myofilament Ca^{2+}-sensitivity (by 0.09 pCa units) in α-MHC fibers. However, $RcTnT_{1-43}$ modestly decreased maximal tension (by ~18%), with no effect on Ca^{2+}-sensitivity in β-MHC fibers. Thus, the desensitizing effect of $RcTnT_{1-43}$ was attenuated by β-MHC. Ca^{2+}-activated maximal tension data were supported by myofiber dynamic stiffness measurements. Another major finding is that $RcTnT_{44-73}$ abolished the sarcomere length-dependent increase in Ca^{2+}-sensitivity in β-MHC, but not in α-MHC fibers. Thus, our data demonstrates that the functional outcome of the N-terminus of cTnT is modulated by the type of MHC isoform present.

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Calponin Cross-Links Unphosphorylated Myosin to Actin and Enhances its Binding Force

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Smooth muscle is unique in its capability to maintain force for long periods of time at low ATP (energy) consumption. This property, called the latch-state, is hypothesized to occur due to the dephosphorylation of myosin while attached to actin. Alternative theories have proposed that dephosphorylated-detached myosin can also re-attach to actin and contribute to force maintenance. The goal of this study was to investigate the role of calponin in regulating and enhancing the binding force of unphosphorylated tonic muscle myosin to actin. To confirm the calponin regulation of our actin filaments at physiological concentrations, we used the in-vitro motility assay to measure the rate ($1/2_{max}$) of actin propulsion by myosin. Our results showed a significant decrease in $1/2_{max}$ (from 0.54 ± 0.01 μm/s (mean ± SE) in the absence to 0.43 ± 0.01 μm/s in the presence of calponin). When calponin was phosphorylated, this inhibitory effect was suppressed (0.50 ± 0.01 μm/s). To measure the force of binding (F_{unb}) of unphosphorylated myosin to actin, we used the laser trap assay. F_{unb} was normalized by the number of myosin molecules estimated per actin filament length. F_{unb} of unregulated actin (0.12 ± 0.01 pN; mean ± SE) was significantly increased in the presence of calponin (0.20 ± 0.02 pN). When calponin was phosphorylated, this enhancement was lost (0.12 ± 0.01 pN). To verify whether this enhancement of F_{unb} is due to cross-linking of myosinto actin by calponin, we repeated the measurements at high [KCl], as the calponin affinity for myosin decreases at high ionic strength. Indeed, the $F_{unb,}$ in presence of calponin, obtained at a [KCl] of 25mM (0.21 ± 0.02 pN; mean ± SE) was significantly decreased at a [KCl] of 150mM, (0.13 ± 0.01 pN). These data demonstrate that calponin enhances the force of binding of unphosphorylated myosin to actin by cross-linking them together.

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Contractile Properties of Human Fetal Skeletal Myofibrils

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Congenital contractures such as clubfoot are present in ~1/250 live births. Several congenital contractures syndromes are caused by mutations in genes that code for skeletal myofilament proteins, including *MYH3* and *MYH8*. Because embryonic (*MYH3*) and perinatal (*MYH8*) myosin heavy chains are unique to the prenatal development of muscle, it is important to understand the contractile properties of human embryonic (HE) myosin and human fetal (HF) muscle to determine how mutations affect performance and development. However, information on HF skeletal muscle function is lacking. We previously reported HE myosin crossbridge cycling (measured by *in vitro* motility) was much slower that rabbit psoas (RP) myosin (Biophys. J. (2010), 98:542a). Here we characterized the contraction and relaxation properties of HF muscle using myofibril mechanics techniques. HF skeletal muscle and myofibrils were isolated from a 15.4 week gestation fetus. During maximal calcium activation (15°C) HF myofibrils produced much lower force (F_{MAX}=5.9 ± 1.2mN/mm^2) as compared to myofibrils from human adult (HA) skeletal muscle (84 ± 34mN/mm^2) or RP muscle (220 ± 40mN/mm^2). Unlike control HA and RP fibrils, no striation pattern was apparent for HF myofibrils, suggesting that immature sarcomeres could explain the lower force production. HF myofibrils had slower kinetics of force development (k_{ACT}=0.66 ± 0.1s^{-1}) vs. HA (7.5 ± 6.3s^{-1}) and RP (5.7 ± 0.5s^{-1}) myofibrils. The initial (slow) phase of relaxation upon return to low calcium solution was slower and prolonged ($k_{REL,SLOW}$=0.59 ± 0.22s^{-1}; $t_{REL,SLOW}$=174 ± 13ms) vs. HA (2.9 ± 1.7s^{-1}; 71 ± 18ms) or RP (2.1 ± 0.4s^{-1}; 73 ± 14ms) myofibrils. The larger, faster phase of relaxation was also slower (HF=1.5 ± 0.2s^{-1} vs. HA=12 ± 5s^{-1} and RP=21 ± 4s^{-1}). Our previous *in vitro* motility experiments indicated a similar inhibition of filament speed by increasing [ADP] for HE and RP myosin, but this was under low load, thus ongoing experiments will determine if ADP release is responsible for the slower kinetics of HF muscle. Funded by F31AR06300(A.R.), 5K23HD057331(A.B.), HD048895(M.B., M.R.).

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Staurosporine Blocks the ATP-Sensitive K^+Channels and Induces Atrophy in Rodent Skeletal Muscles

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The ATP-sensitive K^+channels(KATP) has been proposed as a molecular sensor of atrophy in skeletal muscle(Tricarico et al., 2010). In the present work we evaluated the "in vitro" effects of staurosporine, a well known apoptotic agent, on sarcolemmal KATP channels and on muscle proteins content and fibers diameter in slow-twitch *Soleus*(SOL) and fast-twitch *Exstensor Digitorum Longus*(EDL) and *Flexor Digitorum Brevis*(FDB) muscles of mice. The isolated muscles and the single fibers were incubated with staurosporine (1 μg/mL) for 1-6-24-48 hrs. alone or in combination with diazoxide(100 μM-250 μM), a KATP opener, and the changes in the total proteins content, gene expression, fibers diameter and KATP channel activity were evaluated. We found that staurosporine blocks KATP channels in excised-patch experiments being more effective in SOL rather than in EDL and FDB fibers. The toxin also caused a reduction of the proteins content/muscle of −45%, −25% and −21% for SOL, EDL and FDB, respectively at 24 hrs. A time-dependent reduction of the diameter is observed following incubation of the fibers with staurosporine. An up-regulation of the atrogin-1 gene while a down regulation of the KATP channel subunits genes is observed in SOL. The co-incubation of the muscles with staurosporine+diazoxide for 24 hrs. fully prevented the reduction of the fibers diameter and protein content, and reduction of the KATP channel activity. Our data indicate that staurosporine blocks KATP channels in skeletal muscle leading to muscle atrophy and these effects are phenotype dependent.

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Alleviation of Skeletal Muscle Defects Induced by Huntington's Disease-causing Amyloid by Modulating TOR Pathway in a Drosophila Model

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Huntington's disease (HD) is caused by an expanded polyglutamine (Poly-Q) repeat in the Huntingtin (HTT) protein. Beyond the brain, htt gene is also expressed in skeletal muscle. Muscle atrophy is common in HD patients, likely from accumulation of toxic amyloid. To explore the mechanism of Poly-Q-induced skeletal muscle defects associated with HD-causing amyloid protein, we expressed mutant HTT with short (Httex1-PolyQ25) and expanded (Httex1-PolyQ72) Poly-Q exclusively in the Drosophila indirect flight muscle (IFM). Expression of Httex1-PolyQ-72 in the IFM resulted in severe defects in skeletal muscle function and ultrastructure. Progressive reduction in flight performance was observed in 3-day and 3-week old flies (a 20% and 90% decrease in flight index, respectively) compared to age matched Httex1-PolyQ-25 flies. Further, 3-week old flies expressing Httex1-PolyQ-72 showed amyloid aggregates, whereas expression of Httex1-PolyQ-25 was benign. At the ultrastructural level, the myofibrils of control IFM showed well-organized Z-bands and M-lines, however, IFM of 3-week old flies expressing Httex1-PolyQ-72 showed severe ultrastructural deterioration with broken Z-disks, M-lines and a loss of thick filaments. In addition to myofibril defects, abnormal vacuole-like structures of various sizes and shapes containing mitochondrial remnants were present. Mutant fibers also display rimmed vacuoles fused with additional membranes, suggesting the formation of type 2 autophagic vacuoles. To further explore the mechanism underlying the PolyQ-induced skeletal muscle defects, we treated flies expressing Httex1-PolyQ-72 with rapamycin, a known suppressor of mTOR. Rapamycin-treated flies expressing Httex1-PolyQ-72 showed significantly improved flight function (a 30% increase in flight index) and improved myofibril integrity (organized Z-bands and M-lines) compared to flies expressing Httex1-PolyQ-72 without rapamycin treatment. Thus, we have developed a novel Drosophila model to explore and suppress skeletal muscle defects linked with HD-causing amyloid by modulating the TOR pathway.

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Altered Cross Bridge Kinetics in Skeletal Myofibrils from NEBΔex55, a Novel Mouse Model of Nebulin-Based Nemaline Myopathy

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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 (Tesi 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 Ca^{2+}-activated (pCa 4.5) and fully relaxed (pCa 9.0). Maximal isometric tension was markedly reduced in NEB Δex55 mouse myofibrils (49.7 ± 10.6 mN mm^{-2}n=11) compared to WT (135.3 ± 16.9mN mm^{-2}n=9).The rate constant of active tension generation following maximal Ca^{2+} activation (k_{ACT}) was significantly reduced inNEB Δex55 mouse myofibrils ($1.46 \pm 0.07s^{-1}$) compared to WT (2.75 ± 0.27 s^{-1}). Force relaxation kinetics was remarkably faster in NEB Δex55 mouse myofibrils than in WT, evidence that the apparent rate with which cross-bridges leave the force generating states is accelerated in the NEB Δex55sarcomeres. 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 Δex55sarcomeres. 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 effectiveness as substrates for contraction. We previously demonstrated that complete replacement of ATP with 2 deoxy-ATP (dATP) in activation solutions increases contraction of demembranated rabbit skeletal muscle and that enhanced performance results from increased myosin binding and cycling kinetics, especially during sub-maximal calcium activation. Here we report on a transgenic mouse (Tg-RR) that overexpresses the enzyme, ribonucleotide reductase, which converts ADP to dADP (that is rapidly phosphorylated to dATP). This results in a ~10x increase in dATP content of skeletal muscle, which still constitutes $\leq$ 1% of the adenosine triphosphate nucleotide pool. We are examining the contractile and metabolic properties of skeletal muscle in this transgenic model. Preliminary data indicates the phosphocreatine to ATP (PCr:ATP) ratio of Tg-RR mice is significantly elevated relative to wild type (Tg-WT) mice, suggesting the Tg-RR mice may have an energetic advantage due to increased high energy phosphate reserves. Furthermore, the Tg-RR mice ran for a longer period of time at increased speed and for longer distances in a graded exercise test. Direct *in situ* stimulation of the gastrocnemius muscle indicates improved resistance to fatigue, consistent with both the exercise and the metabolic (PCr:ATP) findings. Interestingly, our preliminary experiments suggest the Tg-RR mice produce significantly less peak force and show signs of atrophy. Ongoing experiments are examining changes in mitochondrial content and function as well as contractile properties of isolated muscles to determine the mechanisms underlying the increased fatigue resistance of the Tg-RR mice. These results suggest a new direction for developing interventions to improve exercise tolerance in human patients. Supported by HL11197 (MR).

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Crossbridge Properties during Fatigue and Recovery in Mouse Skeletal Muscle Fibres

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Fatigue occurring during exercise can be defined as the inability to maintain the initial force or power output. We showed recently that fatigue during repetitive tetanic stimulation at 24 °C of fibre bundles dissected from FDB mouse (C57BL/6) muscle, occurs in two phases: an initial one during which individual crossbridge force decreases, and a later phase during which also crossbridge number decrease (Nocella et al.,2011 J Physiol 589).The present experiments were made on the same preparation to compare the fatigue mechanism at 24°C and at physiological temperature of 35 °C and to investigate the mechanism of force recovery from fatigue at 24°C. Fatigue was induced with 105 consecutive isometric tetani evoked every 1.5 s. Force recovery was followed by tetani evoked every 90s until force recovered to 90-100% of pre-fatigue value. Stiffness was measured with small sinusoidal length oscillations at 6.5 kHz. At both temperatures fatigue occurred initially through the decrease of the individual crossbridge force followed by the reduction of crossbridge number. However the initial phase lasted for ~40 tetani at 35°C and ~20 tetani at 24°C. This suggests a greater resistance to fatigue of this mechanism at high temperature. In contrast, during the second phase the tension loss was faster at 35°C than at 24°C so that after 105 tetani tension was similar at both temperatures. Force recovery also occurred in two phases. The initial phase lasted from ~1.5-4 min and recovered 40-90% of tension loss. The second phase lasted for ~60 min and its amplitude was well correlated with tension decrease during the second phase of fatigue. Thus the mechanism of tension recovery after fatigue seems symmetrical to tension loss during fatigue.

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Localization and Function of Xinα 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. A pair of paralogous genes, Xinα (Xirp1) and Xinβ (Xirp2), is present in mammals. Ablation of the mouse Xinα (mXinα) did not affect heart development but caused late-onset adulthood cardiac hypertrophy and cardiomyopathy with conductive defects. Both mXinα and mXinβ are also found in the myotendinous junctions (MTJs) of skeletal muscle. In the present study, we investigated the structural and functional significance of mXinα in skeletal muscles. In addition to MTJs and the contact sites between muscle and perimysium, mXinα but not mXinβ 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 mXinα with talin, vinculin and filamin but not β-catenin in MTJs of adult skeletal muscle. Complete loss of mXinα in mXinα-null mice had subtle effects on the MTJ structure and the expression of other known MTJ components. Diaphragm muscle fibers of mXinα-null mice showed significant hypertrophy. In comparison with wild type controls, mouse extensor digitorum longus (EDL) muscle lacking mXinα 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 mXinα-null EDL muscle. The results suggest that mXinα 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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Duchene 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 dystophin 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 stress on these proteins in resting cells and in cells stretched with a micropipette. 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