THE EFFECT OF LOADING RATE ON VEGF, VEGFR-1 AND CTGF PRODUCTION IN AN IN VIVO CYCLICALLY LOADED TENDON

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INTRODUCTION:

Soft tissue injuries are common in athletes and workers whose job functions require repetitive, high force hand activities. The exact mechanisms leading to tendinopathy are unknown but both biomechanical and biochemical factors play an important role.

VEGF has been found in human biopsies of degenerated tendons and in cyclically strained fibroblast cell cultures, indicating that it may play a role in overuse injuries leading to tendon degeneration (1). One of the main receptors for VEGF, VEGFR-1, which has the highest affinity for VEGF, has been shown to be up-regulated during angiogenesis and hypoxic conditions (2). Another growth factor in tendons, connective Tissue Growth Factor (CTGF), is a potent inducer of extracellular matrix synthesis (3) and is up-regulated by tensile stresses (4,5). Recently (6), these proteins were shown to increase in cyclically loaded tendons exposed to repetition rates of 60 reps/min.

The purpose of this study was to evaluate changes in VEGF, VEGFR-1 and CTGF cell density in the FDP tendon at the epicondyle following *in vivo* cyclical finger loading at a lower repetition rate (10 reps/min), but the same force and duty cycle, using a rabbit model.

METHODS:

The animal loading model was described previously (6) 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 (6). Image analysis involved digitally photographing six areas of interest at 200x: 3 areas along the enthesis (classified as inner, center and outer) and 3 corresponding areas 1500µm distal to the enthesis. The inner area is that part nearest the bone. Positive staining cells were manually counted in each region (200x400µm^2) and normalized by the area observed (Figure 1). Tissue preparation and cell counting was performed blinded to loading status. A mixed model repeated measures ANOVA was used to analyze differences in cell density by region and by limb loading status. Post hoc analysis was performed using the Tukey method for multiple comparisons.

RESULTS:

The percent changes in cell densities between 10 and 60 reps/min for VEGF, VEGFR-1 and CTGF are shown in Figure 2. At 10 reps/min, VEGF cell densities ranged from 563 to 1292 cells/mm^2 in the loaded tendon and 586 to 1140 cells/mm^2 in the unloaded tendon. VEGFR-1 cell densities ranged from 504 to 800 cells/mm^2 in the loaded tendon and 478 to 860 cells/mm^2 in the unloaded tendon. CTGF cell densities ranged from 668 to 1249 cells/mm^2 in the loaded tendons and 580 to 1219 in the unloaded tendons. For all three proteins, the loaded tendons did not have significantly greater cell densities than the unloaded limbs ($p = 0.62, 0.76, 0.89$, respectively), but regional effects were found ($p < 0.001$). For all three proteins the limbxregion interaction terms were not significant ($p = 0.71, 0.89, 0.77$, respectively).

DISCUSSION:

Previously, we observed that 60 rep/min increased VEGF, VEGFR-1 and CTGF cell densities. In this experiment, 10 rep/min , with the same peak force and duty cycle, led to no change in VEGF, VEGFR-1 and CTGF cell densities. This finding illustrates that if forces (15% MVC, 20% duty cycle) are held constant, repetition rate has an important, independent effect on these growth factors.

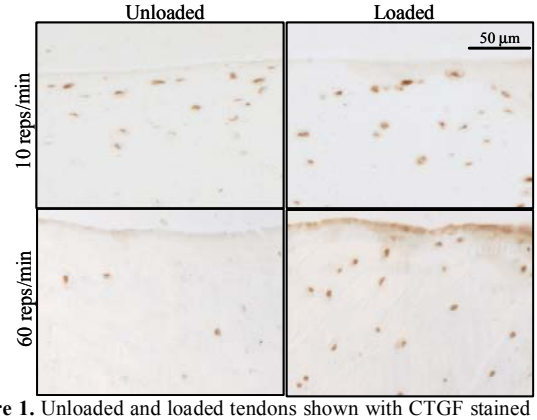

Figure 1. Unloaded and loaded tendons shown with CTGF stained cells at 10 and 60 reps/min.

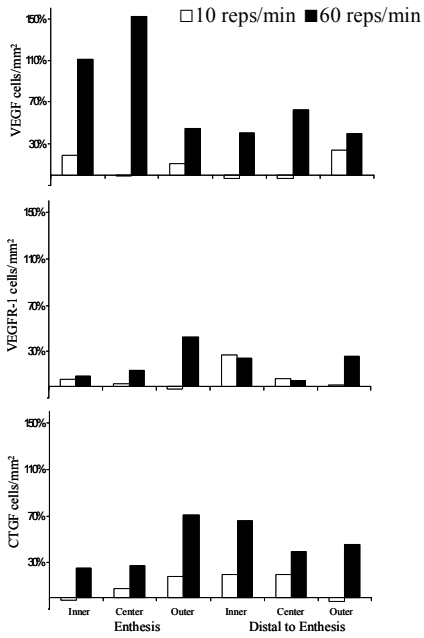

Figure 2. Percent changes in cell densities between loaded and unloaded tendons at different repetition rates.

Regional differences in cell density were present for all three proteins. These differences may be due to a differential strain distribution within the FDP tendon as it is loaded. The changes these regions exhibit may potentially indicate regions of the tendon that may be damaged first with repetitive loading. 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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