Induced Deletion of Biglycan in Mature Tendon Reveals a Surprising Role during Adulthood

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Introduction

Tendon is a highly organized tissue composed of a collagen network linked via small leucine-rich proteoglycans (SLRPs). Biglycan (Bgn) is a SLRP present in the extracellular matrix, is a regulator of collagen fibrillogenesis, and is highly expressed during tendon development [1]. Further, reduction in *Bgn* expression has been shown to alter tendon fibril diameter and viscoelastic properties [2]. However, the role of Bgn on tendon homeostasis has yet to be determined, independent of its influence on development. Therefore, the purpose of this study was to determine the effect of acute, conditional deletion of biglycan on mature, uninjured tendon. Due to its minimal presence in normal adult tendon, we hypothesized that deletion of biglycan expression in mature, uninjured tendon would have no effect on tendon mechanics or structure.

Methods

Female $Bgn^{+/+}$ control (WT, n=16) and bitransgenic conditional $Bgn^{flox/flox}$ mice with a tamoxifen (TM) inducible Cre, (B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J, Jackson Labs) were utilized ($I\text{-}Bgn^{-/-}$, n=16) [3] (IACUC approved). Cre excision of the conditional alleles was induced in mature (120 day) [3] mice via three consecutive daily IP injections of tamoxifen (4.5mg/40g body weight). WT mice received TM injections to control for any potential side effects. Mice were euthanized at 150 days of age. The patellar tendon-bone complex from one limb of each animal was dissected and prepared for biomechanical testing [4]. Tendons (n=16) were subjected to a viscoelastic testing protocol containing three stress relaxations, each followed by frequency sweeps, with the test culminating in a ramp-to-failure. Dynamic collagen fiber realignment was quantified using cross- polarization imaging [4]. Percent relaxation was quantified for each stress-relaxation. Dynamic modulus and phase angle delta were computed for each frequency sweep at multiple strain levels. Fiber realignment and failure stress were computed during the ramp-to-failure. Samples for transmission electron microscopy (TEM) analysis of fibril structure (n=4) were fixed *in situ* [5]. Cross sections through the midsubstance of the patellar tendon were examined at 80 kV. Fibril diameter was measured using images from the center of the tendon. Student's t-test was used to compare groups for mechanical properties. A two-way ANOVA with post-hoc Bonferroni corrections was used to compare across groups and strains for collagen realignment.

Results

Induced deletion of *Bgn* showed decreased elastic modulus (Fig. 1A) at the tibial insertion and decreased max stress (Fig. 1B) when compared to WT. No differences were seen in

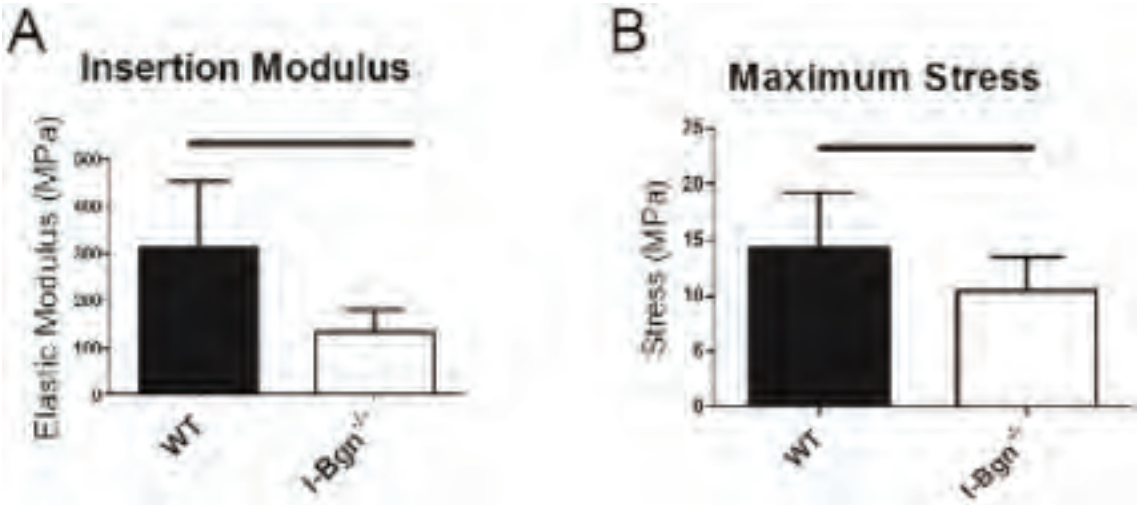

Figure 1. Quasi-static properties of WT and I-$Bgn^{-/-}$ patellar tendons.

midsubstance modulus or transition strain between groups. I-*Bgn*$^{-/-}$ showed decreased dynamic modulus at 3, 4 and 5% strain across all frequencies (Fig. 2). No changes were seen in tan(δ) or percent relaxation. I-*Bgn*$^{-/-}$ tendons exhibited an increase in realignment earlier in the insertion compared to WT (Fig. 3A). I-*Bgn*$^{-/-}$ tendons also displayed increased realignment between the toe and linear regions and greater linear region realignment in the insertion and midsubstance (B) compared to WT (Fig. 3). I-*gn*$^{-/-}$ mice exhibited greater heterogeneity in fibril diameter and larger diameter fibrils compared to WT mice (Fig. 4).

Discussion

Contrary to our hypothesis, acute deletion of *Bgn* expression in mature mice had a surprisingly large impact on tendon mechanics and structure 30 days after knockdown of *Bgn* gene expression. I-*Bgn*$^{-/-}$ tendons showed inferior material properties during quasi-static and dynamic loading in the toe, transition, and linear regions across different rates of loading. I-*Bgn*$^{-/-}$ tendons also displayed an early initiation of realignment in the insertion, a greater magnitude of realignment throughout the linear region, and the ability to continue fiber realignment later into the ramp-to-failure than WT tendons. TEM revealed changes in structure at the fibril level, where I-*Bgn*$^{-/-}$ tendons displayed an increase in fibril heterogeneity and overall diameter, indicating a dysregulation of fibrillogenesis. These results demonstrate a role for Bgn in maintaining tendon mechanical response across a diverse set of loading conditions, and structure across multiple scales. While Bgn is known to play major roles during tendon development and healing, it has been thought to play a relatively minor role in tendon homeostasis in adulthood. This is in contrast to decorin, another class I SLRP that comprises ~90% of tendon proteoglycans in the adult [6], which was hypothesized to have a large effect on tendon properties after knockout in mature tendons. Interestingly, and in direct contrast to the current findings, conditional knockout of decorin in a mature mouse in a similar study resulted in minimal changes in structure and function compared to WT [7]. One possible explanation could lie in the interaction these SLRPs have with each other and with the rest of the tendon matrix. While Bgn and decorin are thought to play similar roles in fibril crosslinking, they likely play different roles in collagen I fibrillogenesis, where decorin inhibits fibrillogenesis via binding of collagen molecules, while

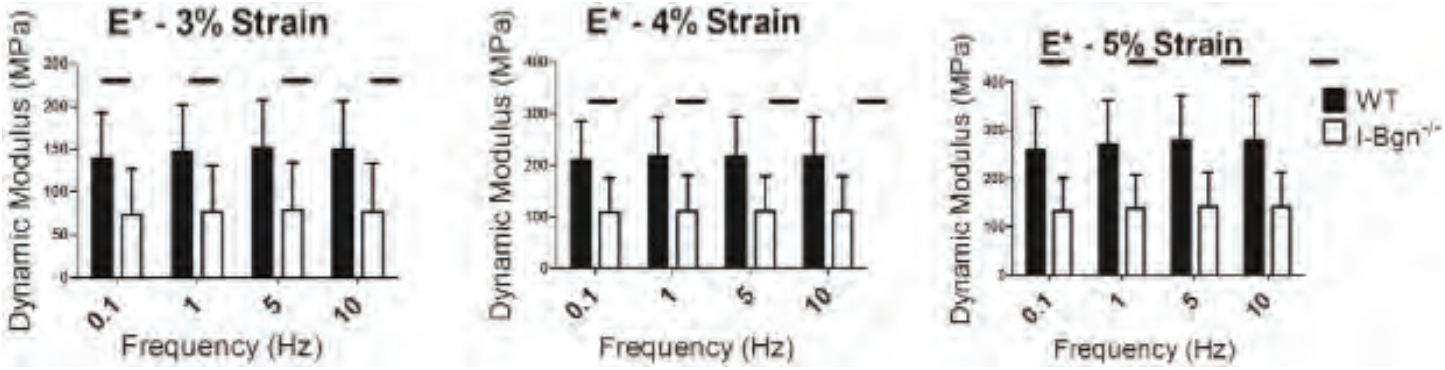

Figure 2. Viscoelastic properties of WT and I-*Bgn*$^{-/-}$ patellar tendons.

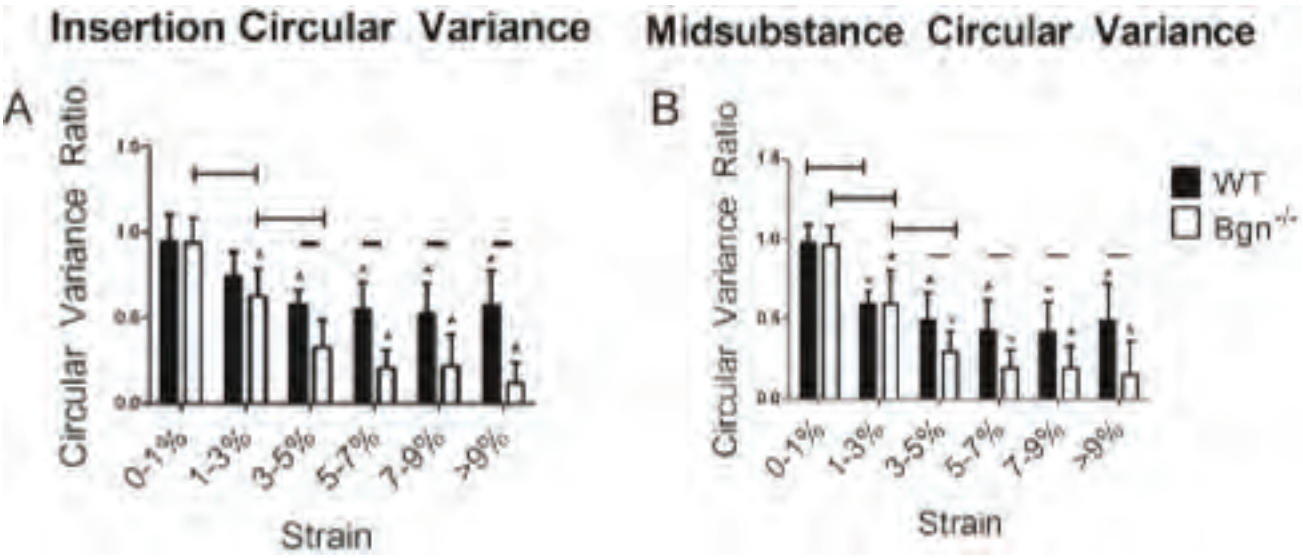

Figure 3. Realignment of WT and I-*Bgn*$^{-/-}$ patellar tendons.

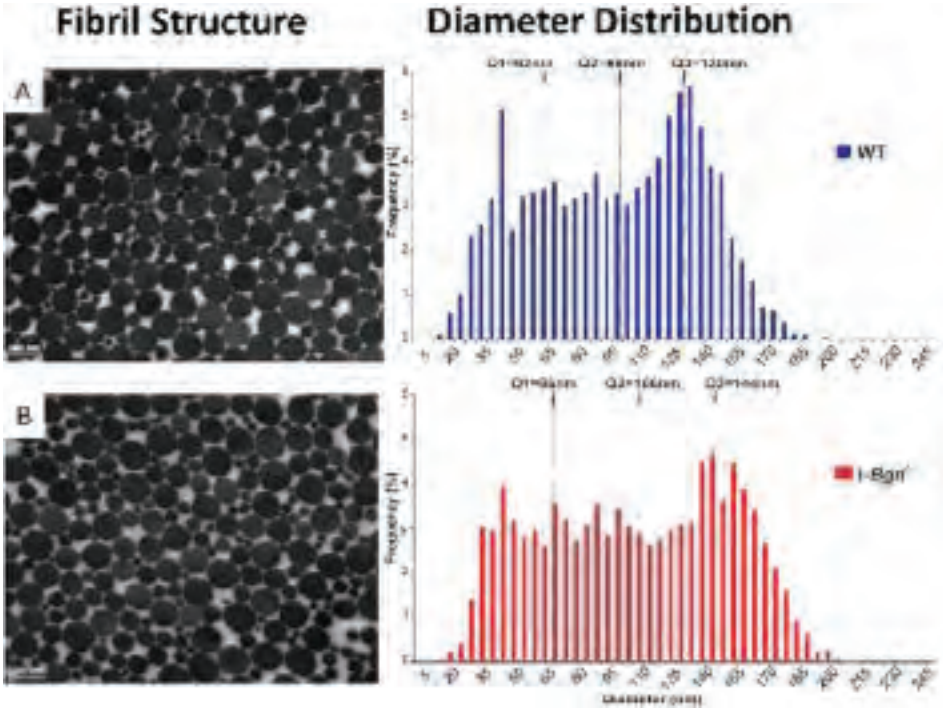

Figure 4. Transmission of electron microscopy analysis of WT and I-*Bgn*$^{-/-}$ patellar tendons.

Bgn does not [8]. Regardless, any compensatory changes that occur in these conditional mice will need to be evaluated in further studies to determine the mechanisms governing the changes demonstrated in this study. Overall, this study provides surprising evidence that Bgn plays an important role in tendon homeostasis.

Significance

This study demonstrates that biglycan plays an important role in mature tendon homeostasis. This knowledge will be used to better understand tendon matrix protein interactions throughout adulthood and aging.

Acknowledgements

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