Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

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Primary CNF Tools Used: GCA 5X Stepper, SÜSS MA6-BA6 contact aligner, photolith spinners, Oxford 81 ion etcher

Abstract:

To measure muscle forces in the nano-Newton range, silicon nitride cantilever pairs were manufactured and used. We investigated history-dependent behaviour in cardiac muscle using a single myofibril model. Our experiments demonstrate for the first time that cardiac myofibrils display the same history-dependent properties observed in skeletal muscle, specifically force enhancement following active stretch. The giant molecular spring titin [1] is thought to play a major role in this enhanced force, possibly through interactions of regions of titin with the actin filament. However, titin in cardiac muscle is much smaller than skeletal muscle titin and is missing regions of titin (specifically N2A region) that are suggested to facilitate interactions with actin. We investigated rabbit cardiac myofibrils to see if enhanced force following stretch is present in eight samples. We observed in all tests performed, greater force after stretch compared to force in the isometric condition and at the same sarcomere length. This provides, for the first time, evidence that cardiac muscle displays history-dependent behaviour.

Summary of Research:

The active force produced by skeletal muscle is well described by the Huxley cross-bridge model [2] and the force-length relationship [3] but these well accepted paradigms cannot adequately explain greater force at the identical muscle length when the muscle is lengthened during the contraction (eccentric contraction), compared to a muscle that does not change length (isometric contraction). This history-dependent force has been observed for over 60 years, but no generally accepted mechanism has been put forth to explain it. Recently, titin interaction with other sarcomeric proteins has been proposed as a mechanism and promising work on a mutation mouse model (mdm) has provided evidence that the N2A region of titin is essential for force enhancement [4]. We hypothesize that cardiac muscle will exhibit enhanced force following active stretch because cardiac muscle contains a domain similar to the N2A region (i.e. N2AB) and so cardiac titin will interact with actin, as has been proposed for skeletal muscle.

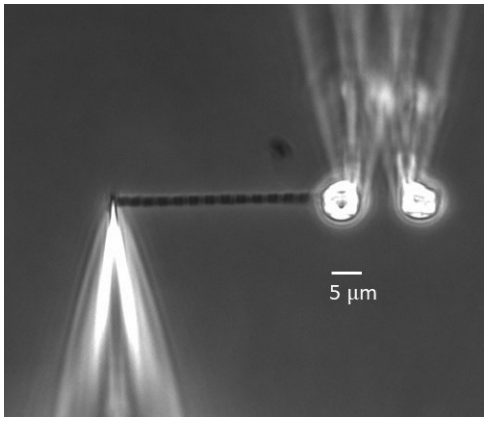

Figure 1: Myofibril attached to a glass needle for stretch-shortening and nano-levers for force measurement. Example of a myofibril with 13 sarcomeres in-series.

Myofibrils were harvested from heart ventricle muscle obtained from New Zealand White rabbits and were chemically and mechanically isolated as described in our previous work [5]. Single myofibrils were attached to nanofabricated silicon-nitride cantilevers (stiffness 150 pN/nm) [6] for force measurement at one end of the myofibril (resolution < 0.5 nN), and at the other end, a glass pipette needle attached to a piezo-motor for controlling specimen length (Figure 1).

Forces were divided by the cross-sectional area of the myofibril and reported as stress (nN/μm^2).

Myofibrils (n=8) were stretched passively from an average sarcomere length (SL) of 1.8 μm to 2 μm, held at that length, and then activated by infusing Ca^{+2} and ATP. After 10 seconds, the specimen was shortened to 1.8 μm and held for 20 seconds to allow the force transients to fade and then the specimen was rapidly stretched to 2.0 μm and held. After the stretch force transients faded, the second force measurement was made.

The isometric (SL=2 μm) and eccentric tests (SL=1.8 μm stretched to 2.0 μm) were combined in a single experiment (Figure 2). The force was recorded at two time-points: once at first vertical-red bar (75 seconds) for the isometric condition and then at the steady-state condition following the active stretch (second vertical bar-blue) at 150 seconds into the test. The eccentric stress was normalized to the isometric stress for each test and the residual force enhancement as a percentage increase (RFE) reported (Figure 3).

In all eight experiments, residual force enhancement following stretch was observed, (average increase 20%).

We show here for the first time, force-enhancement in cardiac myofibrils and this work provides insight into the possible titin-actin interaction in the eccentric condition as the mechanism underlying this phenomenon.

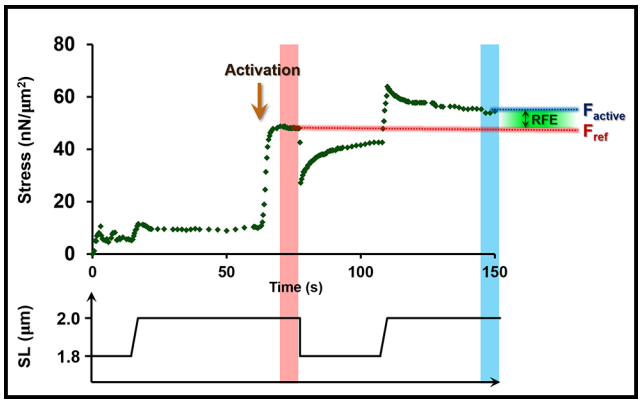

Figure 2: Isometric and steady-state isometric stress following stretch, for a single myofibril test. The residual force enhancement is formalized to the initial isometric stress.

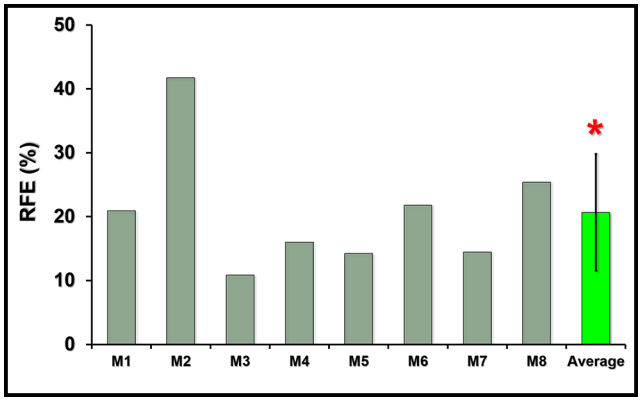

Figure 3: Residual force enhancement values for each of eight myofibrils tested. The increased stress observed following stretch compared to the isometric stress ranged from 11% to 42%, with a mean value of 20%. RFE is statistically greater than the isometric value, Wilcoxon test, a=0.05.

References:

[1] Granzier and Labeit. Muscle Nerve. 36:740-755, 2007.

[2] A.F. Huxley, Prog Biophys Biophys Chem.7:255-318, 1957.

[3] Gordon, et al., J Physiol. 184(1):170-92, 1966.

[4] K. Powers, et al. JEB. 219:1311-1316, 2016.

[5] Joumaa, et al. Eur J Physiol. 455:367-371, 2007.

[6] M. E. Fauver, et al. IEEE Trans Biomed Eng 45(7):891-898, 1998.