the hands and feet, is the most predominant. In one subtype of DA, Freeman Sheldon Syndrome (FSS), 97% of the cases are caused by mutations in the embryonic myosin heavy chain gene, *MYH3*. To assess the effects of this mutation on adult muscle contractility, skeletal muscle was obtained from a needle biopsy of the gastrocnemius muscle in an FSS individual (*MYH3* R672C) and a control subject were performed and skinned single muscle fibers were dissected for measurements of contractile performance as the [Ca^{2+}] of physiological solutions was varied. The magnitude of passive stiffness was 2x greater for patient fibers. There was no difference in maximal Ca^{2+} activated force found in the affected adult muscle fibers (0.204uN ± 0.044) compared to normal adult muscle fibers (0.259uN ± 0.028). However specific force was 69% less ; this was attributable to hypertrophy of the patient fibers (159um ± 8 as compared to normal control myofibers of 87um ± 3). Little to no change was observed in Ca^{2+} sensitivity (pCa_{50}) or in cooperativity of the force-pCa relationship. Relaxation was dramatically slower in patient fibers, taking 4x longer to reach 50% relaxation and 10x longer to reach 90% relaxation. Control experiments suggested this is not due to the larger patient fiber size. Preliminary analysis, using a 12.5% agarose gel, and Western Blots, indicated that these differences were not fiber type dependent. Interestingly, we have identified that embryonic myosin (*MYH3*) is present in single adult muscle cells. This work was supported by HL65497 (Regnier) and HD48895 (Bamshad).

2803-Pos

The Fast Skeletal Troponin Activator, CK-1909178 Reduces Muscle Fatigue in a Model of Peripheral Artery Disease *in Situ*

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CK-1909178 is a member of a class of fast skeletal troponin activators that sensitize skinned skeletal muscle fibers to calcium. In rat muscle preparations *in vitro* and *in situ*, CK-1909178 increased sub-tetanic force without altering maximum force. Given that a major cause of muscle fatigue during repeated muscle contraction is reduced myoplasmic Ca^{2+} due to impaired sarcoplasmic reticulum Ca^{2+} release, we tested whether increased calcium sensitivity with CK-1909178 would slow the development of fatigue. Rat flexor digitorum brevis muscle was pretreated *in vitro* with CK-1909178 and stimulated every 3 seconds at a frequency sufficient to achieve 50% of maximum force for 6 min at 30°C. CK-1909178 diminished the extent of fatigue as compared to control (terminal force 29.5 ± 8% vs. 12.7 ± 4%, $p<0.001$). We next tested whether CK-1909178 treatment would slow the development of muscle fatigue using rat extensor digitorum longus muscle *in situ*, where the muscle was stimulated via the peroneal nerve. To accelerate the development of muscle fatigue, vascular insufficiency was produced by femoral artery ligation (FAL). Muscle fatigue with FAL and sham ligation in the presence and absence of CK-1909178 was assessed. CK-1909178 was administered as a 5mg/kg intravenous bolus before assessment of fatigue at a frequency adjusted to achieve the same force at 30Hz prior to dosing. FAL resulted in significantly reduced terminal tension as compared to sham (33 ± 4% vs. 77 ± 5%, $p<0.01$). CK-1909178 administration significantly attenuated FAL-induced fatigue at 10 minutes (61 ± 7% vs. 33 ± 4%, $p<0.01$). In summary, CK-1909178 increased sub-maximal muscle force development and reduced the extent of fatigue in the presence of limited blood flow *in situ*. We believe that this mechanism may improve muscle fatigue in diseases where blood flow to muscles is compromised such as intermittent claudication.

2804-Pos

Transgenic Replacement of the Myosin S2/hmm Hinge Alters the Rod's Nano-Mechanical Properties and Affects Sarcomeric Organization

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The subfragment 2/light meromyosin "hinge" region of myosin II rods forms a less stable coiled-coil than do flanking regions. Different hinge sequences may contribute to muscle specific properties. Transgenic replacement of a portion of fast muscle myosin hinge A (encoded by exon 15a) in *Drosophila* indirect flight muscle (IFM) with slow muscle hinge B (exon 15b) increases rod coiled-coil propensity, rod and sarcomere lengths and decreases flight performance. To characterize the hinges' nano-mechanical properties we determined persistence length (PL) differences via electron microscopy and molecular dynamic (MD) simulations. Rotary shadowed 15b myosin molecules showed an ~22% higher rod PL relative to 15a (64.2 vs. 50.3 nm) while MD simulations revealed an ~39% greater PL for 15b relative to 15a (85 vs. 52 nm). These data are consistent with a high coiled-coil propensity of exon 15b-containing myosin rods stiffening the hinge and a substantial portion of the myosin tail. We investigated myofibrillar ultrastructure by electron microscopy of ultrathin sections of 15b-expressing IFM and observed some sarcomeres with substantially different Z- to M-line distances on opposing halves of individual sarcomeres. We used confocal microscopy to quantitatively assess the extent of this asymmetry as well as the distribution of sarcomeres lengths (SL). We confirmed an ~8% greater SL, as well as a significant difference between the coefficients of variation in SL, in hinge B- relative to hinge A-containing myofibrils (3.55 ± 0.28 vs. 3.29 ± 0.14 μm) ($F = 3.39$ ($p < 0.001$)).Our data suggest 15b hinge replacement has a stiffening effect on IFM myosin rods. This may decrease local rod flexibility, promote molecular packing during filamentous growth and disrupt the regulation of thick filament lengths, which in turn may account for longer and highly variable SL and for decreased muscle performance.

2805-Pos

Functional Consequences of Large1 Overexpression in Two Distinct Forms of Muscular Dystrophy

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Dystroglycan and the sarcoglycan complex are two essential components of the dystrophin-glycoprotein complex. Mutations that lead to hypoglycosylation of dystroglycan result in various limb-girdle and congenital muscular dystrophies referred to as dystroglycanopathies. Mutations in the genes that encode for sarcoglycans are associated with limb-girdle muscular dystrophies referred to as sarcoglycanopathies. Overexpression of the glycosyltransferase LARGE1 induces hyperglycosylation of dystroglycan and bypasses glycosylation defects present in several distinct dystroglycanopathies. Whether LARGE1 ovexpression improves contractile properties in dystroglycanopathies and whether the efficacy of LARGE1 overexpression extends to sarcoglycanopathy has not been evaluated. We tested the hypothesis that muscle specific LARGE1 overexpression reduces pathology, increases force production, and protects muscles of mice deficient in LARGE1 ($LARGE^{myd}$ mice) or β-sarcoglycan (Sgcb-null mice) from contraction-induced injury. Mice with LARGE1 overexpression driven by the muscle creatine kinase promoter were crossed with $LARGE^{myd}$ and Sgcb-null mice. Extensor digitorum longus muscles were isolated, specific forces measured, and force deficits after lengthening contractions were assessed. Functional expression of LARGE1 overexpression and dystroglycan hyperglycosylation were observed. LARGE1 overexpression in $LARGE^{myd}$ mice reduced pathology and improved specific force and force deficit to wild-type levels. In contrast, overexpression had no beneficial effect for Sgcb-null mice. The results suggest that the efficacy of muscle specific LARGE1 overexpression may be limited to dystroglycanopathies.

2806-Pos

Alterations to Cardiac Muscle Function and Sarcomeric Proteins Following Myocardial Infarction

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Post-translational modifications of the proteins that make up the cardiac sarcomere have been suggested as a source of pathological muscle dysfunction. Reactive oxygen species (ROS) can induce post-translational modifications to proteins, and an increase in ROS levels is recognized as a feature of heart failure following myocardial infarction (MI). The experiments described here test the hypothesis that MI exerts a functional effect through alteration of myofibrillar proteins, which can be detected within days after the infarction. Experimental MI was induced by ligation of the left anterior descending coronary artery in 6-month old female CD1 mice. Samples were collected 3-4 days after ligation or sham surgery ($n = 10$). We performed functional analysis through force-calcium measurements of detergent-extracted fiber bundles ("skinned fibers") dissected from non-infarcted papillary muscle. Our findings included an increase in Ca^{++} sensitivity in fibers from MI hearts compared to those from sham-operated animals and a decreased cooperativity of activation ($p < 0.05$). Biochemical data derived from electrophoresis of isolated myofibrillar proteins from these hearts revealed both oxidation and modified phosphorylation. We used ProQ Diamond phosphoprotein gel stain to analyze myofilament protein phosphorylation, and nonreducing-reducing "diagonal" SDS-PAGE to detect the formation of disulfide products. Total troponin I phosphorylation levels

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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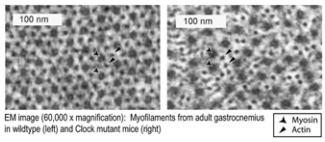
Striated (skeletal and cardiac) muscle is a highly organized and conserved tissue with a molecular structure comprised of bundles of actin (thin filaments) and myosin (thick filaments). We have observed that skeletal muscle from two genetically modified murine models showing disrupted circadian rhythms (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