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