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MYOFIBRIL (AND TITIN) KINETICS DURING PASSIVE STRETCH-SHORTENING CYCLES

[1]Tim Leonard, [1,2]Jens Herzog, [1]Azim Jinha and [1]Walter Herzog
[1]Faculty of Kinesiology, University of Calgary, Alberta, Canada
[2]Mount Allison University, New Brunswick, Canada; email: leonard@ucalgary.ca

SUMMARY
Titin is a giant protein responsible for most of the passive force in muscle. Testing isolated titin molecules is difficult, and so we have investigated titin mechanical properties using myofibrils. Our experiments demonstrate that myofibrils (and therefore titin) behave visco-elastically with repeated stretch-shortening cycles and that full recovery following rest depends on the length of the titin during that rest and not the force. Also we show that at long sarcomere length, titin behaves visco-elastically as expected but if Ig domain refolding during the shortening cycle is prevented, then even at long sarcomere length, titin behaves almost elastically. In summary we suggest that titin is a molecular spring whose properties are governed by the unfolding/refolding kinetics of the Ig domains.

INTRODUCTION
Titin is giant molecular spring present within the sarcomere and is responsible for most of the passive force found in muscle. Titin has two regions, an inextensible region which is bound to the thick filament and an extensible region spanning the I-band from the end of the thick filament to the Z-disc and is composed of spring-like elements arranged in series [1]. These elements have different stiffness and visco-elastic properties and these come into play in an orderly fashion with increasing sarcomere length. In skeletal muscle, the I-band region of titin contains two distinct immunoglobulin (Ig) domains, a small N2A portion, and the PEVK segment. Lengthening a skeletal muscle sarcomere within the physiologically relevant range first causes the Ig domains to straighten out and is then followed by extension of titin's PEVK domain. Both of the Ig regions (proximal and distal) and the PEVK region are thought to be essentially elastic at physiologically relevant sarcomere lengths (SL). However, at SL greater than the physiological range, Ig domains start to unfold during stretch, and this unfolding is thought to be responsible for titin then behaving in a highly visco-elastic manner.
Previous work in our group [2] has shown that stretch-shortening cyclical loading of myofibrils to long SL (>4 µm) results in a large hysteresis from the first to second cycle but that the energy loss is attenuated from the second to third cycle (Figure 1). This loss of energy was attributed to Ig domain unfolding and this work agrees with experiments performed in single titin molecules [3]. We also showed that peak force recovery was not complete even when resting the myofibril at a length where no measurable force was observed. These results suggested that stretching of titin unfolds Ig domains, but shortening alone does not readily allow for refolding. This implies that in repeat stretch-shortening cycles, the number of already unfolded Ig domains becomes increasingly greater and therefore titin becomes increasingly more elastic and hysteresis is reduced. This lead to the work reported here where we hypothesized that while titin is a visco-elastic spring at long sarcomere length, it can change to exhibit essentially elastic behavior with repeated cyclic loading. The purpose of this study was two-fold:

1. To test whether Ig domain refolding during rest between cyclic loading bouts depends on resting sarcomere length.
2. To test whether titin behaves essentially elastically at long sarcomere lengths when effective refolding of Ig domains is prevented.

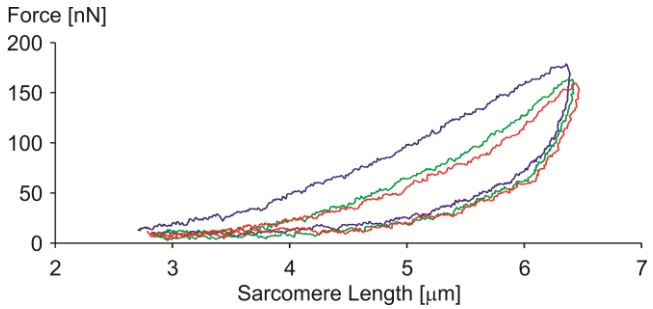

Figure 1: Force-extension curves of three sequential stretch-shortening cycles with no rest. The hysteresis from the first (blue) to last (red) cycle is significant.

METHODS
Myofibrils were harvested from psoas muscle obtained from New Zealand White female rabbit and were chemically and mechanically isolated as described in our previous work [4]. Briefly, single myofibrils were attached to nanofabricated silicon-nitride cantilevers (stiffness 68pN/nm) 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. All testing was done in a relaxing solution which contained ATP but no calcium, pH=7. All experiments were performed at room temperature (20-22 °C).
Ethical approval for these experiments was granted by the University of Calgary Life and Environmental Sciences Animal Use Ethics Committee.
Purpose 1 Test: Myofibrils were passively stretched from a nominal initial average SL of 2.6µm by nominal amounts of 2.0, 2.5, and 3.0 µm/sarcomere at a speed of 0.1µm/s/sarcomere and then released at the same speed to the starting length. Three consecutive stretch-shortening cycles were performed without rest, followed by one or two

subsequent sets of three stretch-shortening cycles separated by a ten minute rest at an average SL either of 2.6µm (n=4) or 1.8µm (n=8). While the resting lengths are different, no measurable force was observed at either of these rest lengths.

Purpose 2 Test: Myofibrils (n=5) were stretched passively from an average sarcomere length of 2.6µm by a nominal amount of 2.0µm/sarcomere at a speed of 0.1µm/s/sarcomere. When the final stretch length was reached, the myofibrils were then subjected to ten stretch-shortening cycles of nominal magnitude 0.5, 1.0, or 1.5µm per sarcomere, and then, following the last of these cycles, released to the starting length.

RESULTS AND DISCUSSION

The first set of experiments, investigating whether a 10 minute rest at different sarcomere lengths, between bouts would allow for full force restoration is shown in Figure 2. Regardless of whether the myofibril is rested at 1.8µm or 2.6µm, no force is observed but rest at 1.8µm allows for full force recovery while rest at 2.6µm does not. This indicates that refolding of Ig domains is dependent not only on the absence of force, but primarily on the length of the titin.

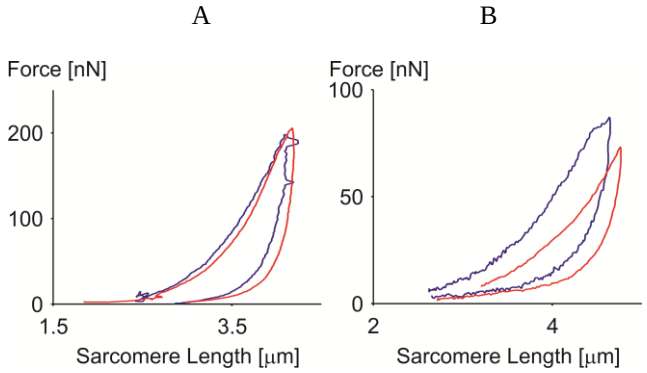

Figure 2: Passive force-elongation curve of a single myofibril. Blue trace is the first cycle. Red trace is first cycle of the second bout after a 10 minute rest. Rest is at a mean SL of 1.8µm (A) or at mean SL of 2.6µm (B).

Experiments for hypothesis 2 were designed to show if inhibition of Ig domain refolding during the shortening cycle would produce a titin behaviour that was essentially elastic. Figure 3 shows a single exemplar myofibril where a small 10 cycle stretch-shortening series is imposed after peak SL is achieved on a single cycle. This small change in mean SL at the end of the stretch would presumably prevent Ig domain refolding since titin is stretched and the force in titin is high. The results indicate that while the myofibril behaves essentially elastically, some small decrease in peak force is observed throughout the 10 cycle sequence, indicating that although there is likely no refolding of Ig domains, there appears to be a small amount of unfolding of Ig domains during the 10 cycle sequence even though forces in the myofibril are lower than the peak force reached at the end of the first (long) stretch.

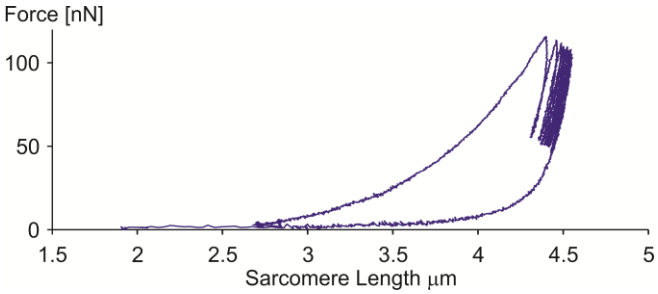

Figure 3: Passive force-elongation curve of a single myofibril stretched from a mean SL of 2.6µm to about 4.4µm and then subjected to 10 repeat stretch/shortening cycles of 0.5µm/sarcomere in amplitude.

The loss in peak force across the ten repeat stretch-shortening cycles at long sarcomere lengths was 11% for nominal magnitudes of 0.5µm (n=5) and was 11% and 5% for single observations at stretch-shortening magnitudes of 1.0 and 1.5µm, respectively. This result suggests the virtually elastic behavior of myofibrils where effective Ig domain refolding was prevented by the experimental protocol design. The minimization of Ig domain refolding for stretch-shortening cycles at long SL, where Ig domains were known to have unfolded in the first stretch cycle, showed substantially less energy loss (hysteresis) relative to the loading energies, than stretch-shortening cycles where the shortening was allowed to return to the initial (short) length. This finding suggests that titin can act almost elastically at long lengths, if Ig domain refolding is prevented, with the result that for repeated passive motion, energy loss can be minimized. The sarcomere lengths where this virtually elastic behavior can be produced can be varied over a large range of sarcomere length provided the Ig domains within the range of the stretch-shortening cycle are not allowed to refold.

CONCLUSIONS

In summary, our results suggest that titin is a molecular spring that behaves highly visco-elastically during passive stretch-shortening cycles provided Ig domain unfolding and refolding is allowed to occur. However, at short SL where Ig domain unfolding does not occur (the physiologically relevant length, SL<3.5µm), or at long SL where Ig domain unfolding has occurred (>3.5µm) but then Ig domain refolding is prevented, titin can act as an essentially elastic spring, thereby minimizing energy losses in passive stretch-shortening cycles.

ACKNOWLEDGEMENTS

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