Chronic Nicotine Exposure Alters Tendon Vascularity and Viscoelasticity

Daniel Gittings, MD[1,2]
Corinne Riggin, PhD[2]
James Boorman-Padgett[2]
Stephanie Weiss[2]
George Fryhofer, MD[1,2]
Daniel Farber, MD[1]
David Steinberg, MD[1]
Louis Soslowsky, PhD[2]

[1]Department of Orthopaedic Surgery
University of Pennsylvania

[2]McKay Orthopaedic Research Laboratory
University of Pennsylvania

Introduction

Tendon injuries are common and lead to significant disability. Smoking is a modifiable risk factor that has numerous adverse health effects, including association with tendon injuries. However, the effect of nicotine and smoking on tendon morphology and function is not well understood.[1] Therefore, the purpose of this study was to investigate the effect of chronic nicotine exposure on Achilles tendon (AT) and supraspinatus tendon (SS) structural and mechanical properties in a rat model. We hypothesized that chronic nicotine exposure would lead to tendinopathic changes as evidenced by altered tendon vascularity, tenocyte and extra-cellular matrix histology consistent with degeneration, and diminished mechanical properties.

Methods

Study Design

Twenty male Sprague-Dawley rats (398±16g) were randomly allocated to groups exposed to either 0.9% saline (n=10) or 36mg/mL of nicotine (n=10) at a rate of 2.5 µL/hr through osmotic pumps for 12 weeks before being euthanized (IACUC approved). Timing for nicotine exposure was based on previous experiments and the nicotine concentration was based on the average tobacco user in the United States (14 cigarettes per day).[2,3] Serum levels of cotinine, the predominant metabolite of nicotine, was measured every 4 weeks with an enzyme-linked immunosorbent assay at 450 nm to monitor the systemic release of nicotine. Osmotic pumps were exchanged after measurement of serum cotinine levels. In vivo assays: At 12 weeks, AT was imaged with contrast enhanced ultrasound (CE-US) to assess for vascularity (n=4/group). CE-US of AT was visualized in B-mode in the sagittal plane with a 21MHz center frequency ultrasound transducer and then video data was converted into echo-power data (linearization). Perfusion parameters were derived from this model including peak enhancement, rise time, time to peak, wash-in rate, wash-in area under the curve, and wash-in perfusion index as described.[4]

Ex vivo assays

Bilateral AT and SS were then harvested for ex vivo histologic structural (n=5/group) and biomechanical analysis (n=8-10/group) as described.[5,6] Briefly, stain lines were used to track optical strain and cross-sectional area was measured using a custom laser device. Tensile testing was performed as follows: preload to 0.08 N, preconditioning (10 cycles of 0.1-0.5 N at a rate of 1% strain/s), stress relaxation at 5% strain for 600 seconds, and ramp to failure at 0.1% strain/s for AT and 0.3% strain/s for SS.

Analysis

Statistical analysis was performed using Student's t-tests and Mann- Whitney U tests to compare parametric and non-parametric variables respectively. Significance was set at $p<0.05$ and trends at $p<0.1$.

Results

AT CE-US demonstrated an increase in contrast wash-in rate and trend to decrease in rise time and time to peak in the nicotine group compared to the saline group, indicating an increase in tissue perfusion rate (Fig 1.). No differences were found in the other amplitude-based CE-US measures.

Nicotine did not alter AT or SS histologic parameters (Figure 2). AT percent relaxation, a measure of tendon viscoelasticity, was significantly increased with nicotine exposure compared to saline (Figure 3a). Similarly, SS percent relaxation had an increased trend with nicotine exposure compared to the saline group (Figure 3b). No differences in maximum load, maximum stress, stiffness, or modulus were observed with nicotine exposure in either AT or SS (n=6-10/group). DISCUSSION: After chronic nicotine exposure at a clinically relevant dose modeling the average US smoker, AT perfusion rate increased and both AT and SS viscoelasticity were altered in this rat model. In a previous clinical study, CE-US detected a significant increase in vascularity in tendinopathic AT when compared to healthy human patients.[7] Furthermore, nicotine has been shown to have pro-angiogenic effects that may represent a compensatory mechanism for nicotine's vasoconstricting effect.[8] In our study, the changes in AT vascularity may suggest early tendinopathic changes to the tendon's structure following chronic nicotine exposure. Despite the changes in vascularity, we did not detect structural changes in cell shape

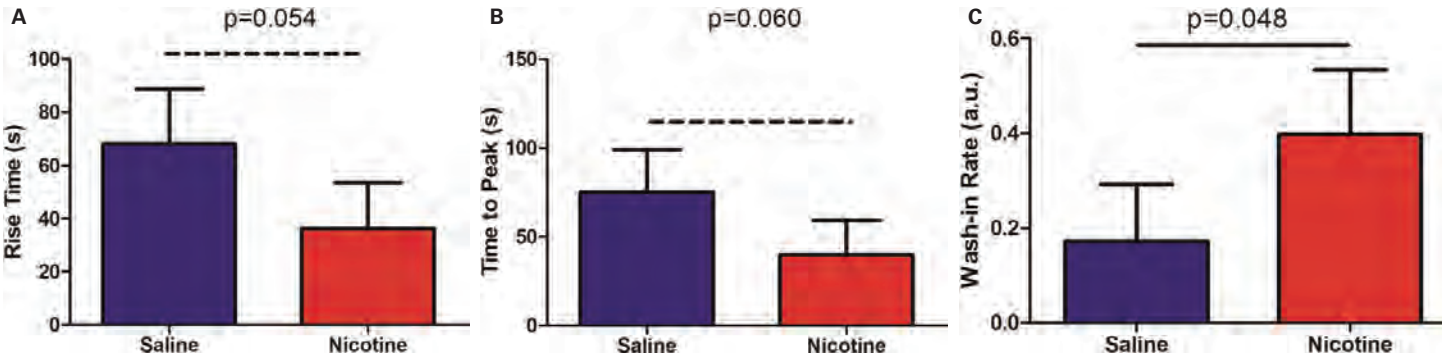

Figure 1. CE-US results of AT. **(A)** Rise Time **(B)** Time to Peak **(C)** Wash-in Rate. Graphs represent mean +/− standard deviation, solid lines denote statistical significance ($p<0.05$), and dashed lines denote trends ($p<0.01$).

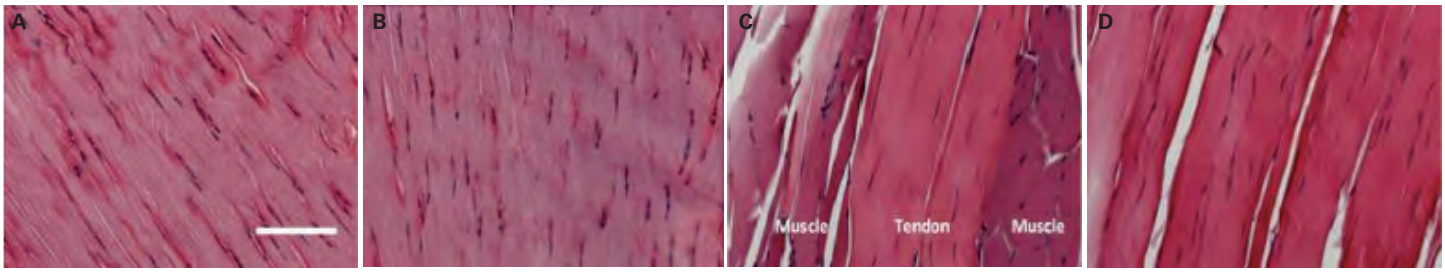

Figure 2. Representative H&E images of saline exposed and nicotine exposed AT **(A,B)** and SS **(C,D)** respectively. All images are at 200x magnification with scale bar representing 100mm. (no scale bar)

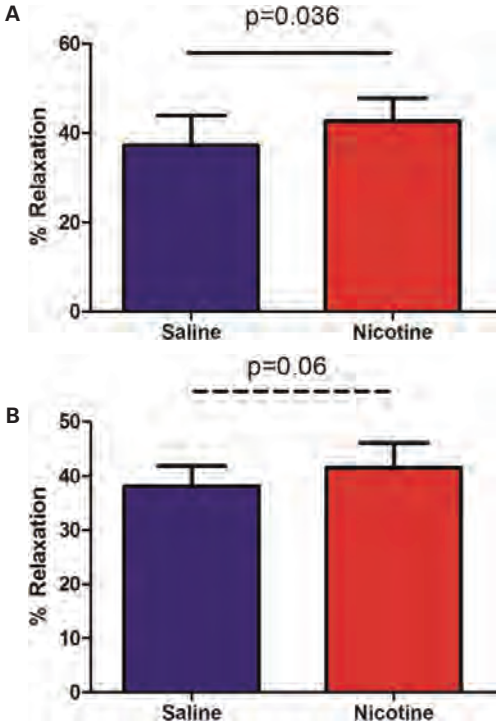

Figure 3. (A) % Relaxation of AT **(B)** % Relaxation of SS. Graphs denote mean +/− standard deviation, solid lines denote statistical significance ($p<0.05$), and dashed lines denote trends ($p<0.01$).

or density. A previous study of porcine tenocytes exposed to nicotine found that alterations in matrix metalloproteinase (MMP) expression were dependent upon exposure to cyclic stretch.[9] Throughout our study, animals were housed in cages and thus were not exposed to a high level of repetitive stimuli. Nicotine exposed tendons also had an increase in percent relaxation, a viscoelastic property, similar to changes seen in fibrotic tendon tissue post-injury. The addition of exercise or overuse as a physical stimulus to these nicotine exposed animals may manifest more dramatic changes to the tendon structure and composition. Further studies are also needed to assess the effect of nicotine dose on tendon properties and on healing of injured tissue.

Significance

Chronic nicotine exposure alters tendon vascularity and viscoelasticity, which may predispose tendons to degeneration and injury.

Acknowledgements

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