Simvastatin Recovers Supraspinatus Tendon Mechanical and Histological Properties in a Diet-Induced Hypercholesterolemia Rat Model

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Introduction

Rotator cuff tendon tears are extremely common, affecting up to 50% of Americans over 50 years of age.[1] Hypercholesterolemia, a condition which affects more than 27% of Americans over 20 years old,[2] has been shown to be a risk factor for tendon rupture, specifically in the supraspinatus and Achilles tendons.[3] Previous studies have used a high-cholesterol diet to induce hypercholesterolemia, which demonstrated an increase in stiffness and elastic modulus of the rat supraspinatus tendon.[4] In the clinic, statins are commonly prescribed to lower cholestero[1,2] but at present, little information is available examining the effect of statin treatment on the musculoskeletal system. Therefore, the objective of this study was to determine the biomechanical and histological effects of statin treatment in a diet-induced hypercholesterolemia model.[4,5] We hypothesized that hypercholesterolemic rats treated with statins would have improved tendon biomechanical and histological properties compared to untreated rats.

Methods

Thirty adult male Sprague-Dawley rats (400-450g) were used in the IACUC approved study. To induce hypercholesterolemia (HC), rats were fed a high cholesterol diet[4] (n = 20) for six months while age-matched control rats (CTL, n = 10) ate standard rat chow for six months. All rats were allowed food and water ad libitum and were weighed weekly throughout the study. After the initial six month treatment, a subset of the HC rats (n = 10) were orally dosed with simvastatin daily (20mg/kg) for three months (HC + S group). The HC and HC + S groups were fed HC chow throughout the study. All rats were sacrificed after a total of 9 months. Blood was collected from all rats at 6 months to confirm high-cholesterol in the HC groups and again at the time of sacrifice to measure total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG) and the ratio of TC to HDL (TC/HDL). Immediately following sacrifice, right shoulders were dissected and fixed in formalin for histological analysis of collagen organization and cell morphology.[6-8] Contralateral limbs were frozen at −20°C and later thawed for mechanical testing.[4,6,9] All comparisons were made between the HC and HC + S groups only (except for serum lipid panels at 6 months to confirm HC and weight comparisons performed over time). Statistical comparisons of mechanical parameters and collagen organization were made using t-tests with significance at $p \leq 0.05$. Comparisons of cell morphology were made using non-parametric Mann-Whitney tests with significance at $p \leq 0.05$.

Results

Animals in the HC and HC + S groups were significantly lighter than the CTL rats after introduction to the HC diet throughout the duration of the study, but no differences in weight were noted between groups after induction of simvastatin treatment (data not shown). *Serum lipid analysis*: After six months, the animals in the HC diet had significantly increased TC, HDL, and TC/HDL, and significantly decreased TG (data not shown). After three months of simvastatin treatment, animals in the HC + S group had significantly decreased HDL and trended toward decreased TC. No differences were noted in TC/HDL or TG (data not shown). *Tendon mechanical properties*: At the insertion site, the HC + S group had significantly increased cross-sectional area and significantly decreased elastic modulus (Figure 1a, b respectively). In the midsubstance, no differences were detected in cross-sectional area or elastic modulus (Figure 1c, d respectively). Additionally, no differences were noted in percent relaxation and stiffness between groups (data not shown). *Histology*: No differences were observed in cell shape (Fig. 2a), cellularity (Figure 2b), or circular standard deviation (data not shown) at the insertion site between the groups. In the midsubstance, the HC + S group had significantly more spindle shaped cells (Figure 2a). No differences were observed in cellularity (Figure 2b) or circular standard deviation (data not shown) between groups.

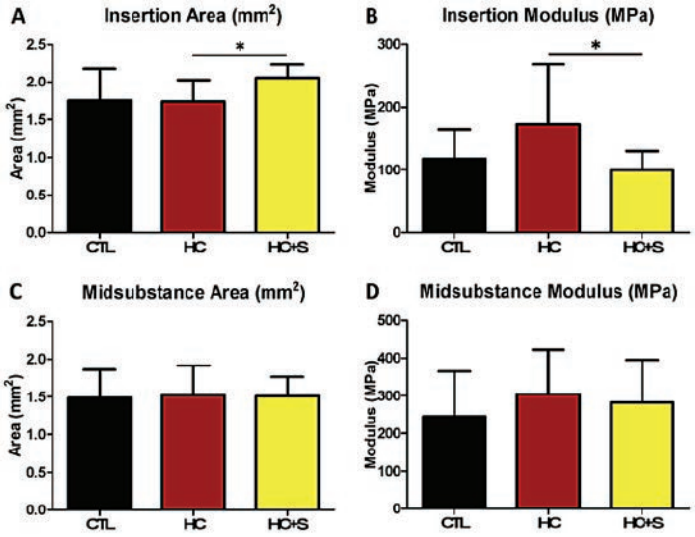

Figure 1. (A) Insertion cross-sectional area was significantly increased in the HC + S group. (B) Insertion modulus was significantly decreased in the HC + S group. (C) No differences were observed in midsubstance cross-sectional area or (D) modulus. Data presented as mean ± SD.

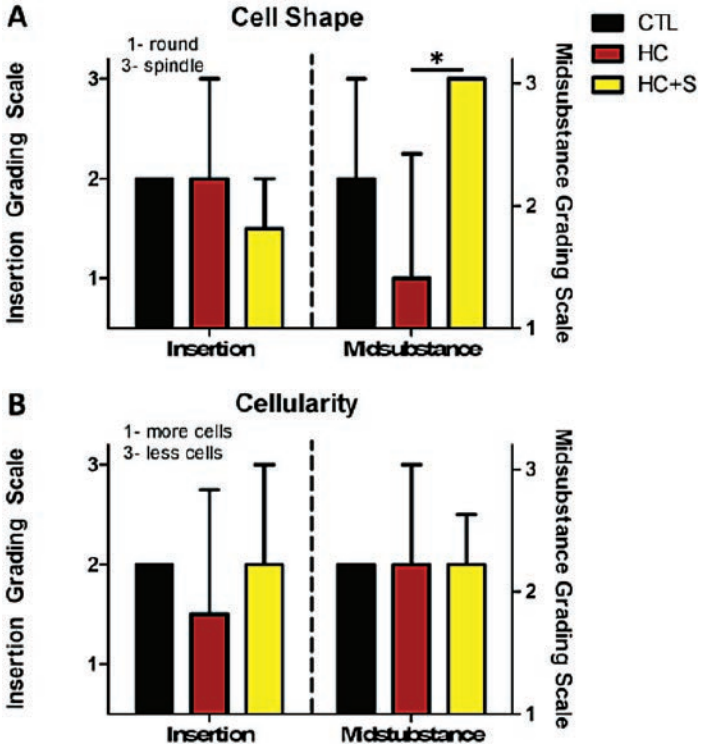

Figure 2. (A) No differences were observed in cell shape at the insertion site. In the midsubstance, the HC + S group had significantly more spindle shaped cells. (B) No differences were observed in cellularity in either region. Data as median ± IQR.

Discussion

Results suggest that treating HC rats with daily simvastatin for 3 months recovers the changes due to HC alone to baseline, control properties. Typically, a decrease in elastic modulus (Figure 1b) might be interpreted as a deleterious outcome, but when considering the modulus values in the CTL and HC + S groups (118 ± 47 MPa and 99 ± 30 MPa, respectively), it is apparent that the simvastatin treatment returned values close to baseline control values, which were elevated in the HC group (172 ± 35 MPa). Histological analysis showed the HC + S group had significantly more spindle shaped cells in the midsubstance, which indicates that the simvastatin intervention is returning the tendons back to normal cell morphology. Rounded cells in the HC group could be associated with tendinopathy and/or a more cartilaginous phenotype, which are risk factors for tendon rupture,[10] or with increased cell activity as a result of high-cholesterol in the tendon. Results suggest that simvastatin treatment tends to bring mechanical and histological parameters closer to baseline values, although the mechanisms governing these findings must still be elucidated. Further studies could also evaluate the effects of different doses or treatments.

Significance

Three months of simvastatin treatment in a diet-induced hypercholesterolemia model returns tendon mechanical and histological properties toward normal in this model system. Additionally, this data suggests that simvastatin use does not negatively affect tendon mechanical properties and might help to reduce the risk of tendon rupture.

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References

1. Jacobs JJ *et al.* *J Bone Joint Surg Am*, 2013.
2. Health, United States, 2010.
3. Klemp P, *et al.* *Ann Rheum Dis*, 1993.
4. Beason DP *et al.* *J Shoulder Elbow Surg*, 2013.
5. Lee *et al.* *J Sex Med*, 2008.
6. Thomopoulos S *et al.* *J Orthop Res*, 2003.
7. Gimbel JA *et al.* *J Biomech*, 2004.
8. Gimbel JA *et al.* *J Biomech Eng*, 2007.
9. Bey MJ *et al.* *J Biomech Eng*, 2002.
10. Soslowsky LJ *et al.* *J Shoulder Elbow Surg*, 1999.