Long-Term Nicotine Exposure Alters Rat Supraspinatus Tendon and Bone Properties

Julianne Huegel, PhD
Courtney A. Nuss
Peter Chan
Adnan N. Cheema, MD
Andrew F. Kuntz, MD
Louis J. Soslowsky, PhD

McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

Introduction

Nicotine is a well-established risk factor for rotator cuff injuries.[1] Several laboratory studies showed that nicotine negatively impacts tendon healing after injury, in both the rat rotator cuff[2] and Achilles.[3] Surprisingly, after twelve weeks of nicotine exposure, material properties of the uninjured rat supraspinatus tendon had increased maximum stress and elastic modulus compared to controls.[4] Conversely, nicotine decreased bone mass due to imbalanced bone turnover.[5] However, an understanding of nicotine effects on rotator cuff tendon-to-bone properties after long term exposure is lacking. Therefore, the objective of this study is to investigate the effects of eighteen weeks of nicotine exposure on tendon-to-bone properties in a rat model via mechanical, μCT, and histological analyses. We hypothesized that long term nicotine exposure would lead to decreased tendon mechanical properties, decreased subchondral bone insertion properties, and decreased trabecular bone properties in the humeral head, as well as altered tendon cell morphology.

Methods

24 adult male Sprague-Dawley rats (350-400g) were used (IACUC approved). Animals were randomized to receive either 0.9% sterile saline (n = 12) or 61 mg/ml nicotine (n = 12) through subcutaneously implanted osmotic pumps, which correlated with appropriate levels of cotinine measured in the blood serum (400-700 ng/ml).[3] Rats were sacrificed after 18 weeks of exposure. Animals were stored at -20°C until supraspinatus tendon-humerus complexes were dissected out and processed for histological analysis (n=5, right limbs) or cross-sectional area measurement and quasistatic mechanical testing (n=12, left limbs). Testing consisted of pre-conditioning, stress relaxation at 5% strain, and a quasi-static ramp to failure at 0.3%/s. Post-test, humeri were μCT scanned at 6μm resolution to assess trabecular properties of the epiphysis proximal to the humeral growth plate, representing the region of rotator cuff attachment on the greater tuberosity. Additionally, the mineralization gradient was calculated (Amira 6.7) across the subchondral plate, defined as the mineralized fibrocartilage of the supraspinatus tendon enthesis and subchondral bone. Briefly, a 100x120x230 voxel volume was identified in the greater tuberosity at the supraspinatus tendon insertion site. After thresholding, the innermost layer of the subchondral bone was defined. Individual layers were then defined outwards towards the mineralized fibrocartilage boundary. Layer intensity values were averaged to construct a mineralization gradient, normalized to the total subchondral plate thickness. Intensity was compared at normalized thickness of 0, 0.5, and 1.0, marking the boundaries between trabecular bone, subchondral bone, mineralized fibrocartilage, and tendon. Statistical comparisons were made between the saline and nicotine groups. Comparisons for mechanics and μCT metrics were made using Student's t-tests. Mineralization intensity was also compared with two-way ANOVA across subchondral thickness. Histological comparisons were made using Mann-Whitney tests. Significance was set at $p < 0.05$ (solid bars), and trends at $p < 0.1$ (dashed bars).

Results

Mechanical properties

Tendons in the nicotine group had a smaller cross-sectional area than the saline group (Fig 1A). There were no differences in stress relaxation (not shown) or tissue modulus measured through the length of the tendon (Fig 1B). However, the nicotine group showed a trend toward decreased modulus at the insertion (first 2 mm proximal to the insertion, Fig 1C), as well as significantly decreased tendon stiffness (Fig 1D).

Histological measures

No differences were seen at the tendon insertion in cellularity or cell shape (Fig 2A,B). However, cell density in the midsubstance was decreased with nicotine exposure (Fig 2C); cell shape was not different (Fig 2D). Representative images of each region are shown in Figure 2E.

μCT parameters

Although no differences were identified in bone volume fraction or trabecular thickness (Fig 3A,B), there was a trend toward increased trabecular number and decreased separation (Fig 3C, D). Additionally, mineralization intensity was significantly different across the subchondral

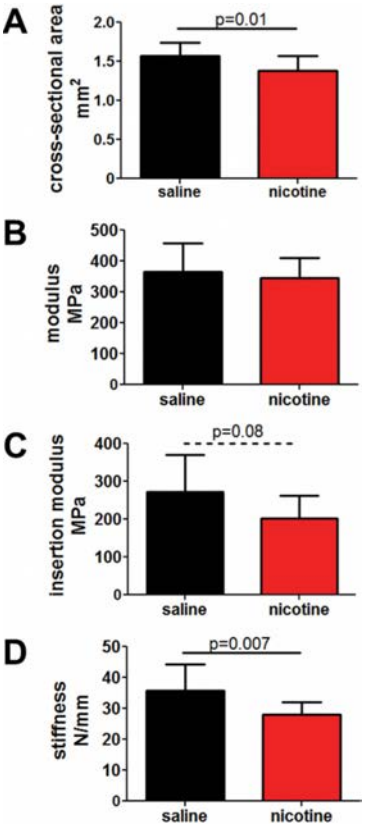

Figure 1. Mechanical Properties. Tendons exposed nicotine had **(A)** decreased tendon area, **(B)** no difference in modulus, **(C)** decreased modulus at the tendon insertion, and **(D)** decreased stiffness. Data shown as mean±SD.

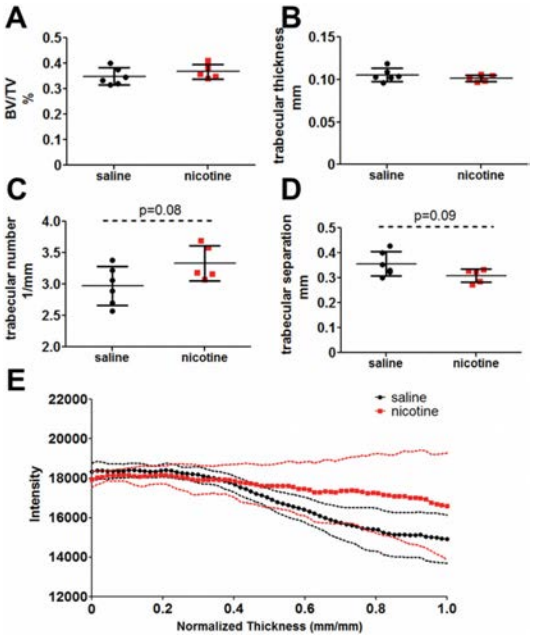

Figure 3. µCT Properties. Nicotine did not have an effect on **(A)** trabecular bone fraction or **(B)** trabecular thickness in the humeral epiphysis. **(C)** Trabecular number was increased and **(D)** trabecular separation was decreased in nicotine treated rats. **(E)** Bone mineralization (intensity) across the subchondral plate from the trabecular boundary (0) to the tendon boundary (1.0) was different between groups ($p < 0.04$). Data shown as mean±SD.

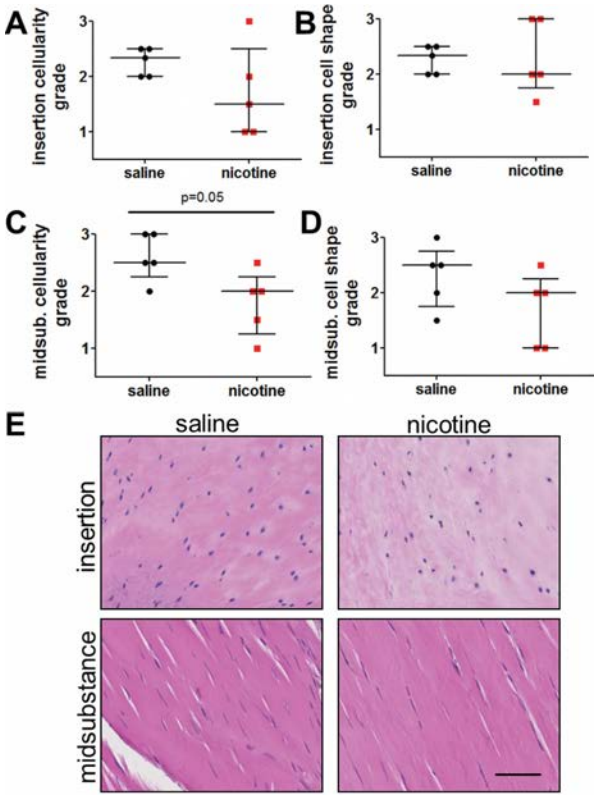

Figure 2. Histological Properties. No differences were found between groups for **(A)** insertion cellularity or **(B)** insertion cell shape. Nicotine tendons had **(C)** decreased cellularity in the midsubstance, but **(D)** cell shape was not changed. **(E)** Representative regions of interest at 200x magnification. Data shown as median±IQR. Scale bar: 100µm.

plate between treatment groups, although not when specific comparisons were made at locations of interest (0, 0.5, and 1.0 thicknesses; Fig 3E).

Discussion

This study measured the effects of long-term nicotine exposure on uninjured supraspinatus tendon and underlying humeral bone properties. Previous work found that nicotine caused decreased Achilles tendon cross-sectional area after injury;[3] similarly, nicotine-exposed animals had smaller uninjured supraspinatus tendons, suggesting a potential decrease in metabolic activity, consistent with decreased cell density in the current study. Contrary to previous reports,[4] this study demonstrated decreased tendon mechanical properties, supporting clinical findings and highlighting the importance of time course studies. Surprisingly, trabecular bone properties were slightly improved with nicotine exposure, suggesting that bone metabolism is also affected, though potentially not as hypothesized. Future studies will investigate additional time points as well as kinetic bone histomorphometry. Although data was variable, increased bone mineralization intensity at the tendon insertion could increase stress concentrations across the tendon-bone interface, increasing risk of tendon rupture.[6] Physical activity such as exercise or overuse may produce more dramatic changes to the tendon structure and composition.

Significance

This study demonstrates that nicotine leads to decreased mechanical properties in uninjured supraspinatus tendons as well as alterations in bone structure. Patients should be

counseled that use of nicotine increases their risk of tendon degeneration and may predispose them to tendon injury.

Acknowledgements

Funding was provided by the NIH/NIAMS supported Penn Center for Musculoskeletal Disorders (P30 AR069619). We thank Ashley Fung for help with μCT assays.

References

1. T. Hu et al. *Tob. Control*, 9:II60-3. 2000.
2. Galatz LM et al. *J Bone Joint Surg (Am)*, 88:2027-34. 2006.
3. Cheema AN et al. *J Orthop Res*, 37: 94-103. 2019.
4. Ininose R et al. *Acta Orthopaedica*, 81:634-638. 2010.
5. Cusano E. Curr Osteoporos Rep, 13:302-309. 2015.
6. Genin GM et al. Biophys K, 2009.