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Novel Application of a μCT Perfusion Technique to Evaluate Achilles Tendon Vessel Microarchitecture in Three Dimensions

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Introduction

Regional variation in Achilles tendon vascularity is believed to contribute to mechanical property deficits, tendinopathy, and rupture; however, this remains controversial.[1] This disagreement likely arises since previous studies using laser Doppler flowmetry, angiography, or histology cannot quantify vessel architecture (i.e., orientation, anisotropy, and topology) with micron resolution in three dimensions. Therefore, the purpose of this study was to demonstrate the reliability of a new perfusion-based μCT laboratory technique, and to quantify Achilles tendon insertion and midsubstance vessel architecture. We hypothesized that the μCT perfusion method would be reliable between limbs, and that the Achilles tendon midsubstance would demonstrate decreased vessel volume, number, and thickness, but increased spacing, anisotropy, and connectivity relative to the insertion.

Methods

18 limbs from 9 female rats were used. A radiopaque perfusion medium (Microfil, Flow Tech) was used to generate vasculature corrosion casting. Following perfusion, the Achilles and plantaris tendons, surrounding soft tissue, and calcaneus were harvested *en bloc*. These tissues were placed in a 7mm diameter tube filled with 1xPBS within a μCT scanner (μCT 35, Scanco Medical AG, Switzerland) and two separate 1.6mm regions representing the insertion and midsubstance were scanned (isotropic voxel size: 3.5μm) (Figure 1A). Vessel volume, thickness, number, spacing, connectivity, and anisotropy were obtained using manufacturer-provided software. Paired T-tests were utilized to evaluate differences between limbs and between insertion and midsubstance vessel architecture ($\alpha = 0.05$). For the latter statistical tests, the two limbs for each animal were averaged together.

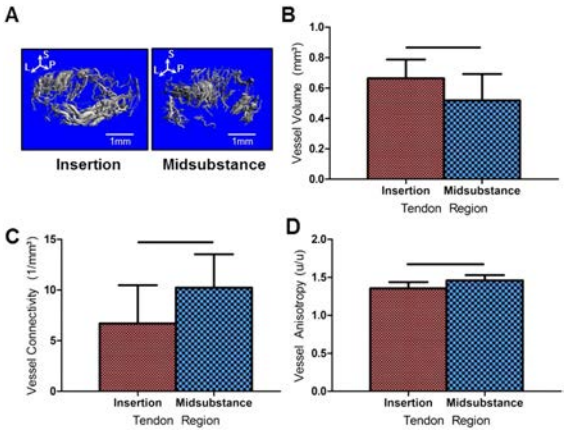

Figure 1. (A) 3D reconstructions of Achilles tendon vessel architecture comparing the insertion and midsubstance regions. L, P, and S indicate the left, posterior, and superior axes of the μCT. Compared to the insertion, vessel volume decreased (B), vessel connectivity increased (C), and vessel anisotropy increased (D) in the midsubstance. Error bars indicate standard deviation and lines indicate significant differences.

Results

There were no differences between limbs for any of the parameters computed when compared within regional subgroups. Compared to the insertion, vessel volume decreased, and connectivity and anisotropy increased in the midsubstance ($p < 0.01$) (Figure 1 B–D). No differences were observed in vessel thickness, number, or spacing between regions.

Discussion

This study provides the first analysis of Achilles tendon microarchitecture in 3D. This work is consistent with previous 2D histological and angiographic studies, and adds to previous research by quantifying regional differences in vessel anisotropy and connectivity. Together, these parameters provide a mechanism that could help explain the specific changes that contribute to tendinopathy and rupture observed in the Achilles tendon midsubstance.

Acknowledgement

This study was supported by the NSF GRFP and NIH/NIAMS.

References

1. Theobald et al. Review of the vascularisation of the human Achilles tendon. *Injury, Int. J. Care Injured,* (36)1267-1272 (2005).