Impact of RAGE inhibitor administration on tendon biomechanical properties in a mouse model of type 2 diabetes

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INTRODUCTION: Disruption of tendon extracellular matrix homeostasis and altered biomechanical properties result in substantial clinical challenges for millions of individuals with diabetes. Compounding the problem, improving blood glucose levels does not normalize tendon properties in those with diabetes. Advanced glycation end-products (AGEs) crosslinking with collagen has been the focus of tendon complications in persons with diabetes. Yet, recent studies have found no evidence of greater collagen crosslinking than those without diabetes and no relationship between tendon AGE content and tensile mechanics. A less explored mechanism of AGE-mediated effects is the interaction of serum AGEs with AGE receptors (RAGE). AGEs accumulate in the serum of individuals with diabetes via hyperglycemia, the intake of AGE-rich foods, and diminished kidney AGE clearance. In cell culture, we have demonstrated that AGEs alter pathways associated with extracellular matrix regulation, cell survival, and connective growth and adaptation. We hypothesized that serum AGEs and activation of RAGE represent a mechanism underlying impaired tendon properties with diabetes. We determined the effect of RAGE inhibition on tendon biomechanical properties in a translational mouse model of type 2 diabetes.

METHODS: Nineteen db/db mice with naturally elevated serum AGEs and impaired tendon function were treated daily with a RAGE inhibitor [Azeliragon (AZ), n=9] or vehicle (n=10) for three weeks. Upon completion of the experiment, mice were euthanized after inhaling CO_2. The patellar tendon of one limb was extracted and tested for biomechanical characteristics. Structural and material properties were calculated from the ramp-to-failure. Stiffness and modulus were calculated from the linear portion of the load-displacement and stress-strain curves, respectively. Biomechanical testing was completed at the Penn Center for Musculoskeletal Disorders Biomechanics Core under the supervision of Dr. Dyment. Serum was collected for analysis of glucose and insulin. The Purdue University Animal Care and Use Committee approved this study (Protocol #: 1905001903).

RESULTS: For the db/db experiments, vehicle and AZ treatment groups were compared using a Mann-Whitney test. Values were considered significant at an α level of $p<0.05$. All data are expressed as mean ± SE and analyzed using Prism 9.5.1 (GraphPad). Serum glucose was not different ($p>0.05$) between the groups (Vehicle: 787±80 mg/dl, AZ-Treated: 830±63 mg/dl). Further, serum insulin was not different between groups (Vehicle: 10±1 µg/L, AZ-Treated: 14±3 µg/L). Patellar tendon stiffness and modulus (Figure 1a and b) were greater ($p<0.05$) in mice receiving AZ (stiffness: 9.6±1.2 N/mm, modulus: 78.2±8.2 MPa) compared to vehicle (5.8±0.9 N/mm, modulus: 49.0±8.3 MPa). Maximum stress tended to be greater in the AZ group (Figure 2a, vehicle: 14.6±2.4, AZ: 23.3±2.9 N/mm^2, $p=0.156$). Maximum load was not different between groups ($p>0.05$. Figure 1c). Maximum strain (vehicle: 0.9±0.1, AZ: 0.8±0.05) and toughness (vehicle: 6.1±1.4, AZ: 6.5±1.2 $J \cdot m^{-3}$) were not different between groups (Figure 2b and c, $p>0.05$).

DISCUSSION: Many studies have examined the impact of crosslinking and advanced end glycation products with mixed results. However, few have investigated the relationship between AGE and RAGE signaling in tendon health using in vivo models. Using an established RAGE inhibitor, we demonstrate that administering a RAGE inhibitor improves tendon properties in an established mouse model for type 2 diabetes.

SIGNIFICANCE/CLINICAL RELEVANCE: These preclinical data suggest that RAGE inhibition could be a viable therapeutical approach to improve tendon properties in individuals with diabetes. While additional work is needed to define the role of RAGE in regulating tendon properties, our preliminary results provide a premise for detailed mechanistic studies and the framework to evaluate therapeutic approaches to prevent tendon complications in people with diabetes with RAGE inhibition.

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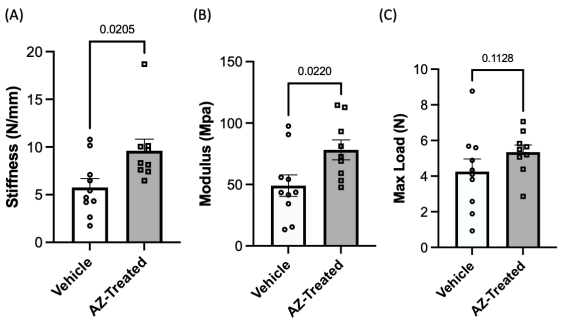

Figure 2. (A) Stiffness (B) Modulus (C) Max Load.

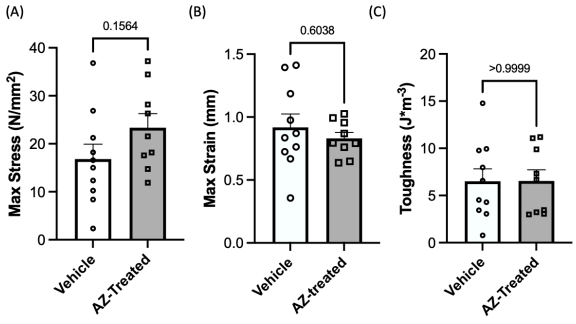

Figure 1 (A) Max Stress (B) Max Strain (C) Toughness