Alterations in the Mechanical Properties of Patellar Tendons in Bone Sialoprotein-Null Mice

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Introduction

Bone sialoprotein (BSP) is a highly conserved multifunctional glycoprotein of the SIBLING family with a flexible structure and a variety of known roles, from cancer biology to tissue mineralization.[1,2,3,4,5] BSP is present in a variety of mineralized tissues such as bone, dentin, and hypertrophic cartilage and is highly expressed in sites of active bone formation, during both development and fracture healing.[2,3,4,5,6,7] BSP-null mice have been shown to exhibit a phenotype of lower body mass, shorter body length and shorter long bones.[4] Although BSP is known to be expressed in some non-mineralized tissues, its effects on tendon are unknown. Therefore, the objective of this study was to use the BSP-null mouse model to assess the role of BSP on the biomechanical properties of the patellar tendon. We hypothesized that the absence of BSP would result in smaller and biomechanically inferior tendons, particularly in locations where tendon inserts into bone.

Methods

Sample Preparation

Wild-type (n=15) and BSP-null (n=15) male mice at 15 weeks of age were used with IACUC approval. Following sacrifice, patella-patellar tendon-tibia complexes were carefully dissected and cross-sectional areas of each tendon were measured using a custom laser-based device. The anterior surface of each tendon was then speckle coated with Verhoeff's stain for local optical strain measurement. Specimens were then potted in custom fixtures using PMMA.

Mechanical Testing

Specimens were tested with the following protocol: 1) preload, 2) preconditioning, 3) stress-relaxation to 5% of gauge-length, 4) return to gauge length, 5) ramp to failure (0.1%/s).

Data Analysis

Linear region modulus was calculated using optical tracking software for each tendon in three tendon regions; at the patellar origin (patella-1mm distal to the patella), tendon mid-substance and tibial insertion (1mm proximal to tibial insertion).

Statistics

Comparisons between the two genotypes were made using Student's t-tests (significance at $p<0.05$).

Results

BSP-null tendons showed a larger cross-sectional area as well as a lower failure stress compared to the wild-type tendons (Figure 1A-B). The moduli showed no statistically significant difference between the BSP-null group and the wild-type. (Figure 2A-C). Failure load, stiffness as well as percent relaxation showed no statistically significant difference between the two genotypes (Figure 2D-F). Tendon failure modality was also analyzed with no significant difference between the two genotypes in location of tendon failure, although the number of tendons in each group was small when stratified in this manner (data not shown).

Discussion

Although the skeletal phenotype of the BSP-null mice is well defined, its role in tendon biology remains largely unknown. We have shown that despite the hypotrophic skeletal phenotype exhibited by BSP-null mice, their patellar tendons are significantly larger in cross-sectional area than wild-type. Given their larger size, one might expect these tendons to exhibit superior mechanical properties, yet they exhibited a failure stress significantly lower than wild-type tendons. Taken together, this set of data describes larger, yet mechanically inferior tendons. However, our data also show no significant effect of BSP knockout on tendon tissue moduli, as well as other parameters, suggesting a limited role for BSP in adult mouse tendon biology. BSP is known to possess a strong binding affinity for fibrillar type I and monomeric collagen in mineralizing tissues[2] and although it is not known to be expressed in tendons, it is possible that BSP also interacts with non-mineralizing type I collagen in tendons as they develop. Thus, knocking out BSP function may lead to mechanically inferior collagen formation or maturation during in-utero or post-natal tendon development, forcing compensatory mechanisms to grow larger tendons during this

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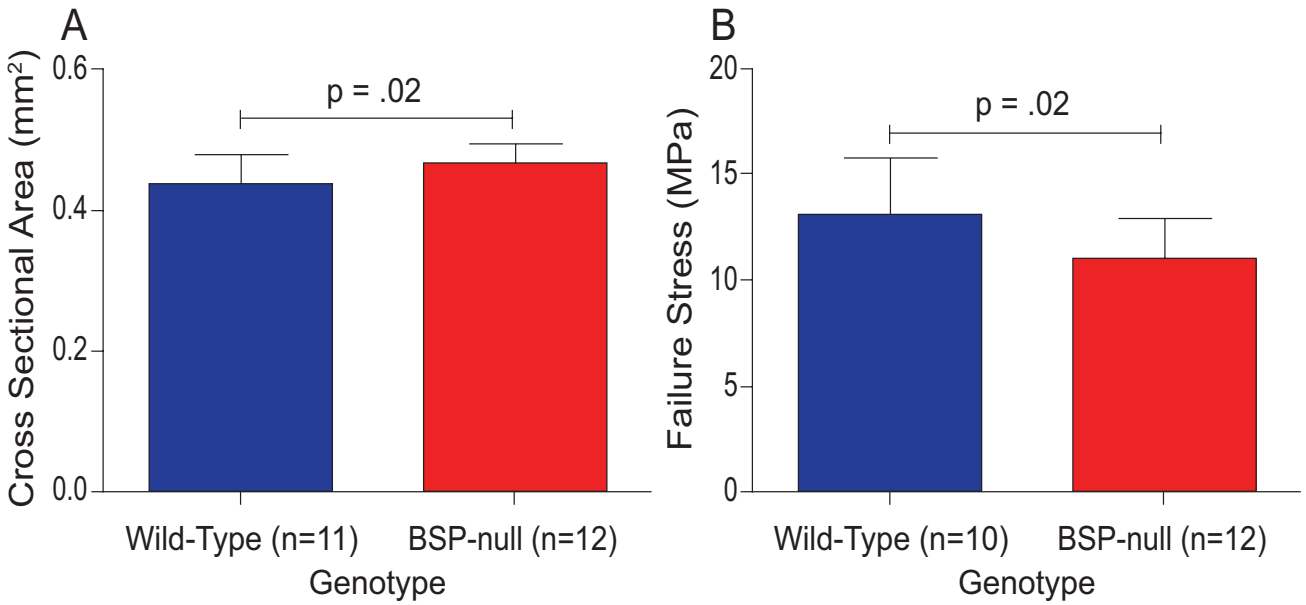

Figure 1. (A) Cross-sectional area was significantly increased in the BSP-null compared to wild-type mice. (B) Failure stress was significantly decreased in the BSP-null compared to wild-type mice. Results are presented as mean +− standard deviation with significance set at $p<0.05$.

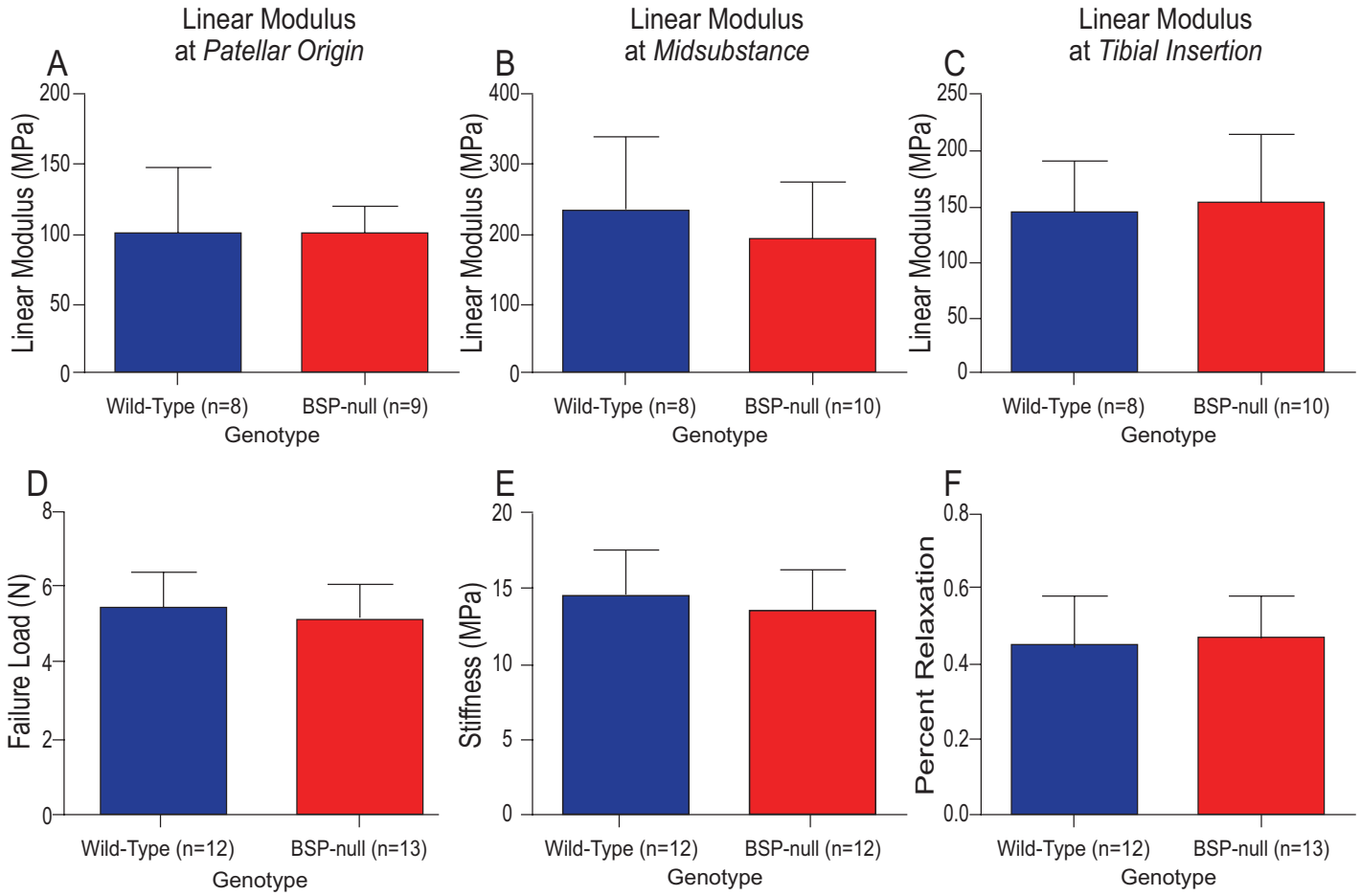

Figure 2. The linear modulus at (A) the patellar origin, (B) tendon mid-substance, and (C) tibial insertion showed no difference between BSP-null and wild-type mice. Similarly, (D) the load at failure, (E) stiffness, and (F) percent relaxation also showed no difference between BSP-null and wild type mice.

period. It is also possible that BSP plays no role or a minimal one in tendon biology and the decrease in some tendon mechanical properties can be attributable to a compensatory response to a significant skeletal phenotype. Surprisingly, there was no greater propensity for knockout tendons to fail at their entheses, given that BSP-null mice are known to have thinner cortices and a lower bone mineral density.[4] This likely indicates that, unlike its better-studied relative, osteopontin, BSP not only displays a more selective phenotype, but one that operates in a milieu with greater redundancy. Further study is necessary to characterize the contribution BSP makes during tendon development to the adult phenotype. In addition, an investigation of the histological properties of BSP-null tendons would shed light on the effect BSP may have on tendon cellularity, fibril morphology and enthesis histomorphology.

Significance

This study demonstrates that BSP likely plays a role in the structure and function of tendon. The multifunctional nature of BSP is highlighted by its continued study as, among others, a possible cancer marker as well as an osteoinductive agent for bone repair and osseous integration of implants[6,7,8]. This study illustrates a putative role for BSP in tendon biology as well as the importance of further study.

Acknowledgments

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