Impact of Cigarette Smoke Exposure on Structural and Mechanical Properties of Rat Supraspinatus Tendon

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INTRODUCTION: The association between smoking and rotator cuff tears has been extensively studied, with smokers having been shown to suffer from tears at younger ages with increased frequency, worse severity, and inferior surgical outcomes following repair. [1-3] Previous studies have examined the effect of nicotine administration on supraspinatus tendon structure and function in animal models, but no study to date has created a cigarette smoke model to study these changes. Additionally, no study has examined the effect of smoking cessation on supraspinatus tendon structure or function. The purpose of the current study is to describe biomechanical and microstructural changes in supraspinatus tendons of rats exposed to tobacco smoke and assess the impact of smoking cessation on these properties. We hypothesize that smoke exposure compromises the structural and mechanical integrity of the supraspinatus tendon.

METHODS: A total of 27 rats were randomized into four groups: a two-month smoke exposure group (n=7), a two-month control group (n=7), a seven-month smoke cessation group (n=7), in which smoke exposure was withdrawn after two months, and a seven-month cessation control group (n=6). Rats selected into the smoke exposure and the smoke-cessation groups were treated with 2 hours of cigarette smoke a day during weekdays and one hour per day on weekends for the stated duration of time. To replicate the smoke dosage in an active heavy smoker (1 pack per day), we exposed the rats to particulate matter in concentrations equivalent to human smokers (~200 mg/m^3) and measured the nicotine metabolite cotinine in the rats to achieve a blood concentration roughly equivalent to that of a normal human smoker (240-300 ng/mL). Supraspinatus specimens consisting of the supraspinatus muscle belly and proximal humerus were harvested from both shoulders on the rat models. The harvested tendons on the right side were then subjected to multi-step stress relaxation and load to failure tensile testing. The harvested tendons from the left side were imaged via second harmonic generation imaging using a custom dual photon microscope. The dimensions of the tendons were assessed by measuring length, width, and thickness with a digital caliper. Statistical tests included two-tailed student t-test, one-way ANOVA, Chi-square test, and Fisher's Exact test as appropriate. An alpha less than or equal to 0.05 defined significance.

RESULTS: Through the above experimental procedure, mechanical properties were obtained for the supraspinatus tendon of rats conducted two-month and seven-month under smoke exposure group and cessation control group, respectively. The result shows that the modulus of elasticity at different strains was difference between the smoke exposure group (ε= 20%; two-month: 14.7±5.65 MPa, seven-month: 19.35±10.26 MPa) and the cessation control group (ε= 20%; two-month: 33.79±8.12MPa, seven-month: 14.07±3.96 MPa) of the rats (**Table 1**). Load to failure testing identified a difference in Young's modulus between groups at the two-month timepoint (smoke: 20.1±5.89 MPa, control: 30.12±8.74 MPa) and in Young's modulus at the seven-month timepoint (smoke: 23.00±4.85 MPa, control: 11.56±4.63 MPa). Other mechanical parameters such as stiffness (smoke: 23.87±5.81 N/mm, control: 15.23±5.66 N/mm), peak tensile strength (smoke: 19.47±2.17 N, control: 11.78±4.02 N), and maximum stress (smoke: 3.65±1.02 MPa, control: 2.02±0.50 MPa) showed large differences at the seven-month timepoint (**Fig. 1**). There is no difference in ultimate tensile strain between smoke exposure group (two-month: 29.53±11.07%, seven-month: 38.23±12.28%) and cessation control group (two-month: 31.22±15.00%, seven-month: 40.45±14.22 %).

DISCUSSION: Chronic smoking adversely affects supraspinatus tendons in rats. Load to failure testing demonstrated that the mechanical parameters such as modulus of elasticity, Young's modulus, stiffness, peak tensile and maximum strength in the smoke exposure group were less than that of the control group at two-month, while those mechanical parameters were significantly larger than that of the cessation control group at seven-month. This result indicates that chronic smoking will result in higher mechanical parameters for the supraspinatus tendon, which means that the supraspinatus tendon become stiffer but also more fragile, supporting the hypothesis that chronic smoke exposure results in less elastic tendons which are more prone to tendinopathy. Meanwhile, it can be noticed that the regions of rupture in the test are not the same, with rupture in the control group occurred in the middle of the supraspinatus tendon but the rupture in the smoke exposure group occurred near the enthesis (**Fig. 2**). The possible mechanism of tendon mechanical performance change could be due to enthesis calcification and degenerative remodeling or the tendon collagen fiber cross-linking.

SIGNIFICANCE/CLINICAL RELEVANCE: One of the key points in addressing the problem of poor postoperative repair in shoulder tendinopathies is the study of adverse factors on the structural mechanisms of the tendon. This study quantitatively describes changes in the mechanical parameters of supraspinatus tendon as a result of cigarette smoking, which provides a scientific basis for the mechanism of structural changes in tendon structure in response to smoking.

REFERENCES: [1] Baumgarten KM, Gerlach D, Galatz LM, et al. Cigarette smoking increases the risk for rotator cuff tears. Clin Orthop Relat Res. Jun 2010;468(6):1534-41. doi:10.1007/s11999-009-0781-2. [2] Carbone S, Gumina S, Arceri V, Campagna V, Fagnani C, Postacchini F. The impact of preoperative smoking habit on rotator cuff tear: cigarette smoking influences rotator cuff tear sizes. J Shoulder Elbow Surg. Jan 2012;21(1):56-60. doi:10.1016/j.jse.2011.01.039. [3] Lundgreen K, Lian OB, Scott A, Nassab P, Fearon A, Engebretsen L. Rotator cuff tear degeneration and cell apoptosis in smokers versus nonsmokers. Arthroscopy. Aug 2014;30(8): 936-41.doi:10.1016/j.arthro.2014.03.027.

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Table 1. Stress relaxation differences between groups

2-month Smoke Exposure vs 2-month Control	*p*-value	7-month Smoke Exposure vs 7-month Control	*p*-value
Elastic Modulus - 5%	>0.999	Elastic Modulus - 5%	>0.999
Smoke: 3.60MPa (SD = 1.42), Control: 3.79MPa (SD = 1.46)		Smoke: 2.42MPa (SD = 3.01), Control: 1.59MPa (SD = 0.53)	
Elastic Modulus - 10%	0.068	Elastic Modulus - 10%	0.960
Smoke: 10.1MPa (SD = 3.9), Control: 11.28MPa (SD = 6.1)		Smoke: 5.95MPa (SD = 6.53), Control: 5.13MPa (SD = 1.45)	
Elastic Modulus - 15%	0.146	Elastic Modulus - 15%	0.573
Smoke: 13.68MPa (SD = 5.27), Control: 19.52MPa (SD = 7.4)		Smoke: 11.57MPa (SD = 7.48), Control: 9.87MPa (SD = 1.34)	
Elastic Modulus - 20%	0.460	Elastic Modulus - 20%	0.236
Smoke: 14.70MPa (SD = 5.65), Control: 33.79MPa (SD = 8.13)		Smoke: 19.35MPa (SD = 10.26), Control: 14.07MPa (SD = 3.96)	

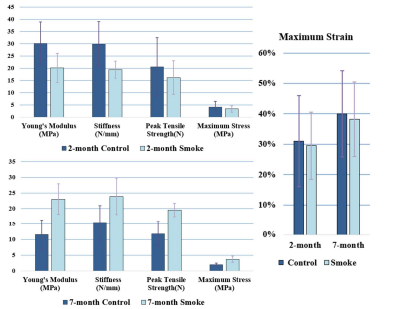

Figure 1. Load to failure outcome differences between groups

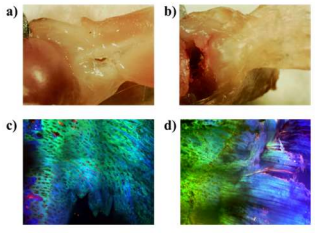

Figure 2. Load to failure testing images. a) A 2-month control rat supraspinatus tendon demonstrating mid-plane failure after load to failure testing . b) A 2-month smoke-exposed rat supraspinatus tendon demonstrating failure at the insertion after load to failure testing. c) A supraspinatus tendon from a control rat imaged via dual-photon microscopy . d) A smoke-exposed rat supraspinatus tendon imaged via dual-photon microscopy.