Effect of Type-2 Diabetes Mellitus on Endplate Microarchitecture, Marrow Cellularity and Intervertebral Disc Creep in Rats

Aaron J. Fields, Ph.D. 1, Britta Berg-Johansen, B.S. 1, Lionel N. Metz, M.D. 1, Brandon La 2, Ellen C. Liebenberg, B.S. 1, Dezba G. Coughlin, Ph.D. 1, James L. Graham, M.S. 1, Peter J. Havel, DVM, Ph.D. 3, Jeffrey C. Lotz, Ph.D. 1.

1University of California, San Francisco, San Francisco, CA, USA, 2Stanford University, Stanford, CA, USA, 3University of California, Davis, Davis, CA, USA.

Disclosures:

Introduction: Intervertebral disc degeneration is a chronic and often-painful spinal condition characterized by complex biochemical, morphological, and biomechanical changes. Although the onset and progression of these changes are multi-factorial, impaired disc nutrition -- perhaps resulting from endplate sclerosis or decreased vascular supply -- is thought to be paramount. Because type-2 diabetes mellitus (T2DM) can result in metabolic bone abnormalities and systemic ischemic injury, it is not surprising that T2DM associates significantly with disc degeneration [1]. However, while the complications of T2DM potentially affect endplate microarchitecture and vascular supply, the extent of these effects as well as their contribution to the increased incidence of T2DM in patients with poor disc health [1] is unknown. Clarifying this contribution would impact patient care by identifying treatable co-morbidities that adversely affect disc health and biomechanical behavior. The goal of this study was to determine the effect of T2DM on endplate microarchitecture, endplate marrow cellularity and disc creep behavior in rats.

We hypothesized that T2DM would diminish endplate microarchitecture, marrow cellularity and disc creep.

Methods: Three coccygeal motion segments (C4-5, C5-6, and C6-7) were harvested from 6-month-old Sprague Dawley rats, non-diabetic obese Sprague Dawley rats, and diabetic obese rats (n = 6 rats/group). The diabetic rats were UCD-T2DM rats [2], a cross between Sprague Dawley rats that display polygenic obesity and insulin resistance and lean Zucker diabetic fatty rats that have an intact leptin receptor and pancreatic beta cell insufficiency. Diabetes severity was quantified with hemoglobin A1c (HgbA1c) measurements using blood obtained after sacrifice.

X-ray micro-computed tomography (Micro-XCT 200; Xradia, Pleasanton, CA) was performed for each C4-5 motion segment to measure endplate microarchitecture. Reconstructed images (4-5 µm resolution) of the motion segments were binarized with a global threshold, and cylindrical volumes of interest (1-mm Ø) adjacent to the nucleus pulposus were evaluated for the following parameters: endplate thickness, endplate porosity, and trabecular bone volume fraction (BV/TV). Parameters derived from cranial and caudal endplates were averaged for each motion segment.

Histology was performed for each C5-6 motion segment to measure the areal fraction of red blood cells in the epiphyses. Motion segments were fixed, decalcified, and sectioned using standard histologic procedures. Three mid-coronal sections from each motion segment were labeled with a custom stain that contains orange G (binds red blood cells) and aniline blue (binds bone matrix). The areas of the epiphyseal marrow space and red blood cells were calculated using Photoshop (Adobe Systems, San Jose, CA). Measurements from cranial and caudal endplates were averaged for each motion segment.

Compressive creep tests were also performed for each C6-7 motion segment to assess the viscoelastic characteristics and osmotic swelling pressure of the discs [3]. Briefly, motion segments were cleaned of soft tissues, radiographed, and mounted into a load frame (ElectroForce 3200; Bose, Eden Prairie, MN). The loading protocol consisted of five cycles of creep for 20 min. at 0.5 MPa followed by recovery for 40 min. at 0.1 MPa. Strain-time data from the final creep cycle were fit to a fluid transport model [4] that yields the following parameters: endplate permeability (K), strain-dependence of nuclear swelling pressure (D), and time-dependence of annular deformation (G). Disc height and cross-sectional area were estimated from the radiographs. All results were compared using ANOVA with a Tukey-Kramer post-hoc test. Data are given as mean ± SD.

Results: HgbA1c levels indicated the UCD-T2DM rats had 67 ± 18 diabetic days. Compared to the weight of the normal rats (413 ± 29 g), the diabetic (550 ± 64 g, p < 0.01) and obese rats (689 ± 76 g, p < 0.001) were significantly heavier. Diabetes but not obesity significantly increased endplate thickness by 21% and tended to decrease endplate porosity by 41% (Fig. 1). Diabetes also decreased trabecular BV/TV by 53% (0.38 ± 0.9 vs. 0.58 ± 0.07, p < 0.001). In contrast, diabetes and obesity both significantly increased the areal fraction of epiphyseal red blood cells by 87% and 78%, respectively (Fig. 1). Creep testing indicated that diabetes nearly doubled the strain-dependence of swelling pressure (Fig 2.), which correlated weakly with endplate thickness (r = 0.49, p = 0.05). Diabetes also decreased the time-dependence of annular deformation and increased disc height, although those changes were only significant compared to the obese rats (Fig. 2). Endplate permeability and swelling pressure (0.170 ± 0.01 MPa for all animals) were unaffected.
Figure 1: (Top) Cross-section (50 μm-thick) from C4-5 motion segment illustrating the location of the 1 mm-diameter volume of interest for microarchitecture assessment. (Bottom) Diabetes diminished endplate microarchitecture and increased the fraction of red blood cells. The total area of epiphyseal red blood cells (data not shown) also tended to be higher in diabetic (p < 0.10) and obese rats (p < 0.10). Bars show mean±SD.

a p < 0.05 vs. Normal
b p < 0.05 vs. Obese
c p < 0.10 vs. Normal
Discussion: In this study we hypothesized that type-2 diabetes mellitus would diminish endplate microarchitecture, marrow cellularity, and disc creep. Our results indicated that diabetes had contrasting effects on endplate microarchitecture and marrow cellularity. Although diabetes increased endplate thickness and tended to decrease endplate porosity, these potential impairments in endplate nutritional function were juxtaposed with an increase in epiphyseal red blood cell fraction, which suggests enhanced nutrient supply. Yet, the creep behavior of the diabetic discs typified that of degenerate discs: results indicated that diabetes diminished the primary phase of disc creep by increasing the strain-dependence of swelling pressure.

This reduction in disc creep is consistent with the decreases in elastic stiffness and viscosity observed with degeneration in human discs [5]. While our data support the conclusion that diabetes may adversely affect disc function by impairing endplate microarchitecture, other complications of diabetes, such as the accumulation of advanced glycation end products as a result of hyperglycemia, could also undermine disc health and biomechanical behavior through direct effects on the disc matrix [6].

Significance: Enhanced care for patients with intervertebral disc degeneration must take into account not only the obvious pathology but also the relevant co-morbidities that affect disc health and biomechanical behavior. Using a rat model that controls for the confounding effects of age, obesity, hyperinsulinemia, and breed, we show for the first time that type-2 diabetes mellitus diminishes endplate microarchitecture and disc creep.

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