

# LOSS OF ISOMETRIC TENSION IN MYOFIBRILS UNDERGOING ACTIVATED STRETCHES

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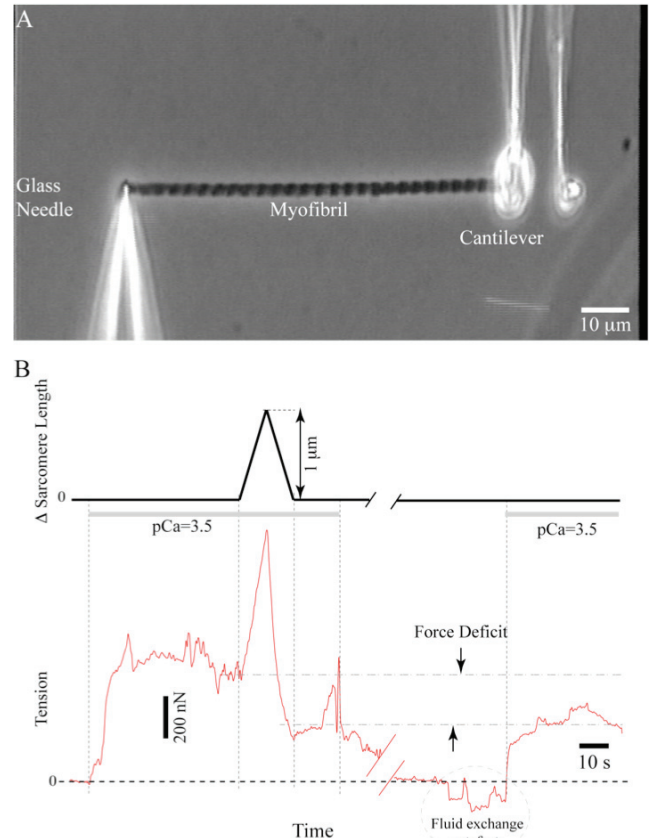
## INTRODUCTION

Stretching of activated skeletal muscle fibers results in immediate loss of isometric tension and focal sarcomere disruption [1]. Loss of tension has been thought to be a direct consequence of the loss of tension generating units, the sarcomeres [2] and the disruption itself was explained with a theoretical model [3]. These two effects, *viz.*, loss of tension and sarcomere disruption, seem to be concurrent on most tissue levels. However, stretch experiments on isolated myofibrils showed evidence neither for sarcomere disruption [4,5], nor for reduction in isometric tension. Consequently, the question whether sarcomere disruption is necessary or not for loss of isometric tension following stretch, remained untested at the myofibril level. We stretched activated myofibrils of rabbit psoas muscles to test the hypothesis that loss of tension following activated stretches does not require sarcomere disruption.

## METHODS

Rabbits were euthanized by an intravenous injection of sodium pentobarbital solution (240 mg/ml), a protocol approved by the University of Calgary Animal Care and Ethics Committee. Small strips of psoas muscle were dissected and stored in 50% rigor and 50% glycerol (v/v) solution at  $-20^{\circ}\text{C}$  for 10 days.

On the day of the experiment, a small sample of the tissue was cut and subsequently blended in a rigor solution. A small amount of the blended mixture was placed in a fluid exchange chamber mounted on an inverted microscope (Zeiss, Axiovert 200M, Germany) and myofibrils were allowed to settle at the bottom of the chamber for  $\sim 5$  min. Then, myofibrils in suspension were washed away by replacing the bathing solution with a relaxing solution leaving only the myofibrils that had settled at the bottom of the chamber.



**Figure 1:** *A.* A Sample myofibril preparation (magnification, 100x). *B.* Myofibrils were stretched and released at constant speed (top). The tension response of a representative myofibril to the stretch-release cycle is shown in the bottom trace. Force deficit was estimated from the isometric tension measurements before and after the stretch.

Myofibrils were mounted (Figure 1A) with one end fixed to the tip of a glass micro needle and the other end attached to a silicon nitride cantilever of known stiffness. The cantilever movement was tracked by projecting it onto a linear CCD array (10,680 elements) to estimate the tension in the myofibril.

Myofibrils were maximally activated by replacing the bathing solution with a high- $[Ca^{2+}]$  activating solution ( $pCa=3.5$ ). At peak isometric tension, myofibrils were subjected to a single stretch-release cycle of magnitude,  $1 \mu m \text{ sarcomere}^{-1}$  and speed,  $0.1 \mu m \text{ s}^{-1} \text{ sarcomere}^{-1}$ . Myofibrils were subsequently relaxed and re-activated to estimate the isometric tension following the stretch-release protocol. Force deficit, the percentage loss in maximum isometric tension, was estimated from the two isometric tension measurements before and after the stretch-release cycle (Figure 1B). Control myofibrils were simply activated twice, but not stretched. All experiments were videotaped throughout the experimental protocol to note any sarcomere disruption.

## RESULTS AND DISCUSSION

At maximal activation, myofibrils produced a peak isometric tension of  $157 \pm 37 \text{ kN m}^{-2}$  (mean  $\pm$  SD;  $n=4$ ) at an average sarcomere length of  $2.61 \pm 0.1 \mu m$ . During the stretch-release cycle, myofibrils reached a peak sarcomere length of  $3.3 \pm 0.1 \mu m$  resulting in  $28.4 \pm 5.6 \%$  strain. Isometric tension immediately following the stretch-release cycle was significantly lower when compared to that before the cycle, and closely matched with the peak isometric tension during the second activation. The stretch-release cycle resulted in a force deficit of  $44 \pm 3 \%$ , whereas the control myofibrils showed only

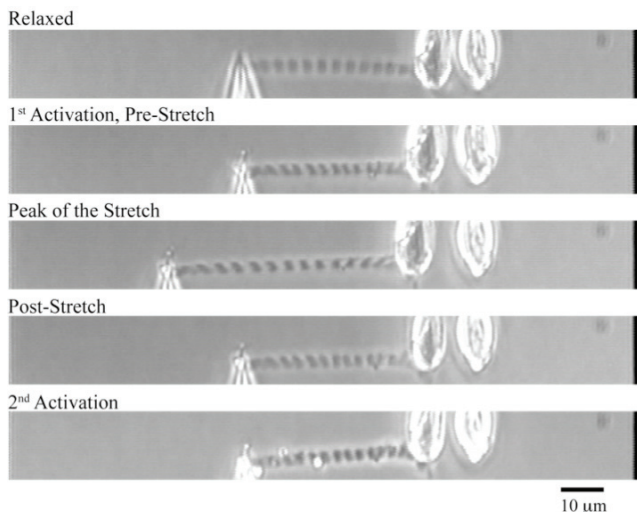
a  $4 \pm 18 \%$  ( $n=3$ ) decrease in isometric force from the first activation to the second activation. Even with such large force deficits, the sarcomeres appeared to be stable and remained intact throughout the experimental protocol (Fig. 2).

Previous experiments [4,5] involving activated stretches of single myofibrils did not show evidence of sarcomere disruption or loss of tension and damage. In contrast, we produced a 44 % reduction in tension, an indication of severe damage, yet without any visible sarcomere disruption. The loss in tension could not be due to force depression [6], because such history-dependent effects should have been abolished by the relaxation and re-activation procedure [6,7].

## CONCLUSIONS

The observation, that activated stretching of myofibrils results in loss of tension without any sarcomere disruption, contradicts the commonly held view that loss of tension is a direct consequence of sarcomere disruption. We conclude that the mechanism for loss of tension in stretched muscles arises mostly from within the sarcomere.

We speculate that activated stretching permanently alters the interaction between active and passive force-transmission mechanisms thereby reducing the isometric tension.



**Figure 2:** A Representative myofibril at 5 different stages of the experiment. Sarcomeres within myofibrils were intact throughout the stretch protocol. No rapid lengthening of sarcomeres was observed.

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