muscle membrane protein that links the contractile proteins to the muscle membrane. Recent studies have suggested that increased Ca$^{2+}$ influx into the muscle and increased production of free radicals (or reactive oxygen species, ROS) are essential for increased susceptibility of mdx muscle to damage. However, the source of the ROS, the Ca$^{2+}$ channels affected, and the mechanism(s) of how mechanical stress results in altered regulation of these signaling pathways have yet to be determined. We hypothesis that NADPH oxidase (Nox2) drives excessive ROS production, increased Ca$^{2+}$ influx, and muscle damage. Manganes (Mn$^{2+}$) quench was used to assess the role of Nox2 activity on sarcoplasmic Ca$^{2+}$ influx in response to a physiological stretch and depolarization. Our results show that stretch-activated Ca$^{2+}$ entry in mdx is significantly increased 4.2-fold (p<0.001) compared to WT, while K$^{+}$-induced depolarization results in 20-fold (p<0.001) increase in Mn$^{2+}$ quench in mdx skeletal muscle. Administration of either gp91ds-TAT, an inhibitor of Nox2, or reduced glutathione ethyl ester, a glutathione analogue, in mdx muscle reduces the Mn$^{2+}$ quench rates back to the WT levels for both passive stretch and K$^{+}$ depolarization. Addition of BTP2, a calcium release activated calcium channel inhibitor, also significantly attenuates Mn$^{2+}$ quench rates in mdx compared to WT. Our data supports a model in which Nox2 dependent redox modifications of stretch and depolarization activated Ca$^{2+}$ channels leads to exuberant Ca$^{2+}$ influx. Our results identify Nox2 as a potential therapeutic approach to minimize or delay the progressive DMD disease.

1483-Pos Board B375
Myostatin Deficient Mice Display Altered Calcium Signaling
Laszlo Csernoch, Dora Bodnar, Olga Ruzsnavszky, Nikolett Geyer, Benedek Dienes, Monika Sztretye, Peter Szentesi.
University of Debrecen, Debrecen, Hungary.
Myostatin, a member of the transforming growth factor (TGF) family, is considered to be a potent negative regulator of skeletal muscle growth. It is strongly expressed in developing muscle and is thought to be a component of the myostatin signaling pathway in muscle cells. We hypothesized that altered calcium signaling pathways may be responsible for the reduced muscle performance in myostatin deficient mice. We investigated the calcium homeostasis of skeletal muscle fibers from mice with the compact myostatin mutation (MstnCmpt-dlAbc).

Skeletal muscle fibers from mice with the compact myostatin mutation (MstnCmpt-dlAbc) were analyzed for calcium signaling pathways. Calcium transients were measured on single FDB fibers with the fluorescent dye Fluo-3, which allowed for the analysis of calcium release from the sarcoplasmic reticulum (SR) in response to a variety of stimuli. The depolarization-evoked calcium transients were smaller in MstnCmpt-dlAbc fibers compared to control fibers. The amplitude of the calcium transient was reduced by 20% (p<0.001) in MstnCmpt-dlAbc fibers compared to control fibers. In addition, the calcium transient decay time constant was increased in MstnCmpt-dlAbc fibers compared to control fibers. The calcium transient decay time constant was increased by 25% (p<0.001) in MstnCmpt-dlAbc fibers compared to control fibers. These results suggest that calcium signaling pathways are altered in skeletal muscle fibers from MstnCmpt-dlAbc mice, which may contribute to the reduced muscle performance observed in these mice.

1485-Pos Board B377
Up-Regulated Autophagy in Skeletal Muscle of Young Amyotrophic Lateral Sclerosis Mouse Model Prior to Disease Onset
Yajuan Xiao1, Chuling Ma1, Jianxun Yi1, Guo Luo1, Frank Yi2, Tian Yu2, Pei-Hui Lin3, Jingsong Zhou1.
1Rush University, Chicago, IL, USA, 2Zunyi Medical College, Zunyi, China, 3The Ohio State University, Columbus, OH, USA.
Autophagy is a cellular process that targets damaged organelles for lysosomal degradation, which may play crucial roles in amyotrophic lateral sclerosis (ALS), a neuromuscular disease characterized by motor neuron and muscle degeneration. Studies from Morimoto (2007) and Zhang (2011) identified increased autophagy in motor neurons of ALS mouse (G93A) only at the age after the disease onset (>90 days). To test if muscle plays an active role in the disease progression, we analyzed autophagy activity in skeletal muscle at three different age groups: before axon withdrawal (<47 days), before disease onset (47-90 days) and around disease onset (>90 days) by expressing LC3-RFP in muscle fibers or loading muscle fibers with LysoTracker. We found that autophagic increases of autophagosome formation and lysosomal vesicles at all age groups of G93A mice compared to the age-matched wild type (WT). The portion of fibers with the ratio of autophagosome-area/fiber-area more than 15*10^{-5} is summarized as G93A (WT): [<46: 19% (3%); 47-90: 43% (8%); >90: 15% (2%); n=31-74 (31-66)]. The portion of fibers with more than 100 lysosome vesicles is summarized as: [<46: 16% (1%); 47-90: 58% (0%); >90 days: 44% (2%); n=39-77 (38-81)]. A majority of autophagosomes are in close contact with lysosome vesicles, indicating a coordinated up-regulation of autophagy-lysosomal pathway in G93A muscle. Western blot analysis also show increased LC3-II level in G93A muscle at all age groups: [<46: 5.4±1.5, n=4 (1.1±0.1, n=4); 47-90 days: 4.4±1.6, n=4 (1.4±0.2, n=12); >90: 7.1±1.8, n=12 (2.2±0.9, n=4)]. Our study identified up-regulated autophagy activity in muscle as an early event before disease onset, suggesting that skeletal muscle may actively contribute to ALS progression. Supported by MDA and NIAMS/NHII.

1486-Pos Board B378
Upregulation of RGK Protein Expression in Aging Mouse Fast Twitch Skeletal Muscle
Whitney Ann Sunner, Donald Begollar, Christin Romberg, Matthias P. Scheele, Roger A. Bannister.
University of Colorado Denver-AMC, Aurora, CO, USA.
A component of muscle weakness in older individuals is directly attributable to compromised excitation-contraction (EC) coupling. In skeletal muscle, the L-type Ca$^{2+}$ channel (Cav1.1) serves as the voltage sensor for EC coupling by triggering Ca$^{2+}$ release from the sarcoplasmic reticulum (SR) in response to plasma membrane depolarization. Although it has been established that impaired EC coupling (termed “EC uncoupling”) by O. Delbono and colleagues in aged individuals is caused by a reduction in the number of L-type channels present in the plasma membrane, the molecular mechanisms responsible for transducing age-dependent cellular signals (e.g., oxidative stress) to decreased Cav1.1 membrane expression remain enigmatic. In this study, we have investigated a role for RGK (Rad, Rem, Rem2; Gen/Kir) family small G proteins in EC uncoupling because: 1) expression of endogenous Rad is enhanced in response to oxidative stress in skeletal muscle, and 2) overexpression of Rem in muscle cells mimics EC uncoupling. We have found that exogenous overexpression of Rad1, but not Rad22, confers long-term resistance to oxidative stress in the presence of myostatin or myotubes also mimics EC uncoupling. Moreover, immuno-staining of whole mount tibialis anterior and mixed gastrocnemius muscles lysates revealed progressive age-dependent enhancement of Rad protein levels while Rad expression was largely absent in slow-twitch soleus muscle.
in all age groups (ranging from 2-36 months). Taken together, our observations raise the possibility that Rad acts as one molecular link between elevated oxidative stress and EC uncoupling in aging fast-twitch muscle. Conversely, the lack of Rad expression in slow twitch muscle suggests that Rad may play a role in the disproportionate retention of slow twitch fibers in aged individuals.

1487-Pos Board B379
Impaired Ca-Calmodulin-Dependent Inactivation of CaV 1.2 Contributes to Loss of SR Ca Release Refractoriness in Mice Lacking Casq2
Dnytro Kryštátl, Bjorn C. Knollmann.
Vanderbilt University, Nashville, TN, USA.
In cardiac muscle, L-type Ca current (CaV 1.2, ICa) induced Ca release from sarcoplasmic reticulum (SR) is reduced with successively shorter coupling intervals of premature stimuli, a phenomenon known as SR Ca release restitution. We previously reported that myocytes lacking the SR luminal Ca binding protein calsequestrin (Casq2 KO) exhibited less SR Ca release restitution and hence largely lack Ca release refractoriness. Here, we test the hypothesis that altered CaV 1.2 channel gating contributes to altered SR Ca release restitution in Casq2 KO myocytes.

Methods and Results: ICa was recorded in voltage-clamped ventricular myocytes isolated from Casq2 KO mice and wild-type (WT) littermates. Cells were pre-treated with ryanodine (50 μM) and thapsigargin (10 μM) to eliminate SR Ca release. Compared to WT, ICa peak currents were unchanged, but the inactivation time course was significantly slower in KO myocytes (τ<sub>i</sub>=157 ms vs. 46 ms in WT, p<0.01). Using Ba as a charge carrier abolished the differences in inactivation, suggesting that the underlying defect lies not in the voltage-dependent but rather in the Ca-dependent ICa inactivation. Addition of apo-calmodulin (CaM, 0.35 mg/ml) to NIH R01-HL-084487 to AE.

Conclusion: Impaired Ca-dependent inactivation of ICa in Casq2 KO mice can be reversed by excess apo-CaM, suggesting that impaired Ca-CaM-dependent inactivation of CaV 1.2 contributes to loss of SR Ca release refractoriness in Casq2 KO mice.

1488-Pos Board B380
Impact of SR Ca<sup>2+</sup> Release on the Cardiac Action Potential Repolarization during Postnatal Development
Azade D. Petrosky1, Rafael Mejia-Alvarez2, Ariel L. Escobar1.
1School of Engineering, University of California Merced, Merced, CA, USA, 2Department of Physiology, Midwestern University, Downers Grove, IL, USA.
In heart, the excitation-contraction coupling (ECC) mechanism changes from sarcomembranous Ca<sup>2+</sup> diffusion in newborn, to Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release in adult. This implies different contribution of the sarcoplasmic reticulum (SR) to ECC during postnatal development is critical in defining the properties of AP repolarization in epimyocardium. Consequently, the AP properties were evaluated during postnatal development in correlation with the SR Ca contribution to ECC. To this end, the temperature dependence of the epimyocardial AP properties was determined with the local-field fluorescence detection method in intact mouse hearts, with/without SRaC, and at various ages after birth (7h, 1d, 2d, 7d, & AD). Our pharmacological (ryanodine+thapsigargin) and thermodynamic (temperature dependence from 21°-37°C) studies confirmed that the SRaC contribution becomes more prominent with development. More importantly, they showed that the impact of SRaC on the AP duration (measured at 25% & 75% of repolarization; APD25 & APD75) exhibits a biphasic effect. At day 2, SRaC significantly reduced the APD25 & APD75 by 38.7% & 31%, respectively; while in adult SRaC prolonged the APD75 by 49.7%; with day 7 being a transitional stage with a 44%-shortened APD25 and 25%-lengthened APD75. Interestingly, the peak-and-dome AP morphology typical of mouse ventricular myocytes, became evident only in adult. A possible explanation for this dual effect on APD is that early after birth, SRaC promotes a Ca<sup>2+</sup>-dependent inactivation of the L-type ICa while in adult, SRaC activates the Na-Ca exchanger forward mode. Altogether, our results demonstrated that differences in SR Ca<sup>2+</sup> release during development not only play a different role in ECC, but greatly impacts plasma membrane excitability as well. Supported by NIH R01-HL-084487 to AE.

1490-Pos Board B382
The Rapid Repolarization of the Action Potential in Twitch Skeletal Muscle Fibers from Frog is due Almost Entirely to a Calcium-Activated K Current
Cedric R.H. Lamboley1, Gabor Gyurkovics2, Paul C. Pape3.
1Victoria University Institute of Sport, Excercise and Active Living, Melbourne, Australia, 2Université de Sherbrooke FMSS, Sherbrooke, QC, Canada.
Action potentials (aps) in cut fibers mounted in a double Vaseline-gap chamber were generated by short current-clamp pulses superimposed on a holding current that maintained the resting potential at −90 mV. Ca<sup>2+</sup> release from the sarcoplasmic reticulum (SR) was assessed with either the EGTA/phenol red method or the SR [Ca<sup>2+</sup>] indicator, tetramethylmurexide. In contrast to the rapid rate of repolarization of the ap with normal SR Ca contents, in the near absence of Ca, the time course of repolarization was approximately exponential with an average exponential time constant of 12.5 ms. Removal of K from the internal and external solutions eliminated the effect of SR Ca<sup>2+</sup> release on the ap indicating that the current is carried by K<sup>+</sup> ions. The time course of total current during the repolarization was assessed from the rate of change of voltage. Taking into account a small Ca-sensitive background current and the relationship between K current and permeability (P<sub>K</sub>) on voltage using the Goldman-Hodgkin-Katz equation, the time course of P<sub>K</sub> is seen to closely match that of the myoplasmic Ca transient. At physiological SR Ca load, the Ca-activated K current, denoted I<sub>KCa</sub>, at its peak was ~80% of the total current. Since almost all of the background current was associated with recharging the membrane capacitance via the holding current passing through ohmic pathways in the cut-fiber preparation, the peak percentage of current responsible for repolarization in an intact fiber carried by I<sub>KCa</sub> should be significantly greater than 90%. While results with voltage-clamp stimulation indicate a complex dependence between I<sub>KCa</sub> on voltage and calcium similar to that displayed by BKCa channels, the I<sub>KCa</sub> current was insensitive to the BKCa channel inhibitor charybdotoxin.

1491-Pos Board B383
Sarcoplasmic Reticulum (SR) Ca<sup>2+</sup> Release during Long-Lasting Depolarizations in Skeletal Muscle Fibers
Gaelle Robin, Bruno Allard.
University Lyon 1, Villeurbanne, France.
High amplitude depolarizations of skeletal muscle fibers induce massive SR Ca<sup>2+</sup> release that progressively declines with time and completely annihilates if depolarization is maintained during several tens of seconds. Several processes may be involved in the decline of SR Ca<sup>2+</sup> release: cytosolic Ca<sup>2+</sup>-dependent closure of SR Ca<sup>2+</sup> release channels, voltage-dependent inactivation of SR Ca<sup>2+</sup> release channels and SR Ca<sup>2+</sup> depletion. This study aimed