Impaired Mechanical Function of Intervertebral Discs in Type 2 Diabetic Rodents

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Disclosures:

Introduction: Intervertebral disc (IVD) degeneration is one of the leading causes of lower back pain and is the second leading cause of disability in the US (1,2). Etiology of IVD degeneration includes a myriad of catalysts such as aging-related processes and/or mechanical injury (2). Characteristic pathophysiology includes the loss of load-bearing proteins and tissue hydration, thereby reducing mechanical efficacy of the disc and increasing susceptibility to pain and injury (3). Degeneration of the IVD has been strongly linked to multiple cardiovascular, genetic, and physical history risk factors, however the metabolic disorder, diabetes mellitus, ranks the highest amongst them (4). Recent work implicates a direct link between these factors, using a type 1 diabetic mouse model, as evidenced by increased matrix metalloproteinase (MMP)-13 expression, reduced IVD height, and increased advanced glycation end-products (5). Additionally, it has been reported that diabetic rats exhibit increased cellular apoptosis and senescence within the IVD (6). Research examining the interplay between metabolic disorders and musculoskeletal tissues continues to gain traction, however the mechanisms and functional outcomes are not well understood, particularly with regard to the spine. Furthermore, many research models of diabetes capitalize on the relative ease of inducing type 1 diabetes; however, only 5% of all diabetics are type 1 in the US (7). Therefore it is imperative to examine models of type 2 diabetes in order to ascertain how both metabolic disorders can lead to degenerative disc disease. Type 2 diabetic rodents have been shown to present biochemical and molecular changes comparable to typical disc degeneration, however, the functional changes have yet to be described (18). The Zucker diabetic Sprague Dawley (ZDSD) rodent exhibits a non-leptin dependent type 2 diabetes that is induced as the result of a high fat diet (17). We hypothesized that the intervertebral discs in these type 2 diabetic rodents may suffer from impaired mechanical function that may increase susceptibility to injuries such as herniation.

Methods: Male ZDSD and non-diabetic control animals, derived from a ZDSD parent strain (CD Rodent, Charles River Laboratory) (n = 5/group), were fed ad libitum, maintained until 33 weeks of age, bled for terminal blood analysis, and then euthanized using CO2 anesthesia. All protocols were approved by the Institutional Animal Care and Use Committee. Biochemical assays were performed on blood samples to confirm diabetic status (17). L1/L2 functional spine units (FSU) were harvested, wrapped in phosphate buffered saline (PBS) soaked gauze and stored at -20°C until testing. IVD height and area were determined from micro-computed tomography (μCT 40, SCANCO Medical AG). Specimens were prepared for dynamic mechanical analysis by removing the transverse processes, sanding the platen contacting surfaces of the vertebral bodies flat, and fixing the FSU to a compression platen using a high viscosity cyanoacrylate. FSUs were immersed in a PBS bath at 37°C and subjected to 20 cycles of sinusoidal compression at +/-4% strain, centered about 20% strain, at four discrete frequencies: 0.1, 0.5, 1, and 2 Hz, using a material test stand (Electropulse E1000, Instron). The material properties storage modulus, loss modulus, and the loss tangent were determined from the stress and strain time histories of the 15th-20th cycles. Disc height, area, and the material properties were check for normality using a Shapiro-Wilk test and then compared between the diabetic and control groups using a student’s t-test (p < 0.05).

Results: Biochemical assays confirmed the type 2-like diabetic status of the animals (17). There were no significant differences in animal weight (p = 0.097), disc height (p = 0.178), or area (p = 0.124) between groups. However, diabetic tissue demonstrated a significantly greater storage modulus and loss tangent than non-diabetic controls for nearly all frequencies (Figure 1A, C). There were no significant differences in loss modulus (Figure 1B).

Discussion: This is the first study to examine functional changes in the intervertebral disc due to a type 2-like model of diabetes. Dynamic mechanical analysis revealed that diabetic tissue had a demonstrable stiffening of the elastic response of the tissue. Additionally, the loss tangent was significantly lower, indicating a decreased capacity of the diabetic tissue to dissipate energy. These findings are analogous to previous investigations of clinically degenerate intervertebral disc tissue (8,9). One potential link between diabetes and disc degeneration are advanced glycation end-products (AGEs). The accumulation of AGEs previously has been associated with an increased prevalence of with IVD degeneration and increased risk of tissue damage (10-14). Interestingly, patients with both type 1 and type 2 diabetes accumulate AGEs in musculoskeletal tissues throughout the body (6,15,16). This is likely due to their atypical metabolism, resulting in chronic hyperglycemia and increased oxidative stress (15). Future work will include quantifying glycosaminoglycan content and AGE accumulation as well as establishing a molecular profile for the degenerative cascade in this model of type 2 diabetes.

Significance: This is the first study to identify a functional change in intervertebral disc mechanics due using a model of type 2
diabetes. Identifying a link between the comorbidity of diabetes mellitus and lower back pain will improve current clinical treatment modalities and lead to the development of novel preventative care for patients with and without diabetes.

Acknowledgments: This study was supported by the National Institutes of Health (AR-047838, 1-R43-AG074242, P30 AR057235, 5T32 AR060719).
