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Fiber Type Transition in Eccentric Exercised Normal vs. Stretch-Activated Channel Blocked New Zealand Rabbits

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Channel Blocked New Zealand Rabbits
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Introduction

The Butterfield lab is focused on investigating the mechanical and biochemical mechanisms that govern skeletal muscle function in order to better understand associated myopathies and dysfunction thereby allowing researchers and physicians to develop more effective pharmaceutical and clinical treatments.

Skeletal muscle is a complexly organized, hierarchical structure composed primary of the three force generating myofilaments: actin, myosin and titin (Figure 1). Skeletal Muscle is under voluntary control and allows for well-articulated biomechanical movements, heat regulation, venous return, posture, and support. Any disruption in the normal function of the skeletal muscle system may therefore be detrimental to the well-being of the organism.
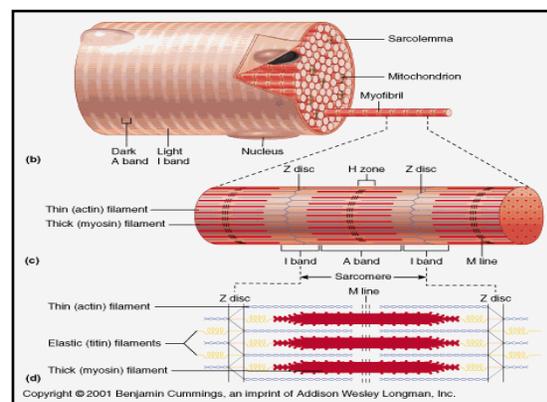

Figure 1. schematic illustration of the hierarchical structure of skeletal muscle.

When the body has committed to making a movement, a signal is propagated through specific nerves that innervate the individual muscles necessary to complete the movement. The signal is terminated at the neuromuscular junction, the site of muscle innervation, at which time the neurotransmitter acetylcholine (ACh) is released into the synaptic cleft. Post-synaptic receptors located on the sarcolemma bind ACh allowing ion-gated channels to be opened causing movement of sodium (Na^+) and potassium (K^+), which induces another depolarization event that is propagated to the transverse tubules.

Upon receipt of post-synaptic electrical stimulation, the transverse tubules stimulate the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum into the cytosol. Ca^{2+} binds to troponin causing a conformational change in the protein tropomyosin that exposes actin-binding sites. The myosin filament that has been waiting in a flexed position with an ATP molecule bound to its head forms a cross-bridge attachment to the actin filament. The ATP bound to myosin is hydrolyzed by the enzyme *myosin ATPase* and the energy released from this hydrolysis causes myosin to take a high-energy "cocked" position. The bound ADP and P_i are released and the myosin undergoes a power stroke that in turn pulls the actin filament toward the M-line by overlapping the

myosin and shrinking the overall size of the sarcomere, the major functional unit of the muscle fiber. When the external load is too great for the forces produced in the muscle, the sarcomeres extend under load, called and eccentric contraction. This active lengthening results in calcium influx into the cell through channels in the fiber membranes, called stretch activated ion channels (SAC), which have been proposed to influence the adaptive changes in muscle due to exercise and potentially the fiber's oxidative capacity.
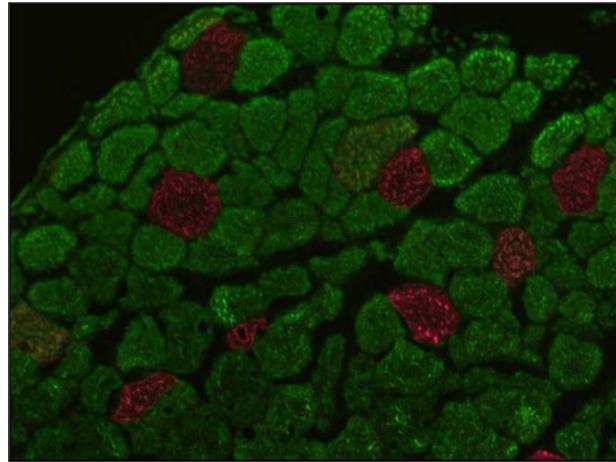

Figure 2. NZW Rabbit tibialis muscle cross section depicting different myosin heavy chain isoforms using Immunohistochemical methodology. Pink fibers = type I fibers; green fibers = Type IIa; red fibers = type IIb; black fibers = type IIx.

Muscle fibers are categorized by their oxidative capacity or ability to utilize O_2. Type 1 fibers (slow oxidative) utilize O_2 as the final electron acceptor in the electron transport chain during the production of adenosine triphosphate (ATP) the energy currency of the cell. Type 1 fibers are utilized during extended periods of exercise and contain a high percentage of mitochondria and myoglobin. In contrast, type II fibers (fast-twitch) undergo anaerobic respiration in order to produce ATP and contain smaller percentage of myoglobin and mitochondria relative to type I fibers. Type II fibers fatigue easily and are typically utilized during short intense periods of physical exertion. An intermediate fiber type also exists. This fiber type has properties of both type I and type II fibers and may shift toward a particular fiber type for given demands.

My summer research is focused on skeletal muscle fiber-type transitioning in eccentrically exercised (muscle contracts as it is lengthened) tibialis anterior muscles of New Zealand white rabbits (*Oryctolagus cuniculus*) and the role that SAC play in mechanotransduction. More specifically we are looking at how inhibition of SAC with streptomycin impacts the transitioning of fiber type normally seen with long-term exercise. We are currently performing biochemical analysis of sectioned muscle tissue to identify specific fiber types and possible transition states in SAC open vs. SAC blocked animal models in order to deduce more specific channel functions and how inhibition relates to specific myopathies such as Duchenne's muscular dystrophy (DMD).

Methods

Over a 4 week period, 12 New Zealand white rabbits underwent an eccentric exercise protocol totaling 12 bouts of exercise in all. The rabbits received daily injections of streptomycin or sham injections during this 4 week period in order to assess skeletal muscle adaption in response to stretch-activated ion channel blockade (Butterfield and Best, 2009). Following the assessment of function on the final training day, animals were euthanized and muscle tissues were harvested and flash frozen in liquid nitrogen before storage at -80℃ for further analysis.

Tissue cross sections were prepared from the lateral-distal portion of the tibialis anterior at a thickness of 8 micrometers (μm) and mounted to positively charged microscope slides in preparation for immunohistochemical staining. Tissue sections were rehydrated using 0.1M phosphate buffered saline (PBS). For immunohistochemistry, sections were incubated in primary antibody overnight at 4°C. The following primary antibodies to myosin II (skeletal muscle myosin) were used, all of which came from the Developmental Studies Hybridoma Bank (DSHB) Iowa: BA.D5 at a 1:75 dilution, SC.71 IgG1 supernatant, and BF.F3 IgM supernatant. After overnight incubation, sections were washed 3 times 5 min in 0.1M PBS. The sections were then incubated in secondary antibody diluted in PBS for 60 min at room temperature. The following secondary antibodies were used: Gt anti-Ms IgG2b, Alexa Fluor 647 conjugated secondary antibody (1:250), (Invitrogen #A21242), Gt anti-Ms IgG1, Alexa Fluor 488 conjugated secondary (1:500), (Invitrogen #A21121), Gt anti-Ms IgM, biotin conjugated secondary (1:150) (Invitrogen #626840). Another 3 times 5 min PBS wash followed incubation in secondary antibody. A fourth secondary antibody diluted in PBS (SA-Texas Red (1:150), Vector #SA-5006) was introduced to the sections and incubated for 15 min at room temperature. The immunohistochemical protocol was completed with a final PBS wash 3 times 5 min. Sections were then viewed with a Zeiss microscope and photos were taken at 100X magnification.

Results

Fiber type staining revealed that exercise did not influence fiber type in the lateral distal portions of rabbit tibialis muscle in the SAC blocked muscles. Both exercised and control muscles in SAC blocked muscles were100% Type IIa fibers. However the SAC open control muscle was 99% Type IIa fibers with 0.8% Type I fibers and 0.03% Type IIa/Type I transitional fibers. The SAC open exercised muscle was 99% Type IIa fibers with no Type I fibers and approximately 0.5% Type IIa/Type I transitional fibers (Figure 3).
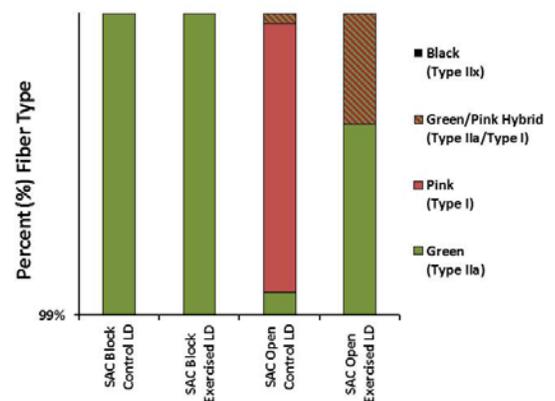

Figure 3. Distribution of fiber types in rabbit lateral distal tibialis anterior muscle.

Discussion

The preliminary data showed that when the stretch-activated ion channels were blocked, 100% of the fibers in the sample were type IIa, and did not undergo a transition with exercise. Interestingly enough, in the models where the SAC remained fully functional, a difference in fiber-type was recorded in both SAC open exercised and SAC open non-exercised. The SAC open non-exercised displayed type IIa along with type I fibers and a minimal amount of transitional fibers. The SAC open exercised tissue

displayed type IIa and transitional fibers with no type I fibers present, indicating an exercise specific remodeling of the fiber morphology.

Butterfield and Best have shown that muscles subject to SAC blockage are unable to adapt to eccentric exercise in terms of sustained force production (Butterfield and Best, 2009). They have also shown that increased isometric torque generated during bouts of eccentric exercise is directly dependent on fully function SAC. Therefore we conclude that exercise has no effect on fiber type transition, in the SAC blocked muscles. This evidence suggests that intact SAC function is indeed essential for fiber morphology and physiology in order to influence fiber-type transition necessary for increased force production and altered function observed by Butterfield and Best. We are continuing to collect data in an effort to show that skeletal muscle is not homogenous throughout the entirety of the tissue in terms of fiber-typing and that different regions of a muscle adapt differently to eccentric exercise through fiber-type transitioning that is driven by intact SAC function.

References

Butterfield, T. A. and T. M. Best (2009). "Stretch-activated ion channel blockade attenuates adaptations to eccentric exercise." Medicine and Science in Sports and Exercise **41**(2): 351-356.