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RESEARCH ARTICLE

Sex Differences in the Response to Exercise Training

Different ramp-incremental slopes elicit similar $\dot{V}O_{2max}$ and fatigability profiles in females and males despite differences in peak power output

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Abstract

The aim of this article is to investigate the effects of different ramp-incremental (RI) slopes on fatigability and its recovery in females and males. Ten females and 11 males performed RI tests with distinct slopes, in separated and randomized sessions, 15 (RI_{15}), 30 (RI_{30}), and 45 (RI_{45}) $W \cdot min^{-1}$. Performance fatigability was assessed by femoral nerve electrical stimuli evoked during and after isometric maximal voluntary contraction (IMVC) of knee extensors at baseline and after task failure at min 0.5, 1.5, 2.5, 5, and 10. Maximal oxygen uptake ($\dot{V}O_{2max}$) and peak power output (POpeak) were also measured. There were significant and similar declines from pre- to post-RI test in RI_{15}, RI_{30}, and RI_{45} for IMVC (−23%; −25%; −25%, respectively; $P < 0.05$) and potentiated single twitch (−46%; −47%; −49%; $P < 0.05$), whereas voluntary activation did not change (−1%; −1%; 0%; $P > 0.05$). There were no RI condition effects, nor time × condition interaction for IMVC, potentiated single twitch and voluntary activation (all $P > 0.05$). $\dot{V}O_{2max}$ was not different among RI_{15}, RI_{30}, and RI_{45} conditions (3.30, 3.29, and 3.26 $L \cdot min^{-1}$, respectively; $P = 0.717$), but POpeak was (272, 304, and 337 W, respectively; $P < 0.001$). Overall, performance fatigability profiles were similar between sexes after the RI tests and during recovery. In addition, during recovery, high-frequency doublets and single twitch recovered faster after RI_{30} and RI_{45} compared with RI_{15}, regardless of sex (all $P > 0.05$ for sex differences). In conclusion, RI tests of different slopes that elicited similar $\dot{V}O_{2max}$ but different POpeak did not affect the profile of performance fatigability at task failure in females and males.

NEW & NOTEWORTHY It was unknown whether performance fatigability and its recovery are affected by different slopes in a ramp incremental (RI) test. It was also uncertain if females and males would respond differently. Performance fatigability was the same regardless of the RI slope adopted and the sex of the population, which was accompanied by similar maximal oxygen uptake but different power output achieved. The recovery of contractile function was similar between sexes but delayed after slower RI slopes.

Central fatigue; cycling; neuromuscular function; peripheral fatigue; sex differences

INTRODUCTION

Ramp-incremental (RI) tests are widely used to assess key parameters of aerobic function, such as maximal oxygen uptake ($\dot{V}O_{2max}$) (1, 2). Generally, attainment of $\dot{V}O_{2max}$ during a cycling RI test is rapidly followed by the inability to sustain the imposed increase in power output (PO), and is accompanied by exercise-induced fatigue development. Interestingly, although $\dot{V}O_{2max}$ remains constant across RI tests of different slopes (i.e., increase of cycling PO by time, $W \cdot min^{-1}$), the achieved peak PO (POpeak) is greater with faster slopes (3, 4). The reason for achieving the same $\dot{V}O_{2max}$ with different POpeak at task failure after RI tests of distinct slopes might be due to the achievement of a given metabolic disturbance and full depletion of the "finite work capacity" (i.e., *W′*) (5, 6), which is independent of the mechanical workload performed (7).

The mechanisms underpinning task failure during RI tests of different slopes can be explored by characterizing the exercise-induced profile of fatigue, which provides further information regarding the etiology of exercise disengagement. Fatigue can be defined as the interaction between perceived fatigability (i.e., increased perceptual responses during exercise) and performance fatigability (i.e., decline in maximal muscle force/power production) (8). Performance fatigability is attributed to reduced voluntary activation and/or contractile function, which are separated by mechanisms occurring above and at/below the neuromuscular

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junction (9), respectively. For example, a recent study (10) has shown that performance fatigability was greater after task failure when the RI slope was fast (50 W·min^{-1}) or medium (25 W·min^{-1}) compared with a slow RI slope (10 W·min^{-1}), despite similar $\dot{V}O_{2max}$ value being achieved, which suggested that contractile function may be less compromised at task failure after a slower RI test. This result might be because slower compared with faster RI slopes could allow more time for matching of oxidative phosphorylation to the increasing metabolic demand (3), which would rely on recruitment of less glycolytic and fatigable muscle fibers (i.e., type I), resulting in diminished impairment in contractile function at task failure (11). However, the characterization of performance fatigability profile in the previous study (10) was based on isokinetic cycling POpeak and surface electromyography, which do not provide direct indications about the changes in maximal voluntary activation and contractile function (12). In addition, to date, perceived responses after different RI protocols have not been characterized adequately, despite their relevance in explaining task failure along with performance fatigability responses (13–15).

In addition, another factor to consider when evaluating the effects of the RI slope on performance fatigability is potential sex differences. Current literature shows that females generally demonstrate lower decline in contractile function at task failure than males (5, 6). It has been suggested that such differences might be due to distinct levels of exercise-induced metabolic disturbance since females compared with their male counterparts rely more on oxidative phosphorylation to replenish ATP thanks to their greater capillarization and overall muscle oxygenation (6, 16, 17). In connection to this idea, previous findings have also shown faster recovery of contractile function in females compared with males (5, 6), which might be due to lower magnitude of exercise-induced contractile function impairment and metabolic disturbance between sexes (5). However, the evidence of sex differences in performance fatigability responses after a RI test are currently lacking. This is a relevant point since RI tests are widely used in both sexes, but previous studies have suggested that potential differences between females and males are diminished as the overall exercise intensity increases (18), partially because of changes in blood flow dynamics to active musculature due to increasing absolute mechanical work near-maximal exercise intensity. For example, the greater absolute mechanical workload generally achieved by males, especially during fast RI slopes, may be associated with impaired blood flow to the active tissue due to the larger internal muscle pressures, thus resulting in greater muscle contractile impairments compared with females (19). Females have also been shown to display a greater vasodilatory local response compared with males toward the mid-portion to the end of a knee extension incremental test (20). In addition, during RI cycling tests, females rely more on oxygen extraction compared with males to support a given increase in $\dot{V}O_2$ toward the end of the test (21). Thus, given the differential hemodynamic responses between females and males reported in the literature during incremental testing, and the effects of the RI slope in the mechanical workload, it could be expected that increasing the RI slope might affect performance fatigability differently in females compared with males. Considering that utilization of distinct RI slopes is expected to directly impact POpeak (i.e., different mechanical workloads) but not $\dot{V}O_{2max}$ (i.e., similar metabolic disturbance), RI testing represents an ideal exercise paradigm to investigate sex differences and recovery profiles of performance fatigability.

Therefore, the aims of the present study were to investigate: *1*) performance fatigability and perceived responses at task failure after three different RI tests of different incrementing rates, namely slow (15 W·min^{-1}), medium (30 W·min^{-1}), and fast (45 W·min^{-1}); *2*) whether the sex of participants would influence the extent and the characteristics of performance fatigability; and *3*) the recovery profile of performance fatigability after each RI protocol in females and males. The hypotheses were that: *1*) the profile of performance fatigability would be characterized by a greater decline in contractile function after the fast and medium compared to the slow RI slopes, despite similar declines in voluntary activation; *2*) females would show smaller impairments in contractile function than males, particularly after the fast RI slope; and *3*) the recovery of performance fatigability would be faster in females compared with males.

METHODS

Participants

Twenty-one recreationally trained participants (10 females and 11 males; see RESULTS section for participants' characteristics) completed the study. None of the participants were undergoing any medical treatment that could potentially alter their cardiorespiratory, neuromuscular, and metabolic responses to maximal exercise. Female participants self-reported a menstrual cycle length of 28 ± 5 days and four participants were taking hormonal contraceptives. All participants signed an informed consent form. All procedures were approved by the Conjoint Health Research Ethics Board at the University of Calgary (REB18-0916).

Experimental Protocol

Figure 1 depicts the overall experimental design, which included three separate sessions, within a 2-wk period, wherein each session took place at least 48 h apart and at a similar time of the day (±1 h). All tests were performed in an environmentally controlled room (temperature: 18°C–21°C; humidity 50%–60%), and the order of the RI tests was randomized, which resulted in an even distribution among RI slopes protocols within the first (RI_{15}, 32%; RI_{30}, 26%; RI_{45}, 42%), second (RI_{15}, 37%; RI_{30}, 32%; RI_{45}, 32%), and third (RI_{15}, 32%; RI_{30}, 42%; RI_{45}, 26%) sessions. Before each visit, all participants were instructed to avoid consumption of food and caffeinated beverages for at least 2 h and 8 h, respectively, and to abstain from vigorous physical activity for 24 h. During the first visit, and before any other testing took place, stature, body mass, and body composition [i.e., % body fat and total muscle mass using dual-energy X-ray absorptiometry (Discovery QDR Series, Hologic, Inc., MA)] were assessed. In each session, participants performed RI tests with different slopes: 15 W·min^{-1} (RI_{15}), 30 W·min^{-1} (RI_{30}), and 45 W·min^{-1}(RI_{45}). Performance fatigability was assessed at baseline and after the RI test at min 0.5, 1.5, 2.5, 5, and 10 (i.e., 30, 90, 150, 300, and 600 s, respectively). Throughout each RI test, different variables were collected, including:

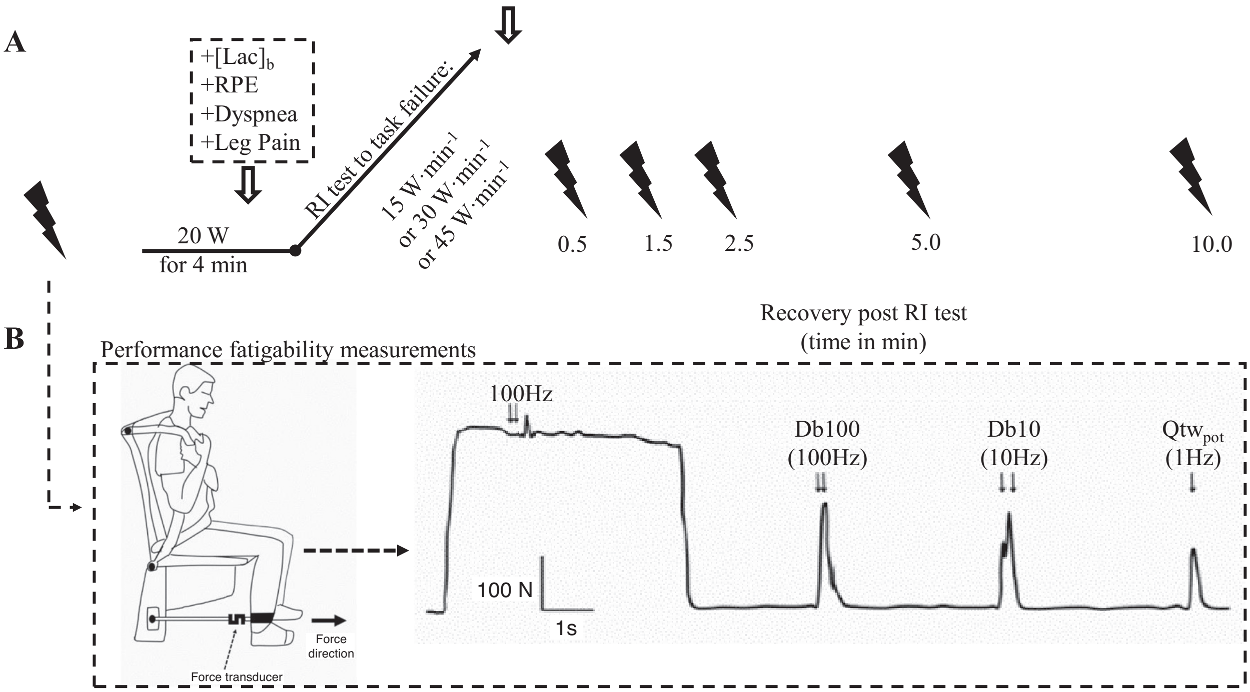

Figure 1. Schematic representation of the study design. *A*: overall experimental study design. *B*: performance fatigability assessments protocol. Ramp-incremental test (RI); blood lactate concentration ([Lac]$_b$); rating of perceived exertion (RPE); 100 Hz paired-pulses evoked torque (Db100); 10 Hz paired-pulses evoked torque (Db10); single pulse evoked torque (Qtw$_{pot}$).

PO, rate of pulmonary oxygen uptake ($\dot{V}O_2$), blood lactate concentration ([Lac]$_b$) and electromyography (EMG) from the vastus lateralis (VL). In addition, before and immediately after each RI test, the rating of perceived exertion (RPE), perceived leg pain (Pain), and dyspnea were also recorded.

Data Collection

Ramp incremental tests.

All testing sessions were performed on an electromagnetically braked cycle ergometer (Velotron, RacerMate, Seattle, WA). The RI tests consisted of a 4 min of cycling at 20 W, followed by different ramp-slopes (i.e., 15, 30, and 45 W·min^{-1} increments). During the 4-min cycling at 20 W, cadence was kept at 60 rpm for all participants in all visits. Thereafter, when the power output started to increase, the preferred cadence was self-selected by the participants, between 60 and 100 rpm, until task failure. Participants were instructed to maintain a similar cadence for all visits, based on the average cadence from the first RI test. Task failure during the RI tests was considered when participants could no longer maintain a cycling cadence of at least 60 rpm for more than 5 s, or at volitional exhaustion despite strong verbal encouragement. In all conditions, participants were blinded to the work rate, elapsed time, and RI slope but received visual feedback on their cadence.

Gas exchange and ventilatory variables.

All gas exchange and ventilatory responses were measured breath-by-breath with a metabolic cart (Quark, Cosmed, Rome, Italy). The system consisted of a low dead space turbine as well as O_2 and carbon dioxide (CO_2) gas analyzers; these were calibrated with a syringe of known volume (3 L) and a gas-mixture of known concentration (16% O_2; 5% CO_2; balance N2), respectively.

Knee extensor muscle torque and performance fatigability assessments.

The isometric maximal voluntary torque (IMVC) of knee extensors from the dominant leg, which was determined based on the preferred leg used to kick a ball (22), and performance fatigability responses were evaluated on an isometric chair (Kin–Com, Chattecx Corporation, Chattanooga, TN) secured by chest and hip straps with knees and hips flexed at 90°. The right ankle was attached perpendicularly to a force transducer (LC101-2K, Omegadyne, Sunbury, OH). To ensure that the upper body did not contribute to the KE torque, straps along the trunk and waist were secured, and participants crossed arms to shoulders during contractions. Force was obtained from a strain gauge (LC101-2K) attached to the isometric chair with a nonextensible strap, sampled at 2000 Hz and digitally converted using the Power Lab acquisition hardware and Lab Chart software (ADInstruments, Bella Vista, Australia), and monitored on a computer. Visual feedback was provided during the performance fatigability protocols.

Peripheral nerve stimulation was performed by delivering percutaneous electrical stimuli using a constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the femoral nerve via cathode electrode (10 mm stimulating diameter; Meditrace 100, Covidien) in the inguinal triangle and 50 × 90 mm rectangular anode electrode (Durastick Plus, DJO Global, Vista, CA) placed between greater trochanter and suprailiac projections. The electrical stimuli intensity was adjusted in each session and prior to any physical exercise by delivering 1-ms rectangular single stimulus incrementally until reaching plateau in twitch torque and maximal compound muscle action potential (M-wave) amplitudes on the vastus lateralis muscle. The stimulation intensities were adjusted to 130% to ensure maximal twitch torque and M-wave amplitudes. Thereafter, participants performed a standardized warm-up consisting of five 3-s contractions of the knee extensors, interspersed by 30-s rest periods, at intensities corresponding to 50%, 60%, 70%, and 80% of the maximum subjective torque, followed by two 5 s IMVC. The performance fatigability assessment protocol consisted of one 5 s IMVC and electrical nerve stimulations of high-frequency (100 Hz, Db100), low-frequency (10 Hz, Db10) paired stimuli, and a single stimulus (Qtw_{pot}). During the 5 s IMVC, a superimposed 100 Hz doublet was applied when the participants produced maximal torque and, once the muscle was relaxed, followed by Db100, Db10, and Qtw_{pot} interspaced by 2 s. The performance fatigability protocol was repeated twice at baseline and once after the RI test at min 0.5, 1.5, 2.5, 5, and 10. A third IMVC at baseline was performed if the peak torque difference before the superimposed twitch between the first two IMVCs was greater than 5%, wherein the highest IMVC force at baseline was considered for performance fatigability analysis.

EMG data acquisition.

EMG of the VL was recorded with pairs of self-adhesive surface electrodes (10 mm recording diameter) (Meditrace 100, Covidien, Mansfield, MA) in bipolar configuration with a 30 mm interelectrode distance and one reference electrode placed on the patella. A low impedance (< 10 kΩ) between electrodes was obtained by shaving and gently abrading the skin with sandpaper and then cleaning with isopropyl alcohol 70%. Signals were analog-to-digitally converted at a sampling rate of 2,000 Hz by PowerLab system (16/35, ADInstruments) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 500) with bandpass filter (5–500 Hz).

Blood lactate concentration.

$[Lac]_b$ measurements were initiated by wiping a finger with an alcohol swab, followed by a finger-prick, and collection of a 20 μL blood sample with a capillary tube, which was mixed in an EKF pre-filled safe lock plastic tube for analysis (EKF Biosen C-Line Analyzer, Barleben, Germany). Samples for evaluation of $[Lac]_b$ were taken at baseline and 3 min after the RI test.

Perceptual variables.

The rating of perceived exertion was assessed using a 15-point category scale (6–20 Borg) (23). The instructions for RPE scale were standardized (24). Briefly, subjects were asked to rate their conscious sensation of how hard, heavy, or strenuous the physical task was. For example, a rating of 9 in the scale corresponded to "very light" exercise (e.g., walking slowly at a self-selected own pace for several minutes). A rating of 17 in the scale corresponded to a "very hard" and strenuous exercise (e.g., a healthy individual must strongly push themselves to continue, as the exercise feels very heavy). Participants were asked to rate their perceived leg pain via 0–10 visual analog scale (25), wherein the "0" in the pain scale represented "no pain," whereas "10" represented "worst possible pain." Participants were asked to verbally answer the question "How is your perceived pain from your legs?". Dyspnea was assessed using a modified Borg CR-10 scale (26) and described as the sensation of breathlessness: participants were asked "how breathless are you?". For the three scales, participants were familiarized during the first session before any cycling exercise. All perceptual responses were collected at baseline and after the RI tests.

Data Analyses

$\dot{V}O_2$ data during the RI tests were cleaned by removing data points laying ± 3 standard deviations from the local mean and linearly interpolated to 1-s intervals (Origin, Origin Lab, Northampton, MA). $\dot{V}O_{2max}$ was computed as the highest value from a 20 s rolling average during the RI test. POpeak corresponded to the highest PO value recorded at the end of the RI test for each participant. In order to estimate the work performed above the critical power (CP) and the "finite work capacity" or W', the two-parameter model was applied to each RI test, as proposed elsewhere (4, 27), given by the following equation:

$$T = \mathrm{CP}/S + \sqrt{2W'/S}$$

where T is the time to exhaustion during the RI test (in seconds), S is the rate of increase of the RI test (in $W \cdot s^{-1}$), and the CP (in W) and W' (kJ) are the parameter estimates of the model. Thereafter, the work done above CP (W > CP) was calculated by the integral of power-time above the estimated CP individual value for each RI test, and expressed in kJ, as proposed elsewhere (28).

Performance fatigability was characterized as the absolute change from baseline (Bsln) to after 0.5 min from the RI task failure. Also, the recovery of performance fatigability variables was assessed as the percent of change from Bsln to min 0.5, 1.5, 2.5, 5, and 10 after RI task failure. Moreover, although IMVC was the marker of global performance fatigability, the voluntary activation (VA) and evoked twitches torques with different frequencies (i.e., contractile function) were markers of changes occurring above and below the neuromuscular junction, respectively. Maximal voluntary torque was calculated as the highest IMVC before the cycling exercise, and the IMVC post the RI tests. The electrically evoked torque from the doublets and single pulse was determined as the peak torque of each stimulation on the relaxed muscle. Low-frequency fatigue was quantified by calculating the ratio between Db10 and Db100 (Db10:100). The M-wave was quantified as peak-to-peak amplitude in mV for VL muscle.

VA was assessed by a superimposed paired-pulse technique as previously described by Strojnik and Komi (29):

$$\mathrm{VA}(\%) = 100 - \mathrm{D} \times \left(\mathrm{IMVC_{Db100}}/\mathrm{IMVC_{peak}}\right)/\mathrm{Db100} \times 100$$

where $\mathrm{IMVC_{Db100}}$ is the voluntary torque when superimposed Db100 was delivered, $\mathrm{IMVC_{peak}}$ is the highest torque during the IMVC before the superimposed Db100, D is the difference between the torque level at the time of $\mathrm{IMVC_{Db100}}$ and the maximum torque during superimposed Db100, and Db100 is the electrically evoked torque on relaxed muscle two seconds after IMVC. Recommendations related to

Table 1. *Sample characteristics for females and males*

	♀ (*n* = 10)	♂ (*n* = 11)	*P* Value
Age, yr	32 ± 10	36 ± 11	0.305
Body mass, kg	63 ± 5	74 ± 13	0.023
Body stature, cm	167 ± 4	179 ± 10	0.002
Body fat, %	28 ± 5	20 ± 7	0.014
LBM, kg	44 ± 4	57 ± 8	0.001
Leg LM, kg	15 ± 1	19 ± 3	0.001

LBM, lean body mass; LM, lean mass; ♀, females; ♂, males.

instructions, practice, visual feedback of performance, and standardized verbal encouragement were adopted in the present study to ensure the maximum effort of participants during the IMVCs and performance fatigability parameters assessments (30).

The root mean square of the EMG signal VL muscle (EMG_{VL}) was calculated during the last 30 s of baseline cycling and RI test. The EMG_{VL} values were represented as the percent of change from baseline to RI task failure.

Statistical Analysis

Normal data distribution was confirmed by Shapiro and Wilk's test and visually checked with Q-Q plots. Results were reported as means ± 1 standard deviation (SD). Females and males' anthropometric data and responses from the RI test were compared by an independent sample *t* test. Intraclass correlation coefficient (ICC) was calculated to quantify the reproducibility for all baseline measurements. The results from RI tests (i.e., $\dot{V}O_{2max}$, POpeak, RI total duration, W > CP, $[Lac]_b$ and EMG_{VL}) were analyzed by a two-factor mixed ANOVA (sex × RI slope). The perceptual responses (i.e., RPE, leg pain, and dyspnea) comparisons were performed using nonparametric data by Friedman's test followed by Wilcoxon's test for multiple comparisons between conditions and sexes, as those variables are ordinal data. Performance fatigability profiles were compared in percent of change from baseline to post RI test in each condition and between sexes by a two-factor mixed ANOVA with between-subject factor defined by sex and within-subject factor defined as RI slope (i.e., $RI_{15} - RI_{30} - RI_{45}$). The recovery of performance fatigability among conditions and between sexes was compared in percent of change from baseline to post performance fatigability assessments (i.e., min 0.5, 1.5, 2.5, 5, and 10) by a three-factor mixed ANOVA with between-subject factor defined by sex and within-subject factor defined as RI slope (i.e., $RI_{15} - RI_{30} - RI_{45}$) × time (i.e., min 0.5 – min 1.5 – min 2.5 – min 5 – min 10). Bonferroni post hoc tests were used to determine where differences existed. Partial eta squared (η_p^2. for ANOVA comparisons were computed and effect sizes were evaluated as small (< 0.02), medium (0.02–0.26) or large (> 0.26) (31). The significance level was set at $P \leq 0.05$. All statistical analyses were performed using a statistical software package (Statistica, version 10.0, Tulsa, OK).

RESULTS

Participants' Characteristics

Table 1 shows that there were no sex differences for age but females had smaller values for body mass, stature, total lean body and leg lean mass, and a greater percentage of fat compared with males (all $P < 0.05$).

Ramp-Incremental Tests Results

Table 2 depicts the overall responses for each RI test in females and males. $\dot{V}O_{2max}$max was not different among conditions (i.e., condition-effect) in absolute values ($L \cdot min^{-1}$; $P = 0.313$, $\eta_p^2 = 0.059$), normalized by body mass ($mL \cdot kg^{-1} \cdot min^{-1}$; $P = 0.237$, $\eta_p^2 = 0.072$) and LBM ($mL \cdot LBM$ in $kg^{-1} \cdot min^{-1}$; $P = 0.221$, $\eta_p^2 = 0.076$). However, females had smaller $\dot{V}O_{2max}$ compared with males (i.e., sex-effect) in absolute values ($P < 0.001$, $\eta_p^2 = 0.716$), normalized by body mass ($P = 0.04$, $\eta_p^2 = 0.354$) and LBM ($P = 0.007$, $\eta_p^2 = 0.327$). POpeak was progressively greater with faster slopes (i.e., condition-effect) in absolute values (W; $P < 0.001$, $\eta_p^2 = 0.890$), normalized by body mass ($W \cdot kg^{-1}$; $P < 0.001$, $\eta_p^2 = 0.909$), and LBM ($W \cdot LBM$ in kg^{-1}; $P < 0.001$, $\eta_p^2 = 0.906$). Females showed smaller POpeak than males (i.e., sex-effect) in absolute values ($P < 0.001$, $\eta_p^2 = 0.615$), normalized by body mass ($P = 0.010$, $\eta_p^2 = 0.298$), and LBM ($P = 0.019$, $\eta_p^2 =$

Table 2. *Ramp incremental tests results at task failure for each condition and sex*

	15 W·min^{-1}		30 W·min^{-1}		45 W·min^{-1}	
	♀	♂	♀	♂	♀	♂
RI time, min	13 ± 3⚥#	20 ± 4#	7 ± 2⚥*	11 ± 2*	6 ± 1⚥*†	8 ± 2*†
$\dot{V}O_{2max}$, L·min^{-1}	2.60 ± 0.44⚥	3.93 ± 0.55	2.51 ± 0.46⚥	4.00 ± 0.70	2.57 ± 0.39⚥	3.89 ± 0.57
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	41.5 ± 8.5⚥	54.4 ± 10.8	40.0 ± 8.8⚥	55.5 ± 12.5	40.9 ± 7.5⚥	53.7 ± 10.0
$\dot{V}O_{2max}$, mL·LBM^{-1}·min^{-1}	57.1 ± 8.6⚥	67.6 ± 9.8	55.0 ± 8.9⚥	68.5 ± 10.3	56.3 ± 7.5⚥	66.7 ± 56.3
POpeak, W	213 ± 39⚥	326 ± 56	243 ± 47⚥*	360 ± 52*	272 ± 42⚥*†	397 ± 69*†
POpeak, W·kg^{-1}	3.4 ± 0.6⚥#	4.5 ± 1.0#	3.9 ± 0.8⚥*	5.0 ± 1.0*	4.3 ± 0.7⚥*†	5.5 ± 1.1*†
POpeak, W·LBM^{-1}	4.8 ± 0.6⚥#	5.8 ± 0.9#	5.5 ± 0.8⚥*	6.4 ± 0.8*	6.2 ± 0.7⚥*†	6.9 ± 0.8*†
W>CP, kJ	15 ± 3	16 ± 5	15 ± 3	16 ± 6	15 ± 1	17 ± 7
$[Lac]_b$, mM	10.2 ± 1.6	11.1 ± 1.9	10.3 ± 1.3	10.3 ± 1.8	10.3 ± 1.3	9.8 ± 1.0
EMG_{VL} (% from baseline)	454 ± 274	734 ± 341	420 ± 249	595 ± 344	456 ± 214	600 ± 395
RPE (Borg 6-20)	19 ± 1	19 ± 1	18 ± 1	19 ± 1	19 ± 1	19 ± 1
Leg pain (0–10)	6 ± 2	8 ± 2	7 ± 3	8 ± 2	6 ± 2	7 ± 2
Dyspnea (0.5–10)	8 ± 1	9 ± 1	8 ± 2	9 ± 1	8 ± 1	9 ± 1

$\dot{V}O_2$, oxygen uptake; LBM, lean body mass; POpeak, peak power output; W > CP, work done above the critical power applied for multiple ramp incremental tests; $[Lac]_b$, blood lactate concentration; EMG_{VL}, electrical myography signal from vastus lateralis muscle belly; RPE, rate of perceived exertion; ♀, females; ♂, males; #different from other conditions; *different from 15 W·min^{-1}; †different from 30 W·min^{-1}; ⚥, sex differences.

0.265). The total time of the RI test was progressively shorter with faster slopes (condition-effect, $P < 0.001$, $\eta_p^2 = 0.937$), wherein males exercised for a longer time compared with females in all conditions (sex-effect, $P < 0.001$, $\eta_p^2 = 0.594$). There were no main effects of condition or sex for $[\text{Lac}]_b$ ($P = 0.402$, $\eta_p^2 = 0.046$ and $P = 0.206$, $\eta_p^2 = 0.082$, respectively), EMG_{VL} ($P = 0.576$, $\eta_p^2 = 0.027$ and $P = 0.117$, $\eta_p^2 = 0.106$, respectively), RPE ($P = 0.529$ and 0.540, respectively), leg pain ($P = 0.930$ and 0.256, respectively), and dyspnea ($P = 0.166$ and 0.188, respectively). The CP and W' estimates applied for multiple RI tests showed lower CP values for females (133 ± 38 W; $R^2 = 0.93 \pm 0.08$; SEE = 9 ± 7 W) compared with males (242 ± 67 W; $R^2 = 0.97 \pm 0.02$; SEE = 5 ± 2 W) ($P < 0.001$) but similar W' between sexes (18 ± 1 and 18 ± 3 kJ, respectively) ($P = 0.859$). Moreover, there was no condition-effect in the work done above CP in kJ among the RI tests ($P = 0.640$, $\eta_p^2 = 0.016$), differences between sexes ($P = 0.354$, $\eta_p^2 = 0.024$) (Table 2). In addition, there was no condition × sex interaction for any of the abovementioned variables (all $P > 0.05$).

Performance Fatigability Responses Among Conditions and Sexes

The performance fatigability responses in each condition and in females and males are shown in Fig. 2. Overall, there was no main effect of condition as shown by percentage of decline from baseline to 0.5 min after the RI task failure for IMVC ($P = 0.551$, $\eta_p^2 = 0.030$), VA ($P = 0.539$, $\eta_p^2 = 0.031$), Db100 ($P = 0.178$, $\eta_p^2 = 0.086$), Db10:100 ($P = 0.389$, $\eta_p^2 = 0.048$), Qtw_{pot} ($P = 0.462$, $\eta_p^2 = 0.039$), and M-wave ($P = 0.247$, $\eta_p^2 = 0.070$). In addition, there was no main effect of sex for IMVC ($P = 0.508$, $\eta_p^2 = 0.023$), VA ($P = 0.498$, $\eta_p^2 = 0.024$), Db100 ($P = 0.65$, $\eta_p^2 = 0.010$), Db10:100 ($P = 0.099$, $\eta_p^2 = 0.136$), Qtw_{pot} ($P = 0.586$, $\eta_p^2 = 0.015$), and M-wave ($P = 0.799$, $\eta_p^2 = 0.003$). In addition, there was no condition × sex interaction for any of the abovementioned variables (all $P > 0.05$).

At baseline, there were no differences between females and males, in absolute values, for IMVC (454 ± 106 vs. 524 ± 99 N, respectively; $P = 0.136$, $\eta_p^2 = 0.113$), VA (93 ± 6% vs. 93 ± 5%, respectively; $P = 0.955$, $\eta_p^2 = 0.001$), Db10:100 (1.02 ± 0.09 vs. 0.97 ± 0.09, respectively; $P = 0.158$, $\eta_p^2 = 0.101$), but there were smaller values in females compared with males for Db100 (195 ± 21 vs. 240 ± 45 N, respectively; $P = 0.009$, $\eta_p^2 = 0.307$), Qtw_{pot} (132 ± 20 vs. 157 ± 30 N, respectively; $P = 0.034$, $\eta_p^2 = 0.214$), and M-wave (10 ± 4 versus 15 ± 5 mV, respectively; $P = 0.005$, $\eta_p^2 = 0.340$) variables.

Recovery of Performance Fatigability Among Conditions and Sexes

The recovery of performance fatigability variables in each condition and between sexes is shown in Fig. 3. The recovery for IMVC values after RI tests only showed a main effect of time ($P < 0.001$, $\eta_p^2 = 0.634$), as its values increased from min 2.5 to min 10 (all $P < 0.05$) without any main effect of condition ($P = 0.814$, $\eta_p^2 = 0.001$). For VA responses, there was no main effect neither for time ($P = 0.884$, $\eta_p^2 = 0.014$) nor for condition ($P = 0.747$, $\eta_p^2 = 0.015$). The recovery of contractile function showed differences throughout time and among conditions, and there were interaction effects for Db100 ($P = 0.005$, $\eta_p^2 = 0.205$) and Qtw_{pot} ($P = 0.009$, $\eta_p^2 = 0.165$). Specifically, in RI_{45} and RI_{30} conditions, Db100 and Qtw_{pot} showed the same recovery at min 1.5, with continuous increase until min 10 (all $P < 0.05$). On the other hand, the recovery after RI_{15} took longer, as Db100 started to increase at min 5 and Qtw_{pot} at min 2.5, thereafter showing a steady increase until min 10 (all $P < 0.05$). There was no recovery for Db10:100 throughout the 10-min period after all RI tests, since there was no main effect of time ($P = 0.160$, $\eta_p^2 = 0.081$) nor condition ($P = 0.182$, $\eta_p^2 = 0.085$). The M-wave amplitude showed a main effect of time ($P = 0.015$, $\eta_p^2 = 0.192$) but no main effect of condition ($P = 0.430$, $\eta_p^2 = 0.043$), where it showed the greatest values at min 2.5 and 5 compared with the remaining data points (all $P < 0.05$). Regarding sex differences, whereas Db10:100 showed less of a change in males compared with females ($P = 0.039$, $\eta_p^2 = 0.206$), there were no sex differences for the change in IMVC ($P = 0.855$, $\eta_p^2 = 0.001$), VA ($P = 0.435$, $\eta_p^2 = 0.015$), Db100 ($P = 0.843$, $\eta_p^2 = 0.002$), Qtw_{pot} ($P = 0.457$, $\eta_p^2 = 0.029$), and M-wave amplitude ($P = 0.995$, $\eta_p^2 = 0.001$) throughout the recovery period. In addition, there was no condition × sex interaction for any of the abovementioned variables (all $P > 0.05$).

Repeatability of Baseline Measurements Among Conditions

There were no differences among visits and high ICC values during the baseline cycling at 20 W before the RI test for $\dot{V}\text{O}_2$ ($P = 0.165$; ICC = 0.886), $[\text{Lac}]_b$ ($P = 0.357$; ICC = 0.779), EMG_{VL} ($P = 0.214$; ICC = 0.680), RPE ($P = 0.630$; ICC = 0.821), leg pain ($P = 0.086$; ICC = 0.633), and dyspnea ($P = 0.214$; ICC = 0.812). Moreover, at baseline, there was no difference among conditions, and high ICC values were found for IMVC ($P = 0.624$; ICC = 0.964), VA ($P = 0.275$; ICC = 0.845), Db100 ($P = 0.891$; ICC = 0.973), Db10:100 ($P = 0.817$; ICC = 0.893), Qtw_{pot} ($P = 0.745$; ICC = 0.962), and M-wave amplitude ($P = 0.861$; ICC = 0.833).

DISCUSSION

The novel findings of this study are that: *1*) there was similar performance fatigability profile and perceived responses after RI tests of different slopes that elicited similar $\dot{V}\text{O}_{2\text{max}}$ values but different POpeak; *2*) females and males showed the same performance fatigability profile and perceived responses regardless the RI slope performed; and *3*) the recovery of contractile function (i.e., Db100 and Qtw_{pot}) in RI_{15} was delayed compared with the RI_{30} and RI_{45} and no sex differences were observed. Taken together, these data indicate that, regardless of the RI slope utilized, the metabolic disturbance rather than the mechanical workload associated with task failure is the main determinant of performance fatigability and perceived responses. In addition, both sexes were equally affected by different RI slopes since there were similar performance fatigability profiles and perceived responses either at task failure or throughout the recovery period investigated.

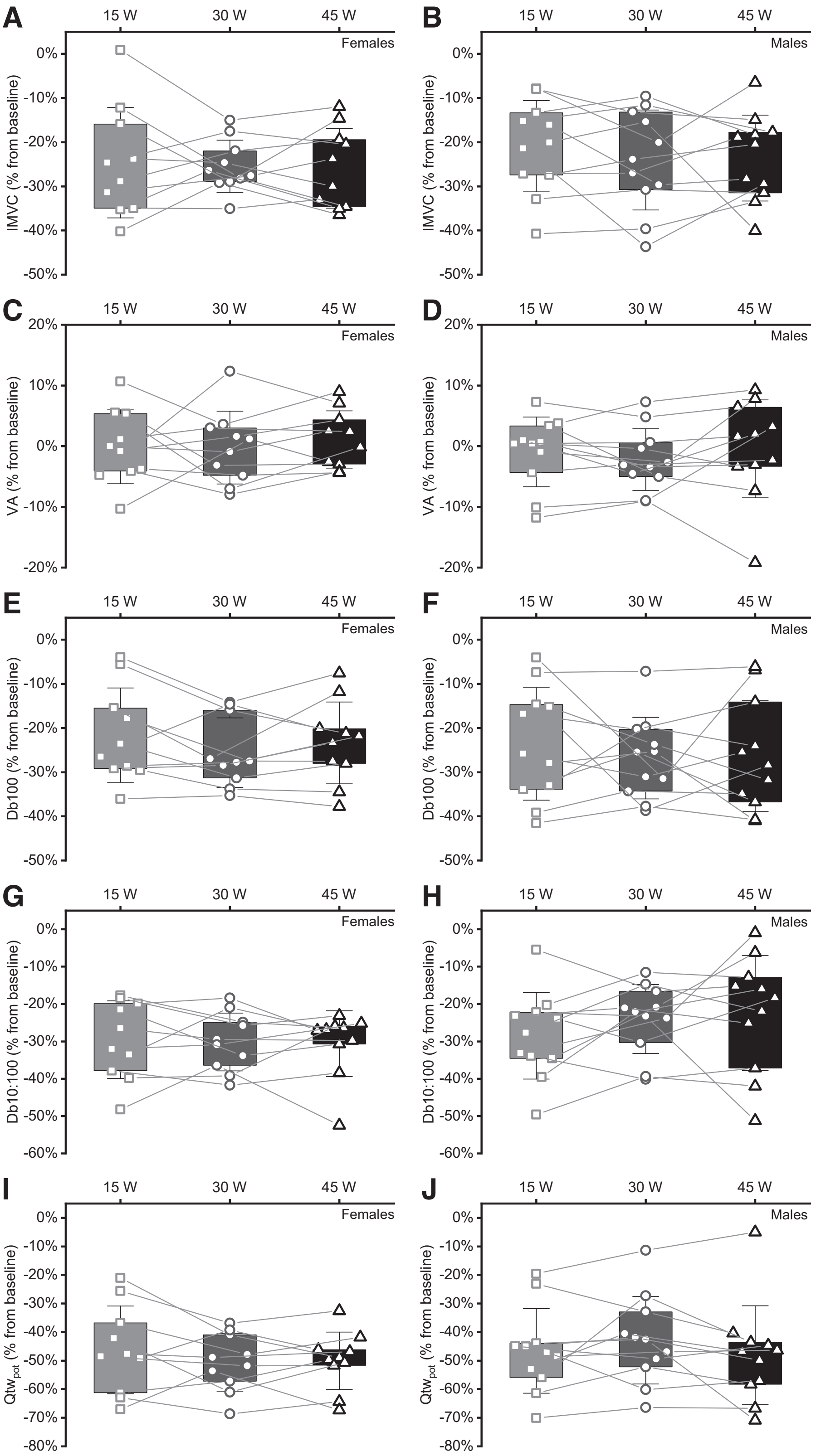

Figure 2. Performance fatigability responses in percentage of baseline values in each condition and sex. *A, B*: isometric maximal voluntary contraction (IMVC) in females and males, respectively; *C, D*: voluntary activation (VA) in females and males, respectively; *E, F*: 100 Hz paired-pulses evoked torque (Db100) in females and males, respectively; *G, H*: ratio between 10 and 100 Hz evoked torques (Db10:100) in females and males, respectively; *I, J*: single pulse evoked torque (Qtw_{pot}) in females and males, respectively. Upper and lower limits of boxes represent first and third quartiles, and whiskers represent one standard deviation.

Profiles of Performance Fatigability and Perceived Responses Among Distinct RI Tests

Performance fatigability responses from baseline to 0.5 min post exercise (i.e., IMVC, VA, Db100, Db10:100, and Qtw_{pot}) were not different after RI_{15}, RI_{30}, and RI_{45} tests (Fig. 2). These performance fatigability profiles were accompanied by similar $\dot{V}O_{2max}$, W > CP, $[Lac]_b$ and EMG_{VL} responses but greater POpeak with progressively faster RI slopes. Together, these findings indicate that performance fatigability responses

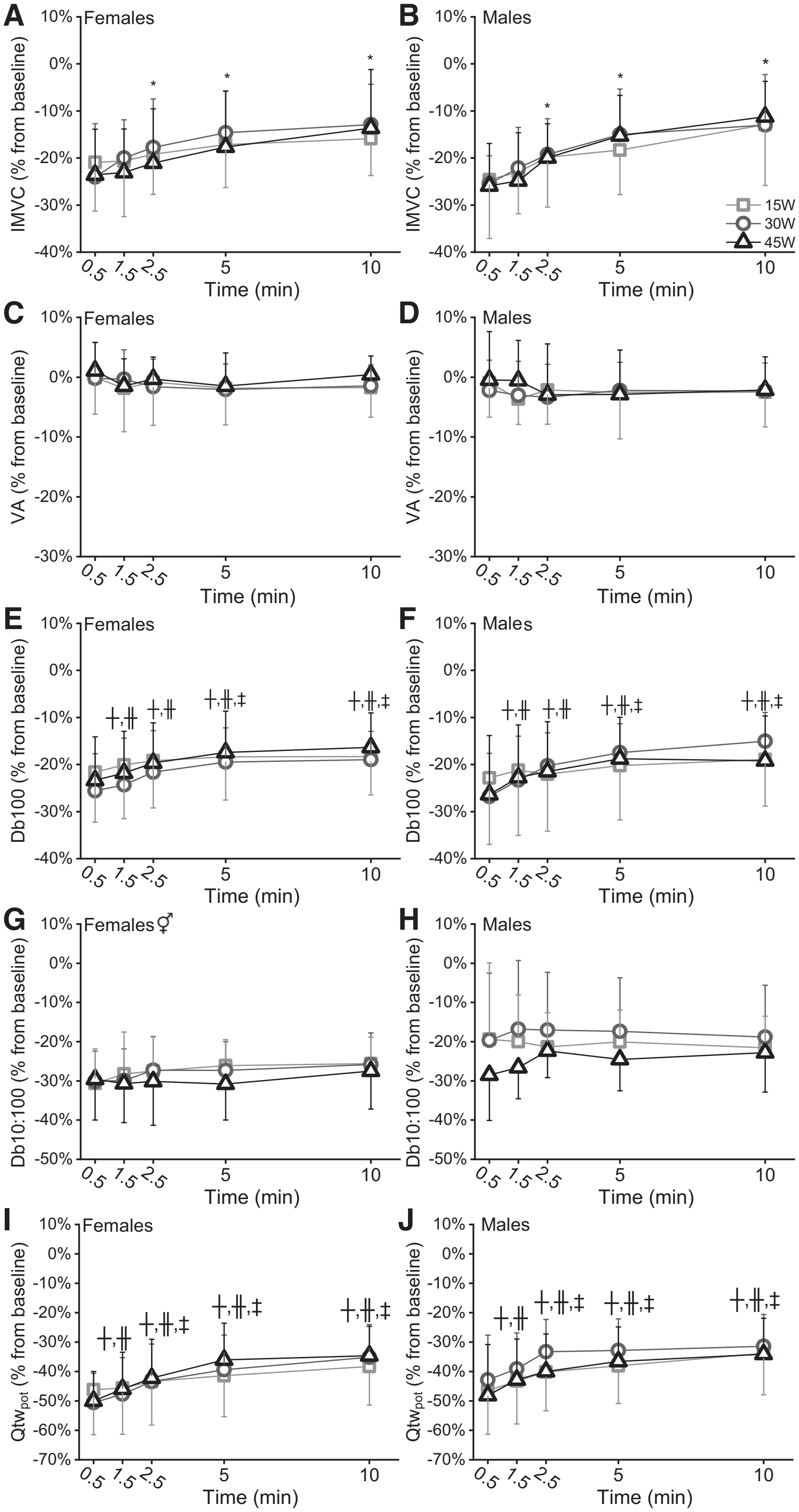

Figure 3. Recovery of performance fatigability responses in percentage of change from baseline in each condition and sex. *A, B*: isometric maximal voluntary contraction (IMVC) in females and males, respectively; *C, D*: voluntary activation (VA) in females and males, respectively; *E, F*: 100 Hz paired-pulses evoked torque (Db100) in females and males, respectively; *G, H*: ratio between 10 and 100 Hz evoked torques (Db10:100) in females and males, respectively; *I, J*: single pulse evoked torque (Qtw$_{pot}$) in females and males, respectively. *different from previous timepoint for all conditions; ‡different from previous timepoint for RI$_{15}$ condition; ┼different from previous timepoint for RI$_{30}$ condition; ╫different from previous timepoint for RI$_{45}$ condition; ⚥, sex-differences.

are influenced by the metabolic disturbance rather than the mechanical workload achieved during the RI protocol. Indeed, exercise-induced metabolic disturbance is suggested to be the cornerstone of performance fatigability responses, especially at the muscle contractile function site (32, 33). At task failure during the RI test, oxidative phosphorylation is at its near maximal rate of ATP resynthesis while utilizing substrate-level phosphorylation to match the exercise-induced metabolic demand, i.e., *W′* depletion (34, 35). As a result, metabolites accumulate within the active musculature, which negatively affects contractile function performance (32, 33). In the present data set, there were similar $\dot{V}O_{2max}$, $[Lac]_b$, and EMG_{VL} responses after RI_{15}, RI_{30}, and RI_{45} tests, which suggest that task failure occurred at a similar level of metabolic disturbance and *W′* depletion (i.e., similar $W > CP$ among RI tests; see Table 2 for details), which likely explained the similar performance fatigability profiles. Overall, these results point out that despite a possible earlier reliance on and faster rate of depletion of *W′* during faster RI compared to slow slopes, all RI tests resulted in a similar *W′* utilization at task failure, and this was accompanied by similar physiological responses (i.e., $\dot{V}O_{2max}$ and $[Lac]_b$). Indeed, task failure for exercise performed within the severe-intensity domain is accompanied by the achievement of a given metabolic disturbance and/or depletion of a finite work capacity (i.e., *W′*) (7), which coincide with the attainment of a consistent level of contractile function impairment. In addition, previous studies (7, 36) have shown a similar decline in contractile function at task failure following constant-load cycling of different durations within the severe intensity domain. For example, Schafer et al. (7) utilized different work rates (i.e., ∼98% vs. ∼78% of POpeak) to deplete all *W′* available (19 ± 6 kJ) and induced task failure within 3- and 12-min of exercise, respectively. There was no difference between bouts regarding the reduction of potentiated twitch force (i.e., Db100, Db10, and Qtw_{pot}), which suggests the achievement of a similar level of metabolic disturbance (37).

In addition to the similar profile in performance fatigability, our data showed that perceived responses were also similar at task failure among RI tests (Table 2). It has been suggested that perceived responses to exercise are driven by a combination of afferent feedback from the active musculature and/or corollary discharge within the cortex (38, 39). Specifically, there was no change in VA regardless of the RI slope utilized, which suggests that there was no deficit in descending motor drive to maximally recruit the active musculature during an isometric contraction of brief duration (i.e., IMVC). In addition, the level of EMG_{VL} signal was the same at task failure among the RI tests, which could suggest similar muscle recruitment and possibly corollary discharge within the motor cortex. However, this result should be interpreted with caution, since surface EMG signal has limitations for muscle recruitment assessment (40) and previous studies have found different results, as greater cycling PO performed was accompanied by higher EMG signal at task failure (10, 11).

Together, these findings suggest that task failure during RI test of different slopes, achieving similar $\dot{V}O_{2max}$ and distinct POpeak, elicits the same perceived and performance fatigability profile. Thus, the present data support the idea that performance fatigability profile and perceived responses during a RI test seem to be determined by the exercise-induced metabolic disturbance, rather than by the absolute mechanical workload achieved.

Sex-Related Effects on Fatigability Development after RI Tests

It has been argued that exercise-induced performance fatigability development is also affected by the sex of the population, since females tend to show smaller amplitude of contractile function impairments than males (41). However, our results showed no main effect of sex and/or condition, nor interaction effect, for any performance fatigability measures and perceived responses at task failure, which suggests that there is no sex-related difference regardless of the RI slope utilized. This finding is somewhat contrary to previous results, which have shown less of a decline in contractile function in females compared with males after a bout of endurance cycling, either within the heavy or severe exercise intensity domains (6, 42). Part of this discrepancy in the results related to sex differences might be due to the characteristics of the task and participants included in this study. For example, the lower amplitude of performance fatigability development in females compared with males in endurance tasks is explained by lower exercise-induced metabolite accumulation, since females rely more on fat oxidation, muscle oxygenation, and activation of type I fibers compared with males (20, 43, 44). However, these sex-related differences in metabolite accumulation and performance fatigability responses are abolished when oxygen delivery and/or extraction within the active musculature is impaired (45), which might occur during maximal endurance exercise such as the final portion of a RI test (46). In addition, our results showed that females and males had similar *W′* estimated from different RI tests and *W′* depletion at task failure in each RI slope, as well as similar $[Lac]_b$ accumulation (i.e., see Table 2 for details). Thus, it is most likely that females and males achieved a similar exercise-induced metabolic disturbance, and thus the same performance fatigability profile, which might have been due to characteristics of the maximal endurance task that abolished potential sex-related physiological differences to be expressed. In relation to the population characteristics included in this study, it is worth mentioning that whereas females had similar IMVC at baseline compared to the literature, the IMVC values for male participants were a slightly smaller than those reported elsewhere (5, 6, 47, 48). As a result, the sex-related differences in muscle properties might have been diminished based on those sample characteristics. Nonetheless, it must be highlighted that, at baseline, males had greater values compared with females for IMVC, Db100, and Qtw_{pot}, which is expected finding and assure that our females and males are likely to represent the overall population.

Recovery of Performance Fatigability Parameters after Different RI Slopes

A novel aspect of the present study was the evaluation of the recovery of performance fatigability following RI testing of different slopes and in both sexes. Our findings showed some differences in recovery patterns after each of the RI slopes, wherein the recovery of high-frequency and single twitch evoked torque (i.e., Db100 and Qtw_{pot}, respectively) started 1.5 min after RI_{30} and RI_{45} but 2.5 min after RI_{15} protocol, whereas the other performance fatigability variables (i.e., IMVC, Db10:100 and M-wave) were not different among conditions. Similar results of recovery in performance fatigability components were also shown after high-intensity cycling trials (49, 50), wherein there was a fast recovery of contractile function within the first 2 min from exercise cessation followed by a slower recovery until ∼10 min. It has been suggested that the rapid recovery phase (i.e., within ∼2 min after exercise) of contractile function occurs due to the washout of metabolites accumulated during the high-intensity exercise, such as inorganic phosphate and adenosine diphosphate (49). In this context, our results suggest that the washout phase might be differently affected according to the RI slope utilized, as there was a slower recovery of contractile function after RI_{15} compared with RI_{30} and RI_{45} conditions. Even though speculative, the different recovery might be due to the duration of the task, since previous findings have shown delayed recovery in performance fatigability as task duration increases (49, 51), which suggests that different contractile function mechanisms were impaired despite a similar metabolic disturbance at task failure. It must be highlighted that the recovery profile of performance fatigability was initiated 30 s after exercise cessation, which might be long enough to underestimate the actual exercise-induced decline in contractile function and voluntary activation (52). However, the recovery period showed similar patterns among conditions, which suggest that the characteristics of the performance fatigability assessments would play a minor role in the overall interpretation of the results.

Another relevant finding in relation to the recovery of performance fatigability was that the low-frequency evoked torque ratio (Db10:100) did not show any sign of recovery regardless of the RI protocol (Fig. 3), which is in line with previous findings after a RI test that utilized a different slope (i.e., 20 $W \cdot min^{-1}$) (50). As the low-frequency fatigue is thought to reflect excitation-coupling failure (53–55), it would be plausible that the intramuscular Ca^{2+}-handling was equally impaired among RI tests. It must be highlighted that changes in muscle contraction properties might have been mostly beyond the sarcolemma, since the action potential (i.e., M-wave peak-to-peak amplitude from potentiated single twitch; Qtw_{pot}) responses were not different among conditions but only throughout time (i.e., greater values at min 2.5 and 5). Finally, there was no sex-related difference during the recovery period in any of performance fatigability variable, which suggests that females and males share similar underpinning mechanism of exercise-induced fatigue development and its recovery after exercise cessation.

Experimental Considerations

First, this study did not assess the phase of the menstrual cycle during which each RI test was performed. Given that sex-related differences in key physiological parameters for the current study (i.e., $\dot{V}O_2max$, POpeak, and performance fatigability variables) are known to be greater than the potential effects of the menstrual cycle fluctuations (42, 56, 57), then the added research cost and burdensome to the participants associated with proper assessment of the menstrual cycle seemed unjustified. Additionally, recent findings indicated no differences among menstrual cycle phases in $\dot{V}O_{2max}$ and POpeak (58). Second, there was no specific familiarization session for RI tests and performance fatigability assessments. However, it must be highlighted that 11 out of 21 participants have already performed RI tests in previous studies in our laboratory and, for the remaining 10 participants, all of them were physically active and familiarized with high-intensity exercise bouts. In terms of performance fatigability assessments, there was extensive familiarization with peripheral nerve stimulation before any physical exercise during the first session, and subsequent tests were only conducted if the IMVC force, with superimposed twitch (i.e., Db100), was consistent during familiarization trials and the participant could completely relax while the other nerve stimulations were delivered (i.e., Db100, Db10, and Qtw_{pot}). Nonetheless, the sessions were randomized, which minimized any familiarization effect and all participants achieved the same $\dot{V}O_{2max}$ value regardless of the order of the RI tests. Finally, there was no assessment of "perceived fatigue" as measurement of perceived fatigability, whereas effort, pain, and dyspnea scales were adopted in the current study. Briefly, this choice was made based on current findings (59) that task failure in exercise bouts within the severe intensity domain might be better associated with perceived effort, pain, and dyspnea than with fatigue, which might be more relevant when exercising within the heavy and moderate intensity domains.

Conclusions

This study showed that RI tests of different slopes did not affect the extent and the characteristics of performance fatigability and perceived responses at task failure in females and males. Thus, metabolic disturbance rather than the mechanical workload associated with task failure might be the main underpinning mechanism determining the decline in contractile function and progression of perceptual responses during this exercise paradigm. This was shown by the lack of differences in the profiles of perceived and performance fatigability variables as well as in $\dot{V}O_{2max}$ and metabolic responses to maximal exercise despite the greater mechanical workload (i.e., POpeak) values observed as the RI slope increased. This conclusion remained true regardless of sex, as females and males showed the same responses independently of the RI slopes performed. Finally, the present study indicated that the recovery pattern of contractile function is partially affected by the slope of the RI test, since there was a slower recovery of high-frequency doublet and single twitch evoked torques after slow compared with medium and fast RI slopes.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.d.A.A., P.R.F.-P., M.T., G.Y.M., and J.M.M. conceived and designed research; R.d.A.A., P.R.F.-P., and M.T. performed experiments; R.d.A.A., P.R.F.-P. and M.T. analyzed data; R.d.A.A., P.R.F.-P., M.T., D.I., G.Y.M., and J.M.M. interpreted results of experiments; R.d.A.A. and D.I. prepared figures; R.d.A.A., P.R.F.-P., M.T., D.I., G.Y.M., and J.M.M. drafted manuscript; R.d.A.A., P.R.F.-P., M.T., D.I., G.Y.M., and J.M.M. edited and revised manuscript; R.d.A.A., P.R.F.-P., M.T., D.I., G.Y.M., and J.M.M. approved final version of manuscript.

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