GLUT-1 immunopositivity is conserved in the nucleus pulposus with age-related lumbar disc degeneration

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INTRODUCTION: Chronic low back pain is the leading cause of disability worldwide and is closely linked to the degeneration of intervertebral discs (IVDs). With age, IVDs undergo substantial changes in their composition, structure, and mechanical properties. Disc degeneration is in part attributed to the avascular nature of the IVD, which limits the supply of nutrients required for normal cellular metabolism [1]. Among these nutrients, glucose is particularly important, serving as the primary energy source for disc cells. Glucose enters discs cells by diffusing from the vascular supply in adjacent vertebrae, across the cartilage endplate, through the disc tissues, and into the cell by crossing the plasma membrane through glucose transporter 1 (GLUT-1) [1]. We hypothesize that disruptions in glucose transport compromise the nutritional environment within the IVD, promoting progressive degeneration. To evaluate this, we examined whether GLUT-1 expression varies with age, disc level, and age-related disc degeneration in rat model. Histological analyses were used to evaluate disc degeneration across age, while we assessed changes in GLUT-1 immunopositivity within the nucleus pulposus via immunofluorescence.

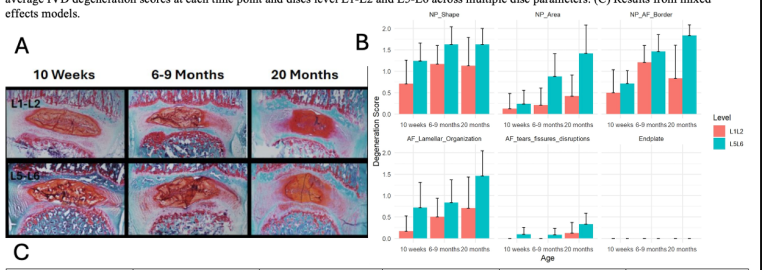
METHODS: *Histological analysis* Rats (Fischer 344, N=24) from three age groups were evaluated histologically: 10 weeks (4 male, 4 female, Charles River), 6-9 month (4 male, 4 female, Charles River) and 20 months (4 male, 4 female, National Institute of Aging colony). Levels L1-L2 and L5-L6 were extracted and harvested from the rats and bisected in the mid-coronal plane. Sections were decalcified, fixed in formalin, and embedded in paraffin wax. Embedded samples were sectioned on a microtome at 8 µm, deparaffinized and stained with Safranin O/Fast Green/Meyers Hematoxylin. *Immunofluorescence analysis* IVD sections underwent a 3-day immunofluorescence protocol to stain for GLUT-1. The sections were heated in a dry oven for 60 minutes at 60°C and cooled for 20 minutes at room temperature. Once cooled, the slides were immersed in xylene three times for 10 minutes and then dehydrated with a graded ethanol series. The sections were immersed overnight in a 0.009X Citrate-based (H-3300) Vector® Antigen Unmasking Solution at 60°C. After deparaffinization and antigen retrieval, sections were washed in phosphate-buffered saline (PBS) for 5 minutes and placed in a 0.2% Triton-X in PBS solution for cell membrane permeabilization. Then, sections were washed in phosphate-buffered saline (PBS) for 5 minutes and then incubated in a Bovine Serum Albumin (BSA) blocking solution that contained 4% Fetal Bovine Serum, 4% BSA, and 0.4% Fish gelatin for one hour. Slides were incubated overnight in the primary antibody, rabbit anti-GLUT-1 antibody [SP168] (1:200, #150299, abcam). Then slides were washed three times for 10 minutes each in PBS with 0.1% Tween and then incubated with a secondary antibody, donkey anti-rabbit IgG, Alexa Fluor™ 555 (1:400, Fisher) for 1 hour. Slides were then washed three times for 10 minutes each in PBS with 0.1% Tween and mounted with DAPI Fluoromount-G® (Southern Biotech). *Image Analysis* The safranin o/fast green/meyers hematoxylin stained IVD slides were imaged with Nikon Eclipse 50i microscope at 20x using the Nikon NIS-elements imaging software (Nikon Corporation, Tokyo, Japan) and scored for semi-quantitative degeneration grading using the JOR Spine system [2]. Scores were evaluated statistically R via mixed effects models. Immunofluorescence images were acquired using a Zeiss Confocal microscope equipped with 20x objective lens and two scans were taken per image. GLUT-1 expression was analyzed using QuPath to quantify the proportion of DAPI-positive nucleus pulposus cells that were GLUT-1 positive, with mean fluorescence intensity measured per cell. Differences across age and disc level were assessed statistically R via linear regression.

RESULTS: Histological analysis revealed mild age-related IVD degeneration with age, where 20-month aged rats had evidence of degeneration in the inner annulus fibrosis/outer nucleus pulposus [Fig. 1A]. Degeneration scores increased across multiple parameters with age, with the most pronounced changes observed in the nucleus pulposus shape and annulus fibrosis lamellar organization in the 20-month aged rats compared to the 10-week aged rats [Fig. 1B-C]. Representative immunofluorescence images demonstrate stable GLUT1 immunopositivity (red) in nucleus pulposus cells with age and spinal level [Fig. 2A]. Despite this increase in degeneration, GLUT-1 immunopositivity in the nucleus pulposus cells was not significantly different between all age groups and levels [p=0.476, Fig. 2B].

DISCUSSION: While mild intervertebral disc degeneration develops with age, GLUT-1 expression in the nucleus pulposus remains stable, suggesting that glucose transport is likely conserved and tightly regulated. This developmental and anatomical stability further validates GLUT-1's utility as a reliable phenotypic marker for nucleus pulposus cells [3]. In contrast, GLUT-1 expression in human disc cells increases with degeneration severity [4]; differences may be due to greater transport gradients. In rats, age-related changes in disc integrity may be driven by other factors such as age-related senescence or inflammation, rather than altered GLUT-1-mediated glucose uptake.

SIGNIFICANCE: GLUT-1 expression remains stable with age and disc level, indicating that glucose transport is tightly regulated and not the primary driver of disc degeneration. This stability validates GLUT-1 as a reliable phenotype marker for nucleus pulposus cells, supporting its use in disc research. Identifying alternative mechanism underlying disc degeneration could help develop strategies to prevent or slow the progression of chronic low back pain.

Figure 1 - (A) Representative histological images from rats at each time point and disc level stained in Safranin O/Fast Green/Meyers Hematoxylin. 20-month rat discs appear to be mildly degenerated at levels L1-L2 and L5-L6, especially in the AF region. (B) Series of boxplots represents the average IVD degeneration scores at each time point and discs level L1-L2 and L5-L6 across multiple disc parameters. (C) Results from mixed effects models.



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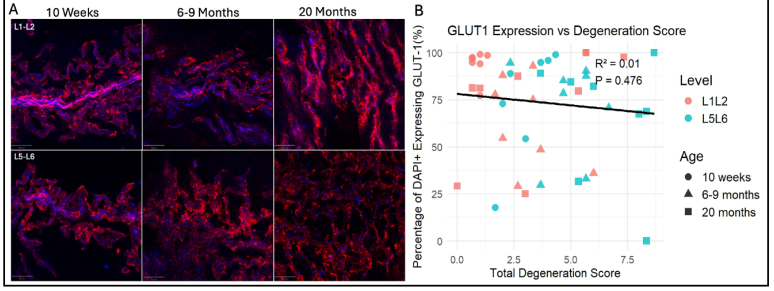
	NP Area			NP Shape			NP AF Border			AF Lamellar Organization			AF tears/fissures/disruptions		
Predictors	*β*	*95% CI*	*p-value*	*β*	*95% CI*	*p-value*	*β*	*95% CI*	*p-value*	*β*	*95% CI*	*p-value*	*β*	*95% CI*	*p-value*
(Intercept)	-0.24	-0.60 – 0.12	0.191	0.59	0.23 – 0.95	**0.002**	0.27	-0.08 – 0.62	0.132	-0.02	-0.41 – 0.38	0.937	-0.08	-0.19 – 0.03	0.132
Sex [Male]	0.26	-0.08 – 0.60	0.127	0.26	-0.09 – 0.60	0.138	0.20	-0.12 – 0.52	0.214	0.35	-0.02 – 0.73	0.065	0.12	0.03 – 0.22	**0.011**
Age6-9 months	0.35	-0.07 – 0.76	0.097	0.43	0.01 – 0.85	**0.047**	0.72	0.32 – 1.11	**0.001**	0.24	-0.22 – 0.70	0.303	-0.00	-0.12 – 0.11	0.960
Age [20 months]	0.72	0.31 – 1.14	**0.001**	0.41	-0.02 – 0.83	0.059	0.72	0.32 – 1.11	**0.001**	0.65	0.19 – 1.12	**0.007**	0.18	0.07 – 0.30	**0.002**
Level [L5L6]	0.60	0.36 – 0.84	**<0.001**	0.49	0.31 – 0.67	**<0.001**	0.49	0.23 – 0.76	**0.001**	0.54	0.31 – 0.76	**<0.001**	0.13	0.03 – 0.22	**0.009**
Observations	47			47			47			47			47		
Marginal R^2 / Conditional R^2	0.452 / 0.635			0.353 / 0.723			0.431 / 0.537			0.398 / 0.687			0.389 / NA		

Figure 2 – (A) Representative immunofluorescence images from rats of each age group and disc level. Across all age groups, nucleus pulposus regions were immunopositive for GLUT-1. Scale bar = 50 µm. (B) Scatterplot represents the average percentage of cells expressing GLUT1 compared to its total degeneration score at each time point and disc level L1-L2 and L5-L6. The line represents a linear regression model (R^2 = [0.01], p = [0.476]).



REFERENCES: 1) Johnson+ *Arthritis Res Ther 2008*; 2) Lai+ *JOR Spine 2021*; 3) Risbud+ *J Orthop Res. 2015*; 4) Richardson+ *Histochem Cell Biol 2008*.