

# Impact of serum advanced glycation end-products and RAGE inhibitor administration on patellar tendon healing in a mouse model

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**Introduction:** Inadequate tendon healing is a significant challenge for millions with diabetes. The mechanistic underpinnings of poor tendon healing with diabetes remain a critical knowledge gap limiting the delivery of beneficial treatment approaches. We have shown that advanced glycation end-products (AGEs) impair cell proliferation and migration, critical aspects of tendon healing. AGEs accumulate in the serum of persons with diabetes and interact with AGE receptors (RAGE). We hypothesized that increasing serum AGEs would impair tendon biomechanical properties in healing tendons and that inhibition of RAGE [Azelaigron (AZ)] would improve tendon mechanics.

**Methods:** BKS.Cg-Dock7<sup><m></sup> <sup><db></sup>/J M02 Heterozygous for Dock7<sup><m></sup> Heterozygous for Lepr<sup><db></sup>, Strain 000642 were purchased from The Jackson Laboratories. Mice were maintained on a 12:12-h light-dark cycle and had free access to standard pellets and water. Ten-week-old mice were assigned to receive daily injections of bovine serum albumin (BSA-only, n=6), BSA and 100µg/day AZ (BSA-AZ, n=5), 200 µg/ml glycated BSA (AGE-BSA, n=4), or AGE-BSA with AZ (AGE-AZ, n=6). Under general anesthesia (1.5% isoflurane, 1.0 L/min O<sub>2</sub>), a full-thickness, partial-width defect was created in both patellar tendons. Treatments were started one week before surgery and continued for three weeks after surgery. Three weeks after surgery, mice were euthanized after inhaling CO<sub>2</sub>. 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. Dymment. Serum was collected for analysis of glucose and insulin. Groups were compared using a Kruskal-Wallis test. Significant p values were further explored using multiple comparison testing. Values were considered significant at an  $\alpha$  level of  $p < 0.05$ . All data are expressed as mean  $\pm$  SE and analyzed using Prism 9.5.1 (GraphPad, La Jolla, CA). This study was approved by the Purdue University Animal Care and Use Committee (Protocol #: 1905001903).

**Results:** Serum glucose was not impacted by AGE or AZ treatments ( $p > 0.05$ , BSA-only:  $246 \pm 27$  mg/dl, BSA-AZ:  $227 \pm 21$ , AGE-BSA:  $280 \pm 43$ , AGE-AZ:  $298 \pm 27$ ). Further, serum insulin was not impacted by AGE or AZ treatments ( $p > 0.05$ , BSA-only:  $1.9 \pm 0.4$  mg/dl, BSA-AZ:  $0.8 \pm 0.2$ , AGE-BSA:  $1.4 \pm 0.3$ , AGE-AZ:  $1.8 \pm 0.8$ ). Tendon stiffness was lower (Figure 1a) in mice treated with AGEs ( $p < 0.05$ ,  $10.8 \pm 1.4$  N/mm) compared to BSA-only ( $17.6 \pm 1.3$  N/mm). Further, tendon stiffness in AGE-treated mice given AZ was not different from AGE-BSA ( $p > 0.05$ ,  $12.7 \pm 1.8$  N/mm Figure 1a). Tendon modulus was lower in mice treated with AGEs ( $p < 0.05$ ,  $28.0 \pm 7.0$  MPa) compared to BSA-only ( $63.5 \pm 9.0$  MPa). Additionally, modulus in AGE-treated mice given AZ was not different from AGE-BSA ( $p > 0.05$ ,  $47.6 \pm 10.4$  N/mm). Maximum stress (Figure 2b) was not different between groups ( $p > 0.05$ , BSA-only:  $14.4 \pm 2.5$  MPa; BSA-AZ:  $12.0 \pm 1.6$ ; AGE-BSA:  $9.1 \pm 2.0$ ; AGE-AZ:  $12.4 \pm 1.7$ ). We found no statistically significant difference between groups in maximum load, stress, strain, or toughness ( $p > 0.05$ , Figure 2a and 3ab).

**Discussion:** In this initial pilot cohort, we demonstrate that increasing serum AGEs in healthy mice impairs recovery of tendon biomechanical properties after injury. Treatment with AZ in the presence of elevated AGEs tended to improve tendon modulus ( $p = 0.139$ , Figure 1b). However, with the limited sample size, we did not detect a significant effect of RAGE inhibition. These data link serum AGEs to impaired tendon healing and support our hypothesis that elevated serum AGEs, as seen with diabetes, contribute to delayed tendon healing. Further work with a larger sample size may implicate the RAGE receptor as mediating the effect of elevated serum AGE on tendon injury recovery.

**Significance:** These preclinical data suggest elevated AGE, as seen with diabetes, could impact tendon healing *in vivo*. If, with a large sample size, we find that RAGE inhibition limits the impact of AGEs on tendon healing, then local RAGE inhibition could be a viable therapeutic approach to improve tendon properties in individuals with elevated AGEs. 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. Our initial work in mice provides a framework to evaluate the potential of RAGE inhibition or approaches to lower serum AGEs to improve tendon healing.

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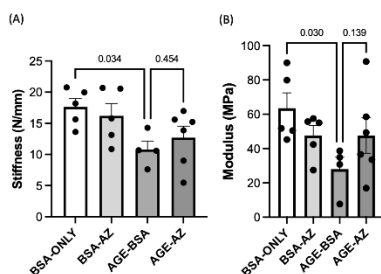


Figure 1 (A) Stiffness (B) Modulus

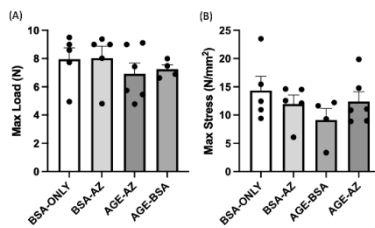


Figure 2 (A) Max Load, (B) Max Stress

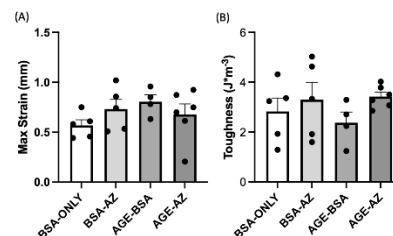


Figure 3 (A) Max Strain (B) Toughness