. In terms of compensatory mechanisms for the decreased cell viability and disc height as well as changes on gene expression level, the results do not provide a clear observation. One could assume that the remaining cells would try to compensate for the lower cell density and disc height. Indeed, a first sign for an early response of the stressed cells is a change of the gene expression pattern. But no further signs for responding were obtained regarding the ECM turnover. Seven days of culture might be too short to induce already a change on the translations level. Increasing the cultivation time may allow clarifying this issue.

REFERENCES:


ACKNOWLEDGEMENTS:

The authors would like to thank M. van der Werf and S. Zeiter. Funding was received from the Swiss National Science Foundation, grant # 3100A0-109722.