Tendon Strain Stiffening is Reduced During Healing and High Magnitude Long Duration Dynamic Loading

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Introduction

Tendons transfer stresses and strains from muscle to bone during loading, resulting in multi-scale changes to their extracellular matrix (ECM). For example, tendon stiffness increases with strain, as disorganized ECM at the microscopic level becomes more aligned and less crimped[1]. Previous work has shown that tissue strains correlate with cellular and nuclear strains in uninjured tendons during *quasi-static* tensile loading[2]. However, it remains unknown how ECM stresses are altered in clinically relevant situations, such as high *dynamic* loading and *healing*, which may propagate to alter strain transfer to cellular components. Therefore, the objective of this study was to investigate the role of tendon healing and dynamic loading on mechanical strain stiffening and fiber recruitment. We hypothesized that healing and high magnitude long duration dynamic loading would reduce strain stiffening and fiber recruitment compared to uninjured tendons and low magnitude long duration dynamic loading.

Materials and Methods

Study Design

Female C57BL/6 mice at 150 days of age were randomized into uninjured controls (n=60 mice) and those that received bilateral partial width (60%), full thickness excisional injury (n=120 mice) to their patellar tendons (Figure 1A) (IACUC approved)[3]. Animals injured were randomized into groups euthanized at 2 or 6 weeks post-injury.

Ex vivo Assays

Following sacrifice, tendons were harvested immediately and carefully prepared for mechanical testing under aseptic conditions to maintain cell viability. The patellar tendon was stamped into a “dog-bone” shape to isolate the injury site, and cross sectional area measured at the injury site[4]. To maintain tenocyte viability during loading, tissues were immersed in a bath containing sterile DMEM supplemented with 5% FBS, maintained at 37ºC integrated with a tensile testing device (Instron 5848; Norwood, MA). Cell viability was evaluated following each type of testing protocol using an MTT assay. To evaluate the effect of healing on strain stiffening, tendons (n = 10-13/group) were preconditioned and ramped at constant strain rate (0.1% strain/s) until 1% or 10% strain prior to a frequency sweep (Figure 1B). To evaluate the effect of dynamic loading and healing on strain stiffening, tendons were randomized into a zero, low, or high magnitude loading protocol (corresponding to the toe or linear regions of the force-displacement curve) for either 10 or 1000 cycles at 1Hz. During loading, force and displacement data were acquired and analyzed using MATLAB (Mathworks, Natick, MA).

Analysis

The change in equilibrium stress (force divided by the cross sectional area) between 1 and 10% strain was used to indicate the amount of strain stiffening, and the dynamic modulus assessed during dynamic loading were computed. Using quasi-static ramp data, we applied a structurally based elastic model [5, 6] to quantify the non-linear force-displacement behavior as fibers uncrimp to their slack length. Data were evaluated with either one-way ANOVAs with post hoc t-tests or with two-way ANOVAs with post hoc Fisher’s tests.

Results

Cell viability was maintained throughout mechanical testing. Tendon healing affected strain stiffening, as the change in equilibrium stress was reduced at both 2- and 6-weeks post-injury compared to uninjured control tendons (Figure 2A). This decrease in strain stiffening was

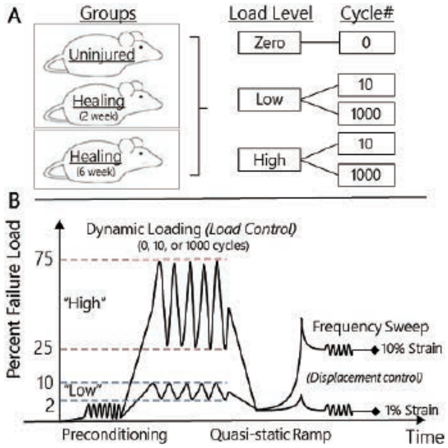

Figure 1. Study Design. **(A)** Mice were randomized into three groups before **(B)** quasi-static and dynamic loading were completed. The dynamic loading protocols varied the magnitude (low or high load) and duration (0, 10, or 1000 cycles) of loading.

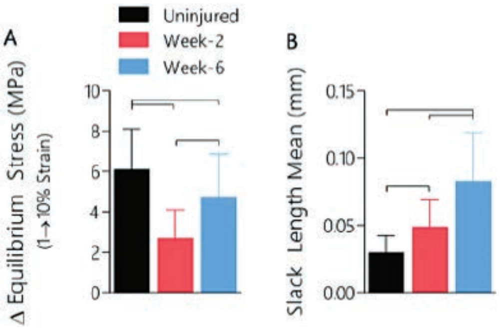

Figure 2. Effect of healing on tendon stress and slack lengths. **(A)** The change in equilibrium stress was reduced in tendons 2- and 6-weeks pos-injury. **(B)** Tendon mean slack length increased in healing tendons. Data shown as mean ± SD. Bars indicate p < 0.05.

coupled with increased fiber slack lengths (Figure 2B). Strain stiffening was also reduced due to high magnitude loading in uninjured and 6-week post-injury tendons, but not 2-week post-injury tendons (Figure 3A). Cycle duration only affected strain stiffening for high magnitude loading in uninjured tendons. Although neither loading magnitude nor cycle duration altered the change in equilibrium stress in tendons at 2-weeks post-injury, an increase in these factors increased slack lengths in all groups (Figure 3B). The dependence of slack length on cycle duration in high magnitude loading was mirrored by increases in the secant modulus, which was also affected by cycle duration during high magnitude loading (Figure 3C).

Discussion

This study evaluated stress transfer in uninjured and healing tendons during quasi-static and dynamic loading. Although mechanical properties are well established to be inferior in healing tendon[6], the relationship to strain stiffening and additional effects of dynamic loading are poorly understood. Multi-scale strain transfer (i.e., relationship of strain between structural hierarchies) ultimately affects cell proliferation, differentiation, and matrix production[7]. The stress-strain response was greatly reduced in tendons at 2-weeks post-injury, which suggests that the multi-scale response to loading may be abnormal. Interestingly, although there were no significant differences in the change in stress with varying loading protocols 2-weeks post-injury, tendons exhibited elevated slack lengths, suggesting that the toe region is elongated, but the overall change in stress from 1 to 10% strain remains similar. Slack length data may reveal structural changes responsible for the mechanical response. Additionally, the role of changing material properties with loading may provide insight into the dynamic functional nature of tendons. Future studies will be designed to specifically assess changes in collagen structure due to injury and dynamic loading, and measure strain transfer to cells in these loading paradigms.

Conclusions

Defining the mechanical implications for loading and tendon healing on the ECM may provide important insight into material behavior and ultimate strain transfer to resident cells. This study showed that healing and dynamic loading alters the tendon strain stiffening, which may be due to fiber uncrimping and the change in material modulus during cyclic loading.

Acknowledgements

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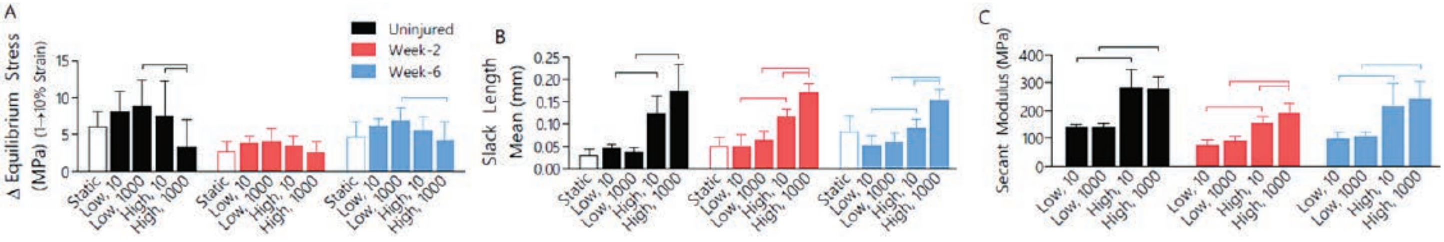

Figure 3. Effect of magnitude and duration of loading on tendon stress, fiber recruitment and macromechancis. **(A)** The change in equilibrium stress with loading was dependent on load magnitude and healing. **(B)** Mean fiber slack length increased following high magnitude, long duration loading. **(C)** The secant modulus increased with long duration loading in 2-week post-injury tendons. Data shown as mean ± SD, with clear columns indicating quasi static loaded tendons. Bars indicate p < 0.05.