

# “Energy storing” tendon structure may vary significantly between small and large animal models

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**INTRODUCTION:** It is recognized that two distinct classes of tendons exist within humans: energy storing tendons (ESTs) that sustain high stresses and assist in locomotion, such as the Achilles or quadriceps tendons, and positional tendons (PTs) that have non-locomotive function and operate under much lower stresses whilst positioning limbs. Both energy storing and positional tendons are recognized to occur within large animal models (equine and bovine, for instance), with tendons from the two classes sustaining significantly different *in vivo* maximum stresses, and having differing structures and mechanics [1-3]. Tendons from small animal (rats, mice) are frequently used as tissue models for ESTs, to both explore structure-function relationships and development of mechanically-induced or mediated pathology or disease. Yet, how representative tendons from small animals are to ESTs from large animals has not been thoroughly examined. The present research aimed to determine structural differences and similarities between the most commonly used PTs and ESTs in three animal models: bovine, ovine, and rat.

**METHODS:** Superficial digital flexor and common digital extensor tendons were dissected from adult bovine and ovine forelimbs, while Achilles and tibialis anterior tendons were dissected from the hindlimbs of adult rats. There were 5 tendon pairs for each model, totaling 10 samples per animal and 30 tendons in total for each method of testing. Tendons were structurally evaluated by four methods: (i) Hydrothermal Isometric Tension (HIT) analysis, used to evaluate molecular stability and intermolecular crosslinking via denaturation temperature ( $T_d$ ) and the temperature of maximum force ( $T_{max}$ ); (ii) sodium borohydride reduction with HIT (NaBH<sub>4</sub>HIT), used to examine intermolecular network connectivity via half-time of load decay ( $t_{1/2}$ ); (iii) Transmission Electron Microscopy, used to evaluate collagen fibril diameter; and (iv) Scanning Electron Microscopy (SEM), used to characterize interfibrillar webbing within the fascicular collagen matrix. Statistical analyses were performed using JMP.

**RESULTS:** In HIT, all three models displayed distinct differences between tendon types, with PTs having lower  $T_d$  than ESTs in both bovine and ovine but higher  $T_d$  in rat ( $p=0.0025$ ,  $p=0.001$ , and  $p=0.0090$ , respectively) (Figure 1A). Similarly, the PTs had a lower  $T_{max}$  than ESTs in bovine and ovine but there were no significant differences between the two in rat ( $p=0.0367$ ,  $p=0.0216$ ) (Figure 1B). Distinctions were also found across models: both bovine and ovine ESTs were found to have significantly higher  $T_d$  than rat ESTs ( $p=0.0001$ ), and bovine PTs had higher  $T_d$  than the rat PTs ( $p=0.0001$ ). Additionally, bovine and ovine ESTs were found to have a higher  $T_{max}$  than the rat ESTs ( $p=0.0112$ ,  $p=0.0112$ ). There was no difference found in the  $T_{max}$  of the PTs across species. In NaBH<sub>4</sub>HIT, there were distinct differences in  $t_{1/2}$  between control and treatment groups of PTs in all three models: bovine, ovine, and rat ( $p=0.0216$ ,  $p=0.0122$ ,  $p=0.0122$ ). A significant difference also existed between control and treatment groups for the rat ESTs, but this difference did not exist in the other two models ( $p=0.0122$ ). TEM results to date show significant differences in fibril diameter between the PTs and the ESTs in all three models (Figure 1C). Between models, the bovine and ovine PTs showed similar fibril diameters. For ESTs, tendons from all three animal models had similar fibril diameters. In SEM, differences in the abundance of interfibrillar webbing existed between tendon types and across models (Figure 1D). Bovine and ovine ESTs contained more interfibrillar webbing than PTs, while rat ESTs and PTs contained similar levels of webbing.

**DISCUSSION:** The current results indicate that class-based differences between tendon structure in ESTs and PTs varies between animal models, with structures in rat tendons showing differences to those present in large animals. These difference likely influence how these tendons respond to mechanical treatment, and how damage is accrued as the result of excessive load.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Numerous studies have used rats as a model to examine mechanically-induced or mediated tendon pathology. The present study has found that the tendons typically used to represent ESTs in rats differ significantly to those ESTs usually studied in large animals. Knowledge of such differences is important when considering the translatability or applicability of tendon research to humans.

**REFERENCES:** [1] Ker & Bennett. *Journal of Zoology* 216, 309–324 (1988). [2] Thorpe et al. *Journal of The Royal Society Interface* 9, 3108–3117 (2012). [3] Herod et al. *Acta Biomaterialia* 42, 296–307 (2016).

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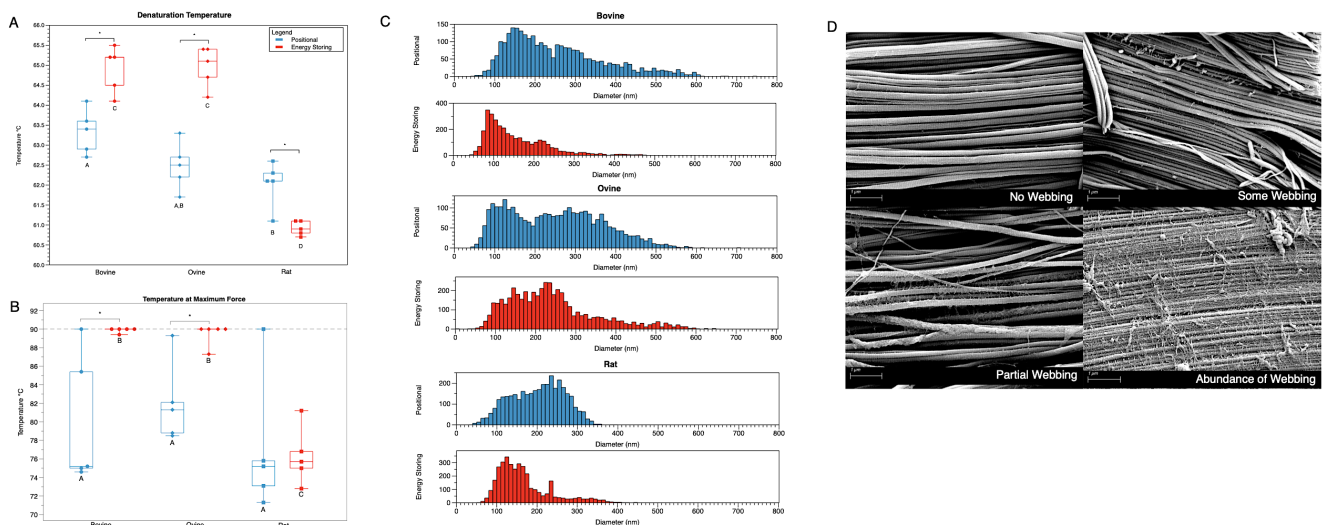


Figure 1: Three structural assessments of the positional and energy storing tendons of bovine, ovine, and rat models. (A) HIT based structural assessment using denaturation temperature. (B) HIT structural assessment using temperature at maximum force, the dashed line represents the maximum possible temperature for this analysis. (C) Fibril diameter assessment performed by using images from Transmission Electron Microscopy. (D) Images of ovine tendon taken on a Scanning Electron Microscope at 15,000X magnification. Images are representation of the four categories of webbing that the tendon sites were placed into: no webbing, some webbing, partial webbing, and an abundance of webbing.