Newly Identified Intervertebral Disc Fat Pad Degenerates After Injury in a Rat Model of Disc Degeneration

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INTRODUCTION:

Intervertebral disc (IVD) degeneration (IVDD) is a leading underlying pathology of chronic low back pain, a condition causing major health burdens in the United States and globally. Rodents have been used as an effective preclinical model of IVDD, and histologic analysis of these samples remains a gold standard for studying injury and IVDD progression. However, such approaches tend to focus solely on IVD tissues, often discarding tissue anterior and posterior to the IVD. In this process, key structures surrounding the IVD may be overlooked.

Fat pad structures have been identified as key contributors to joint pathology, such as cytokine release and stem cell harboring by the infrapatellar fat pad of the knee. IVD degeneration has also been related to obesity and high fat diets, though these mechanisms are not well understood. Despite this, the existence of a fat pad around IVDs, and its possible contributions to low back pain, remain unknown.

Therefore, this study aimed to 1) assess if an IVD fat pad exists in rats, 2) quantify how this fat pad changes following IVD puncture injury, and 3) evaluate mouse and human IVDs for the presence of a fat pad.

METHODS:

Naïve and injured IVDs were analyzed from a previously established Sprague Dawley rat model, in which 5-month-old rats underwent a triple-puncture injury to the anterior annulus fibrosus (AF) of discs L3-4, L4-5, and L5-6. After injury, rats were euthanized at 3 days (n=18), 7 days (n=21), 14 days (n=12), and 56 days (n=21), with sample size (n) being defined as the number of IVDs per time point. Spines were fixed, decalcified, processed for paraffin histology without adjacent tissue removal, and stained with safranin-O/fast-green/hematoxylin. The disappearance of the fat pad in injured rats following IVD injury was quantified by two experienced reviewers evaluating the percentage of fat pad remaining (from 0% to 100%). Normal distribution was tested using the Shapiro Wilk test. Statistical analysis of fat pad percent presence scores was performed using a nonparametric one-way ANOVA (Kruskal-Wallis test) followed by Dunn's post-hoc pairwise comparison.

RESULTS:

In all naïve IVDs (n=12), a fat pad structure was identified anterior to the IVD, bounded by the anterior AF and anterior longitudinal ligament (ALL) (**Figure 1**). After puncture injury, a gradual disappearance of the IVD fat pad occurred (**Figure 2A**). Percent fat pad presence at day 3 and day 7 post-injury did not differ significantly from naïve. However, fat pads at day 14 and day 56 post-injury were significantly lower than naïve (**Figure 2B**). Qualitative analysis of the fat pad after injury demonstrated an increased number of small fat lobules and an infiltration of fibrous tissue. IVD fat pads were also identified in mouse and human IVD samples (**Figure 3**).

DISCUSSION AND CONCLUSION:

This study identified a previously uncharacterized fat pad structure located anterior and immediately adjacent to rat IVDs, with preliminary evidence suggesting its presence in mice and humans as well. IVD fat pads disappeared progressively and were gone at 14 days following severe anterior IVD puncture injury. The gradual disappearance and morphology during fat pad degradation, including reduced fat lobule size and fibrous tissue proliferation, suggests potential metabolic activity and adipocyte dedifferentiation into fibroblasts. By considering the functions of known fat pads in other anatomical sites, these results suggest the IVD fat pad may serve similar mechanical and/or metabolic roles. Taken together, we highlight this newly identified IVD fat pad to draw attention to its potentially important mechanistic roles in spinal pathologies or treatments.

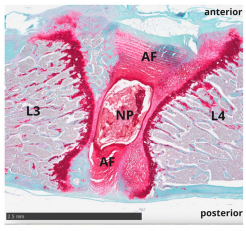

Figure 1. Representative safranin O/fast green image of a naive intervertebral fat pad at level L3-4, composed of homogeneous adipose tissue and bounded anteriorly by the anterior longitudinal ligament (ALL) and posteriorly by the anterior annulus fibrosus (AF). NP = nucleus pulposus

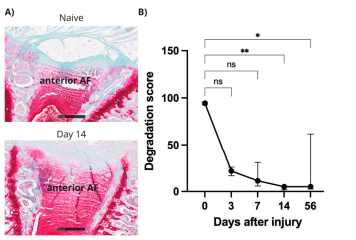

Figure 2. A) Representative histology of the intervertebral fat pad at naive and day 14 time points. Scale bars represent 500μm. **B)** Fat pad percent presence decreased over a period of weeks. Naive rats were plotted at 0 days after injury, and error bars represent 95% confidence intervals. No comparisons between post-injury time points were significant.

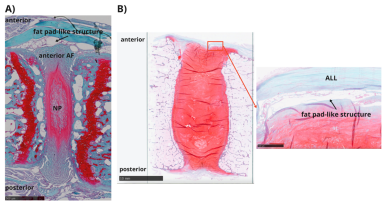

Figure 3. Evidence of an anterior intervertebral (IVD) fat pad in: **A)** sagittal section of lumbar IVD from 4-month-old mouse. **B)** sagittal section of human lumbar IVD from 66-year-old female.