EFFECT OF REPETITION RATE ON BLOOD VESSEL FORMATION IN THE PARATENON OF A REPETITIVELY LOADED TENDON IN VIVO

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INTRODUCTION:

Soft tissue injuries are common in athletes and workers whose job functions require the use of repetitive high force hand activities. The exact mechanisms leading up to tendinopathy is unknown but both biomechanical and biochemical factors play a significant role.

Previously Vascular Endothelial Growth Factor, VEGF, had been found in human biopsies of degenerated tendons and in cyclically strained fibroblast cell cultures (1). Recently, this protein was shown to increase with loading (2) using a repetition rate of 60 reps/min, where the highest levels were found in the outer regions of the tendon, next to the paratenon. VEGF is responsible for stimulating the proliferation of microvascular endothelial cells and inducing angiogenesis. The purpose of this study was to evaluate changes in blood vessel number in the paratenon of the FDP tendon at the epicondyle following *in vivo* cyclical finger loading at different repetition rates (10 versus 60 reps/min), but the same force and duty cycle using a rabbit model.

METHODS:

The animal loading model was described previously (2) with the use of 60 reps/min. To summarize eight female, New Zealand White rabbits weighing 3.58 kg (± 0.84) were used. Under general anesthesia, the FDP muscle on one limb was electrically stimulated with a train of pulses at 0.17Hz (10reps/min), with a train duration of 1200ms and a pulse rate of 100 pulses/s. The stimulation voltage was adjusted [6-12V] to maintain a peak fingertip force of 0.42N (15% of P_o). The muscle was to contract repetitively for 2 hours per day, 3 days a week, for 14 weeks (80h of cumulative loading) at a rate of 10 reps/min. The contralateral limb, although restrained in the same manner as the loaded limb, did not receive a stimulus and as a result served as the control. This study was approved by the University's Committee on Animal Research.

At 14wks, the animals (4.00 ± 0.55kg) were euthanized and the epicondyles were processed identical to the protocol used in a previous study (2). Sections were stained with Safranin O, Fast Green and Iron Hemotoxlin. Image analysis involved digitally photographing the paratenon at 200x from the enthesis to the muscle (Figure 1) and manually counting blood vessels and categorizing them as an arteriole or capillary. A capillary was identified if it was smaller than 10 µm in diameter. Tissue preparation and image analysis was performed blinded to loading status. A mixed model repeated measures ANOVA was used to analyze differences in vessel density by limb loading status.

RESULTS:

The 10 repetitions/min group had an average paratenon area of 0.64mm^2 in the loaded tendon and 0.63mm^2 in the unloaded tendon. There were 4.3 capillaries/mm^2 and 15.7 arterioles/mm^2 in the paratenon of the loaded tendon and 9.7 capillaries/mm^2 and 18.0 arterioles/mm^2 in the paratenon of the unloaded tendon. The 60 repeitions/min group had an average paratenon area of 0.81mm^2 in the paratenon of the loaded tendon and 1.54mm^2 in the paratenon of the unloaded tendon. There were 7.1 capilarries/mm^2 and 14.8 arterioles/mm^2 in the paratenon of the loaded tendon and 8.6 capilarrries/mm^2 and 18.0 arterioles/mm^2 in the paratenon of the unloaded tendon. There was no significant differences between unloaded and loaded tendons of the same loading group ($p > 0.05$).

DISCUSSION:

The repetition rate of our loading model was reduced to understand the effects it has on tendon degeneration, particularly neovascularation. Previous *in vivo* loading models have shown an increase in both paratenon area and number of capillaries (3) and an increase in angiogenic factors (2,4) with loading. The biggest changes that were found for VEGF, a main angiogenic component, at 60 reps/min were located on the outer edges of the tendon, which is adjacent to the paratenon (2).

Although previously it was found that loading increased angiogenic components (VEGF, VWF, etc,) with loading, there are no vessel changes in the paratenon associated with our model of *in vivo* loading at 80 hours. It may indicate that the onset of capillary formation is further downstream in the disease progression of tendinopathy.

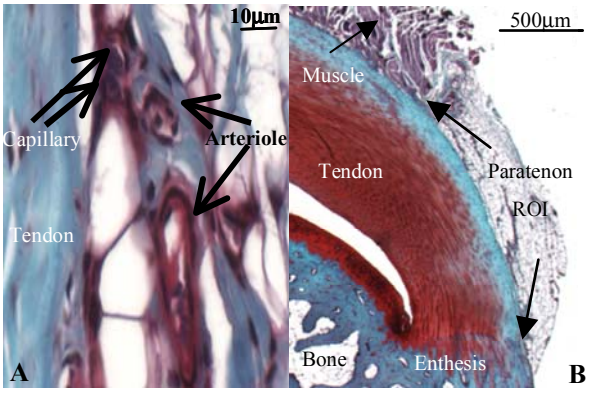

Figure 1 (A) Capillaries and arterioles shown within paratenon. (B) Paratenon shown with surrounding bone, tendon and muscle tissue. The region of interest is between the enthesis and the tendon-muscle junction.

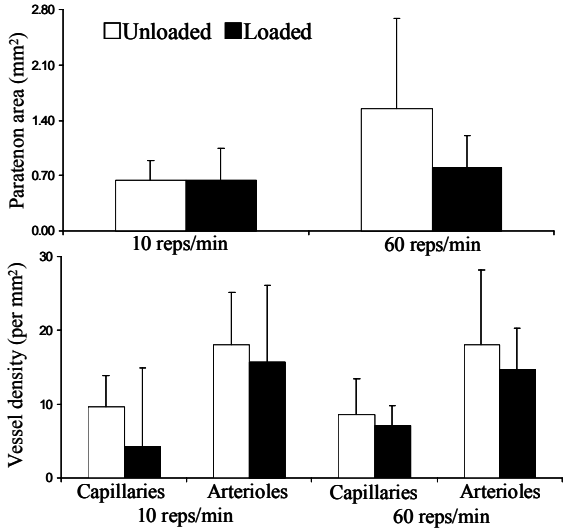

Figure 2. Graphs showing paratenon area, capillary and arteriole density in loaded and unloaded limbs loaded at 10 and 60 repetitions/min.

The load applied to the finger was well within the physiologic range of the muscle and the number of repetitions and the duration of loading are less than that experienced by athletes and workers who perform repeated tasks.

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