Adverse Mechanical Consequences from Abnormal Activation and Deactivation of the mTORC1 Pathway in Tendons

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Disclosures

None

Introduction

Tendon, a collagen-rich tissue, is the primary component that transmits loads from muscle to bone. It is relatively avascular and hypocellular, and thus can be afflicted with tendinopathy or ruptures without a clear solution towards complete recovery. Thus, recent efforts have strived to understand the mechanistic basis of tendon development and maturation, which might help guide better treatment options. The mechanistic target of rapamycin complex 1 (mTORC1) regulates multiple cellular biological processes such as metabolism, growth, proliferation, and survival. Recently, we showed that both deactivation and activation of mTORC1 in tendon caused impaired postnatal tendon development with immature collagen fibrillogenesis, which suggest that fine-tune regulation of mTORC1 signaling is essential for normal postnatal tendon development. While these data highlight the molecular effects of deactivation and activation of the mTORC1 pathways, downstream macroscale effects on tendon mechanical function remain unclear. Therefore, the objective of this study was to examine the mechanical response of mouse Achilles tendons after activation or deactivation of mTORC1, via tendon-specific deletion of *Tsc1* (gain-of-function) or *Raptor* (loss-of-function), respectively. We hypothesized that any deviations from physiological levels of mTORC1 signaling will adversely affect the structural and material properties of tendons, with a pronounced effect due to impaired collagen fibrillogenesis.

Methods

All procedures were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee. Mouse hindlimbs (n = 10/group) were collected from the *ScxCre; Raptor$^{f/f}$* mice at P60 for loss-of-function study. Mouse hindlimbs (n 5 7/group) were collected from the *ScxCre;Tsc1$^{f/f}$* mice for gain-of-function study at P30. All mice assigned for mechanical testing were frozen at −20° C until the day of testing. Mice were thawed at room temperature and calcaneal bone-Achilles tendon-muscle complexes were grossly dissected. All extraneous soft tissues and muscles were finely dissected, and a custom laser device was used to measure the cross-sectional area (CSA) of the Achilles tendon. The myotendinous junction was sandwiched between two sandpaper tabs with cyanoacrylate glue to prevent any slippage. The calcaneal bone was gripped with a custom fixture and the construct was mounted onto a material testing machine (Instron 5542, Instron Inc., Norwood, MA). All testing was conducted in phosphate buffered saline bath at room temperature. Each sample was preloaded to 0.02 N followed by 10 cycles of preconditioning between 0.02 to 0.04 N. After a resting period of 60 seconds at 0 N, the sample was quasi-statically ramped to failure at a strain rate of 0.03 %/s. All data were collected at 100 Hz. Ensuing force-displacement curves were analyzed to obtain failure load (N) and tissue stiffness (N/mm, defined as the slope of the linear region). Cross-sectional areas (mm^2) and gauge length (mm) values were used to obtain stress-strain curves for each sample. Modulus (MPa) was calculated as the slope of the linear region of the stress-strain curve and failure stress (N/mm^2) as the maximum stress value observed.

Results

Consistent with previous histological studies, Achilles tendons from *ScxCre;Raptor$^{f/f}$* mice had structural deficits with a significantly reduced CSA (Figure 1A). This further resulted in a significantly reduced failure load (Figure 1B) and significantly lower tendon stiffness (Figure 1C). *ScxCre; Raptor$^{f/f}$* tendons did not show material deficits with no significant differences observed in tendon modulus (Figure 1D) and failure stress (data not shown). Consistent with previous histological study, Achilles tendons from *ScxCre;Tsc1$^{f/f}$* did not have any macroscale structural changes with no significant difference in tendon CSA (Figure 2A). Unexpectedly, structural properties including failure load (Figure 2B) and tendon stiffness (Figure 2C) were significantly lower. Further, material effects were evident in *ScxCre;Tsc1$^{f/f}$* tendons

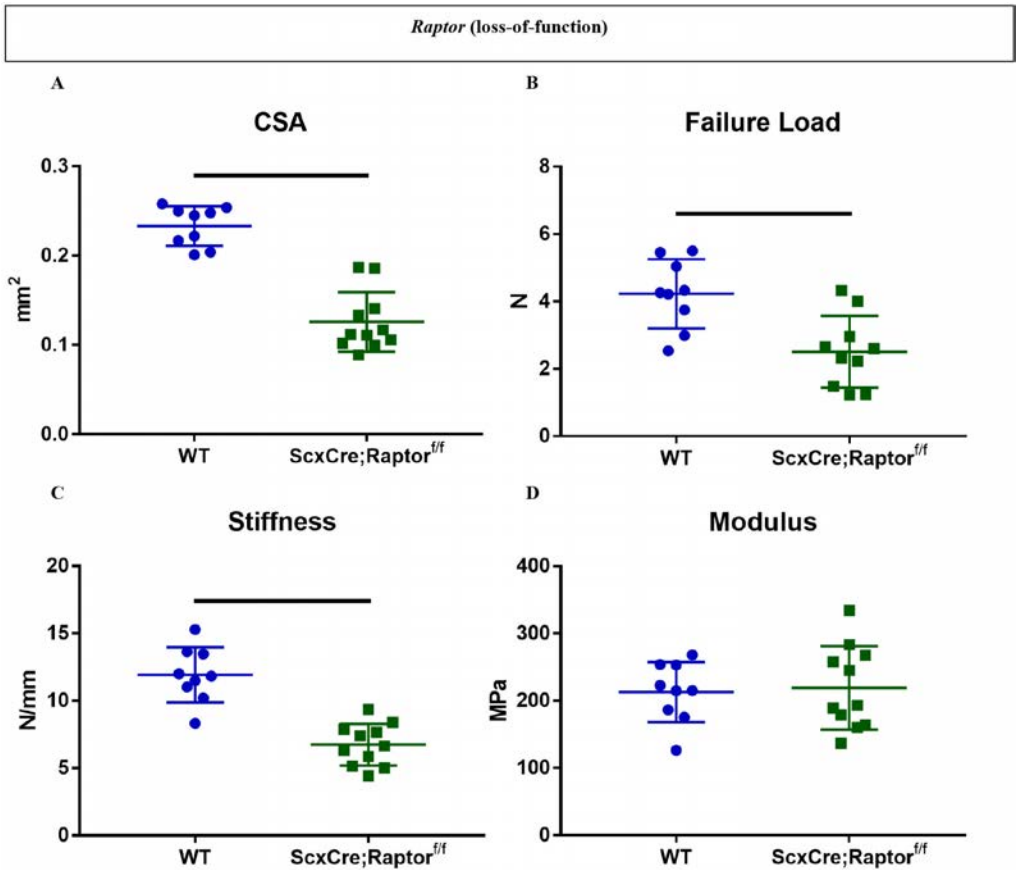

Figure 1. Comparison of mechanical properties of control and *ScxCre;Raptor*$^{f/f}$ mouse Achilles tendons **(A)** Cross-sectional area; **(B)** Failure load; **(C)** Stiffness; **(D)** Modulus. Bars indicate a significant difference at $p < 0.05$ after unpaired two-tailed t-tests.

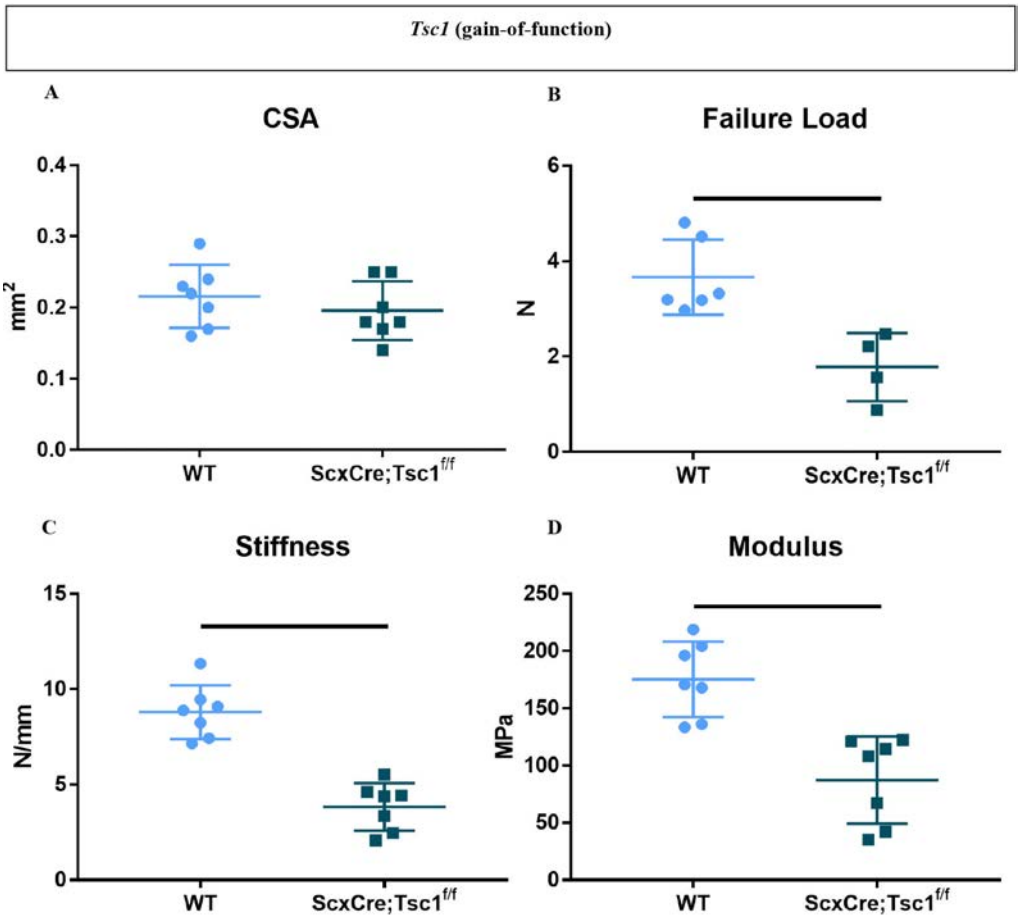

Figure 2. Comparison of mechanical properties of control and *ScxCre;Tsc1*$^{f/f}$ mouse Achilles tendons: **(A)** Cross-sectional area; **(B)** Failure load; **(C)** Stiffness; ***(D)*** Modulus. Bars indicate a significant difference at $p < 0.05$ after unpaired two-tailed t-tests.

with a significantly reduced tendon modulus (Figure 2C) and a trending difference ($p < 0.1$) in failure stress (data not shown).

Discussion

This study investigated the macroscale mechanical sequelae from abnormal activation and deactivation of the mTORC1 pathway in murine Achilles tendons. Each of these perturbations resulted in substantial, but interestingly divergent disruption of tendon mechanical response. Our previous work with *ScxCre;Raptor$^{f/f}$* tendons showed the loss of the typical bimodal fibril diameter distribution with abrogation of all large diameter fibrils. Surprisingly, this resulting unimodal distribution of smaller diameter fibrils did not affect the material properties of these tendons with not differences observed in tendon modulus or failure stress. Transmission electron microscopy of *ScxCre;Tsc1$^{f/f}$* tendons showed a more severe effect on collagen fibrils with most diameters in the 40-50 nm range. Further, histological analysis depicted a very disorganized matrix and increased neovascularization. These structural and extracellular matrix (ECM) disruptions may explain the significant material property effects seen here in *ScxCre;Tsc1$^{f/f}$* tendons. However, it was surprising that even with these ECM deficiencies, macroscale structure *i.e.,* cross-sectional area was not affected. Future studies will explore microscale structural changes in tendon ECM via second harmonic generation imaging of collagen fibrils, and atomic force microscopy imaging to measure fibril sliding and deformation, which might explain the macroscale mechanical response reported here.

Significance

The findings reported here suggest that *optimal* mTORC1 signaling is crucial for postnatal tendon development and abnormal activation or deactivation has deleterious effects on tendon mechanics. Clinically, temporal control of this pathway might allow for improved tendon healing outcomes.