Correlated Expression of Type 1 Collagen and Aggrecan with Exercise of Fatigue Damaged Tendons

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Disclosures:

Introduction: Tendinopathy is a debilitating disease caused by repetitive damage leading to an impaired repair response. While exercise can effectively induce adaptation in healthy tendons [1], it remains unclear whether exercise can promote adaptive changes and induce remodeling in fatigue damaged tendons. We have previously examined the effect of 6-weeks of daily 30 and 60 minute exercise protocol that is initiated immediately after fatigue loading and found both running protocols to similarly lead to increased structural damage compared to the group that underwent cage activity following fatigue loading [2]. Since our previous findings showed that the molecular response of fatigue damage tendons peaks 7-days after fatigue loading and mostly return to baseline thereafter, we suspected that the time of initiation of exercise after fatigue loading will impact the effect of exercise on fatigue loaded tendons. In addition, while our previous finding showed that 1-week of intense exercise induces a molecular response that is indicative of adaptation [3], the long-term effect of exercise on fatigue loaded tendons that exhibit a range of damage severities remains unknown. Consequently, the objectives of this study were (1) to determine the effect of initiation of an exercise protocol during the period of heightened biologic response or after its dissipation in response to fatigue loading and (2) to determine the effect of amount of induced damage on the ability of the fatigue damaged tendon to adapt to exercise. We hypothesize (1) Initiating exercise after 14-days from induction of fatigue loading (‘delayed exercise’) will reduced the damage in the tendon compared to immediate initiation of exercise or cage activity (2) Delayed exercise will induce adaptive changes in fatigue damaged tendons that is characterized by upregulation of type 1 collagen and decorin and downregulation of aggrecan.

Methods: Following IACUC approval, 75 retired breeder female Sprague-Dawley rats were fatigue loaded as previously described [4]. The patellar tendons (PT) of 24 rats were fatigue loaded for 500 or 7200 cycles between 1N and 40N at 1Hz. To complete the 1st objective, rats were divided into 3 groups (n=8/group) of 60 min. runners, 30 min. runners or cage rest controls. Running protocols were initiated 1-day or 14-days after fatigue loading, resulting in a total of 6 groups (n=48). Rats ran for 6-weeks (5 days/ week at 17 meters/min for 30 or 60 minutes per day). At sacrifice, left PTs were harvested and fixed under 2N tension in zinc buffered formalin. Tendons were decalcified and split longitudinally into 2 halves. One half of the tendon was used for micro-structural analysis using Second Harmonic Generation (SHG) imaging. Damage area fraction (DAF) was determined for each SHG sample. All analysis was conducted at the origin, midsubstance and insertion. Analyzed data was used to guide the 2nd objective of the study. Subsequently, left patellar tendons were loaded for either 500 or 7200 cycles (n=11-12/ group). Additional animals were used as Naïve controls (n=6). Half of the animals in each of the 500 and 7200 cycle groups maintained cage activity and the other half commenced running 14-days post-fatigue loading for 14-days. At 4 weeks post-fatigue all animals were sacrificed and the PTs were harvested and flash-frozen. Half of each tendon was pulverized and RNA was isolated and converted to cDNA. The other half is being analyzed for protein content. Primers for four genes (GAPDH, Col1a1, Acan and Dcn) were used. The data is presented as -ΔΔCt values in order to appropriately reflect the direction of fold change in gene expression. Student’s t-test, ANOVA, and linear regression were used. Statistical significant was set at p≤0.05 and a trend at p≤0.1. K-means cluster analysis was used to separate the severity of damage to high and low damaged groups.

Results: No differences were found in DAF between the 30 and 60 min. runners, leading to subsequent pooling of this data. DAF was significantly increased in the PT insertion of animals that initiated running 1-day but not 14-days after fatigue loading. As expected, DAF (Fig 1) was decreased (trend) in the PT midsubstance of animals that initiated running 14-days but not 1-day after fatigue loading, suggesting that a short delay in initiating treatment after onset of injury could be essential. Based on this data the second set of animals began treadmill running 14-days post fatigue loading for 30 min/day. Surprisingly, no significant difference between exercise and cage activity on fatigue damaged tendons was observed for the low damaged or high damaged groups. Since there were no differences based on the severity of damage, the low and high damaged groups were combined. The expressions of the genes were correlated separately for the exercised group and the caged group. Positive correlations were found between col 1 and aggrecan for the exercised animals but not the caged animals (Fig 2). Col 1 and decorin had a positive correlation for both exercised and caged animals (Fig 3). There was no correlation for any of the groups between aggrecan and decorin.

Discussion: Since exercise can induce adaptation in healthy tendons, it was expected to promote remodeling in fatigue damage tendons. However, damaged tendons could further degenerate with exercise, requiring a careful approach to determine the appropriate exercise protocol to induce adaptation. In the first objective, we found that the tendon midsubstance exhibited
recovery in damage from 6-weeks of exercise that was initiated 14-days after fatigue loading, but exhibited further accumulation of damage from 6-weeks of exercise that was initiated 1-day after fatigue loading. Consequently, to complete the second objective, we initiated exercise 14-days post fatigue. We expected that the observed recovery in DAF at 8 weeks post fatigue (6 weeks of exercise) would be a result of increased protein levels of collagen I and decorin and decreased protein levels of aggrecan, and therefore expected that the gene expression would show similar patterns at the timepoint evaluated (14-days of exercise). Surprisingly, there was no significant differences for any of the genes between exercised and cage. This is also in contrast with our previous finding that showed [3] an increase of col 1 with 1-week exercise of fatigue damaged tendons, suggesting that the gene expression likely returned to baseline levels at 14-days after initiation of exercise. We expect that protein content (ongoing studies) of these tendons will provide great insight since our previous study has indicated a high level of activity earlier [3]. Interestingly, aggrecan and col 1 expression were positively correlated in the exercised group but not in the cage group. Since there was a trend for both of these genes to be downregulated, we expect that both were upregulated at an earlier timepoint leading to increased protein at this timepoint and resulting in downregulation of their expression through a negative feedback loop. While increase in aggrecan protein levels is contrary to our hypothesis, since the tissue analyzed included the insertion and origin, the increase of aggrecan could be necessary for protection/recovery of the insertion and origin. Further studies are needed to explore these potential mechanisms and will include quantification of protein levels at this timepoint as well as determining the structural and functional properties at this and a later timepoint. 

Significance: Delaying exercise as a treatment could be more beneficial than immediate start of exercise after injury. Collagen 1 and aggrecan respond similarly to exercise in fatigue damaged tendons suggesting that an increase in aggrecan could be beneficial in early adaptation to exercise in fatigue damaged tendons.

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Fig 1: DAF of tendons from rats that initiated 6-weeks of exercise 1-day or 14-days after fatigue loading normalized to time-matched controls. ‘**’: p<0.05; ‘#’: p≤0.1.
Fig 2: Correlation between expression of aggregan and type 1 collagen levels in exercised (Top) and cage (Bottom) rats.
Fig 3: Correlation between expression of decorin and type 1 collagen levels in exercised (Top) and cage (Bottom) rats.