the procedure. The other electrode was used to dialyze the fiber with a solution containing either 6-15 mM BAPTA, or up to 60 mM EGTA (pCa=7.0), and adjusted to pH=7.0 with 20 or 60 mM MOPS, respectively. Free myoplasmic $Ca^{2+}$ concentration ($[Ca^{2+}]$) changes were measured with the low affinity indicator OGB-5N (200 μM). SR $Ca^{2+}$ release was elicited by either 20 mM caffeine, or 1 mM 4-chloro-methy-phenol (4CmC), added to the external solution. The maximal fluorescence change of the $Ca^{2+}$ indicator was assessed at the end of the protocol by exposing the fibers to saponin (0.1 mg/ml in isotonic $CaCl_2$, pH=7.0 with 20 mM MOPS). To prevent changes in shape of the fibers under these conditions, they were pretreated for 1 min with 1% formaldehyde (in Tyrode). The experiments were conducted at 20 °C. A single model compartment was used to estimate (from the $[Ca^{2+}]$ changes) the total $Ca^{2+}$ released in response to caffeine/4CmC application. We obtained values of SR $Ca^{2+}$ content in the range of 15-27 mM for normal FDB fibers. Interestingly, comparable values were obtained in fibers from *mdx* mice.

**1211-Pos Board B55**

**The Absence of Utrophin Does Not Further the Impairment of $Ca^{2+}$ Release Displayed by *mdx* Muscle**

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The double mutant utr$^{-/-}$/mdx mouse has been postulated to be a better model of Duchenne Muscular Dystrophy than *mdx* mouse because it displays a progression of pathological features comparable to that in humans. We previously demonstrated that FDB fibers from *mdx* mice show limitations in action potential (AP) elicited $Ca^{2+}$ release. Here we investigated the properties of the APs and the ability to release $Ca^{2+}$ (30 mM [EGTA], 20ºC) in response to single and trains of APs (20 pulses, at 33Hz and 100Hz) in FDB fibers isolated from control, *mdx* and utr$^{-/-}$/mdx mice. Single APs of normal amplitude but longer duration were recorded in utr$^{-/-}$/mdx fibers, whereas the amplitude of the $Ca^{2+}$ release was ~37% smaller than in normal fibers, but comparable with that found in *mdx* fibers. Fibers from the three strains sustained trains of Ca release at 33 Hz in which the amplitude of individual $Ca^{2+}$ release transients decayed exponentially towards a sustained release amplitude with two time constants ($\tau_1$=10ms, $\tau_2$=200ms). In response to 100Hz trains, the amplitude of $Ca^{2+}$ release in normal fibers decayed still with a double exponential ($\tau_1$=3.5ms, $\tau_2$=41ms) in which the amplitude of the 2$^{nd}$ and 20$^{th}$ transient along the train were ~50% and ~35% that of the first one, respectively. In contrast, fibers isolated from both *mdx* and *utr$^{-/-}$/mdx* mice could be divided in two groups according to their tetanic response to 100Hz trains: approximately 75% of *mdx* and 33% of utr$^{-/-}$/mdx fibers showed a behavior similar to that observed in normal fibers; in the remaining 25% of *mdx* and 67% of utr$^{-/-}$/mdx fibers, respectively, the amplitude of the 2$^{nd}$ $Ca^{2+}$ release transient was ~20% of the first one, and this amplitude was sustained throughout the train.

**1212-Pos Board B56**

**Deficits in Ca2+ Release and in vivo Muscle Strength in Heterozygous I4895T RyR1 Knock-In Mice**

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The mutation from isoleucine to threonine of the skeletal isoform of the ryanodine receptor (RyR1) at residue 4898 results in severe Central Core Disease (CCD). Under homozygous expression (IT/IT), we reported a lack of $Ca^{2+}$ release in response to electrical and pharmacological activation despite SR $Ca^{2+}$ store content indistinguishable from control. Here we used heterozygous knock-in mice for the I4895T (IT/+; analogous to human I4898T) RyR1 mutation to determine the effects of the mutation on muscle strength and $Ca^{2+}$ handling in flexor digitorum brevis (FDB) and interosseous muscle fibers.
We compared *in vivo* muscle strength of wild-type (WT) and IT/+ mice. IT/+ mice exhibited significant weakness in both upper body and grip strength assays (4-paw peak grip force: 2400 ± 70 mN, n=8 and 2040 ± 80 mN, n=14 in WT and IT/+ mice, respectively). We also determined the magnitude of action potential- and ligand-evoked $Ca^{2+}$ release in single intact FDB fibers using $Ca^{2+}$ fluorometry. The magnitude of both electrically- and ligand-evoked $Ca^{2+}$ release was significantly reduced in IT/+ fibers. Moreover, the maximum rate of change in mag-fluo-4 fluorescence during the rising phase of the electrically-evoked $Ca^{2+}$ transient was significantly reduced in IT/+ fibers (WT 0.17 ± 0.01 ΔF/F/ms vs IT/+ 0.11 ± 0.01 ΔF/F/ms, n= 53, 56, respectively). Finally, the frequency (1.9 ± 0.5 and 0.8 ± 0.3 events/scan) and $Ca^{2+}$ spark mass (5.9 ± 0.3 and 4.6 ± 0.2 μm$^3$) of local $Ca^{2+}$ release induced by osmotic shock (440 mOsm with sucrose, 750 lines/sec) were reduced in acutely dissociated IT/+ interosseous fibers compared to that of WT fibers. Together, these findings are consistent with the hypothesis that the IT mutation in the putative RyR1 selectivity filter significantly reduces $Ca^{2+}$ flux through the channel.

**1213-Pos Board B57**

**Malignant Hyperthermia Mutation Alters Excitation-coupled Ca2+ Entry In MH RyR1-R163C Knock-in Myotubes**

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Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle triggered insusceptible individuals by inhalation anesthetics and depolarizing skeletal muscle relaxants. This syndrome has been linked to a missense mutation in the type 1 ryanodine receptor (RyR1) in more than 50% of cases studied to date. We have examined how the R163C MH-RyR1 mutation alters the $Ca^{2+}$ transient during $[K^+]_e$ depolarization using Fluo4 and excitation-coupled $Ca^{2+}$ entry (ECCE) using manganese-quench of Fura2 Wt and $RyR1_{R163C}$ knock-in myotubes. Exposure of WT and $RyR1_{R163C}$ myotubes to low $Ca^{2+}$ solution ($8.7x10^{-6}$ M) and then to high $[K+]_e$ did not modify initial $Ca^{2+}$ transient ($Ca^{2+}{}_{peak}$), but dramatically altered the time course of the $Ca^{2+}$-transient, making the duration shorter and the rate of decay faster in all genotypes. However, these changes were more evident in $RyR1_{R163C}$ than Wt myotubes. The rate of $Mn^{2+}$ quench of Fura2 associated with $K^+$ depolarization (ECCE), was membrane potential dependent and always greater and faster in $RyR1_{R163C}$ myotubes than in Wt. Incubation of Wt and MH $RyR1_{R163C}$ myotubes with 15 μM ryanodine overnight, to block RyR1 $Ca^{2+}$ release, enhanced the amplitude but did not significantly change in the rate of ECCE in either genotype. However, the increment in amplitude was greater in Wt than $RyR1_{R163C}$ knock-in myotubes. We conclude that the pre-existing conformational change caused by the $RyR1_{R163C}$ MH mutation alters the properties ECCE as consequence of significant changes in the retrograde signaling between RyR1 and DHPR making it less sensitive to the conformational change caused of ryanodine.

**1214-Pos Board B58**

**Malignant Hyperthermia and Heat Stroke in Calsequestrin-1 Knockout Mice**

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Malignant hyperthermia (MHS) and environmental heat stroke (EHS) in humans present as similar life threatening crises triggered by volatile anesthetics and strenuous exercise and/or high temperature, respectively. Many families (70-80%) diagnosed with MH susceptibility (MHS), and a few with EHS, are linked to mutations in the gene that encodes the type 1 ryanodine receptor (RYR1) located in the sarcoplasmic reticulum (SR) of skeletal muscle. However, mutations in the RYR1 gene are not found in all MH families, suggesting that alternative genes remain to be identified. Here we investigated whether a MH/EHS-like phenotype results from deficiency in skeletal muscle calsequestrin (CASQ1), a SR $Ca^{2+}$-binding protein that modulates RYR1 function. Exposure of CASQ1-null mice to halothane or heat stress triggers lethal episodes characterized by elevated core temperature, whole body contractures, and severe rhabdomyolysis. Both heat- and halothane-induced episodes are prevented by prior dantrolene administration, the standard antidote used to treat MH episodes in humans. *In vitro* studies indicate that CASQ1-null muscle exhibits increased contractile sensitivity to caffeine, temperature-dependent increases in resting $Ca^{2+}$, and an increase in the magnitude of depolarization-induced $Ca^{2+}$ release. These findings validate CASQ1 as a candidate gene for linkage analysis in MH/EHS families where mutations in RYR1 are excluded.

**1215-Pos Board B59**

**Expression Of Calsequestrin-1 (CS1) In CS1-null Mice: Restoration Of $Ca^{2+}$ Release Unit Architecture And Amplitude Of $Ca^{2+}$ Transient In Fast-twitch Muscle Fibres**

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Amplitude of calcium ($Ca^{2+}$) transients and width of the Sarcoplasmic Reticulum (SR) lumen in $Ca^{2+}$ release units (CRUs) are significantly reduced in Calsequestrin1 (CS1)- null mice, moreover increase in fatigue resistance is characteristic of the CS1-null-model (*Paolini et al 2007*). We extend the study of the null model at molecular level: decrease in expression of CS2, Triadin, Sarcalumenin were detected in CS1-null FDB muscles in comparison to wild type (wt) and differential FDB (null/wt) expression of 13400 mRNAs was assayed by microarray profiling. To rescue the CS1-null phenotype, exogenous mouse CS1 was expressed in adult null-FDBs by *in vivo* DNA electrotransfer. CS1 expression and correct targeting to CRUs was verified by confocal

microscopy, whereas restoration of CRUs architecture - i.e. shape and width of junctional SR (jSR) containing CS1 - was assessed by electron microscopy. Exogenous CS1 was correctly targeted to CRUs and positioned at the jSR, in close proximity of $Ca^{2+}$ release sites. Size of the SR lumen was increased. At proteomic level CS2, Sarcalumenin, Triadin and Junctin did not change upon CS1 expression. $Ca^{2+}$ transients induced by electrical stimulation were recorded in mock-transfected , and CS1-transfected fibres: successfully, average peak height and baseline showed significant increase upon CS1 expression resembling wt fibres. The present results provide strong evidences that expression of CS1 directly controls size of jSR terminal cisternae, influences resting cytosolic $Ca^{2+}$ and modulates the amplitude of $Ca^{2+}$ transient in response to electrical stimulation in fast-twitch muscles.

Paolini, C et al. 2007 J Physiol, 583: 767.

**1216-Pos Board B60**

**Deletion Of Triadin Results In Marked Alterations In Tetanic Contraction And Global Calcium Handling**

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In our recent work (Shen et al., JBC, 282(52), 2007), we examined the role of triadin in skeletal muscle EC coupling in a pan-triadin null mouse. No contractile dysfunction was evident during single twitches *in vitro*, however a reduction in $Ca^{2+}$ transient magnitude was observed. Analysis of single myofibers revealed a decrease in SR $Ca^{2+}$ content and an increase in sarcoplasmic $[Ca^{2+}]$, suggesting that triadin is not critical to EC coupling, yet might play a modulatory role at the RyR. In this investigation, we test our hypothesis that an increase in functional demands will unmask an EC coupling phenotype in muscle null for triadin (Tdn). We used tetanic stimulation to examine the contractile characteristics of EDL muscles *in vitro* and TA muscles *in situ*, as well as global calcium transients. The tetanic force vs. stimulation frequency (FF) relationship (250msec train; 0.5ms pulse @ 1-300Hz) between WT and Tdn EDLs was evaluated *in vitro*. Tdn EDLs were unable sustain the initial peak achieved during each 250msec train. The magnitude of the tetanic fade was progressive with increasing stimulation frequency. At peak tetanic stimulation (300 Hz) this tetanic fade resulted in a ~23% decrease in the tension-time integral; a finding that was replicated with nerve evoked tetanic stimulation of the tibialis anterior muscle *in-situ*. Similarly, our assay of myoplasmic $[Ca^{2+}]$ in FDB myofibers (MagFluo4AM) demonstrated that the plateau of the tetanic $[Ca^{2+}]$ was not sustained in triadin null FDBs, even though the peak $[Ca^{2+}]$ amplitude during a 250msec tetanic train (80Hz; field stim.) was not different between genotypes. We believe that the contractility deficits in the Tdn muscles are due to a decrease in SR $Ca^{2+}$ release. Funded by grants from NIH-NIAMS to C.W.W, R.M.L, P.D.A, and C.P.

**1217-Pos Board B61**

**Triadin Function In Sarcoplasmic Reticulum Structure?**

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Muscle contraction is achieved when an efficient excitation signal at the plasma membrane triggers intracellular calcium release. This process called ''excitation-contraction (E-C) coupling'' relies on a multimolecular protein complex, spanning the plasma membrane and the sarcoplasmic reticulum (SR), containing the calcium channel of the SR, the ryanodine receptor (RyR). Triadin is a member of this complex, present in the SR membrane and interacting with RyR in skeletal muscle. We have shown that overexpression of triadin in cultured myotubes abolishes E-C coupling, although RyR is still functional. Moreover in knock-out mice, deletion of the triadin gene leads to disorganisation of SR membranes in skeletal muscles. In the present work, we have expressed triadin in COS-7 cells to dissect its intrinsic properties on membrane organisation. We show that triadin expression leads to important modification of the endoplasmic reticulum (ER) morphology, already observed with the expression of proteins regulating ER morphology, and known as ''rope-like structures''. These modifications of ER morphology are correlated to alteration of the microtubule network. Indeed, in cells expressing triadin, microtubules are bundled, often running parallel to the plasma membrane, and more stable than in untransfected cells. Surprisingly, suppression of the cytosolic N-terminal part of triadin did not reverse this phenotype. Using splice versions of the triadin protein and C-terminal deletion mutants, we show that ER/microtubules modifications depend on an intra-lumenal sequence. Altogether, our work lead to the hypothesis that modifications of ER morphology and microtubule dynamics observed in cells expressing triadin are mediated by an intermediate protein currently under investigation. Expression of triadin in COS-7 cells can modify endoplasmic reticulum morphology. It thus suggests that in skeletal muscle, triadin could play a role in the structure of sarcoplasmic reticulum to allow efficient E-C coupling.

**1218-Pos Board B62**

**Molecular Basis Of Protein Localization To The Junctional Sarcoplasmic Reticulum Of Skeletal Muscle Cells**

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The sarcoplasmic reticulum (SR) of skeletal muscle cells is a continuous network of membranes in which some specific domains (i.e. the longitudinal SR and the junctional SR) can be clearly distinguished. Although each of these domains is characterized by a specific protein composition the mechanisms leading to protein targeting to these domains are still unknown. In particular, specific targeting mechanisms to the junctional SR are likely to be present in triadic proteins, yet no specific localization signal has been defined. In order to investigate this point we expressed wild type and deletion mutant GFP-tagged triadic proteins in rat primary myotubes and followed their localization during in vitro development. In parallel the dynamic properties of these proteins were investigated by FRAP technique. Analysis of triadin, junctin, Ryanodine Receptor type 1 and junctophilin-1 allowed us to identify specific sequences that might be responsible for targeting of these proteins to the junctional SR. Furthermore, FRAP analysis showed that deletion of some, but not all, of these sequences resulted in a significant increase in the mobility of triadic proteins. This would suggest that some of these regions, in addition to mediate protein targeting to the junctional SR, could also contribute to the establishment of protein-protein interactions within the multi-molecular complex associated with the calcium release channel. Experiments are being performed to further dissect these interactions.

**1219-Pos Board B63**

**Intra-sarcoplasmic Reticulum $Ca^{2+}$ Depletion In Adult Skeletal Muscle Fibers Measured With The Biosensor D1ER**

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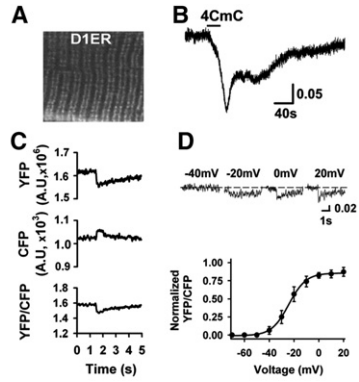
The endoplasmic/sarcoplasmic reticulum (ER/SR) plays a crucial role in cytoplasmic signaling in a variety of cells. It is particularly relevant for skeletal muscle fibers, where this organelle constitutes the main $Ca^{2+}$ store for essential functions, such as contraction. In this work, we expressed the cameleon biosensor D1ER by in vivo electroporation in the mouse flexor digitorum brevis (FDB) muscle to directly assess intra-SR $Ca^{2+}$ depletion in response to electrical and pharmacological stimulation. The main conclusions are: (1) D1ER is expressed in the SR of FDB fibers according to both di-8-ANEPPs staining and reductions in FRET; (2) the amplitude of intra-SR $Ca^{2+}$ release evoked by either 4-CmC or electrical stimulation is directly proportional to resting SR $Ca^{2+}$, which indicates that intra-$Ca^{2+}$ modulates RyR1-meditated SR $Ca^{2+}$ release in the intact muscle fiber; (3) intra-SR $Ca^{2+}$ release, as measured with D1ER, is voltage-dependent and follows a Boltzmann function; (4)




**A) The triad striation pattern is evident in FDB fibers expressing D1ER; B) Calcium release evoked by 30s exposure to the RyR agonist 4CmC; C) Typical D1ER response to electrical stimulation to 20mV for 200 ms; D) Voltage-dependence of intra-SR calcium release**