Title: Locomotor muscle fatigue is not critically regulated after prior upper body exercise

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ABSTRACT

This study examined the effects of prior upper body exercise on subsequent high-intensity cycling exercise tolerance and associated changes in neuromuscular function and perceptual responses. Eight males performed 3 fixed work-rate (85% peak power) cycling tests: (1) to the limit of tolerance (CYC); (2) to the limit of tolerance after prior high-intensity arm-cranking exercise (ARM-CYC); (3) without prior exercise and for an equal duration as ARM-CYC (ISOTIME). Peripheral fatigue was assessed via changes in potentiated quadriceps twitch force during supramaximal electrical femoral nerve stimulation. Voluntary activation was assessed using twitch interpolation during maximal voluntary contractions. Cycling time during ARM-CYC and ISOTIME (4.33 ± 1.10 min) was 38% shorter than CYC (7.46 ± 2.79 min) ($P < 0.001$). Twitch force decreased more after CYC (-38 ± 13%) than ARM-CYC (-26 ± 10%) ($P = 0.004$) and ISOTIME (-24 ± 10%) ($P = 0.003$). Voluntary activation was 94 ± 5% at rest and decreased after CYC (89 ± 9%, $P = 0.012$) and ARM-CYC (91 ± 8%, $P = 0.047$). Rating of perceived exertion for limb discomfort increased more quickly during cycling in ARM-CYC (1.83 ± 0.46 AU·min^{-1}) than CYC (1.10 ± 0.38 AU·min^{-1}, $P = 0.003$) and ISOTIME (1.05 ± 0.43 AU·min^{-1}, $P = 0.002$), and this was correlated with the reduced cycling time in ARM-CYC ($r = -0.72$, $P = 0.045$). In conclusion, cycling exercise tolerance after prior upper body exercise is potentially mediated by central fatigue and intolerable levels of sensory perception rather than a critical peripheral fatigue limit.

INTRODUCTION

A consistent reduction (~35%) in the potentiated quadriceps twitch force is observed after high-intensity cycling (4-6, 66, 73, 79). It is proposed that this reduction represents an "individual critical threshold" of peripheral locomotor muscle fatigue beyond which the degree of associated sensory perception would not be tolerable (3). The observation of similar intramuscular metabolic perturbation at the end of exhaustive exercise irrespective of the work-rate (20, 77) supports the notion that it is probably not peripheral fatigue *per se* that is monitored / regulated but the associated fatigue-inducing biochemical changes within the muscle (3). The critical limit of peripheral fatigue observed under "normal" conditions is also unchanged when exercise tolerance is reduced (i.e. the critical limit is reached more quickly) due to moderate hypoxia (F_IO_2 0.13-0.15) (7, 66), superimposed inspiratory muscle loading (67), volitionally-induced inspiratory or expiratory muscle fatigue (73, 80), prior high-intensity cycling exercise (5), and prior electrically-induced quadriceps muscle fatigue (34). Conversely, the degree of peripheral fatigue observed after cycling exercise in severe hypoxia (F_IO_2 0.10) is about two-thirds of that observed in normoxia, suggesting that the major determinant of exercise tolerance switches from a peripheral to central origin, possibly due to brain hypoxia (8). Individual critical limits to peripheral fatigue are thought to be mediated by thin fiber group III/IV muscle afferents (3, 31), which may influence central motor drive, and thereby exercise tolerance, by providing inhibitory feedback to the central nervous system in response to intramuscular metabolic perturbation (1, 10, 25, 51). However, despite growing support for an important role of peripheral fatigue in determining exercise tolerance, this notion has been challenged (53, 54). Marcora (53) has proposed a psychobiological model of endurance exercise tolerance, which primarily attributes exercise intolerance to a conscious decision to stop exercise due to perception of effort, mediated exclusively by feed-forward mechanisms (i.e. corollary discharge), reaching a level that the individual is

unwilling to tolerate. A pivotal role for the rating of perceived exertion (RPE) in limiting exercise tolerance is also depicted in the 'flush model' proposed by Millet (57). However, this model differs from the psychobiological model because it attributes RPE to both feed-forward *and* feedback (i.e. peripheral) mechanisms, thereby also emphasizing the importance of intramuscular metabolic perturbation and peripheral fatigue. The importance of sensory perception in influencing exercise tolerance is also evident in the striking ability of the RPE to predict the tolerable duration of exercise after prior fatiguing exercise (32), and at various exercise intensities (64), muscle glycogen concentrations (59) and ambient temperatures (24). Thus the rate of increase in RPE (ΔRPE/Δtime), and possibly dyspnea (Δdyspnea/Δtime), may be considered major contributors to the attainment of a "critical sensory tolerance limit" (35, 57) and subsequent cessation of exercise.

Several studies have also shed light on the determinants of exercise tolerance by showing reduced lower body exercise tolerance after prior high-intensity upper body exercise (12, 17, 36, 44, 46, 47, 61). This has been attributed to an accelerated development of peripheral locomotor muscle fatigue secondary to faster intramuscular metabolite (i.e. K^+, H^+, and La^-) accumulation resulting from the prior upper body exercise. However, this explanation remains conjecture because peripheral fatigue was not evaluated in these studies. An alternative explanation is that rather than accelerating the development of peripheral fatigue, prior upper body exercise might reduce lower body exercise tolerance by accelerating the attainment of an intolerable level of sensory perception that is mediated, in part, by the ensemble input of group III/IV muscle afferents. Specifically, since group III/IV muscle afferent input may remain elevated for up to 15 min after high-intensity upper body exercise (25, 45), the ensemble group III/IV muscle afferent input would be elevated during subsequent high-intensity lower body exercise. Subsequently, increases in ΔRPE/Δtime and/or Δdyspnea/Δtime may reduce exercise tolerance with *less* lower body peripheral

fatigue incurred. This notion is supported by the observation of increased RPE and reduced exercise tolerance with less peripheral fatigue incurred during single-leg knee extensor exercise preceded by fatiguing knee extensor exercise using the contralateral leg (10).

Therefore, the present study aimed to elucidate the mechanism(s) by which prior high-intensity upper body exercise reduces subsequent leg cycling exercise tolerance. Specifically, we tested the hypothesis that prior upper body exercise reduces subsequent leg cycling exercise tolerance and that this is associated with less peripheral fatigue, but an accelerated rise in ΔRPE/Δtime and Δdyspnea/Δtime .

METHODS

Participants

Eight healthy, non-smoking, moderately trained males (age: 26 ± 4 years; height: 182 ± 4 cm; body mass: 83 ± 4 kg; peak oxygen uptake: 50 ± 10 $mL \cdot kg^{-1} \cdot min^{-1}$) provided written informed consent to participate in the study. Five of the participants had previously taken part in investigations that included assessment of neuromuscular function using the methods described in the present study (38-41). Participants refrained from strenuous exercise and alcohol the day preceding and the day of an exercise test, abstained from caffeine on test days, and reported to the laboratory at least 2 h post-prandial. The study was approved by the Nottingham Trent University Human Ethics Committee, and all procedures were conducted in accordance with the Declaration of Helsinki.

Experimental design

Participants attended the laboratory on five separate occasions, at a similar time of day, separated by at least 48 h. The initial visit comprised a maximal incremental cycling test for the determination of peak oxygen uptake and peak cycling power ($\dot{W}_{peak}$). The second visit comprised familiarization with the knee extensor neuromuscular function assessments

and arm-cranking protocol. The subsequent three visits comprised the experimental trials. The first two experimental trials were performed in a randomized order and comprised a fixed work-rate cycling test at 85% $\dot{W}_{peak}$, and exercise was performed to the limit of tolerance. These two cycling tests were performed without (hereafter termed CYC) and with (hereafter termed ARM-CYC) prior high-intensity arm-cranking exercise. For the third experimental trial, the CYC protocol was repeated except that the cycling test was terminated after an identical duration to that achieved during ARM-CYC (hereafter termed ISOTIME). Knee extensor force and surface electromyographic (EMG) signals were recorded during a series of electrically-evoked and voluntary isometric contractions of the dominant leg to quantify the presence and magnitude of central and peripheral locomotor muscle fatigue. For an illustration of the protocol for the experimental trials please refer to Figure 1.

Neuromuscular Function

Dynamometer Participants were seated in a rigid, custom built dynamometer adapted from Hannah et al. (40), with hip and knee joint angles of 100° and 95° (180° = full extension) respectively. Adjustable strapping across the pelvis and shoulders prevented extraneous movement during muscle activation. A non-compliant strap was attached to the dominant leg of the participant ~2 cm proximal to the medial malleolus and was in series with a linear strain gauge (615, Tedea-Huntleigh, Herzliya, Israel) oriented perpendicular to the tibia. The dynamometer configuration was established during the familiarization session and replicated thereafter. The force signal was amplified (×1000) in the frequency range 0-500 Hz, and sampled at 2000 Hz using an external A/D converter (1401; CED, Cambridge, UK) interfaced with a personal computer using Spike 2 software (CED). Force data were low-pass filtered in both directions at 450 Hz using a fourth-order zero-lag Butterworth filter prior to analysis. Baseline resting force was subtracted from all force recordings to correct for the effect of gravity.

Electromyography EMG signals were recorded from the superficial quadriceps (rectus femoris, vastus medialis, and vastus lateralis) and hamstring (biceps femoris) muscles, as described previously (40). After preparation of the skin by shaving, light abrasion, and cleaning with alcohol, bipolar surface electrodes (2.5 cm inter-electrode distance; silver/silver chloride, 95 mm^2 area, Ambu Blue Sensor; Ambu, Ballerup, Denmark) were attached over each muscle at standardized percentages of thigh length measured from the knee joint space to the greater trochanter (rectus femoris, 55%; vastus medialis, 25%; vastus lateralis and biceps femoris, 45%). These sites were selected to avoid the innervation zones of each muscle (65). Electrodes were positioned parallel to the presumed orientation of the muscle fibers. EMG signals were pre-amplified by active EMG leads (input impedance 100 MΩ, common mode rejection ratio > 100 dB, base gain 500, 1st order high pass filter set to 10 Hz; Noraxon, Scottsdale, USA) connected in series to a custom-built junction box and subsequently to the same A/D converter and computer software that enabled synchronization with the force data. The signals were sampled at 2000 Hz. Prior to analysis EMG data were band-pass filtered in both directions between 20 and 450 Hz using a fourth-order zero-lag Butterworth filter (26, 27, 55).

Electrical stimulation Equipment and procedures for electrical stimulation have been described previously (41). A constant current variable voltage stimulator (DS7AH; Digitimer Ltd, Welwyn Garden City, UK) was used to assess knee extensor contractile properties whilst the participant was voluntarily passive. Square-wave pulses (0.2 ms duration) were delivered via supramaximal femoral nerve stimulation to evoke maximal potentiated twitch and triplet (3 pulses at 300 Hz) contractions (24, 41). Stimulation of the femoral nerve was achieved via a 1 cm diameter cathode stimulation probe (Electro Medical Supplies, Wantage, UK) pressed into the femoral triangle. The surface of the anode, a 4 × 7 cm carbon rubber electrode (Electro Medical Supplies), was coated in electrode gel and located over the greater

trochanter. The precise location of the cathode was determined as the position that evoked the greatest twitch response for a particular submaximal electrical current (typically 30–50 mA), and was marked on the skin using indelible ink to ensure accurate repositioning within each trial.

Procedure Initially discrete electrical stimuli were delivered via percutaneous stimulation of the femoral nerve in the femoral triangle to elicit twitch contractions of the quadriceps. Stepwise increments in the current were delivered, separated by 10 s to allow for neuromuscular recovery, until plateaus were reached in the amplitude of twitch force and compound muscle action potentials (M-waves). The stimulus intensity was then increased by 25% above the value required to elicit a plateau to ensure supramaximal stimulation. Participants subsequently performed sub-maximal warm-up contractions of the knee extensors, lasting ~3 s and interspersed by ~30 s rest, at ~50, 75 and 90% of their perceived maximal force. Thereafter, and following baseline measurements for heart rate and $[La^-]_B$, participants performed four maximum voluntary contractions (MVCs) lasting 3-4 s and interspersed by ~30 s rest. Participants were instructed to extend the knee "as hard and as fast as possible". During and after each contraction participants received strong verbal encouragement. Online feedback of the force signal was provided and a marker showing maximum force during that session was displayed onscreen in order to assist participants in attempting to maintain a high and stable force level. Each MVC was followed within 1-2 s by two supramaximal electrical stimuli, separated by 1 s, delivered to the femoral nerve to elicit maximal potentiated twitches (49). Single electrically-evoked triplet contractions (3 supramaximal stimuli delivered at 300 Hz) were superimposed on the 3rd and 4th MVC, and at rest ~1-2 s after the two potentiated twitch contractions (29, 48). Triplets were used in the calculation of voluntary activation (see below), because the detection of single superimposed twitches becomes increasingly difficult at high forces as a result of the decreasing signal-to-

noise ratio. This may lead to the erroneous conclusion that voluntary activation is maximal (i.e. 100%). Consequently, some studies have suggested the use of multiple stimuli (48, 63, 72). Furthermore, triplets may offer advantages over potentiated twitches as an indicator of peripheral fatigue since pilot data from our lab and that of de Haan (28, 29) has found the force evoked by 300 Hz bursts (3-8 pulses) to be insensitive to potentiation, and because they evoke much greater force than potentiated twitches they may better reflect the functional changes observed during maximal voluntary contractions.

The maximum voluntary force (MVF) of the quadriceps was defined as the greatest instantaneous force produced during the relevant series of MVCs. The root mean square (RMS) amplitude of the EMG signal for each agonist muscle (vastus medialis, vastus lateralis and rectus femoris) was calculated over a 500 ms epoch surrounding MVF (250 ms either side) (19). Agonist EMG RMS values were averaged to calculate a mean quadriceps ($QEMG_{max}$) value and normalized to the peak-to-peak amplitude of the M-wave (see below) to provide a measure of neuromuscular activation. Potentiated twitches were measured for peak force and the amplitude of M-wave response for the three quadriceps electrodes, which were then averaged across the three sites to provide a mean quadriceps value. Mean quadriceps M-wave amplitude and potentiated peak twitch force were averaged across the latter four twitch contractions (i.e. after the 3^{rd} and 4^{th} MVC) within each time period because it typically takes three MVC's to fully potentiate twitches (49). The mean quadriceps M-wave amplitude across the four potentiated twitches was defined as the maximal M-wave amplitude (M_{max}) and was used for normalization of voluntary quadriceps EMG RMS (19). Measures of triplet peak force were averaged across the two contractions within each time period. To evaluate the presence and magnitude of central fatigue voluntary activation was evaluated for the 3^{rd} and 4^{th} MVC using the formula for the twitch interpolation technique (56) as described previously (29, 48):

Voluntary activation (%) = 100 – [(triplet force increment / resting triplet force) × 100]

where the triplet force increment refers to that produced by superimposed triplet stimulation. The highest voluntary activation of the two MVCs was retained for analysis. Assessment of neuromuscular function (from the first MVC to the last triplet) took ~2 min.

Maximal incremental cycling test

Participants initially performed a maximal incremental cycling test using an electromagnetically-braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). Tests began at 0 W and power was increased by discrete 20 W increments every 60 s and exercise was performed to the limit of volitional tolerance or task failure (i.e. cycling cadence below 60 rpm) (46). Participants wore a facemask (model 7940; Hans Rudolph, Missouri, USA) connected to a flow sensor (ZAN variable orifice pneumotach; Nspire Health, Oberthulba, Germany) that was calibrated using a 3 L syringe. Gas concentrations were measured using fast responding laser diode absorption spectroscopy sensors, which were calibrated using gases of known concentration (5% CO_2, 15% O_2, balance N_2; BOC, Guilford, UK), and ventilatory and pulmonary gas exchange variables were measured breath-by-breath (ZAN 600USB; Nspire Health) as described previously (46). Peak oxygen uptake was defined as the highest recorded value over any 30 s period, and $\dot{W}_{peak}$ was calculated as the sum of the power output in the last completed stage plus the product of ramp increment (20 W) and the fraction of the final stage actually completed.

Experimental trials

During the experimental trials (CYC, ARM-CYC, and ISOTIME) heart rate was measured using short-range telemetry (Polar S610; Polar, Kempele, Finland) and fingertip

capillary blood samples were taken and analyzed for blood lactate concentration ($[La^-]_B$) using an automated analyzer (Biosen C_line Sport; EKF Diagnostics, Barleben, Germany).

An illustration of the timing of measurements taken during the experimental trials is shown in Figure 1. Each experimental trial comprised a fixed work-rate cycling test at 85% $\dot{W}_{peak}$ preceded by a standardized 23.5 min period. During the first 6 min of this period baseline measures of heart rate, $[La^-]_B$, and neuromuscular function were taken.

After baseline measurements, participants remained seated in the dynamometer and then either rested (CYC and ISOTIME) or performed intense intermittent arm-cranking exercise (ARM-CYC) using an electromagnetically-braked arm-cranking ergometer (Angio; Lode). The arm-cranking protocol comprised 8 × 1 min arm-cranking bouts, interspersed with 30 s rest, at a fixed work-rate of 1.0-1.5 $W \cdot kg^{-1}$ body mass (mean: 1.2 ± 0.2 $W \cdot kg^{-1}$ body mass, 100 ± 15 W) (46). As in our previous study (46), the arm-cranking work-rate for each individual was selected based on their habitual upper body exercise regimen. This work-rate was trialed during the familiarization session and, based on successful completion by all participants, was deemed suitable for subsequent testing. Cadence was maintained between 90-110 rpm. Heart rate was measured at the end of each arm-cranking bout during ARM-CYC, and $[La^-]_B$ was also measured after the final arm-cranking bout. These measurements were taken at equivalent time points whilst participants rested during CYC and ISOTIME. Quadriceps and hamstring muscle EMG was recorded throughout the arm-cranking protocol and displayed online with a high gain to aid visual detection of EMG activity. Participants received verbal feedback regarding EMG activity in order to ensure minimal activation of the leg muscles.

Arm-cranking (ARM-CYC) or seated rest (CYC and ISOTIME) was followed by another 6 min period before the start of the fixed work-rate cycling test. During this period

measures of neuromuscular function were taken and participants then transferred to the cycle ergometer (positioned ~2 m from the dynamometer). Immediately before the start of the cycling test heart rate and $[La^-]_B$ were measured along with dyspnea (defined as breathing "effort") and RPE for leg discomfort using Borg's modified CR10 scale (18).

Participants adopted a self-selected cadence between 80-100 rpm during the first cycling test and this was replicated during subsequent tests. Quadriceps and hamstring muscle EMG was synchronized with the cycle ergometer crank position via a reed switch attached to the crank and ergometer. The RMS amplitude of the EMG signal of the quadriceps muscle was measured at the start and end of each minute during each arm-cranking exercise bout and normalized to $QEMG_{max}$ to quantify quadriceps neuromuscular activation. EMG RMS amplitude of the quadriceps and hamstring muscles was also measured over 10 consecutive pedal revolutions at the end of the first, third and final minute of cycling exercise and normalized to M_{max} (quadriceps only) to quantify changes in neuromuscular activation during cycling. Onsets and offsets of EMG bursts were determined visually by the same investigator according to a previously published method (22, 23). Threshold methods for determining EMG onsets and offsets are sensitive to changes in background EMG (42) and are unsuitable for this type of analysis because bursts of EMG activity occur with background activity already present in the muscles and the amplitude of background activity varies between muscles (22, 23). Heart rate, RPE and dyspnea were measured after 3 min of cycling. During CYC and ARM-CYC, cycling exercise was performed to the limit of volitional tolerance. An additional criterion for terminating a cycling test was a fall in cadence below 60 rpm. During ISOTIME, cycling exercise was terminated by the investigators after an identical duration to that achieved during ARM-CYC.

290 Upon cessation of cycling exercise heart rate, $[La^-]_B$, RPE and dyspnea were
291 measured immediately and participants were assisted to the dynamometer for neuromuscular
292 function evaluation with the first MVC initiated after 2 min (± 9 s).

293 **Statistical analyses**

294 Data were analyzed using SPSS for Windows (IBM, Chicago, IL). Trial-to-trial
295 variation in baseline neuromuscular function was calculated as the within-participant
296 coefficient of variation (CV). Measurement error and reproducibility of baseline
297 neuromuscular function were calculated, and the smallest meaningful change was
298 subsequently determined (16, 43). A one-way repeated measures ANOVA followed by
299 Tukey's post-hoc test was used to analyze differences between trials for cycling exercise
300 duration and rates of change in perceptual responses expressed relative to absolute exercise
301 time (ΔRPE/Δtime and Δdyspnea/Δtime) and when normalized to total cycling exercise
302 duration (ΔRPE/%time and Δdyspnea/%time). All other data were analyzed using a two-way
303 (trial × time) repeated measures ANOVA. Significant interactions were further explored by
304 performing one-way repeated measures ANOVA: (i) within each trial, and (ii) across trials at
305 individual time-points, followed by Tukey's post-hoc test. When differences were observed
306 within or between trials, 95% confidence intervals (CI) for the difference were calculated (2).
307 Pearson's correlation coefficient was used to determine the relationship between selected
308 variables. Statistical significance was set at $P < 0.05$. Results are presented as mean ± SD.

309 **RESULTS**

310 **Cycling exercise tolerance**

311 There was an effect of trial on cycling exercise duration at 85% $\dot{W}_{peak}$ (273 ± 26 W)
312 [$F(2,14) = 16.8$, $P < 0.001$], which was, as expected, identical (4.33 ± 1.10 min) during

ARM-CYC and ISOTIME and 38 ± 17% shorter than CYC (7.46 ± 2.79 min) (mean difference = 3.13 ± 2.15 min, 95% CI = 1.50 to 4.75 min, $P < 0.001$). Cycling cadence at the termination of cycling exercise in CYC (68 ± 6 rpm, range: 61-78 rpm), ARM-CYC (66 ± 5 rpm, range: 60-74 rpm) and ISOTIME (92 ± 10 rpm, range: 85-104 rpm) was always ≥60 rpm. Therefore, cycling exercise during CYC and ARM-CYC was always performed to the limit of volitional tolerance rather than being terminated by the investigators.

Neuromuscular function

Baseline measures of neuromuscular function are shown in Table 1 and these were highly reproducible between trials. Raw traces of force from a representative participant at baseline performing a MVC with superimposed triplet, followed by twitch and triplet contractions, are shown in Figure 2. In all trials measures of neuromuscular function were unchanged from baseline to pre-cycling (data not shown). Thus arm-cranking *per se* did not result in central or peripheral locomotor muscle fatigue.

For MVF, there was a trial × time interaction [$F(4,28) = 6.2$, $P < 0.001$] and an effect of time in CYC [$F(2,14) = 14.3$, $P < 0.001$], ARM-CYC [$F(2,14) = 11.5$, $P = 0.001$] and ISOTIME [$F(2,14) = 8.5$, $P = 0.003$]. MVF decreased from baseline to post-cycling in CYC (mean difference = 95 ± 70 N, 95% CI = 45 to 145 N, $P < 0.001$), ARM-CYC (mean difference = 56 ± 39 N, 95% CI = 22 to 89 N, $P = 0.002$) and ISOTIME (mean difference = 49 ± 48 N, 95% CI = 13 to 85 N, $P = 0.008$). Furthermore, there was an effect of trial on the decrease in MVF [$F(2,14) = 8.3$, $P = 0.004$], which was greater in CYC than ARM-CYC (mean difference = 39 ± 38 N, 95% CI = 7 to 71 N, $P = 0.02$) and ISOTIME (mean difference = 46 ± 43 N, 95% CI = 14 to 78 N, $P = 0.005$) (Fig. 3A).

For voluntary activation, there was a trial × time interaction [$F(4,28) = 3.8$, $P = 0.013$] and an effect of time in CYC [$F(2,14) = 8.0$, $P = 0.005$] and ARM-CYC [$F(2,14) = 4.7$, $P = 0.027$], but not ISOTIME [$F(2,14) = 0.8$, $P = 0.46$]. Voluntary activation decreased from baseline (see Table 1) to post-cycling in CYC (89 ± 9%, mean difference = 5.0 ± 4.8%, 95% CI = 1.4 to 8.7%, $P = 0.012$) and ARM-CYC (91 ± 8%, mean difference = 3.8 ± 4.7%, 95% CI = 0.1 to 7.4%, $P = 0.047$). Furthermore, there was an effect of trial on the decrease in voluntary activation [$F(2,14) = 5.2$, $P = 0.021$], which was greater in CYC than ISOTIME (mean difference = 4.4 ± 4.9%, 95% CI = 0.7 to 8.0%, $P = 0.019$) (Fig. 3B).

For potentiated twitch force, there was a trial × time interaction [$F(4,28) = 8.8$, $P < 0.001$] and an effect of time in CYC [$F(2,14) = 49.4$, $P < 0.001$], ARM-CYC [$F(2,14) = 48.3$, $P < 0.001$], and ISOTIME [$F(2,14) = 22.7$, $P < 0.001$]. Potentiated twitch force decreased from baseline to post-cycling in CYC (mean difference = 77 ± 30 N, 95% CI = 55 to 98 N, $P < 0.001$), ARM-CYC (mean difference = 52 ± 21 N, 95% CI = 38 to 66 N, $P < 0.001$) and ISOTIME (mean difference = 50 ± 24 N, 95% CI = 30 to 70 N, $P < 0.001$). Furthermore, there was an effect of trial on the decrease in potentiated twitch force [$F(2,14) = 10.9$, $P = 0.001$], which was greater in CYC than ARM-CYC (mean difference = 25 ± 17 N, 95% CI = 8 to 41 N, $P = 0.004$) and ISOTIME (mean difference = 27 ± 22 N, 95% CI = 10 to 43 N, $P = 0.003$) (Fig. 3C).

For potentiated triplet force, there was a trial × time interaction [$F(4,28) = 9.1$, $P < 0.001$] and an effect of time in CYC [$F(2,14) = 11.1$, $P = 0.001$], ARM-CYC [$F(2,14) = 5.4$, $P = 0.018$], and ISOTIME [$F(2,14) = 7.2$, $P = 0.007$]. Potentiated triplet force decreased from baseline to post-cycling in CYC (mean difference = 63 ± 50 N, 95% CI = 26 to 100 N, $P = 0.001$), ARM-CYC (mean difference = 37 ± 40 N, 95% CI = 7 to 66 N, $P = 0.014$) and ISOTIME (mean difference = 31 ± 26 N, 95% CI = 9 to 52 N, $P < 0.001$). Furthermore, there

was an effect of trial on the decrease in potentiated triplet force [$F(2,14) = 9.0$, $P = 0.003$], which was greater in CYC than ARM-CYC (mean difference = 27 ± 18 N, 95% CI = 5 to 48 N, $P = 0.015$) and ISOTIME (mean difference = 33 ± 28 N, 95% CI = 11 to 54 N, $P = 0.004$). (Fig. 3D).

Quadriceps M_{max} and neuromuscular activation (i.e. RMS EMG normalized to M_{max}) at MVF remained unchanged in all trials.

Leg muscle EMG during cycling

Quadriceps EMG RMS during arm-cranking was ≤3% of the $QEMG_{max}$ during an MVC (data not shown), thus demonstrating minimal leg activation. For quadriceps neuromuscular activation (EMG RMS normalized to M_{max}) during cycling, there was a trial × time interaction [$F(4,28) = 6.1$, $P = 0.001$] and an effect of time in CYC [$F(2,14) = 36.3$, $P < 0.001$], ARM-CYC [$F(2,14) = 11.6$, $P = 0.001$] and ISOTIME [$F(2,14) = 36.3$, $P = 0.012$]. There was also an effect of trial on neuromuscular activation in the final minute of cycling [$F(2,14) = 6.2$, $P = 0.012$], which was greater in CYC than ARM-CYC (mean difference = 0.76 ± 0.84 $\%M_{max}$, 95% CI = 0.03 to 1.5 $\%M_{max}$, $P = 0.040$) and ISOTIME (mean difference = 0.91 ± 0.87 $\%M_{max}$, 95% CI = 0.18 to 1.64 $\%M_{max}$, $P = 0.014$). (Fig. 4). The absolute hamstrings EMG RMS remained constant during cycling and was not different between trials (pooled data: 0.08 ± 0.05 mV).

Heart rate and blood lactate concentration

For heart rate, there was a trial × time interaction [$F(24,168) = 81.7$, $P < 0.001$] and an effect of trial on the mean of the eight heart rate measurements taken during the 11.5 min period of arm-cranking in ARM-CYC or seated rest in CYC and ISOTIME (see Fig. 1) [$F(2,24) = 144.3$, $P < 0.001$]. The mean heart rate during this period was higher in ARM-

CYC (153 ± 19 bpm) than CYC (72 ± 14 bpm) (mean difference = 81 ± 20 bpm, 95% CI = 67 to 96 bpm, $P < 0.001$) and ISOTIME (73 ± 11 bpm) (mean difference = 80 ± 16 bpm, 95% CI = 66 to 95 bpm, $P < 0.001$). There was also an effect of trial on heart rate measured pre-cycling [$F(2,14) = 21.7$, $P < 0.001$], after 3 min of cycling [$F(2,14) = 17.8$, $P < 0.001$], and post-cycling [$F(2,14) = 12.3$, $P < 0.001$]. Pre-cycling, heart rate was higher in ARM-CYC than CYC (mean difference = 34 ± 21 bpm, 95% CI = 18 to 49 bpm, $P < 0.001$) and ISOTIME (mean difference = 34 ± 15 bpm, 95% CI = 18 to 49 bpm, $P < 0.001$). After 3 min of cycling, heart rate was higher in ARM-CYC than CYC and ISOTIME (mean difference from both trials = 10 ± 6 bpm, 95% CI = 5 to 15 bpm, $P < 0.001$). Post-cycling, heart rate was lower in ISOTIME than CYC (mean difference = 10 ± 6 bpm, 95% CI = 3 to 16 bpm, $P = 0.005$) and ARM-CYC (mean difference = 12 ± 8 bpm, 95% CI = 5 to 18 bpm, $P = 0.001$) (Fig. 5A).

For $[La^-]_B$, there was a trial × time interaction [$F(6,42) = 79.7$, $P < 0.001$] and an effect of trial on $[La^-]_B$ measured immediately after the period of arm-cranking in ARM-CYC or seated rest in CYC and ISOTIME [$F(2,14) = 167.2$, $P < 0.001$]. Immediately after this period, $[La^-]_B$ was higher in ARM-CYC than CYC and ISOTIME (mean difference from both trials = 10.3 ± 2.2 mmol·L^{-1}, 95% CI = 8.6 to 12.0 mmol·L^{-1}, $P < 0.001$). There was also an effect of trial on $[La^-]_B$ measured pre-cycling [$F(2,14) = 158.2$, $P < 0.001$], which was higher in ARM-CYC than CYC (mean difference = 8.5 ± 2.0 mmol·L^{-1}, 95% CI = 7.0 to 10.0 mmol·L^{-1}, $P < 0.001$) and ISOTIME (mean difference = 8.6 ± 1.8 mmol·L^{-1}, 95% CI = 7.2 to 10.1 mmol·L^{-1}, $P < 0.001$). Furthermore, there was an effect of trial on $[La^-]_B$ measured post-cycling [$F(2,14) = 31.9$, $P < 0.001$], which was higher in ARM-CYC than CYC (mean difference = 2.3 ± 1.0 mmol·L^{-1}, 95% CI = 0.8 to 3.9 mmol·L^{-1}, $P = 0.003$) and ISOTIME (mean difference = 4.6 ± 1.8 mmol·L^{-1}, 95% CI = 3.1 to 6.1 mmol·L^{-1}, $P < 0.001$). Post-

406 cycling, $[La^-]_B$ was also higher in CYC than ISOTIME (mean difference = 2.3 ± 2.0 mmol·L^{-1},
407 95% CI = 0.8 to 3.8 mmol·L^{-1}, P = 0.004) (Fig. 5B).

408 **Rating of perceived exertion and dyspnea**

409 There was a trial × time interaction for RPE [F(4,28) = 14.7, P < 0.001] and an effect
410 of trial on RPE measured after 3 min of cycling [F(2,14) = 11.7, P = 0.001], which was
411 higher in ARM-CYC than CYC (mean difference = 2.4 ± 1.7 AU, 95% CI = 1.0 to 3.9 AU, P
412 = 0.002) and ISOTIME (mean difference = 2.3 ± 1.9 AU, 95% CI = 0.8 to 3.8 AU, P =
413 0.003). There was also an effect of trial on RPE measured post-cycling [F(2,14) = 18.4, P <
414 0.001], which was lower in ISOTIME than CYC (mean difference = 3.3 ± 1.9 AU, 95% CI =
415 1.4 to 5.2 AU, P = 0.001) and ARM-CYC (mean difference = 4.1 ± 2.6 AU, 95% CI = 2.2 to
416 5.9 AU, P < 0.001) (Fig. 6A).

417 There was a trial × time interaction for dyspnea [F(4,28) = 5.8, P < 0.001] and an
418 effect of trial on dyspnea measured after 3 min of cycling [F(2,14) = 16.3, P < 0.001], which
419 was higher in ARM-CYC than CYC (mean difference = 1.9 ± 1.4 AU, 95% CI = 0.9 to 3.0
420 AU, P < 0.001) and ISOTIME (mean difference = 2.1 ± 1.1 AU, 95% CI = 1.0 to 3.2 AU, P <
421 0.001). There was also a main effect of trial on dyspnea measured post-cycling [F(2,14) =
422 11.8, P = 0.001], which was lower in ISOTIME than CYC (mean difference = 2.8 ± 2.4 AU,
423 95% CI = 0.9 to 4.6 AU, P = 0.004) and ARM-CYC (mean difference = 3.1 ± 2.6 AU, 95%
424 CI = 1.3 to 5.0 AU, P = 0.002) (Fig. 6B).

425 There was an effect of trial on ΔRPE/Δtime [F(2,14) = 11.7, P = 0.001], which was
426 higher in ARM-CYC than CYC (mean difference = 0.72 ± 0.63 AU·min^{-1}, 95% CI = 0.25 to
427 1.21 AU·min^{-1}, P = 0.003) and ISOTIME (mean difference = 0.79 ± 0.55 AU·min^{-1}, 95% CI
428 = 0.31 to 1.27 AU·min^{-1}, P = 0.002) (Table 2). There was also an effect of trial on

429 Δdyspnea/Δtime [$F(2,14) = 4.5$, $P = 0.031$], which was higher in ARM-CYC than ISOTIME
430 (mean difference = 0.46 ± 0.58 AU·min^{-1}, 95% CI = 0.02 to 0.90 AU·min^{-1}, $P = 0.038$) (Table
431 2).

432 There was an effect of trial on ΔRPE/Δ%time [$F(2,14) = 19.1$, $P < 0.001$] (Fig. 6C
433 and Table 2), which was lower in ISOTIME than CYC (mean difference = 0.03 ± 0.01
434 AU·%time^{-1}, 95% CI = 0.01 to 0.05 AU·%time^{-1}, $P < 0.001$) and ARM-CYC (mean
435 difference = 0.04 ± 0.02 AU·%time^{-1}, 95% CI = 0.02 to 0.05 AU·%time^{-1}, $P < 0.001$). There
436 was also an effect of trial on Δdyspnea/Δ%time [$F(2,14) = 7.5$, $P = 0.006$] (Fig. 6D and Table
437 2), which was lower in ISOTIME than CYC (mean difference = 0.03 ± 0.01 AU·%time^{-1}, 95%
438 CI = 0.01 to 0.05 AU·%time^{-1}, $P = 0.006$) and ARM-CYC (mean difference = 0.02 ± 0.01
439 AU·%time^{-1}, 95% CI = 0.001 to 0.04 AU·%time^{-1}, $P = 0.036$).

440 When data from CYC and ARM-CYC were pooled, ΔRPE/Δtime was negatively
441 correlated with the time to the limit of cycling exercise tolerance ($r = -0.74$, $P = 0.001$).
442 Furthermore, the reduction in cycling exercise tolerance during ARM-CYC compared with
443 CYC was negatively correlated with the increases in ΔRPE/Δtime ($r = -0.72$, $P = 0.045$) and
444 Δdyspnea/Δtime ($r = -0.80$, $P = 0.018$).

445 **DISCUSSION**

446 The present study examined the effects of prior high-intensity upper body exercise on
447 subsequent high-intensity leg cycling exercise tolerance and associated changes in
448 neuromuscular function and perceptual responses. Our main findings were threefold: (I) prior
449 upper body exercise in ARM-CYC reduced subsequent cycling exercise tolerance by 38%; (II)
450 the reduced cycling exercise tolerance in ARM-CYC was associated with less peripheral
451 muscle fatigue incurred but a similar reduction in voluntary activation compared with CYC;

and (III) the reduced cycling exercise tolerance in ARM-CYC was related to increases in ΔRPE/Δtime and Δdyspnea/Δtime. These findings suggest that exercise tolerance is not regulated by a critical level of peripheral fatigue. Instead, central fatigue and an exacerbation of perceptual responses are the potential mechanisms underlying the reduced cycling exercise tolerance after prior upper body exercise.

We recently showed that high-intensity cycling exercise tolerance was reduced by a strikingly similar extent after an identical upper body exercise protocol (46). Several authors suggest that reduced lower limb exercise tolerance after prior upper body exercise occurs because of accelerated development of peripheral fatigue caused by greater intramuscular metabolic perturbation (12, 17, 36, 44, 46, 61). This notion is supported, indirectly, by the observation that prior high-intensity upper body exercise elevated leg muscle [La^-] and [H^+] at the onset of isolated knee extensor exercise (11, 12), accelerated the exercise-induced increase in interstitial [K^+] (61), and reduced exercise tolerance (12, 61). However, although such metabolite accumulation has been implicated in the etiology of peripheral fatigue (21, 33), previous prior upper body exercise studies did not measure peripheral fatigue or neuromuscular activation. Comparisons of our work with isolated knee extensor exercise studies are also complicated by the task-specificity of fatigue etiology (13, 74). Two observations from the present study suggest that peripheral fatigue during cycling exercise was not accelerated by prior upper body exercise. Firstly, the extent of peripheral fatigue in ARM-CYC and ISOTIME was the same even though there was considerable systemic metabolic perturbation in ARM-CYC. Indeed, in our previous study the same upper body exercise protocol reduced the strong ion difference by 15%, increased plasma [H^+] by 33%, reduced [HCO_3^-] by 29%, and accelerated the increase in plasma [K^+] during subsequent cycling exercise by 56% (46). Secondly, if peripheral fatigue during cycling exercise was accelerated this would be expected to result in greater neuromuscular activation (i.e.

increased motor unit recruitment and/or firing frequency) to compensate for the reduced force generating capacity (14, 30); however, this was not observed. Collectively, these observations therefore suggest that systemic metabolite perturbation plays a minor role in peripheral fatigue generation.

Our findings contrast previous cycling exercise studies in which moderate hypoxia (7, 66), superimposed inspiratory muscle loading (67), volitionally-induced inspiratory or expiratory muscle fatigue (73, 80), prior high-intensity cycling exercise (5), and prior electrically-induced quadriceps muscle fatigue (34), reduced exercise tolerance but resulted in the same degree of peripheral fatigue incurred compared with control conditions. These observations are taken as evidence for inhibitory group III/IV muscle afferent feedback to the central nervous system regulating central motor drive to confine the development of peripheral fatigue to a critical threshold (3, 31). However, although the 38% reduction in twitch force after CYC is comparable to the proposed critical threshold of peripheral fatigue previously reported after high-intensity fixed work-rate cycling exercise (4, 5, 66, 73, 80), this degree of peripheral fatigue was not reached during ARM-CYC (26% reduction in twitch force). This finding is similar to the observation of less peripheral fatigue incurred after high-intensity cycling exercise in severe hypoxia (F_IO_2 0.10) compared with normoxia (8). The notion that peripheral fatigue is not critically regulated is also supported by two recent isolated muscle studies: Rossman et al. (68) observed greater quadriceps muscle fatigue during single-leg compared with double-leg knee extensor exercise, whereas Amann et al. (10) observed less quadriceps muscle fatigue during single-leg knee extensor exercise after fatiguing knee extensor exercise with the contralateral leg. The present study thus extends these observations to whole-body exercise by providing novel evidence that peripheral fatigue is not independently regulated during high-intensity fixed work-rate cycling exercise to volitional tolerance.

Whether peripheral fatigue plays an important role in governing exercise tolerance remains controversial (9, 53, 54, 60). Consistent with previous observations (5, 7, 54, 71, 75), submaximal quadriceps muscle recruitment was observed at the limit of exercise tolerance in CYC and ARM-CYC (~55% and 50%, respectively, of the $QEMG_{max}$) and Noakes (58) argues that this negates peripheral fatigue as the single limiting factor to exercise tolerance. Furthermore, Decorte et al. (30) have shown that peripheral fatigue during cycling exercise at 80% W_{peak} develops mostly during the first half of the test, such that the limit of tolerance approaches without further peripheral fatigue, but with a significant reduction in voluntary activation. The similar reduction in voluntary activation after CYC and ARM-CYC indicates that central fatigue developed more quickly in ARM-CYC, possibly due to a 'spill-over' of central fatigue from the exercised upper body muscles to the leg locomotor muscles. In support, it was recently demonstrated that fatiguing leg cycling exercise resulted in a 'spill-over' of central fatigue (i.e. reduced voluntary activation) to the remote unexercised elbow flexors (69). This effect was attributed to inhibitory group III/IV muscle afferent feedback originating in fatigued leg muscle since attenuating this feedback using intrathecal fentanyl abolished the decline in voluntary activation of the elbow flexors. Whether a fall in voluntary activation limits cycling exercise that is characterized by submaximal muscle contractions remains uncertain (74). However, it is also recognized that a limiting influence of central fatigue on exercise tolerance may be manifest by changes in sensory perception (57, 74).

The conscious perception of fatigue is thought to reflect the complex integration and interpretation of central motor drive and an associated corollary discharge, somatosensory feedback (4, 50, 70, 78), and cognitive functions such as motivation and emotional state (70). After 3 min of cycling, RPE for leg discomfort was greater in ARM-CYC compared with CYC and ISOTIME despite similar levels of quadriceps neuromuscular activation, which supports observations made during single-leg knee extensor exercise after fatiguing knee

extensor exercise with the contralateral leg (10). Furthermore, at the end of cycling, RPE was greater in ARM-CYC compared with ISOTIME despite similar levels of peripheral fatigue incurred, whereas RPE was similar at the end of cycling in CYC and ARM-CYC despite less peripheral fatigue incurred during ARM-CYC. Collectively, our findings suggest that the perception of leg discomfort during cycling exercise does not exclusively reflect the extent of quadriceps neuromuscular activation or degree of peripheral fatigue incurred. Similar observations have been made in COPD patients who sometimes stop exercise because of leg discomfort and in the absence of quadriceps muscle fatigue (52). These observations suggest that the conscious perception of leg discomfort likely reflects a complex interplay between multiple factors other than peripheral fatigue and neuromuscular activation (70). Minute ventilation was not measured in the present study and thus it cannot be ruled out that the greater Δdyspnea/Δtime during ARM-CYC resulted, in part, from a greater ventilatory response (10). However, afferents involved in the perception of dyspnea and limb discomfort project to the same sensorimotor brain areas (62) and, therefore, a heightened level of one perception may potentiate the other. In support, quadriceps fatigue induced by sustained contractions increased dyspnea during a subsequent inspiratory loaded breathing challenge without affecting breathing pattern or pleural pressure swings (37). Thus although we could not elucidate the precise causative mechanism(s), we propose that the greater ΔRPE/Δtime and Δdyspnea/Δtime during ARM-CYC reflects, in part, greater ensemble group III/IV afferent projections to integrated sensorimotor brain structures due to cycling commencing with pre-existing afferent input originating from the previously exercised respiratory (50) and upper body musculature (10, 25, 45), lungs (50), and heart (78). During cycling exercise the pre-existing afferent input would have been added to the prevailing inputs related to central motor drive, and locomotor muscle (10) and cardiorespiratory (50, 78) activity, thereby accelerating the increase in perceptual responses and reducing exercise tolerance. The

correlation between increased ΔRPE/Δtime and Δdyspnea/Δtime and reduced cycling exercise tolerance in ARM-CYC also supports sensory perception as an important mediator of exercise tolerance. Our findings are therefore consistent with the 'flush model' proposed by Millet (57), which suggests that exercise tolerance is mediated primarily by ΔRPE/Δtime which, in turn, depends mainly on feedback (i.e. peripheral) and feed-forward (i.e. central) mechanisms.

The greater ΔRPE/Δtime and Δdyspnea/Δtime during ARM-CYC compared with CYC, but similar ΔRPE/Δ%time and Δdyspnea/Δ%time, suggests that the pre-existing afferent input at the onset of cycling in ARM-CYC affected perceptual responses by increasing their gain. Similar effects on the absolute and normalized RPE are observed when exercise tolerance is reduced by muscle glycogen depletion (59), warm and cold ambient temperatures (24), and prior fatiguing activity using the same muscle groups (32). These observations underpin the notion that perceptual responses are set in anticipation, otherwise known as teleoanticipation (76), so that exercise terminates at a critical sensory tolerance limit (32, 58, 60, 75). By limiting exercise tolerance the sensory tolerance limit will, therefore, also mediate the degree of peripheral fatigue incurred, which is consistent with the findings of recent studies using the isolated knee extensor exercise model (10, 68). We note, however, that the limit of cycling exercise tolerance during CYC and ARM-CYC was sometimes associated with submaximal RPE and dyspnea, suggesting that additional influences, such as psychological factors (15, 54), were also mediating the limit of exercise tolerance.

In conclusion, reductions in cycling exercise tolerance due to prior upper body exercise are associated with an acceleration of central fatigue and greater perceptual responses rather than an accelerated development of peripheral fatigue. These findings suggest that peripheral fatigue is not independently regulated during high-intensity fixed work-rate cycling exercise to volitional tolerance, and that exercise tolerance, and thus the

degree of peripheral fatigue incurred, is potentially determined by intolerable levels of sensory perception.

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Table 1. Baseline neuromuscular function and between-trial reproducibility. Measured variables are shown as mean ± SD. CV, coefficient of variation; SMC, smallest meaningful change.

	CYC	ARM-CYC	ISOTIME	Within-participant CV (%)	Measurement error	Reproducibility	SMC
MVF (N)	616 ± 75	602 ± 84	616 ± 73	4	19	53	27
Potentiated twitch force (N)	201 ± 33	200 ± 20	203 ± 22	3	11	29	15
Potentiated triplet force (N)	339 ± 35	328 ± 37	337 ± 33	3	10	28	14
Voluntary activation (%)	94 ± 5	95 ± 6	94 ± 6	2	1.8	5.1	2.6
Quadriceps M-wave amplitude (mV)	6.3 ± 2.1	6.1 ± 1.9	5.8 ± 2.5	10	0.6	1.7	0.8
Quadriceps EMG RMS at MVF ($\%M_{max}$ amplitude)	8.8 ± 2.9	8.5 ± 2.9	8.9 ± 2.5	11	1.0	2.7	1.3

MVF, maximal voluntary force.

Table 2. Rates of change in the rating of perceived exertion (RPE) and dyspnea expressed relative to absolute exercise time and when normalized to total cycling exercise duration. Data are mean ± SD.

	CYC	ARM-CYC	ISOTIME
ΔRPE/Δtime (AU·min^{-1})	1.10 ± 0.38	1.83 ± 0.46**	1.05 ± 0.43
ΔRPE/Δ%time (AU·%time^{-1})	0.07 ± 0.02	0.08 ± 0.02	0.04 ± 0.02**
Δdyspnea/Δtime (AU·min^{-1})	0.93 ± 0.39	1.33 ± 0.55*	0.87 ± 0.03
Δdyspnea/Δ%time (AU·%time^{-1})	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.02$^{\#\dagger}$

**P < 0.01 vs. other two trials; *P < 0.05 vs. ISOTIME; $^{\#}P$ < 0.01 vs. CYC; $^{\dagger}P$ < 0.05 vs. ARM-CYC.

Figuress

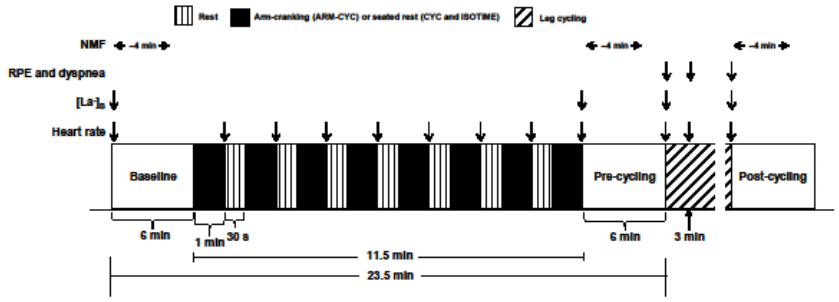


Fig. 1. Experimental protocol. Arrows denote timing of measurement. Note that $[La^-]_B$, heart rate, rating of perceived exertion (RPE) and dyspnea were measured immediately before the start of leg cycling exercise.

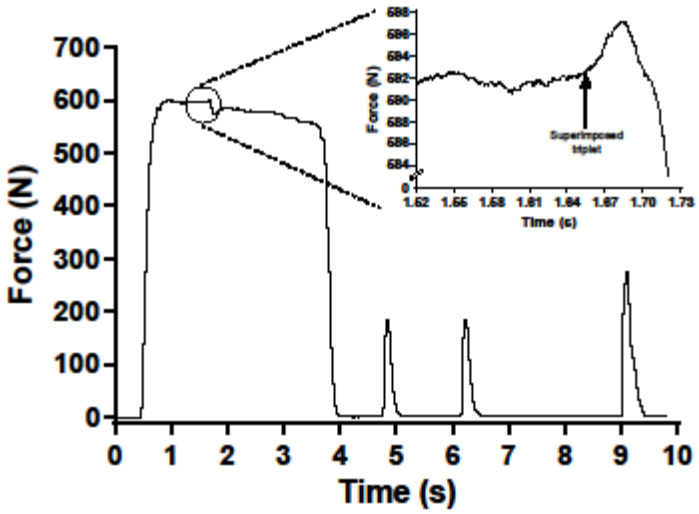
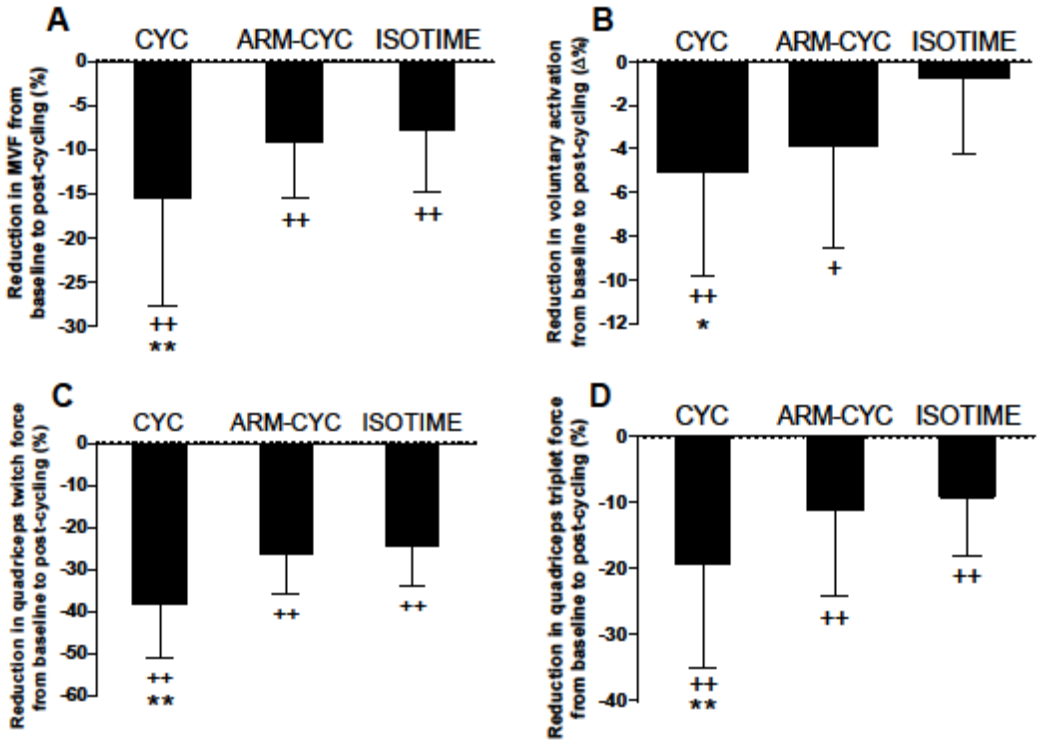


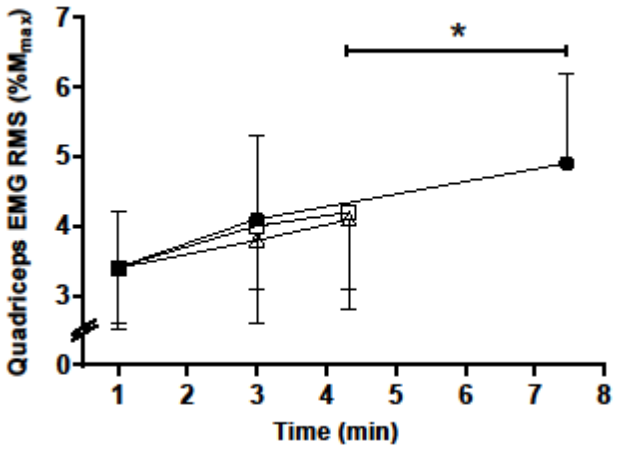
Fig. 2. Raw traces of force at baseline from a representative participant. Force was measured during a maximal voluntary contraction with superimposed triplet, and subsequently during two potentiated twitch contractions and one triplet contraction. Inset figure provides a close up view of changes in force with the superimposed triplet.



832

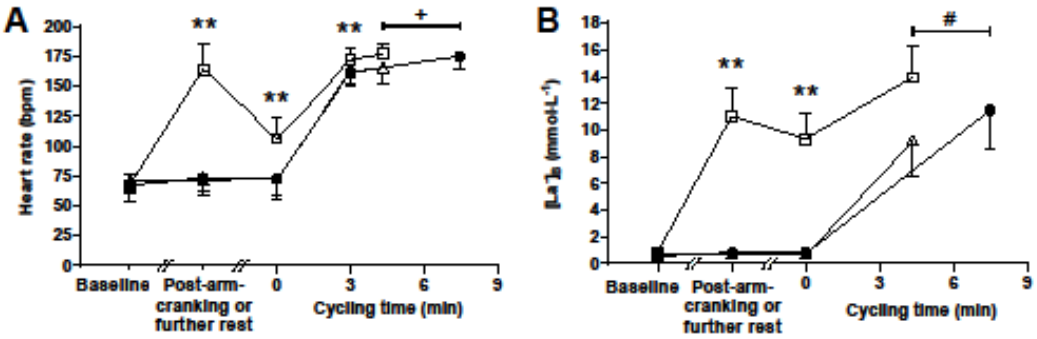
833 Fig. 3. Reductions in maximal voluntary force (MVF) (A), voluntary activation (B), and
834 electrically-evoked potentiated twitch (C) and triplet (D) force after cycling exercise. Data
835 are mean ± SD. Reduction from baseline ($^{+}P < 0.05$, $^{++}P < 0.01$). *Greater reduction
836 compared with ISOTIME ($P < 0.05$). **Greater reduction compared with ARM-CYC and
837 ISOTIME ($P < 0.01$).

838



839

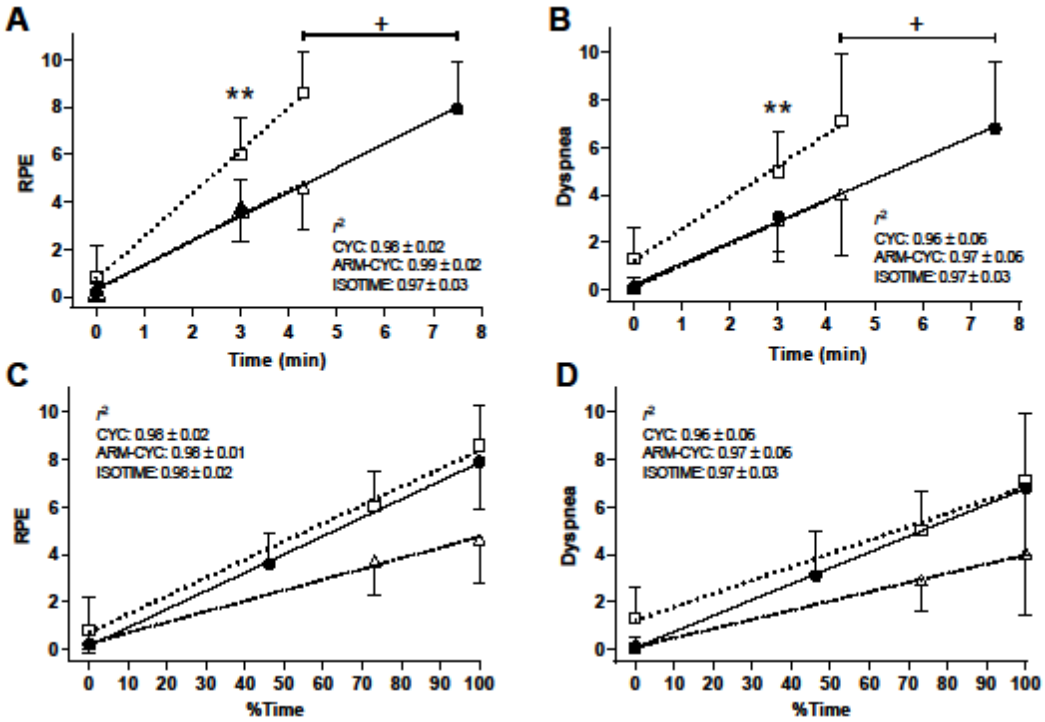
840 Fig. 4. Quadriceps neuromuscular activation measured as EMG RMS normalized to M_{max}
841 during cycling in CYC (●), ARM-CYC (□) and ISOTIME (Δ). Data are mean ± SD with x-
842 error bars omitted at the end of cycling exercise to improve clarity. *Significant difference:
843 CYC vs. ARM-CYC and ISOTIME at the end of cycling ($P < 0.05$).



844

845 Fig. 5. Heart rate (A) and blood lactate concentration ($[La^-]_B$) (B) during CYC (●), ARM-
846 CYC (□) and ISOTIME (Δ). Data are mean ± SD and x-error bars are omitted at the end of
847 cycling exercise to improve clarity. Measurements at 0 min were taken immediately before
848 the start of cycling exercise. Significant difference between trials ($P < 0.01$): **ARM-CYC
849 vs. CYC and ISOTIME; $^+$CYC and ARM-CYC vs. ISOTIME at the end of cycling; $^\#$all trials
850 at the end of cycling.

851



852

853 Fig. 6. Rating of perceived exertion (RPE) and dyspnea during cycling exercise in CYC (●),
854 ARM-CYC (□) and ISOTIME (Δ). Data are mean ± SD and expressed relative to absolute
855 exercise time (A and B) and when normalized to total cycling exercise duration (C and D).
856 Measurements at 0 min and 0 %time were taken immediately before the start of cycling
857 exercise. X-error bars in A and B are omitted at the end of cycling exercise to improve clarity.
858 Significant difference ($P < 0.01$): **ARM-CYC vs. CYC and ISOTIME; $^+$CYC and ARM-
859 CYC vs. ISOTIME at the end of cycling.