Estrogen Deficiency and Intermittent Parathyroid Hormone Treatment Affect Regional Achilles Tendon Vessel Microarchitecture

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Introduction

The Achilles tendon is frequently injured and vascularity has been implicated as a predictor of Achilles tendon injury and healing potential[1]. Estrogen deficiency and intermittent parathyroid hormone (iPTH) treatment have been shown to differentially affect tendon healing response[2-7]. In rat models of tendon injury, estrogen-deficiency results in decreased Achilles tendon mechanical properties[3], while iPTH treatment increases fibrocartilage formation near the tendon insertion during the healing response[2,6]. Despite the significant effects that estrogen deficiency and iPTH treatment have shown on the tendon healing response in animal models, there has been little research investigating their effect on vascularity of the Achilles tendon. Therefore, the objective of this study was to evaluate how estrogen deficiency and iPTH treatment modulate vessel microarchitecture in a rat Achilles tendon. We hypothesized that estrogen deficiency, simulated by bilateral ovariectomy surgery (OVX), would cause a decrease in Achilles tendon vessel microarchitecture throughout the length of the tendon, while iPTH treatment would result in increased tendon vessel microarchitecture, particularly near the insertion.

Methods

Study Design

At 3 months of age, female Sprague-Dawley rats (n =14) were divided into three groups (IACUC approved): VEH (n = 4), iPTH (n = 3), and OVX (n = 7). The OVX rats received OVX surgeries at 3 months of age to simulate estrogen deficiency for 4 weeks. At 3.5 months of age, VEH and iPTH rats received subcutaneous injections of saline solution and iPTH (PTH 1-34, 60μg/kg/day, Bachem, Bubendorf, Switzerland), respectively for 5 days a week for 2 weeks.

Vascular casting

At 4 months of age, a vascular casting procedure was performed by infusion of Microfil mixture (MV122, Flow Tech Inc., Carver, MA) in the rat vascular network as described[8-10]. Briefly, 50 mL heparin sodium solution, followed by 100 mL 0.9% normal saline and 50 mL 4% PFA into the abdominal aorta at 4.4 mL/min via a perfusion pump (Bio-Rad, Hercules, CA) while the animals were under anesthesia. A syringe was used to inject 5 mL Microfil® mixture with 3% catalyst at 0.3 mL/min and the animals were stored at 4°C for 24 hours to allow complete polymerization. Afterwards, both the left and right Achilles tendons were harvested and μCT-scanned at 3.5μm voxel size (μCT 35, Scanco Medical AG, Brüttisellen, Switzerland) at a 1.6 mm region of the tendon insertion proximal to the calcaneus and another 1.6 mm long region near the midsubstance of the tendon, 3.6 mm proximal from the end of the insertion region (Figure 1). A custom MATLAB (Mathworks, Natick, MA) script was used to apply a local thresholding technique to segment casted blood vessels from surrounding soft tissue[11]. Finally, the vascular microarchitecture parameters vessel volume (VV), vessel number (Ves.N), vessel thickness (Ves.Th), vessel separation (Ves.Sp), and connectivity density (Conn.D) were evaluated.

Analysis

Separate two-way ANOVAs for tendon region and treatment were performed comparing VEH and iPTH, and VEH and OVX. If the ANOVAs determined a significant effect ($p < 0.05$),

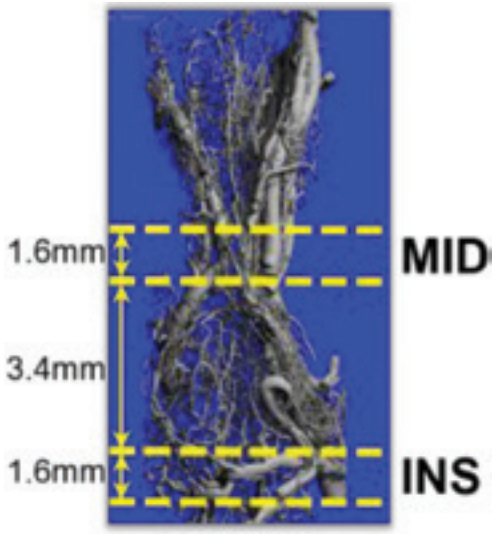

Figure 1: Achilles tendon vascularity, denoting insertion and midsubstance region.

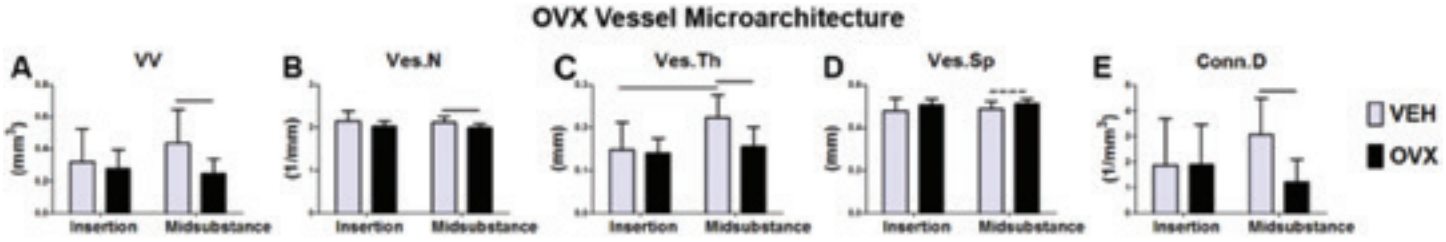

Figure 2. OVX vessel microarchitecture comparisons to the VEH group for VV **(A)**, Ves.N **(B)**, Ves.Th **(C)**, Ves.Sp **(D)**, and Conn.D **(E).** OVX shows detrimental effects on Achilles tendon vascular microarchitecture, particularly in the midsubstance region.

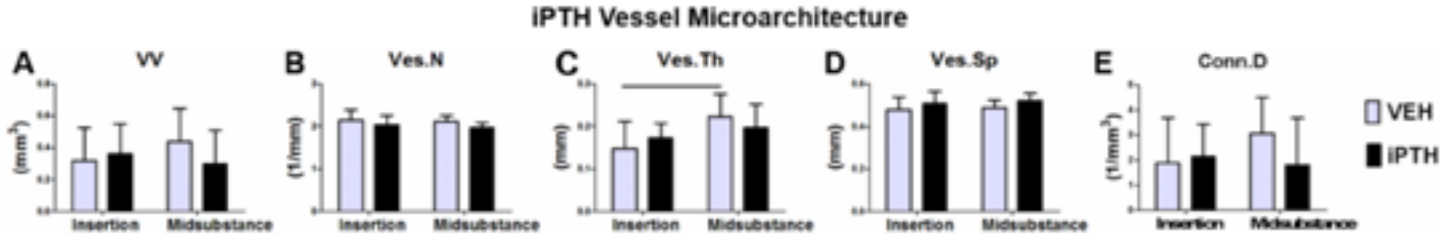

Figure 3. iPTH vessel microarchitecture comparisons to the VEH group for VV **(A)**, Ves.N **(B)**, Ves.Th **(C)**, Ves.Sp **(D)**, and Conn.D **(E)**. iPTH treatment shows no differences compared to the VEH group except for eliminating the difference in Ves.Th between the insertion and midsubstance.

Student's t-tests were performed to compare region and/or treatment between specific groups. Significant interaction terms were also evaluated.

Results

When comparing VEH-OVX, treatment was a significant factor in VV, Conn.D, Ves.N, and Ves.Th, while region was a significant factor in Ves.Th. There was a significant interaction term for Conn.D. Further, the midsubstance region of the OVX tendons had significantly lower VV, Ves.N, Ves.Th, and Conn.D, with a trend toward greater Ves.Sp relative to the VEH group (Figure 2). In addition, Ves.Th was significantly greater in the midsubstance relative to the insertion in the VEH tendons. When comparing parameters for VEH-iPTH, there were significant effects of region on Ves.Th. However, there were no significant differences between the VEH and iPTH groups (Figure 3).

Discussion

We investigated the effects of estrogen deficiency and iPTH treatment on vascular microarchitecture in the rat Achilles tendon. As hypothesized, OVX resulted in reduced vascular microarchitecture of the Achilles tendon, with the most profound effects in the tendon midsubstance. While OVX has limited effects on rat Achilles tendon homeostatic function[4], it has significant detrimental effects on Achilles tendon healing response, resulting in decreased mechanical properties, including reduced max stress and secant modulus during fatigue loading, as well as decreased joint range of motion, cell proliferation, and GAG content[3,5]. As vascularity has been implicated as a predictor of healing potential[1], the reduced Achilles tendon vessel microarchitecture observed in this study provides a potential explanation for the reduced healing potential seen in estrogen-deficient Achilles tendons. Contrary to our hypothesis, iPTH treatment did not have a drastic effect on vessel microarchitecture. In previous studies in bone, iPTH treatment did not result in osteoangiogenesis but rather relocated the vascular structure closer to the sites of new bone formation, thereby providing a favorable microenvironment for growth[12]. It may be possible that a similar effect happens in tendon, though it is also possible that the increased fibrocartilage formation in the tendon insertion observed previously is specific to the healing response[2,6]. Further studies should evaluate the effects of estrogen deficiency and iPTH treatment on vascular microarchitecture in an Achilles rupture model.

This study highlights estrogen deficiency and iPTH treatment effects on vascular microarchitecture in the rat Achilles tendon. The decrease in tendon vascular microarchitecture in the estrogen-deficient rats could be a possible explanation for the reduced healing potential with estrogen deficiency.

References

1. Theobald P *et al.*, *Injury*, 2005.
2. Hettrich CM *et al.*, *J Orthop Res*, 2012.
3. Fryhofer GW *et al.*, *J Appl Phsiol*, 2016.
4. Pardes AM *et al.*, *Ann Biomed Eng*, 2016.
5. Circi E *et al.*, *Int Orthop*, 2009.
6. Duchman KR *et al.*, *J Shoulder Elbow Surg*, 2016.
7. Lee DJ *et al.*, *J Orthop Res*, 2015.
8. Wang Y *et al.*, *J Clin Invest*, 2007.
9. Razavi H *et al.*, *Microvasc Res*, 2012.
10. Duvall CL *et al.*, *Am J Physiol Heart Circ Physiol*, 2004.
11. Waarsing JH *et al.*, *J Bone Miner Res*, 2004.
12. Prisby R *et al.*, *J Bone Miner Res*, 2011.