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Free communications: Muscle

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Chair: Anthony Bakker

The effects of arsenic on the two major fibre types in the chelae of the freshwater crayfish *Cherax destructor* (Clarke)

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Arsenic is a proven carcinogen found in the soil in gold mining regions at concentrations that can be thousands of times greater than gold. During mining arsenic is released into the environment, easily entering surrounding water bodies. The main chemical forms of arsenic found in the environment are arsenite (As(III)) and arsenate (As(V)), which are known to be the more toxic and available arsenic compounds.

It has been found that *Cherax destructor* (the yabby) can accumulate arsenic at levels comparable to those in the sediment of their environment (Williams *et al.*, 2008).

This study determined the effects of arsenic contamination on the yabbies themselves. Individual muscle fibres with long- or short-sarcomeres from the chelae of the yabbies were used to determine if arsenic exposure altered muscle function e.g. Ca^{2+} sensitivity and Ca^{2+}-activated force production. Yabbies were exposed to arsenic at 10 ppm Sodium Arsenite, $AsNaO_2$ (5.7 ppm As(III)) and 10 ppm Arsenic Acid, $Na_2HAsO_4 \cdot 7H_2O$ (2.6 ppm As(V)) for 40-60 days. After exposure either their left or right chela was removed and cut open to expose the muscle fibres. Individual muscle fibres were dissected from the chela and "skinned" (membrane removed) before being attached to the force recording apparatus. Muscle fibres were then placed in solutions of increasing $[Ca^{2+}]$ until a maximum Ca^{2+}-activation was obtained (pCa 4.47). Various parameters (e.g. pCa_{50} the $[Ca^{2+}]$ required to produce 50% of the maximum. Ca^{2+}-activated force) were derived from these activation profiles to determine the effect of arsenic on Ca^{2+} sensitivity and maximum Ca^{2+}-activated force.

Exposure to As(III) produced a small yet significant leftward shift in the Ca^{2+}-activation curve of both long- and short-sarcomere fibres. As(V) exposure however, caused a more substantial leftward shift in the Ca^{2+}-activation curve (by 0.64 pCa units). These results indicate the muscle fibres have become more sensitive to Ca^{2+} after long term exposure to As(V) and As(III). Single fibres from the chela of As(V) exposed animals produced significantly more force (N/cm^2) (65.91 ± 11.66 Long-sarcomere; 25.73 ± 2.607 Short-sarcomere fibres) than muscle fibres from control animals (30.88 ± 3.34 Long-sarcomere; 14.73 ± 2.52 Short-sarcomere fibres).

Histological examination of the muscle fibres from the chelae of the yabby showed no alterations to the appearance of the muscles at a light microscopy level. Therefore it appears that the difference in Ca^{2+} sensitivity seen in the muscle fibres from arsenic exposed animals is a result of alterations at a molecular level. It has been shown that Ca^{2+} sensitivity in muscle fibres can be increased in the presence of amino acids (Powney *et al.*, 2003). Therefore an explanation for this altered sensitivity of the muscle fibres exposed to arsenic could be a change in the activity of the amino acid transporters in the muscle which may alter intracellular amino acid concentrations. Alternatively arsenic exposure may affect the binding affinity of troponin C (the Ca^{2+} binding subunit) to Ca^{2+} thus altering the sensitivity of the muscle fibres to Ca^{2+}. Thus, long-term exposure of the animals to arsenic alters the activation profiles of these two major fibre types in the chelae.

Powney EL, West JM, Stephenson DG & Dooley PC (2003) *Journal of Muscle Research & Cell Motility* **24:** 461-469.

Williams G, West JM & Snow ET (2008) *Environmental Toxicology & Chemistry* **27:** 1332-1342.

Development of the diaphragm and the effects of maternal creatine supplementation on birth hypoxia in a novel precocial species, the spiny mouse (*Acomys cahirinus*)

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The diaphragm is the main respiratory muscle that enables us to breathe. It is the only skeletal muscle that must function at the onset of birth. Even though the importance of the diaphragm is well established, little is known about the transition the diaphragm undergoes when the lungs are filled with embryonic fluid to when they are filled with air when breathing is established. Breathing difficulties are common amongst newborns, particularly in cases of pre-mature birth or when development has been compromised by hypoxia. Regardless of the origin of these difficulties, in these conditions the demand on the diaphragm is high, thus it is conceivable that respiratory failure can be due to the diaphragm being unable to meet demands of the respiratory system.

Skeletal muscle fibres are generally categorised as either slow- or fast-twitch, distinguishable by their contractile and activation properties (West & Stephenson, 1993), and expression of unique proteins and ATPase isoforms. In some species (e.g. sheep), the diaphragm is composed of 'hybrid' fibres that express both slow- and fast-twitch ATPase isoforms and exhibit activation properties characteristic of both fibre types. The aim of this study was to examine the characteristics of diaphragm muscle fibres before and after birth as well as the effect of hypoxia in a small precocial animal, the spiny mouse (*Acomys cahirinus*). In addition, maternal creatine supplementation was administered to determine if creatine resulted in an increase survival rate of neonates subjected to birth hypoxia.

Diaphragm muscle was collected from fetal, newborn and adult spiny mice, pinned onto dental wax at an approximate *in vivo* length, and stored in a relaxing solution containing glycerol at -20°C until use. Single fibres were then isolated by dissection, chemically 'skinned' (membrane removed), and Ca^{2+}- and Sr^{2+}-activation profiles obtained. Samples were also snap frozen and stored at -80°C to determine myosin ATPase, $NADH^{+}$ and succinate dehydrogenase activity. For the hypoxia model, neonates were placed under hypoxic conditions for 8 minutes before they were able to take their first breath at birth. Samples were collected 24 hours after birth as described above. Half the pregnant dams were given a 5% creatine diet during their pregnancy.

In the diaphragm of fetal spiny mouse, the Ca^{2+}- and Sr^{2+}-activation profiles were characteristic of a fast-twitch fibre (large difference in sensitivity to Ca^{2+} and Sr^{2+}). However, a transition occurs in the last few days of gestation providing evidence that slow-twitch isoforms are activated. There is an increased sensitivity to Sr^{2+}. (pCa_{10}-pSr_{10} value decreasing by 0.28 units). ATPase and MHC expression also showed a similar transition at this time with the number of Type I (slow-twitch) fibres doubling (6% to 12%), and the presence of both I and IIa MHC isoforms (oxidative isoforms). Thus considerable re-modelling of the diaphragm occurs just before birth. This suggests that the metabolic characteristics of the diaphragm shift to become more 'oxidative/fatigue resistant'. In male animals, the effect of hypoxia decreased the survival rate (20%), however the survival rate increased with creatine supplementation (80%). In these conditions creatine may provide extra structural support to the diaphragm. Cross-sectional area (CSA) of diaphragm fibres in newborns decreased under hypoxic conditions ($510\mu m^2 \pm 28.6$), unless the mother was supplemented with creatine, to which CSA was normal ($950\mu m^2 \pm 30.4$). The survival rate of female animals that underwent hypoxia, remained relatively high (80%). Creatine supplementation did not increase the survival rate (80%) of the female newborns.

West JM, Stephenson DG (1993) *Journal of Physiology*, **462**: 579-596

Unique actions of junctin and triadin on skeletal muscle ryanodine receptor calcium release channels

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Excitation contraction (EC) coupling is the process which initiates skeletal muscle contraction. Depolarisation of the sarcolemma triggers Ca^{2+} release through ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum (SR) of skeletal muscle. The Ca^{2+} binding protein calsequestrin (CSQ1) interacts with the skeletal muscle type 1 ryanodine receptor (RyR1) in the lumen of the SR and strongly inhibits RyR1 channel activity. The membrane associated proteins triadin and junctin are thought to mediate a functional interaction between CSQ1 and the RyR1 which conserves Ca^{2+} stores in the lumen of the sarcoplasmic reticulum (SR). The individual actions of triadin and junctin on RyR1 and their contribution to CSQ1 interactions with RyR1 are reported here. Our aims were to determine: 1) the influence of triadin and junctin (either individually or together) on purified RyR1 activity and 2) the ability of the anchoring proteins (either individually or together) to mediate the inhibitory effect of CSQ1 on RyR1 and to prevent an increase in RyR1 activity when the concentration of Ca^{2+} in the SR falls.

New Zealand male white rabbits were euthanized by a captive bolt and back and leg muscle used to prepare skeletal muscle proteins. We obtained highly purified CSQ1, triadin, junctin and RyR1 from skeletal muscle using a combination of skeletal SR protein solubilization, gradient centrifugation and SDS and native preparative gel electrophoresis. RyR channel activity was measured in lipid bilayers. Purified RyR1 channels were reconstituted into artificial planar lipid bilayers which separate two chambers which are equivalent to the cytoplasmic and SR luminal compartments of the fibres and we examined the actions of purified triadin, junctin and CSQ on channel activity.

Addition of either triadin or junctin to the luminal side of RyR1 channels embedded in lipid bilayers increased channel open probability by increasing channel open time and stabilised channel openings to the maximum conductance. Junctin/triadin competition studies showed an additive effect of adding these proteins to the RyR, indicating that triadin and junctin interacted with independent sites on RyR1, confirming previous observations from our laboratory. Purified CSQ1 inhibited the reconstituted triadin/junctin/RyR1 complex without reducing the number of maximum conductance openings. In addition the CSQ1/triadin/junctin/RyR1 complex responded to low luminal $[Ca^{2+}]$ in the same way as native RyR1 channels. Therefore, triadin and junctin alone are sufficient to transmit the inhibitory effect of CSQ1 to RyR1 channels and other associated proteins in the native SR are not essential for this action. CSQ1 inhibited RyR1 channels associated with junctin alone, but not RyR1 channels associated only with triadin. In addition the complex between purified CSQ1, junctin and RyR1 supported the inhibition seen when native RyR1 channels are briefly exposed to low luminal Ca^{2+} which may occur during repetitive activity. The CSQ1/triadin/RyR1 complex did not support channel inhibition indicating that triadin is not essential for communication between CSQ1 and the RyR1.

These novel results show that, contrary to expectation, junctin alone is responsible for mediating signals between CSQ1 and RyR1. The rapid Ca^{2+} efflux *in vivo* may be reinforced by the actions of triadin and junctin in stabilizing RyR1 channel gating to enhance channel function. The activation achieved by triadin and junctin binding to RyR1 is negated by the antagonistic effect of CSQ1 on junctin activation. Therefore junctin underlies the role of CSQ1 as a key regulator of the amount of Ca^{2+} released from the SR and is thus an essential component of a tightly interacting machine that *in vivo* ensures functional EC coupling and SR Ca^{2+} release.

The force-generating attachment of myosin heads (cross-bridges) to the actin filaments is controlled differently in fast- and slow-muscle fibre types

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The dynamic functional diversity of skeletal muscle fibres is generally thought to be predominantly related to the molecular species of the myosin heavy chain (MHC) isoform present, which determines the speed of myosin head (cross-bridge) pulling cycles. The molecular mechanisms of force activation and generation are believed to be essentially the same in different muscle fibre types. Thus, the binding of Ca^{2+} released from the sarcoplasmic reticulum to troponin C is thought to cause a lateral shift of the inhibitory troponin complex and increase mobility of tropomyosin (TM) molecules on the surface of the actin filaments between positions that either block or expose myosin head attachment sites on actin. Strong attachment of myosin heads to exposed binding sites on the actin filament stabilises the TM in an 'open' position, uncovering more sites for cross-bridge formation. Here we show evidence of qualitative differences between the mechanisms of myosin head attachment in slow and fast vertebrate skeletal muscle fibres, where the term "attachment" refers to the binding of the myosin head to the actin filament with subsequent force generation. This is contrary to the belief that dynamic contractile differences between fibre types relate mainly to differences in the speed of cross-bridge pulling cycles.

In this study, force-generating attachments of myosin heads were investigated by applying small perturbations of myosin head pulling cycles in stepwise stretch experiments on segments of mechanically skinned single skeletal muscle fibres of different type activated to different levels. Fibres were obtained from freshly dissected muscles from the female clawed frog *Xenopus laevis* (105-230 g) and the rat (Fisher 344 strain, 3-6 months old) after the animals were killed in accordance with animal ethics procedures approved at the University of Salzburg, where the experiments were conducted. The frogs were cooled to 0-2°C and killed by decapitation and double pithing and the rats were anaesthetized with sodium pentobarbitone and killed by bleeding. Single fibres were mechanically skinned, attached to a force transducer and activated in solutions of different [Ca^{2+}] and [Sr^{2+}] as previously described (Andruchova *et al.*, 2006; Bortolotto *et al.*, 2000). After reaching a stable activation level, fibres were subjected to small stepwise stretches to induce a small perturbation in the interaction of myosin heads with actin. The rapid stretches result in characteristic force transients which include a simultaneous rise in force with the stretch (phase 1), a rapid decay (phase 2) and a subsequent delayed force rise (phase 3). The delayed force rise (characterized by the time to peak, **t3**) is tightly correlated with the MHC isoform composition of a fibre (see Andruchova *et al.*, 2006) and can be used as a measure of kinetics of myosin head attachment. After the mechanical experiments, the fibres were collected for analysis of the MHC isoform content. This was carried out using a refined SDS-PAGE protocol described earlier (see Andruchova *et al.*, 2006; Bortolotto *et al.*, 2000).

Slow fibres (frog tonic and rat slow-twitch) exhibited only one 'slow-type' of myosin head attachment over the entire activation range (10 – 100% maximum Ca^{2+}-activated force (Tmax)) which was characterized by a gradual decrease in **t3** as the level of activation increased. In contrast, the fast fibres from both frog and rat displayed two types of myosin head attachment: a 'slow-type' of myosin head attachment at low levels of activation (< 25% Tmax), which was not dissimilar from that observed in the slow fibres, and an up to 30-times faster type at high levels of activation (>75% Tmax). Importantly, at intermediate levels of activation (25 – 75% Tmax) the force traces for fast fibres typically displayed both the 'slow-type' and the 'fast-type' of stretch-induced delayed force rises.

These results indicate that there are two qualitatively different types of myosin head attachment in fast fibers, but only one type in slow fibres, demonstrating that the dynamic contractile properties of different fibre types differ not only with respect to the speed of myosin-head pulling cycles, but also with respect to molecular mechanisms of myosin head attachment.

Andruchova O, Stephenson GM, Andruchov O, Stephenson DG, Galler S. (2006) *Journal of Physiology*, **574:** 307-7.

Bortolotto SK, Cellini M, Stephenson DG, Stephenson GM. (2000) *American Journal of Physiology* **279:** C1564-77.

Conformational coupling of store-operated Ca^{2+} entry in skeletal muscle

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Store-operated Ca^{2+} entry (SOCE) is a mechanism that allows the entry of extracellular Ca^{2+} upon depletion of the internal stores in order to refill them. This mechanism has been described in excitable and non-excitable cells and the two main molecular players, Stim1 and Orai1, have been identified. In non-excitable cells the response to depletion of internal Ca^{2+} stores involves aggregation of Stim1 on the endoplasmic reticulum (ER) membrane and translocation of the ER towards the plasma membrane to allow Orai1 to conduct the entry of Ca^{2+} in a process taking tens of seconds (Lewis, 2007). In contrast, the skeletal muscle cell is built for the rapid delivery of Ca^{2+} to the contractile proteins. The cell microarchitecture allows this with the surface membrane invaginating into the cell forming the tubular (t-) system which apposes the sarcoplasmic reticulum (SR) for rapid signalling. In skeletal muscle SOCE has been shown to occur within 1 s of Ca^{2+} release (Launikonis & Rios, 2007) but this should be significantly faster if the molecular agonists are prepositioned for activation.

The Animal Ethics Committee at The University of Queensland approved the use of animals in this study. 7-12 week old C57 mice were killed by asphyxiation and the extensor digitorum longus (EDL) muscles were removed. Intact fibres were exposed to a Na^{+}-based physiological solution containing fluo-5N salt. Fibres were mechanically skinned, trapping the dye in the t-system, and transferred to a chamber containing a K^{+}-based internal solution with 1 mM EGTA (100 nM Ca^{2+}), 1 mM Mg^{2+} and 0.05 mM rhod-2. Release of SR Ca^{2+} was evoked by substitution of the bathing solution with a 'low Mg^{2+}' solution, containing 0.01 mM Mg^{2+} and being nominally free of Ca^{2+}. Cytoplasmic rhod-2 and t-system fluo-5N were continuously imaged on an Olympus FV1000 confocal microscope in xyt mode during Ca^{2+} release. The net change in t-system fluo-5N signal was used as an indicator of SOCE activity (Launikonis & Rios, 2007).

As described previously, low Mg^{2+} solution induced cell-wide SR Ca^{2+} release that was accompanied by an initial uptake of Ca^{2+} by the t-system, followed by a depletion (due to SOCE) and a reuptake of Ca^{2+} as the cytoplasmic Ca^{2+} transient declined and the SR refilled with Ca^{2+} (SOCE deactivation). In some experiments we observed subsequent Ca^{2+} waves. These waves were associated with a lower SR release flux than cell-wide release and allowed observation of the latency between Ca^{2+} release, which had a defined front and the defined onset of SOCE. The line acquisition rate of 2 ms along the y-axis of each image allowed for analysis of Ca^{2+} fluxes at this temporal resolution. Thus SOCE "Coupling delay" following the initiation of SR Ca^{2+} release was determined to be 27 ± 3.6 ms ($n = 6$). This rapid activation of SOCE is too fast to involve diffusible messengers and is consistent with conformational coupling of SOCE in skeletal muscle. A rapid deactivation mechanism in this cell (Friedrich *et al.*, 2008) also supports this conclusion.

Friedrich O, Edwards JN, Murphy RM, Launikonis BS. (2008) *Proceedings of the Australian Physiological Society*, **39:** 19P.

Launikonis BS, Rios E (2007) *Journal of Physiology*, **583:** 81-97.

Lewis RS (2007) *Nature*, **446:** 284-287.

Store-operated Ca^{2+} entry in dystrophic skeletal muscle is not a source for Ca^{2+} overload due to robust deactivation

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Store-operated Ca^{2+} entry (SOCE) has been shown to be fully operational in intact and skinned skeletal muscle fibres. Previous work, mainly on myotubes, suggested that SOCE may become deregulated in dystrophic skeletal muscle and result in Ca^{2+} overload of the cell. The final result of such a process would be activation of proteolytic enzymes, cell necrosis and/or apoptosis. The assumption of studies exploring this is that an increase in SOCE flux or proteins, such as the Ca^{2+} sensing protein Stim1 or the Ca^{2+} channel, Orai1, will lead to Ca^{2+} overload. SOCE is a low conductance Ca^{2+} current feeding into the triad junction. On the other hand, the muscle cell can readily handle SR Ca^{2+} fluxes into the same microdomain that are much larger during excitation-contraction coupling. More importantly and generally less well characterized, a SOCE that deactivates properly following store refilling would prevent Ca^{2+} overload via this pathway regardless of flux rate. Therefore, under the same conditions, we compared the SOCE deactivation properties as well as the Stim1 and Orai1 protein levels in normal and dystrophic muscle.

7-12 weeks old C57 and mdx mice were killed by asphyxiation, according to the guidelines laid down by the Local Animal Care Committee, and the extensor digitorum longus (EDL) muscles were removed. Intact fibres were exposed to a Na^{+}-based physiological solution containing fluo-5N salt and were then mechanically skinned, trapping the dye in the t-system. Cytoplasmic SR Ca^{2+} release waves and tubular Ca^{2+} SOCE fluxes in response to exposure to 'low Mg^{2+}' solutions were monitored with a confocal microscope (Launikonis *et al.*, 2008). From the line averages in successive images, the time course of SOCE activation and deactivation with myoplasmic Ca^{2+} oscillations could be resolved at a much faster temporal resolution of 500 Hz. Western blotting was used to determine Stim1 protein levels in the same fibres used for individual physiological recordings as well as fibres collected specifically for protein detection from wt and mdx mice. Orai1 protein was measured in whole muscle homogenates from wt and mdx mice.

Stim1 and Orai1 protein amounts were 2-3 times higher in dystrophic compared to healthy muscle fibres and homogenates, respectively. Interestingly, during both cell-wide release and Ca^{2+} waves, SOCE activation rates were similar in wt and mdx muscle (see Launikonis *et al.*, previous abstract). Furthermore, upon inactivation of SR Ca^{2+} release, there was always a reuptake of Ca^{2+} by the t-system indicating that SOCE deactivated (Launikonis & Rios, 2007) in both healthy and dystrophic muscle. SOCE deactivation already started to occur when myoplasmic Ca^{2+} levels dropped only by 10 % suggesting an intact switch-off signal for SOCE from the store in both wt and mdx fibres. Also, SOCE deactivation rate depended upon SR Ca^{2+} refilling rate in a sigmoidal manner, indicating that binding of luminal Ca^{2+} to Stim1 effectively decoupled from Orai1 during refilling in both wt and mdx fibres. These results show that overexpression of Stim1 does not alter the SOCE mechanism in dystrophic muscle. Indeed a robust deactivation mechanism continues to exist in dystrophic muscle preventing this pathway to contribute to any Ca^{2+} overload in these cells.

Launikonis BS, Rios E. (2007) *Journal of Physiology*, **583:** 81-97.

Launikonis BS, Edwards JN, von Wegner, F, Friedrich, O. (2008) *Proceedings of the Australian Physiological Society*, **39:** 18P.

Calcium transients in fast-twitch FDB skeletal muscle from old dystrophic mice

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Duchenne muscular dystrophy (DMD) is the second most common fatal genetic disease in humans with an occurrence of 1:3500 live male births. The disease is characterised by a cyclic degeneration and regeneration of the skeletal musculature, there is also a CNS involvement with the boys having an average IQ of 85 and associated cognitive defects. The *mdx* mouse is the most commonly used model of DMD, it has a point mutation in the dystrophin gene and lacks all long forms of the protein dystrophin. By 6 weeks of age the majority of the skeletal musculature in the *mdx* mouse has undergone at least 1 cycle of degeneration and regeneration. The regenerated muscle fibres have centrally located nuclei and the fibres exhibit a range of deformities from simple splits to more complex syncytiums of branching. There are several reports that the dystrophic process is more pronounced in old *mdx* mice, with old muscles being more susceptible to damage by lengthening contractions. Here I examine Ca^{2+} transients in single isolated fast-twitch fibres from FDB muscles from old *mdx* mice 20-24 months of age. The *mdx* mice (C57BL/10ScSn-DMD) have been produced by a backcross in order to provide age matched littermate controls (C57BL/10ScSn) on identical genetic backgrounds. This circumvents deficiencies in studies which use separate colonies as wild-type controls which have been bred separately from the *mdx* mouse for over 20 years and contain an unknown number of additional mutations. Immediately before experimentation, animals were killed with an overdose of halothane. The FDB muscle was dissected out and digested to yield individual fibres. The digestion solution was Krebs solution containing 3 mg/ml collagenase Type I (Sigma) and 1 mg/ml trypsin inhibitor (Sigma), continuously bubbled with 95% O_2–5% CO_2 and maintained at 37°C. After 30 min, the muscle was removed from this solution and rinsed in Krebs solution. Fibres were plated onto a chamber placed on a Nikon inverted microscope attached to a Cairn spectrophotometer to monitor calcium transients using a PMT, during the experiment fibres were videotaped using infrared illumination to monitor their contraction and viability. Fibres were continually superfused with bubbled Krebs. After fibres had attached to the glass coverslip the central area of the fibre was bracketed to minimise any movement artefacts. An intracellular microelectrode was used to ionophoresed the free acid form of fura-2 (molecular probes) to give a final concentration 5-50 μm. Fibres were rejected if they had a RMP more depolarised than -65 mV. Fibres were electrically stimulated using a bipolar concentric electrode positioned near the neuromuscular junction. In some cases after the first Ca^{2+}ratio/freq had been constructed the fibre was immobilised with BDM to stop contractions, the BDM Ca^{2+}ratio/freq curve was essentially the same. Fibres were fatigued by stimulating at 50 Hz, 1 second on 1 second off, for 3 minutes. The resting ratio (Ca^{2+}) was the same in old *mdx* and old littermate controls; 0.46 ± 0.02 (n = 5), 0.45 ± 0.03 (n = 4). Ca^{2+}ratio /freq curves were produced by stimulating fibres in the range 2-100 Hz.

Freq(Hz)	2	5	10	15	20	25	30	50	60	75	100
Mdx ratio n=5	0.59±.03	0.66±.02	0.75±.02	0.88±.04	1.00±.02	1.16±.04	1.20±.02	1.38±.02	1.42±.05	1.44±.04	SAG*
Cont. ratio n=4	0.65±.02	0.68±.01	0.77±.03	0.95±.05	1.02±.02	1.12±.08	1.19±.01	1.37±.01	1.39±.04	1.44±.04	1.49±.02

The table shows ratio/freq curves for rested *mdx* (*mdx* fibres were centrally nucleated but had no splits) and control fibres. There was no statistical difference at any frequency apart form 100 Hz where the plateau of the tetanus peaked (1.62 ± 0.02) and then sagged to a new stable level (1.46 ± 0.03) for the duration of the stimulus. The peak in the *mdx* at 100 Hz was significantly higher than in littermates. When fatigue fibres were stimulated at 100 Hz immediately after fatigue *mdx* tetanus sag was more pronounced, (peak 1.34 ± 0.05 plateau 0.75 ± 0.04 n = 5) *c.f.* littermate control which had no sag (peak 1.21 ± 0.03 n = 4). The resting ratio (Ca^{2+}) during fatigue was the same in *mdx* and control. When deformed split fibres were examined there was a dramatic difference in that they did not survive the fatiguing stimulus and the base line ratio (Ca^{2+}) rose to 0.7 ± 0.12 and the fibres stopped contracting, although ratio (Ca^{2+}) transients were still present on stimulation. It appears that there is a pathology of Ca^{2+} kinetics in old dystrophin deficient *mdx* fast-twitch fibres.

Blocking TNF (using cV1q) reduces the severity of stretch-induced muscle damage in dystrophic, mdx mice

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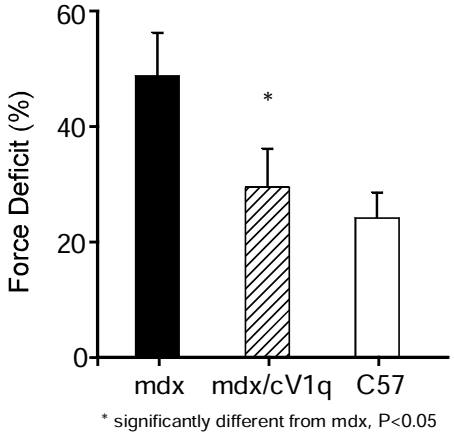
Duchenne muscular dystrophy (DMD) is a disorder characterized by progressive loss of muscle mass and function due to damage, necrosis and incomplete regeneration. The lack of dystrophin in skeletal muscle of DMD patients and in the mdx mouse model of DMD, render these muscles highly susceptible to contraction induced damage leading to an exacerbated inflammatory response which may further increase muscle necrosis. It has been suggested that excessive inflammation in dystrophic muscle, in particular the pro-inflammatory cytokine tumor necrosis factor (TNF), is a major contributor to the dystrophic pathology. This study investigated the effect of *in vivo* blockade of TNF using cV1q, a mouse specific TNF antibody, on the extent of muscle damage and subsequent necrosis following a damaging eccentric exercise protocol.

Experiments were performed using 10-13 week old male mdx and C57Bl/10 (C57) mice. Mice were anaesthetized by inhalation of a gaseous mixture of isoflurane (isoflurane, 0.4 L/min N_20, 0.4 L/min O_2) and the right hindlimb attached to an *in vivo* mouse dynamometer to quantify the contractile properties of the anterior crural muscles and to induce eccentric muscle damage (Ridgley *et al.*, 2008). Anterior crural muscles were activated by stimulation of the common peroneal nerve and the peak torque and optimal joint angle for torque production were determined. A damaging eccentric exercise protocol was performed consisting of 20 lengthening contractions (15°-55° plantar flexion at 1000°s^{-1}). Mice were sacrificed by cervical dislocation 48 h after the dynamometer protocol and the tibialis anterior (TA) muscle excised, snap frozen in isopentane cooled by liquid nitrogen and sectioned on a cryostat for histological analysis. Muscle damage was quantified by the decrease in joint torque immediately after the dynamometer exercise and the area of necrosis was determined from H&E staining. The effect of blocking the pro-inflammatory cytokine TNF on the extent of muscle damage following eccentric exercise was evaluated in mdx mice using the mouse specific anti-TNF antibody cV1q (4.0 mg/ml; Centocor U.S.A.), injected at 1 week and one day before the dynamometer exercise.

The peak isometric torque was significantly lower ($p < 0.05$) in mdx (60.5 ± 5.0 Nm^2/kg) compared to C57 mice (77.5 ± 5.4 Nm^2/kg). The peak torque in cV1q treated mdx mice (67.2 ± 3.3 Nm^2/kg) was not significantly different from mdx. The deficit in peak joint torque induced by the eccentric exercise protocol was two-fold greater in mdx compared to C57 mice. However, blocking TNF significantly reduced this force deficit in cV1q treated mdx mice (see figure). Furthermore, cV1q treatment significantly reduced the amount of necrosis in mdx mice (mdx 13.6 ± 2.4%, mdx/cV1q 5.0 ± 1.8%). There was no detectable necrosis in C57 mice.



The ability of cV1q to reduce the force deficit and subsequent necrosis following exercise-induced muscle damage raises interesting questions about the early events in dystrophic muscle that occur in response to such exercise and the precise mechanism responsible for the adverse effects of TNF on force production. The results further support the proposal that increased TNF contributes to the dystrophic pathology and that blockade of TNF is a potential protective therapy to reduce the severity of DMD.

Ridgley, J.A., Pinniger, G.J., Hamer, P.W. & Grounds, M.D. (in press) *Pflügers Archive – European Journal of Physiology*, DOI 10.1007/s00424-008-0568-4.