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Force Transients during Stretch of Myofibrils Activated with MgADP
Fabio C. Minozzo, Dilson E. Rassier.
McGill University, Montreal, QC, Canada.
When muscle fibres are subjected to a fast stretch while activated, the force increases rapidly to attain a peak, hereby referred to as critical force (P_c). Although there is still uncertainty on the mechanism responsible for P_c, it has been commonly associated with the state of crossbridges attached to actin. In the present study, we used fast stretches to investigate myofibrils activated with Ca^{2+} or MgADP, which biases crossbridges into a strongly bound state. Isolated myofibrils were attached between an atomic force cantilever and a microneedle using micromanipulators. A multi-channel perfusion system connected to a double-barreled pipette was used to activate myofibrils with calcium (pCa^{2+} 4.5) or MgADP (20mM). After force was fully developed, myofibrils were stretched by 3% of SL_o at 10 $SL_o.s^{-1}$. MgADP activation resulted in a higher P_c (1.37 ± 0.07 P/P_o) than Ca^{2+} activation (P_c = 1.23 ± 0.03 P/P_o) without changing the critical extension required to reach such force (MgADP = 25.01 ± 1.59 nm/HSL; Ca^{2+} = 24.99 ± 2.68 nm/HSL). This result suggests that myofibrils activated with MgADP produce less force, but are stiffer than myofibrils activated with Ca^{2+}. Crossbridges induced to strongly bound state via MgADP activation may cause further crossbridge formation while generating less force in comparison with crossbridges that undergo the power-stroke. However they can resist stretching when the myofibrils are stretched.

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Passive Force Analysis of Single Sarcomeres from Muscles Lacking Arginyl-tRNA-Protein Transferase (Ate1)
Felipe de Souza Leite[1], Fabio C. Minozzo[1], Neal Trecarten[1], Xuemei Han[2], John R. Yates[3], Anna Kashina[4], Dilson E. Rassier[1].
[1]Kinesiology and Physical Education, McGill University, Montreal, QC, Canada, [2]Department of Animal Biology and Scripps Research Institute, university of Pennsylvania, Philadelphia, PA, USA, [3]department of Animal Biology and Scripps Research Institute, University of Pennsylvania, philadelphia, PA, USA, [4]Department of Animal Biology, University of Pennsylvania, Philadelphia, PA, USA.
Introduction: Arginylation is a post-translational process mediated by the enzyme arginyl-tRNA transferase (Ate1). Recent findings from our laboratory suggest an important role of arginylation on the architecture and function of skeletal and cardiac muscles. In this study, we investigated the passive force development of single sarcomeres isolated from soleus muscles of mice lacking Ate1.
Methods: Soleus muscles from Ate1 KO and wild type (WT) mice were homogenized to myofibrils. Single sarcomeres in relaxing solution were tested using a pair of micro-needles. The sarcomeres were stretched in steps of 300 nm, starting in a sarcomere length of 2.2μm (±0.2μm). The force produced by the sarcomeres was plotted against the sarcomere length. Mass spectrometry was used to evaluate the presence of arginylation sites on titin. Gel electrophoresis was used to test for isoform modifications due to the lack of Ate1.
Results: Single sarcomeres of Ate KO developed less passive force than sarcomere from WT. Mass Spectrometry reviewed 4 sites for arginylation within titin's A-band structure. Electrophoresis gels showed no difference in the titin N2A isoform.
Conclusion: Post-translation arginylation of titin A-band does not change titin's isoform, but is required for normal passive force development in soleus muscles.

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Direct Evidence for the Effect of Titin Stiffness on Thick-Filament Mechanical Properties in Stretched Muscle Sarcomeres
Yong Li, **Wolfgang A. Linke**.
Institute of Physiology, Ruhr Univ. Bochum, Bochum, Germany.
The titin springs of muscle sarcomeres determine tensile muscle stress. However, they run not strictly in parallel with the sarcomeric axis, being anchored to actin at Z-disks and myosin at A-bands. On tensile sarcomere strain, which stretches the titin springs, a force component arises both longitudinally and laterally. The latter could contribute to increased sarcomeric transversal stiffness, decreased lateral myofilament spacing, and length-dependent activation of stretched muscle. We aimed to quantify by AFM force-mapping the titin contribution to sarcomeric transversal stiffness in comparison to the contribution from electrostatic forces modulated by osmotic compression. Single myofibrils were isolated from skeletal muscles expressing stiff titin-isoform (rabbit psoas) or compliant titin-isoform (rabbit diaphragm). Myofibrils were placed in relaxing buffer under the MFP-3D-BIO-AFM (Asylum Research) and force curves (50x50) were performed over a region-of-interest encompassing a whole sarcomere. Measurements were done in the absence or presence (5%) of dextran, and in the non-stretched ('slack') or stretched state (140-150% of slack). Force mapping revealed distinct transversal stiffness patterns along the sarcomere, suggesting relatively higher Z-disk stiffness and somewhat lower A-band stiffness. A-band lateral stiffness did not differ between psoas and diaphragm sarcomeres at slack. With stretch, this stiffness was increased significantly greater in psoas than in diaphragm. Osmotic compression by 5% dextran at slack increased the A-band lateral stiffness to a degree matching (psoas) or exceeding (diaphragm) that seen after stretch in 0% dextran. The stretch-induced and compression-induced contributions to A-band lateral stiffness were additive. Thus, stiff titin contributes more to A-band stiffness than compliant titin, confirming a role for titin in lateral force generation during sarcomere stretching. The electrostatic and the titin-based lateral forces compressing the A-band lattice are of the same order of magnitude in stretched muscle.

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Residual Force Enhancement in Cardiac Myofibrils
Kevin R. Boldt, Venus Joumaa, Walter Herzog.
Kinesiology, University of Calgary, Calgary, AB, Canada.
Residual force enhancement (RFE) is a property of muscle where an activated muscle is stretched from a short to a long length resulting in greater force than is produced isometrically at the long length. This history-dependent property has been identified across skeletal muscle hierarchy including whole muscle, fascicles, fibres, and myofibrils. However, RFE has not been investigated in cardiac muscle. Therefore, the purpose of this study was to determine if RFE was present in cardiac myofibrils.
Rabbit hearts were dissected and strips of left ventricle were skinned overnight with 1% Triton skinning solution and stored at −20°C. On the day of experiments, the cardiac tissue was blended and a myofibril with a good striation pattern was identified and suspended between a glass needle and a nanolever allowing for length changes and force measurement. The myofibril was set at a sarcomere length (SL) of 2.4 μm and activated to establish a reference contraction before being passively stretched to a SL of 3.2 μm. After a rest period, the myofibril was activated at a SL of 2.4 μm, actively stretched to a SL of 3.2μm, held for one minute and then relaxed. RFE was calculated as the difference between the steady-state force obtained after active stretch and the corresponding predicted isometric force at 3.2 μm based on the reference force, calculated according to the force-length relationship scaled to the filament lengths in rabbit muscles, and accounting for passive force.
All myofibrils (n=5) produced more force when stretched actively compared to the calculated reference isometric force, indicating the presence of RFE in cardiac myofibrils. The average RFE was 54.8 ± 10.8%.
The presence of RFE in cardiac myofibrils supports that RFE is a universal muscle property. Investigation in cardiac muscle may shed new light into the mechanisms underlying RFE.

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The Effects of Titin Degradation on Passive Stiffness Properties of Skinned Rabbit Psoas Fibers during Osmotic Compression
Ian C. Smith, Walter Herzog.
Kinesiology, University of Calgary, Calgary, AB, Canada.
Muscle is disproportionately stiff over short stretch distances. This effect is largely calcium-sensitive, and can be eliminated in actively contracting skinned fibers by inhibiting crossbridge formation with pharmacological agents such as 2,3-butanedione monoxime (BDM). However, elevated short range stiffness can be induced in the absence of calcium in relaxed skinned muscle fibers by osmotic compression, an effect which is not attenuated by BDM. This study aimed to determine if titin, the primary source of tension and stiffness in relaxed muscle, could account for the elevated short range stiffness in osmotically compressed, relaxed skinned fibers. Accordingly, skinned rabbit psoas fibers (n=9) were held at 2.8 μm sarcomere length and osmotically compressed using 7.5% dextran-T-500 in calcium-free relaxing solution. Muscles were stretched by 2% (~0.057 μm/sarcomere) over 0.66 s, and returned to initial length over another 0.66 s. Dextran treatment resulted in a 5.6 ± 0.6 fold increase in short range stiffness, with an apparent length limit of 4.5 ± 0.2 nm per sarcomere (Phase 1). Beyond this limit (Phase 2), stiffness was not different (100.4 ± 3.0%) than dextran-free stiffness. To selectively degrade titin, fibers were held in relaxing solution containing both dextran and trypsin (0.25 μg/mL) until mechanical failure, with periodic application of the stretch protocol. In the stretch immediately preceding failure, Phase 2 stiffness was 86 ± 3% lower than initial values, while Phase 1 stiffness only decreased by 31 ± 3%. Additionally, tension at peak stretch increased 12.5 ± 2.1 μN (33 ± 3%) upon osmotic compression, matching the 12.6 ± 1.1 μN intercept of the Phase 2 regression line on the Δtension versus Δlength graph. Thus, short range stiffness appears additive to Phase 2, titin-based stiffness, and is best accounted for as a separate entity acting in parallel to titin.