INTRODUCTION:
Skeletal muscle atrophy can be a serious consequence resulting from both immobilization and musculoskeletal trauma such as tendon injuries. Although difficulties with mobility and other daily complications are witnessed by orthopaedic patients, the biomechanical properties of muscle dysfunction vary under these two models. In this study, we investigated the muscle functional change under two clinically relevant mouse models as representations of tendon injury and muscle disuse: Achilles tendon transection (TT) and hindlimb suspension (HS). More specifically, we compared the functional loss of gastrocnemius (GA) through in situ biomechanical testing as one of our main assessments. Our results provided evidence to the hypothesis that tendon injury and muscle disuse may result in different levels of muscle weight and functional loss.

METHODS:
Surgical Procedure: Adult FVB/NJ male mice (N=6) underwent complete transection of Achilles tendons on the right side legs. Contralateral sides were used as control.

Hindlimb Suspension: Disuse of hindlimb was achieved in six wild type FVB mice through hindlimb suspension (reference of the HS model). The mice were able to use their forelimbs for support, movement, feeding, and grooming. Control mice were kept in identical cages but without hindlimb suspension. All procedures were approved by Animal Component of Research Protocol (ACORP).

Muscle Biomechanical Testing: All mice underwent in situ muscle functional testing 2 weeks after surgery/suspension. The Achilles tendon was exposed and released from the calcaneus, allowing the gastrocnemius muscle to attach to a force transducer (Grass Tech FT03 Transducer, Astro-Med Inc) by using a suture silk tied to the Achilles tendon. The femur was secured to eliminate all motion artifacts from other muscles.

A bipolar electrode was placed in the sciatic nerve to stimulate muscle contraction. Animals were under isoflurane anesthesia during the entire procedure, which was approved by our Institutional Animal Care and Use Committee.

Muscle Harvesting: Gastrocnemius muscles were harvested and weighted immediately after the mechanical testing. They were then flash frozen for histological analysis.

Histology: Gastrocnemius muscle was sectioned using a cryostat. Picrosirius red staining for collagenase connective tissue was performed as described previously (Liu et al. 2011 JOR).

Statistical Analysis: Student T-test was used to compare data between control and treatment groups. Significance was defined as a p value of less than 0.05.

RESULTS:
Significant GA muscle weight loss was found in between atrophic and control mice after HS and TT (Figure 1). Significant maximum GA force loss was found between atrophic and control mice only in the TT model, not in the HS model (Figure 2).

Picrosirius red staining showed a greater amount of connective tissue in the atrophic GA compared to control GA after 2-week TT, but the difference of connective tissue content was not as visible between atrophic and control muscles after HS (Figure 3).

DISCUSSION:
Our results provide interesting findings on different gastrocnemius biomechanics following muscle disuse and tendon injury. Disuse atrophy after 2 weeks of hindlimb suspension significantly reduces the weight of the gastrocnemius. However, the reduction of muscle isometric contractile force after the 2-week period is not significant. On the contrary, tendon injury-related atrophy displays a much greater muscle functional loss in addition to its weight loss. Histological results demonstrate a greater amount of connective tissue only after Achilles tendon transection, suggesting muscle fibrosis after tendon rupture as opposed to muscle disuse. The presence of fibrosis after tendon rupture may explain the greater muscle maximum force reduction seen in muscle atrophy after tendon transection.

In summary, both gross level and histological findings provide evidence that disuse-induced and tendon injury-induced muscle atrophy has different impact on the functional properties of gastrocnemius. The accumulation of intramuscular collagenous connective tissue in atrophic muscle after tendon rupture may be responsible for the difference.

SIGNIFICANCE:
Skeletal muscle atrophy has high clinical relevance to the orthopaedic patient population. In this study, we have shown that the muscle force generation differs under disuse atrophy and tendon injury atrophy. These findings may help us understand the functional role of muscle contractions after these conditions.

REFERENCES:

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