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DIFFERENCES BETWEEN ACTIVE AND PASSIVE FAILURE IN WHOLE SKELETAL MUSCLE

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SUMMARY
Extensive research has been performed examining eccentric contraction and related muscle injury. Only a few studies have examined muscle failure due to an imposed tissue strain. Those that have examined failure tend to investigate active muscle failure and define failure as complete tearing of the muscle. However, muscle damage is known to occur well before a tear is apparent, and failure may be better defined by a drop in force during stretch. Leonard *et al.* showed that isolated rabbit myofibrils fail (as defined by a drop in force during stretch) beyond actin-myosin overlap in both active and passive states; however, active myofibrils failed at much higher forces than passive ones. This study, carried out on frog (*Rana pipiens*) tibialis anterior muscles, was performed to see if this difference between actively and passively stretched myofibrils persisted in whole muscles.

Contrary to the results of Leonard *et al.*, there was no difference between active and passive muscles in either failure force or sarcomere length at failure. Furthermore, average sarcomere lengths were within the range of myofilament overlap.

INTRODUCTION
Lengthening contractions of skeletal muscle are known to cause damage [1]. The majority of studies examining the effect of lengthening on skeletal muscle do so by imposing a repetitive strain on the tissue and assessing the extent of injury by calculating the deficit in isometric force post-stretch compared to pre-stretch. Damage tends to be manifest as misalignment of sarcomeres and disruption of sarcomere structure (streaming Z-lines, damaged A-bands). Studies have been performed on whole muscle (e.g. [2]), intact single fibres (e.g. [3]), permeabilized fibres (e.g. [4]), and single myofibrils (e.g. [5]). Only a limited number of studies have examined complete failure of muscle, defined by a tearing of the muscle. It has been shown that active muscles require a greater force-to-tear than their passive counterparts, and that strain-to-tear is the same [6].

Recently, Leonard *et al.* showed that actively stretched myofibrils from the rabbit psoas failed at much higher stresses than passively stretched ones, and that both active and passive myofibrils failed at similar lengths well beyond myofilament overlap [7]. These results are puzzling in that, despite being at a length beyond myofilament overlap where cross-bridge activity is presumably non-existent, higher failure forces in active myofibrils persist compared to passive myofibrils. These results suggest that active forces (i.e. cross-bridge-dependent forces) somehow alter interactions and/or structures within the myofibril to produce high forces during stretch that persist beyond myofilament overlap. However, it is not known if this difference is realized at higher tissue levels.

METHODS
Rana pipiens (n=24) were anesthetized by initial immersion in a 0.3% tricane methylsulfonate solution and maintained by application of MS-222-soaked gauze strips placed over the body. The tibialis anterior muscle was isolated, the lateral head was cut, and the remaining medial head was severed distally with a piece of remnant tarsal bone. The sciatic nerve was exposed with an incision on the dorsal thigh through which a hook-type electrode was placed against the nerve. The frog was placed in a stereotaxic frame and fixed at the knee, while the remnant tarsal bone was clamped into a force transducer that was mounted on a linear table motor.

Prior to commencing the failure test, the muscle was activated over a range of lengths in 0.5 mm increments for determination of the force-length relationship. Optimal length (L_0) was determined by calculating the stationary point of the active forces' best fit polynomial. The muscle was positioned at its optimal length and then pulled to failure either actively (n=12) or passively (n=12) at a rate of 5% L_0 per second (Figure 1). The criterion for failure was appearance of a negative slope in the force-time curve [7], and the motor was stopped if force dropped by more than 2%.

Following the failure test, the frog was sacrificed and the muscle was surrounded with a custom acrylic bath which was filled with 10% neutral buffered formalin. This allowed the muscle to be fixed at the failure length without removing it from the setup. Samples from the proximal and distal ends and the midbelly were then analyzed using transmission electron microscopy (n=3 passive, n=3 active), or were used to estimate sarcomere lengths at failure using laser diffraction (n=3 passive, n=3 active, 10 fascicles per muscle) [8]. One-way ANOVA was performed to evaluate differences in failure forces and sarcomere lengths between active and passive samples ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Passively- and actively-failed muscles showed no significant difference in force-to-failure or in sarcomere length at failure (Figure 2). Mean sarcomere lengths at failure occurred towards the end of the descending limb of the force-length relationship (loss of filament overlap in frog occurs at 3.65 µm based on thick filament length of 1.6 µm, thin filament length of 0.975 µm and Z-disk thickness of 0.05 µm; [9]).

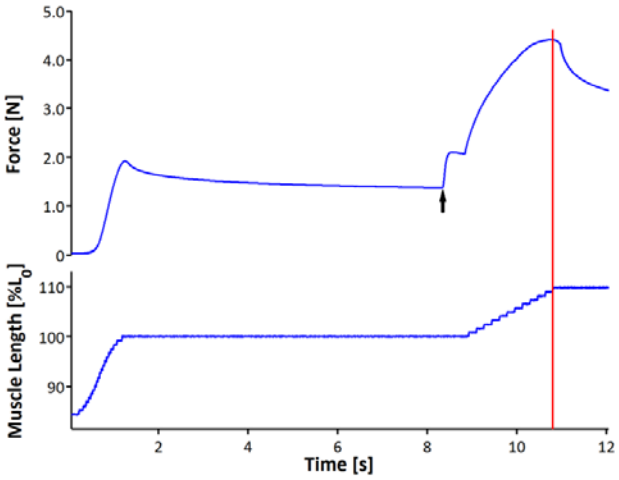

Figure 1: Exemplar force- and length-time history of a muscle actively pulled to failure. The arrow indicates the onset of activation and the vertical line indicates the point of failure.

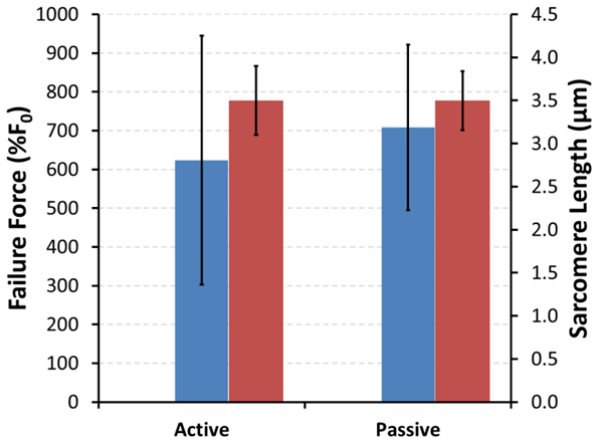

Figure 2: Active (n=12) and passive (n=12) normalized mean failure forces (blue bars) and mean sarcomere lengths (red bars). The error bars indicate ±1 standard deviation.

Both actively- and passively-failed muscles showed evidence of damage under transmission electron microscopy (Figure 3). Damage was manifested primarily as streaming Z-lines and misaligned sarcomeres. Active samples tended to show slightly more evidence of damage than passive ones when evaluated by eye. Actively-failed muscles usually showed more evidence of damage in samples taken from the midbelly of the muscle, while passively-failed muscles usually showed more signs of damage in samples taken from the distal end.

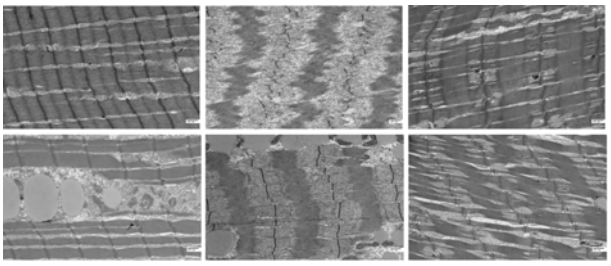

Figure 3: Electron micrographs of active (top) and passive (bottom) failed muscles. Samples taken from proximal (left), midbelly (middle), and distal (right) regions.

CONCLUSIONS

No differences were observed between passive and active muscles in terms of failure force or sarcomere length at failure. The behavior observed by Leonard *et al.* in isolated myofibrils was not found in our whole muscle preparation, and the onset of damage was not activation-dependent. However, qualitative differences were observed in the transmission electron micrographs. Future work will focus on examining the same properties in intact single fibres and myofibrils obtained from the same muscle.

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