reduced efficacy in affecting the skeletal myopathy. P188 belongs to the tri-block copolymer family, which comprises molecules made of a hydrophobic polypropylene oxide (PPO) core flanked by hydrophilic chains of polyethylene oxide (PEO) moieties. These copolymers exist at various molecular weights and PPO to PEO ratios and it is unknown what structural properties of P188 confer its membrane protecting functionality. Mechanistic knowledge is requisite for a deeper understanding of muscle membrane protection by copolymer sealants to enable therapeutic application. Interestingly, our data shows that P188 is efficacious in isolated dystrophic skeletal myofibers suggesting that poor delivery and low diffusion into the core of dystrophic whole muscle in vivo limits P188 effectiveness. Ultimately, fully effective therapeutic strategies must simultaneously target both cardiac and skeletal muscle tissues. We will present data on copolymer structure-function understanding and discuss how these new data will shed light into the structural requisite for more efficacious and potent membrane sealants for dystrophic skeletal muscle in vivo.

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Enzymatic Dissociation Makes Skeletal Muscle Fibers Susceptible to Osmotic Stress and More Prone to Mitochondrial Calcium Uptake

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Introduction: Enzymatic dissociation using collagenases is a frequently used method when studying function in individual adult skeletal muscles fibers. A potential problem with this method is that enzymatic disruption of the extracellular matrix may alter the anchorage of the intracellular cytoskeleton to the cell membrane and hence affect cellular structure and function.

Methods: We used single mouse flexor digitorum brevis (FDB) fibers isolated either by enzymatic dissociation with collagenase or by mechanical dissection, which leaves the immediate extracellular matrix intact. The gross structure of isolated fibers was assessed with Second Harmonic Generation (SHG) microscopy. Cytosolic and mitochondrial free [Ca^{2+}] were measured with fluo-3 and rhod-2, respectively.

Results: In comparison to dissected fibers, enzymatically dissociated fibers show: (1) less elaborate gross structure; (2) increased susceptibility to develop defective intracellular Ca^{2+} handling (increased basal cytosolic [Ca^{2+}], spontaneous Ca^{2+} waves and Ca^{2+} sparks) in response to a hypo-osmotic shock (3 min exposure to 50% of the normal NaCl concentration); (3) increased mitochondrial Ca^{2+} uptake during repeated tetanic contractions.

Conclusion: Disruption of the extracellular matrix results in marked changes in muscle fiber structure and function. This raises concerns when interpreting results obtained in experiments performed with enzymatically dissociated fibers. Moreover, our results support a role of altered cellular Ca^{2+} homeostasis in the disease process of muscle dystrophies caused by mutations in extracellular matrix proteins.

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Human Diaphragm Single Fiber Function after Unilateral Phrenic Nerve Stimulation During Mechanical Ventilation

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Patients undergoing open chest surgery depend on mechanical ventilation (MV) for breathing. Although a life-saving measure, prolonged MV can cause a weaning problem due to diaphragm weakness because the muscle is unloaded and quiescent. Unloading and inactivity of the diaphragm causes sarcomeric protein dysfunction. However, there is limited understanding and no countermeasures to the sarcomeric dysfunction that contributes to diaphragm weakness after MV in humans. The purpose of our ongoing investigation is to examine the impact of intermittent unilateral phrenic nerve stimulation during surgery on diaphragm single fiber contractile properties. One phrenic nerve was stimulated (30 contractions per bout, every 30 minutes) during open chest surgery. Shortly before the end of surgery diaphragm biopsies were obtained from stimulated (STIM) and non-stimulated (NO-STIM) hemidiaphragms. We tested contractile properties of permeabilized single fibers from both hemidiaphragms. Preliminary results show that, in type I fibers, there was no difference in specific force (sF_o) from STIM and NO-STIM hemidiaphragms. The rate constant of tension redevelopment (K_{tr}) was faster by ~7% in STIM hemidiaphragm than in NO-STIM. Calcium sensitivity was improved in STIM hemidiaphragm, such that the calcium concentration (-log of pCa50) that elicits 50% maximum force was reduced by ~18%. In type II_a fibers, the three parameters (sF_o, K_{tr} and pCa_{50}) were similar in STIM and NO-STIM hemidiaphragm fibers. Our preliminary data suggest beneficial effects of intermittent phrenic nerve stimulation, and preferential effects on type I fibers.

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Random Myosin Loss along Thick-Filaments Increases Myosin Attachment Time and the Proportion of Bound Myosin Heads to Mitigate Force Decline in Skeletal Muscle

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Diminished skeletal muscle performance with aging, disuse, and acute or chronic disease may be partially attributed to the loss of myofilament proteins. Several laboratories have found a disproportionate loss of myosin protein content relative to other myofilament proteins, but due to methodological limitations, the structural manifestation of this loss is unknown. In addition to loss of thick-filaments with severe physiological or pathological conditions, recent ultrastructural measurements suggest that myosin loss can occur randomly along the length of thick-filaments, in keeping with models for thick-filament remodeling. To investigate how ensemble cross-bridge behavior and force production might be influenced by variation in myosin content, uniform and random myosin loss was simulated at different sarcomere lengths and cross-bridge stiffness values using a computational model of the half-sarcomere. Uniformly removing myosin (up to 50% of normal) from the Z-line end of thick-filaments showed force decrements that were slightly below the decrease predicted from simply losing available cross-bridges, due to minimal changes in myosin-actin cross-bridge kinetics. In contrast, randomly reducing myosin content along thick-filaments resulted in greater force production compared to uniform loss, largely due to increased myosin attachment time (t_on) and the proportion of bound cross-bridges. Force production and cross-bridge behavior was less affected by variations in sarcomere length and cross-bridge stiffness than reductions in myosin content. These findings support our prior observations that prolonged t_on may increase single fiber force production when myosin content decreased in chronic heart failure patients. These simulation results are consistent with the idea that myosin loss from skeletal muscles occurs randomly along thick-filaments and illustrate that the pattern of myosin loss along thick-filaments influences ensemble cross-bridge behavior and maintenance of force throughout the sarcomere.

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Molecular Chaperone Mediated Inhibition of the Myosin Power Stroke may be Critical for Sarcomere Assembly

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Molecular chaperones are required for successful folding and assembly of sarcomeric myosin. Here, we show that the chaperone UNC-45B stabilizes a non-translocation competent, actin-bound conformation of myosin that preserves its normal ATPase function. This novel locked actomyosin conformation may be necessary for assembly into myofibrils during development and after mechanical stress, and is fully reversible by the general chaperone Hsp90. We propose that a major function of UNC-45B is to prevent the power stroke from occurring during myosin biogenesis. In the forming sarcomere, this would prevent untimely force from being applied to thin filaments, which could disrupt their orderly assembly into the semi-crystalline sarcomere. Hsp90 could then serve both to assist in the completion of the myosin fold and to release the UNC-45B-mediated block, allowing the now fully formed myofibrils to contract.

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Localization and Binding Partners of SESTD1 in Skeletal Muscles

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Myofibrillogenesis is a complex production system of the striated muscles, which are correctly built by a lot of structural and signaling proteins. Some insights about the molecular mechanism of myofibrillogenesis were elucidated but most of them are not uncovered. SESTD1 is a novel protein that consists of SEC14 domain, three spectrin repeats and unique sequences and is expressed in various tissues including striated muscles. One research suggests that SESTD1 is involved in the planar cell polarity pathway during mammalian embryonic development and another research suggests that SESTD1 regulates some transient receptor potential channels in smooth muscle. However, it remains unclear about the function of SESTD1 in striated