Isolated Rotator Cuff Supraspinatus Tendon Tear Does Not Induce Muscle Pathophysiological Changes In Mice

Kaya A. Adelzadeh¹, Christian E. Awah¹, Kevin Wang¹, GaYoung Park¹, Damien Laudier¹, Leesa M. Galatz¹, Woojin M. Han^{1,2,3,4}

¹Leni and Peter W. May Department of Orthopaedics, ²Department of Cell, Developmental and Regenerative Biology, ³Black Family Stem Cell Institute,

⁴Institute for Regenerative Medicine, Icahn School of Medicine at Mount Sinai, New York, NY

kaya.adelzadeh@icahn.mssm.edu

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INTRODUCTION: Rotator cuff (RC) tears account for a significant proportion of shoulder injuries seen in orthopedic clinics. RC tears often lead to secondary muscular pathologies characterized by irreversible muscle atrophy and fatty infiltration. These complications reduce muscle function and negatively impact reparability, enthesis healing, and repair prognosis. ^{1,2} Mouse models are commonly used to study RC injury-induced muscle degeneration because of their anatomical similarity to humans, availability of transgenic and knockout models. ³ However, these models to date include a suprascapular nerve injury and/or induction of massive tears to both the supraspinatus (SS) and infraspinatus (IS) tendons, significantly limiting its clinical relevance. ^{4,5} It is unknown if an isolated full-thickness tear to SS tendon only, a more common mode of injury seen in younger individuals, induces muscle atrophy and fatty infiltration in mice. Therefore, the objective of this study is to determine whether isolated SS tears induce clinically significant levels of pathophysiological changes in the muscle, including fatty infiltration.

METHODS: Animal studies were conducted under approved IACUC protocol. Three-month-old female C57BL/6J mice underwent unilateral transverse SS tendon tear (n=9) or unilateral transverse SS and IS tendon tear (n=9), serving as a positive control, without tendon repair. Sham surgeries were performed on contralateral RCs and served as negative controls. DigiGait (Mouse Specifics) analysis was conducted to measure shoulder function of healthy (pre-injury) controls (n=11) as well as at 6 weeks (n=13) and 12 weeks (n=12) after injury. Mice were euthanized at 12 weeks after injury, and SS and IS muscles were dissected, harvested, and flash-frozen in 2-methylbutane cooled in liquid nitrogen. SS muscles were cryosectioned transversely at a thickness of 10 μ m at depths of 25%, 50% and 75% distance of sagittal tissue length from the myotendinous junction (Fig. 1A), stained with Oil Red O (ORO), and imaged under brightfield to assess lipid content indicative of fatty infiltration in the muscle tissue. Statistical analysis was performed using GraphPad Prism. Two-way ANOVA with Dunn's test was used to determine the effect of injury severity (SS vs. SS+IS injury) and sagittal tissue depth on 1) morphology and 2) intramuscular lipid content. Ordinary one-way ANOVA with Tukey's test was used to determine effects of injury severity on gait parameters. Statistical significance was set at p < 0.05.

RESULTS: At 12 weeks post-injury, percentage area fraction of fat was significantly higher, indicating more intramuscular fatty infiltration, in the SS muscle following SS+IS tears than in both sham (p = 0.004, 0.001, 0.002) and SS tears (p = 0.015, 0.003, 0.014) at all respective tissue depths (Fig. 1C). Sham and SS tears did not significantly differ in percentage area fraction of fat (p = 0.33), indicating minimal pathophysiological changes (Fig. 1C). From preliminary qualitative analysis, lipids were often located at relatively greater levels near the tendinous connective tissue (Fig. 1B), though lipids were not restricted to this region within the tissue. At 6 weeks post-injury, stride length was significantly decreased in both SS injury (p = 0.004) and SS+IS injury (p = 0.0006) compared to the pre-injury condition, while stance width did not differ for SS injury (p = 0.936) and SS+IS injury (p = 0.651) in relation to pre-injury (Fig. 2A). The trends found in each gait parameter were consistent at 12 weeks post-injury, where stride length decreased, albeit less significantly, between the pre-injury and SS tear (p = 0.003) and the SS+IS tear (p = 0.002; Fig. 2B).

DISCUSSION: Our findings indicate that a SS tendon tear alone is not enough to induce degenerative pathophysiologic changes in the SS muscle in mice. Muscle lipid content in SS injury and sham groups resemble one another at all depths. However, gait analysis indicates functional deficit resulting from the RC tendon injury regardless of its severity. Thus, we conclude that fatty infiltration is not significantly induced by a full thickness SS tear only in mice, and that massive SS+IS tears are required to induce fatty infiltration. Limitations of our study include our method of functional assessment. While gait analysis is a commonly used measure of functional outcomes, this method does not directly test muscle function in an isolated manner. To improve our understanding of functional outcomes, we are currently conducting *in-situ SS* muscle testing and grip strength testing.

SIGNIFICANCE/CLINICAL RELEVANCE: Our work to elucidate that isolated SS tear does not lead to injury-induced fatty infiltration progression expands knowledge of injury models in mice that may be leveraged in developing targeted therapies designed to promote physiological and functional recovery in patients with tendon tears.

REFERENCES: [1] Frich+ J Shoulder Elbow Surg, 2020 [2] Gladstone+ Am J Sports Med, 2007 [3] Mathewson+ J Exp Biol, 2013 [4] Wang+ Am J Sports Med, 2018 [5] Kim+ J Shoulder Elbow Surg, 2012

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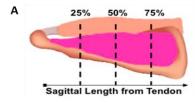
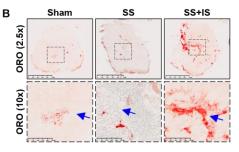
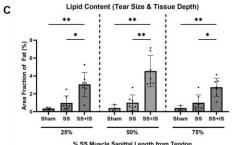


Fig 1: SS muscle maintains quality without significant increases in fatty infiltration following SS tendon tear.

(A) Diagram representing three muscle depth groups: 25%, 50%, and 75%. (B) Representative ORO-stained SS sections at 50% and of each tear size: sham, SS, and SS+IS, including high-magnification ORO images with arrows indicating tendon location, used to calculate muscle fiber diameter, area fraction of fat, and qualitatively identify intramyocellular fat droplets, respectively. (C) Percentage area fraction of fat comparisons by tear size and tissue depth. *p < 0.05, **p < 0.01





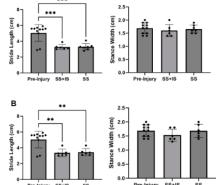


Fig 2: Functional outcomes are somewhat impaired after either injury size (SS tear and SS+IS tear).

Functional assessment performed via gait analysis including stride length and stance width parameters measured at (A) 6 weeks post-injury and (B) 12 weeks post-injury. These values are compared to the same preinjury values corresponding with each parameter as represented in each graph. ** p < 0.01, *** p < 0.001