were decreased after MI ($p < 0.01$). We observed evidence of increased tropomyosin oxidation by reversible modification of sulfhydryls, and confirmed the oxidized protein's identity using mass spectrometry. These data characterize the relatively unexplored structural and functional modifications to sarcomeres in the early aftermath of MI, and may provide insight into the initial changes that trigger remodeling and heart failure, as well as the contribution of ROS to this process.

2807-Pos

Automated Image Analysis of Electron Micrographs of Structurally Compromised Striated Muscle

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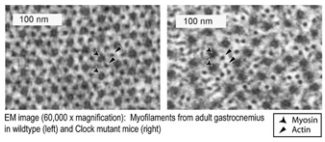
Striated (skeletal and cardiac) muscle is a highly organized and conserved tissue with a molecular structure comprised of bundles of actin (thin filaments) and myosin (thick filaments). We have observed that skeletal muscle from two genetically modified murine models showing disrupted circadian rhythms (Bmal knockout and Clock$^{\Delta 19}$), exhibit significant muscle weakness defined by a reduction in specific tension. Electron micrographs (EMs) of cross-sections from adult gastrocnemius in these mice reveal obvious divergences from the normal hexagonal arrangement of thin filaments around thick filaments.

The goal of this project is to develop a tool for the high-throughput analysis of myofilament architecture. Image processing software written in MATLAB identifies myofilaments in EMs of muscle cross-sections as intensity peaks in the gray-scale image. Filaments are categorized as thick or thin depending on the cross-sectional area of the peaks after thresholding. Structural properties, such as the ratio of thin to thick filaments, the distance to closest neighbors, the angular distribution and the diameter of filaments will be determined for different muscle samples. This quantitative analysis should lead to improved understanding of structure-function relationships in striated muscle.



EM image (60,000 x magnification): Myofilaments from adult gastrocnemius in wildtype (left) and Clock mutant mice (right)

2808-Pos

Myosin-Based Inclusion Body Myopathy Type 3 Decreases Muscle Power Generation and Kinetics

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Dominant inclusion body myopathy type 3 (IBM-3) results from a point mutation, Glu706Lys, in the SH1 helix of the myosin head in skeletal muscle. The mutation leads to progressive myofibrillar disorganization, rimmed vacuoles and muscle weakness. We are studying *Drosophila* transgenically expressing this myosin mutation in their indirect flight muscle (IFM) and jump muscle. Wing beat frequency (WBF) and jump ability assays were performed on 2-3 day old flies at 25°C. Heterozygous fly WBF was reduced to 123 ± 14 Hz compared to control fly WBF of 181 ± 13 Hz. This decrease contributes to a completely flightless phenotype. Homozygous flies showed no ability to beat wings. A jump ability assay was executed to gauge any changes in myosin function of the *Drosophila* jump muscle, which is similar to very fast vertebrate muscle. No significant impairment of jumping ability was observed in heterozygous mutants, 5.93 ± 0.41 cm compared to control flies, 5.61 ± 1.48 cm. However, homozygous flies were not able to jump. Homozygous skinned IFM fibers at 2-days of age failed to produce power. Mechanical analysis of < 2 hour old skinned heterozygous fly IFM fibers revealed an 85% decrease in maximum oscillatory power generation (P_{max}) and an ~6-fold decrease in the frequency at which maximum power was generated (f_{max}) compared to controls. We hypothesize that the mutation increases the time myosin spends in a strongly actin bound state, leading to muscle myofibrillar disorganization and decreased power output.

2809-Pos

Titin Isoform Size is not Correlated with Thin Filament Length in Rat Skeletal Muscle

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The striated muscle sarcomere is dependent upon the precise interactions of a variety of myofibrillar proteins for its proper formation. As the largest and third most abundant protein in this milieu, titin plays a number of functions in the sarcomere, including assembly of the thick filaments and preventing overstretch. The titin gene is expressed as multiple splice variants in skeletal muscle, generating a continuum of titin protein sizes. Recently it was reported that thin filament length was related to titin size, and that the latter might be involved in determining thin filament length. We tested this hypothesis using several muscles from wild type rats and from a mutant rat model (Greaser et al J Mol Cellul Cardiol 44:983, 2008) which results in increased titin size. Myofibrils were isolated from skeletal muscles (diaphragm, extensor digitorum longus, gastrocnemius, longissimus dorsi, psoas major, rectus abdominis, and tibialis anterior) using both adult wild type (WT) and homozygous mutant (HM) rats (n=6 each). Thin filament length was estimated using fluorescent dye labeled phalloidin and relaxed sarcomere length was determined by phase contrast microscopy after adding ATP and BDM. No differences in thin filament lengths were found between WT muscles with titin sizes ranging from 3.2 to 3.7 MDa. Similarly the thin filament lengths in the mutant rats did not differ from the paired WT muscles in spite of large differences in titin size with several muscles. However, the relaxed sarcomere length was correlated to titin size in muscles from WT rats, and it was significantly increased relative to WT for within muscle comparisons. The data indicates that, although titin performs many functions, its relationship to thin filament length could not be demonstrated in the rat. Supported by HL77196.

2810-Pos

Conformation of A-Band Titin

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The giant protein titin has important roles in the assembly, signalling and passive mechanical properties of muscle sarcomeres. Titin is formed by a single polypeptide with isoforms ranging between 3 and 4 MDa. This folds into ~300 immunoglobulin (Ig) and fibronectin (Fn3) domains in a beads-on-a-string-like chain more than 1 μm long. The N-terminal half of the molecule forms an elastic connection between the end of the thick filament and the Z-line. The C-terminal half is bound to the thick (myosin) filament. Through most of the thick filament region, the Ig and Fn3 domains are arranged in a distinctive eleven domain 'large super-repeat', Ig-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn. Eleven copies of the large super-repeat make up ~0.5 μm of the titin molecule length. In an attempt to reconstruct the structure of this region, we have studied a set of two- and three-domain recombinant fragments forming a large super-repeat using electron microscopy, synchrotron X-ray solution scattering and analytical ultracentrifugation. The data illustrate different average conformations in different domain pairs, correlating with differences in lengths of the inter-domain linkers. They also illustrate a level of flexibility between domains in all pairs around average states. Overall, the results suggest the large super-repeat forms an irregular helix, and is also likely to be dimerized *in situ*.

2811-Pos

Effect of Excision of Titin's PEVK Exons 219-225 on Skeletal Muscle Structure and Function

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We recently published a PEVK KO mouse model in which all exons that constitute the PEVK region of cardiac titin (N2B cardiac isoform), exons 219-225, had been excised, and reported cardiac hypertrophy and increased passive stress (Granzier et al., 2009 *Circ Res*., 11, 557). Here we investigated the phenotype of EDL (fast twitch) and soleus (slow twitch) skeletal muscle of wildtype and homozygous PEVK KO mice. Muscle mass was significantly increased in both soleus (51 ± 5%) and EDL (21 ± 6%) muscles; we are currently studying whether the underlying hypertrophy mechanisms are similar to those previously found in the heart. Because the excised exons make up a small portion of the PEVK segment of skeletal muscle titins we expected modest differences, if any at all, in passive stress. Unexpectedly, passive stress was significantly increased in soleus and EDL muscles, both when measurements (SL 3.0μm) were made at the whole muscle level (40 ± 7% and 67 ± 16%, respectively) and at the fiber bundle level (50 ± 14% and 81 ± 1%). Gel electrophoresis revealed, in both EDL and soleus, expression of a single titin isoform in wildtype muscle, but surprisingly, co-expression of two isoforms in the KO muscles. The larger isoform co-migrated with the isoform expressed in wt muscle and thus is likely to

represent KO titin (exons 219-225 represent ~30kDa, which is insufficient to cause a mobility difference). The smaller isoform, found in both EDL and soleus KO muscle, is likely to represent a splicing adaption that correlates with increased passive tension. We are currently investigating the identity of these exons using titin exon microarray analysis. In summary, excision of titin exons 219-225 in skeletal muscle results in elevated passive stress with the underlying cause likely being due to an adaption in titin splicing.

2812-Pos

Developmentally Regulated Differential Splicing of the Large Sarcomeric Proteins Titin and Nebulin

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Titin (3-4MDa) and nebulin (700-900kDa) are large sarcomeric proteins of skeletal muscle. Both proteins contain sites of differential splicing throughout their length. To study splicing during development we used a home-made exon microarray representing all of titin's and nebulin's exons and compared exon expression between mice of different ages in numerous muscle types. We found that a gradual reduction in titin's transcript size in exons encoding the elastic PEVK segment located in the I-band correlates with a reduced titin protein (typically ~0.2 MDa smaller in adults) and stiffer sarcomere in all skeletal muscles studied (soleus, m. gastrocnemius, tibialis cranialis, extensor digitorum longus, and quadriceps). Nebulin undergoes less dramatic differential splicing than titin, but it was found that exons encoding the M177/M178 repeat in the z-disc are upregulated in the adult versus embryonic samples. Additionally, the soleus muscle exhibited a 4-fold increase in expression level of the SH3 domain of nebulin and a 3-fold increase in the serine rich exons. These findings correlate with structural changes in the z-disc which are wider in the adult (133nm) versus the neonatal soleus samples (121nm, pvalue 0.04). Thus titin and nebulin transcripts are tuned during development with changes in titin altering the I-band properties and changes in nebulin affecting the Z-disc.

2813-Pos

MybP-C Slow: a Novel Phosphoprotein of Skeletal Muscles

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Myosin Binding Protein-C slow (MyBP-C slow) is a thick filament associated protein, consisting of seven immunoglobulin (Ig) and three fibronectin-III (Fn-III) motifs, arranged in tandem. Four alternatively spliced forms of MyBP-C slow, referred to as variants 1-4, have been characterized in skeletal muscles. These differ in three regions; variants 1 and 2 contain a 25-residues long insertion at the extreme, non-modular NH2-terminus, variant 3 carries an 18-amino acid long segment within Ig7 and variant 1 contains a unique COOH-terminus consisting of 26-amino acids, while variant 4 does not possess any of these insertions. Our recent work has indicated that variants 1-4 are expressed in different amounts in distinct skeletal muscles and exhibit diverse topographies. To date, the regulatory mechanisms that modulate the activities of MyBP-C slow are unknown. We used proteomics tools to examine the profile of MyBP-C slow in flexor digitorum brevis (FDB) muscle. To this end, protein homogenates prepared from adult FDB muscles of mouse origin were treated with different kinases, phosphatases or their inhibitors, and analyzed by isoelectric focusing and 2-dimensional SDS-PAGE. We found that MyBP-C slow, similar to its cardiac counterpart, is phosphorylated by Protein Kinase A, and additional, yet unidentified, kinases. Using high-stringency phosphorylation prediction software, we also found that the three novel insertions present in variants 1-3 contain consensus phosphorylation motifs for PKA and other Ser/Thr kinases. We are currently mutagenizing the predicted sites and examining their ability to be phosphorylated in vitro by PKA, as well as two other major kinases associated with the thick filaments: titin kinase and obscurin kinase. Our studies are the first to show that MyBP-C slow is a phosphoprotein and a substrate of PKA, and to suggest a novel mechanism that may regulate its activities in skeletal muscle.

2814-Pos

A C-terminal Truncation of Flightin Slows Actomyosin Cycling, Elevates Passive Tension, and Decreases Power Output in Drosophila Flight Muscle Fibers

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Flightin is a 182 amino acid myosin rod binding protein that is essential for thick filament stability and function in the indirect flight muscles (IFM) of *Drosophila melanogaster*. Flightin is not homologous to any known protein in vertebrates, but recent phylogenetic analyses revealed that flightin is widespread among insects and crustaceans. Amino acid sequence analysis suggests that flightin consists of three functional domains: a fast evolving and highly phosphorylated N-terminal domain (residues 1-83), a conserved central domain (residues 84-134) and a semi-conserved C-terminal domain (residues 135-182). To interrogate the function of the C-terminal domain, we created a transgenic Drosophila that expresses a truncated flightin, missing the last 43 residues (fln^{142t}). These transgenic flies are unable to beat their wings, but their myofilament lattice and sarcomere structure appear normal compared to a flightin-null rescued control line (fln^{R}). Active (pCa 5) and rigor isometric tensions in skinned IFM fibers were higher for fln^{142t} versus fln^{R}, which occurs due to a 2-fold increase in passive (pCa 8) isometric tension. Small amplitude sinusoidal perturbation analysis showed that fln^{142t} fibers produced approximately 30% of the oscillatory work and power of fln^{R} fibers. The fln^{142t} also showed a decreased frequency of maximum work (123 Hz vs. 154 Hz) and power (139 Hz vs. 187 Hz) compared to fln^{R}, suggesting slower myosin kinetics even though myosin attachment time (=0.5 ms) was unchanged. These results suggest that the C-terminus of flightin plays a limited role in thick filament integrity and normal sarcomere structure of the IFM, but is essential for maintaining the muscle's passive properties and tuning the kinetic properties during contraction.

2815-Pos

Intermediate Filament and Ecm Mechanics Deduced from Desmin Knockout Muscles

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Desmin is the skeletal muscle intermediate filament protein that forms a mesh-like network around Z-disks and helps transmit force to the extracellular matrix (ECM). It generally functions as a mechanical integrator of the cell, maintaining lattice connectivity and enabling efficient force transmission. Desmin related myopathies have debilitating effects that range from muscle weakness and atrophy to cardiac and respiratory failure. Studies performed on desmin knockout muscles have begun to elucidate the physiological and biological roles of desmin, but the mechanical properties of the desmin network are still unknown.

We performed incremental stress-relaxation tests on fibers and fiber bundles from desmin knockout and wildtype mouse EDL muscles to investigate how the absence of desmin affects the fiber and fiber bundle viscoelastic properties. Using these data, we developed a structural model with explicit elastic and viscous elements representing the desmin, the rest of the fiber and the ECM. Single fibers from desmin knockout muscles were significantly more compliant (linear modulus $= 122 \pm 61$ kPa) compared to wildtype fibers (176 ± 49 kPa, $p<0.05$). These data demonstrate that the desmin matrix bears 30-40% of the passive load in muscle cells, a much greater fraction than previously believed. In contrast to fibers, bundles of fibers were nonlinear and demonstrated the opposite trend_bundles from desmin knockout muscles were over twice as stiff (440 ± 237 kPa) compared to wildtype bundles (214 ± 97 kPa) at 60% strain ($p<0.0001$). Time constants for stress-relaxation were larger for fibers than bundles and were significantly larger for knockout bundles compared to wildtype bundles. These data suggest a biological and biomechanical interaction between muscle cells and the ECM and may indicate that the ECM becomes stiffer in desmin knockout muscles in response to the more compliant fibers.