Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

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Primary CNF Tools Used: GCA 5X stepper, photolith spinners, Oxford 81 ion etcher

Abstract:

To measure muscle forces in the nano-Newton range, silicon nitride cantilever pairs were manufactured using the GCA 5x-stepper photolithography system and the Oxford 81 ion etching system at the CNF, and then used in our lab in Canada. We investigated titin mechanical properties using a skeletal muscle myofibril model. Our experiments demonstrate sarcomeres in skeletal muscle are not unstable when suddenly made weaker relative to the sarcomeres adjacent to them.

Summary of Research:

Muscle (the smallest functional unit being the sarcomere) generates active force through cyclic interactions between myosin and actin and the amount of active isometric force generated is proportional to the length of the sarcomere [1]. Passive forces in the sarcomere are also length dependent and are supported by the molecular spring-like protein titin [2]. Instability of sarcomeres has been proposed as a mechanism for injury in muscle since sarcomeres arranged in-series must sustain the same force along a myofibril and sarcomeres that are weaker than adjacent ones are expected to over-lengthen since there is a disparity in active force producing potential. This instability results in a sarcomere that is rapidly lengthened (termed "popping") until only passive structures (titin) sustain the in-series force, with damage occurring to that sarcomere [3]. The purpose of this study was to measure the length of each sarcomere in a single myofibril during activation and then follow these sarcomeres with time as portions of the sample are deactivated to see whether weaker sarcomeres behave as predicted, and do in fact, over-lengthen.

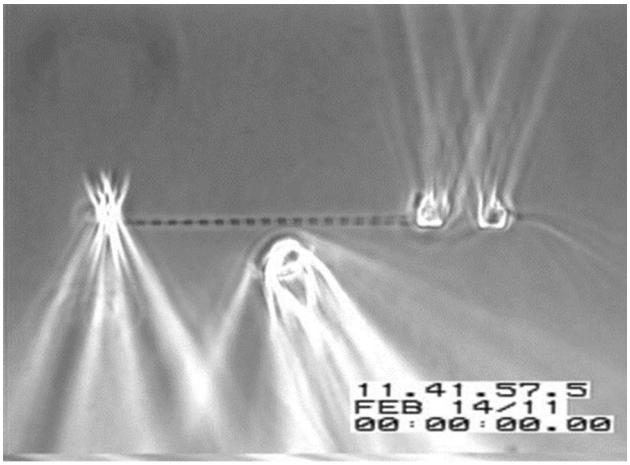

Figure 1: Myofibril attached to a glass needle for stretch-shortening and cantilevers for force measurement. An example of a single myofibril with 19 sarcomeres in-series. The glass tube (center) is used to deliver a focused stream of deactivating solution.

Methods:

Skeletal muscle myofibrils were generated using rabbit psoas muscle as previously described in reference [4] and were placed in an experimental chamber atop an inverted microscope. Single myofibrils (n=6) were attached at one end to a glass needle/motor assembly for specimen length control and at the other end to a micro-fabricated silicon nitride cantilever pair (68 nN/μm stiffness) for measuring force. High-resolution (88 nm per pixel) video data (30 fps) were collected continuously during the experiment and analyzed using custom MATLAB

analysis code. The myofibril was initially in a relaxed state and the myofibril length adjusted to an average sarcomere length (SL) of approximately 2.4 μm. Then the Ca+2 rich activating solution was delivered and once the myofibril was fully activated, a second stream of relaxing solution was targeted to the left side of the myofibril (Figure 1). This resulted in a wave of deactivation that started at the left and propagated rightward until it encompassed the entire myofibril, and the myofibril returned to the relaxed state.

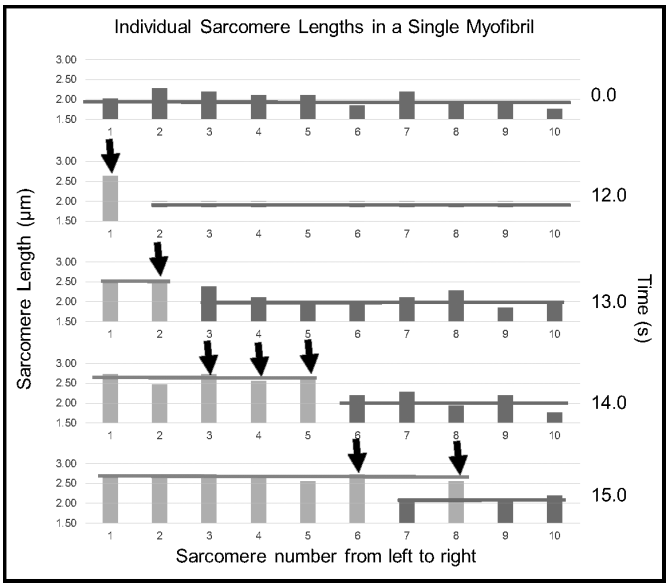

Figure 2: Sarcomere length in an activated myofibril as a wave of deactivation solution moves from left to right. Black arrows highlight activated sarcomeres (dark grey) that transition rapidly to passive (light grey). Dark grey and light grey horizontal bars indicate the mean SL for sarcomeres belonging to groups classified as active or passive.

Results and Discussion:

In Figure 2, for one typical experiment, the mean SL upon activation was 2.1 μm and the stress measured (not shown) was 209 nN/μm^2. The infusion of the localized stream of relaxing solution at time-point 12s resulted in the first sarcomere (sarcomere #1) rapidly lengthening from a SL of 2.47 μm to 2.65 μm. At 13s, the next sarcomere (#2) lengthened from 2.21 μm to 2.47 μm and at 14s, #3 lengthens from 2.29 μm to 2.74 μm. At time point 15s, more than half of the sarcomeres have relaxed, the total stress is 110 nN/μm^2 and the mean relaxed SL (light grey horizontal line; Figure 1) is 2.65 μm and the remaining active sarcomeres have a mean SL of 2.15 μm (dark grey horizontal line). At time 15s, seven sarcomeres out of ten have lengthened from their initial active length and are positioned on the descending limb of the force-length relationship. Instability theory would predict these sarcomeres to over-lengthen. In fact, these deactivated sarcomeres would need to lengthen considerably if they were to passively support the 110 nN/μm^2 of stress still detected; A SL of near 4.0 μm would be required and passive force does not appear in this preparation until SL of about 2.8 μm. These passive sarcomeres are presumably sustaining this tension by titin alone, and in this example, with the titin stiffness being modulated so that a relaxed sarcomere at 2.6 μm can sustain the load.

Conclusions:

Weak (deactivated) sarcomeres do not "pop". We speculate that popping is prevented by a "stiffening" of the molecular spring titin. The mechanisms underlying this stiffening of titin need further elucidation.

References:

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