SKELETAL MUSCLE MYOFIBRILS FAIL AT DIFFERENT FORCES BUT SIMILAR SARCOMERE LENGTHS FOR ACTIVE AND PASSIVE STRETCHING.

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INTRODUCTION

Lengthening contractions can cause significant injury to muscle fibres (McCully and Faulkner, 1985) and severe mechanical injury or failure is associated with forced lengthening of activated muscle (Faulkner et al., 1993). Previous work has shown that the force required to fail activated muscle fibres was 30% greater than that required to fail fibres stretched passively. Furthermore, the extra force measured at failure was attributed to both the displacement of cross bridges during stretch and to sarcomere length non-uniformities at the beginning of stretch resulting from connective tissue compliance (Tidball et al., 1993). Patel and Lieber (1997) suggested that three parallel force transmission systems exist in skeletal muscle fibres; the cross bridge pathway, the titin pathway and the costamere pathway (Z disc-costamere-basement membrane system). Recent work by Claflin and Brooks (2008) used this model in their work on sarcomere failure with incomplete activation in the mdx mouse, where the costamere pathway is impaired, and they concluded that in inactive sarcomeres, the cross bridge (inactive) and costamere (absent) pathways are not able to support stretch forces and hence the titin system must support all the force and sometimes it fails. We decided to determine failure forces in single myofibrils since myofibrils have no costamere pathway and inactive myofibrils would support stretch forces solely through the titin pathway. Activated myofibrils would support stretch forces via the titin pathway and also the cross bridge pathway. However, at sarcomere lengths beyond actin-myosin filament overlap, the force supporting system would revert back to the titin pathway alone. Therefore, we hypothesized that forces in active myofibrils would be greater than those for inactive myofibrils if failure occurred at sarcomere lengths less than the “no overlap” length, but would be the same if failure was observed at sarcomere lengths beyond myofilament overlap.

METHODS AND PROCEDURES

Rabbit psoas muscle myofibrils were obtained and tested on an inverted microscope system described elsewhere (Joumaa et al., 2007). Myofibrils with 3 to 8 sarcomeres in series were used. Myofibrils were stretched actively (n=7), passively (n=12) or passively with titin deleted (n=6) at 0.1 µm/sarcomere/s from the plateau region of the force length relationship to a length where failure occurred. Failure was defined at the point when the force-time curve changed from a positive to a negative slope (Tidball et al., 1993). All forces were normalized by the cross sectional area of the myofibrils and results are reported as stress (nN/µm²). Mean sarcomere length for the myofibril was determined by measuring the length of the specimen and dividing it by the number of sarcomeres. Individual sarcomere lengths were determined during stretch using the striation pattern of the myofibrils which was projected onto a high resolution linear photo-diode array. The plateau region of rabbit psoas occurs between sarcomere lengths of 2.26µm and 2.43µm and loss of actin-myosin overlap occurs at 3.91µm (Herzog et al., 1992).
RESULTS & DISCUSSION

Figure 1. Stress and sarcomere length during continuous stretching for active, passive and titin depleted myofibrils (means ± 1 S.D.)

Figure 1 shows stress values versus sarcomere length during stretch. At sarcomere lengths above 4µm active force traces should have mirrored those observed for purely passive lengthening because the active cross bridge pathway of force production is no longer able to support force (Gordon et al., 1966).

Figure 2. Stress and sarcomere lengths of myofibrils at failure (means and ± 1 S.D.)

Figure 2 shows failure stress and sarcomere lengths for active stretching, passive stretching, and the titin depleted passive myofibrils. The average sarcomere lengths at failure were the same for all three experimental conditions (p=0.42, α=0.05), while the failure stresses were significantly different (p<0.0005, α=0.05). The titin depleted myofibrils show drastically reduced failure stresses compared to the normal passive myofibrils, suggesting that titin is the primary passive force constraint in myofibrils. The activated myofibrils show the greatest failure stresses. Since actin-myosin overlap, and therefore active cross-bridge force, is lost at sarcomere lengths greater than 4.0µm, this result suggests that titin stiffness is increased in an activation (calcium) dependent manner. It has been suggested previously that titin is be a calcium-dependent molecular spring (Labeit et al., 2003). Our results support this idea, but demonstrate that this is true not only for physiological sarcomere lengths but also for sarcomere lengths beyond actin-myosin overlap. Furthermore, increases in titin stiffness have been found to be modest within the physiological range of sarcomere lengths, while the present results suggest a dramatic increase in titin stiffness in the presence of calcium, a result that will need explanation in the future.

REFERENCES


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