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Authors: Paul Ansdell
Jakob Škarabot
Elliott Atkinson
Sarah Corden
Amber Tygart
Kirsty Hicks
Kevin Thomas
Sandra Hunter
Glyn Howatson
Stuart Goodall

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Sex differences in fatigability following exercise normalised to the power-duration relationship

Paul Ansdell[1], Jakob Škarabot[1,2], Elliott Atkinson[1], Sarah Corden[1], Amber Tygart[1], Kirsty M Hicks[1], Kevin Thomas[1], Sandra K Hunter[3], Glyn Howatson[1,4], Stuart Goodall[1]

[1] Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK

[2] School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK

[3] Department of Physical Therapy, Marquette University, Milwaukee, WI, USA

[4] Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa

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Correspondence:

Dr Stuart Goodall

Faculty of Health and Life Sciences

Department of Sport, Exercise and Rehabilitation

Northumbria University

Newcastle upon Tyne

NE1 8ST

UK

Email: stuart.goodall@northumbria.ac.uk

Key Points

- Knee-extensors demonstrate greater fatigue-resistance in females compared to males during single-limb and whole-body exercise. For single-limb exercise, the intensity-duration relationship is different between sexes, with females sustaining a greater relative intensity of exercise.

- This study established the power-duration relationship during cycling, then assessed fatigability during critical power-matched exercise within the heavy and severe intensity domains.

- When critical power and the curvature constant were expressed relative to maximal ramp test power, no sex difference was observed. No sex difference in time to task failure was observed in either trial.

- During heavy and severe intensity cycling, females experienced lesser muscle de-oxygenation. Following both trials, females experienced lesser reductions in knee-extensor contractile function, and following heavy intensity exercise, females experienced less reduction in voluntary activation.

- These data demonstrate that whilst the relative power-duration relationship is not different between males and females, the mechanisms of fatigability during critical power-matched exercise are mediated by sex.

Abstract

Due to morphological differences, females demonstrate greater fatigue resistance of locomotor muscle during single-limb and whole-body exercise modalities. Whilst females sustain a greater relative intensity of single-limb, isometric exercise than males, limited investigation has been performed during whole-body exercise. Accordingly, this study established the power-duration relationship during cycling in 18 trained participants (8 females). Subsequently, constant-load exercise was performed at critical power (CP)-matched intensities within the heavy and severe domains, with the mechanisms of fatigability assessed via non-invasive neurostimulation, near-infrared spectroscopy, and pulmonary gas exchange during and following exercise. Relative CP (72±5 vs. 74±2% P_{max}, p=0.210) and curvature constant (51±11 vs. 52±10 $J \cdot P_{max}^{-1}$, p=0.733) of the power-duration relationship were similar between males and females. Subsequent heavy (p=0.758) and severe intensity (p=0.645) exercise time to task failures were not different between sexes. However, females experienced lesser reductions in contractile function at task failure ($p \leq 0.020$), and greater *vastus lateralis* oxygenation ($p \leq 0.039$) during both trials. Reductions in voluntary activation occurred following both trials ($p<0.001$), but were less in females following the heavy trial (p=0.036). Furthermore, during the heavy-intensity trial only, corticospinal excitability was reduced at the cortical (p=0.020) and spinal (p=0.036) levels, but these reductions were not sex-dependent. Other than a lower respiratory exchange ratio in the heavy trial for females (p=0.039), no gas exchange variables differed between sexes ($p \geq 0.052$). Collectively, these data demonstrate that whilst the relative power-duration relationship is not different between males and females, the mechanisms of fatigability during CP-matched exercise above and below critical power are mediated by sex.

Key Words: cycling, females, integrative, locomotor, males, neuromuscular, respiratory

Introduction

The exercise intensity-duration relationship is a phenomenon that permits mechanistic insight into the metabolic demands and physiological consequences of exercise within distinct intensity domains (Jones *et al.*, 2010; Poole *et al.*, 2016; Burnley & Jones, 2018). The relationship between exercise intensity and maximal sustainable duration is hyperbolic at severe intensities, with the asymptote of the curve, the so-called critical power, representing the threshold between exercise that is sustainable via a steady-state of substrate utilisation and re-synthesis, and exercise that requires ATP re-synthesis from substrate-level phosphorylation (Poole *et al.*, 2016). Exercising above critical power therefore leads to a progressive loss of intramuscular homeostasis, and impairment of the contractile apparatus (Vanhatalo *et al.*, 2010; Schäfer *et al.*, 2019). Below the critical power, substrate-level phosphorylation and the associated accumulation of metabolites are maintained at a steady rate, permitting a 4-5 times slower rate of fatigability (Burnley *et al.*, 2012; Thomas *et al.*, 2016).

Recent evidence showed the power-duration relationship differs between males and females for intermittent, isometric knee-extensor exercise (Ansdell *et al.*, 2019*a*). The mechanism for this is likely a result of morphological differences within the exercising musculature in comparison to males. For instance, within the *vastus lateralis* (VL) the proportional area of type I muscle fibres is greater in females (Simoneau & Bouchard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006), and differences in sarcoplasmic reticulum calcium activity in response to fatiguing exercise are evident between sexes (Harmer *et al.*, 2014). Furthermore, the female VL has a greater capillary density per unit of skeletal muscle (Roepstorff *et al.*, 2006), and an augmented vasodilatory response of the femoral artery to exercise (Parker *et al.*, 2007); collectively, these physiological differences could augment the delivery of oxygen to the working muscle. Indeed, VL type I fibre proportion (Vanhatalo *et al.*, 2016) and capillarisation (Mitchell *et al.*, 2018*a*) is positively correlated with aerobic exercise performance indices such as critical power (CP) during cycling in males and mixed-sex samples, providing a potential explanation as to why females are able to sustain a greater relative exercise intensity than males during an isometric exercise paradigm (Ansdell *et al.*, 2019*a*).

Whilst the data from Ansdell *et al.* (2019*a*) provide mechanistic insight into the sex difference in fatigability during single-limb exercise (e.g. Hunter *et al.*, 2006; Yoon *et al.*, 2007*b*; Russ *et*

al., 2008; Ansdell *et al.*, 2018), it does not fully explain why a similar sex difference is demonstrated during whole-body exercise (Glace *et al.*, 2013; Temesi *et al.*, 2015). To date, it remains unclear whether the power-duration relationship is different between sexes, and whether a sex difference in fatigability exists if exercise to exhaustion is performed relative to a critical power for whole-body exercise. Sundberg *et al.* (2016) provided an initial investigation into this topic, assessing the power-duration relationship during bouts ranging from 8-283 s, and average power during a three-minute all-out test. The authors suggested no sex difference in the time constant of 'performance loss', or maximum sustainable power. However, exercise bouts spanned both the severe and extreme intensity domains; with the latter being defined by the attainment of task failure prior to the attainment of maximal oxygen uptake ($\dot{V}O_{2max}$, Burnley & Jones, 2018). Therefore, limited conclusions can be drawn regarding fatigability within and between the heavy and severe exercise intensity domains.

It is well established that the mechanisms of fatigability differ for whole-body and single-limb exercise (Hureau *et al.*, 2018; Thomas *et al.*, 2018). Indeed, Poole *et al.* (2016) suggested that parameters of the intensity-duration relationship, such as W′, are likely influenced by different factors in the two modalities of exercise. For example, termination of whole-body exercise above CP coincides with the attainment maximal cardiopulmonary responses (e.g. Vanhatalo *et al.*, 2010; Murgatroyd *et al.*, 2011), whereas for single-limb exercise, equivalent variables do not reach maximal values (e.g. Goodall *et al.*, 2010; Ansdell *et al.*, 2019*a*). Collectively, this evidence suggested that in healthy humans, whole-body severe-intensity exercise performance is limited by a combination of convective and diffusive factors, whereas equivalent intensities of single-limb exercise are solely limited by diffusive factors. As described by Hureau *et al.* (2018) and Thomas *et al.* (2018), during whole-body exercise afferent feedback from other physiological systems (e.g. respiratory) contributes to the attainment of a 'sensory tolerance limit', in addition to accumulation of intramuscular metabolites and depletion of energy stores (Broxterman *et al.*, 2015*b*). Therefore, conclusions based on data from single-limb exercise (Ansdell *et al.* (2019*a*) are limited in explaining exercise tolerance in a whole-body model.

The fact that females have more fatigue-resistant locomotor (Hunter *et al.*, 2006; Yoon *et al.*, 2007*b*; Russ *et al.*, 2008; Ansdell *et al.*, 2017) and respiratory musculature (Guenette *et al.*, 2010; Welch *et al.*, 2018) might lead to the hypothesis that females are able to sustain a greater relative work rate during cycling compared to males. However, morphological sex

differences within the respiratory system have the potential to reduce high-intensity exercise tolerance in females. For example, when height-matched, females have smaller lung volumes and airway size, weaker respiratory muscles, and smaller alveolar surface area for gas exchange compared to males (Mead, 1980; Crapo *et al.*, 1982; Martin *et al.*, 1987; Sheel *et al.*, 2009). Combined, these factors elicit a greater expiratory flow limitation in females at near-maximal ventilatory capacity during cycling (Guenette *et al.*, 2007). Furthermore, females demonstrate a greater work of breathing (W*b*) than males (Witt *et al.*, 2007), and at peak exercise, the oxygen cost of breathing (expressed at a fraction of whole-body $\dot{V}O_2$), is greater compared to males; 14 vs. 9%, respectively (Dominelli *et al.*, 2015). Additionally, females typically demonstrate a lower haemoglobin mass compared to males (Murphy, 2014), which is considered to limit endurance exercise performance (Joyner, 2017). Collectively, these potentially deleterious factors could counteract the greater fatigue-resistance of locomotor and respiratory muscles during whole-body exercise, and negate the sex difference in critical torque demonstrated in single-limb exercise, where central factors are not a limitation to exercise (Ansdell *et al.*, 2019*a*). Additionally, these physiological sex differences could lead to different contributing mechanisms to exercise intolerance, or the so-called sensory tolerance limit (Hureau *et al.*, 2018; Thomas *et al.*, 2018).

Accordingly, the present study had two primary aims: 1) to compare the power-duration relationship between males and females during cycling; 2) to determine whether a sex difference in fatigability (time to task failure, TTF), and the mechanisms (neuromuscular fatigue, muscle oxygenation, and pulmonary gas exchange), existed when exercise intensity was normalised to the power-duration relationship. To do so, non-invasive neurostimulation was employed to quantify the neural and contractile adjustments to cycling exercise, and near-infrared spectroscopy (NIRS) was recorded during exercise to monitor changes in knee-extensor oxygenation. It was hypothesised that: 1) due to the poorer convective aspects of oxygen transport negating the superior diffusive aspects that females demonstrate, no sex difference in the power-duration relationship would exist when expressed relative to maximum exercise performance, and 2) when exercise was CP-matched TTF would not differ, but females would exhibit greater fatigue resistance, and lesser deoxygenation of the knee-extensors compared to males in both heavy and severe intensity domains.

Methods

Ethical Approval

The study received institutional ethical approval from the Northumbria University Health and Life Sciences Research Ethics Committee (submission reference: 12241) and was conducted according to all aspect of the Declaration of Helsinki, apart from registration in a database. Participants provided written informed consent to volunteer for the study.

Participants

Using the effect size from Ansdell *et al.* (2018) for the sex difference in fatigability during isometric exercise, a power calculation (alpha = 0.05, power = 0.80) determined that a sample size of 16 participants was required. Thus, ten males (mean ± SD age: 25 ± 5 years, stature: 178 ± 9 cm, mass: 67.0 ± 8.8 kg) and eight females (age: 25 ± 6, stature: 169 ± 9 cm, mass: 63.3 ± 7.2 kg) gave written informed consent to participate. The females that volunteered were all using monophasic hormonal contraceptives (>6 months), and those using combined contraceptive pills were tested in the 21-day consumption period of the pill cycle in order to negate the effects of endogenous hormones on neuromuscular function and fatigability (Ansdell *et al.*, 2019*c*). To ensure homogeneity in the training status of participants, minimum criteria were set for relative $\dot{V}O_{2max}$ and maximal ramp test power (P_{max}) attained in the first visit (see below). These values were based upon recommendations by De Pauw *et al.* (2013) for males, and Decroix *et al.* (2016) for females and were as follows: minimum $\dot{V}O_{2max}$ of 55 and 48 $mL \cdot kg^{-1} \cdot min^{-1}$, and P_{max} of 4.6 and 3.8 $W \cdot kg^{-1}$ for males and females, respectively, and a minimum weekly training duration of ≥5 $h \cdot week^{-1}$. Participants had to achieve one of the aforementioned criteria in order to proceed to the subsequent experimental visits. In total, 18 males and 16 females were screened to achieve the resultant sample size.

Experimental Design

All participants visited the laboratory six or seven times, completing a familiarisation visit, three or four constant intensity trials to estimate CP, then subsequent trials 10% above and below CP. Testing took place over a three to five week period, with a minimum of 48 h between visits to allow recovery (Carroll *et al.*, 2016). The time of day for each testing session was controlled (± 1 h) to account for diurnal variations in maximal force generating capacity and corticospinal excitability (Tamm *et al.*, 2009). All visits were conducted in an environmentally controlled laboratory facility (TIS Services, Environmental Control

Specialists, Hampshire, UK), where the conditions were pre-set to 20°C, 40% relative humidity.

Experimental Protocol

Familiarisation and Incremental Exercise Test

Upon providing written informed consent, participants performed a 5 minute warm up (80-100 W) on a cycle ergometer (Velotron Pro, RacerMate Inc, Seattle, Washington, USA) at a self-selected cadence (60-100 rpm). Participants were then given 2 minutes of rest, during which they remained stationary on the cycle ergometer, before an incremental exercise test began. For both sexes, the test started at 100 W, then for males the intensity increased gradually by 25 $W \cdot min^{-1}$ (0.416 $W \cdot sec^{-1}$), and for females by 20 $W \cdot min^{-1}$ (0.333 $W \cdot sec^{-1}$). The different rate of intensity increase was intended to produce ramp tests of similar duration in both sexes, due to lower absolute power outputs demonstrated in females (Sundberg *et al.*, 2016), in an attempt to negate the effects of test duration on cardiopulmonary outcomes (Yoon *et al.*, 2007*a*). Mean ramp test duration was not different between males and females (10.5 ± 1.2 vs. 9.1 ± 2.1 mins, p = 0.103, respectively). The test was terminated once the participant's self-selected cadence decreased by 10 rpm, despite strong verbal encouragement. During the test, expired gas was analysed breath-by-breath using an online system (Vyntus CPX, Jaeger, CareFusion, Germany). The outcome variables from the ramp test were $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) and P_{max} (W). Following the incremental exercise test, participants rested for 15 minutes, before a neuromuscular familiarisation was performed, including all forms of non-invasive neurostimulation and a full neuromuscular function assessment and maximal respiratory pressure assessments (see below).

Critical Power Estimation Trials

To estimate CP, participants completed a minimum of three constant-load exercise trials to task failure. The intensities for the initial three trials were set at 110, 90, and 80% of P_{max} and were performed on separate days (minimum 24 h between trials) in a randomised order, designed to elicit task failure within 2-15 minutes (Poole *et al.*, 1988). Time to task failure (s) was recorded as the first time at which participants' cadence fell by 10 rpm. Although strong verbal encouragement was provided throughout the test, no feedback was provided to participants about the power output and time elapsed during the trials. Gas exchange was recorded continuously throughout each trial, and a criterion of an end-exercise $\dot{V}O_2$ of >95% $\dot{V}O_{2max}$ was set; all trials used for estimation achieved this. The parameters of the power-duration relationship (CP and W′) were estimated using the inverse linear model (equation 1), the linear work-time model (equation 2), and the hyperbolic model (equation 3). The

equation with the highest r^2 and lowest standard error (SE) was selected for each individual and used for all further analysis (Mitchell *et al.*, 2018*a*). The hyperbolic fit was used for 10 participants, the linear fit for 5, and the 1/time fit for 3:

$$1.\quad P = W' \cdot \left(\frac{1}{\mathrm{t}}\right) + CP$$

$$2.\quad W = CP \cdot t + W'$$

$$3.\quad t = W'/(P - CP)$$

Where t is time to task failure, P is power output, and W is total work done. If three estimation trials resulted in a large SE for CP (>%5 of the mean) and W′ (>10%), a fourth trial was performed (Mitchell *et al.*, 2018*a*). This occurred for three out of the 18 participants (two males, one female).

Severe and Heavy Intensity Trials

Once CP and W′ were estimated, severe (110% CP) and heavy (90% CP) intensity trials were performed on separate days in a randomised order. Each session began with electrical nerve stimulation and transcranial magnetic stimulation (TMS) thresholds being determined. Participants then completed a standardised isometric warm up (Gruet *et al.*, 2014), before a baseline assessment of neuromuscular function. Following this, NIRS optodes were attached to the VL and baseline measures were recorded for 5 minutes on the cycle ergometer with the right leg relaxed in the fully extended position (crank angle 180° from top dead centre). Resting measures of gas exchange were also recorded in this period, then both NIRS and gas exchange were continuously sampled until task failure. Participants completed a five-minute warm up (80-100 W), followed by one minute of seated rest on the ergometer. In the 5-10 s prior to the trial, participants were instructed to obtain their self-selected cadence against no resistance, then when achieved, the resistance was applied in a square wave fashion. Time to task failure was recorded for the severe intensity trial, whereas for the heavy intensity trial participants cycled to task failure, or for 60 minutes, whichever occurred sooner. Immediately upon task failure (<20 s) participants transitioned from the cycle ergometer to the dynamometer and commenced a neuromuscular assessment which was completed within 2.5 min post-exercise (described below).

Experimental Procedures

Pulmonary Gas Exchange

Breath-by-breath pulmonary gas exchange and ventilation were measured continuously during all trials. With minute ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER) quantified. Prior to each visit, the Vyntus CPX was calibrated for oxygen (O_2) and carbon dioxide (CO_2) with gas of known concentration (16.00% O_2 and 4.97% CO_2) using an electrochemical fuel cell and non-dispersive infrared cell, respectively. Ventilatory volumes were calibrated using a digital turbine transducer at high (2 $L \cdot s^{-1}$) and low (0.2 $L \cdot s^{-1}$) flow rates.

Neuromuscular Function Assessments

Measures of neuromuscular function were assessed pre- and post-exercise, starting within 30 s of task failure. Pre-exercise neuromuscular assessments began with two practice MVCs to ensure potentiation of subsequent evoked measures, followed by three ~3 s MVCs, all separated by 30 s. During these 3 MVCs, motor nerve stimulation (MNS) was delivered when peak force plateaued, and then ~2 s after the MVC to measure voluntary activation (VA_{MNS}) and quadriceps potentiated twitch amplitude ($Q_{tw.pot}$) of the knee-extensors. Single-pulse TMS was subsequently delivered during two sets of five 3-5 s contractions at 100, 87.5, 75, 62.5 and 50% MVC, with 5 s rest between contractions and 10 s rest between sets, to determine VA_{TMS} (Dekerle *et al.*, 2019*b*). Finally, ten single- and ten paired-pulse TMS stimulations were delivered during a 10% MVC contraction in an alternate order to determine corticospinal excitability and short-interval cortical inhibition (SICI), respectively. Measures of neuromuscular function (MVC, $Q_{tw.pot}$, VA_{MNS}) were measured within 30 s of task failure, and VA_{TMS} measured within 2-2.5 minutes, in an attempt to minimise the dissipation of fatigue (Gruet *et al.*, 2014).

Transcranial Magnetic Stimulation

Single and paired-pulse stimuli (1 ms duration) were delivered to the contralateral (left) motor cortex via a concave double cone coil oriented to induce a posterior-to-anterior cortical current (110 mm diameter, maximum output 1.4 T) powered by two linked monopulse stimulators (Magstim Bistim and Magstim^{200}, The Magstim Company, Whitland, UK). Optimal coil placement was determined as the position that elicited the greatest *rectus femoris* (RF) motor evoked potential (MEP) with concomitant smallest antagonist (*biceps femoris*, BF) MEP during a 10% MVC at 50-70% stimulator output. This position was marked on the scalp

with indelible marker to ensure consistent placement during trials. Stimulator intensity for VA_{TMS} was determined as the intensity that elicited the greatest superimposed twitch (SIT) during a 50% MVC. Stimulator intensity was increased in 5% intervals from 50% stimulator output and two stimuli were delivered during a ~5 s isometric contraction, with the mean of two SITs recorded (Dekerle *et al.*, 2019*a*). Mean stimulator intensity was not different between males and females (65 ± 6 vs. 64 ± 5%, $p = 0.791$) or between visits (66 ± 6 vs. 64 ± 6%, $p = 0.100$). The intensities used, activated a large proportion of the motoneuron pool for the RF with no difference in the RF MEPs between trials at baseline (51 ± 15 vs. 53 ± 11% M_{MAX}, $p = 0.314$). The TMS pulse also avoided substantial activation of the antagonist (BF) with small MEPs recorded at baseline (0.44 ± 0.23 vs. 0.47 ± 0.23 mV, $p = 0.476$).

Active motor threshold (AMT) was determined as the stimulator intensity that elicited a MEP of > 200 μV in three out of five stimulations during a 10% MVC contraction. Stimulator intensity was increased in 5% steps from 35% of stimulator output until a consistent MEP amplitude >200 μV was found. Thereafter, stimulus intensity was reduced in 1% steps until the lowest intensity to elicit a MEP of >200 μV was found. Mean AMT was not different between males and females (43 ± 6 vs. 40 ± 5%, $P = 0.392$), or between visits (42 ± 5 vs. 43 ± 7%, $P = 0.245$). Short-interval intracortical inhibition (SICI) was assessed with ten paired- and ten single-pulse stimulations delivered. Paired-pulse TMS consisted of a conditioning pulse at 70% of AMT, and a test pulse at 120% AMT, with an inter-stimulus interval of 2 ms. Two sets of 10 stimuli were used, with a 10 s rest between contractions. All stimuli were delivered during a 10% contraction. This stimulus paradigm has previously been demonstrated as the optimal configuration for measuring SICI in the RF (Brownstein *et al.*, 2018).

Lumbar Electrical Stimulation

To assess spinal motoneuron excitability, lumbar-evoked potentials (LEPs) were measured with a constant-current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK). The cathode was centred over the first lumbar spinous process (5 × 9 cm; Nidd Valley Medical Ltd., Bordon, UK) with the electrode aligned to the centre of the vertebral column. The surface area of the cathode covered two spinous processes above and below the centre point (*T*11–*L*3). A cathode of large area was chosen as it produced less discomfort and greater tolerance by participants (Ugawa *et al.*, 1995; Kuhn *et al.*, 2010). The anode (2.5 cm^2) was placed 5 cm above the upper edge of the cathode (Ugawa *et al.*, 1995), corresponding to the level of the eighth thoracic spinous process (*T*8) as this stimulating site

has recently been shown to activate corticospinal axons at the level of lumbar spinal segments (Škarabot *et al.*, 2019*a*). The pre-exercise LEP was standardised to 15-25% of M_{MAX}. Lumbar stimulation was performed during a 10% MVC contraction alone (unconditioned), and 100 ms into a 200 ms SP (conditioned) to determine excitability of the spinal cord without the presence of background neural drive (Finn *et al.*, 2018). The mean stimulus intensity for unconditioned LEPs was 172 ± 47 mA for males and 166 ± 24 mA for females, (p = 0.732). For conditioned LEPs (SP-LEPs), the TMS intensity to produce a SP of 200 ms was not different between males and females (49 ± 8 vs. 51 ± 6% MSO, p = 0.605), likewise the intensity of subsequent lumbar stimulation was not different (176 ± 46 vs. 172 ± 22 mA, p = 0.747).

Motor Nerve Stimulation

Single electrical stimuli (200 μs duration) were delivered to the right femoral nerve using a constant current stimulator (DS7AH Digitimer Ltd, Welwyn Garden City, UK) via adhesive surface electrodes (CF3200; Nidd Valley Medical Ltd., Harrogate, UK). The cathode was placed over the nerve, high in the femoral triangle, in the position that elicited the greatest twitch amplitude (Q_{tw}) and M-wave in the RF at rest. The anode was placed halfway between the greater trochanter and iliac crest. Optimum stimulus intensity was determined as the minimum current that elicited maximum values of Q_{tw} and M-wave (M_{max}) at rest and then subsequently multiplied by 1.3 to ensure a supra-maximal stimulus was delivered. Mean stimulus intensity was not different between sexes (189 ± 62 vs. 210 ± 57 mA, p = 0.438) or between visits (194 ± 61 vs. 202 ± 61 mA, p = 0.620).

Force and Electromyography

During assessments of neuromuscular function, participants sat on a custom-built chair with knee and hip angles kept constant (both 90° flexion). A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was attached via a non-compliant cuff positioned 2 cm superior to the ankle malleoli on the participants' right leg, to measure knee extensor force (N). Electromyographic signals were recorded continuously throughout the final two trials using wireless sensors (10 mm inter-electrode distance; Trigno Avanti, Delsys, MA, USA). Sensors were placed over the right RF, VL, and BF, consistent with SENIAM guidelines (Hermens *et al.*, 2000), as well as the sternocleidomastoid (SCM), and seventh intercostal space (IC). Prior to placement, the skin-electrode contact area was shaved, abraded, and cleaned using a 70% IPA alcohol wipe (FastAid, Robinson Healthcare,

Worksop, UK). Signals were amplified: gain ×100 for EMG (Delsys Trigno Wireless EMG systems, Boston, MA, USA) and ×300 for force (CED 1902; Cambridge Electronic Design, Cambridge, UK), bandpass filtered (EMG only: 20–450 Hz), digitized (EMG: 2 kHz; Force: 5 kHz; CED 1401, Cambridge Electronic Design), and analysed offline (Spike2 v8, Cambridge Electronic Design).

Near Infrared Spectroscopy

A multi-distance, continuous-wave, single channel NIRS system (NIRO-200NX, Hamamatsu, Hamamatsu City, Japan) evaluated changes in VL oxy- (HbO_2), and deoxy- (HHb) haemoglobin concentrations [μmol/L], as well as tissue oxygenation index (TOI = HbO_2 ÷ [HbO_2 + HHb] × 100), sampled at a rate of 1 Hz. The light-emitting probe comprised of diodes operating at three wavelengths (735, 810, and 850 nm), and an emitter-detector distance of 3 cm. The probe was placed on the vastus lateralis, 20 cm above the fibular head lateral side of the patella (Keane *et al.*, 2018). Optodes were held in place by an elasticised, tensor bandage and covered by an opaque, dark material to avoid motion and ambient light influences. During the fatiguing tasks, the 30 s window around 25, 50, 75% of the task, as well as the final 30 s of the task (100%) were analysed.

Maximal Inspiratory and Expiratory Pressure Measurement

Maximum static inspiratory mouth pressure (MaxInsp) was measured from residual lung volume, while maximum static expiratory mouth pressure (MaxExp) was measured from total lung volume. Manoeuvres were performed using a handheld device (MicroRPM, CareFusion, Hampshire, UK) attached to a phlanged mouthpiece with a 1 mm leak to prevent glottic closure during the MaxInsp manoeuvre, and to reduce the use of buccal muscles during the MaxExp manoeuvre (American Thoracic Society/European Respiratory Society, 2002). Measures were taken while participants were seated, with strong verbal encouragement given to maintain a maximal effort for ~3 s, participants were given 30 s rest between efforts. Post-exercise values were taken immediately after the neuromuscular function assessments (~2.5 mins after task termination). The largest of three values within 5% variability was used for analysis (Wen *et al.*, 1997). The coefficient of variation (CV = [standard deviation ÷ mean] × 100) between baseline assessments in the severe and heavy trials for MaxInsp was 3.7% and 4.6%, and the CV for MaxExp was 7.9% and 3.3% for males and females, respectively.

Data Analysis

One female participant did not complete any assessment incorporating TMS (e.g. VA_{TMS}, MEP, SP-LEP, or SICI) due to a contraindication (metal object in the skull), however, she did complete all other measures. Voluntary activation using MNS was determined using the twitch interpolation method (Merton, 1954) by comparing the amplitude of the superimposed twitch (SIT) with the amplitude of the potentiated resting twitch ($Q_{tw.pot}$) using the following formula: VA_{MNS} (%) = (1 – [SIT ÷ $Q_{tw.pot}$]) × 100]. Voluntary activation using TMS (VA_{TMS}) was assessed during two sets of contractions at 100, 87.5, 75, 62.5 and 50% MVC (Dekerle *et al.*, 2019b). Single pulse TMS was delivered during each contraction, and the linear regression between SIT amplitude and contraction intensity was extrapolated to the *y* intercept to obtain an estimated resting twitch (ERT; Todd *et al.*, 2003). In order to achieve significant linearity ($r^2 > 0.80$, $p < 0.05$), a total of three out of 720 SITs across all trials were excluded (0.4%), which led to three regressions containing 9 data points rather than 10 (all post-exercise). As a result, mean r^2 values for ERTs were linear throughout the study (0.92 ± 0.07). The SIT during 100% MVC was compared with the ERT using the following formula: VA_{TMS} (%) = (1 – [SIT ÷ ERT]) × 100.

Short-interval intracortical inhibition was quantified as the percentage ratio between the amplitude of conditioned MEPs to the amplitude of unconditioned MEPs. Corticospinal excitability was determined by expressing the mean MEP amplitude during the 10% MVC as a percentage of M_{max} (MEP/M_{max}). The rmsEMG was recorded for the 50 ms prior to each stimulation and compared pre-post exercise to measure background muscle activity. The NIRS (O_2Hb, HHb, and TOI) and gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and RER) data were expressed as a percentage of baseline, and the 30 s epochs throughout exercise are presented as Δ%. Gas exchange data was also expressed as a % of final ramp test values, to facilitate comparisons between sexes.

Statistical Analysis

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. The significance level for all statistical tests was set at $p < 0.05$. For variables assessed prior to and during exercise (NIRS and gas exchange) a two-way (2×5) repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Pre, 25, 50, 75, and 100% TTF). For variables assessed only during exercise (rmsEMG) a two-way (2×5) repeated measures

ANOVA was used to assess differences between sex (male vs. female) and over time (Start, 25, 50, 75, and 100% TTF). For variables that were assessed pre and post-exercise (neuromuscular function) a two-way 2×2 repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Pre vs. Post). If significant main or interaction effects were observed, these were followed up by post-hoc Tukey's pairwise comparisons. Paired-samples t tests were performed to compare end-exercise $\dot{V}O2$ to $\dot{V}O2_{max}$ in both exercise intensity domains.

Results

Incremental Ramp Test

The variables recorded during the ramp test are displayed in Table 1. As shown, males recorded greater values for $\dot{V}O_{2max}$, and P_{max} when expressed in absolute units and also when normalised to body mass (all $p < 0.001$). The average performance level *(*De Pauw *et al.,* 2013, Decroix *et al.,* 2016) was similar between males and females (3.4 ± 0.7 vs. 3.3 ± 0.5, $p = 0.609$).

Table 1 here

Power-Duration Relationship

The parameter estimates for the power-duration relationship are presented in Table 1. The range of times to task failure for the shortest estimation trial was 105-185 s, while the range for the longest trial was 568-1,192 s. When data were expressed in absolute units, males demonstrated greater values than females ($p \leq 0.009$), however, when CP and W′ were normalised to P_{max}, no differences between the sexes were observed ($p \geq 0.210$).

Severe Intensity Exercise

Fatigability

All participants reached task failure, and there was no difference in time to task failure between sexes during the trial at 110% CP (males: 752 ± 329 vs. females: 681 ± 277 s, $p = 0.645$). The power-duration relationship accurately predicted time to task failure for both males and females, with no difference between predicted values (719 ± 213 vs. 713 ± 146 s, $p \geq 0.678$).

Figure 1 here

The changes in neuromuscular variables are displayed in Figure 1. MVC, $Q_{tw.pot}$, VA_{MNS}, and VA_{TMS} decreased pre to post exercise at 110% CP ($p \leq 0.002$), and this decrease was less in females compared to males for $Q_{tw.pot}$ (sex × time interaction: −36 ± 17 vs. −15 ± 10%, $F_{1,16}$ = 8.4, p = 0.010, ηp^2 = 0.344). When the percentage change in $Q_{tw.pot}$ was normalised to W′, no sex difference was observed (−2.1 ± 1.3% decline per kJ vs. −1.2 ± 0.8% decline per kJ, p = 0.119).

No other variables demonstrated sex × time interaction effects ($p \geq 0.058$). The amplitude of MEPs, LEPs, and SP-LEPs did not change from pre to post exercise ($p \geq 0.094$, Figure 2), and similarly, M_{max} did not change (p = 0.980). Maximum inspiratory and expiratory pressures decreased from pre-post exercise ($p \leq 0.005$), with no sex difference in the magnitude of decrease ($p \geq 0.565$, Table 8.2).

Figure 2 here

Oxygenation

Both HbO_2 and TOI decreased throughout severe intensity exercise (Figure 3, time effect p < 0.001), whilst HHb increased (p = 0.017). A lesser decrease in TOI (sex × time interaction: $F_{1.3, 21.1}$ = 16.6, p < 0.001, ηp^2 = 0.509) was observed for females compared to males, as well as a reduced increase in HHb (sex × time interaction: $F_{1.7, 26.5}$ = 5,3, p = 0.024, ηp^2 = 0.254).

Figure 3 here

Respiratory and Locomotor Muscle Electromyography

The rmsEMG for VL, SCM and IC all increased throughout the task (Table 2, all time effects p < 0.001). Females demonstrated a lesser increase in rmsEMG for the VL (sex × time interaction: $F_{4,64}$ = 2.7, p = 0.041, ηp^2 = 0.142), but not the SCM (p = 0.079), or IC (p = 0.255).

Pulmonary Gas Exchange

Oxygen consumption, $\dot{V}CO_2$, and $\dot{V}_{E,}$ increased while RER decreased throughout the task (Table 2, $p < 0.001$). The sex × time interaction effect for $\dot{V}_E$ was not significant ($p = 0.052$), and no other sex differences were observed ($p \geq 0.114$). End-exercise $\dot{V}O_2$ was not significantly different from the $\dot{V}O_{2max}$ measured during the incremental test ($p = 0.442$).

Heavy Intensity

Fatigability

Three males (1843 ± 498 s) and three females (1831 ± 568 s) reached task failure prior to the 60 min (3600 s) cut-off and were included in subsequent analyses (whole group mean duration, 3073 ± 835 vs. 2937 ± 964 s, $p = 0.758$).

Figure 4 here

There were significant decreases in MVC, $Q_{tw.pot}$, VA_{MNS}, VA_{TMS}, MEP, and SP-LEP following exercise at 90% CP ($p \leq 0.039$, Figures 4 & 5). Females demonstrated less of a decrease in $Q_{tw.pot}$ (sex × time interaction: −10 ± 11% vs. −24 ± 11, $F_{1,16} = 31.8$, $p = 0.020$, $\eta p^2 = 0.655$) and VA_{MNS} (sex × time interaction: −4 ± 3% vs. −9 ± 6%, $F_{1,16} = 5.2$, $p = 0.036$, $\eta p^2 = 0.246$) compared to males, but no sex difference was demonstrated for VA_{TMS}, MEP, and SP-LEP ($p \geq 0.051$). No change in M_{max} was observed following exercise (p = 0.980). Maximum inspiratory pressure decreased from pre-post exercise ($p = 0.001$), whereas maximum expiratory pressure did not ($p = 0.063$, Table 2). No sex × time interaction in the magnitude of decrease for the former was observed ($p = 1.000$).

Figure 5 here

Oxygenation

Decreases in HbO_2 ($p < 0.019$) and TOI ($p < 0.001$) were observed during heavy exercise, with females demonstrating less of a decrease for TOI (sex × time interaction: $F_{1.7,26.9} = 41.0$, $p < 0.001$, $\eta p^2 = 0.719$). An increase in HHb was observed for both sexes ($p = 0.008$), with

females demonstrating less of an increase than males (Figure 6, sex × time interaction: $F_{1.4,22.8} = 20.8$, $p < 0.001$, $\eta p^2 = 0.565$).

Figure 6 here

Respiratory and Locomotor Muscle Electromyography

The rmsEMG signal from the VL, SCM, and IC all increased throughout the task (Table 2, all time effects $p \leq 0.033$). However, there were no sex differences in the rate of increase for any muscle (sex × time interactions: $p \geq 0.063$).

Pulmonary Gas Exchange

Oxygen consumption, $\dot{V}CO_2$, and $\dot{V}_E$ all increased throughout the heavy intensity exercise task ($p < 0.001$, Table 2) while RER decreased ($p = 0.001$). A sex × time interaction was not observed for $\dot{V}_E$ ($p = 0.052$), but was for RER ($F_{1,16} = 5.08$, $p = 0.039$, $\eta p^2 = 0.241$; see Table 8-2). *Post-hoc* tests indicated that females had a lower RER (mean difference: −0.05) throughout the trial. End-exercise $\dot{V}O_2$ was 19 ± 5% lower than $\dot{V}O_{2max}$ ($p < 0.001$).

Discussion

The present study explored the sex difference in fatigability during locomotor exercise by comparing the power-duration relationship, then muscle oxygenation and neuromuscular responses to CP-matched exercise intensities. The novel findings were that, while males demonstrated a greater absolute critical power and W′, there was no sex difference when these parameters were normalised to the absolute maximal power (P_{max}). Time to task failure/completion was not different in either heavy or severe exercise intensity domains, however, females demonstrated lesser reductions in knee-extensor contractile function ($Q_{tw.pot}$) immediately after exercise. These sex differences are likely related to differences in skeletal muscle size and composition influencing the physiological response to exercise in both intensity domains. The change in corticospinal excitability appeared to be domain- but not sex-specific, with a decrease in MEP and SP-LEP amplitude observed in the heavy domain for both males and females. Together, this integrative data set suggests that the mechanism(s) for greater neuromuscular fatigue-resistance in females reside within the musculature.

Incremental Test & Power-Duration Relationship

As expected, males produced greater absolute power outputs during cycling for variables such as P_{max} and CP. Female P_{max} was 66% of male values, similar to previous reports of 68% (Sundberg *et al.,* 2016). This sex difference in maximum power was still evident when P_{max} was normalised to body mass ($W \cdot kg^{-1}$), which is likely a result of differences in body composition (Pate & O'Neill, 2007). A similar sex difference was observed for $\dot{V}O_{2max}$, with males having greater absolute values and values relative to body weight (Pate & O'Neill, 2007). However, allometric scaling of $\dot{V}O_{2max}$ to fat-free mass typically eliminates some of this sex difference, with differences in haemoglobin mass explaining the remainder of the sex differences (Joyner, 2017). The normalisation of CP with P_{max} presents a method of making inter-individual comparisons of the power-duration relationship. Indeed, when relative CP (% P_{max}) was compared, no sex difference was demonstrated. Previously, Sundberg *et al.* (2016) demonstrated no sex difference when profiling the power-duration relationship during cycling bouts across the extreme and severe intensity domains (bout durations: 8-283 s), as well as the three-minute 'all-out' test. The present study corroborates this evidence and extends the conclusion to when CP is assessed using multiple severe intensity exercise trials to exhaustion. Collectively, these data obtained from cycling assessments conflict with equivalent data obtained in an isometric exercise setting where females had a ~7% greater critical torque compared to males (Ansdell *et al.*, 2019*a*, 2019*b*) . This discrepancy is likely explained by the modality of exercise.

Critical power during cycling is considered to be limited by oxygen delivery to the working muscle, a result of convective and diffusive capacity (Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012; Broxterman *et al.*, 2015*a*; Goulding *et al.*, 2017), therefore, during single-limb exercise, where convective factors are not a limiting factor (i.e. Ansdell *et al.*, 2019*a*), the sex difference in critical torque could be a result of greater diffusive capacity of female muscle. For example, it is well established that females have greater capillarisation and type I fibre proportional area of the knee-extensors (Roepstorff *et al.*, 2006), which could permit a greater rate of oxygen extraction and utilisation, and a greater relative critical torque. Whereas during locomotor exercise in the present study, knee-extensor blood flow is limited due to the modality of exercise (Calbet, 2000), therefore, convective capacity becomes the primary determinant of CP, rather than diffusive capacity of the muscle, leading to the lack of sex difference in relative CP. Other contributing factors to the lack of sex difference in CP could be that haemoglobin concentrations are typically ~12% lower (Murphy, 2014), and

lung volumes are smaller (Schwartz *et al.*, 1988) in females than males. The negative consequence of these factors is that females are more prone to exercise-induced arterial hypoxemia (Harms *et al.*, 1998), meaning that when cardiac output is near maximal (i.e. at task failure within the severe intensity domain), there is no possibility for increased oxygen extraction. This leads to a reduced arterio-venous oxygen difference, which has been suggested to negate the sex difference in muscle fatigability (Dominelli *et al.*, 2017) and could conceivably oppose the positive aspects of greater type I muscle fibre proportion on CP (Mitchell *et al.*, 2018*a*), leading to a lack of sex difference.

Severe Intensity Exercise

The power-duration relationship successfully predicted time to task failure in the exercise trial at 110% CP (Table 2), and the $\dot{V}O_2$ response to exercise confirmed that exercise was indeed in the severe intensity domain. The end-exercise $\dot{V}O_2$ was equivalent with $\dot{V}O_{2max}$ and demonstrated a gradual increase throughout exercise, indicating the presence of a considerable slow component. Fatigability within the severe intensity domain is consistently associated with depletion of high-energy phosphates and an accumulation of metabolites within the exercising musculature (Jones *et al.*, 2008; Black *et al.*, 2016; Vanhatalo *et al.*, 2016), which may reduce the contractile capacity of exercised musculature until the attainment of a limiting degree of disruption (Amann, 2011; Burnley & Jones, 2018). In contrast to our hypothesis, and despite no difference in time to task failure when exercise intensity was CP-matched, females demonstrated greater fatigue-resistance of the knee-extensors compared to males immediately after the exercise (21% difference in $Q_{tw.pot}$ reduction). There are multiple factors that could explain this sex difference from the present study and previous data. For example, as previously mentioned, females typically have a greater proportional area of type I muscle fibres (Staron *et al.*, 2000; Roepstorff *et al.*, 2006), and whilst in the context of this study it might not contribute to differences in the power-duration relationship, it could provide females with the capacity to tolerate deleterious metabolites when exercising above CP. Similarly, previous studies using pMRS during 'all-out' exercise have shown lower decreases in muscle pH, PCr, and attenuated increases in ADP (Russ *et al.*, 2005; Willcocks *et al.*, 2010). Currently, it is unknown whether a lesser accumulation of metabolites occurs in the severe intensity domain for females, or whether the sex differences in contractile properties allows lesser peripheral fatigue for equivalent metabolic stress. Slower sarcoplasmic reticulum calcium ATPase and uptake activity in females (Harmer *et al.*, 2014) could reflect a more fatigue-resistant contractile apparatus. Additionally, females demonstrated a smaller increase in rmsEMG during the severe intensity task, and while this has some limitations as a measure of neural activity (Farina *et al.*, 2004), it could represent a reduced rate of increase in neural drive because of less

fatigue within the already-recruited motor units than males (Vigotsky *et al.*, 2018). Indeed, this finding mirrors previous data (Ansdell *et al.,* 2019*a*), and exists when rmsEMG is normalised to M_{max} to negate the influence of subcutaneous fat on the EMG signal (Lanza *et al.*, 2018).

Another potential explanation of the lesser degree of $Q_{tw.pot}$ decrease in females could be unearthed when exercise is considered using absolute (i.e. 286 vs. 197 W), rather than relative (110% CP) values, given that the power-duration relationship was not different between sexes (i.e. similar relative values, Table 1). The power output for CP, and W′ values were both ~30% lower for females compared to males, and therefore TTF was not different, despite the lower amount of work done for females. Evidence suggests that W′ is positively related to the cross-sectional area (CSA) of the exercising muscle (Miura *et al.*, 2002; Kordi *et al.*, 2018), and whilst the present study did not measure CSA of the thigh musculature, it is established that females have 25-30% smaller knee extensors and flexors (Behan *et al.*, 2018). Similarly, Schäfer *et al.* (2019) demonstrated a positive relationship between W′ and $Q_{tw.pot}$ decrease following severe intensity exercise. Therefore, in the present study, it is possible that the larger muscle CSA in males likely permitted a greater absolute W′, which consequently elicited a greater decrease in $Q_{tw.pot}$ when severe intensity exercise was performed to task failure. Indeed, when the decrease in $Q_{tw.pot}$ was normalised to W′, no sex difference was observed, providing some support to the possibility that the sex difference in $Q_{tw.pot}$ decrease was a result of the greater absolute workload performed by males.

A final potential factor contributing to the sex difference observed in $Q_{tw.pot}$ decline, could be the greater oxygenation within the VL for females during exercise (Figure 3). In both Ansdell *et al.* (2019*a*) and the present study, this manifested predominantly as a lesser rise in HHb concentration for females during both heavy and severe intensity cycling, which could be a result of the fibre type difference between males and females. Specifically, the greater HHb increase in males could be a result of greater oxygen extraction (Grassi *et al.*, 2003), which could be related to a greater oxygen cost within the muscle ($m\dot{V}O_2$) compared to females. Indeed, when assessed at a pulmonary level, individuals with greater type I fibre proportion of the VL demonstrate a lower $\dot{V}O_2$ for a given exercise intensity (Coyle *et al.*, 1992). This is speculative, although potentially fertile ground for future research as $m\dot{V}O_2$ can be non-invasively quantified with a combination of NIRS and muscle occlusion (Ryan *et al.*, 2012); a combined approach to pulmonary and muscle $\dot{V}O_2$ kinetics could permit further insight into to the integrative response to exercise in males and females (Poole & Jones, 2012). One might expect pulmonary $\dot{V}O_2$ to reflect a potential sex difference in $m\dot{V}O_2$; however, females experienced a similar $\dot{V}_E$ to males during severe intensity cycling, which has previously been linked with a greater oxygen cost of breathing in females (Witt *et al.*, 2007). When measured

at the pulmonary level, the $\dot{V}O_2$ response to exercise is an amalgamation of all physiological systems, therefore the elevated W*b* might have counterbalanced the reduced m$\dot{V}O_2$ in females, leading to no sex difference in pulmonary $\dot{V}O_2$ values attained in the present study. Together, the aforementioned data present evidence that whilst exercise performance (TTF) in the severe intensity domain is not affected by sex, the integrative response differs between males and females. Females experience lesser decline in $Q_{tw.pot}$, potentially because of differences in muscle oxygenation and Ca^{2+} kinetics. Despite this, females likely have a greater W*b* when exercise intensity is CP-matched. Collectively, these data imply that even though TTF was not different, the mechanisms underpinning severe intensity exercise tolerance might differ between males and females.

Although a reduction in voluntary activation occurred during this trial for both sexes, excitability of the corticospinal tract was unaltered at the cortical and spinal level, suggesting that responsiveness of descending neurons did not change post-exercise (Weavil & Amann, 2018). Therefore, the central nervous system adjustments might have been a result of impaired neural drive, or synaptic input into the corticospinal tract (Amann, 2011). Regardless, these central adjustments are not considered to be the limiting factor to exercise within the severe intensity domain during cycling (Burnley & Jones, 2018). One caveat of the present study, and other locomotor neuromuscular fatigue studies is that responses were assessed post exercise during an isometric contraction (Sidhu *et al.*, 2013; Place & Millet, 2020). Responses evoked *during* exercise could elucidate further details about the time-course and magnitude of fatigue-related changes in activation.

Heavy Intensity Domain

The $\dot{V}O_2$ response to exercise at 90% CP was typical of heavy intensity exercise. The $\dot{V}O_2$ response exhibited a slow component, however only reached ~83% $\dot{V}O_{2max}$ at task termination, indicating that energy provision from aerobic sources was not maximal (i.e. exercise intensity was less than CP). In terms of the pre-post exercise change in neuromuscular function, the fatigue observed was not due to an accumulation of disruptive metabolites, or an exhaustion of high-energy phosphates as substrate-level phosphorylation reaches a steady-state (Black *et al.*, 2016; Vanhatalo *et al.*, 2016). Rather, neuromuscular fatigue in the heavy intensity domain is a result of both central and peripheral adjustments, with the latter occurring in response to depletion of intramuscular glycogen concentration and the associated negative consequences for excitation-contraction coupling (Ørtenblad *et al.*, 2013). Furthermore, reactive oxygen species generation, and extracellular accumulation of K^+ might also impair contractile function (Allen *et al.*, 2008). The net result in the present

study is a decrease in $Q_{tw.pot}$ (Figure 4B), which was less profound in females. Given that the mechanisms of peripheral adjustments differ above and below CP, this greater fatigue-resistance of female knee-extensors below CP must be a result of different physiological processes as well. One explanation could be that, given RER was lower in females compared to males during the 90% CP trial, the rate of fatty acid utilisation as a substrate was greater, eliciting a glycogen-sparing effect. This notion is supported by previous evidence demonstrating that males utilise ~25% more muscle glycogen at exercise intensities matched below CP (Tarnopolsky *et al.*, 1990; Roepstorff *et al.*, 2002, 2006; Devries *et al.*, 2006). The greater reliance on fat oxidation in females (RER), yet similar exercise economy ($\dot{V}O_2$) between males and females also implies a lower oxygen cost of exercise, as fat oxidation is less efficient compared to carbohydrate oxidation. To further support this, and similar to 110% CP, the decrease in muscle oxygenation was less in females at 90% CP, potentially reflecting a lower oxygen cost of contraction as a result of greater type I muscle fibre proportion.

The central nervous system adjustments occurring below CP are thought not to be a result of group III/IV afferent feedback, as there is no progressive metabolite accumulation (Burnley & Jones, 2018), instead, repetitive activation of motoneurons can alter their intrinsic properties, rendering them less responsive to activation (Carpentier *et al.*, 2001). This phenomenon is reflected in the present study as a decrease in VA_{MNS} and VA_{TMS}, with a greater decline in VA_{MNS} only for males. This discrepancy might indirectly suggest that the aetiology of the sex difference in central fatigue would be located at a sub-cortical level. Indeed, a decrease in MEP and SP-LEP was observed (Figure 5), and is likely a result of reduced strength of persistent inward currents (Heckman *et al.*, 2008). However, the sex × time interaction for these evoked variables was not significant ($p \geq 0.132$). Multiple studies have provided evidence to show reduced motoneuronal excitability with fatigue in single-limb (Kennedy *et al.*, 2016; Finn *et al.*, 2018) and whole-body exercise modalities (Weavil *et al.*, 2016; Sidhu *et al.*, 2017), however, the present study is the first to match exercise intensity to critical power and assess the neural response. Interestingly, the decrease in LEP was only evident during the SP, with no change in unconditioned LEP (Figure 5). Finn *et al.* (2018) demonstrated a similar phenomenon in an isometric modality and suggested that SP-LEPs were more sensitive to intrinsic changes in motoneuronal properties, as inhibiting descending drive from the motor cortex (i.e. the TMS-SP Škarabot *et al.* 2019*b*) removes a confound of excitatory synaptic input to the motoneuron. The present data support this notion, as the unconditioned LEPs did not change, due to the compensatory effects of neural drive (rmsEMG in Table 2) on net motoneuronal output. Therefore, as only SP-LEPs changed, the central fatigue observed at 90% CP in the present study is likely a result of a

change in intrinsic properties of motoneurons, rendering them less responsive to synaptic input. As mentioned above, this occurred independent of sex for any evoked responses. It is possible that due to far smaller measurement error for VA_{MNS} compared to evoked potentials (Ansdell *et al.*, 2019*c*), a sex difference in motoneuronal excitability was not discernible due to a lack of statistical power. Collectively, these data suggested that the neuromuscular response to cycling at 90% CP is underpinned by decreases in central nervous system function and contractile impairment. Similar to severe intensity exercise, there was no sex difference in exercise duration, but the neuromuscular adjustments were different between males and females, with greater oxygenation and less contractile dysfunction observed in females.

Further Considerations

Fatigability of both inspiratory and expiratory muscle groups was demonstrated above CP, which was not sex-dependent. This contrasts with previous evidence suggesting that the diaphragm is a more fatigue-resistant muscle in females (Guenette *et al.*, 2010; Welch *et al.*, 2018), however, the assessment modality employed in the present study was not able to provide information on the individual muscles or mechanisms responsible for the reduced pressures observed post-exercise. The rise in rmsEMG for respiratory musculature was similar between sexes in both trials, which also contradicts previous findings suggesting females activate ‘accessory’ respiratory muscle such as the SCM to reduce the diaphragmatic load (Mitchell *et al.*, 2018*b*). Whilst no sex difference in respiratory muscle fatigability or gas exchange were observed in the present study, the similar $\dot{V}_E$ values attained in the present study in males and females during both severe and heavy intensity cycling likely led to females experiencing a greater relative work of breathing during both trials (Dominelli *et al.*, 2015), which could contribute to greater exertional dyspnea (Schaeffer *et al.*, 2014; Cory *et al.*, 2015). When taken into consideration with the lesser degree of peripheral adjustments in locomotor muscles in females, it could conceivably be suggested that the ‘sensory tolerance limit’ consists of different magnitudes of afferent feedback from different physiological systems in males and females (Hureau *et al.,* 2018, Thomas *et al.,* 2019); such that the locomotor muscle component is less, but the respiratory component is greater in females (Cory *et al.*, 2015).

To compare fatigability in different populations, it is necessary to match both the intensity of exercise and the training status of the populations. The former was addressed in the present study by normalising exercise intensity to CP. Attempts were made to recruit populations of

males and females of equivalent training status (De Pauw *et al.*, 2013; Decroix *et al.*, 2016), which resulted in similar average performance levels between groups. However, the sex difference in relative $\dot{V}O_{2max}$ was ~25%. This is larger than the sex difference suggested for sexes of equivalent training status (~10%, Joyner 2017), although this was based off a mixture of studies and a small sample of n = 8 male and 15 female elite distance runners (Pate & O'Neill, 2007). Other sources have previously described larger magnitudes in this sex difference (e.g. 17%, Froberg & Pedersen, 1984), although similarly rely on small sample sizes (n = 6 females and n = 7 males). Indeed, a meta-analysis of 440 male and 381 female participants that demonstrated an average sex difference of 28% in $\dot{V}O_{2max}$ when expressed relative to body mass; this difference remained in trained vs. untrained populations when body composition was accounted for (Sparling, 1980). Nevertheless, there appears to be a discrepancy in what researchers deem to be an appropriate magnitude for the sex difference in $\dot{V}O_{2max}$. The present study used a minimum performance level (De Pauw *et al.*, 2013; Decroix *et al.*, 2016) to account for $\dot{V}O_{2max}$, relative P_{max}, as well as training history (hours·week^{-1}), and the sex differences demonstrated are therefore assumed to be independent of training status. However, as is well established, training status influences aerobic fitness, therefore this discrepancy in the appropriate magnitude of sex difference in $\dot{V}O_{2max}$ highlights a potential limitation in the present study, if the differences in indices of aerobic fitness are considered to be of too great a magnitude. The precise measurement of, and normalisation of values to fat-free mass could be an area for future research in order to uncouple the effects of sex and muscle mass in the field of integrative exercise physiology.

Finally, NIRS signals can be influenced by subcutaneous adipose tissue thickness, which manifests as a reduction in the concentration of haeme compounds (Van Beekvelt *et al.*, 2001; Bopp *et al.*, 2011; Bowen *et al.*, 2013). It is well-established that females typically have a greater amount of subcutaneous adipose tissue (Westerbacka *et al.*, 2004). The system used in the present study was a spatially-resolved spectroscopy system, which enhances the signal from deeper tissues, whilst reducing the contribution from superficial tissues (i.e. skin and subcutaneous fat; Messere & Roatta, 2013). Additionally, this form of NIRS system provides a relative index of tissue oxygenation (TOI), in which both the numerator (HbO_2) and denominator (HbO_2 + HHb) are equally affected by adipose tissue thickness, therefore a correction might not be necessary (Barstow, 2019). Despite adipose tissue's established effects on HbO_2 and HHb values, it is currently unknown whether this also affects the sensitivity of the technique to changes induced by exercise. Therefore, the NIRS data presented in this study must be considered within this context.

Conclusions

This study demonstrated that the power-duration relationship for cycling did not differ between males and females when expressed relative to P_{max}. Subsequent exercise performance in the severe and heavy intensity domains was not different, however the integrative response of cardiopulmonary, respiratory, and neuromuscular systems differed. Specifically, muscle de-oxygenation and contractile impairment was less in females during both tasks, potentially related to skeletal muscle size and composition. Additionally, the decline in central nervous system function was attenuated for females in the heavy intensity domain. Collectively, the present data show that the integrative response of physiological systems differs between males and females, which has important implications for acute and chronic exercise prescription.

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Additional Information

Author Contributions

PA, KMH, KT, SKH, GH, and SG contributed to the conception and design of the work; PA, JS, AT, SC, EA collected the data; PA analysed the data; PA, JS, KMH, KT, SKH, GH and SG interpreted the data; PA drafted the manuscript; PA, JS, AT, SC, EA, KMH, KT, SKH, GH, and SG all revising the manuscript.

All authors approved the final version of the manuscript, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Competing Interests

The authors declare no conflict of interest, financial or otherwise.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Tables

***Table 1**. Participant demographics, comparison of the results from the incremental exercise test, and power-duration relationship modelling in males and females.*

	Males	Females	P value
N	10	8	n/a
Age (years)	25 ± 5	25 ± 6	0.836
Stature (cm)	178 ± 9	169 ± 9	0.054
Mass (kg)	67.0 ± 8.8	63.3 ± 7.2	0.325
Training (h.week^{-1})	9 ± 3	10 ± 5	0.581
	Incremental Test		
$\dot{V}O_{2max}$ (L·min^{-1})	4.02 ± 0.47	2.85 ± 0.51	*< 0.001*
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	60.5 ± 8.2	45.1 ± 6.3	*< 0.001*
P_{max} (W)	362 ± 29	241 ± 42	*< 0.001*
P_{max} (W·kg^{-1})	5.5 ± 0.6	3.8 ± 0.5	*< 0.001*
	Power-Duration Relationship		
CP (W)	260 ± 28	179 ± 32	*< 0.001*
CP (W·kg body mass)	3.9 ± 0.7	2.8 ± 0.5	*< 0.001*
CP (% P_{max})	72 ± 5	74 ± 2	0.210
W′ (J)	18,515 ± 4,831	12,684 ± 3,155	*0.009*
W′ (J·kg body mass)	276 ± 65	197 ± 41	*0.009*
W′ (J·P_{max}^{-1})	51 ± 11	52 ± 10	0.733
r^2	0.98 ± 0.02	0.96 ± 0.02	
CP SE (%)	2 ± 1	3 ± 2	
W′ SE (%)	7 ± 4	8 ± 3	

$\dot{V}O_{2max}$ = maximal oxygen uptake; P_{max} = maximal power; CP = critical power; W′ = curvature constant; SE = standard error

***Table 2**. Changes throughout exercise above critical power for pulmonary gas exchange, EMG, and pulmonary function variables. * indicates significantly different from pre-exercise (P < 0.05). $ = significantly different from females.*

		Severe Intensity					Heavy Intensity				
Time to Task Failure/Termination	Males	752 ± 329					3073 ± 835				
(s)	Females	681 ± 277					2937 ± 964				
		Pulmonary Gas Exchange									
		Pre-Exercise	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*	*Pre-Exercise*	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*
$\dot{V}O_2$	Males	19 ± 4	87 ± 6*	93 ± 5*	95 ± 7*	98 ± 4*	17 ± 3	76 ± 6*	78 ± 6*	78 ± 5*	81 ± 5*
(%$\dot{V}O_{2max}$)	Females	18 ± 3	82 ± 6*	87 ± 5*	93 ± 6*	98 ± 4*	18 ± 3	76 ± 7*	79 ± 7*	81 ± 7*	84 ± 6*
$\dot{V}CO_2$	Males	15 ± 2	76 ± 12*	78 ± 12*	79 ± 12*	81 ± 12*	15 ± 2*	64 ± 9*	63 ± 9*	64 ± 9*	65 ± 9*
(%$\dot{V}O_{2max}$)	Females	16 ± 2	77 ± 6*	82 ± 6*	84 ± 7*	86 ± 6*	16 ± 4*	63 ± 8*	63 ± 6*	66 ± 6*	68 ± 6*
$\dot{V}_E$	Males	15 ± 3	76 ± 12*	78 ± 12*	79 ± 12*	81 ± 12*	14 ± 3	52 ± 8*	56 ± 8*	59 ± 7*	64 ± 10*
(%$\dot{V}O_{2max}$)	Females	16 ± 2	77 ± 6*	82 ± 6*	84 ± 7*	86 ± 6*	20 ± 2	66 ± 5*	68 ± 8*	72 ± 7*	77 ± 7*
RER	Males	0.92 ± 0.04	1.01 ± 0.11	0.98 ± 0.09	0.95 ± 0.06	0.95 ± 0.07	1.00 ± 0.10	0.96 ± 0.05$	0.94 ± 0.07	0.95 ± 0.07	0.93 ± 0.05$
($\dot{V}O_2$ / $\dot{V}CO_2$	Females	0.95 ± 0.06	1.02 ± 0.06	1.00 ± 0.05	0.97 ± 0.06	0.94 ± 0.05	0.97 ± 0.14	0.90 ± 0.05	0.88 ± 0.06	0.89 ± 0.05	0.88 ± 0.05
		Muscle Activation									
		Start-Exercise	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*	*Start-Exercise*	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*
Vastus Lateralis	Males	3.3 ± 1.6	4.2 ± 2.0*	4.5 ± 2.2*	5.2 ± 2.0*	5.7 ± 1.9*	4.3 ± 3.8	4.7 ± 3.8	4.6 ± 3.3	5.3 ± 4.2*	5.1 ± 3.7*
(rmsEMG·M_{max}^{-1})	Females	3.7 ± 1.4	4.5 ± 1.6*	5.0 ± 1.6*	5.0 ± 1.7*	5.2 ± 1.9*	2.9 ± 1.1	3.2 ± 1.2	3.4 ± 1.2	3.4 ± 1.5	3.4 ± 1.4
Sternocleidomastoid	Males	11.6 ± 8.8	18.4 ± 9.4	26.2 ± 14.1	33.3 ± 16.0	44.5 ± 22.2	10.6 ± 8.0	11.6 ± 8.2	11.9 ± 8.8	15.1 ± 13.6	15.1 ± 11.1
(% rmsMaxInsp)	Females	16.9 ± 10.5	23.4 ± 12.3	24.9 ± 10.0	28.4 ± 11.7	36.2 ± 11.7	12.9 ± 5.0	19.9 ± 12.8	19.9 ± 12.3	17.9 ± 9.4	19.5 ± 10.8
External Intercostal	Males	19.5 ± 9.9	27.9 ± 13.4	34.4 ± 16.3	37.1 ± 17.9	49.6 ± 26.1	10.3 ± 6.5	12.6 ± 13.5	13.5 ± 6.5	13.6 ± 7.8	15.2 ± 7.1
(% rmsMaxExp)	Females	38.5 ± 18.4	55.5 ± 38.9	54.9 ± 38.9	62.15 ± 38.6	61.0 ± 34.7	24.9 ± 8.3	31.0 ± 11.7	30.2 ± 12.5	29.5 ± 14.2	33.9 ± 17.6
		Maximal Pulmonary Pressures									
		Pre-Exercise			*Post-Exercise*		*Pre-Exercise*			*Post-Exercise*	
Maximum Expiratory Pressure	Males	197 ± 52			171 ± 48*		174 ± 39			157 ± 31	
(mmHg)	Females	143 ± 37			129 ± 38*		138 ± 34			136 ± 40	
Maximum Inspiratory Pressure	Males	130 ± 37			118 ± 33*		140 ± 53			135 ± 50*	
(mmHg)	Females	113 ± 29			104 ± 25*		138 ± 34			136 ± 40	

$\dot{V}O_2$ = respiratory oxygen uptake; $\dot{V}CO_2$ = carbon dioxide production; $\dot{V}_E$ = expired ventilation; RER = respiratory exchange ratio; rmsEMG·M_{max}^{-1} = root-mean-square electromyographic activity normalised to maximal compound action potential amplitude; MaxInsp = maximal inspiratory pressure; MaxExp = maximal expiratory pressure, TTF = time to task failure

Figure Legends

Figure 1. *Neuromuscular function before and after exercise at 110% CP. Panel A: Maximum voluntary contraction (MVC), Panel B: Potentiated quadriceps twitch ($Q_{tw.pot}$), Panel C: Voluntary activation with motor nerve stimulation (VA_{MNS}), Panel D: Voluntary activation with transcranial magnetic stimulation (VA_{TMS}). * indicates a greater decrease in males than females ($p < 0.05$). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

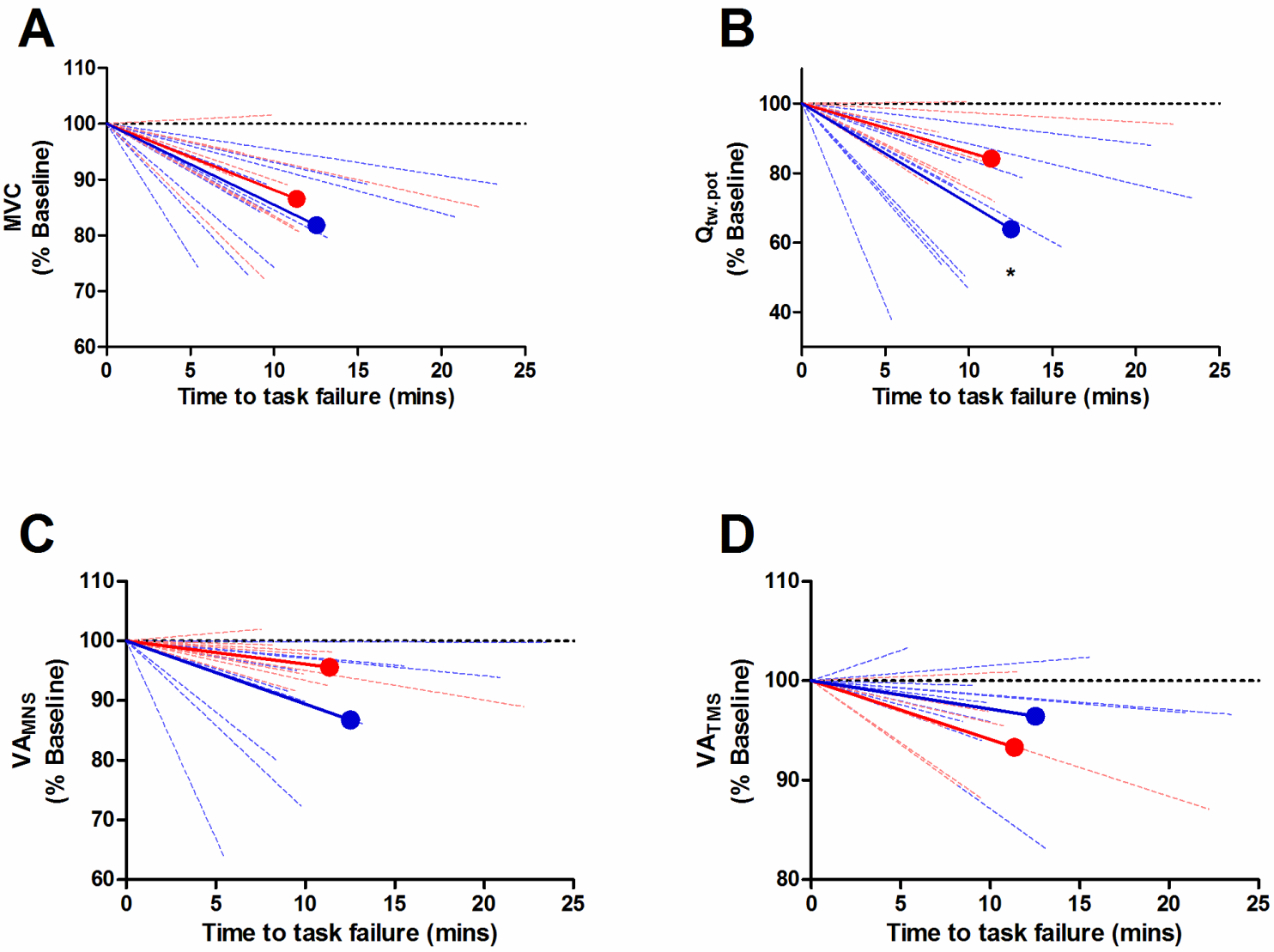
Figure 2. *Indices of neural excitability before and after exercise at 110% CP. Panel A: Motor evoked potentials (MEP) amplitude normalised to maximal compound action potential amplitude (MEP/Mmax), Panel B: Lumbar evoked potentials (LEP) amplitude normalised to Mmax (LEP/Mmax), Panel C: Conditioned lumbar evoked potentials (SP-LEP), Panel D: Short-interval intracortical inhibition (SICI). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

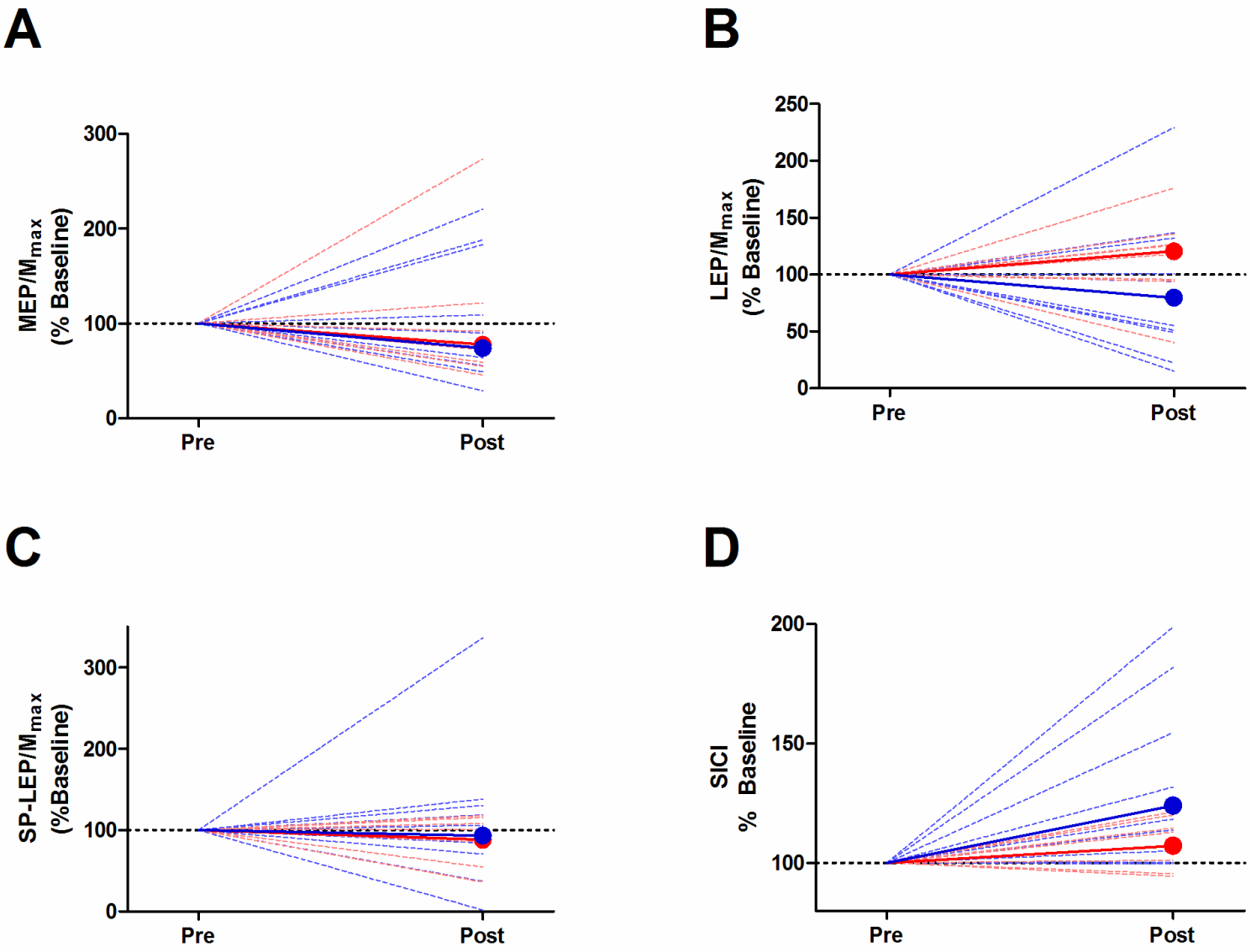
Figure 3. *Indices of muscle oxygenation throughout exercise at 110% CP. Panel A: Oxyhaemoglobin (O_2Hb), Panel B: Deoxyhaemoglobin (HHb), Panel C: Tissue oxygenation index (TOI). * = greater in males than females ($p < 0.05$). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

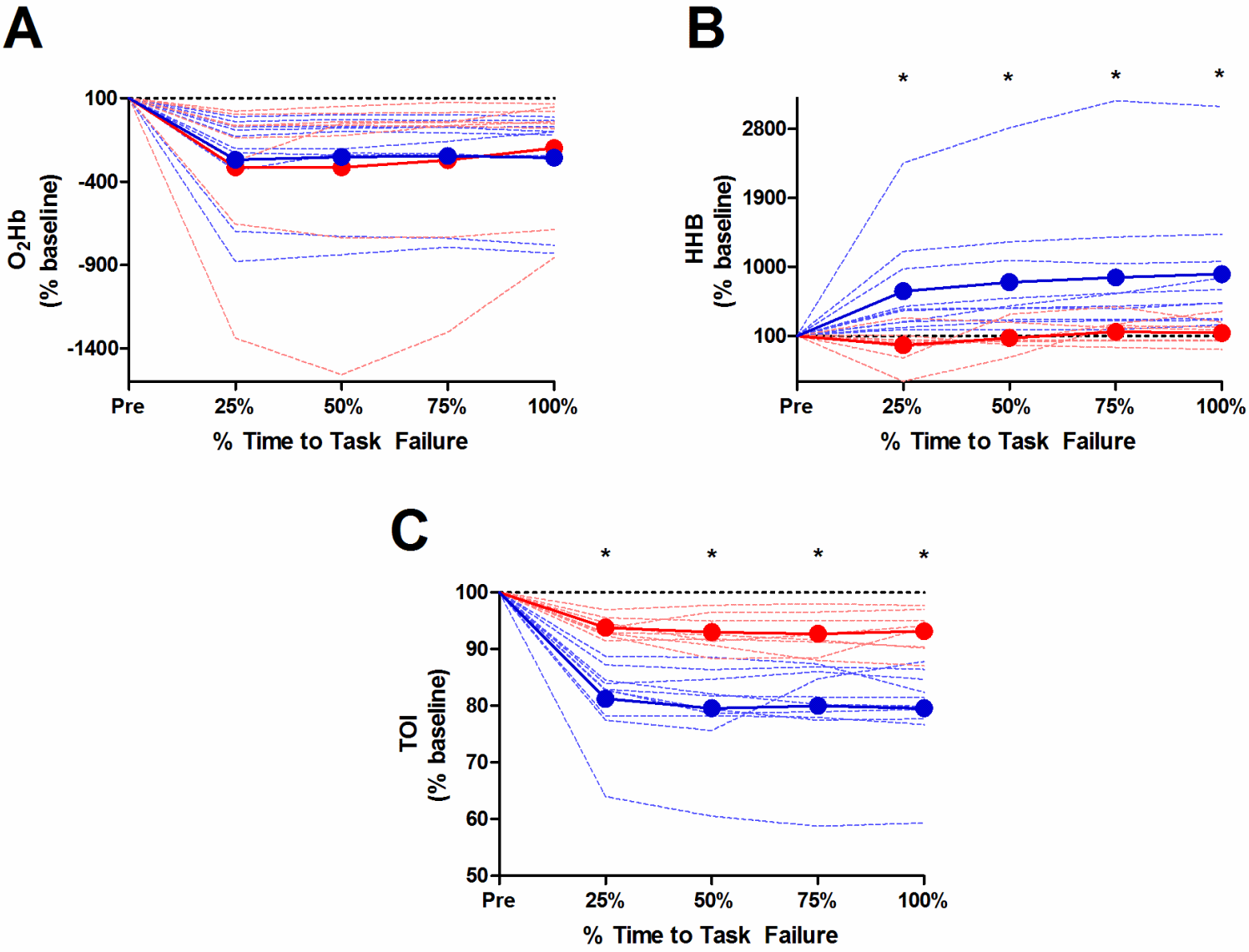
Figure 4. *Neuromuscular function changes relative to baseline for exercise at 90% CP. Panel A: Maximum voluntary contraction (MVC), Panel B: Potentiated quadriceps twitch ($Q_{tw.pot}$), Panel C: Voluntary activation with motor nerve stimulation (VA_{MNS}), Panel D: Voluntary activation with transcranial magnetic stimulation (VA_{TMS}). * indicates a greater decrease in males than females ($p < 0.05$). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

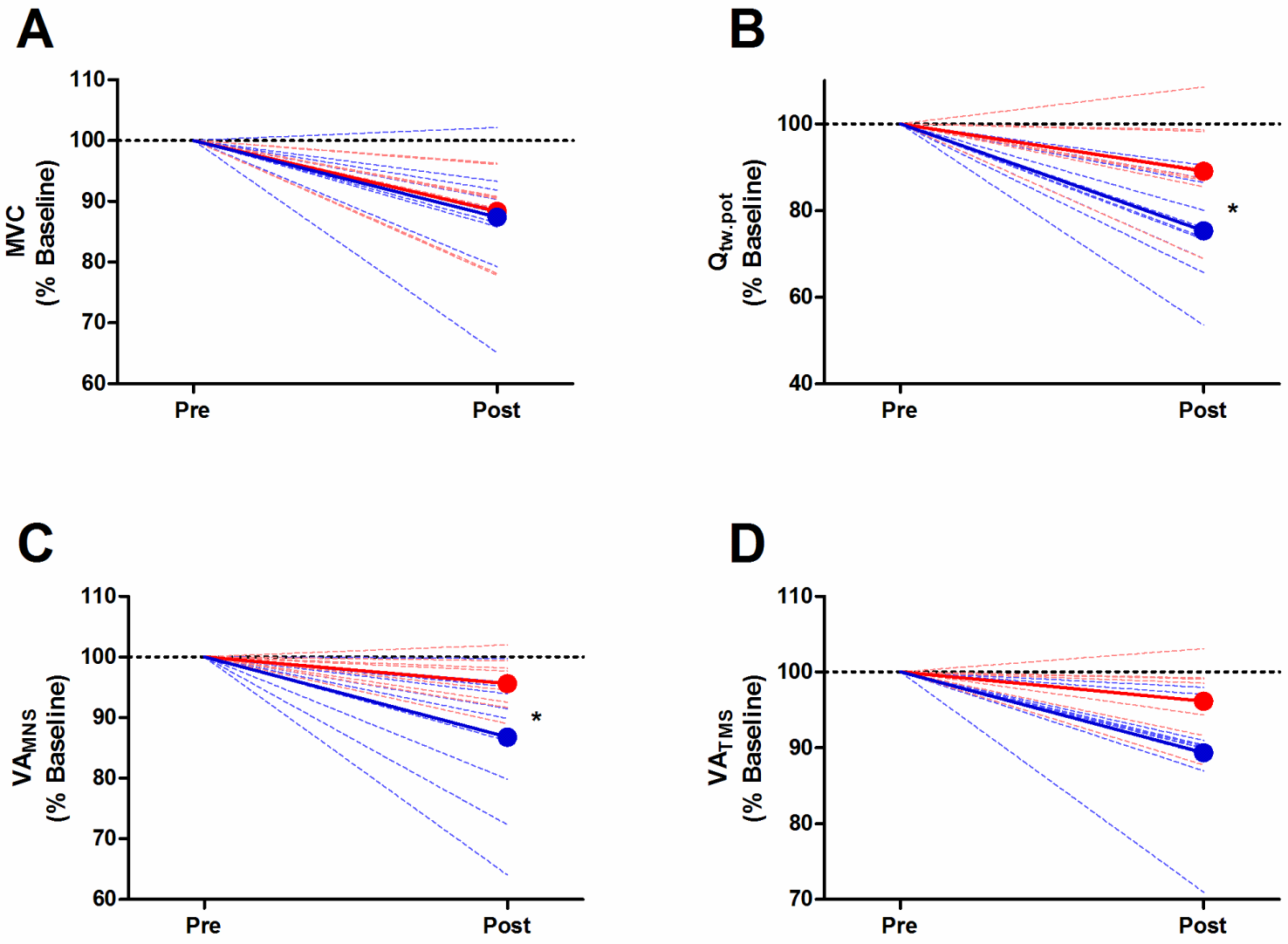
Figure 5. *Indices of neural excitability before and after exercise at 90% CP. Panel A: Motor evoked potentials (MEP) amplitude normalised to maximal compound action potential amplitude (MEP/Mmax), Panel B: Lumbar evoked potentials amplitude normalised to Mmax amplitude (LEP/Mmax), Panel C: Conditioned lumbar evoked potentials amplitude (SP-LEP), Panel D: Short-interval intracortical inhibition (SICI). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

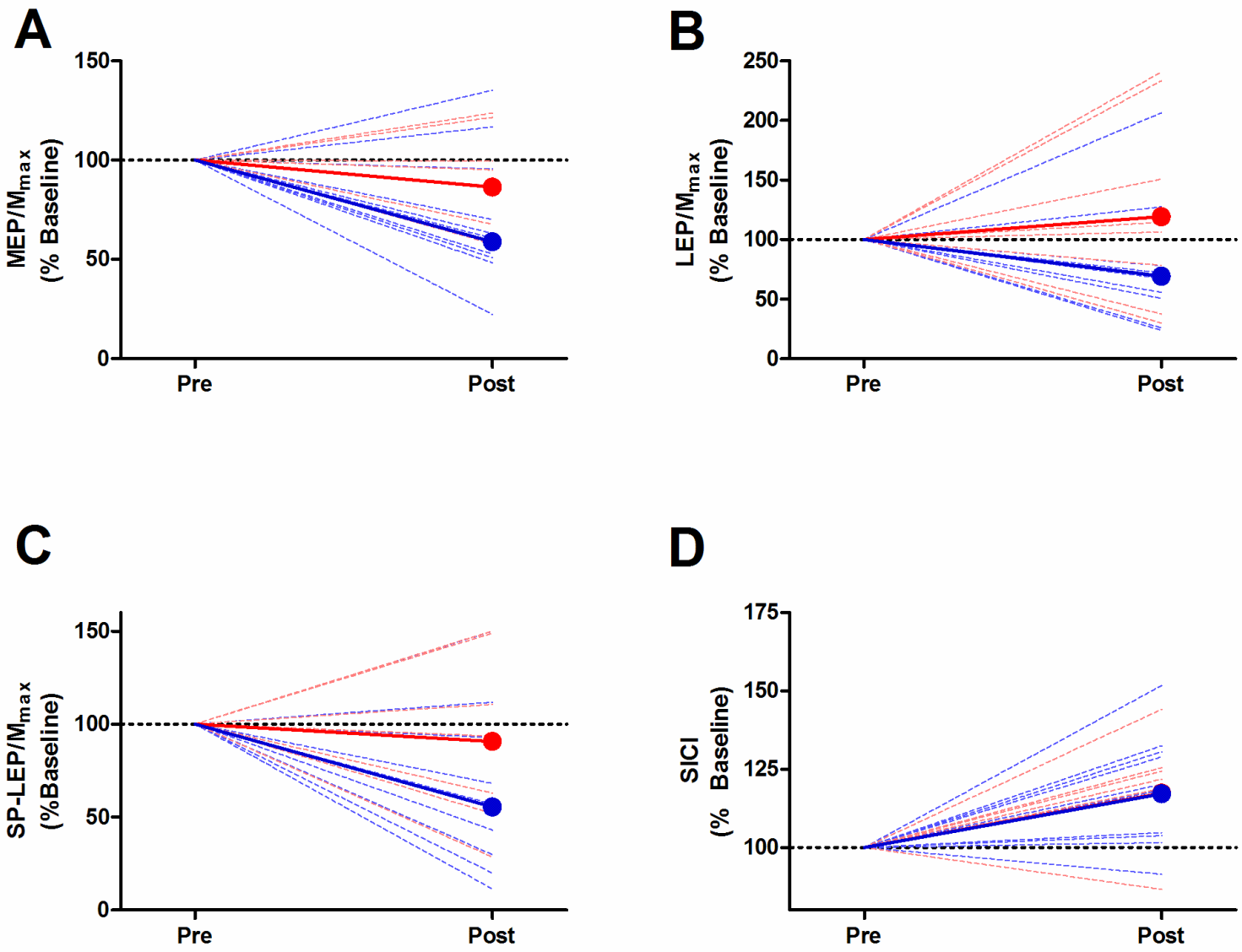
Figure 6. *Indices of muscle oxygenation throughout exercise at 90% CP. Panel A: Oxyhaemoglobin (O_2Hb), Panel B: Deoxyhaemoglobin (HHb), Panel C: Tissue oxygenation index (TOI). * = greater in males than females ($p < 0.05$). Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.*

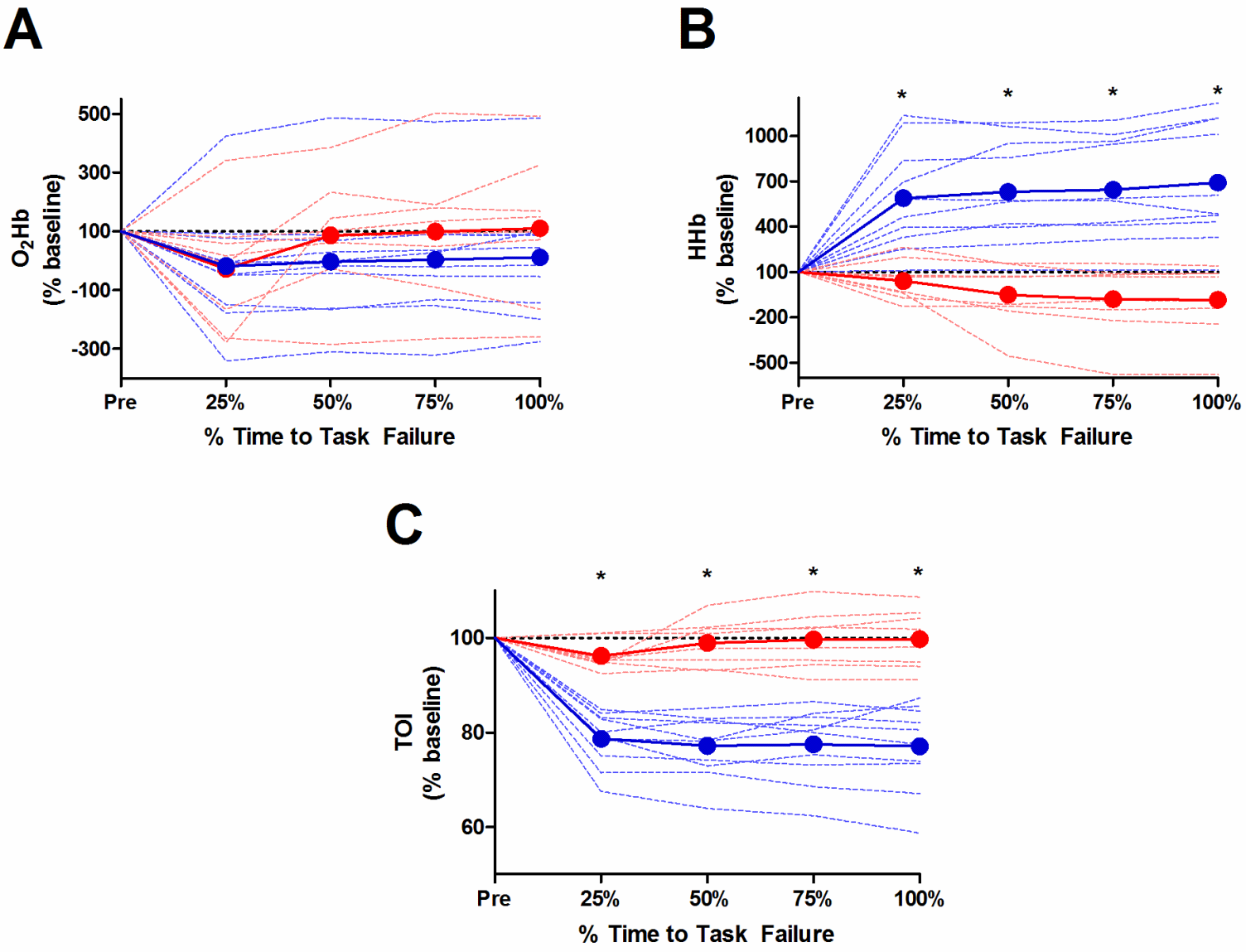

A
MVC
(% Baseline)
110
100
90
80
70
60
0
5
10
15
20
25
Time to task failure (mins)
B
Qtw.pot
(% Baseline)
100
80
60
40
*
0
5
10
15
20
25
Time to task failure (mins)
C
VAMNS
(% Baseline)
110
100
90
80
70
60
0
5
10
15
20
25
Time to task failure (mins)
D
VATMS
(% Baseline)
110
100
90
80
0
5
10
15
20
25
Time to task failure (mins)


A
MEP/Mmax
(% Baseline)
300
200
100
0
Pre
Post
B
LEP/Mmax
(% Baseline)
250
200
150
100
50
0
Pre
Post
C
SP-LEP/Mmax
(%Baseline)
300
200
100
0
Pre
Post
D
SICI
% Baseline
200
150
100
Pre
Post


A
100
-400
-900
-1400
O2Hb
(% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure
B
*
*
*
*
2800
1900
1000
100
HHB
(% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure
C
*
*
*
*
100
90
80
70
60
50
TOI
(% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure


A
MVC
(% Baseline)
110
100
90
80
70
60
Pre
Post
B
$Q_{tw.pot}$
(% Baseline)
100
80
60
40
*
Pre
Post
C
VA_{MNS}
(% Baseline)
110
100
90
80
70
60
*
Pre
Post
D
VA_{TMS}
(% Baseline)
110
100
90
80
70
Pre
Post


A
MEP/Mmax
(% Baseline)
150
100
50
0
Pre
Post
B
LEP/Mmax
(% Baseline)
250
200
150
100
50
0
Pre
Post
C
SP-LEP/Mmax
(%Baseline)
150
100
50
0
Pre
Post
D
SICI
(% Baseline)
175
150
125
100
Pre
Post


A
500
300
100
-100
-300
O_2Hb (% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure
B
*
*
*
*
1000
700
400
100
-200
-500
HHb (% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure
C
*
*
*
*
100
80
60
TOI (% baseline)
Pre
25%
50%
75%
100%
% Time to Task Failure