Biomechanical Properties of Achilles Tendons in db/db Mice Treated with Spironolactone or Rosiglitazone

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INTRODUCTION
Diabetes is a well-studied disease that has an impact on multiple systems. Little however is known about tendon alterations due to diabetes, nor the effect of treating the diabetes. What has been observed is an association of diabetes with tendinopathy in multiple tendon sites.1 The tendinopathy is believed to be due to advance glycation end products. Studies in humans have been primarily limited to examination of gross alterations such as altered gait analysis and increased tendon thickness, and could greatly benefit from analysis of animal models.

In this study we examined the effect of diabetes on Achilles tendon biomechanical properties in db/db mice, which have a genetic mutation in the leptin gene receptor leading to diabetes and obesity by 6 weeks of age. To examine the baseline effect of diabetes on the tendon biomechanical properties, mice were housed until 16 weeks and then euthanized for analysis. We hypothesized that there are structural and material declines in the Achilles tendons in db/db mice.

Also examined was the potential for two drugs to improve biomechanical properties in the db/db mice. Rosiglitazone is an anti-diabetic drug in the thiazolidinedione class of drugs. It works as an insulin sensitizer, by binding to the PPAR receptors in fat cells and making the cells more responsive to insulin. Spironolactone is a diuretic and is used as an anti-androgen. Spironolactone inhibits the effect of aldosterone by competing for intracellular aldosterone receptors in the cortical collecting duct. We hypothesized that treatment of diabetes with rosiglitazone or spironolactone would protect the tendons from the biomechanical decline.

METHODS
Male db/db [5 week old; background strain C57BL/1KsJ (BKS-Cg-Dock7™/++ Lepr™/J)] and age-matched non-diabetic lean control mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were singly housed in shoebox cages with wood chips (sani-chips) in a temperature controlled room (22–23 °C) with 12:12 h light: dark cycle. All experimental procedures were approved by the Wright State University Animal Care and Use Committee.

Drug treatment After 1 week acclimatization to room conditions, mice were randomly assigned to six different groups based on food type: (1) Lean control [standard Teklad rodent diet 8640], (2) db/db [standard Teklad diet] (3) Lean control [Rosiglitazone (20 mg/kg) in standard Teklad rodent diet], (4) db/db + [standard Teklad diet + Rosiglitazone], (5) Lean control [standard Teklad diet + Spironolactone 100mg/kg], and (6) db/db [standard Teklad diet + Spironolactone]. After 10 weeks of treatment, mice were euthanized. The Achilles tendons from both legs was dissected and stored at -70°C until biomechanical testing.

Glucose measurements Blood samples were taken from a cut made on the tip of the tail and the glucose concentration was determined using a Free Style Lite®(Abbott Diabetes Care, CA) Blood Glucose Monitor.

Biomechanical testing Tendon diameter and initial length were measured using a laser micrometer (Keyence, Elkwood Park, NJ) and digital calipers, respectively. Following an established procedure2, a custom grip was used to secure the calcaneous during testing. The tendon was threaded through a small opening (1.75 mm dia) in the block using a suture. The intramuscular tendon fibers were then glued between a folded piece of sandpaper and clamped in a grip secured to a 50 N load cell on a materials testing system (E3000;Instron, Norwood, MA). The block was secured in pneumatic jaws. The tendon was manually preloaded to 0.5 N and then loaded in tension at a rate of 0.1 mm/sec until tendon failure (60% decrease in load). Maximum load, tensile stress and tensile strain were calculated. Two-way ANOVA analysis was used to detect statistical differences with significance level set at p≤0.05. A Tukey’s post-hoc analysis followed to test for multiple comparisons.

RESULTS
At 16 weeks, body weight was increased for all db/db mice compared to controls (p≤0.001). There was a significant increase in weight in the rosiglitazone treated db/db mice compared to standard diet db/db mice (p≤0.001). There was a significant reduction in blood glucose levels in Rosiglitazone treated db/db mice to below control levels (p≤0.001) (Table 1).

Significant differences in maximum failure load (p<0.001), tensile stress (p<0.001), and tensile strain (p<0.001) were detected between the diabetic and control mice Achilles tendons (Figure 1). On average, tendons from diabetic mice were 34% percent weaker than the normal mice. Treatment type did not have a significant effect on tendon strength. However, a significant difference (p=0.014) in tendon tensile strain was found between the two drug treatments, with greater tendon elongation in spironolactone-treated mice.

Table 1. Body weight and blood glucose measurements in 16 week old mice treated with rosiglitazone or spironolactone for 10 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rosiglitazone</th>
<th>Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Wt</td>
<td>27.3±3.9</td>
<td>31.7±1.6</td>
<td>27.3±1.1</td>
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<tr>
<td>db/db</td>
<td>39.7±1.6</td>
<td>62.8±2.3</td>
<td>41.4±6</td>
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<tr>
<td>Blood Glucose</td>
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<tr>
<td>Standard Diet</td>
<td>135±9</td>
<td>140±18</td>
<td>142±5</td>
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<tr>
<td>Rosiglitazone</td>
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<td>578±41</td>
<td></td>
</tr>
<tr>
<td>Spironolactone</td>
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Figure 1. Significant differences were found for maximum load, tensile stress and tensile strain between control and diabetic mice Achilles tendons. In addition, significant differences in tensile strain were found between mice that were treated with rosiglitazone and those treated with spironolactone. *p<0.001; *p=0.014

DISCUSSION
Exercise is an essential component for control of diabetes. Tendinopathy and muscle atrophy can negatively contribute to activity. This is the first study to analyze the biomechanical effects of diabetes on tendons in db/db mice. In this study we found that there are significantly decreased material and structural properties in the mouse Achilles tendon. Treatment of the diabetes with either of two commonly used drugs had no effect on the tendon parameters compared with db/db mice without treatment. A limitation of the study is the relatively short treatment duration. A longer time beyond 10 weeks may be required for tendon improvements to be observed with the treatment. db/db mice have significant biomechanical impairment; future studies addressing both treatment of the diabetes and tendon deficits would be valuable.

SIGNIFICANCE
17.5 million people have diabetes mellitus in the United States. This debilitating disease has widespread impact on society. Research into the musculoskeletal features of this disease is in its infancy. Identifying methods to improve tendon health will contribute to improving the quality of life of diabetic patients.

REFERENCES