Identification of Tendon as an Insulin Target Tissue: Impaired Flexor Tendon Gliding and Attenuated Insulin Receptor Signaling in a Murine Model of Type II Diabetes Mellitus

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Introduction: The dramatic increase in Type II Diabetes Mellitus (T2DM), as part of the obesity epidemic [1], is one of the most pressing health challenges facing the U.S. There is a clear link between T2DM and musculoskeletal pathologies; T2DM accelerates the progression of osteoarthritis [2], and increases fracture risk [3]. T2DM also dramatically effects the baseline function of flexor tendons; rates of tenosynovitis, and carpal tunnel syndrome are increased in diabetic patients [4]. However, there remains a paucity of information regarding the molecular mechanisms of T2DM-induced changes in tendon function. Flexor tendons (FT) facilitate digit range of motion (ROM) and movement of the hand. FT gliding can be impaired by tendon fibrosis and an inability to fit within the surrounding synovial sheath, or due to the formation of fibrous adhesions between the tendon and synovial sheath during healing. Fibrosis and increased disorganization of the extracellular matrix are hallmarks of the diabetic hand phenotype observed in T2DM patients [4]. In the present study we identify tendon as an insulin target tissue and demonstrate that a murine model of diet induced obesity and T2DM mimics the pathological changes in tendon structure and function that are seen in human diabetic flexor tendons including impaired gliding and loss of strength [4]. Moreover, we identify attenuated insulin receptor (IR) signaling in tendon cells as a key transition from homeostasis to tendinopathy.

Methods: Murine Model of Diet Induced Obesity and Type II Diabetes: Male C57Bl/6J mice were fed either a high fat diet (HFD; 60% Kcal from fat) or low fat diet (LFD; 10% Kcal from fat) beginning at four weeks of age. Metabolic dysfunction and T2DM were confirmed in the HFD-fed mice based on significant increases in body weight (+65%, p<0.0001), and fasting blood glucose levels (HFD: 229.1±21.51 mg/dL; LFD: 147.1 ± 19.73 mg/dL, p=0.015), as well as impaired glucose tolerance, relative to LFD-fed control mice. Tendons were harvested after 12 and 48 weeks on the respective diets to assess changes in tendon function and IR signaling as a function of disease duration.

Tendon Gliding Function and Mechanical Testing: Changes in tendon excursion were quantified using the ‘Gliding Coefficient’, a measure of the degree of metatarsophalangeal joint range of motion (MTP ROM) when incremental loads (0-19g) were applied to the tendon. A higher gliding coefficient is indicative of impaired tendon excursion, due to tendon fibrosis or adhesion formation between the tendon and synovial sheath [5]. MTP joint ROM, which is the flexion angle corresponding to the maximum applied load of 19 grams, was also quantified, with higher MTP ROM corresponding to increased tendon gliding function. Following the MTP flexion test, biomechanical testing was used to determine maximum load at failure of the tendons.

Insulin receptor signaling: Primary tenocytes were isolated from the Flexor Digitorum Longus (FDL) tendon of C57Bl/6J mice, which were maintained on normal chow. Tenocytes were stimulated with
10nm insulin for 15 minutes and changes in activated insulin receptor signaling (including phospho-Akt/total Akt, and p-IRβ/total IRβ) were assessed by western blotting. To examine changes in insulin receptor signaling in control and diabetic tendons, FDL tendons were isolated from HFD and LFD-fed mice after 48 weeks. Whole tendons were stimulated ex-vivo with 10nm insulin for 15 minutes. Changes in insulin receptor signaling were assessed by western blot and immunohistochemistry.

**Results:** Type II Diabetes Impairs Tendon Gliding Function and Strength: No significant change in gliding coefficient, MTP ROM or max load at failure was observed between T2DM and control mice after 12 weeks on their respective diets. However, after 48 weeks, flexor tendons from T2DM mice had a significant increase in gliding coefficient (+53%, p<0.0001), and significant decreases in MTP ROM (-40%, p<0.001) and max load at failure (-38%, p<0.001), relative to non-diabetic control tendons (Figure 1A-C). No significant differences in MTP ROM or Gliding coefficient were observed between LFD-fed control mice at 12 vs. 48 weeks; however, max load at failure was significantly decreased in control mice from 12 to 48 weeks. MTP ROM, gliding coefficient, and max load at failure were significantly impaired in T2DM mice at 48 weeks relative to T2DM mice at 12 weeks, indicative of progressive loss of tendon gliding function and strength.

Identification of tendon as an insulin target tissue: Following stimulation with 10nm insulin, primary tenocytes expressed abundant phospho-Akt protein, with a significant 3.5-fold increase in p-Akt/total Akt expression (p<0.001), and a 2.3-fold increase (p<0.05) in phospho-IRβ/total IRβ, relative to vehicle treated cells (Figure 2A), indicating the expression of functional insulin receptors in tenocytes. Insulin receptor signaling is impaired in T2DM Tendons: Insulin stimulation resulted in a robust 6-fold induction of p-Akt signaling in lean control mice, and a 4-fold increase in p-IRβ; in contrast, no change in p-Akt or p-IRβ was observed in insulin stimulated T2DM tendons relative to vehicle control (Figure 2B).

**Discussion:** The effects of T2DM on tendon function are a challenging clinical problem, however, the cellular and molecular changes in tendon homeostasis due to T2DM remains an under-studied and not well-understood pathology. We have used a murine model of diet-induced obesity and T2DM to recapitulate many aspects of the diabetic hand syndrome that is observed clinically, including impaired gliding function and loss of strength. Decrements in strength make the tendon more susceptible to rupture; not only is unsatisfactory healing of flexor tendon injuries a major challenge for hand surgeons due to the formation of fibrous adhesions [6], but healing is further impaired in T2DM patients [7], indicating the importance of mitigating the effects of T2DM on tendon homeostasis. This murine model will allow for intensive investigation in to the mechanisms of diabetic tendinopathy and will identify new therapeutic approaches to deal with these disruptions in tendon homeostasis.

Activation of IR signaling, including phosphorylation of Akt upon insulin stimulation provides the first direct evidence of tendon as an insulin target tissue. Additionally, we have demonstrated that T2DM blunts insulin sensitivity and impairs IR-signaling in tendon tissue, suggestive of a direct role for IR-signaling in tendon homeostasis and attenuated IR-signaling as a novel molecular mechanism for diabetic tendinopathy.

**Significance:** Impaired gliding function in flexor tendon of the hands is an immense clinical challenge in T2DM patients, resulting in compromised hand function. This study provides the first evidence of attenuated IR signaling in diabetic tendons, suggesting a novel mechanism of diabetic tendinopathy and provides a platform for future studies to identify previously unknown therapeutic approaches to treat these common tendon pathologies.
Figure 2. Insulin receptor (IR) signaling in tendon. [A] Primary tenocytes were isolated from WT C57Bl/6 mice maintained on normal chow. Tenocytes were stimulated with 10nm insulin for 15 minutes. Changes in activation of IR signaling, including the immediate downstream target Akt were assessed by western blotting. Phosphorylation of Akt (p-Akt) is indicative of activated IR signaling. These data identify tendon as an insulin target tissue with functional insulin receptors. [B] Whole FDL tendons were isolated from C57Bl/6J mice that were fed either a high fat diet resulting in type II diabetes mellitus (T2DM), or a lean control diet for 48 weeks. Whole tendons were stimulated with 10nm insulin and assessed for activation of IR signaling. Robust phosphorylation of Akt is observed in control tendons, however, blunted p-Akt is observed in T2DM tendons, indicating loss of insulin sensitivity.
Figure 1. C57Bl/6J mice were maintained on either a high fat (HFD) or low fat (LFD) diet for 48 weeks. The HFD-fed mice develop systemic metabolic dysfunction, and Type II diabetes mellitus (T2DM). Changes in flexor tendon gliding function and strength were assessed in T2DM and control mice. [A] The MTP flexion angle, and indicator of digit range of motion, was significantly decreased in T2DM mice relative to control. [B] The Gliding coefficient quantifies resistance to tendon excursion through the synovial sheath; an elevated gliding coefficient is indicative of impaired gliding and tendon fibrosis or adhesion formation. The Gliding coefficient was significantly increased in T2DM tendons relative to control. [C] Max load at failure, an indicator of tendon strength, was significantly decreased in T2DM tendons relative to control. Data are presented as mean ± SEM, (*) indicates p<0.05; n=5 per group.