

## LOCATION DEPENDENT MECHANICAL BEHAVIOR OF APONEUROSIS TISSUE UNDER UNIAXIAL TENSILE STRETCH

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### INTRODUCTION

Aponeurosis is a connective tissue that attaches muscle fibers to tendon [1]. The aponeurosis covers the surface of a muscle in a sheath-like manner and fans out with decreasing thickness away from tendon [2]. Although aponeurosis is commonly found in muscle-tendon units throughout the body, there have been comparably few studies on the mechanical role of aponeurosis and fewer still on the material properties of aponeurosis. Specifically, there are no reported investigations on the interaction of aponeurosis thickness, distance from the tendon, and mechanical response to load. Previous studies measured material properties of aponeurosis samples assuming that there is a constant thickness throughout the tissue, when it is known to be variable [2-5].

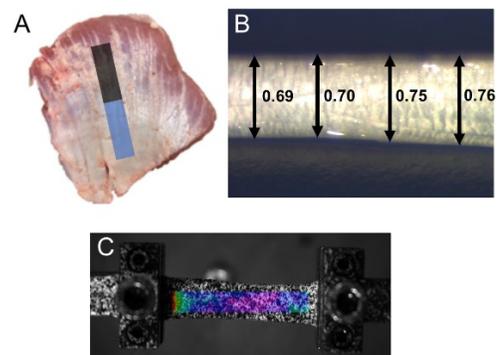
This unknown relationship between aponeurosis regional structure and function makes aponeurosis tissue a poorly understood component of muscle-tendon units. For example, it remains unclear if aponeurosis acts as a material extension of tendon, or if it transitions from a higher or lower stiffness near the tendon to a different stiffness near the muscle midbelly. Computational models regularly neglect aponeurosis tissue or aponeurosis variability due to lack of information, rather than confidence in assumptions. The goal of this work is to perform uniaxial tensile testing and digital image correlation analysis to measure the mechanical response of aponeurosis tissue as a function of thickness and location from tendon to muscle. We hypothesize that aponeurosis tissue maintains consistent material properties, as measured by stress-strain curves, as it transitions from tendon to muscle.

### METHODS

Triceps brachii tissue was extracted from porcine shoulder for testing (Figure 1A). The aponeurosis was separated from the muscle tissue using standard dissection tools such as scalpel and forceps. Ten

(n=10) samples were then cut into rectangular strips measuring ~60 mm in length and ten mm in width with custom dissection equipment, with fibers oriented longitudinally. Five mm increments were marked on each sample with permanent marker. Each sample was placed under a dissection microscope with the marked edge facing up. Four evenly spaced thickness measurements were taken between each marking in the middle 40 mm, for a total of 32 total thickness measurements for each sample (Figure 1B).

Uniaxial tensile testing was completed on a custom planar biaxial material testing system (ADMET, Inc., Norwood, MA). Ten mm sections at both ends of the sample were clamped with serrated grips and the strain rate for the tensile test was set to  $0.03 \text{ min}^{-1}$  elongation to failure. A charcoal powder was lightly dusted onto the sample through a sifter for digital image correlation (DIC) (Figure 1C).



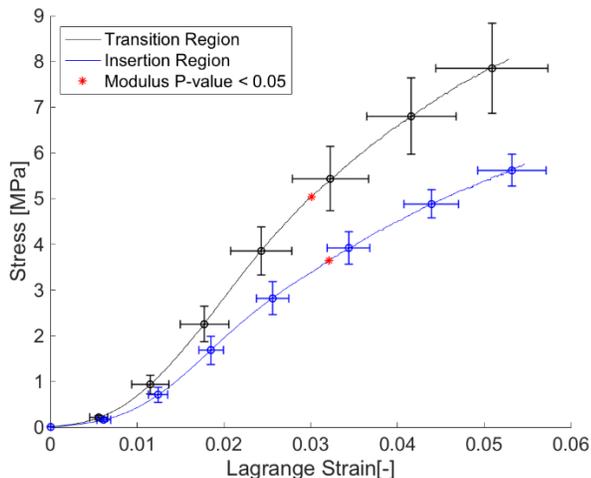
**Figure 1.** A) Triceps brachii muscle with attached aponeurosis and representative sample regions (black – transition region, blue – insertion region) [2] B) dissection light microscope image for

measuring thickness [mm] C) rectangular sample used for tensile testing, including charcoal speckle pattern and representative DIC analysis strain color contour plot.

Image capture rate was at an interval of 0.5 seconds. Using the uniaxial force data, inhomogeneous 2D DIC data, and thickness measurements, the average nominal (engineering) stress and Lagrange strain values were determined for two sections of each sample, one considered the 'transition region', where the aponeurosis tissue connects to muscle fibers and is thinner, and the other considered the 'insertion region', where the aponeurosis tissue connects to the tendon tissue and is thicker. Linearized moduli were determined at each time point by dividing nominal stress by Lagrange strain of both regions. Paired t-tests ( $p < 0.05$ ) were performed on the Lagrange strain and linearized modulus at each time point to determine any statistically significant differences in the material behavior of tissue of varying thickness and location.

## RESULTS

The average nominal stress and Lagrange strain data for both regions showed a nonlinear response, with an initial toe-region, a linear region, and a softening region (Figure 2). The transition region had an average thickness of  $0.703 \pm 0.073$  mm, and the insertion region had an average thickness of  $0.952 \pm 0.061$  mm. The Lagrange strain t-tests remained above  $p=0.05$  for each data point. At 131 seconds, indicated by a red asterisk in Figure 2, the p-value of the linearize modulus t-test drops below  $p=0.05$  and remains below that threshold until the test is complete at 200 seconds (Figure 3).

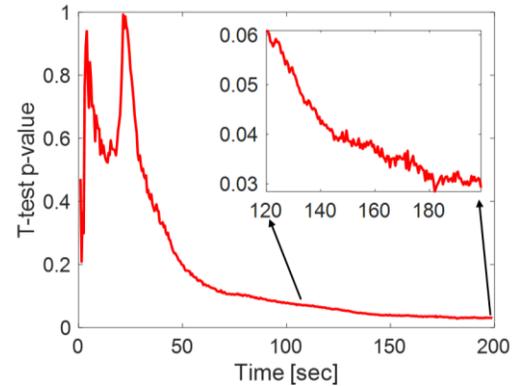


**Figure 2.** Average stress-strain curves for each quarter of aponeurosis tissue samples. Error bars in Figure 2 are shown at time points with intervals of 55 seconds.

## DISCUSSION

Previous studies have investigated the material properties of aponeurosis tissue assuming an average thickness across samples, while this study investigated how variations in thickness may affect stress-strain behavior. Aponeurosis thickness measurements from this study showed a decrease in thickness from the tendon insertion region to the muscle transition region. This varying thickness may be a function of the inhomogeneous load the aponeurosis bears *in vivo*. A paired t-test of the strain of the two regions indicated that there is no significant difference in the strain of an aponeurosis sample at different thicknesses. However, a paired t-test of the linearized modulus shows

that the moduli of the regions may diverge as the stress increases over time (Figure 3). Specifically, the transition (thinner) region exhibited a stiffer stress-strain response than the insertion (thicker) region, disproving our hypothesis. This suggests that under load, aponeurosis may exhibit higher moduli in thinner sections.



**Figure 3.** P-values of t-test comparing insertion and transition region linearized modulus (stress/strain) versus time.

When working with and analyzing the mechanical properties of many biological materials, including aponeurosis tissue, it is important to consider how natural variations in the material may affect the observed behavior. The collagen fibers in aponeurosis are likely to have some inhomogeneous alignment and/or crimping, which is shown in the existence of a toe region at the beginning of stress-strain curves (Figure 2). Additionally, individual collagen bundles are likely to have varying geometry, meaning they experience different stresses under the same force or uncrimp and fail at different loads and strains, and may naturally have different stiffness. This study assumed that there were consistent material properties and thicknesses across the width and thickness of the sample, with variations only occurring lengthwise, when the DIC imaging showed varying strain measurements in both directions (Figure 1C). Future work to better characterize the stress-strain response of the two regions, such as with tangent moduli or hyperelastic modeling would benefit this work.

## CONCLUSIONS

Variations in aponeurosis tissue thickness and material properties are likely to affect muscle-tendon unit mechanics and subsequent modeling efforts [3]. This work suggests thinner aponeurosis regions near the muscle midbelly may exhibit a stiffer stress-strain response than regions closer to the tendon. The mechanical consequences of this are not known but could impact the function of muscle-tendon units. These data help to create a more complete understanding of the material properties of aponeurosis, with the goal of improving future computational studies of muscle-tendon unit mechanics.

## ACKNOWLEDGEMENTS

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