Nemaline Myopathy is the most common non-dystrophic congenital myopathy, clinically characterized by muscle weakness. The disease is associated with mutations in the nebulin gene and the nebulin-based disease is referred to as NEM2. Recent work on skinned muscle fibres from NEM2 patients revealed remarkable phenotypic similarities to fibres from nebulin KO mice (Ottenheijm et al, 2012). Here we investigated mechanics and kinetics of single myofibrils from a novel NEM2 mouse model (NEB Δex55) that mimics a deletion in the nebulin gene found in a large group of NEM2 patients. We used rapid solution switching (Tes et al,2002) to compare maximal tension and kinetics of contraction and relaxation of myofibrils isolated from frozen skeletal muscles (tibialis cranialis of neonatal mice) of WT and NEB Δex55 mice. Myofibrils, mounted in a force recording apparatus (15 °C), were maximally Ca²⁺-activated (pCa 4.5) and fully relaxed (pCa 9.0). Maximal isometric tension was markedly reduced in NEB Δex55 myofibrils (49.7 ± 10.6 μN mm⁻²) compared to WT (135.3 ± 16.9 μN mm⁻²). The rate constant of active tension generation following maximal Ca²⁺ activation ($k_{act}$) was significantly reduced inNEB Δex55 myofibrils (1.46 ± 0.07 s⁻¹) compared to WT (2.75 ± 0.27 s⁻¹). Force relaxation kinetics was remarkably faster in NEB Δex55 myofibrils than in WT, evidence that the apparent rate with which cross-bridges leave the force generating states is accelerated in the NEB Δex55 sarcomeres. Reduction of the rate with which cross-bridges enter force generating states and of cross bridge dissociation can markedly contribute to reducing maximal tension. This is expected to increase the energetic cost of tension generation of the NEB Δex55 sarcomeres. Results suggest that nebulin plays a significant role in contraction regulation and that altered cross bridge kinetics contribute to NEM2 pathogenesis.

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Increased Fatigue Resistance of Skeletal Muscle with Elevated 2-Deoxy-ATP following Ribonucleotide Reductase Overexpression
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Skeletal muscle myosin can use a variety of nucleotides to varying effective-ness as substrates for contraction. We previously demonstrated that complete replacement of ATP with 2 deoxy-ATP (dATP) in activation solutions in-creases the rate of cross-bridge formation with actin relative to ATP. We now report on a transgenic mouse (Tg-RR) that overexpresses the enzyme, ribonucleotide reductase. This is expected to increase the energetic cost of tension generation of the NEB Δex55 sarcomeres. Reduction of the rate with which cross-bridges enter force generating states and of cross bridge dissociation can markedly contribute to reducing maximal tension. This is expected to increase the energetic cost of tension generation of the NEB Δex55 sarcomeres. Results suggest that nebulin plays a significant role in contraction regulation and that altered cross bridge kinetics contribute to NEM2 pathogenesis.

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Localization and Function of Xinz in Mouse Skeletal Muscle
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Xin repeat-containing proteins were originally found in the intercalated discs of cardiac muscle with proposed roles in cardiac development and function. Of the paralogous genes, Xin1 (Xirp1) and Xin2 (Xirp2), is present in mammals. Ablation of the mouse Xin1 (mXinz) did not affect heart development but caused late-onset adulthood cardiac hypertrophy and cardiomyop-athy with conductive defects. Both mXinz and mXin2 are also found in the myotendinous junctions (MTJs) of skeletal muscle. In the present study, we investigated the structural and functional significance of mXinz in skeletal muscle. In addition to MTJs and the contact sites between muscle and peri-mysium, mXinz but not mXin2 was found in the blood vessel walls, whereas both proteins were absent in neuromuscular junctions or the nerve fascicles. Co-localization and co-immunoprecipitation suggested association of mXinz with talin, vinculin and filamin but not β-catenin in MTJs of adult skeletal muscle. Complete loss of mXinz in mXinz-null mice had subtle effects on the MTJ structure and the expression of other known MTJ components. Dia-phragm muscle fibres of mXinz-null mice showed significant hypertrophy. In comparison with wild type controls, mouse extensor digitorum longus (EDL) muscle lacking mXinz exhibited no overt change in contraction and relaxation velocities or in maximum force development. Its fatigability and recovery from fatigue were similar to that of wild type control. Loaded fatigue contractions generated stretch injury in wild type EDL muscle as indicated by an adaptive restrictive truncation of troponin T. However, this effect was blunted in mXinz-null EDL muscle. The results suggest that mXinz may play a role in MTJ conductance of contractile and stretching forces, essential to skeletal muscle function.

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Cytoskeletal Tension Differences between Normal and Dystrophic Myotubes Probed with FRET Based Stress Sensors
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Duchenne muscular dystrophy is caused by the loss of the cortical cytoskeletal protein dystrophin. These muscle cells have abnormally structured cortical cytoskeleton that leads to impaired ability to control stress in the cell cortex. When a stress bearing cytoskeletal element is removed from a system other proteins rearrange to adapt to the changed stress distribution and must absorb stresses that they were not intended to bear. This could affect a number of downstream mechanically sensitive receptors and enzymes. Knowing which cytoskeletal elements absorb the stress in the system that was intended for dystrophin would be useful in understanding what mechanically based sensory systems will be affected most and can help in the design of treatments and in assessing therapies to treat muscular dystrophy. We created chimeric cytoskeletal proteins containing the cpstFRET stress sensing cassette and expressed them in developing normal and dystrophic mouse myotubes. These proteins included actinin, filamin, spectrin, vinculin and dystrophin. These chimeric proteins all showed distinct spatial distributions in the myotubes. We measured the stresses these proteins in resting cells and in cells stretched with a micro-pipette. All proteins had different resting stress levels. Filamin, an important component of focal adhesion plaques, showed the most significant difference in resting stress levels between normal and dystrophic myotubes. It also showed