The effect of sex on the cardiopulmonary and neuromuscular response to high-intensity interval exercise

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Abstract:

Sex differences exist in the integrative response to exercise, however, these are typically researched during incremental and constant-load exercise. Interval exercise involves high-intensity efforts interspersed with recovery periods to repeatedly stress physiological systems, and it is currently unknown whether the response to this form of exercise differs between sexes.

Ten males and ten females (age: 25±3 years) completed two experimental visits. First, an incremental treadmill exercise test was performed to obtain submaximal (lactate threshold) and maximal ($\dot{V}O_{2peak}$) data. Thereafter, visit two involved 4 × 3-min running intervals at 90% of the final incremental test velocity ($v\dot{V}O_{2peak}$), with 90 secs rest between intervals. Before exercise and after each interval, maximal voluntary contraction (MVC), quadriceps potentiated twitch ($Q_{tw.pot}$), and voluntary activation (VA) were recorded. The rates of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and ventilation ($\dot{V}_E$) were continuously recorded throughout.

There was no sex difference in relative $\dot{V}O_{2peak}$ (males: 47.2±6.0 vs. females: 44.4±5.8 $ml \cdot kg^{-1} \cdot min^{-1}$, p=0.292). When expressed relative to peak values, there were no sex differences in the $\dot{V}O_2$ or $\dot{V}CO_2$ response to the interval task (p≥0.781). Females had greater $\dot{V}_E/\dot{V}O_2$, and $\dot{V}_E/\dot{V}CO_2$ values during the first (p≤0.034) and second ($\dot{V}_E/\dot{V}CO_2$, p=0.006) intervals, with a sex × time interaction effect (p≤0.046). There were no sex differences in the reductions in MVC, $Q_{tw.pot}$, and VA during the interval task (p≥0.150), however females had lesser reductions in $Q_{tw.pot}$ values post-exercise (-24±9 vs. -15±8%, p=0.044).

Sex differences exist in the physiological response to interval exercise. Compared to males, females experienced greater hyperpnoea during the initial stages, and had lesser decreases in contractile function post-exercise.

New and Noteworthy:

This study determined that males and females differ in the physiological response to high-intensity interval exercise. Specifically, females had poorer ventilatory efficiency during the first half of the task, but greater knee-extensor fatigue resistance following the task. These data build upon previous observations from constant-load exercise, demonstrating that physiological sex differences are observed during an ecologically valid exercise task commonly prescribed by practitioners in clinical and athletic populations.

Key words: Fatigue, female, gender, HIIT, male, ventilation.

Introduction

Females have historically been under-represented in sport science studies for a multitude of reasons [1, 2]. As a result, assumptions about exercise training have been generalised from male-dominated research and applied to females. It is becoming increasingly evident that the acute physiological responses to various modalities of exercise differ between the sexes [3], meaning this approach might not be optimal for the prescription of exercise to females. Research is needed to identify whether males and females respond to commonly prescribed forms of exercise similarly, in order to optimise training and performance for both sexes.

Morphological and anatomical sex differences in key physiological systems are thought to lead to the differences in the integrative response to exercise between sexes. For instance, males typically have a greater quantity of muscle mass and can generate larger maximal force, but experience greater proportional occlusion of limb blood flow during muscle contraction [4]. Whilst not consistent across all muscle groups [5, 6], females have consistently demonstrated a higher proportional area of type I muscle fibres of the knee-extensors [7], as well as greater capillary density [8], and vasodilatory response of the femoral artery [9]. These physiological sex differences have previously been suggested to aid in oxygen delivery and help delay the onset of fatigue in females [3, 10]. Despite the potentially superior aerobic muscular phenotype, it has been established that females have smaller lung volumes, airway size, and alveolar surface for gas exchange, even when matched for stature [11-14]. These morphological differences lead to a greater work and oxygen cost of breathing at elevated ventilatory rates [14, 15], resulting in an increased fraction of whole-body oxygen uptake ($\dot{V}O_2$) originating from the respiratory musculature in females compared to males [16]. This, combined with lower cardiac output and haemoglobin concentrations [17], means that females have a poorer O_2 carrying capacity during exercise [18, 19]. The balance between the importance of O_2 delivery and utilisation depends on the physiological determinants of the task being performed, which is one reason why sex differences in the integrative response to exercise are not uniform and require further investigation [3, 20].

Previous studies considering single-limb contractions found that males experienced greater rates of fatigue than females [21-23] as well as males demonstrating a slower recovery time than females [23, 24]. Indeed, more recent evidence suggests that these sex differences in the response to single-limb exercise appear to be related to the proportion of myosin heavy chain I isoform and mitochondrial protein abundance [25]. Despite this evidence in single-limb models, these findings do not necessarily translate into whole-body locomotion [26]. In particular, the literature comparing the physiological response to locomotor exercise

between sexes is less comprehensive. Several studies have demonstrated that female knee-extensors experience less fatigue following cycling to task failure at equivalent relative exercise intensities [27-29], or following fixed duration running [30] and cycling [31] tasks. Although these studies point towards a consistent mechanism for the sex difference in knee extensor fatigability, the tasks employed are limited in generalisability to athletic training outside of the controlled lab environment. Constant-load tasks, particularly to task failure, are rarely employed by those prescribing exercise for athletic enhancement. Recently, we demonstrated that the sex difference in knee extensor fatigability was evident following a self-paced 5 km running time trial [32], whilst others have demonstrated similar sex differences following longer distance running tasks longer than 40 km [33, 34]. Although Boccia, Dardanello [35] did not observe a sex difference in fatigability following a half marathon, suggesting that the difference is intensity and/or duration dependent.

High intensity interval training is a method often prescribed to enhance aerobic and anaerobic capacity [36]. Research that has previously investigated sex differences in the response to interval exercise typically utilises repeated sprints (5-6 secs bouts) interspersed with prolonged (25-30 secs) rest [37, 38]. This modality of training has implications for intermittent and team sport athletes, and researchers have previously suggested that the lesser fatigue experienced by females is related to lower absolute mechanical work [39]. Longer duration intervals are typically prescribed at submaximal, yet supra-threshold intensities (e.g., 2-6 min at 3 km-10 km race pace, Parmar *et al.,* 2021). In terms of exercise intensity domains, the intention of such intervals is to intersperse severe intensity bouts with periods of moderate intensity recovery or rest, and repeatedly elicit a state of metabolic stress [41]. Given that evidence from constant-load exercise indicates that female skeletal muscle is more resistant to fatigue during metabolically challenging tasks [27-29], it is important to understand how interspersing high-intensity bouts with recovery periods mediates the sex difference in fatigability and the integrative physiological response to exercise. The little evidence that does exist from cycling exercise suggests that there might also be perceptual, but not physiological sex differences in the response to high-intensity interval exercise [42]. As highlighted, both exercise modality and intensity mediate the influence of sex on the responses to exercise, therefore this study aimed to compare the physiological responses to, and recovery from, a bout of high-intensity interval running between sexes. We hypothesised that both sexes would experience a similar metabolic response to exercise, but female knee-extensors would be less fatigable.

Methods

Ethical Approval

This study received institutional ethical approval from the Northumbria University Health and Life Sciences Research Ethics Committee (submission reference: 2022-0094-368) and was conducted according to all aspects of the Declaration of Helsinki, apart from pre-registration in a public database. Participants volunteered for the study and provided written informed consent.

Sample Size Calculation

An *a priori* sample size calculation was performed using GPower (v3.0.0) using the effect size from Ansdell, Škarabot [27] for the sex difference in contractile dysfunction following high-intensity cycling ($\eta p^2 = 0.344$), and reliability data for the same variable from Ansdell, Brownstein [43]. With the parameters of $\alpha = 0.001$ and $1-\beta = 0.99$, the minimum sample size required was 12 participants (6 males, 6 females). Therefore, to maximise statistical power, 10 participants of each sex were recruited.

Participant Characteristics

Ten healthy males (mean ± SD age: 27 ± 4 years, stature: 180 ± 6 cm, body mass: 83.5 ± 12.1 kg) and ten healthy females (age: 23 ± 2 years, stature: 163 ± 6 cm, body mass: 62.9 ± 9.1 kg) whose gender matched their sex assigned at birth, volunteered for the study. All participants were free from musculoskeletal conditions, as well as neurological, respiratory, and cardiovascular disease. Hormonal status was not an exclusion criterion or controlled for in this study, based on evidence that the menstrual cycle or hormonal contraceptive usage do not influence the cardiopulmonary or neuromuscular responses to whole-body exercise [44, 45]. However, it must be acknowledged that perceptual responses to exercise might be influenced by menstrual cycle phase [46]. Five females were naturally cycling, with their second visits occurring on self-reported days 5, 8, 16, 19, and 23 of their menstrual cycle. Three females were using combined oral contraceptive pills; one female had a non-hormonal (or copper) intrauterine device, and one had a contraceptive implant. A screening questionnaire was used to ensure the participants met the inclusion criteria and training/activity status. Male and female participants reported 6.5 ± 4.1 hours and 5.8 ± 2.1 hours of structured physical activity per week (respectively) and 4.3 ± 1.8 hours and 2.8 ± 1.9 hours of unstructured physical activity per week (respectively). All participants were considered to be recreationally active as per the U.K. Chief Medical Officer's guidelines of at least 150 min of moderate intensity exercise or 75 min of vigorous intensity exercise.

Experimental Design

Participants visited the laboratory on two occasions. The first visit involved familiarisation with neuromuscular measures, followed by a treadmill incremental exercise test to the limit of tolerance. The second visit was the performance of a high-intensity interval exercise protocol, involving 4 × 3-min intervals at 90% of final incremental test velocity ($v\dot{V}O_{2peak}$), interspersed with 90 sec rest periods. This exercise paradigm was chosen as it has high ecological validity [40] as well as being an effective method of improving aerobic capacity over 4-8 weeks [47, 48]. Before exercise, and in each rest period, participants performed a neuromuscular assessment while blood lactate was sampled, and heart rate and rating of perceived exertion (RPE) were recorded. Following exercise, the neuromuscular measures were repeated at 10, 20, and 30 min.

Visit 1: Familiarisation & Incremental Exercise Testing

Participants were firstly familiarised with the neuromuscular stimulation techniques. This began with the determination of the femoral nerve stimulation threshold at rest (see details below), then the performance of warm up contractions increasing from 50% perceived effort to 90%. Participants then performed a neuromuscular function assessment (see details below).

Prior to the incremental exercise test, participants provided a capillary blood lactate sample from the earlobe, then performed a five-min warm up at an intensity corresponding to the first stage of the incremental test. Depending on each participant's sex and training history, the speed for the first stage was set between 6 – 10 $km \cdot h^{-1}$. This permitted a similar number of subsequent stages to be completed prior to the limit of tolerance for males and females (6 ± 2 vs. 6 ± 1, respectively, $p = 0.756$). After the warmup, participants were given one minute rest, where another blood lactate sample was taken. The incremental test then involved three-min stages, with the treadmill speed increased by 1 $km \cdot h^{-1}$ each stage [49]. Between stages, participants had 1 min of rest, where they provided further blood lactate samples. The treadmill was kept at a constant elevation of 1% throughout all testing and subsequent visits. Instructions were provided to "complete as many stages as possible", and participants were informed that they should jump to the sides of the treadmill if they could not maintain the running speed any longer. The speed of the final complete stage was recorded as $v\dot{V}O_{2peak}$, however, if participants were able to run for longer than 90 secs during the final incomplete stage, 0.5 $km \cdot h^{-1}$ was added to the speed at the final complete stage when recording $v\dot{V}O_{2peak}$. Throughout the incremental exercise test, breath-by-breath gas exchange was recorded continuously, with heart rate (Polar T31-coded chest strap & FT1 watch, Polar O.Y., Finland) and RPE (6-20 scale) recorded at the end of each stage.

Blood lactate samples were analysed immediately to determine lactate threshold and turnpoint (Biosen C-Line, EKF Diagnostics, Cardiff, UK). Lactate threshold was determined as the first work rate at which a non-linear increase in blood lactate concentration was observed, while lactate turnpoint was identified as the work rate that elicited a sudden and sustained increase in blood lactate concentration [50]. The two lactate thresholds were analysed independently by two experimenters, and if any disagreement occurred, a third experimenter was consulted in order to mediate.

Visit 2: High Intensity Interval Exercise

This visit began with the determination of the femoral nerve stimulation threshold, then warm up contractions increasing from 50% perceived effort to 90% were performed. Hereafter, a neuromuscular assessment was performed (see details below), before participants moved to the treadmill and provided a resting blood lactate sample.

Participants began the high-intensity interval exercise with a warmup consisting of a 5-min stage at 50% $v\dot{V}O_{2peak}$, and the final minute at 90% $v\dot{V}O_{2peak}$. After the warmup, participants rested for one min, while providing a blood lactate sample. The interval exercise involved 4 × 3-min intervals at 90% $v\dot{V}O_{2peak}$, interspersed with 90 secs of rest. At the end of each interval, participants moved from the treadmill, with the gas exchange mask still attached, to an isometric dynamometer, and performed three MVCs with femoral nerve stimulation, as well as providing a blood lactate sample, before returning to the treadmill. Heart rate and RPE were recorded at the end of each interval. Upon completion of the intervals, participants repeated the neuromuscular assessments immediately, as well as at 10-, 20-, and 30-min post-exercise. All participants completed the four intervals.

Experimental Techniques

Breath by Breath Gas Exchange

During all visits, expired gas was analysed breath-by-breath using an online system (Vyntus CPX, Jaeger, CareFusion, Germany). Oxygen (O_2) and carbon dioxide (CO_2) concentrations were analysed via a paramagnetic chemical fuel cell and non-dispersive infrared cell respectively. Before each test, the analysers were calibrated using ambient air and a gas of known O_2 (15.00%) and CO_2 (4.97%) concentrations. Ventilatory volumes were inferred from measurement of gas flow using a digital turbine transducer (volume 0 to 10 L, resolution 3 mL, flow 0 to 15 $L \cdot s^{-1}$), which was calibrated prior to each visit (Hans Rudolph Inc. Kansas City, USA).

Neuromuscular Assessments

Pre-exercise neuromuscular assessments consisted of five MVCs separated by 30 secs. During the final three MVCs, femoral nerve stimulation was delivered at peak force of the MVC and 2 secs after. This was used to quantify MVC force, voluntary activation (VA), and potentiated twitch force ($Q_{tw.pot}$). Following each interval, the same neuromuscular assessment was repeated but with three MVCs instead of five, as prior potentiation of twitches was not required [51]. For the assessments 10-, 20-, and 30-min post exercise, the baseline assessment was repeated including the two additional MVCs for potentiation of resting twitches.

Femoral Nerve Stimulation

Electrical stimuli (200 μs duration) were delivered to the femoral nerve via 32 mm-diameter surface electrodes (CF3200; Nidd Valley Medical, North Yorkshire, UK) using a constant-current stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode was placed high in the femoral triangle over the nerve, and the anode was positioned midway between the greater trochanter and the iliac crest. The cathode was repositioned if necessary, to the location that elicited the largest quadriceps twitch amplitude (Q_{tw}). Stimuli began at 20 mA, and increased by 20 mA until a plateau in Q_{tw} occured; this stimulus intensity was then increased by 30% to ensure supramaximal stimulations during the neuromuscular assessments.

Force and Electromyography

For neuromuscular assessments, participants were seated on a custom-built MVC chair, with force (N) measured using a calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway). The load cell was attached to the participant's dominant leg, 2 cm superior to the ankle malleoli, using a non-compliant cuff. The load cell height was adjusted to ensure a direct line with the applied force for each participant. Participants were sat upright with knee and hip angles kept at 90° flexion. Force was sampled continuously (1000 Hz), and acquired for off-line analysis (Spike 2, Cambridge Electronic Design, Cambridge, UK).

Blood Lactate Sampling

Blood lactate was sampled via capillary puncture technique with a 10 µl sample taken from the earlobe of each participant. Samples were immediately analysed for the concentration of lactate ($mmol \cdot L^{-1}$) and used for the calculation of lactate threshold and lactate turnpoint.

293
294 *Data Analysis*
295 All MVCs were recorded, with the average of the peak forces during the three contractions
296 used for further analyses. Voluntary activation assessed with nerve stimulation was
297 calculated using the twitch interpolation method: VA (%) = (1 – [SIT/$Q_{tw,pot}$] × 100), where SIT
298 is the amplitude of the superimposed twitch force measured during MVC, and $Q_{tw,pot}$ is the
299 amplitude of the resting potentiated twitch force assessed 2 secs post-MVC.
300
301 Pulmonary gas exchange during both visits was exported in 5 secs bins, and the greatest 30
302 secs average recorded in visit 1 was used as peak values for each variable ($\dot{V}O_2$, $\dot{V}CO_2$, and
303 $\dot{V}_E$). Data from visit two was exported in the same manner, with the final 30 secs of data
304 during each interval expressed in absolute ($L \cdot min^{-1}$) and relative (%peak) units. Ventilatory
305 equivalents of $\dot{V}O_2$ and $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$) and respiratory exchange ratio
306 ($\dot{V}CO_2/\dot{V}O_2$) were also calculated.
307
308 *Statistical Analysis*
309 Data are presented as mean ± standard deviation within the text and figures. Normal
310 distribution of data was confirmed with the Shapiro-Wilk test. As all variables had normally
311 distributed data, males and females were compared with independent samples t tests for
312 variables with a single value or time point. For repeated measures variables assessed
313 during and after exercise, a two-way (sex × time) repeated measures ANOVA was
314 performed. To assess fatigue during exercise, neuromuscular variables (normalised to %
315 baseline) were assessed using a 2 × 5 (sex × time) ANOVA (time points: pre, interval 1, 2, 3,
316 and 4). Whereas to assess recovery, neuromuscular variables (normalised to % baseline)
317 were assessed with a 2 × 4 (sex × time) ANOVA (time points: post, +10, +20, and +30 min).
318 Main and interaction effects were adjusted according to the Greenhouse-Geisser correction
319 if the assumption of sphericity was violated, and significant effects were followed with
320 Bonferroni-corrected *post-hoc* tests. The significance level for all statistical tests was set at α
321 < 0.05.
322

Results

Incremental Exercise Testing

Absolute and relative data recorded during the incremental exercise test are presented in Table 1. As expected, males had greater values for absolute $\dot{V}O_{2peak}$ ($p < 0.001$), however, when values were normalized to body mass, there was no sex difference in $\dot{V}O_{2peak}$ ($p = 0.292$). Both sexes completed a similar number of incremental test stages before reaching exhaustion (males: 6.3 ± 1.6 stages vs females: 6.5 ± 1.3 stages, $p = 0.756$). The velocity at which lactate threshold occurred, expressed as a percentage of $v\dot{V}O_{2peak}$, was not different between sexes (males: 67 ± 7% vs females: 68 ± 9%, $p = 0.773$).

Table 1: Submaximal and peak data recorded during the incremental exercise test.

	Males (n = 10)	Females (n = 10)	P value
$\dot{V}O_{2peak}$ ($l \cdot min^{-1}$)	3.901 ± 0.433	2.763 ± 0.323	< 0.001
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	47.2 ± 6.0	44.4 ± 5.8	0.292
$\dot{V}_{Epeak}$ ($l \cdot min^{-1}$)	152.9 ± 20	105.8 ± 11.3	< 0.001
RER_{peak}	1.06 ± 0.05	1.05 ± 0.07	0.798
HR_{peak} (bpm)	200 ± 4	200 ± 6	0.874
$v\dot{V}O_{2peak}$ ($km \cdot h^{-1}$)	14.9 ± 1.7	13.6 ± 1.6	0.090
Lactate threshold ($km \cdot h^{-1}$)	10.0 ± 1.8	9.2 ± 1.5	0.317

HR: heart rate; RER: respiratory exchange ratio; $\dot{V}_E$: minute ventilation; $\dot{V}O_2$: rate of oxygen uptake; $v\dot{V}O_{2peak}$: velocity at the maximal rate of oxygen uptake.

Metabolic & Cardiopulmonary Responses to Interval Exercise

During the interval task, heart rate, RPE, and blood lactate progressively increased (main effects of time: all $p < 0.001$). No main effects of sex were observed for heart rate ($p = 0.601$), RPE ($p = 0.497$) or blood lactate ($p = 0.203$). A sex × time interaction effect was observed for heart rate (Figure 1A, $F_{2.7, 45.5} = 3.470$, $p = 0.028$, $\eta p^2 = 0.170$), with *post-hoc* comparisons revealing females had greater heart rate at the end of the warmup ($p = 0.041$), but no other time points ($p \geq 0.696$). No sex × time interaction effects were observed for either RPE (Figure 1B, $p = 0.137$) or blood lactate (Figure 1C, $p = 0.183$).

**** Figure 1 here ****

While males demonstrated greater absolute values ($p < 0.001$), when $\dot{V}O_2$ and $\dot{V}CO_2$ were expressed as a percentage of peak values (Figure 2A and 2B) from the incremental exercise

test, no sex ($p \geq 0.631$) or sex × time interaction effects ($p \geq 781$) were observed. Similarly, no sex ($p = 0.330$) or sex × time interaction effects ($p = 0.710$) were observed for RER.

*** Figure 2 here ***

In absolute values, males had greater $\dot{V}_E$ throughout the interval task ($p < 0.001$). When expressed as a percentage of peak values (Figure 2C), no main effect of sex was observed ($p = 0.187$), however, there was a sex × time interaction ($F_{3,54} = 3.269$, $p = 0.042$, $\eta p^2 = 0.154$) for $\dot{V}_E$. *Post-hoc* comparisons revealed no significant sex differences at either the first ($p = 0.067$), second ($p = 0.050$), third ($p = 0.212$), or fourth interval ($p = 1.000$). Similarly, $\dot{V}_E/\dot{V}O_2$ demonstrated no main effect of sex (Figure 3A, $p = 0.317$), but a sex × time interaction effect ($F_{3,54} = 4.831$, $p = 0.005$, $\eta p^2 = 0.212$); with *post-hoc* comparisons revealing greater female values compared to males during the first interval ($p = 0.034$). $\dot{V}_E/\dot{V}CO_2$ also demonstrated no main effect of sex (Figure 3B, $p = 0.096$), but a sex × time interaction effect ($F_{3,54} = 2.853$, $p = 0.046$, $\eta p^2 = 0.137$). *Post-hoc* comparisons revealed that females had greater values than males during the first interval for both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ ($p \leq 0.034$) and second ($p = 0.006$) intervals for $\dot{V}_E/\dot{V}CO_2$.

*** Figure 3 here ***

Fatigability and Recovery

At baseline, males produced greater maximal force than females (667 ± 35 vs. 471 ± 98 N, $p < 0.001$). Throughout the interval task, a main effect of time was observed for MVC ($F_{2.8,51.6} = 9.897$, $p < 0.001$, $\eta p^2 = 0.355$), $Q_{tw.pot}$ ($F_{2.0,36.1} = 30.481$, $p < 0.001$, $\eta p^2 = 0.629$), and VA ($F_{2.4,43.0} = 10.884$, $p < 0.001$, $\eta p^2 = 0.377$). *Post-hoc* comparisons revealed significant reductions in each variable after interval one compared to baseline ($p \leq 0.016$), however, after the first interval, no further reductions were observed ($p \geq 0.506$). No sex or sex × time interaction effects were observed for MVC ($p \geq 0.150$), $Q_{tw.pot}$ ($p \geq 0.184$), or VA ($p = 0.461$).

*** Figure 4 here ***

In the 30-min recovery period after the interval task, no main effect of time was observed for MVC ($p = 0.226$), whereas as main effect of time was observed for $Q_{tw.pot}$ ($F_{2.3,42.0} = 12.824$, $p < 0.001$, $\eta p^2 = 0.416$) and VA ($F_{2.1,37.9} = 6.531$, $p = 0.003$, $\eta p^2 = 0.266$). No sex or sex × time interaction effects were observed for MVC ($p \geq 0.256$) or VA ($p \geq 0.598$), and while there was no sex × time interaction for $Q_{tw.pot}$ ($p = 0.567$), there was a main effect of sex ($F_{1,18} = 4.679$, p 0.044, $\eta p^2 = 0.206$). *Post-hoc* comparisons indicated that females had

greater $Q_{tw.pot}$ amplitudes than males at 20 ($p = 0.027$) and 30 minutes ($p = 0.030$) after the interval task.

Discussion

This study aimed to compare the neuromuscular and cardiopulmonary responses to high-intensity interval running exercise between sexes. It was hypothesised that there would be no sex differences in the metabolic response to the task, but females would experience lesser declines in voluntary and evoked contractions. The lack of sex differences in the relative $\dot{V}O_2$, RER, and blood lactate response to the task confirm the similarity of the metabolic response, whilst the sex difference in $Q_{tw.pot}$ following the interval task indicated more fatigue-resistant female knee extensors. In addition, females demonstrated poorer ventilatory efficiency in the first half of the interval task compared to males, which was not evident in the second half of the task. Combined, these data demonstrate that the cardiopulmonary and neuromuscular responses to high-intensity interval running differ between sexes, adding to the growing evidence base that suggests practitioners should consider sex when prescribing exercise.

Fatigability & Recovery Following Interval Exercise

The lack of sex difference in the decline in neuromuscular function during exercise contradicts previous literature utilising high-intensity constant load [27-29] and self-paced [32] locomotor exercise. Additionally, the data contradict those reported following repeated sprint exercise [37, 38], reinforcing the notion that sex differences in fatigability are task-specific [52]. A recent study that employed a similar task to the present study (4 minute intervals with 3 minutes rest) also demonstrated similar fatigability between sexes [42], suggesting that the specific demands of high-intensity interval exercise do not permit sex differences in fatigability from manifesting. The duration of the intervals used in the present study (3 min) likely resulted in a substantial metabolic disturbance within the working muscles. Jones, Wilkerson [53] demonstrated that after 3.6 min of exercise at 110% of critical power, depletion of phosphocreatine (PCr) stores, inorganic phosphate accumulation, and pH decreases were all exaggerated compared to exercise below critical power. The rest periods between intervals (90 secs) likely permitted a degree of metabolic recovery of the working muscles, with near complete recovery of PCr stores and intramuscular pH being observed 90-120 secs after single-limb exercise [54, 55] and 6 minutes following all-out sprinting [56]. However, it should be acknowledged that the rest periods in the present study included the performance of MVCs, which may have delayed the metabolic recovery. The magnitude of knee-extensor fatigability that this repeated metabolic stress and recovery

elicited was moderate, with MVC reductions (between 5-10%) less than was reported in males following a 5 km running time trial [32], and substantially less than typically reported reductions following severe intensity cycling (~20% reduction in MVC, [57, 58]). One potential explanation why the fatigability induced by high-intensity interval running was not different between sexes is that the magnitude was too small to detect sex differences. Indeed, compared to previous data in single-limb and cycling exercise [27-29], where sex differences were observed, the present study observed approximately half the degree of MVC reduction in both sexes.

Although sex differences in fatigability were not evident during the interval task, a sex difference was observed in the 30 min recovery period, whereby females demonstrated greater $Q_{tw.pot}$ amplitudes relative to pre-exercise. Although this post-exercise period was termed the 'recovery period', no recovery was observed for either sex for MVC or $Q_{tw.pot}$. Although central and peripheral contributions to fatigability are thought to mostly recover within 30 minutes following short duration exercise, recovery from running is further complicated by the presence of muscle damage caused by repeated stretch-shortening cycles [58, 59]. Studies employing high- and low-frequency electrical stimulation to profile fatigue and recovery following damaging exercise reveal that low-frequency evoked contractions remain depressed, whereas high-frequency contractions recover [60]. Depression of low-frequency evoked contractions is thought to be underpinned by impaired intracellular calcium ion (Ca^{2+}) release and/or reduced Ca^{2+} sensitivity of myofibrils [61, 62], with the former being mechanistically linked to the reduced contractility of muscle fibres following damaging exercise [63]. It is likely that the prolonged depression of MVC and $Q_{tw.pot}$ in the present study was underpinned by altered Ca^{2+} handling within the knee-extensors, which also provides insight into the lesser relative reductions experienced by females compared to males. Evidence from Harmer, Ruell [64] demonstrated sex differences in Ca^{2+} regulation before and after high-intensity exercise, with Ca^{2+}ATPase activity reduced in males, but increased in females following exercise. Therefore, it is possible that the lesser relative reductions in $Q_{tw.pot}$ experienced by females following high-intensity interval exercise in the present study were related to a lesser disruption to Ca^{2+} regulation within the knee-extensors. One additional consideration is that the patellar tendon stiffness has been demonstrated to be lower in females, which results in a greater mechanical buffer, and lesser knee-extensor fascicle lengthening for females during eccentric contractions [65]. Conceivably, this could also contribute to the lesser reductions in contractile function observed post-exercise in the present study.

Metabolic and Cardiopulmonary Responses to Interval Exercise

460 The $\dot{V}O_2$ recorded during all four intervals exceeded 90% of $\dot{V}O_{2peak,}$ with values of ~95% in
461 both sexes by the final interval. The lack of sex difference in relative $\dot{V}O_2$, $\dot{V}CO_{2,}$ RER, and
462 blood lactate provides evidence that the metabolic response to high-intensity interval
463 exercise was similar between males and females. This agrees with similar data recorded
464 during constant-load exercise in the severe intensity domain, where no sex differences in the
465 aforementioned variables were observed [27]. While sex differences in substrate utilisation
466 have been observed previously, these appear to be limited to steady-state exercise (i.e.,
467 moderate and heavy intensity domains) rather than the intensities typically utilized during
468 high-intensity interval exercise [66, 67]. In response to the similar metabolic demands, males
469 and females employed different respiratory strategies across the interval task, with females
470 demonstrating poorer ventilatory efficiency during the first two intervals. While it must be
471 acknowledged that females did not reach criteria for clinical diagnosis of 'ventilatory
472 inefficiency' [68], poorer ventilatory efficiency is thought to reflect poorer matching of lung
473 ventilation to perfusion, and therefore impaired gas exchange [69]. Sex differences are well-
474 established in the structure and function of the respiratory system [70], with larger airway
475 diameters and lung volume, as well as greater alveolar surface area observed in males,
476 even when height matched [71]. The lesser alveolar surface area in females results in
477 poorer oxygen diffusing capacity during exercise compared to height-matched males,
478 although matching for lung volume negates this sex difference [72]. The lower lung volume
479 necessitates greater relative $\dot{V}_E$ in females to achieve the same relative $\dot{V}O_2$ and $\dot{V}CO_2$,
480 which is driven primarily through increases in breathing frequency in females [73]. In addition
481 to the present study that observed this sex difference during high-intensity interval exercise,
482 greater relative $\dot{V}_E$ were observed in females during constant-load exercise in the heavy and
483 severe intensity domains [27]. Interestingly, in the present study this ventilatory sex
484 difference had dissipated by the third and fourth intervals, implying that both sexes ultimately
485 experienced a loss of ventilatory efficiency as exercise progressed. Furthermore, the sex
486 differences in ventilatory efficiency did not appear to affect perceptual variables in the
487 present study, although future research should investigate dyspnoea more directly.
488
489
490
491 *Further Considerations*
492 High-intensity interval exercise is often prescribed to improve an individual's $\dot{V}O_{2max}$ via
493 positive adaptations to skeletal muscle capillary density, maximum stroke volume and
494 cardiac output, and blood volume [74]. How sex influences physiological adaptation to
495 exercise is unclear, with some evidence suggesting that $\dot{V}O_{2max}$ adaptation is blunted in
496 females compared to males [75]. Physiological adaptation to exercise is multi-factorial, with

a variety of signalling pathways activated by disruptions to homeostasis in various physiological systems [76]. In the present study, the relative cardiovascular and metabolic perturbations were similar between sexes, but females experienced lesser contractile impairment following the task. Disruptions to intramuscular Ca^{2+} homeostasis that cause contractile impairment also activate Ca^{2+}-calmodulin-dependent kinases (CaMK, [77]). CaMKs play an important role in regulating oxidative enzyme expression, as well as peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1a) and therefore mitochondrial biogenesis [78]. While speculative, it could be suggested that the lesser reductions in contractile function experienced by females in the present study might result in a lesser stimulus for adaptation, however this hypothesis should be directly tested with appropriate methodologies.

Limitations

The present study employed a high-intensity interval task that was performed at a percentage of each individual's $v\dot{V}O_{2peak}$, which has previously been suggested to lead to greater heterogeneity of physiological responses compared to threshold-based approaches [79]. The approach of threshold-anchored exercise prescription has led to sex differences in fatigability and cardiopulmonary function being observed previously [27-29]. However, the present study observed similar sex differences to previous research when work rate was normalized to maximum capacity, rather than submaximal thresholds. Given that both sexes performed the task at ~140% of their lactate threshold (Table 1), and reached $\dot{V}O_2$ values of ~95% $\dot{V}O_{2peak}$, peak blood lactate concentrations of ~ 8 $mmol \cdot l^{-1}$, and peak RERs >1.05 during the interval task, it is likely that the task took place in the severe domain, and observed sex differences were not related to differences in the relative work rate of the task.

Participants completed an incremental exercise test to exhaustion in visit 1, however they did not complete a verification phase to determine whether the peak $\dot{V}O_2$ data were a valid estimate of $\dot{V}O_{2max}$ [80]. While the concern here is that $\dot{V}O_{2peak}$ might not be an accurate reflection of $\dot{V}O_{2max}$ and its associated variables, Chidnok, DiMenna [81] demonstrated that in healthy, active participants, $\dot{V}O_{2peak}$ does consistently provide equivalent outcomes when compared to verification tests.

Finally, our *a priori* sample size calculation estimated that n=12 participants would be necessary to detect a sex difference in fatigability, yet that calculation used an effect size from constant-load cycling [27]. As discussed above, the magnitude of fatigability experienced by participants was smaller than previous research, for a variety of reasons, therefore one potential reason as to why a sex difference in fatigability *during* interval

exercise was not observed could be that the study was underpowered, and a sample size greater than the n=20 we tested would be required to detect differences. Similarly, the anticipated sex difference in relative $\dot{V}O_{2peak}$ (~10%, [82]) was not statistically detected in this study (6% difference, p = 0.292). Given the study was powered to detect changes in fatigability, rather than between group differences in $\dot{V}O_{2peak}$, as well as the similar self-reported training volume in males and females, as well as similar relative velocities at submaximal thresholds, it is unlikely that training status was different between groups in the present study.

Conclusions

This study demonstrated that males and females experienced similar cardiovascular and metabolic responses to a high-intensity interval exercise task, however females demonstrated poorer ventilatory efficiency in the first half of the task, and lesser reductions in knee-extensor contractile function following the task. Much like previous research that observed integrative sex differences during constant-load exercise, this study demonstrated that females and males do not experience the same responses to interval exercise. The exercise task employed in the present study is akin to those typically used for the enhancement of athletic performance, highlighting that those prescribing exercise should be cognisant that the sex of participants will influence the acute physiological responses.

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Conflicts of Interest

The authors report no conflicts, financial or otherwise.

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Data Availability

Data are available upon request to the corresponding author.

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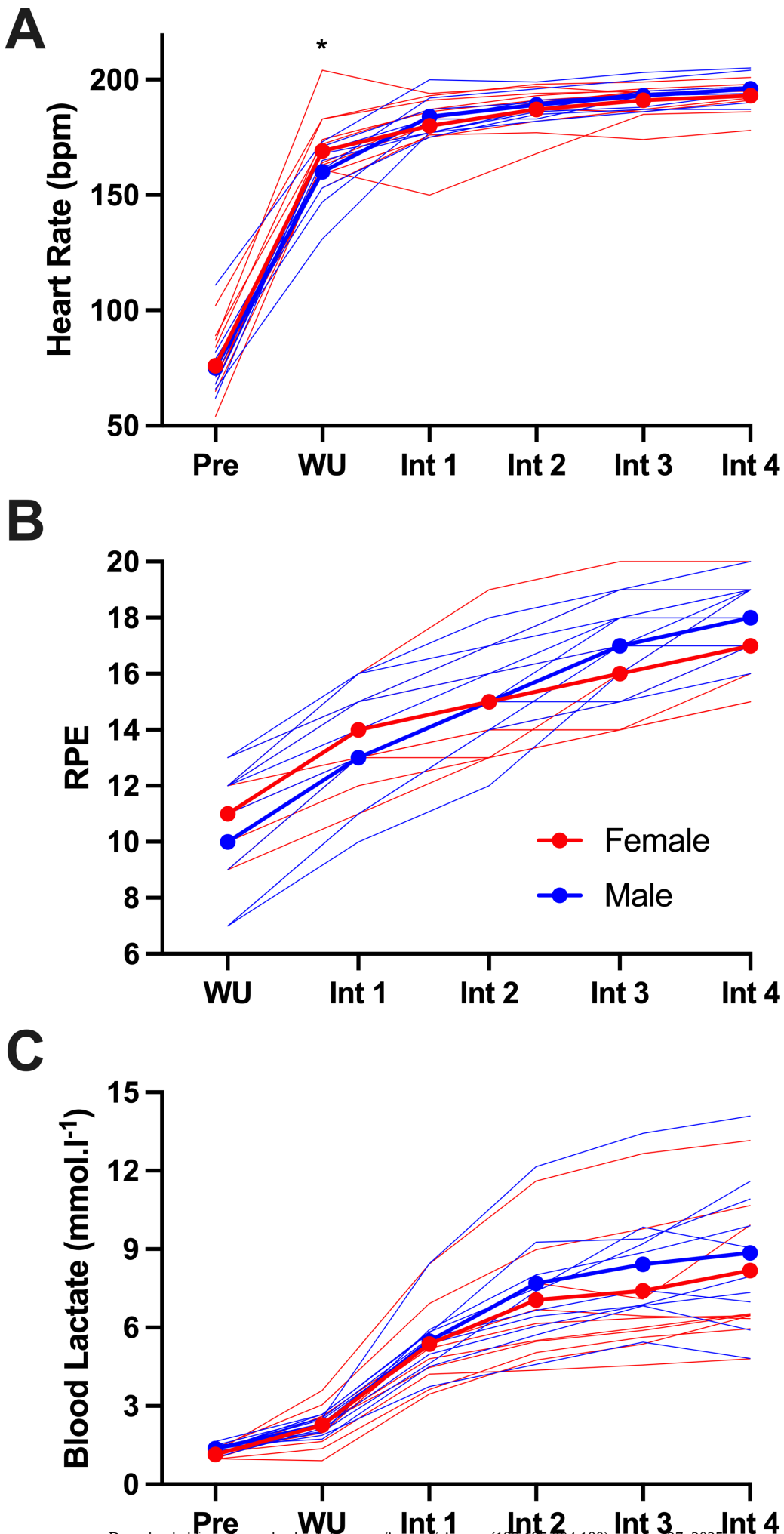
List of Figures:

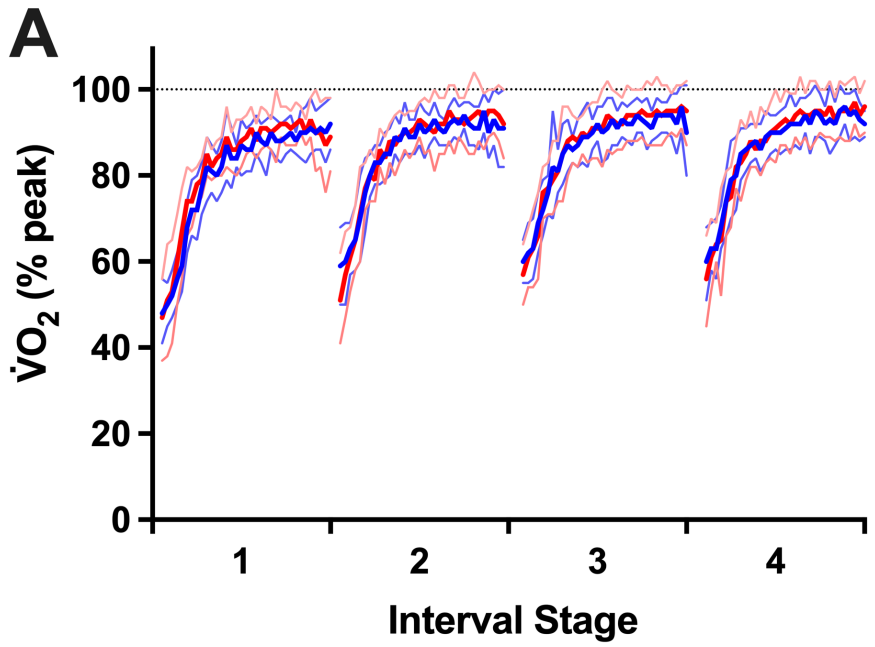
Figure 1: Heart rate (Panel A), rating of perceived exertion (RPE, Panel B), and blood lactate concentration (Panel C) at rest, following the warmup (WU), and at the end of each interval (Int). * = females greater than males ($p < 0.05$).

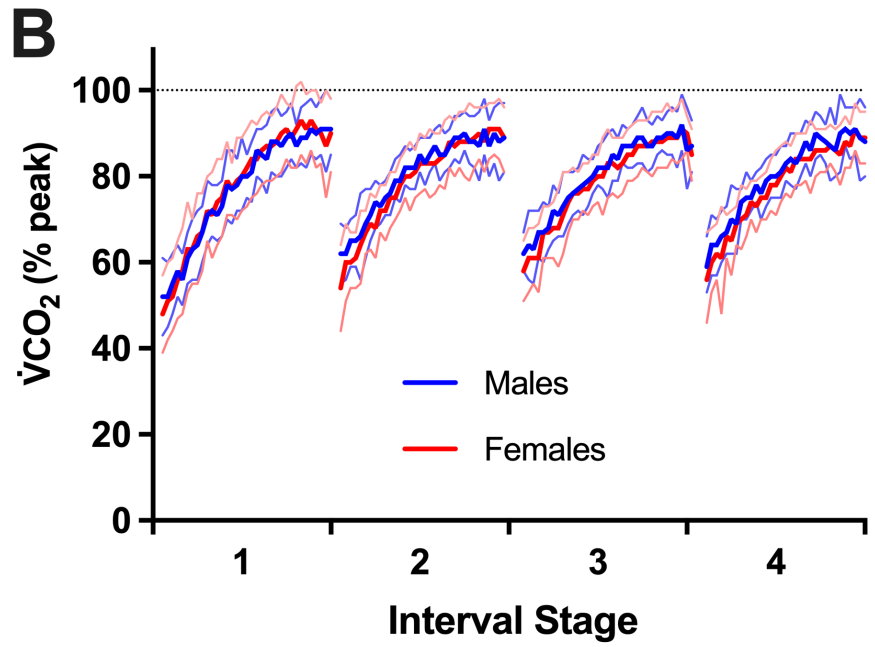
Figure 2: Rate of oxygen uptake ($\dot{V}O_2$, Panel A), carbon dioxide production ($\dot{V}CO_2$, Panel B), and ventilation ($\dot{V}_E$, Panel C) during the four interval stages. Thick lines represent the mean data for each sex, while thin lines represent the standard deviation. Horizontal dashed lines indicate peak values attained during the incremental exercise test. * = sex x time interaction effect ($p < 0.05$).

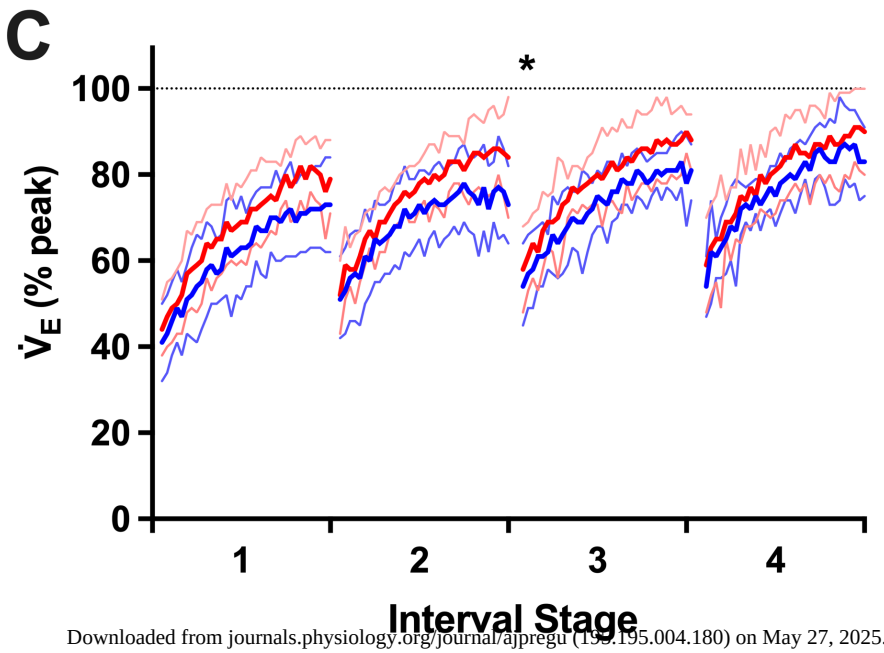
Figure 3: Ventilatory equivalents of oxygen uptake ($\dot{V}_E/\dot{V}O_2$, Panel A) and carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$, Panel B) during the final 30 secs of each interval (Int). Blue dots represent individual males, while red dots represent individual females. * = females greater than males ($p < 0.05$).

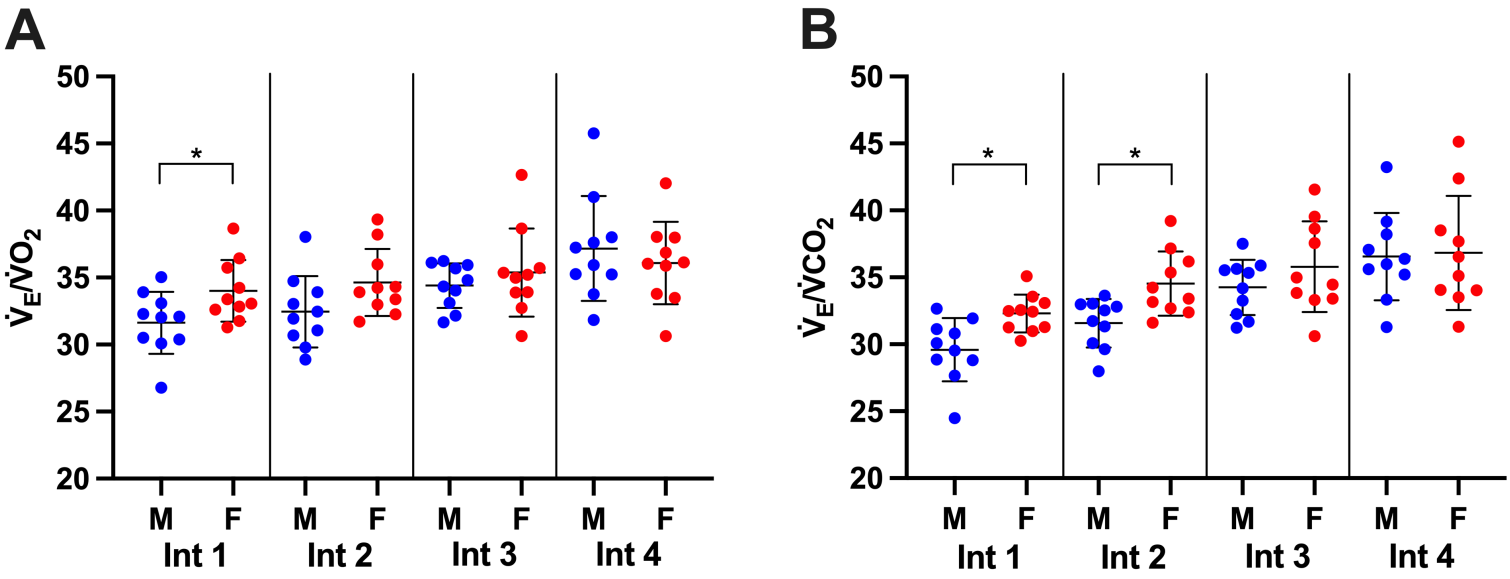
Figure 4: Neuromuscular variables recorded during and following the interval task. Thin lines represent individual participants, whereas the thick lines represent group mean data. Maximal voluntary contraction (MVC, Panel A); quadriceps potentiated twitch (Qtw.pot, Panel B); and voluntary activation (VA, Panel C). * = females greater than males ($p < 0.05$).

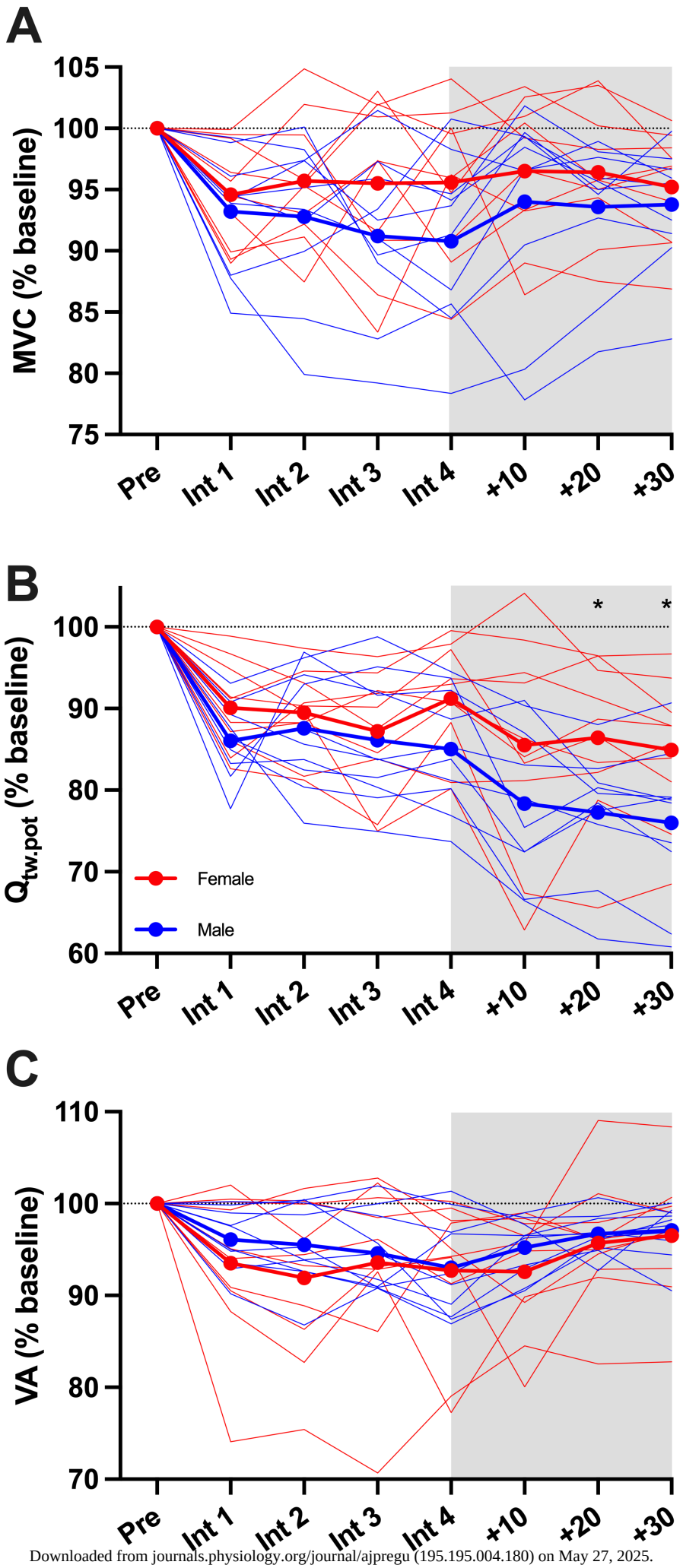




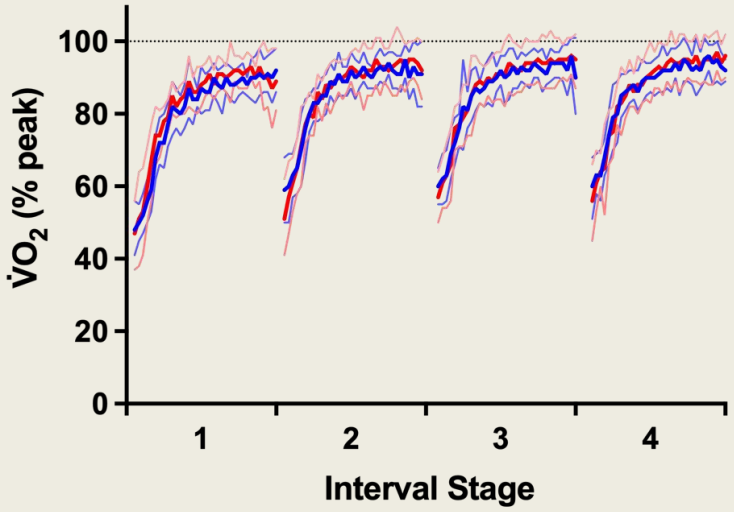


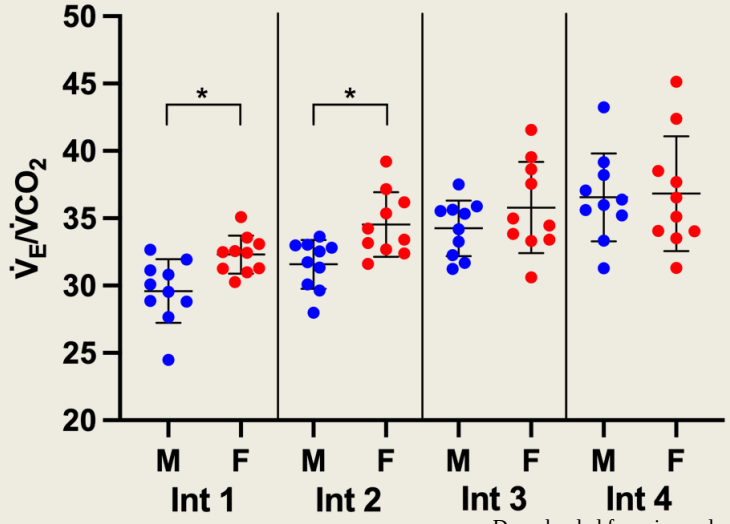


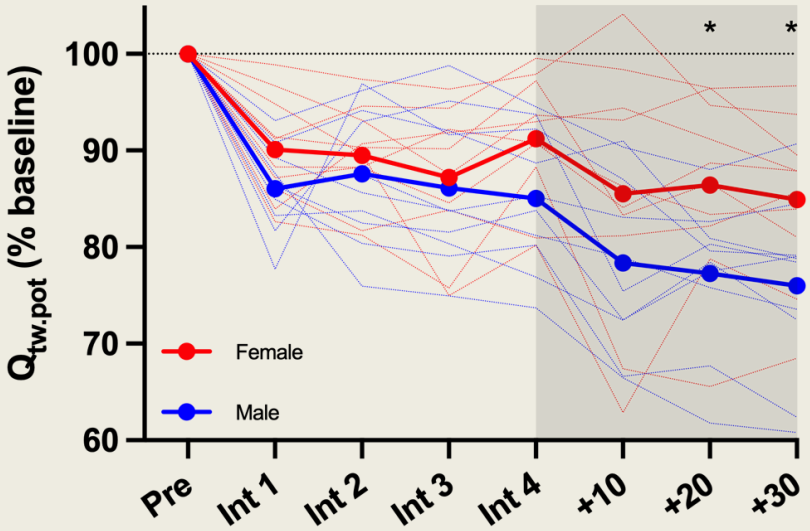




Sex differences in the cardiopulmonary and neuromuscular response to high-intensity interval exercise







RESULTS

In response to 4 × 3-min running intervals at 90% of the final incremental test velocity ($v\dot{V}O_{2peak}$), females experienced:

- A similar metabolic response
- Greater hyperpnoea
- Lesser contractile dysfunction post-exercise