A Novel Murine Model for Muscle Atrophy and Fat Infiltration after Rotator Cuff Tears

INTRODUCTION:
Rotator cuff tears (RCT) are one of the most common orthopaedic conditions treated. Chronic rotator cuff tears lead to poor shoulder function, pain, and decreased quality of life. In the setting of chronic rotator cuff tears, studies have demonstrated that muscle atrophy and fatty infiltration are independent predictors of poor outcome following surgical repair [1,2]. Understanding the factors that are responsible for muscle degeneration and atrophy as well as fatty infiltration may lead to pharmacologic treatments that will improve the outcomes of patients following a large rotator cuff tear. Significant and consistent muscle atrophy and fat infiltration were observed as early as 6 weeks after surgery in this model.

It has been hypothesized that denervation of supraspinatus and infraspinatus due the entrapment of suprascapular nerve by the retraction of supraspinatus after RCT may contribute to the atrophy and fatty infiltration in these muscles [4]. In this study, we have established a new method to denervate SS and IS by transecting suprascapular nerve from its nerve root. This new method allows us to study the role of denervation on muscle atrophy and fat infiltration after RCT with and without combination of tendon transection. Denervated SS and IS showed nearly 3 fold of muscle weight loss compared to those with simple tendon rupture. This data suggested that denervation has a significant effect on muscle atrophy and may play an important role in muscle atrophy after RCT. In a short period of 6 weeks, we did not observe a role of denervation on muscle fat infiltration. However, longer time points in future works are needed further evaluate this model.

The limitations of this model includes: 1) the mouse shoulder differs considerably from the human shoulder; 2) the RCT heals much better in mice compared to humans; 3) the severity of fat infiltration in mice muscles after RCT was significantly milder than that found in human. However, our gross and histologic analysis has showed consistent muscle atrophy and fat infiltration after RCT in this model, suggesting this is a valid mouse model for future study for mechanisms and possible treatment of muscle atrophy and fat infiltration after RCT.

METHODS:
Surgical Procedure Adult female FVB/N mice at 3 months (Charles River Laboratories Inc.) were used in this study. Mice were randomly assigned to the following 3 groups: (1) tendon transection only (TT); (2) denervation only (DN); and (3) tendon transection plus denervation (TT+DN). In the TT group, the deltoïd muscle of the mice was sharply divided with a blade to expose the rotator cuff tendons after the skin is opened. The supraspinatus (SS) and the infraspinatus (IS) tendons were sharply transected with a sharp blade. In the DN group, trapezius muscle of the mice was sharply divided to expose the suprascapular nerve. One cm long fragment of suprascapular nerve was then removed in order to prevent nerve regeneration after the surgery. In TT+DN group, both procedures were conducted. Sham surgery was performed on the opposite shoulder to serve as internal control. All procedures were approved by our Institutional Animal Care and Use Committee.

Muscle Harvesting and Histology Mice were sacrificed 6 weeks after surgery. Both SS and IS muscles were harvested and weighted immediately. Muscles were then flash frozen in liquid nitrogen cooled isopentane and sectioned at the thickness of 10µm using a cryostat. Hematoxylin & Eosin staining was performed to study the morphology change of muscles. In order to evaluate possible muscle fat infiltration, Oil Red O [3] staining was performed.

Statistical Analysis Paired T-test was used within groups and one-way ANOVA was used among groups for data analysis. Statistical significant difference was considered when P<0.05.

RESULTS:
Six weeks after surgery, the wet weight of the SS decreased 18.8 ± 9.6%, 63.4 ± 6.1% and 53.5 ± 4.7% in TT, TT+DN and DN group respectively. IS decreased 21.2 ± 8.9 %, 60.4 ± 4.5% and 59.4 ± 5.4% in TT, TT+DN and DN group respectively. Significant difference was found in both SS and IS in TT, DN and TT+DN groups compared to the shams (P<0.05). However, no significant difference was found between SS and IS in any groups. Denervation significantly exaggerated muscle atrophy in both SS and IS. For both SS and IS, there was a significant higher muscle weight loss in DN and TT+DN groups compared to TT only group (P<0.05). However, no significant difference was found between DN and TT+DN groups (Figure 1).

Muscle Oil Red O staining showed significantly increased fat in both SS and IS 6 weeks after tendon transection (Figure 2). Atrophic SS and IS in DN and TT+DN groups have similar amount of fat infiltration compared to those in TT group (pictures not shown).

DISCUSSION:
The development of small animal models is a critical tool in advancing orthopaedic research. In this study, we have successfully created a novel mouse model for muscle atrophy and fat infiltration following a large rotator cuff tear. Significant and consistent muscle atrophy and fat infiltration were observed as early as 6 weeks after surgery in this model.

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REFERENCES:

Figure 1. Relative muscle weight loss in SS and IS in TT, TT+DN and DN groups 6 weeks after surgery (N=6). Significant more muscle weight loss was found in both SS and IS in DN and TT+DN groups compared to TT group (*, P<0.05)

Figure 2. Typical pictures of Oil Red O staining for control SS and IS (sham) and those with tendon transection (TT) 6 weeks after surgery.