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Free communications 11: Skeletal muscle 2

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Chair: Gordon Lynch

The effect of altering the rest period during interval training on adaptations to muscle metabolism, ion regulation and exercise performance

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Exercise training has been shown to reduce the ionic and metabolic disturbance within skeletal muscle during exercise. This may be beneficial to athletes involved in various sporting pursuits, as a greater ionic/metabolic disturbance during a given exercise task is linked with early muscle fatigue. Interval training is a popular training method used by athletes to improve both power and endurance performance. While the adaptations to various interval-training programs have been documented, little research has compared the affects of manipulating the rest period between intervals, on muscle and performance adaptations. The purpose of the present study was to determine the effects of altering the rest period between intense, exercise intervals (during 5 weeks interval training), on adaptations to repeated sprint performance, aerobic fitness and muscle metabolism and ion regulation.

Methods. Twelve, recreationally trained females (mean ± SD: age 20 ± 3 y, mass 62.3 ± 10 kg), participated in this study. Tests consisted of a graded exercise test (GXT) to determine VO_{2peak} and the lactate threshold (LT), followed 48 and 96 h later, by two, high-intensity exercise tests. On these days, subjects performed a constant intensity cycle test (CIT: 45 s at 200% VO_{2peak}). On test day one, 60 s after the CIT, subjects performed a repeated-sprint test (5 × 6 s sprints, 24 s rest between sprints). On day two, subjects had muscle biopsies before and after the CIT and did not perform the repeated-sprint test. Capillary blood was sampled at the end of each stage of the GXT and before and after the CIT and repeated-sprint test to determine blood lactate and hydrogen ion (H^+) concentration. Muscle biopsies (vastus lateralis) were taken to determine muscle ATP, PCr, lactate and H^+ accumulation and muscle buffer capacity. Subjects were randomly assigned to one of two training groups. Group one performed high-intensity interval training, with 1 min rest periods between intervals (HIT-1), while group two performed high-intensity interval training with 3 min rest periods between intervals (HIT-3). Each subject had a matched partner (matched on the LT) in the opposing group, with whom they were required to complete an equal amount of work during each training interval and session (10 × 2 min at 150% LT, 3 $d.wk^{-1}$ × 5 weeks).

Results. There were significant increases in VO_{2peak} (11% HIT-1 *vs* 9% HIT-3; $p<0.05$) and the LT (8% HIT-1 *vs* 15% HIT-3; $p<0.05$) for both groups, with no differences between groups. There were also significant improvements in mean peak power ($W.kg^{-1}$; 9% HIT-1 *vs* 10% HIT-3; $p<0.05$) and total work ($J.kg^{-1}$, 13% HIT-1 *vs* 11% HIT-3; $p<0.05$) completed during the repeated sprint-test for both groups with no differences between groups. There were no significant changes in muscle buffer capacity or immediate post-CIT blood lactate or H^+ following the training period ($p>0.05$). There were significant reductions in the changes to muscle ATP (~30%), PCr (~30%), lactate (~80%) and H^+ (~60%) following training for both groups ($p>0.05$), however, no differences between groups.

Discussion/Conclusions. Similar to others using endurance activity (1-2 h), we have shown that interval training (3 days per week × 5 weeks) can significantly reduce the metabolic disturbance during short-term, high-intensity exercise (45 s). Our results also show that, when intense interval training (~100% of VO_{2peak} intensity), is interspersed with either short (1 min) or longer (3 min) rest periods between each interval, there is little difference in the metabolic and ionic adaptations within the muscle or changes to VO_{2peak}, the LT or repeated-sprint performance. It is likely that the reduced metabolic disturbance during the 45 s of high-intensity exercise, contributed to the improved repeated-sprint performance, as the concentration of PCr and H^+ within muscle has previously been shown to affect repeated-sprint performance. It appears that the workload during each interval may be more important to training adaptations following very high-intensity exercise, than the length of the rest period between efforts. This may have important implications for those wanting to improve exercise performance or health, as a greater training stress during intense intervals (shorter rest periods), did not result in a greater training adaptation.

Long lasting muscle fatigue: partial disruption of EC-coupling by the elevated cytosolic calcium during contractions

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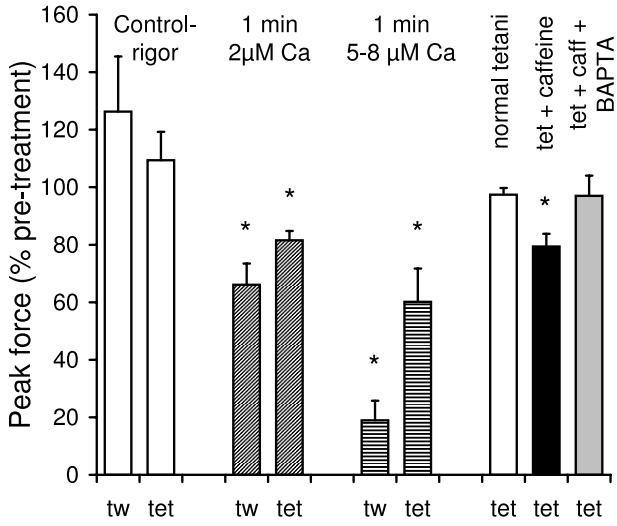
We have previously shown that a 10s period of very high cytosolic [Ca^{2+}] (20μM) can disrupt excitation-contraction (EC)-coupling at the signal transduction between the t-tubuli and the Sarcoplasmic Reticulum (SR) Ca^{2+}-release channels in the triad junction (Lamb *et al.*, 1995). It has also been shown that the repeated periods of elevated cytosolic [Ca^{2+}] during repeated tetani are associated with reduced Ca^{2+}-release and long-lasting fatigue (Chin & Allen, 1996). It is however unclear how low levels of [Ca^{2+}] can disrupt EC-coupling in mammalian muscle, and what aspects of the increased levels of cytosolic [Ca^{2+}] during contractions are causing the disruption of EC-coupling.

In this study we have used a mechanically-skinned fibre preparation, in which the normal EC-coupling system remains intact. Extensor Digitorum Longus muscles were excised from 4-11 month old Long-Evans Hooded rats that had been killed by an overdose of halothane. Single fibres were dissected from the muscle and skinned. The fibre was then transferred to a solution mimicking the cytosol. Twitch and tetanic force responses were triggered by depolarising the T-system with electrical field stimulation. Periods of elevated cytosolic [Ca^{2+}] were induced by transferring the fibre to a 'Ca^{2+}-rigor'-solution containing a set [Ca^{2+}]. In this solution no ATP or CrP was present, preventing Ca^{2+}-uptake by the SR, and thus applying a homogenous [Ca^{2+}] throughout the fibre. Alternatively, elevated [Ca^{2+}] was achieved by eliciting four or five 50Hz-tetani in the presence of 5 mM Caffeine.



The figure shows that even a concentration as low as 2μM free Ca^{2+} throughout the fibre can disrupt EC-coupling, with a bigger effect at concentrations that elicit > 90% of maximal force ($n \geq 4$ in each case). The time of elevated [Ca^{2+}] required for the effect was however quite long, 1 min. Fifteen normal 0.2 s long tetani, or 4-5 tetani with caffeine and 0.2 mM BAPTA present, did not result in a significant decrease in peak tetanic force. However, the total time at high [Ca^{2+}] eliciting > 90% force would have been ≤ 2s in the normal tetani. Only in the presence of caffeine, when tetani were at least twice as long as the normal ones and peak [Ca^{2+}] in the triad junction probably a lot higher, was EC-coupling partially disrupted. This shows that the [Ca^{2+}] has to be raised to a very high level and/or be applied for a relatively long period in order to have a deleterious effect. It also suggests that the relatively high [Ca^{2+}] attained locally in the triad junction is more important than the concentration attained in the bulk of the cytoplasm. During normal contractile activity, it can be expected that calcium-induced disruption of EC-coupling would have a significant impact after a very large number of contractions, and hence it may be one of the mechanisms causing the long-lasting muscle fatigue observed after prolonged hard exercise.

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The role of reactive oxygen species on stretch-induced muscle damage in dystrophic mice

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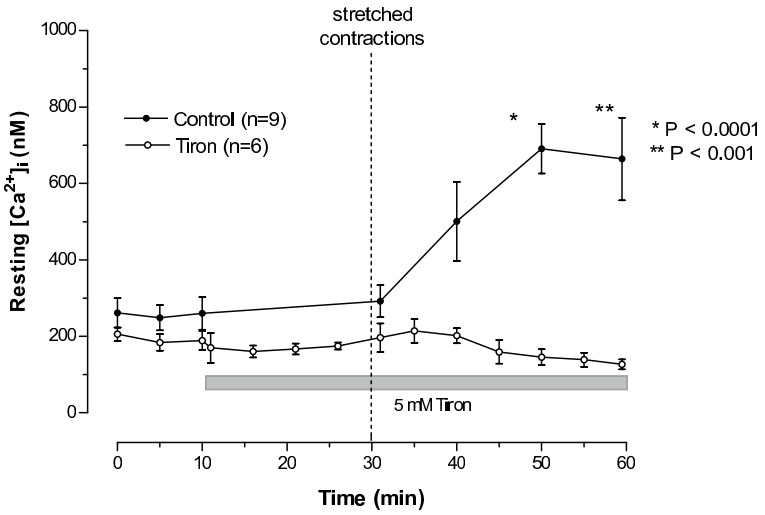
Recently we showed that *mdx* (animal model of Duchenne muscular dystrophy) muscle fibres are more susceptible to stretch-induced muscle damage and there is an associated rise in resting $[Ca^{2+}]_i$ (Yeung *et al*., 2005). We propose that elevated $[Ca^{2+}]_i$ causes reactive oxygen species (ROS) production, leading to muscle damage. Thus treatment with ROS scavenger may exert a protective effect against stretch-induced muscle damage. To test this hypothesis, single fibres isolated from the flexor digitorum brevis of the *mdx* mice were subjected to 10 stretched contractions (eccentric contractions), stretched by 30 % of optimal length (L_o) during each tetanus. Measurements of intracellular calcium with fluo-4 were obtained using confocal microscopy. Calibration of fluo-4 intensities were performed using the procedure described by Kao *et al*. (1989).

The resting $[Ca^{2+}]_i$ in the *mdx* fibres was 227 ± 44 nM (n = 5), significantly higher than that in the wild-type fibres (100 ± 6 nM, n=3, P < 0.05). Under control conditions in the *mdx* muscle, $[Ca^{2+}]_i$ increased slowly following stretched contractions to 690 ± 64 nM (n= 9) after 20 min. The ROS scavenger 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron, 5 mM) was applied during and for 30 min following the stretched contractions in 6 *mdx* fibres. Not only did Tiron prevent the rise in $[Ca^{2+}]_i$ (145 ± 21 nM, P<0.0001) at 20 min, it also improved the force following stretched contractions from 35 ± 4% to 59 ± 7 % (P<0.05).

These results indicate that production of ROS play a role in stretch-induced muscle damage in *mdx* fibres and, further, suggest that ROS may have a role in the activation of stretch-activated channels which produce the Ca^{2+} entry.



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Effects of raising the temperature from 25°C to 37°C on twitch responses in fast-twitch mechanically skinned muscle fibres of the rat

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It has been known for many years that intact mammalian muscle fibre preparations brought to the normal body core temperature (37°C) rapidly and irreversibly deteriorate in their ability to produce force (Lännergren & Westerblad, 1987). Consequently, most experiments on isolated mammalian skeletal muscle are conducted at sub-physiological temperatures. In a previous study (van der Poel & Stephenson, 2004) we showed that as the temperature is brought to 37°C, the rate of mitochondrial production of superoxide ($O_2\cdot^-$), the parent molecule in the reactive oxygen species (ROS) cascade, rises and that a relatively large fraction of the superoxide produced in the mitochondria can be measured extracellularly. In this study we investigated the effect of raising the temperature to 37°C on the twitch responses induced by triggering action potentials in the sealed transverse tubular (t-) system of single fast-twitch mechanically skinned fibres of the rat by electric stimulation (Posterino *et al.*, 2000).

Long-Evans hooded rats were killed by an overdose of halothane in accordance with the procedure approved by La Trobe University Animal Ethics Committee. Extensor digitorum longus (EDL) muscles were dissected out at room temperature, attached to a force transducer and placed in physiological solution at room temperature containing (mM): HEPES, 90; Mg^{2+}, 1; HDTA, 49.95; ATP, 10; CP, 8; Na^+, 36; K^+, 126; Ca^{2+}, $<10^{-6}$; pH 7.1. Fibres were initially equilibrated at 25°C and then transferred to equivalent solutions at 37°C. Twitch force responses at 37°C were obtained by electrically stimulating the fibres every 2 mins with supramaximal square pulses until the fibre failed to produce any force. After 7 min at 37°C, the amplitude of single twitches dropped to only 17.33 ± 10.22% (n = 5) of initial response. To test if this decrease was associated with $O_2\cdot^-$ production, an uncharged, membrane permeable SOD mimetic Tempol (1 mM), which effectively removes $O_2\cdot^-$ without being used as a substrate, was applied. In its presence, Tempol prevented to a large extent this decrease to only 61.31 ± 8.72% (n = 3).

The ability of the contractile apparatus to produce maximum Ca^{2+} activated force was not different between treatments as shown by the similar force responses per cross sectional area obtained at the end of each experiment in maximally Ca^{2+}-activating solutions at 22ºC (ANOVA, P = 0.82). Also the sarcoplasmic reticulum (SR) Ca^{2+} content was not different between treatments as indicated by the similarity of force responses elicited following direct activation of the SR Ca^{2+}-release channels in the presence of low [Mg^{2+}] (0.015 mM) ((ANOVA, P = 0.61) (low [Mg^{2+}] responses at 22°C as % of maximum Ca^{2+}-activated force at 22°C: 91 ± 14% max force for control fibres that were kept only at 22°C (n = 6) *vs* 96 ± 15% max force for fibres that became unresponsive to electrical stimulation at 37°C (n = 6)). Separate experiments indicated that neither the SR Ca^{2+} handling properties nor the sensitivity to Ca^{2+} of the contractile apparatus were affected by exposure to 40°C for up to 10 min. Thus, the results imply that $O_2\cdot^-$ production inside mitochondria at 37ºC is associated with the depression in Excitation-Contraction coupling at a step preceding the SR involvement.

The most likely interpretation of our results is that the intracellular $O_2\cdot^-$ production in the mitochondria decreases the excitability of the t-system in muscle fibres, thus explaining the deterioration in ability of the intact muscle fibre preparations to produce force at 37°C. The results also show that the use of Tempol, a membrane permeant $O_2\cdot^-$ dismutase (SOD) mimetic can markedly prevent muscle function deterioration at 37°C.

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Exposure of mammalian skeletal muscle to sub-physiological temperatures reduces its ability to function at physiological temperatures

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Most studies using isolated mammalian skeletal muscle preparations are conducted at temperatures well below physiological temperatures (17-25°C) because the performance of isolated mammalian skeletal muscle preparations dramatically and irreversibly drops when preparations are re-exposed to normal body core temperatures around 37°C (Lännergren & Westerblad 1987; Ranatunga 1998; Coupland & Ranatunga 2003). This loss in force may be the result of re-heating the preparation during experimental procedures.

In order to test the hypothesis that re-heating isolated skeletal muscle fibre preparations to physiological temperature causes damage to the muscle, rat EDL fibre bundles were excised (30-50 fibres) either at 22°C or in a temperature-controlled room at 37°C from Long Evans (Hooded) rats killed by halothane overdose in accordance with the LTU Animal Ethics Committee. The muscles were then attached to a force transducer, stretched to optimum length and tetanically stimulated every 10 min until force could not be measured whilst immersed in a Krebs-Ringer solution (KRS) maintained at 37°C. KRS contained (mM); NaCl 122, KCl 2.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and D-glucose 5, (constantly bubbled with carbogen: 95% oxygen, 5% carbon dioxide).

The results show that after 30 min of exposure to solution maintained at 37°C, tetanic force dropped dramatically to 3.4 ± 0.1% of initial tetanic force in muscle preparations that were dissected at 22°C and then re-heated, whereas after the same length of time, tetanic force dropped to only 68.0 ± 7.8% of initial tetanic force in muscle preparations dissected and kept throughout at 37°C. This marked decrease in tetanic force appears to be associated with an increase in free radical $O_2^{\bullet -}$ production when preparations are re-heated. These results show that preventing isolated mammalian skeletal muscle from dropping below core body temperature during dissection helps maintain its function when working at 37°C.

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