Effects of high-intensity intermittent exercise on the contractile properties of human type I and type II skeletal muscle fibers

Effects of high-intensity intermittent exercise on the contractile properties of human type I and type II skeletal muscle fibers

C.R. Lamboley [1,2], D.M. Rouffet [1,3], T.L. Dutka [2], M.J. McKenna [1] & G.D. Lamb [2]

[1] *Institute for Health and Sport, Victoria University, PO Box 14428, Melbourne, VIC, 8001, Australia*

[2] *School of Life Sciences, La Trobe University, Melbourne 3086, Victoria, Australia*

[3] *Department of Health and Sport Sciences, Kentucky Spinal Cord Injury Research Center, University of Louisville, Louisville, KY, USA*

Running Head: Repeated sprints modulate muscle contractile properties

Correspondence: Dr. Cedric R. Lamboley
School of Biomedical Sciences, University of Queensland, St Lucia, Queensland, 4072, Australia
e-mail: c.lamboley@uq.edu.au

Keywords: High-intensity intermittent exercise, reactive oxygen species, troponin I, Ca^{2+}-sensitivity, contractile apparatus, fatigue

Abstract

In vitro studies have shown that alterations in redox state can cause a range of opposing effects on the properties of the contractile apparatus in skeletal muscle fibers. To test whether and how redox changes occurring *in vivo* affect the contractile properties, *vastus lateralis* muscle fibers from seven healthy young adults were examined at rest (PRE) and following (POST) high-intensity intermittent cycling exercise. Individual mechanically-skinned muscle fibers were exposed to heavily buffered solutions at progressively higher free [Ca^{2+}] to determine their force-Ca^{2+} relationship. Following acute exercise, Ca^{2+} sensitivity was significantly decreased in type I fibers (by 0.06 pCa unit) but not in type II fibers (0.01 pCa unit). Specific force decreased after the exercise in type II fibers (-18%), but was unchanged in type I fibers. Treatment with the reducing agent dithiothreitol (DTT) caused a small decrease in Ca^{2+}-sensitivity in type II fibers at PRE (by ~0.014 pCa units) and a significantly larger decrease at POST (~0.035 pCa units), indicating that the exercise had increased S-glutathionylation of fast troponin I. DTT treatment also increased specific force (by ~4%) but only at POST. In contrast, DTT treatment had no effect on either parameter in type I fibers at either PRE or POST. In type I fibers, the decreased Ca^{2+}-sensitivity was not due to reversible oxidative changes and may have contributed to a decrease in power production during vigorous exercises. In type II fibers, exercise-induced redox changes help counter the decline in Ca^{2+}-sensitivity while causing a small decline in maximum force.

New and Noteworthy

This study identified important cellular changes occurring in human skeletal muscle fibers following high-intensity intermittent exercise: (i) a decrease in contractile apparatus Ca^{2+} sensitivity in type I but not type II fibers, (ii) a decrease in specific force only in type II muscle fibers, and (iii) a redox-dependent increase in Ca^{2+} sensitivity occurring only in type II fibers, which would help maintain muscle performance by countering the normal metabolite-induced decline in Ca^{2+} sensitivity.

Introduction

Repeated or intense activity of skeletal muscle leads acutely to decreased muscle performance, referred to as muscle fatigue, owing to decreases in the Ca^{2+}-sensitivity and maximum force production of the contractile apparatus, and/or to decreases in Ca^{2+} release from the sarcoplasmic reticulum (SR) (see (1) for review). These changes stem primarily from direct deleterious effects of the altered intracellular conditions, in particular, increased inorganic phosphate and free Mg^{2+} concentrations and decreased pH, ATP and glycogen levels (1). In addition, exercise might acutely modify the underlying properties of the contractile apparatus or SR Ca^{2+} release process, either in a negative or positive way, by altering their redox or phosphorylation state or other aspect. These latter types of changes can be studied by 'skinning' muscle fibers and examining the fiber properties under standardized intracellular conditions, thereby removing the strong confounding effects produced by direct actions of the altered intracellular conditions in fatigue.

Many types of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during muscle contractions (8, 11, 23, 24, 28, 40, 42-44). The range of possible effects of redox alterations on the contractile apparatus is extremely diverse, and the effects can be reversible or irreversible. *In vitro* studies in rested muscle fibers from rodents and humans have shown that application of particular ROS (e.g. H_2O_2) can either reversibly increase or decrease the Ca^{2+}-sensitivity of the contractile apparatus in type II (fast-twitch, FT) fibers depending on the duration of application, with little effect on maximum Ca^{2+}-activated force, whereas nitric oxide (NO) seemingly only decreases the Ca^{2+}-sensitivity (2-4, 14, 15, 26, 35, 36, 48). The increases and decreases in contractile Ca^{2+}-sensitivity appear to be mainly caused by S-glutathionylation and S-nitrosylation, respectively, of a specific cysteine residue in the FT isoform of troponin I (TnI_f) (14, 35). With longer and stronger exposures, ROS and RNS however can also cause irreversible decreases in both Ca^{2+}-sensitivity and maximum force in both type I and type II fibers, depending on the particular species of ROS or RNS applied, the amount and duration of the exposure, and the activation state of the fiber (7, 10, 14, 26, 36, 41, 49).

Little is known about the acute effects of short-term exercise on the contractile apparatus properties in human muscle, and in particular whether the contractile properties are appreciably modified by any of the many possible redox actions of the ROS and RNS generated during the exercise. Hvid et al (20) examined the contractile properties of chemically skinned muscle fibers from the *vastus lateralis* muscle of highly trained athletes, obtained before or ~12 min or 24 hr

following a 4 hr bout of strenuous cycling. It was found that the mean specific force in both type I and type II fibers was decreased by ~10 to 15% immediately following the exercise, but specific force had recovered to the pre-exercise level following a 24-hour rest period. Ca^{2+}-sensitivity was also significantly decreased immediately after the exercise in type II fibers ($pCa_{50,}$ pCa at 50% maximum force, decreased by 0.07 pCa units), but was unchanged in type I fibers. The study did not specifically examine whether the observed effects were due to reversible redox changes. A later study by the same group (18) examined the contractile properties of chemically skinned fibers from biopsies of the *triceps brachii* muscle of elite cross-country skiers taken before and ~10 min following four maximal bouts of treadmill skiing, each bout lasting ~4 min with 45 min rest in-between. There was no significant change in the mean specific force between pre- and post-exercise in either the type I or type II fibers, but the mean Ca^{2+}-sensitivity was increased (mean pCa_{50} increased ~0.07 pCa units) in both fiber types. A further set of fibers was subjected to strong reducing treatment with DTT before examining the contractile properties, and in these cases the mean pCa_{50} was not significantly different between the fibers obtained pre- versus post-exercise, in either type I or type II fibers. These results appear to indicate that redox effects occurring during the exercise caused an increase in the Ca^{2+}-sensitivity in both the type I and type II fibers of the subjects, which was reversed by the DTT treatment. However, it is possible that the apparent effect of the reducing treatment, particularly in the type I fibers, was actually due to fiber sampling variability, given that the pCa_{50} values with and without DTT treatment were determined in different pools of fibers, and unusually, the Ca^{2+}-sensitivity (pCa_{50}) in the particular pool of type I fibers sampled pre-exercise was relatively low, showing no significant difference from that in the type II fibers. The findings also differ from a recent study in exercising rats, where exercise was found to cause a reversible increase in Ca^{2+}-sensitivity only in type II fibers (54), likely due to S-glutathionylation of TnI_f (14, 35, 53), an effect specific to type II fibers.

To further investigate this, the present study examined whether the contractile properties in type I and type II fibers in young heathy and recreationally active humans were modified by repeated brief bouts of intense cycling exercise, and in particular, whether the fiber properties were modified by redox effects induced by the exercise. Biopsies were obtained from *vastus lateralis* muscle before and immediately after the exercise in each participant, and the contractile properties examined in fibers freshly skinned by microdissection. To avoid possible problems with fiber sampling variability, the properties of each pre- and post-exercise fiber were examined both before and after a strong reducing treatment with DTT. In this way, each fiber acted as its own control, which is a sensitive and accurate way to identify any effects of the reducing

treatment. Furthermore, each fiber was subsequently subjected to a standardized S-glutathionylation treatment, as the Ca^{2+}-sensitivity response to such treatment is an indicator of whether TnI_f had undergone some irreversible oxidative change during the exercise (36). It was found that the exercise elicited a reversible redox-dependent increase in Ca^{2+}-sensitivity only in type II fibres, an increase that would help counter the decrease in Ca^{2+}-sensitivity occurring due to increased metabolite levels in the contracting fibers. The findings highlight an important compensatory redox action occurring in the fast-twitch muscle fibers in exercising humans and other mammals.

Materials and Methods

Participant details and ethical approval

All protocols and procedures were approved by the Human Research Ethics Committee at Victoria University. Informed consent was obtained in writing from all subjects and the studies conformed to the standards set by the Declaration of Helsinki. All the experiments on human skinned fibers were performed on fibers obtained from *vastus lateralis* muscle biopsies from 7 participants, comprising four males and three females (age 27 ± 8 years; height, 173 ± 11 cm; body mass, 77 ± 15 kg; mean ± SD). All participants were healthy and recreationally active but were not involved in regular training.

High-intensity intermittent exercise

Participants visited the laboratory on two occasions, with the two visits being scheduled within 2 to 7 days for all participants. During their first visit, participants completed a Force-Velocity test using the iso-inertial method (45) on a custom-built bike ergometer equipped with instrumented cranks (Axis, Swift Performance Equipment, Australia). The mechanical signals recorded by the cranks were sampled at 100 Hz and processed off-line to calculate average crank power (W), crank torque (N.m) and cadence (rpm) from all the pedal cycles completed by the participants during the force-velocity test. Participants performed a total of 79 ±32 (SD) pedal revolutions during the force-velocity test. For each participant, a power vs. cadence relationship was modelled using a 3rd order polynomial with a fixed y-intercept set at zero (45) using an average of 21 ±5 data points. During their second visit, participants performed a high intensity intermittent cycling exercise protocol on the same custom-built bike ergometer that consisted of a series of 15 s maximal efforts produced every 3 min. Cycling exercises were completed against a constant external resistance that was individually selected so that cadence would plateau between 130 and 150rpm during their first 15-s maximal effort. Between each maximal effort, participants cycled at 80 rpm and 15% of the maximal power predicted at this cadence. Ratings of perceived exertion (RPE) were obtained using the original 6-20 point Borg scale (5). Maximal heart rate (HRmax) was estimated for each participant using the age-predicted equation proposed by Tanaka et al. (51) for healthy adults; i.e. 208 – (0.7 x age), HRmax was 189 ±5 bpm across participants. We continuously recorded heart rate (HR) during the cycling exercise using a Polar FT1 heart rate monitor system (Polar Electro Oy, Kempele, Finland). Both RPE values were recorded immediately after each sprint. The series of maximal efforts was stopped when participants reported an RPE value >17 and HR was >150 bpm (~80% HRmax) immediately after a maximal effort. We computed average values of cadence (rpm) and crank power at the start (first 3 s), end (last 3 s) and over the entire duration of the first and last 15-s maximal efforts

completed by the participants. Crank power was expressed both in $W.kg^{-1}$ as well as in percentage of the maximal fatigue-free power calculated for the corresponding cadence, using results from the Force-Velocity test (17, 45).

Muscle biopsies

One biopsy was taken at pre-exercise (PRE) and a second post-exercise (POST) from all participants. The protocol to collect the muscle biopsy was similar for both conditions. Briefly, after injection of a local anaesthetic (1% lidocaine (Xylocaine, AstraZeneca, Macquarie Park NSW, Australia)) into the skin and fascia, a small incision was made in the middle third of the *vastus lateralis* muscle of each subject and a muscle sample taken using a Bergström biopsy needle (34). The PRE and POST muscle biopsies were taken from the *vastus lateralis* of the same leg, with separate incisions ~1 cm apart and from distal to proximal direction. An experienced medical practitioner took all biopsies at approximately constant depth and general location. The PRE and POST biopsies were obtained approximately 10 min prior to and ~1 min following the exercise, respectively. The excised muscle sample was rapidly blotted on filter paper to remove excess blood and placed in room temperature paraffin oil (Ajax Chemicals, Sydney, Australia) then gradually cooled to ~10°C for 45 min before individual muscle fibers were dissected.

Fiber mounting and force recording

The muscle biopsy was pinned at resting length in a petri dish lined with Sylgard 184 (Dow Corning, Midland, MI) and immersed in paraffin oil (Ajax Chemicals, Sydney, Australia) and kept cool (~10°C) on an icepack. As described previously (12, 27, 29), segments of individual fibers were mechanically skinned using jeweler's forceps and pinned out unstretched under oil, with the diameter being measured at three places along the fiber. Fiber cross-sectional area was calculated assuming an ellipsoidal profile with dimensions corresponding to the largest and smallest diameter measurements. The skinned fiber was then mounted at 120% of resting length on a force transducer (AME801, Horten) with a resonance frequency of >2 kHz before being transferred to a 2-ml Perspex bath containing standard K^+-based solution that broadly mimicked the intracellular milieu (see below). Force responses were recorded using a Bioamp pod and Powerlab 4/20 series hardware (ADInstruments, Sydney, Australia).

Skinned fiber solutions

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless specified otherwise. As described previously (26, 27, 29), the properties of the contractile apparatus were

examined using a mixture of two heavily Ca^{2+}-buffered solutions, namely the relaxing solution and the maximal Ca^{2+}-activating solution. The relaxing solution contained (in mM) 50 EGTA, 90 Hepes, 10.3 total Mg^{2+} (giving 1 mM free), 126 K^+, 36 Na^+, 8 total ATP and 10 creatine phosphate, pH 7.10, pCa (=-$\log_{10}[Ca^{2+}]$) ~9. Maximal Ca^{2+}-activating solution contained (in mM) 50 CaEGTA, 90 Hepes, 8.1 total Mg^{2+} (giving 1 mM free), 126 K^+, 36 Na^+, 8 total ATP and 10 creatine phosphate, pH 7.10 and pCa ~4.7.

The relaxing solution and maximal Ca^{2+}-activating solutions were mixed in appropriate ratios so as to produce a series of solutions with the free [Ca^{2+}] heavily buffered over an intermediate range (pCa 6.7 to 4.7). In addition, a strontium-based solution (at pSr 5.2, pSr = -$\log_{10}$ [Sr^{2+}]) was made by mixing relaxing solution with a maximal Sr-activating solution containing (mM): 40 SrEGTA, 10 EGTA, 90 Hepes, 8.5 Mg^{2+} (giving 1 mM free), 126 K^+, 36 Na^+, 8 ATP, 10 creatine phosphate, pH 7.10 and pSr ~3.7. Where required, 10 mM dithiodithreitol (DTT) was added to relaxing solution from a 1 M stock prepared in distilled water. A 100 mM stock of reduced glutathione (GSH) was made in relaxing solution with pH re-adjusted to 7.10 with KOH, and then diluted 20 fold to give 5mM in the final relaxing solution. A 100 mM stock solution of 2,2'-dithiodipyridine (DTDP) was made in absolute ethanol and diluted 1000-fold in the final relaxing solution to 100 μM. These stock solutions of DTT, GSH and DTDP were all freshly prepared just before the experiment.

Force-Ca^{2+} relationship and analysis

All measurements on skinned fibers were performed at room temperature (~23 ±1°C). The force–Ca^{2+} relationship in each individual muscle fiber was assessed by exposing the skinned fiber segment to a series of solutions with the [Ca^{2+}] strongly buffered at progressively higher levels (at pCa 6.7 to 4.7, the latter eliciting maximum force) and then the fiber was fully relaxed again in the relaxing solution. As described previously (30), this sequence was performed twice for each of the four different conditions: (a) Control, before any treatment, (b) after 10 min exposure to 10 mM DTT, (c) after S-glutathionylation treatment, by 2 min exposure in 100 μM DTDP followed by 2 min exposure in 5 mM GSH, and finally (d) after a further 10 min exposure to DTT. The fiber was washed for 1 min in relaxing solution between the different conditions. This procedure allows verification of the reproducibility of the responses and also assessment of the small "rundown" occurring with repeated activation of the fiber (14, 30). Finally, each fiber was also tentatively assessed as being type I (slow-twitch) or type II (fast-twitch) according to its response to Sr^{2+} activation at pSr 5.2, so as to give a preliminary indication of the fiber type, which was subsequently checked by dot blotting of MHC (see below). Fibers containing the

slow-twitch isoform of troponin C (TnC) give close to the maximum Ca^{2+}-activated force level at pSr 5.2, whereas fibers containing the fast-twitch isoform of TnC produce <5% of maximum force, and fibers with a mixture of the fast and slow isoforms of TnC produce an intermediate level of force (6, 29, 30, 38).

Isometric force responses produced at each [Ca^{2+}] within a given sequence were expressed as a percentage of the corresponding maximum force generated in that same sequence, and analyzed by fitting a Hill curve using GraphPad Prism 6 software, to ascertain values of pCa_{50} (pCa at half-maximum force) and the Hill coefficient (*h*) for each sequence. The maximum force reached during each sequence (at pCa 4.7) was expressed relative to the control level before any treatment in the given fiber, after correcting for the small rundown occurring with each repetition of the sequence.

Fiber typing

Dot blotting was subsequently performed to determine the fiber type of each muscle fiber segment examined, as described previously (9, 31). Briefly, PVDF membrane was activated with 95% ethanol and equilibrated in transfer buffer, 1 µL of each sample was applied to the wet membrane and allowed to dry. The dry membrane was then reactivated with 95% ethanol, equilibrated in transfer buffer, washed in TBST for 5 min, and then placed in blocking buffer for 5 min. The presence of myosin heavy chain (MHC) types IIa, and I were determined by sequential probing of the membrane with antibodies specific to MHC IIa (mouse monoclonal IgG, clone A4.74, Developmental Studies Hybridoma Bank [DSHB], 1 in 200 in 1% BSA/PBST) and MHC I (mouse monoclonal IgM, clone A4.840, DSHB, 1 in 200 in 1% BSA/PBST). Lastly, the membrane was probed for MHC IIx (mouse monoclonal IgM, clone 6H1 DSHB, 1 in 100 in 1% BSA/PBST).

Statistics

Values are presented as mean ±SD (or ±SEM where indicated), with n denoting the number of fibers examined and N the number of participants. Statistical significance ($P < 0.05$) was determined with two-tailed Student's t test with repeated measures unless specified otherwise. Pearson's correlation analyses were performed with GraphPad Prism version 8 (La Jolla, California, USA).

Results

High-intensity intermittent exercise performance

Results from the Force-Velocity test showed that all individual power *vs.* cadence relationships were well described by third order polynomial regressions (r^2=0.940 ±0.016; standard error of the estimate=24.8 W), with participants producing maximal levels of crank power of 12.3 ±3.2 $W.kg^{-1}$ or 963 ±363 W at cadences of 116 ±12 rpm. During the main experimental session, participants completed between three and seven 15-s maximal cycling efforts (see Materials and Methods). HR and RPE measured immediately after the maximal efforts increased between the first and the last efforts (HR: 150 ±14 bpm vs. 164 ±17 bpm, respectively; P<0.05; RPE: 14.6 ±3.5 vs. 19.1 ±1.2; P<0.001). For both the first and last maximal efforts, significant decrease in cadence-specific relative levels of power were seen between the start and the end of the efforts (89.0 ± 2.8% vs. 49.6 ±4.8 %, P<0.05) (Fig. 1). Additionally, participants reached lower cadences at the end of the 15-s maximal efforts during the last effort compared to the first one (144 ±5 rpm vs. 134 ± 6 rpm, respectively; P<0.05), reducing the gap to their optimal cadences (i.e. 116 ±12 rpm). However, we observed a significant reduction in power production at the end of the last maximal effort compared to the first one (6.2 ±0.9 $W.kg^{-1}$ vs. 5.0 ±0.8 $W.kg^{-1}$; P<0.001). Finally, the average cadence-specific relative levels of power calculated over the entire duration of the 15-s maximal effort were lower during the last maximal effort compared to the first one (37.1 ±5.0 % vs. 23.6 ±3.3 %, respectively; P<0.05).

Specific force and contractile properties of fibers

Force responses were measured in a total of 37 skinned muscle fibers prior to exercise (PRE) and 52 muscle fibers following the exercise (POST). Subsequent dot blotting of MHC (see Materials and Methods) showed that the sample of PRE fibers consisted of 19 type I, 16 type II and 2 ‘mixed’ (type I/II) fibers, and the sample of POST fibers consisted of 26 type I, 22 type II and 4 ‘mixed’ fibers. All type II fibers were IIa or IIax, with no pure IIx. Results for the ‘mixed’ fibers are not presented here because the proportions of MHCI and MHCII varied greatly between the different fibers; only results for ‘pure’ type I or type II fibers are presented. The force response of contractile apparatus to the Sr^{2+} solution at pSr 5.2 (see Materials and Methods) was found to be fully in accord with the MHC typing in each fiber, with the TnC isoform evidently being largely or exclusively the slow isoform in all type I fibers and the largely or exclusively the fast isoform in all type II fibers, similar to our previous studies (12, 30).

316 The specific force (i.e. maximum Ca^{2+}-activated force per unit cross-sectional area) and
317 Ca^{2+} sensitivity of the contractile apparatus in each skinned fiber were assessed by activating
318 each fiber in a series of solutions with the free [Ca^{2+}] heavily buffered at progressively higher
319 levels, from < 1nM up to 20 μM (i.e. pCa > 9 to pCa 4.7), as in Fig. 2. Specific force was
320 examined in at least one type I and two type II fibers from each participant both PRE and POST
321 exercise (Fig. 3); note that each of these 7 subjects showed similar decrease in average power
322 output between their first and last maximal cycling efforts (see above). In the type II fibers the
323 specific force was on average ~18% lower at POST compared to PRE (P=0.037) (with similar
324 results seen in fibers of all 7 participants), whereas the specific force in type I fibers was not
325 significantly different before and after exercise (P=0.803) (Fig. 3). On average the cross-
326 sectional area (CSA) of the POST type II fibers was ~16% higher than in the PRE type II fibers
327 (see Table 1), although this difference was not statistically significant owing to the large spread
328 in values between the different individual skinned fibers; in contrast, the average CSA of the
329 type I fibers was very similar POST and PRE.
330
331 The Ca^{2+} sensitivity of the type I fibers was found to be lower at POST relative to PRE
332 (pCa_{50} ~0.06 pCa units lower, P=0.008), whereas in type II fibers the Ca^{2+} sensitivity was not
333 significantly different between POST and PRE (P=0.440) (Fig. 4 and Table 2). The Hill
334 coefficient (*h*) in the type I fibers at POST was on average slightly steeper than at PRE, whereas
335 in type II fibers there was no difference (Table 2). As expected from previous work, before the
336 exercise, type II fibers had a lower Ca^{2+} sensitivity (lower pCa_{50}) and steeper *h* than type I fibers
337 (Table 2).
338

Effects of DTT and S-glutathionylation

In order to examine whether the exercise had affected the contractile properties by some reversible oxidative modification, the properties were tested both before and after a 10 min strong reducing treatment in 10 mM DTT (e.g. Fig. 2). In the PRE fibers, such DTT treatment had no significant effect on maximal force production in either type I or type II fibers (-0.4 ±0.2 % and 0.0 ±0.8 %, respectively). However, in the POST fibers, the reducing treatment increased maximal force production by ~4 % in the type II fibers (P=0.003) but had no significant effect in the type I fibers (P=0.723) (Table 2). Importantly, the DTT treatment also caused a significant decrease in the Ca^{2+} sensitivity in the type II fibers, with the decrease being substantially greater at POST than at PRE (P<0.001) (Fig. 5 and Table 2); similar results were seen in the fibers from all 7 subjects. In contrast, the DTT treatment had no effect on the Ca^{2+}-sensitivity in the type I fibers in either condition (P=0.921).

We have previously shown that treating mammalian type II fibers successively with the sulphydryl-specific oxidant DTDP (100 μM, 5 min) and then reduced glutathione (GSH) (5 mM, 2 min) (e.g. Fig 2), results in S-glutathionylation of the troponin I fast isoform (TnI_f) (14), which induces a large increase in myofibrillar Ca^{2+} sensitivity (14, 30, 35). The increase in Ca^{2+} sensitivity induced by this treatment is seen only in type II fibers and not in type I fibers. In the present study, this S-glutathionylation treatment (applied after the fibers had been subjected to the first DTT reducing treatment, Fig. 2) was found to cause a very similar large increase in Ca^{2+} sensitivity in both the PRE (+0.183 pCa units) and POST type II fibers (+0.179 pCa units) (Table 2), which was fully reversed by treating the fibers with DTT again (e.g. Fig. 2). In contrast, in the type I fibers such S-glutathionylation treatment had very little or no effect in either condition (Table 2) (e.g. Fig. 2).

Finally, i) the size of the decrease in pCa_{50} to DTT treatment, and ii) the size of the increase in pCa_{50} to subsequent S-glutathionylation treatment, in the type II POST fibers, showed no apparent dependence of either parameter upon the length of time that the given fiber had been kept in the cool paraffin oil before being skinned and examined. Furthermore, Pearson's correlation analysis of that data showed no significant relationship of either DTT treatment (r = -0.19, p=0.44, n=18) or S-glutathionylation treatment (r= -0.21, p=0.47, n=15) with time.

Discussion

High-intensity intermittent exercise

Irrespective of the exact number of maximal efforts they completed before reaching the exhaustion endpoint (between 3 and 7), each participant was able to successfully produce repeated high-intensity efforts, as shown by the near-maximal power levels of ~90% elicited at the start of each 15-s maximal effort (45). During each 15-s maximal effort and in all participants, the levels of power markedly dropped to ~50% during the last 3s of the sprints (17). With the resistance kept constant across the maximal efforts, cadence was reduced by ~10rpm at the end of the last maximal effort relative to the first one. The decrease in cadence was accompanied by a >1 W/kg decrease in power production between the first and last maximal efforts, even though the participants operated over a more favorable portion of their power-cadence relationship in terms of power production (closer to their optimal cadences) during their last maximal effort. Ultimately, the level of cadence-specific power was decreased by ~15% at the end of the last maximal effort relative to the first one, evidencing an accumulation of fatigue which was expressed by participants who reported RPE values of ~19 immediately following that last maximal effort. In view of the changes in joint powers reported across the hip, knee and ankle joints after a similar 15-s maximal cycling effort (33), changes in the contractile properties of the vastii muscles likely made a large contribution to the decreases in power induced by our exercise protocol.

Changes in contractile properties with exercise

In order to determine whether the underlying properties of the contractile apparatus were altered by the repetition of maximal cycling efforts, muscle fibers from biopsies obtained just before and immediately after the first and last 15-s maximal efforts, respectively, were skinned by microdissection and examined under set intracellular conditions, in order to remove any direct effects of altered cytoplasmic metabolites on the fiber properties. It was found that the Ca^{2+}-sensitivity in type I fibers post exercise was significantly lower (by ~0.07 pCa units) than in the type I fibers obtained before exercise (Fig. 4), but the specific force was not significantly different (Fig. 3). The reason for the decrease in Ca^{2+}-sensitivity in the type I fibers is unknown; it was evidently not due to reversible oxidative changes as it was not reversed by DTT treatment (see next section). It may have been the result of some structural change or damage in the fibers, or it might simply be the result of sampling issues; Gejl et al (18) in contrast found an increase in Ca^{2+}-sensitivity in type I fibers in trained athletes following repeated high intensity exercise. In contrast to the type I fibers, in the type II fibers here, the Ca^{2+}-sensitivity was not significantly

406 different pre- and post-exercise, but the specific force was ~18% lower following the exercise
407 (Fig.s 3 & 4). Although the latter change outwardly seems a profound reduction, it is likely that
408 the functional effect in the subjects was far less pronounced. Here it needs to be borne in mind
409 that specific force is calculated as the maximum Ca^{2+}-activated force divided by the fiber CSA.
410 In the study here, the CSA of each fiber was measured under paraffin oil with the fiber still in a
411 similar state as it was *in vivo* when the biopsy was taken, with any exercise-generated
412 metabolites still trapped within the fiber. During very intense exercise, there is a very large
413 increase in inorganic phosphate levels within each type II fiber owing to the breakdown of most
414 of the creatine phosphate and ATP present in the cytosol (22), as well as the generation of large
415 amounts of lactate ions (46), which together constitute a large increase in the number of
416 osmotically active particles inside the muscle fiber. This increase causes the osmotically-driven
417 influx of extracellular water, leading to substantial fiber swelling, as seen by the ~10 to 15%
418 increase in intracellular water content in the quadriceps muscle of humans following exhaustive
419 cycling exercise or maximal dynamic knee extensions (46, 47), which only returns to the rested
420 level 20–30 min after the exercise. Such swelling has also been visualized directly in isolated
421 *Xenopus* fibers, where single fiber cross-sectional area was increased ~18% after forty 0.5 s
422 tetani (32). As the amount of creatine phosphate and ATP broken down (and lactate produced)
423 during intense exercise is substantially higher in type II fibers than in type I fibers (22), it is
424 expected that the extent of fiber swelling is substantially greater in the type II fibers. In the
425 present study, the decrease in specific force seen in the type II fibers (Fig. 3) was largely
426 attributable to such fiber swelling, given that the CSA of the type II fibers examined post-
427 exercise was on average ~16% greater than in the pre-exercise fibers (Table1). (Note that the
428 difference in mean CSA values did not reach significance simply because there was large
429 variability in size of the individual fibers, but the specific force difference was significant
430 because the force in each fiber was normalised to its own CSA). Two previous studies that
431 examined specific force in human muscle fibers before and after short term intense exercise
432 (Gejl et al (18), see Introduction, and Place et al. (39)) did not find any significant change in
433 specific force in either type I or type II fibers. However, in both studies the fibers were
434 chemically skinned and kept for a prolonged period before measuring the CSA of each fiber in a
435 standard solution, and so the values did not reflect the actual CSA of the fibers *in vivo* pre- and
436 post-exercise, but did facilitate direct comparison of specific force under standardised conditions.
437 In summary, it seems that even though the specific force in type II fibers *in vivo* declines to a
438 marked extent during very intense exercise, this is largely due to fiber swelling, and the absolute
439 maximum force that each fiber can produce in standard conditions (i.e. in the absence of any
440 effects of raised metabolites levels etc.) is changed comparatively little. The swelling occurring

441 *in vivo* does, however, have small direct deleterious effects on both contractile Ca^{2+}-sensitivity
442 and Ca^{2+} release - see (52), though it is possible that the swelling nevertheless might aid the
443 speed of contraction by reducing internal drag of the contractile elements (16).
444
445 **Redox effects on contractile apparatus**
446 It was evident, nevertheless, that the intense exercise did cause a small oxidation-dependent
447 decrease (~4%) in maximum force production in the type II fibers, which was reversed by the
448 reducing treatment with DTT (Table 2). No such decrease was seen in the type I fibers. This
449 inhibitory effect on maximum force production could have been due to action of one or more of
450 the many ROS and RNS known to be generated during exercise and previously observed to have
451 a depressing effect *in vitro* on maximum force production of the contractile apparatus (or myosin
452 MgATPase rate), including superoxide and H_2O_2 (7, 26, 41), NO (37), and peroxynitrite (13, 49).
453 The lack of effect in the type I fibers was possibly because antioxidant enzyme activity (e.g.
454 superoxide dismutase activity, and total glutathione level) is substantially higher in type I fibers
455 than in type II fibers (e.g. ~ fivefold higher gluthathione content in type I fibers) (19, 21).
456
457 Importantly, the present study further found that the intense exercise caused a reversible
458 redox-dependent increase in the Ca^{2+}-sensitivity of the contractile apparatus, but only in type II
459 fibers (Fig. 5). This effect was evident from the decrease in Ca^{2+}-sensitivity occurring with
460 strong reducing treatment with DTT in every type II fiber. Although the size of the sensitivity
461 shift found in the subjects here was comparatively small, it did produce a substantial (>10-15%)
462 increase in the force elicited at submaximal Ca^{2+} levels (e.g. see force difference before and after
463 DTT in Fig. 2A). It was also evident that there was a very small level of redox-enhancement of
464 Ca^{2+}-sensitivity (~0.01 pCa units) in the type II fibers even before the exercise regime (Fig. 5),
465 which has also been seen previously in type II fibers from non-exercised muscle in both young
466 and old human subjects (30) and rats (54). In marked contrast to the type II fibers, the intense
467 exercise regime used here did not elicit any reversible change in Ca^{2+}-sensitivity in type I fibers
468 (Fig. 5), with the DTT reducing treatment having no effect on the sensitivity either before or
469 after exercise. These results in human fibers are all in close accord with recent findings in rat
470 muscle before and after exercise, where DTT reversed an exercise-dependent increase in Ca^{2+}-
471 sensitivity in type II fibers but had no effect at all on Ca^{2+}-sensitivity in type I fibers either
472 before or after exercise (54).

473 The observed redox-dependent increase in Ca^{2+}-sensitivity in the type II fibers here was
474 most likely due to S-glutathionylation of Cys134 on TnI_f, because i) of all the redox-induced

changes examined to date in muscle fibers *in vitro*, it is one of only two processes seen to induce an increase in Ca^{2+}-sensitivity (26) and the only process to do so exclusively in type II fibers, and ii) such S-glutathionylation of TnI_f is seen to occur with exercise in humans (35) and with *in vivo* stimulation of muscles in rats (53) and mice (25). Interestingly, the increase in Ca^{2+}-sensitivity seen here with exercise was only ~20% of the maximal increase in sensitivity occurring with S-glutathionylation of TnI_f (~0.035 vs 0.18 pCa units, Table 2) (and see (14, 35)), and less than half the size of the increase in sensitivity reported in type II fibers in the study by Gejl et al (18) in trained athletes (~0.08 pCa units) and in a study on rat muscle stimulated *in vivo* (53) (~0.10 pCa units). The reasons for this are not known. It was not the result of some of the level of irreversible oxidation of TnI_f (36) that had occurred during the intense exercise, because direct S-gluathionylation treatment (applied after first reversing any existing S-glutathionylation/S-nitrosylation by DTT treatment (Fig. 2)) induced a similar large increase in Ca^{2+}-sensitivity in type II fibers obtained before or after the exercise (both ~0.18 pCa units, Table 2). It is possible that there was some reversal of the increased sensitivity between the end of the exercise and the time the muscle biopsy was cooled down sufficiently to hinder any such reversal (though note that there was no evidence of any reversal occurring in the later period whilst the muscle preparation was maintained cool - see Results). Certainly, the increase in Ca^{2+}-sensitivity occurring in type II fibers of exercising rats persists to some extent *in vivo* for at least an hour after the exercise, but it is fully reversed within 24 hr (54). An alternative reason why only a relatively small increase in Ca^{2+}-sensitivity was observed here might be because the experiments are reporting the *net* change in sensitivity, and it is possible that another reversible oxidative process occurring during the exercise was *decreasing* the Ca^{2+}-sensitivity. This other oxidative process could have been acting on TnI_f or instead on some entirely different target. Here we note that NO donors produce a reversible decrease in Ca^{2+}-sensitivity of the contractile apparatus (3, 14, 15, 48), due to S-nitrosylation of TnI_f (14), with S-nitrosylation and S-glutathionylation acting competitively on the same cysteine residue. Thus, inhibitory effects of the increased NO levels produced in the skeletal muscle fibers during the exercise (40) may have partially countered the potentiating effect on the Ca^{2+}-sensitivity of S-glutathionylation of TnI_f in the subjects here. In this regard it is interesting to note that the intensively exercising subjects in the present study were healthy and recreationally active but were not involved in regular training, whereas in the study by Gejl et al (18), where a much larger increase in Ca^{2+}-sensitivity was seen, the subjects were highly trained. In view of this, it would be interesting in a future study to use the experimental design employed here to directly compare the extent of the Ca^{2+}-sensitivity increase occurring with different levels of exercise intensity in participants who are sedentary or recreationally active or highly trained, as a greater response in the highly trained

participants could be an important part of the training response, possibly reflecting increased S-glutathionylation or decreased S-nitrosylation in the highly trained participants.

The redox-dependent increase in Ca^{2+}-sensitivity in the type II fibers reported in this study is separate from, and would act in addition to, any Ca^{2+}-sensitivity increase arising from phosphorylation of regulatory myosin light chain (see (50) for review). It seems likely that there would have been myosin light chain phosphorylation in the fibers of the exercising subjects here, though it is unclear whether this would have been still present at the conclusion of the exercise bouts, given that S-glutathionylation of TnI_f, but no myosin light chain phosphorylation, was found at the end of a prolonged bout of intensive *in vivo* muscle stimulation in rats (53).

Conclusion

This study examined the contractile properties of mechanically-skinned skinned muscle fibers freshly obtained from the *vastus lateralis* muscle of healthy young adults before and immediately after they performed an exhausting series of high intensity cycling exercises. The properties of the fibers in each subject were examined on the day of exercise under controlled conditions, with the ATP, phosphate and other intracellular constituents set close to the normal resting levels, so as to identify any changes in fiber properties occurring independently from those due to the accumulation of exercise-related metabolites. The study established that brief bouts of high-intensity cycling exercise in recreationally active humans elicit a substantial decrease in single fiber specific force, attributable largely to fiber swelling, and a reversible redox-dependent increase in Ca^{2+}-sensitivity, but only in type II fibres. This increase in Ca^{2+}-sensitivity would act to help counter the decrease in Ca^{2+}-sensitivity that occurs due to raised metabolite levels in the contracting fibers (principally inorganic phosphate and H^+). The findings give a consistent picture of what is likely an important compensatory redox action occurring in the fast-twitch muscle fibers of exercising humans and other mammals that acts to help minimize reduction in muscle performance in the face of unavoidable biochemical and ionic changes occurring in the fibers.

Acknowledgements

We thank the participants for their time in completing the study, and also thank Maria Cellini and Heidy Latchman for technical assistance. The monoclonal antibodies directed against adult human MHC isoforms (A4.840, A4.74 and 6H1) used in the present study were developed by Dr H. Blau and obtained from the Development Studies Hybridoma Bank, under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, USA.

Grants

This project was supported by the National Health and Medical Research Council of Australia (grant numbers 1051460 & 1085331).

Disclosures

No conflict of interests, financial or otherwise, are declared by the author(s).

Author contributions

Author contributions: C.R.L., D.M.R., M.J.M. and G.D.L. conception and design of research; C.R.L., D.M.R., and T.L.D. performed experiments; C.R.L., D.M.R., T.L.D. and G.D.L. analyzed data; C.R.L., D.M.R., and G.D.L. interpreted results of experiments; C.R.L. and D.M.R. prepared figures; C.R.L., D.M.R., and G.D.L drafted the manuscript; all authors edited and revised the manuscript and approved the final version of the manuscript.

References

1. Allen DG, Lamb GD and Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287–332, 2008.
2. Andrade FH, Reid MB, Allen DG, Westerblad H. Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J Physiol* 509 565-575, 1998.
3. Andrade FH, Reid MB, Allen DG, Westerblad H. Effect of nitric oxide on single skeletal muscle fibres from the mouse. *J Physiol* 509 577-586, 1998.
4. Andrade FH, Reid MB and Westerblad H. Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. *Faseb J* 15: 309-311, 2001.
5. Borg G. Perceived exertion as an indicator of somatic stress. *Scandinavian journal of rehabilitation medicine* 2: 92-98, 1970.
6. Bortolotto SK, Cellini M, Stephenson DG, Stephenson GM. MHC isoform composition and Ca(2+)- or Sr(2+)-activation properties of rat skeletal muscle fibers. *Am J Physiol Cell Physiol* 279: C1564-1577, 2000.
7. Callahan LA, She ZW and Nosek TM. Superoxide, hydroxyl radical, and hydrogen peroxide effects on single-diaphragm fiber contractile apparatus. *J Appl Physiol* 90: 45-54, 2001.
8. Cheng AJ, Yamada T, Rassier DE, Andersson DC, Westerblad H, Lanner JT. Reactive oxygen/nitrogen species and contractile function in skeletal muscle during fatigue and recovery. *J Physiol* 594: 5149-5160, 2016.
9. Christiansen D, MacInnis MJ, Zacharewicz E, Xu H, Frankish BP, Murphy RM. A fast, reliable and sample-sparing method to identify fibre types of single muscle fibres. *Sci Rep* 9: 6473, 2019.
10. Darnley GM, Duke AM, Steele DS, MacFarlane NG. Effects of reactive oxygen species on aspects of excitation-contraction coupling in chemically skinned rabbit diaphragm muscle fibres. *Exp Physiol* 86: 161-168, 2001.
11. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 107: 1198-1205, 1982.
12. Dutka TL, Lamboley CR, McKenna MJ, Murphy RM, Lamb GD. Effects of carnosine on contractile apparatus Ca2+ sensitivity and sarcoplasmic reticulum Ca2+ release in human skeletal muscle fibers. *J Appl Physiol* 112: 728-736, 2012.
13. Dutka TL, Mollica JP and Lamb GD. Differential effects of peroxynitrite on contractile protein properties in fast- and slow-twitch skeletal muscle fibers of rat. *J Appl Physiol* 110: 705-716, 2011.
14. Dutka TL, Mollica JP, Lamboley CR, Weerakkody VC, Greening DW, Posterino GS, Murphy RM, Lamb GD. S-nitrosylation and S-glutathionylation of Cys134 on troponin I have opposing competitive actions on Ca(2+) sensitivity in rat fast-twitch muscle fibers. *Am J Physiol Cell Physiol* 312: C316-C327, 2017.
15. Dutka TL, Mollica JP, Posterino GS, Lamb GD. Modulation of contractile apparatus Ca2+ sensitivity and disruption of excitation-contraction coupling by S-nitrosoglutathione in rat muscle fibres. *J Physiol* 589: 2181-2196, 2011.
16. Fitts RH, Riley DR and Widrick JJ. Functional and structural adaptations of skeletal muscle to microgravity. *J Exp Biol* 204: 3201-3208, 2001.
17. Gardner AS, Martin DT, Jenkins DG, Dyer I, Van Eiden J, Barras M, Martin JC. Velocity-specific fatigue: quantifying fatigue during variable velocity cycling. *Med Sci Sports Exerc* 41: 904-911, 2009.
18. Gejl KD, Hvid LG, Willis SJ, Andersson E, Holmberg HC, Jensen R, Frandsen U, Hansen J, Plomgaard P, Ortenblad N. Repeated high-intensity exercise modulates Ca(2+) sensitivity of human skeletal muscle fibers. *Scandinavian journal of medicine & science in sports* 26: 488-497, 2016.
19. Higuchi M, Cartier LJ, Chen M, Holloszy JO. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. *J Gerontol* 40: 281-286, 1985.
20. Hvid LG, Gejl K, Bech RD, Nygaard T, Jensen K, Frandsen U, Ortenblad N. Transient impairments in single muscle fibre contractile function after prolonged cycling in elite endurance athletes. *Acta Physiol (Oxf)* 208: 265-273, 2013.

609 21. Ji LL, Fu R and Mitchell EW. Glutathione and antioxidant enzymes in skeletal muscle: effects of
610 fiber type and exercise intensity. *J Appl Physiol* 73: 1854-1859, 1992.
611 22. Karatzaferi C, de Haan A, Ferguson RA, van Mechelen W, Sargeant AJ. Phosphocreatine and ATP
612 content in human single muscle fibres before and after maximum dynamic exercise. *Pflugers Archiv* 442:
613 467-474, 2001.
614 23. Kobzik L, Reid MB, Bredt DS, Stamler JS. Nitric oxide in skeletal muscle *Nature* 372: 546-548,
615 1994.
616 24. Kolbeck RC, She ZW, Callahan LA, Nosek TM. Increased superoxide production during fatigue in
617 the perfused rat diaphragm. *American journal of respiratory and critical care medicine* 156: 140-145,
618 1997.
619 25. Kramer PA, Duan J, Gaffrey MJ, Shukla AK, Wang L, Bammler TK, Qian WJ, Marcinek DJ. Fatiguing
620 contractions increase protein S-glutathionylation occupancy in mouse skeletal muscle. *Redox Biol* 17:
621 367-376, 2018.
622 26. Lamb GD and Posterino GS. Effects of oxidation and reduction on contractile function in skeletal
623 muscle fibres of the rat. *J Physiol* 546: 149-163, 2003.
624 27. Lamb GD and Stephenson DG. Measurement of force and calcium release using mechanically
625 skinned fibers from mammalian skeletal muscle. *J Appl Physiol (1985)* 125: 1105-1127, 2018.
626 28. Lamb GD and Westerblad H. Acute effects of reactive oxygen and nitrogen species on the
627 contractile function of skeletal muscle. *J Physiol* 589: 2119-2127, 2011.
628 29. Lamboley CR, Murphy RM, McKenna MJ, Lamb GD. Endogenous and maximal sarcoplasmic
629 reticulum calcium content and calsequestrin expression in Type I and Type II human skeletal muscle
630 fibres. *J Physiol* 591: 6053–6068, 2013.
631 30. Lamboley CR, Wyckelsma VL, Dutka TL, McKenna MJ, Murphy RM, Lamb GD. Contractile
632 properties and sarcoplasmic reticulum calcium content in type I and type II skeletal muscle fibres in
633 active aged humans. *J Physiol* 593: 2499-2514 2015.
634 31. Lamboley CR, Xu H, Dutka TL, Hanson ED, Hayes A, Violet JA, Murphy RM, Lamb GD. Effect of
635 androgen deprivation therapy on the contractile properties of type I and type II skeletal muscle fibres in
636 men with non-metastatic prostate cancer. *Clin Exp Pharmacol Physiol* 45: 146-154, 2018.
637 32. Lannergren J. Volume changes of isolated Xenopus muscle fibres associated with repeated
638 tetanic contractions. *J Physiol* 420: 116P, 1990.
639 33. Martin JC and Brown NA. Joint-specific power production and fatigue during maximal cycling. *J*
640 *Biomech* 42: 474-479, 2009.
641 34. McKenna MJ, Medved I, Goodman CA, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC,
642 Sostaric S, Gong X. N-acetylcysteine attenuates the decline in muscle Na+,K+-pump activity and delays
643 fatigue during prolonged exercise. *J Physiol* 576: 279-288, 2006.
644 35. Mollica JP, Dutka TL, Merry T, Lamboley C, McConell GK, McKenna MJ, Murphy RM, Lamb GD. S-
645 glutathionylation of Troponin I (fast) increases contractile apparatus Ca2+-sensitivity in fast-twitch
646 muscle fibres of rats and humans. *J Physiol* 590: 1443-1463, 2012.
647 36. Murphy RM, Dutka TL and Lamb GD. Hydroxyl radical and glutathione interactions alter calcium
648 sensitivity and maximum force of the contractile apparatus in rat skeletal muscle fibres. *J Physiol* 586:
649 2203-2216, 2008.
650 37. Nogueira L, Figueiredo-Freitas C, Casimiro-Lopes G, Magdesian MH, Assreuy J, Sorenson MM.
651 Myosin is reversibly inhibited by S-nitrosylation. *Biochem J* 424: 221-231, 2009.
652 38. O'Connell B, Stephenson DG, Blazev R, Stephenson GM. Troponin C isoform composition
653 determines differences in Sr(2+)-activation characteristics between rat diaphragm fibers. *Am J Physiol*
654 *Cell Physiol* 287: C79-87, 2004.
655 39. Place N, Ivarsson N, Venckunas T, Neyroud D, Brazaitis M, Cheng AJ, Ochala J, Kamandulis S,
656 Girard S, Volungevicius G, Pauzas H, Mekideche A, Kayser B, Martinez-Redondo V, Ruas JL, Bruton J,
657 Truffert A, Lanner JT, Skurvydas A, Westerblad H. Ryanodine receptor fragmentation and sarcoplasmic
658 reticulum Ca2+ leak after one session of high-intensity interval exercise. *Proc Natl Acad Sci U S A* 112:
659 15492-15497, 2015.
660 40. Powers SK and Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact
661 on muscle force production. *Physiol Rev* 88: 1243-1276, 2008.

662 41. Prochniewicz E, Lowe DA, Spakowicz DJ, Higgins L, O'Conor K, Thompson LV, Ferrington DA,
663 Thomas DD. Functional, structural, and chemical changes in myosin associated with hydrogen peroxide
664 treatment of skeletal muscle fibers. *Am J Physiol Cell Physiol* 294: C613-626, 2008.
665 42. Pye D, Palomero J, Kabayo T, Jackson MJ. Real-time measurement of nitric oxide in single
666 mature mouse skeletal muscle fibres during contractions. *J Physiol* 581: 309-318, 2007.
667 43. Reid MB. Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic Biol Med*
668 44: 169-179, 2008.
669 44. Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L, West MS. Reactive oxygen in skeletal
670 muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol* 73: 1797-1804, 1992.
671 45. Rudsits BL, Hopkins WG, Hautier CA, Rouffet DM. Force-velocity test on a stationary cycle
672 ergometer: methodological recommendations. *J Appl Physiol (1985)* 124: 831-839, 2018.
673 46. Sahlin K, Alvestrand A, Brandt R, Hultman E. Intracellular pH and bicarbonate concentration in
674 human muscle during recovery from exercise. 45: 474-480, 1978.
675 47. Sjogaard G, Adams RP and Saltin B. Water and ion shifts in skeletal muscle of humans with
676 intense dynamic knee extension. *Am J Physiol* 248: R190-196, 1985.
677 48. Spencer T and Posterino GS. Sequential effects of GSNO and H2O2 on the Ca2+ sensitivity of the
678 contractile apparatus of fast- and slow-twitch skeletal muscle fibers from the rat. *Am J Physiol Cell*
679 *Physiol* 296: C1015-1023, 2009.
680 49. Supinski G, Stofan D, Callahan LA, Nethery D, Nosek TM, DiMarco A. Peroxynitrite induces
681 contractile dysfunction and lipid peroxidation in the diaphragm. *J Appl Physiol* 87: 783-791, 1999.
682 50. Sweeney HL, Bowman BF and Stull JT. Myosin light chain phosphorylation in vertebrate striated
683 muscle: regulation and function. *Am J Physiol* 264: C1085-1095, 1993.
684 51. Tanaka H, Monahan KD and Seals DR. Age-predicted maximal heart rate revisited. *Journal of the*
685 *American College of Cardiology* 37: 153-156, 2001.
686 52. Watanabe D, Dutka TL, Lamboley CR, Lamb GD. Skeletal muscle fibre swelling contributes to
687 force depression in rats and humans: a mechanically-skinned fibre study. *J Muscle Res Cell Motil* 40: 343-
688 351, 2019.
689 53. Watanabe D, Kanzaki K, Kuratani M, Matsunaga S, Yanaka N, Wada M. Contribution of impaired
690 myofibril and ryanodine receptor function to prolonged low-frequency force depression after in situ
691 stimulation in rat skeletal muscle. *J Muscle Res Cell Motil* 36: 275-286, 2015.
692 54. Xu H, Ren X, Lamb GD, Murphy RM. Physiological and biochemical characteristics of skeletal
693 muscles in sedentary and active rats. *J Muscle Res Cell Motil* 39: 1-16, 2018.
694

695 Fig. 1. Representative changes in power production and cadence measured during each of the
696 five 15-s maximal efforts performed by one female participant. *A*: Crank power (expressed in
697 $W.kg^{-1}$) increased during the first portion of the maximal bouts before decreasing as participants
698 reached the end of their maximal efforts, while a lower average level of power was measured
699 during the last maximal effort compared to the first one. *B*: Cadence increased to a plateau
700 between the start and the end of each maximal effort while lower plateau cadences were reached
701 at the end of the last maximal effort, with cadence changes contributing to the variations in
702 power production seen on the top panel. *C*: Crank power (expressed as % of the maximal power
703 at the same cadence) decreased during each sprint, while successive sprints led to power
704 decreases at the start of the next sprint.

705

Fig. 2: Effects of DTT and DTDP-GSH exposure on maximal activated force and Ca^{2+}-sensitivity of contractile apparatus in human *vastus lateralis* fibers. Representative force responses in a type II (*A*) and a type I fiber (*B*) (both Post-exercise) elicited by directly activating contractile apparatus with heavily Ca^{2+}-buffered solutions with progressively higher free [Ca^{2+}] (pCa of successive solutions: >9, 6.7, 6.4, 6.22, 6.02, 5.88, 5.75, 5.48, 4.7, then back to >9, marked by ticks under each force trace). Force-pCa staircases elicited twice successively for each of four different conditions: (1) Control, (2) after 10 min exposure to 10 mM DTT, (3) after 2 min exposure to 0.1 mM DTDP followed by 2 min exposure to 5 mM GSH, and again (4) after 10 min exposure to DTT (only one force-pCa staircase shown). Fiber washed in relaxing solution for 1 min between different conditions. Horizontal arrows show force levels produced at pCa 5.88 and pCa 6.02 in type II (A) and type I fiber (B), respectively, in the different conditions. Average Ca^{2+}-sensitivity of contractile apparatus (pCa_{50}) values in conditions 1 to 4 were 5.87, 5.83, 6.02 and 5.81 respectively in type II fiber, and 6.00, 5.99, 5.99 and 5.98 in type I fiber.

Fig. 3. Mean +SEM of specific force in type I and type II fibers from Pre and Post-exercise; specific force assessed by exposing skinned fiber to maximal activation solution. 'n' denotes number of fibers and 'N' the number of participants from which biopsies taken. '*' indicates value significantly different from type I fiber in matching condition; '#' indicates value is significantly different from Pre-exercise in same fiber type (Student's two tailed t test). Mean force and CSA for each case shown in Table 1.

729 Fig. 4. Type I muscle fibers are less sensitive to Ca^{2+} following high-intensity intermittent
730 exercise. Average force-Ca^{2+} relationship in type I (*A*) and type II fibers (*B*) from *vastus*
731 *lateralis* muscle biopsies in PRE and POST exercise. Mean (±SEM) of pCa_{50} (pCa at half
732 maximal force) of best-fit Hill curves for each individual fiber was 6.05 ±0.02 in PRE and 5.98
733 ±0.01 in POST ($P<0.05$) for the type I fibers, and 5.92 ±0.01 in PRE and 5.91 ±0.01 in POST for
734 the type II fibers (not significantly different); corresponding *h* coefficient values respectively
735 were 4.3 ±0.3 and 4.8 ±0.2 ($P<0.05$), and 4.7 ±0.2 and 4.8 ±0.2.
736
737

738 Fig. 5. Reducing treatment induces a larger decrease in Ca^{2+} sensitivity in type II muscle fibers
739 following high-intensity intermittent exercise. Mean (and SEM) of change (Δ) in Ca^{2+}
740 sensitivity (pCa_{50}) value following exposure to DTT (e.g. Fig. 3). 'n' denotes number of fibers
741 and 'N' the number of subjects from which the biopsies were taken. '*' indicates value is
742 significantly different from the type I fiber in the matching condition; '#' indicates that POST
743 value is significantly different from PRE value in same fiber type (Student's two tailed t test).
744
745
746

747 Table 1: Maximum force and diameter before and after exercise

748 Mean (±SEM) of maximum Ca^{2+}-activated force and CSA in single skinned fibers sampled PRE
749 and POST exercise. No significant difference between PRE and POST values in either fiber type.

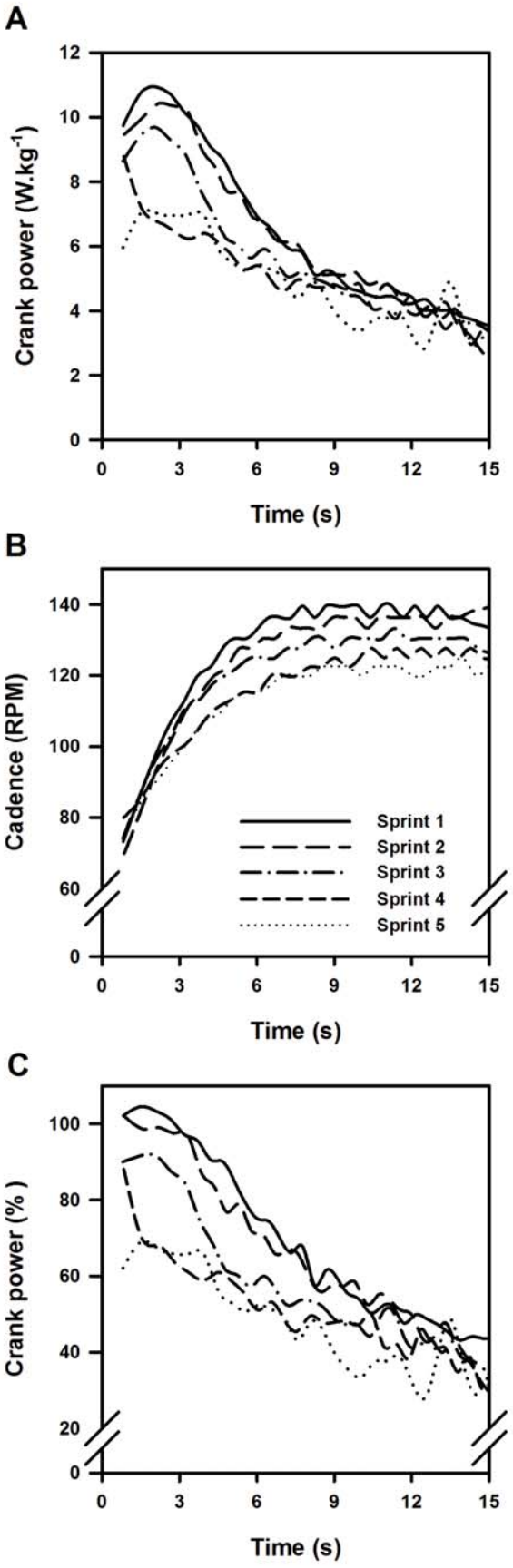
750

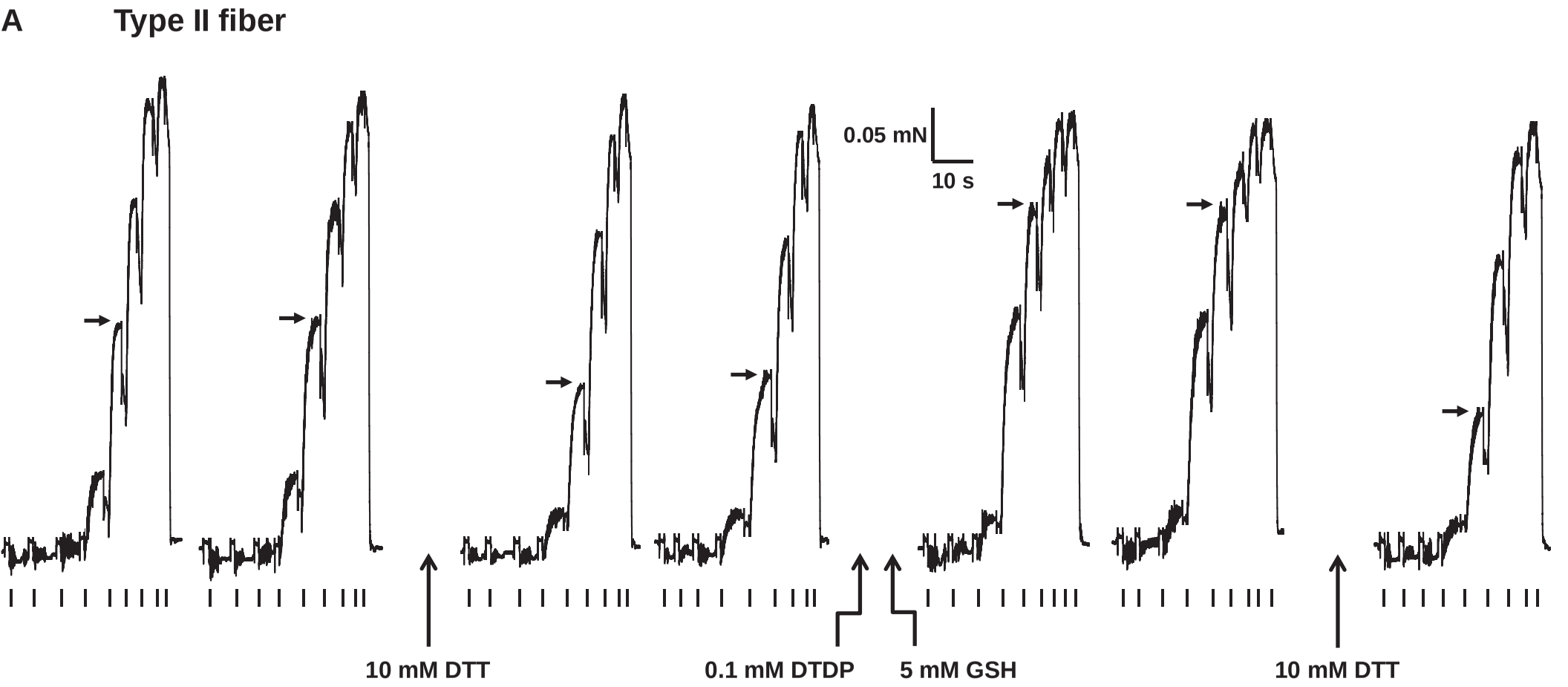
751

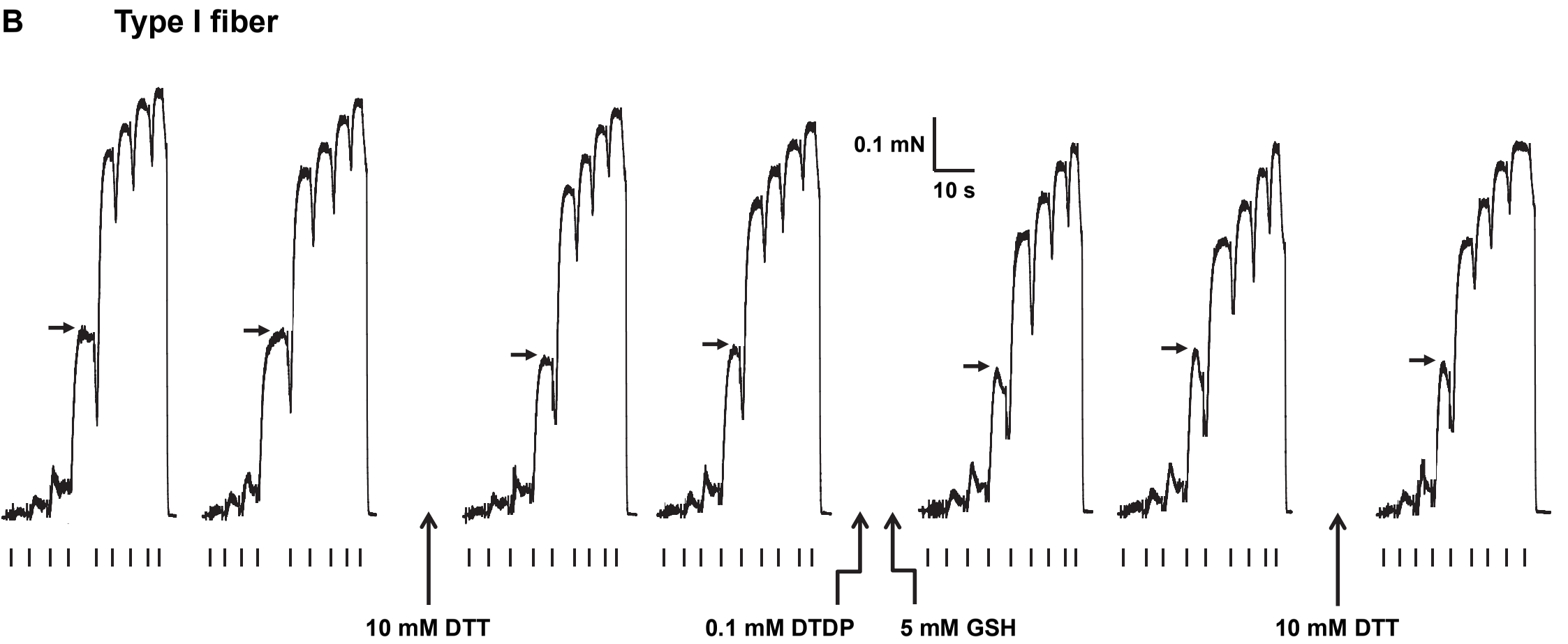
Table 2: Contractile apparatus properties before and after exercise.

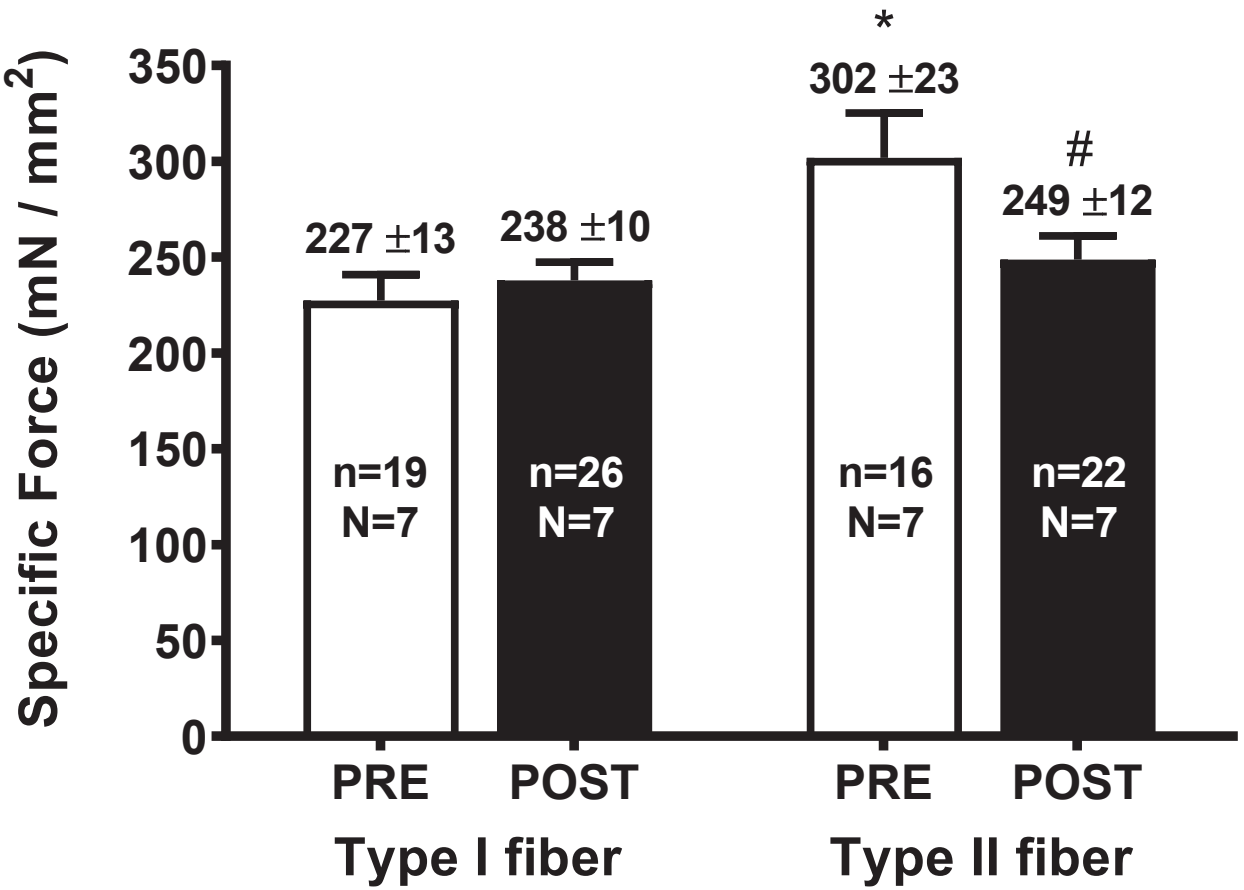
Means ± SEM of pCa_{50}, Hill coefficient (*h*), and change (Δ) in pCa_{50} and maximum force (F_{Max}) following DTT treatment in type I and type II fibers, and change following S-glutathionylation treatment (S-Glut) (as in Fig. 2). Values corrected for small decline in maximum force and pCa_{50} occurring upon repeated examination of force–pCa staircase, as gauged by values obtained by repeating controls and with bracketing treatments with DTT. n denotes number of fibers and N the number of subjects. # Value in POST significantly different from matching value in PRE; ∗ value for type II fibers significantly different from that in type I fibers in matching condition (Student's two-tailed t tests)

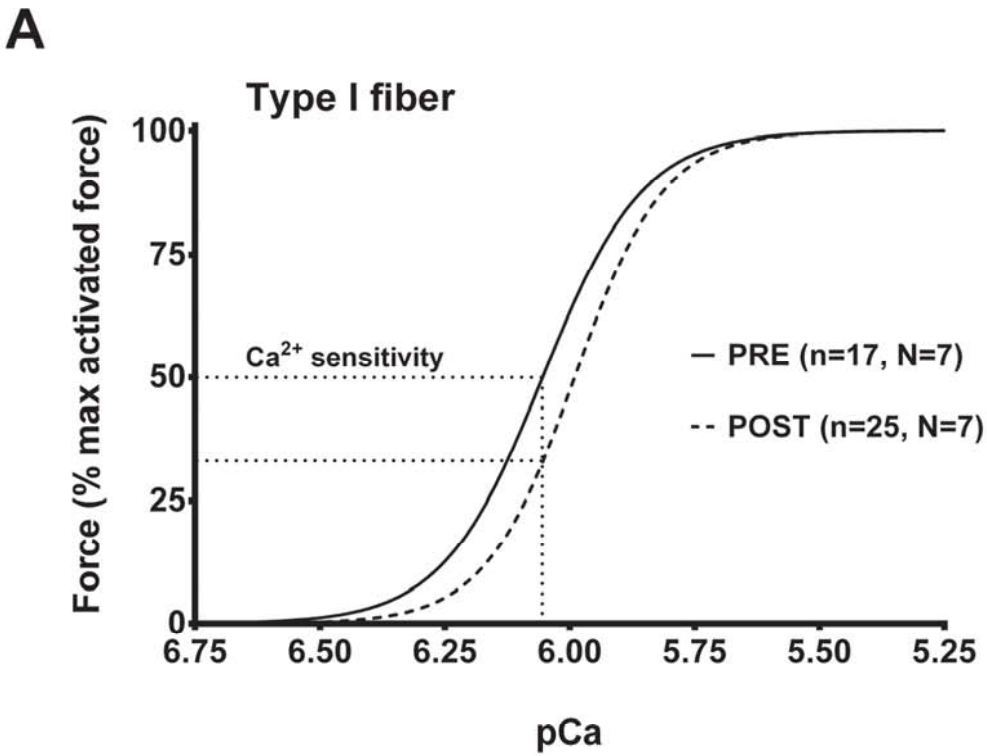
761

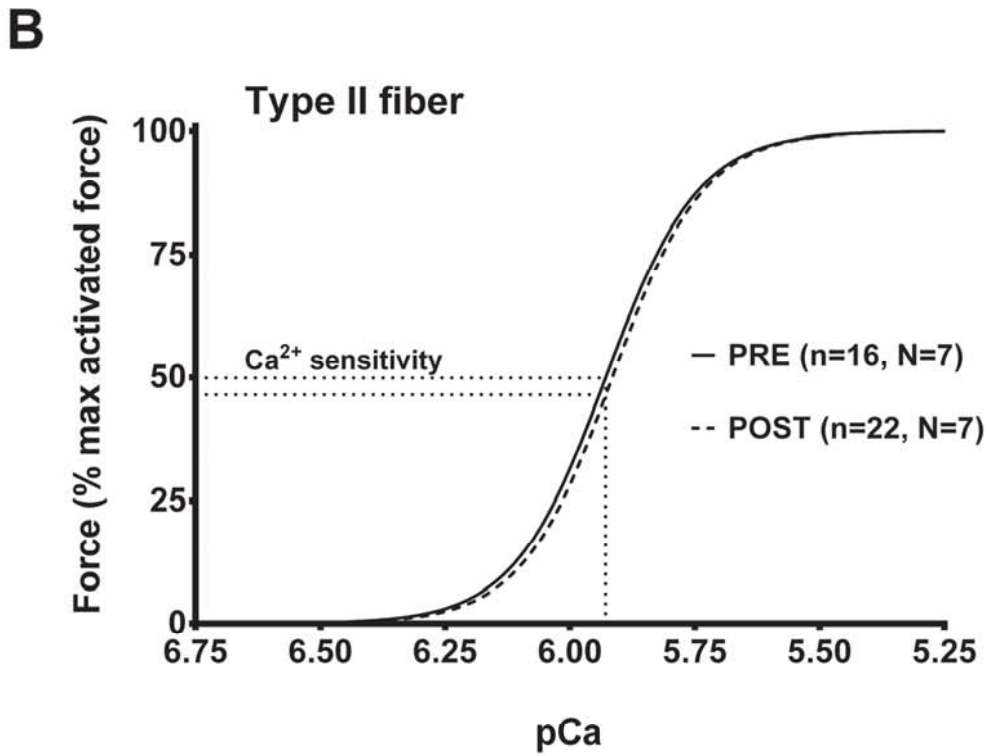

A
Crank power (W.kg-1)
Time (s)
B
Cadence (RPM)
Sprint 1
Sprint 2
Sprint 3
Sprint 4
Sprint 5
Time (s)
C
Crank power (%)
Time (s)


A
Type II fiber
0.05 mN
10 s
10 mM DTT
0.1 mM DTDP
5 mM GSH
10 mM DTT


B
Type I fiber
0.1 mN
10 s
10 mM DTT
0.1 mM DTDP
5 mM GSH
10 mM DTT


Specific Force (mN / mm2)
350
300
250
200
150
100
50
0
227 ±13
238 ±10
*
302 ±23
#
249 ±12
n=19
N=7
n=26
N=7
n=16
N=7
n=22
N=7
PRE
POST
PRE
POST
Type I fiber
Type II fiber


A
Type I fiber
Force (% max activated force)
100
75
50
25
0
Ca2+ sensitivity
— PRE (n=17, N=7)
-- POST (n=25, N=7)
6.75
6.50
6.25
6.00
5.75
5.50
5.25
pCa


B
Type II fiber
Force (% max activated force)
100
75
50
25
0
Ca2+ sensitivity
— PRE (n=16, N=7)
-- POST (n=22, N=7)
6.75
6.50
6.25
6.00
5.75
5.50
5.25
pCa

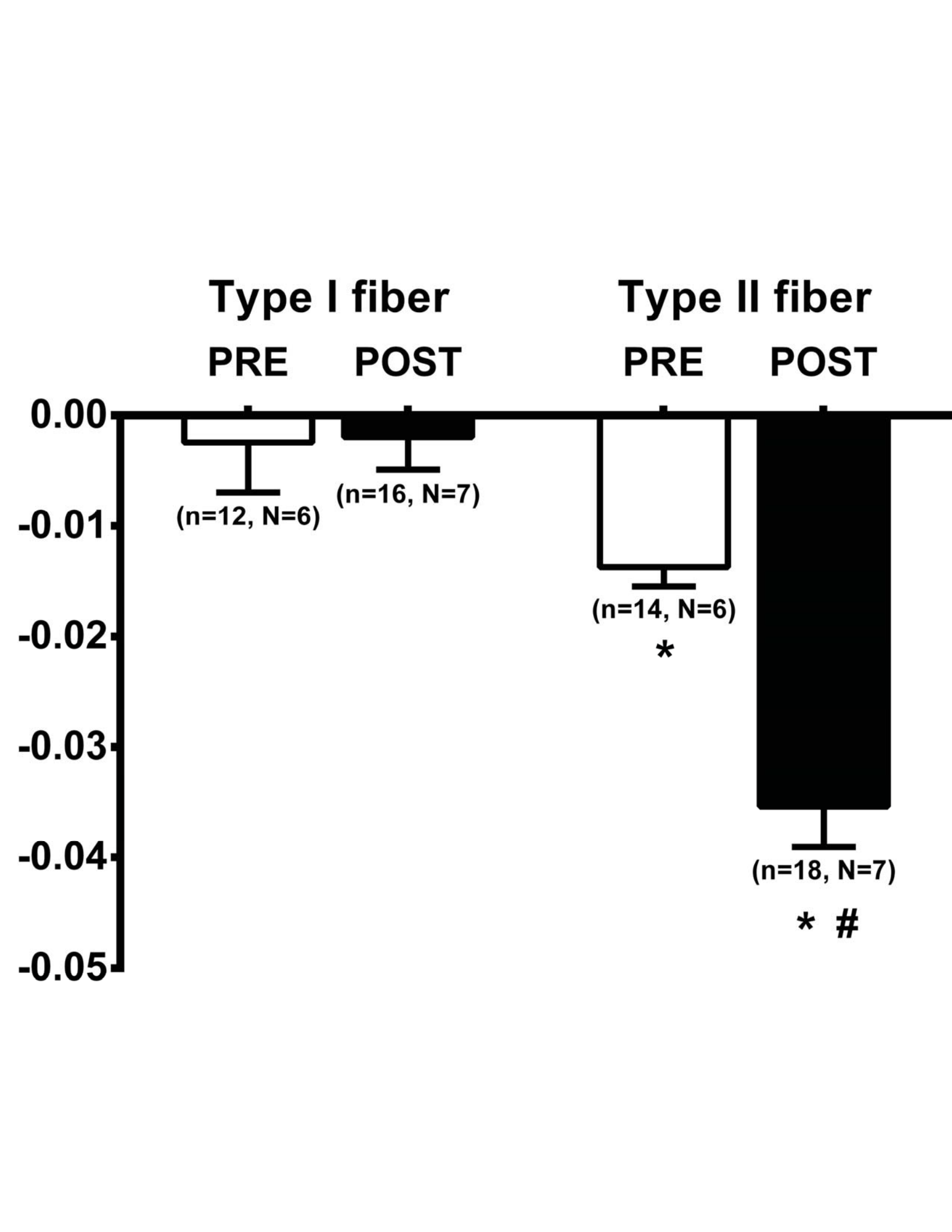

Type I fiber
Type II fiber
PRE
POST
PRE
POST
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
(n=12, N=6)
(n=16, N=7)
(n=14, N=6)
*
(n=18, N=7)
* #

Table 1: Maximum force and diameter before and after exercise

Mean (±SEM) of maximum Ca^{2+}-activated force and CSA in single skinned fibers sampled PRE and POST exercise. No significant difference between PRE and POST values in either fiber type.

	Type I			Type II		
	PRE	**POST**	**% Diff**	**PRE**	**POST**	**% Diff**
CSA (μm^2)	3591 ±283	3607 ±281	+0.5%	4026 ±359	4685 ±520	+16.4%
Force (mN)	0.79 ±0.06	0.83 ±0.06	+6.2%	1.23 ±0.15	1.16 ±0.13	-6.0%
	n = 19	n = 26		n = 16	n = 22	

Table 2: Contractile apparatus properties before and after exercise.

Means ± SEM of pCa_{50}, Hill coefficient (*h*), and change (Δ) in pCa_{50} and maximum force (F_{Max}) following DTT treatment in type I and type II fibers, and change following S-glutathionylation treatment (S-Glut) (as in Fig. 2). Values corrected for small decline in maximum force and pCa_{50} occurring upon repeated examination of force–pCa staircase, as gauged by values obtained by repeating controls and with bracketing treatments with DTT. n denotes number of fibers and N the number of subjects. # Value in POST significantly different from matching value in PRE; ∗ value for type II fibers significantly different from that in type I fibers in matching condition (Student's two-tailed t tests).

Parameter	**Type I fiber**		**Type II fiber**	
	PRE	POST	PRE	POST
	(n = 17, N = 7)	(n = 25, N = 7)	(n = 16, N = 7)	(n = 22, N = 7)
pCa_{50}	6.05 ± 0.02	5.99 ± 0.01 #	5.92 ± 0.01 *	5.91 ± 0.01 *
h	4.3 ± 0.3	4.8 ± 0.2 #	4.7 ± 0.2 *	4.8 ± 0.2
	PRE	POST	PRE	POST
	(n = 12, N = 6)	(n = 16, N = 7)	(n = 14, N = 6)	(n = 18, N = 7)
ΔpCa_{50} DTT	-0.002 ± 0.004	-0.002 ± 0.003	-0.014 ± 0.002 *	-0.035 ± 0.004 * #
ΔF_{Max} DTT (%)	-0.4 ± 0.2	-0.6 ± 0.3	0.0 ± 0.8	4.2 ± 0.9 * #
	PRE	POST	PRE	POST
	(n = 2, N = 2)	(n = 7, N = 6)	(n = 15, N = 7)	(n = 15, N = 6)
ΔpCa_{50} S-Glut	0.000 ± 0.002	0.005 ± 0.001	0.183 ± 0.004 *	0.179 ± 0.004 *