

Integrin- $\alpha 5$ Inhibition and α SMA Positive Cells Improve Exercise Mediated Repair of Tendon Fatigue Damage

Lainie E. Eisner¹, Benjamin B. Johnston¹, Diane Stonestreet¹, Nelly Andarawis-Puri, PhD^{1,2}
¹Cornell University, Ithaca, NY ²Hospital for Special Surgery, New York, NY
Lee45@cornell.edu

Disclosures: L.E. Eisner (N), B.B. Johnston (N), D. Stonestreet (N), N. Andarawis-Puri (N)

INTRODUCTION: Tendinopathies result from accumulation of sub-rupture damage that does not innately repair. Therapeutics to halt or reverse the progression of damage are thwarted by paucity of data on effective repair mechanisms. We previously established an *in vivo* model of sub-rupture tendon fatigue injury to study early-stage tendon pathogenesis¹ and demonstrated that one bout of moderate fatigue loading disrupts the tendon's aligned extracellular matrix (ECM) structure¹ and diminishes remodeling-associated gene expression.² Further, we showed that loading in the form of treadmill exercise facilitated repair of fatigue damage when initiated 2-weeks post-injury (therapeutic exercise) but exacerbated damage when initiated 1-week post-injury,³ providing a platform to interrogate mechanisms of repair versus progressions of degeneration of fatigue injury. We observed that therapeutic exercise was particularly associated with an enhanced population of α -Smooth Muscle Actin-positive (α SMA+) cells and integrin- $\alpha 5$ + cells;⁴ an integrin that enhances the capacity of cells to withstand loads thereby preventing cell death. We hypothesized that (H1) α SMA+ cells, which are known for their role in depositing matrix and imparting contractile forces in the context of wound healing,⁵ facilitate exercise-mediated repair of tendon fatigue damage by remodeling ECM damage and depositing new aligned collagen matrix; and (H2) integrin- $\alpha 5$ enhances the capacity of cells to withstand loads thereby promoting cell survival and enhancing the population of cells that can repair the damaged matrix.

METHODS: With IACUC approval, left patellar tendons (PTs) of 8-9 month-old female Sprague Dawley rats (n = 4-5/group; Charles River Laboratories) underwent 7200 cycles of fatigue loading per our established protocol.¹ At 14-days post-injury, animals began 2 weeks of treadmill running for 30 min/day, 5 days/week, modifying our established therapeutic exercise protocol.³ Concurrently, one group received a daily oral dose of simvastatin (10 mg/kg; Midwest Veterinary Supply) in 10% sucrose solution to deplete α SMA+ cells, or 10% sucrose alone (vehicle control); a second group received a twice daily subcutaneous bolus injection of ATN-161 (1.67 mg/kg; Cayman Chemical) in phosphate buffered saline (PBS) vehicle to deplete integrin- $\alpha 5$, or PBS alone (vehicle control). Animals were euthanized 28-days post-injury and PTs were fixed in zinc buffered formalin under 2N of load and decalcified in formic acid. **Second Harmonic Generation (SHG) imaging:** PTs were imaged at 63x using an inverted Zeiss LSM880 confocal/multiphoton microscope at 900 nm excitation. A 150-pixel square grid was applied to each image and a blinded observer assigned each grid square a damage severity score of 0 (no damage), 1 (low damage), 2 (moderate damage), or 3 (severe damage). **Immunohistochemistry (IHC):** Following imaging, PTs were embedded in paraffin and cut into 6 μ m-thick sections. Sections were stained with primary antibodies against α SMA (1:1000, Abcam), integrin- $\alpha 5$ (1:2000, Abcam), and procollagen-I (1:100, Biorbyt) and counterstained with Toluidine Blue. A blinded observer counted positive and negative cells using ImageJ. **Statistics:** Multiple Mann-Whitney tests. Significance was set at p < 0.05.

RESULTS: Treatment with simvastatin led to 20% reduction in α SMA+ cells in the PTs (p = 0.11; **Fig. 1A**). Depletion of α SMA+ cells had no effect on total damage area fraction (DAF) but increased the percentage of severe matrix damage compared to the control group (p = 0.03, **Fig. 2**). Supporting H1, α SMA+ cell depletion also decreased the percentage of procollagen-I+ cells in the tendon (p = 0.03, **Fig. 1B**), indicating diminished deposition of new aligned collagen matrix in α SMA+ cells depleted tissue. Contrary to H2, inhibition of integrin- $\alpha 5$ via ATN-161 treatment led to a striking decrease in total damage (p = 0.03) and low (p = 0.01) and moderate (p = 0.03) damage regions compared to control (**Fig. 2**).

DISCUSSION: We showed that inhibition of α SMA+ cells increases the area of high matrix damage and decreases procollagen-I in fatigue damaged tendons that undergo typically therapeutic exercise, suggesting that the small increase in α SMA+ cells could be highly impactful because it decreases the most severe manifestations of damage thereby inhibiting the catabolic cascade that would subsequently spread to the entire tendon. Furthermore, the finding that overall DAF does not change, but severe matrix damage is increased following α SMA+ cell depletion suggests that these cells preferentially repair regions of high damage. This observation is supported by previous work which has shown that cells in regions of severe matrix damage experience aberrant mechanical cues,^{1,6} which may stimulate α SMA+ cell differentiation or recruitment. Additionally, the concurrent decrease in procollagen-I expression with α SMA+ cell depletion during exercise suggests that α SMA+ positive cells contribute to exercise mediated tendon repair in multiple ways, by repairing damage kinks through high contractile forces as well as synthesizing new matrix. We hypothesized that integrin- $\alpha 5$ would be critical to exercise-led repair of fatigue damaged tendons because it imparts cells with the capacity to withstand greater loads thereby improving cell survival.^{7,8} We found that therapeutic exercise is associated with an increase in integrin- $\alpha 5$ and a decrease in apoptosis,³ which is consistent with our hypothesis that its increase may protect cells in damaged ECM under the high stress of exercise. However, our finding that inhibition of integrin- $\alpha 5$ further improves the repair outcome of therapeutic exercise is reminiscent of our published studies where we found that pharmaceutical inhibition of apoptosis ultimately leads to an increase in cell stress markers of the surviving cells and exacerbates the induced fatigue damage.⁴ We expect that the therapeutic versus degenerative effect of integrin- $\alpha 5$ may be cell-specific since we found a large population of both α SMA+ cells and tenocytes to be positive for integrin- $\alpha 5$, and have shown that α SMA+ cells are essential to the observed therapeutic outcome.⁴ We will investigate the cell-type specific role of integrin- $\alpha 5$ in the response to loading of fatigue damaged tendons through a combined application of ATN-161 (blocking of integrin- $\alpha 5$) and simvastatin (inhibition of α SMA+ cells).

SIGNIFICANCE/CLINICAL RELEVANCE: Identification of the mechanism by which α SMA+ and integrin- $\alpha 5$ + cells contribute to repair during therapeutic exercise will contribute to the development of early stage tendinopathic therapeutics and improve exercise-driven clinical outcomes.

REFERENCES: [1] Fung+ *J Biomech* 2010 [2] Andarawis-Puri+ *J Orthop Res* 2012 [3] Bell, Boniello+ *J Orthop Res* 2015 [4] Bell+ *Sci Rep* 2018 [5] Gabbiani *J Pathol* 2003 [6] Sun+ *J Orthop Res* 2010 [7] Zhang+ *PNAS* 1995 [8] Schaffner+ *Cancers* 2013

ACKNOWLEDGEMENTS: Cornell-HSS T32 NIAMS AR078751, NIH R56AR077239, Cornell BRC (NIH S10RR025502) shared Zeiss LSM880 Confocal

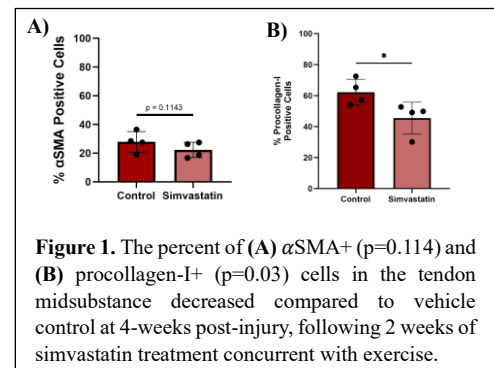


Figure 1. The percent of (A) α SMA+ (p=0.114) and (B) procollagen-I+ (p=0.03) cells in the tendon midsubstance decreased compared to vehicle control at 4-weeks post-injury, following 2 weeks of simvastatin treatment concurrent with exercise.

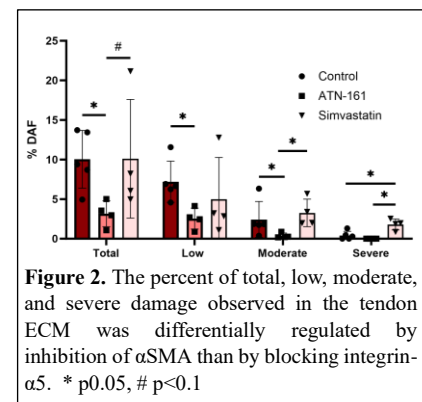


Figure 2. The percent of total, low, moderate, and severe damage observed in the tendon ECM was differentially regulated by inhibition of α SMA than by blocking integrin- $\alpha 5$. * p<0.05, # p<0.1