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Differential Effects of Exercise Intensity and Tolerable Duration on Exercise-Induced Diaphragm and Expiratory Muscle Fatigue

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Running head
Exercise intensity, duration, and respiratory muscle fatigue.

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ABSTRACT

We investigated the effect of exercise intensity and tolerable duration on the development of exercise-induced diaphragm and expiratory muscle fatigue. Ten healthy adults (25 ± 5 y; 2 females) cycled to intolerance on three separate occasions: 1) 5% below critical power (<CP; *heavy intensity*); 2) ~25% of the difference (Δ) between CP and peak ramp-incremental power (Δ25; *severe intensity 'longer'*); and 3) ~50% Δ (Δ50; *severe intensity 'shorter'*). Diaphragm and expiratory muscle fatigue were quantified as a pre- to 5 min post-exercise reduction in magnetically evoked transdiaphragmatic (Pdi_{tw}) and gastric (Pga_{tw}) twitch pressures, respectively. Exercise time was 34.5 ± 6.2 min, 10.2 ± 2.6 min, and 4.9 ± 0.7 min for <CP, Δ25, and Δ50 conditions, respectively. Oxygen uptake ($\dot{V}O_2$) at end-exercise was lower during <CP (87 ± 6% $\dot{V}O_{2peak}$) relative to Δ25 (97 ± 4% $\dot{V}O_{2peak}$) and Δ50 (99 ± 4% $\dot{V}O_{2peak}$) ($P < 0.001$). The pre- to post-exercise decrease in Pdi_{tw} was greater after Δ25 (−22 ± 12%) vs. <CP (−13 ± 8%; $P = 0.0499$) and Δ50 (−14 ± 12%; $P = 0.045$). Conversely, the decrease in Pga_{tw} from pre- to post-exercise was not different between trials (<CP: −23 ± 15%; ∆25: −29 ± 15%; ∆50: −25 ± 16%) ($P > 0.05$). In conclusion, the magnitude of exercise-induced diaphragm fatigue was greater after longer duration severe exercise than after shorter duration severe and heavy exercise. By contrast, the magnitude of exercise-induced expiratory muscle fatigue was unaffected by exercise intensity and tolerable duration.

Key words: diaphragm fatigue; expiratory muscle fatigue; magnetic nerve stimulation; exercise intensity; critical power.

NEW & NOTEWORTHY:

Exercise-induced respiratory muscle fatigue contributes to limiting exercise tolerance. Accordingly, better understanding the exercise conditions under which respiratory muscle fatigue occurs is warranted. While heavy intensity as well as short- and long-duration severe intensity exercise performed to intolerance elicit diaphragm and expiratory muscle fatigue,

78 we find, for the first time, that the relationship between exercise intensity, exercise duration,
79 and the magnitude of exercise-induced fatigue is different for the diaphragm compared to the
80 expiratory muscles.

INTRODUCTION

Whole-body exercise performed at ≥80-85% of maximal oxygen uptake ($\dot{V}O_{2max}$) that is sustained to intolerance elicits fatigue of the diaphragm and the expiratory abdominal muscles, as shown by a transient reduction in electrically or magnetically evoked diaphragm and gastric twitch pressures (1-5). Based on this evidence, it is often stated that respiratory muscle fatigue can occur during and/or in response to heavy intensity exercise in healthy humans (6). However, the assertion that *heavy* intensity exercise causes respiratory muscle fatigue is questionable when considering the intensity domain schema (7), defined primarily by the pulmonary $\dot{V}O_2$ and blood lactate dynamics. The heavy intensity domain encompasses work rates between lactate threshold (LT) and critical power (CP), and is characterized by an initial increase followed by a stabilization of pulmonary $\dot{V}O_2$ and arterial blood lactate concentration ($[La^-]_B$) at submaximal levels (i.e., $\dot{V}O_{2max}$ is not obtained) (7, 8). Conversely, during severe exercise (>CP), pulmonary $\dot{V}O_2$ and $[La^-]_B$ increase progressively without attainment of a steady state and $\dot{V}O_{2max}$ is ultimately reached at, or in close proximity to, the point of intolerance (7, 8). Crucially, and reflecting the cardiometabolic and neuromuscular demands of the exercise, another differentiating characteristic of heavy- vs. severe-intensity exercise is the tolerable duration of the exercise task, wherein exercise performed above CP is typically limited to ≤30 minutes (8). Given that a number of prior studies have observed exercise-induced respiratory muscle fatigue when the exercise task engenders exercise intolerance within roughly 10 to 20 minutes (2-4, 9), it may be more accurate to conclude that severe rather than heavy intensity exercise elicits respiratory muscle fatigue based on the available evidence.

The development of respiratory muscle fatigue plays a role in limiting exercise tolerance (10, 11). As such, a more detailed and precise understanding of the exercise conditions under which respiratory muscle fatigue occurs is warranted. Mechanistically, it is considered that a consequence of respiratory muscle fatigue during ‘high-intensity’ whole-body exercise is the triggering of a reflexively-mediated sympathoexcitation that is associated with an increase in

muscle sympathetic nerve activity (MSNA) (12, 13). The consequence of this reflex is peripheral vasoconstriction characterised by an increase in mean arterial pressure (MAP), and a reduction in blood flow and oxygen delivery in the exercising limbs (13-15). During submaximal exercise (50% and 75% of $\dot{V}O_{2max}$), however, it has been suggested that the associated ventilatory demand and power of breathing (Pb) are insufficient to cause such a cardiovascular adjustment, and do not trigger vasoconstriction in the locomotor muscles (i.e., the respiratory muscle metaboreflex is not activated) (16). This observation is true even when the Pb is experimentally increased by 50-70% during such exercise (16). That exercise at ~50 or 75% of $\dot{V}O_{2max}$ does not evoke sympathetically mediated alterations in limb vascular resistance and locomotor muscle blood flow could, in theory, indicate that inspiratory muscle fatigue *does not* occur during or in response to such heavy-intensity exercise. In addition, it has also been suggested that diaphragm fatigue does not occur in response to short-term incremental exercise performed to intolerance, presumably because the time period of very high Pb is not long enough to engender such fatigue (17). That is, it is possible that there is a minimum exercise intensity and tolerable duration required for the development of exercise-induced respiratory muscle fatigue.

To our knowledge, using the intensity-domain schema (7) to systematically assess the effect of exercise intensity on the development of exercise-induced respiratory muscle fatigue has not been investigated. Accordingly, our primary aim was to determine the effect of exercise intensity domain (i.e., heavy vs. severe) on the presence and magnitude of exercise-induced respiratory muscle fatigue. A secondary aim was to determine the effect of tolerable duration in the severe intensity domain on the presence and magnitude of exercise-induced respiratory muscle fatigue. We hypothesized that both the magnitude and incidence of respiratory muscle fatigue would be greater following longer-duration severe-intensity exercise compared to heavy-intensity exercise and shorter-duration severe-intensity exercise.

METHODS

Participants

Ten physically active adults participated in the study (2 females, 8 males; mean ± SD: age 25 ± 5 years, stature 1.77 ± 0.07 m, body mass 72 ± 10 kg). All participants were healthy, had no history of respiratory, cardiovascular, or metabolic disease, and had normal pulmonary function (forced vital capacity: 5.48 ± 0.87 L, 103 ± 12% of predicted; forced expiratory volume in 1 s: 4.32 ± 0.51 $L \cdot s^{-1}$, 98 ± 10% of predicted; maximum voluntary ventilation – extrapolated from 12 s of maximal ventilation: 191 ± 35 $L \cdot min^{-1}$, 113 ± 18% predicted). The participants abstained from food for 3 h, caffeine for 12 h, and alcohol and exercise for 48 h before each laboratory visit. The experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee (approval REF: BIOSCI 17-016) and conformed to the Declaration of Helsinki. Each participant provided written informed consent prior to commencement of any testing procedures.

Experimental Procedures

An overview of the experimental procedures is presented in Figure 1. Each participant visited the laboratory on four different occasions, each separated by at least 48 h. At the first visit, resting pulmonary function was assessed according to standard procedures (18). Next, the participants performed maximal ramp incremental exercise followed immediately by an all-out 3 min effort against a fixed resistance (ramp incremental sprint test, RIST) (19) on an electromagnetically braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands). This allowed for the determination of peak pulmonary oxygen uptake ($\dot{V}O_{2peak}$) and associated cardiometabolic variables, peak ramp power (P_{peak}), and estimation of critical power (CP). By convention, CP was defined as the asymptote of the power-duration relationship and represents the physiological threshold between heavy intensity exercise and severe intensity exercise (7). The participants were then familiarized thoroughly with the

protocols used to assess respiratory neuromuscular function, described below. During the next three visits, the participants performed constant-power cycle exercise to the limit of tolerance at: 1) 5% <CP (*heavy intensity exercise;* <CP); 2) ~25% of the difference (Δ) between CP and P_{peak} (‘*longer-duration’ severe intensity exercise*; Δ25); or 3) ~50% Δ (‘*shorter-duration’ severe intensity exercise*; Δ50) (Figure 1). The order of visits 2, 3, and 4 was pseudo-randomized between the participants such that three participants performed <CP first, three participants performed ∆25 first, and four participants performed ∆50 first. Diaphragm and expiratory abdominal muscle contractility were assessed before, 5 min after, and 30 min after each constant-power exercise test by measuring the transdiaphragmatic (Pdi_{tw}) and gastric (Pga_{tw}) twitch pressure response to magnetic stimulation of the phrenic nerve roots and thoracic nerve roots, respectively.

Ramp Incremental Sprint Test

Participants cycled at 20 W for 4-6 min before power was increased as a linear function of time at a rate of 25-30 $W \cdot min^{-1}$. The participants increased their pedal cadence progressively until they reached a sustainable self-determined rate (between 80 and 100 rpm). Each participant maintained this pedal cadence to within ± 5 rpm until the limit of tolerance, defined as the point at which pedal cadence fell below 60 rpm despite strong verbal encouragement. At the point of exercise intolerance, the cycle ergometer was switched instantaneously to cadence-dependent (linear) mode, in which mechanical power then became the product of cadence and a ‘linear factor’ (i.e., flywheel resistance). The participants accelerated their pedal cadence as fast as possible, and performed 3 min of maximal effort cycling (19). The ‘linear factor’ (i.e., power/$cadence^2$) was pre-determined for each participant with the aim of eliciting a cadence of ~80 rpm (i.e., the optimum of the parabolic relationship between power and cadence in the fatigued state) during the sprint phase while participants were cycling at CP (20). Using data from previous investigations (21) combined with pilot work in our laboratory it was determined that a power output of 2.5 and 3.0 times body mass in untrained and trained females, and 3.0 and 3.5 times body mass

192 in untrained and trained males, respectively, would result in a cadence of ~80 rpm during the
193 sprint phase. Because the time taken for power output to stabilize varies between
194 participants, the sprint phase was split into 30 s time bins. Mean power output during the
195 entire sprint was initially calculated, and any 30-s time-bin that was not within 5% of this
196 value was excluded. The recalculated mean power output was defined as sprint power (SP)
197 which is a direct estimate of CP (19). Using this technique, the time taken for a plateau in
198 power output to be established ranged from 0 to 90 s.
199
200 **Diaphragm and Expiratory Abdominal Function via Magnetic Nerve Stimulation**
201 *Respiratory pressures*
202 Gastric (Pga) and esophageal pressure (Pes) were measured using two balloon-tipped
203 catheters (47-9005; Ackrad Laboratories, Cooper Surgical, CT, USA) that were passed via
204 the nares into the stomach and lower one-third of the esophagus, respectively, following
205 application of 2% lidocaine to the naris. The esophageal balloon was filled with 1 ml of air
206 and the balloon depth was altered until the change in Pes relative to the change in mouth
207 pressure (Pm) ($\Delta Pes/\Delta Pm$) during resisted breaths against an occluded mouthpiece was
208 >0.97 to <1.03 (22). The gastric balloon was filled with 2 ml of air and positioned so that Pga
209 was positive throughout eupneic breathing with the participant in the seated position. The
210 final position of each catheter was documented and used for subsequent trials. Each
211 catheter was connected to a differential pressure transducer (DP15; Validyne, Northridge,
212 CA, USA) that was calibrated across the physiological range using a digital pressure
213 manometer (no. 621, Test Products International Inc., Beaverton, OR, USA).
214 Transdiaphragmatic pressure (Pdi) was obtained by numerical subtraction of Pes from Pga.
215
216 *Electromyography*
217 Electromyograms (EMG) were recorded from the right hemi-diaphragm (EMG_{DI}) and rectus
218 abdominis (EMG_{RA}) using bipolar surface electrodes with a fixed 10 mm inter-electrode

distance (Trigno Avanti, Delsys Inc.; Natick, MA, USA). For EMG_{DI}, the electrodes were positioned between the 6th and the 8th intercostal space along the anterior axillary line on the right-hand side of the thorax (23). For EMG_{RA}, the electrodes were positioned over the muscle belly on the right-hand side of the abdomen, 2-4 cm lateral and ~2 cm superior to the umbilicus, and in the orientation of the muscle fibers (24). Final electrode positions were determined according to the optimal M-wave response to magnetic stimulation. After verification of correct positioning, all electrodes were secured in place using double-sided adhesive interfaces and hypoallergenic medical tape.

Magnetic nerve stimulation

Magnetic stimuli (1-Hz) were delivered to the nerve roots supplying the respiratory muscles using a 90 mm circular coil powered by a magnetic stimulator (Magstim BiStim^2; The Magstim Company Ltd, Whitland, UK). For the diaphragm, the participants sat upright with the neck flexed and the coil was positioned between the 3rd (C3) and 7th (C7) cervical vertebrae (25). For the expiratory abdominal muscles, the participants sat facing an inclined bench (~30° past vertical) with their chest and abdomen supported, and the coil was positioned between the 8th (T8) and 11th (T11) thoracic vertebrae (26). The areas of stimulation that evoked the greatest Pdi_{tw} and Pga_{tw} were located and marked for use for all subsequent stimulations. All stimulations were delivered at 100% of the stimulator's power output and at a consistent relaxed end-expiratory lung volume (i.e., functional residual capacity), as judged by end-expiratory Pes. To determine whether the diaphragm and the expiratory abdominal muscles were maximally activated in response to magnetic stimulation of their nerve roots, three single twitch stimulations were delivered at progressively increasing stimulator intensities (50%, 60%, 70%, 80%, 85%, 90%, 95%, and 100%) and the twitch amplitude inspected as described previously (27). There was a plateau in Pdi_{tw} between 90 and 100% of stimulator intensity in eight of the 10 participants, and between 95 and 100% of stimulator intensity in all 10 participants (Supplemental Fig 1

[https://figshare.com/s/b7ad94f0a948d345e4aa]). The change in Pdi_{tw} from 90 to 100% stimulator power was <5% in 7/10 participants. Conversely, there was a proportional increase in Pga_{tw} with increasing stimulator intensity (Supplemental Fig 1). To ensure consistency of stimulation throughout the study, 1) all twitch stimulations we delivered at 100% of the stimulator's power output, 2) the stimulating coil position was marked with indelible ink to ensure accurate repositioning, and 3) all stimulations were delivered at a stable end-expiratory Pes. The consistency of our approach is evidenced by an excellent within-day test-retest CV for Pdi_{tw} (3.7%) and Pga_{tw} (3.8%) (Supplemental Table 1 [https://figshare.com/s/fc8038655a3835525d33]).

Respiratory muscle function

Diaphragm and expiratory abdominal muscle contractility were assessed approximately 10 min before, and at 5 and 30 min after each of the constant-power exercise tests (Figure 1). The potentiated twitch is a more sensitive measure of muscle fatigue in comparison to the non-potentiated twitch, particularly when the degree of fatigue is small (28). Accordingly, Pdi_{tw} was measured ~5 s after a maximal Müeller maneuver that was initiated from residual volume and maintained for ~5 s. This procedure was repeated six times such that six measures of potentiated Pdi_{tw} were obtained. Similarly, Pga_{tw} was measured 5 s after a maximal expulsive maneuver. These expiratory maneuvers were initiated from total lung capacity, maintained for ~5 s, and repeated six times such that six measures of potentiated Pga_{tw} were obtained. The average of the highest 3 valid twitches was used for analysis. Any twitch response that was initiated from an unstable end-expiratory Pes, Pdi, and/or Pga, or in the presence of participant 'bracing' (evidenced by EMG_{DI} and/or EMG_{RA} activity immediately prior to the stimulation) were excluded from subsequent analysis. The order of diaphragm and expiratory abdominal muscle assessment was randomized and counterbalanced between participants but remained constant within each participant across each of the constant-power exercise trials.

For both Pdi_{tw} and Pga_{tw}, each potentiated twitch was analyzed for amplitude (baseline to peak), contraction time (CT), one-half relaxation time ($RT_{0.5}$) maximal rate of pressure development (MRPD), and maximal relaxation rate (MRR). Membrane excitability was determined by measuring the magnetically evoked peak-to-peak amplitude (mV), duration (ms), and area (mV·ms) of the EMG_{DI} and EMG_{RA} M-waves. In addition, maximal inspiratory mouth pressure (MIP) and maximal expiratory mouth pressure (MEP) were determined during each Müeller maneuver and maximal expulsive maneuver, respectively, using a handheld mouth pressure meter (MicroRPM, Carefusion, CA, USA). The average of the highest three values for MIP and MEP was reported for analysis. The within-day, between-occasion reproducibility of the measures of diaphragm and expiratory abdominal muscle function was determined by assessing the participants before and after 30 min of quiet rest. There were no systemic differences in the evoked pressures, contraction and relaxation rates, membrane excitability, and maximal volitional pressures at the mouth before vs. after the 30 min rest period. All reproducibility coefficients were <6.2% for the coefficient of variation (CV) and >0.88 for the intraclass correlation coefficient (ICC) (Supplemental Table 1). At the individual participant level, the presence of respiratory muscle fatigue was defined as a reduction in Pdi_{tw} or Pga_{tw} from pre-exercise values of ≥8% (i.e., twice the within-day between-occasion CV).

Constant-power Exercise Tests

Following 5 min of quiet rest in the cycling position, the participants cycled for 2 min at 20 W and 2 min at 30% of peak power before power output was increased to either: 1) 5% <CP; 2) Δ25; or 3) Δ50 (Figure 1). Each participant pedaled at a self-selected cadence (80-100 rpm) and maintained this cadence during all constant-power exercise tests. During each test, the participants exercised until intolerance, which was defined as the point at which pedal cadence fell below 60 rpm despite strong verbal encouragement. Inspiratory and expiratory gas flow was measured breath-by-breath using a non-heated linear pneumotachometer (model 4813, Hans Rudolph, Kansas City, MO, USA). The inspiratory and expiratory flow

signal was captured within the data acquisition system to determine periods of inspiration and expiration for subsequent calculation of pressure-time products (PTP). Additionally, ventilatory and pulmonary gas exchange indices were measured breath-by-breath using a calibrated bidirectional Pitot tube sensor, connected in series with the linear pneumotachometer, for volume measurement and galvanic (O_2) and non-dispersive infrared (CO_2) sensors for gas analysis (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA). Heart rate (HR) was measured beat-by-beat via 12-lead electrocardiogram (X12, Montara Instrument; Milwaukee, WI, USA). Capillary blood was sampled from an earlobe at rest, every 2.5 min for the first 10 min of exercise, at 20 and 30 min (i.e., during <CP exercise), and within 15 s of exercise termination during all exercise tests for the determination of blood lactate concentration ($[La^-]_B$) (Lactate Pro 2, Arkray Factory Inc., Shiga, Japan). Ratings of perceived leg discomfort and breathing discomfort were obtained at rest, at the end of the 'warm-up', every 2 min for the first 14 min of exercise, at 20, 26, 32, 38 min (i.e., during <CP exercise) and within 15 s of exercise termination using a modified Borg CR10 scale. Pga, Pes, and Pdi were measured throughout exercise and time-aligned to the gas flow signal.

Data Collection

The raw pressure signals (Pes and Pga) were passed through a carrier demodulator (Validyne model CD15, Northridge, CA, USA) and the EMG signals recorded at a band-width of 10-850 Hz (Trigno Avanti, Delsys Inc.; Natick, MA, USA). The pressure, gas flow (from the non-heated linear pneumotachometer), and EMG signals were digitized at sampling rates of 150 Hz and 2 kHz (EMG only) (Micro 1401-3, Cambridge Electronic Design, Cambridge, UK), and captured and analyzed using commercially available software (Spike 2 version 8.0, Cambridge Electronic Design, Cambridge, UK).

Data Analysis

Cardiopulmonary variables including pulmonary oxygen uptake ($\dot{V}O_2$), pulmonary carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), respiratory frequency (f_R), tidal volume (V_T),

end-tidal CO_2 tension ($P_{ET}CO_2$), and heart rate (HR) were averaged at rest, during each entire minute of exercise, and during the final 60 s of exercise (*end-exercise*) for all trials. In addition, $\dot{V}O_{2peak}$ was determined during the ramp incremental phase of the RIST and during all constant-power exercise tests as the highest consecutive 12 breath average from the final 25 breaths. Diaphragm and expiratory abdominal pressure-time products were calculated by integrating Pdi and Pga from end-inspiratory and end-expiratory pressure of each preceding breath over the periods of inspiratory flow (PTP_{di}) and expiratory flow (PTP_{ga}), respectively. PTP_{di} and PTP_{ga} were determined per minute as the sum of every breath performed during each 60 s time bin, and cumulative PTP_{di} and PTP_{ga} were determined as the sum of all breaths performed during each exercise trial.

Statistical Analyses

The within-participant test-retest CV of Pdi_{tw} and Pga_{tw} was used to estimate the sample size required to detect meaningful changes in Pdi_{tw} and Pga_{tw} (approximately double the CV) from before to after exercise. Based on the previously reported within-participant CV for Pdi_{tw} (5.6%) and Pga_{tw} (3.6%) from our group (27), we determined that a sample size of 8 would allow us to detect an 8% change in Pdi_{tw} and a 6% change in Pga_{tw}, at a statistical power of 0.8 and an alpha level of 0.05 (29). Changes in respiratory muscle contractility in response to the constant-power exercise trials were assessed using two-way repeated measures ANOVA (exercise trial × time). Pairwise comparisons were adjusted using the Holm-Sidak correction. One-way repeated measures ANOVA with a Holm-Sidak correction were used to compare the change in respiratory muscle contractility from pre-exercise to 5 min post-exercise (i.e., the magnitude of exercise-induced respiratory muscle fatigue) between the exercise trials (<CP vs. ∆25 vs. ∆50). Paired samples *t*-tests were used to compare the pre- to post-exercise change in Pdi_{tw} vs. Pga_{tw} for each exercise trial, and Cohen's D effect sizes (*ES*) were computed to determine the magnitude of effect as small (<0.5), medium (≥0.5 to <0.8) or large (≥0.8). To compare differences in cardiopulmonary exercise responses over time within each trial, one-way repeated-measures ANOVA with Holm-Sidak correction were

performed across 1 min bins and end-exercise values for Δ25 and Δ50, and at 5, 10, 15, 20 min and end-exercise for <CP. A one-way repeated-measures ANOVA with a Holm-Sidak correction was used to assess differences in final min exercise responses between trials (<CP vs. Δ25 vs. Δ50). A Friedman's ANOVA was used to assess differences in end-exercise perceptual responses between trials (<CP vs. Δ25 vs. Δ50). The acceptable type I error was set at $P < 0.05$. Data are expressed as group means ± SD. Statistical analyses were performed using SPSS version 26 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Physiological Responses to the Ramp Incremental Sprint Test

During the ramp incremental phase of the RIST, peak values for $\dot{V}O_2$, $\dot{V}_E$, HR, respiratory exchange ratio (RER), and power output were 4.15 ± 0.92 $L \cdot min^{-1}$ (57.1 ± 8.8 $ml \cdot kg^{-1} \cdot min^{-1}$), 157 ± 36 $L \cdot min^{-1}$, 184 ± 11 $beats \cdot min^{-1}$, 1.20 ± 0.09 and 321 ± 57 W, respectively. During the sprint phase of the RIST, pedal cadence was maintained at 76 ± 8 rpm and CP was estimated as 202 ± 55 W. Mean $\dot{V}O_2$ during the sprint phase was 96 ± 3% of $\dot{V}O_{2peak}$ determined during the ramp phase, indicating that a maximal cardiometabolic effort was maintained.

Physiological Responses to the Constant-power Exercise Trials

Cardiopulmonary, metabolic, and perceptual responses

The participants cycled at 191 ± 52 W, 234 ± 53 W, and 263 ± 53 W during <CP, ∆25, and ∆50, respectively. The tolerable duration of constant-power exercise decreased with increasing exercise task power: <CP 34.5 ± 6.2 min (range: 25.0 to 45.0 min), ∆25 10.2 ± 2.6 min (range: 7.5 to 15.8 min), and ∆50: 4.9 ± 0.7 min (range: 3.7 to 6.1 min) (all $P < 0.001$). The cardiopulmonary, metabolic, and perceptual responses to the constant-power exercise tests are shown in Table 1 and Figure 2. There were significant main effects of time ($P < 0.05$) within each exercise trial for all cardiopulmonary, metabolic and perceptual responses to exercise. During <CP, there was a progressive rise in pulmonary $\dot{V}O_2$ that

386 reached a steady-state after 15 min (P = 0.111 vs. end-exercise) (Figure 2) and remained
387 submaximal (peak $\dot{V}O_2$: 3.62 ± 0.82 L·min^{-1}, 87 ± 6% of $\dot{V}O_{2peak}$). By comparison, $\dot{V}O_2$
388 increased more rapidly and reached a peak value of 4.02 ± 0.86 L·min^{-1} (97 ± 4% of $\dot{V}O_{2peak}$)
389 and 4.08 ± 0.82 L·min^{-1} (99 ± 4% of $\dot{V}O_{2peak}$), respectively, in Δ25 and Δ50. At end exercise,
390 $[La^-]_B$ was greater in Δ25 (P = 0.040) and Δ50 (P = 0.033) vs. <CP (Table 1, Figure 2).
391 During <CP, the rate of $[La^-]_B$ increase was slower than during the severe-intensity exercise
392 trials, and $[La^-]_B$ was not different at 20 min vs. end-exercise in <CP (8.0 ± 2.0 vs. 8.9 ± 2.3,
393 P = 0.459). End-exercise $\dot{V}_E$ was greater in Δ25 and Δ50 vs. <CP (both P < 0.001), but not
394 different in Δ25 vs. Δ50 (P = 0.092) despite a significantly greater V_T in Δ50 (Table 1, Figure
395 2). Group mean HR, breathing discomfort, and leg discomfort were not different at end-
396 exercise between the three constant-power exercise trials (P > 0.05) (Table 1, Figure 2).
397
398 *Respiratory muscle pressure production*
399 There were significant main effects of time within each exercise trial for PTP_{di} and PTP_{ga} per
400 min and cumulative PTP_{di} and PTP_{ga} (all P < 0.05). End-exercise PTP_{di} was greater during
401 Δ50 (P = 0.006) and Δ25 (P = 0.033) vs. <CP (Figure 3) and end-exercise PTP_{ga} was greater
402 during Δ50 (P = 0.005), but not different in Δ25 (P = 0.168), vs. <CP (Figure 3). Cumulative
403 PTP_{di} and PTP_{ga} increased with tolerable exercise duration, and both were higher during
404 <CP vs. Δ25 (P < 0.01) and Δ50 (P < 0.001), and during Δ25 vs. Δ50 (P < 0.01) (Table 1).
405
406 **Exercise-induced Respiratory Muscle Fatigue**
407 The mechanical and electromyographical responses to magnetic stimulation of the cervical
408 and thoracic nerve roots before and after the three constant-power exercise trials in a
409 representative participant are presented in Figure 4.
410
411 *Exercise-induced diaphragm fatigue*
412 Magnetically evoked M-wave amplitude, duration, and area for the diaphragm were not
413 different before vs. after exercise in <CP, Δ25, and Δ50 (Supplemental Table 2

[https://figshare.com/s/d52a70db952ed565024e]). There was a significant of time ($F = 18.155$, $P < 0.001$) as well as a significant exercise trial × time interaction effect ($F = 3.646$, $P = 0.014$) for Pdi_{tw}. At 5 min after exercise in <CP, Δ25, and Δ50, the group mean Pdi_{tw} was reduced below pre-exercise baseline values (Supplemental Table 2, Figure 5). Diaphragm fatigue was present at 5 min post-exercise in 8 (80%), 10 (100%), and 7 (70%) individuals for <CP, Δ25, and Δ50, respectively (Figure 5). At 30 min post-exercise, Pdi_{tw} had recovered fully in <CP (−6 ± 8% vs. baseline, $P = 0.065$) and Δ50 (−10 ± 10%, $P = 0.055$) but remained reduced relative to pre-exercise values in Δ25 (−13 ± 9%, $P = 0.008$). The magnitude of the exercise-induced decrease in Pdi_{tw} was greater in Δ25 (−22 ± 12%) compared to <CP (−13 ± 8%; $P = 0.0499$) and Δ50 (−14 ± 12%; $P = 0.045$) (Figure 5). In response to all exercise trials, there was a pre- to post-exercise decrease in diaphragm twitch contraction and relaxation times, and an increase in MRPD and MRR corrected for Pdi_{tw} amplitude (Supplemental Table 2). There was a pre- to post-exercise decrease in MIP in response to <CP (−10 ± 8%, $P = 0.012$) and Δ25 (−8 ± 7%, $P = 0.025$), but not Δ50 (−5 ± 7%, $P = 0.252$) (Supplemental Table 2).

Exercise-induced expiratory muscle fatigue

There was no pre- to post-exercise change in magnetically evoked M-wave amplitude, duration, and area for the rectus abdominis in <CP, Δ25, and Δ50 (Supplemental Table 2). There was a significant main effect of time ($F = 37.022$, $P < 0.001$), but no exercise trial × time interaction effect ($F = 0.819$, $P = 0.521$) for Pga_{tw}. From before to 5 min after exercise, there was a reduction in group mean Pga_{tw} for <CP, Δ25, and Δ50 (Figure 5). Expiratory muscle fatigue was present at 5 min post-exercise in 10 (100%), 10 (100%), and 7 (70%) individuals for <CP, Δ25, and Δ50, respectively. Despite some recovery, Pga_{tw} remained below baseline values at 30 min after exercise following all three constant-power exercise trials (<CP: −18 ± 9%; Δ25: −18 ± 13%; Δ50: −17 ± 12%, all $P < 0.05$ vs. baseline). Unlike for the magnitude of exercise-induced diaphragm fatigue, the pre- to post-exercise percent reduction in Pga_{tw} was not different between <CP vs. Δ25 vs. Δ50 (−23 ± 15% vs. −29 ± 15%

vs. −25 ± 16%, $P > 0.05$) (Figure 5). There was no change in gastric twitch contractile parameters (CT, MRPD/Pga_{tw}), but the amplitude-corrected maximum relaxation rate (MRR/Pga_{tw}) was increased, and relaxation time was reduced in all trials (Supplemental Table 2). There was a pre- to post-exercise decrease in MEP in response to <CP (−10 ± 6%, $P = 0.003$), ∆25 (−13 ± 6%, $P = 0.002$) and ∆50 (−10 ± 8%, $P = 0.013$) (Supplemental Table 2); the reduction in exercise-induced reduction in MEP was not different between trials ($P = 0.536$).

Exercise-induced diaphragm vs. expiratory abdominal muscle fatigue

The magnitude of exercise-induced expiratory abdominal muscle fatigue was not different than the magnitude of exercise-induced diaphragm fatigue in <CP (−23 ± 15% vs. −13 ± 8%, $P = 0.051$; $ES = 0.90$), ∆25 (−29 ± 15% vs. −22 ± 12%, $P = 0.157$; $ES = 0.74$), or ∆50 (−25 ± 16% vs. −14 ± 12%, $P = 0.057$; $ES = 0.51$).

DISCUSSION

Main Findings

In the present study, we show that the magnitude of exercise-induced fatigue of the diaphragm, but not the expiratory muscles, varies with exercise intensity domain and the tolerable duration of exercise. The principal findings of this study were that: 1) both the diaphragm and the expiratory muscles fatigued in response to heavy intensity exercise (<CP; T_{LIM} ~35 min) as well as shorter- (Δ25; T_{LIM} ~5 min) and longer-duration (Δ50; T_{LIM} ~10 min) severe intensity exercise continued to intolerance; 2) the magnitude of exercise-induced diaphragm fatigue was greater after the Δ25 trial compared to the <CP and ∆50 trials; and 3) conversely, the pre- to post-exercise reduction in expiratory muscle contractility was comparable between the three exercise trials. These findings provide the first direct evidence that the relationship between exercise intensity, exercise duration, and the magnitude of exercise-induced fatigue may be different for the diaphragm and the expiratory muscles.

Comparison with Previous Studies

Prescribed Exercise Intensity. Most previous studies investigating exercise-induced respiratory muscle fatigue have prescribed exercise at a percentage of $\dot{V}O_{2max}$ or peak power output. Such normalization procedures may fail to induce uniform physiological responses (i.e., consistency of exercise intensity) as there is the potential for substantial between-individual differences in the point at which physiological thresholds occur relative to peak values (30). Indeed, the occurrence of lactate threshold (40-85% of $\dot{V}O_{2max}$) and CP (50-95% of $\dot{V}O_{2max}$) can vary drastically between individuals (19, 30). Prescription of exercise intensity via estimation of CP in the present study ensured more consistent cardiopulmonary and metabolic responses, characteristic of exercise in the heavy and severe intensity domains (Table 1, Figure 2). In both severe exercise trials (Δ25 and Δ50), pulmonary $\dot{V}O_2$ and $[La^-]_B$ increased progressively without attainment of a steady state, and $\dot{V}O_{2max}$ was ultimately reached. Conversely, during the <CP trial, a steady state was attained in pulmonary $\dot{V}O_2$ and $[La^-]_B$, pulmonary $\dot{V}O_2$ remained below peak in all participants (average 87 ± 6% of $\dot{V}O_{2peak}$), and T_{LIM} was 34.5 ± 6.2 min. Although it was previously considered that exercise performed at work rates <CP could be sustained 'indefinitely', it is now increasingly well established that CP actually separates power outputs that are predictably limited (e.g., for a maximum of ~20-30 min >CP) from those that can be sustained for longer durations (8).

Exercise-induced muscle fatigue in the heavy vs. severe domain. The role of exercise intensity in the development of peripheral locomotor muscle fatigue is well-established (31-33). For example, peripheral neuromuscular fatigue is evident in response to single-limb maximal voluntary contractions sustained to task failure above and below critical torque, although the rate of fatigue development and mechanism of fatigue differ between severe and heavy intensity bouts (33). Similarly, peripheral neuromuscular fatigue is evident in response to cycling below (heavy) and above CP (severe), although the magnitude of

peripheral muscle fatigue is greater with increasing exercise intensity (31). In our study, we report that both diaphragm and expiratory muscle fatigue is observed following longer-duration severe intensity exercise (Pdi_{tw}: −22 ± 12%; Pga_{tw}: −29 ± 15%). This finding is in agreement with previous investigations on the effect of whole-body exercise performed at ≥80-85% of $\dot{V}O_{2max}$ and sustained for ~10 to 20 minutes on inspiratory and/or expiratory neuromuscular function (2-4, 9). However, we also report that exercise sustained for ~35 min but at a lower intensity (i.e., heavy) elicits a smaller reduction in Pdi_{tw} (−13 ± 8%), expanding our understanding of the relationship between exercise intensity domain, exercise duration, and the severity of exercise-induced respiratory muscle fatigue. Similarly, exercise performed in the severe intensity domain that was tolerable for only ~5 min resulted in a −14 ± 12% reduction in Pdi_{tw}. Conversely, the magnitude of exercise-induced expiratory muscle fatigue (i.e., the pre- to post-exercise reduction in Pga_{tw}) was not different across the three exercise trials and thus appears to be somewhat independent of exercise intensity and exercise duration. So, the question arises: why does exercise intensity and tolerable duration play a role in the magnitude of exercise-induced diaphragm but not expiratory muscle fatigue?

Exercise-Induced Diaphragm Fatigue

The magnitude of diaphragm fatigue in response to heavy vs. severe intensity exercise. The prerequisite factors that contribute to exercise-induced *diaphragm* fatigue have been investigated extensively (6, 34, 35). Experimental attenuation of the inspiratory Pb during exhaustive exercise that can be tolerated for ~10-13 minutes (i.e., severe intensity exercise) effectively abolishes the pre- to post-exercise reduction in evoked diaphragm pressure (35). Conversely, in healthy humans who are otherwise at rest, volitional mimicking of the diaphragmatic power engendered during such exhaustive severe intensity exercise does not, with a few exceptions, elicit fatigue of the diaphragm (34). Together, these data suggest that the inspiratory Pb is a key but not exclusive determinant of diaphragmatic fatigue during exercise. Indeed, it is considered that the large demand for cardiac output reserve from both

526 the limb-locomotor and the respiratory muscles during severe intensity exercise creates a
527 fatigue-favoring imbalance between the magnitude of diaphragm power and the adequacy of
528 diaphragm blood and O_2 supply. This may explain the consistent finding of diaphragm
529 fatigue following whole-body exercise that is performed to the limit of tolerance at or above
530 this 'high-intensity' (1, 3, 4, 9).
531
532 While it has been shown that the inspiratory muscles can fatigue in response to submaximal,
533 non-exhaustive and prolonged duration endurance exercise (e.g., marathon running) (36,
534 37), we are unaware of any prior work that has directly and systematically determined the
535 effect of heavy intensity exercise continued to intolerance on diaphragmatic contractility. It is
536 commonly held that a major consequence of inspiratory muscle fatigue is an increase in
537 MSNA, with a consequent vasoconstriction, increase in MAP and a reduction in blood flow
538 and oxygen delivery in the resting or exercising limbs (12-15). Previously, it was reported
539 that the ventilatory demands and associated Pb of exercise performed at ~75% of $\dot{V}O_{2max}$
540 are insufficient to cause such a cardiovascular adjustment and do not trigger
541 vasoconstriction in the locomotor muscles (i.e., the respiratory muscle metaboreflex is not
542 activated), even when the Pb is experimentally increased by 50-70% during such exercise
543 (16). That exercise at ~75% of $\dot{V}O_{2max}$ did not evoke sympathetically mediated alterations in
544 limb vascular resistance and locomotor muscle blood flow could, in theory, indicate that
545 inspiratory muscle fatigue *does not* occur during or in response to such heavy-intensity
546 exercise. However, in that study inspiratory muscle fatigue was not assessed and exercise
547 was not performed until intolerance (16); therefore, conclusions regarding the role of heavy-
548 intensity exercise on diaphragm contractility are unclear. In our study, the end-exercise $\dot{V}_E$,
549 $\dot{V}O_2$ and PTP_{di} were ~16%, ~11%, and ~25% lower during the heavy intensity (<CP) trial
550 compared with the longer duration severe intensity Δ25 trial (Figure 2 and Figure 3). These
551 observations likely reflected a lower inspiratory Pb during heavy- compared with severe-
552 intensity exercise bouts. Although somewhat speculative, it is possible that the lower
553 diaphragm force output combined with a greater cardiac output reserve secondary to a

554 smaller cardiometabolic demand may have ‘preserved’ diaphragm blood flow during heavy
555 exercise. This may have resulted in a lower magnitude of diaphragm fatigue following the
556 heavy compared to the longer duration severe trial. Importantly, however, although the
557 magnitude of exercise-induced diaphragm fatigue was lower following heavy vs. longer
558 duration severe exercise, diaphragm fatigue *was still present* in 70% of participants after the
559 <CP trial (Figure 5), during which the average $\dot{V}O_2$ across the entire exercise bout was only
560 ~75% of $\dot{V}O_{2peak}$. Therefore, the present data suggest that the O_2 uptake threshold proposed
561 for the development of exercise-induced diaphragm fatigue in response to exhaustive
562 exercise may be lower than that previously reported (i.e., 85% of $\dot{V}O_{2max}$) (1).
563
564 **Tolerable Exercise Duration and Diaphragm Fatigue**
565 In response to shorter-duration severe intensity exercise (Δ50; T_{LIM} ~5 min) diaphragm
566 fatigue was less prevalent (70% vs. 100% of participants) and lower in magnitude (−14 ±
567 12% vs. −22 ± 12%) compared to prolonged severe intensity exercise (Δ25; T_{LIM} ~10 min)
568 (Figure 5). These data support the hypothesis that the duration for which a high Pb is
569 sustained during exercise, and therefore cumulative force output of the diaphragm,
570 influences the magnitude of exercise-induced diaphragm fatigue. Indeed, $\dot{V}O_2$ was sustained
571 at more than 85% of $\dot{V}O_{2peak}$ for ~4 min longer and cumulative PTP_{di} was ~3700 $cmH_2O{\cdot}s$
572 higher during exercise at Δ25 vs. Δ50 (Figure 2 and Figure 3). Previous reports on the effect
573 of short duration constant power exercise or maximal ramp incremental exercise that evokes
574 a short duration of very high ventilatory work on diaphragm contractility have yielded
575 inconsistent findings (9, 17, 38). For example, Romer et al. (17) suggested that ramp
576 incremental cycling did not elicit diaphragm fatigue; however, it is of note that the reported
577 reduction in Pdi_{tw} amplitude closely approximated significance (~12%; P = 0.051).
578 Conversely, and more recently, diaphragm fatigue (−24 ± 6% reduction in Pdi_{tw}) was
579 reported after only 6 min of severe-intensity constant power exercise that was stopped prior
580 to intolerance (9). Moreover, the normally occurring Pb during constant power cycling at

581 >95% of $\dot{V}O_{2max}$ has been associated with sympathetically mediated peripheral
582 vasoconstriction and an attenuation in locomotor blood flow and O_2 delivery after only ~2.5-3
583 min of exercise (14). This observation may signify the early development of respiratory
584 muscle fatigue, or at least the ongoing accumulation of fatigue-associated metabolites, and
585 the consequent stimulation of group III and IV phrenic afferents (13). Therefore, in
586 combination with previous literature, the present data suggest that short-duration severe
587 intensity exercise is sufficient to evoke diaphragm fatigue, albeit of a lower magnitude and
588 consistency in comparison to prolonged severe intensity exercise.
589
590 **Exercise-Induced Expiratory Muscle Fatigue**
591 *Effect of Exercise Intensity and Tolerable Duration on the Development of Expiratory Muscle*
592 *Fatigue.* To the authors' knowledge, this study is the first report that expiratory abdominal
593 muscle fatigue is present and of a similar magnitude across a range of exercise intensities
594 and durations. The observed magnitude of expiratory abdominal muscle fatigue is within the
595 range of values (15-33%) previously reported in response to cycling at >85% $\dot{V}O_{2max}$ and
596 comparable to reductions in MEP (20-28%) following long-distance (≥42 km) running events
597 (2, 5, 36, 37, 39). In contrast to the diaphragm, the similar magnitude of fatigue across trials
598 is somewhat surprising considering the vastly different cumulative and average PTP_{ga} (Table
599 1 and Figure 3). Moreover, while statistical significance was not reached, we observed large
600 and moderate effect sizes when comparing the magnitude of exercise-induced expiratory vs.
601 diaphragm fatigue for the heavy intensity (ES = 0.90) and the short-duration severe intensity
602 (ES = 0.74 [Δ25] and 0.51 [Δ50]) trials (Figure 5). In combination, the present findings
603 suggest that the fatigue threshold of the expiratory muscles may require a lower Pb and
604 cumulative force output compared to the diaphragm. These differences may be in part
605 explained by phenotypical and morphological differences between the diaphragm and
606 expiratory abdominal muscles, and the consequent effect on their resistance to fatigue. The
607 expiratory muscles possess a lower but more variable proportion of type I muscle fibers (30-
608 60% vs. 50-55%) (40, 41), a reduced oxidative capacity (42), and a lower metabolic

efficiency in comparison to the diaphragm (43). This muscle phenotype likely explains the greater decline in the ability to volitionally generate expiratory vs. inspiratory mouth pressure in response to a series of sustained maximal contractions (44). Moreover, in addition to contributing substantially to the hyperpnea of exercise by augmenting expiratory airflow and expanding tidal volume secondary to a decrease in end-expiratory lung volume (45), the abdominal muscles perform important non-ventilatory roles during cycling. Tonic expiratory abdominal muscle activity serves to maintain body posture, stabilize the torso (46), and reduces abdominal compliance providing an 'abdominal fulcrum' which enhances diaphragm contractility (47). These additional non-ventilatory roles likely contribute to the development of expiratory abdominal muscle fatigue independent of the ventilatory demand of exercise. Compared to peripheral skeletal muscle, muscle afferents isolated from the diaphragm in a rat preparation demonstrate a reduced sensitivity to adrenergic stimuli, which may theoretically aid the preservation of diaphragm blood flow during exercise-induced increases in global sympathetic nerve activity (48). Although speculative, it is possible that such attenuated responsiveness to adrenergic stimuli is not ubiquitous among the expiratory and accessory inspiratory muscles which could contribute to differences in the susceptibility of fatigue; however further evidence is required.

Limitations and Considerations

Submaximal depolarization of the cervical and thoracic nerve roots

In the present study, cervical magnetic stimulation evoked a near-maximal Pdi_{tw} response whereas the Pga_{tw} response to magnetic stimulation of the thoracic nerve roots was submaximal (Supplemental Fig 1). This is in agreement with previous reports (2, 4, 9, 26). Typically, two concerns regarding submaximal activation of motor nerves have been considered: 1) poor stimulus reproducibility, and 2) an increase in the activation threshold of motor axons, secondary to axonal hyperpolarization, with reduced response to the same stimulus. However, in the present study, the consistency of our method is evidenced by an excellent within-day test-retest CV for Pdi_{tw} and Pga_{tw} of ~4%. Moreover, axonal

637 hyperpolarization appears to occur in response to repetitive maximal voluntary contractions
638 (49), with evidence that submaximal voluntary contractions (i.e., 50% of MVC) do not alter
639 motoneuronal discharge rates (50). During exercise, within-breath transdiaphragmatic and
640 gastric pressures typically do not exceed 20-40% of Pdi_{MAX} and Pga_{MAX}. This consideration,
641 combined with our finding of similar M-wave responses before and after exercise, as well as
642 previous findings of unchanged Pga_{tw} recruitment curves from before to after exercise (5),
643 suggest that axonal hyperpolarization is unlikely to be a major contributor to the exercise-
644 induced reduction in diaphragm and expiratory muscle contractility that we report.
645
646 More recently, Angus et al. (51) reported that the degree of diaphragm fatigue following an
647 inspiratory muscle loading protocol tended to be overestimated when using one rather than
648 two magnetic stimulators to interrogate the diaphragm. This is likely because the magnetic
649 field generated is ~13% greater when using two rather than one magnetic stimulator,
650 resulting in greater nerve root depolarization. We believe that this consideration likely *did not*
651 impact our finding that the magnitude of exercise-induced fatigue of the diaphragm, but not
652 the expiratory muscles, varies with exercise intensity domain and the tolerable duration of
653 exercise. However, given that cervical magnetic stimulation was near-maximal but magnetic
654 stimulation of the thoracic nerve roots was submaximal, it is theoretically possible that the
655 magnitude of exercise-induced expiratory muscle fatigue may have been overestimated
656 relative to the magnitude of exercise-induced diaphragm muscle fatigue.
657
658 ***Sex Differences***
659 Although investigation of sex differences was not an aim of this study, it is acknowledged
660 that the number of male and female participants was unbalanced (8:2). In females, the
661 magnitude of exercise-induced diaphragm fatigue is lower than males during severe intensity
662 exercise (4). While it is possible that inclusion of females may have resulted in a lower
663 magnitude of fatigue within each exercise trial compared to a male-only population, we are

confident that the repeated-measures approach prevented any influence on the interpretation of findings.

Conclusions

Heavy intensity exercise continued to intolerance, as well as short- (~5 min) and long-duration (~10 min) severe intensity exercise elicits diaphragm and expiratory abdominal muscle fatigue. The magnitude of exercise-induced fatigue was different between trials for the diaphragm, but not the expiratory muscles. These findings demonstrate that the relationship between exercise intensity, exercise duration, and the magnitude of fatigue is different between the key inspiratory and expiratory muscles. For the diaphragm, the greatest magnitude of exercise-induced fatigue is observed under exercise conditions that likely engender a high and sustained inspiratory Pb combined with a high demand for cardiac output reserve (i.e., prolonged severe intensity exercise). In contrast, a similar magnitude of expiratory abdominal muscle fatigue was observed in response to exercise of vastly different intensities and durations. These differences in the susceptibility of exercise-induced fatigue between the primary inspiratory and expiratory muscles may relate to the less fatigue-resistant phenotype and additional non-ventilatory roles during exercise for the expiratory abdominal muscles versus the diaphragm.

Data Availability

Data are subject to privacy/ethical restrictions: Source data for this study are not publicly available due to privacy or ethical restrictions. The source data are available to verified researchers upon request by contacting the corresponding author.

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The graphical abstract was created using Biorender.com.

Supplemental Material

Supplemental Fig 1. Diaphragm and expiratory abdominal muscle supramaximality curves. https://doi.org/10.6084/m9.figshare.22068956

Supplemental Table 1. Within-day test-retest reproducibility of respiratory muscle function measurements. https://doi.org/10.6084/m9.figshare.22069094

Supplemental Table 2. Mechanical and electrical twitch characteristics and volitional muscle function before and (~5 min) after exercise. https://doi.org/10.6084/m9.figshare.22070117

Disclosures

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

Author Contributions

T.A.H. and B.J.T. conceived and designed the research; T.A.H., M.R.C. and B.J.T performed experiments; T.A.H and B.J.T. analyzed data; T.A.H., M.R.C., C.F., T.J.C. and B.J.T. interpreted results of experiments; T.A.H. prepared figures; T.A.H. drafted manuscript; T.A.H., M.R.C., C.F., T.J.C. and B.J.T. edited and revised manuscript; T.A.H., M.R.C., C.F., T.J.C. and B.J.T. approved final version of manuscript.

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862 **FIGURE & TABLE LEGENDS**

863 **Figure 1.** Experimental exercise protocols. RIST, ramp incremental sprint test; TF, task
864 failure; P_{peak}, peak power output; CP, critical power; Pga_{tw}, gastric twitch pressure; Pdi_{tw},
865 diaphragm twitch pressure.

866
867 **Figure 2.** Cardiopulmonary responses to constant-power exercise trials. A, pulmonary
868 oxygen uptake ($\dot{V}O_2$) (*n* = 10); B, blood lactate concentration (*n* = 8); C, minute ventilation
869 ($\dot{V}_E$) (*n* = 10); D, tidal volume (V_T) (*n* = 10); E, respiratory frequency (f_R) (*n* = 10); F, heart
870 rate (HR) (*n* = 10); G, leg discomfort (*n* = 10); F, breathing discomfort (*n* = 10). A one-way
871 repeated-measures ANOVA with a Holm-Sidak post-hoc correction was used to assess
872 differences in final min exercise responses between trials (<CP vs. Δ25 vs. Δ50). **P* < 0.05,
873 significantly different to <CP at end-exercise; † *P* < 0.05, significantly different to Δ25 at end-
874 exercise.

875

876 **Figure 3.** Pressure-time product (PTP) per minute during constant-power exercise trials. A,
877 esophageal PTP (PTP_{es}) (*n* = 10); B, gastric PTP (PTP_{ga}) (*n* = 10); C, diaphragm PTP_{di} (*n* =
878 10); D, PTP_{di}/PTP_{es} (*n* = 10). Values are group means ± SD. Data were analyzed for
879 between group differences at end-exercise by one-way repeated measures ANOVA with
880 Holm-Sidak post-hoc correction. **P* < 0.05, significantly different to <CP at end-exercise.

881

882 **Figure 4.** Individual representative ensemble average traces of diaphragm twitch pressure
883 (A) (Pdi_{tw}) and gastric twitch pressure (B) (Pga_{tw}) at baseline (mean value across all time
884 points), and in response to <CP, Δ25, and Δ50 exercise trials. Diaphragm (EMG_{DI}) and
885 rectus abdominis (EMG_{RA}) electromyography signals are also shown.

886

887 **Figure 5.** A comparison of the magnitude of exercise-induced inspiratory (Pdi_{tw}, black
888 symbols) and expiratory muscle fatigue (Pga_{tw}, white symbols) across trials (<CP vs. Δ25 vs.
889 Δ50) and between muscle groups (diaphragm vs. expiratory muscle). Symbols represent

individual participants (n = 10 in all conditions). Data were analyzed by two-way repeated measures ANOVA with Holm-Sidak post-hoc correction. One-way repeated measures ANOVA with a Holm-Sidak correction were used to compare the change in respiratory muscle contractility from pre-exercise to 5 min post-exercise (i.e., the magnitude of exercise-induced respiratory muscle fatigue) between the exercise trials (<CP vs. ∆25 vs. ∆50). Paired samples t-tests were used to compare change from pre-exercise to 5 min post-exercise in Pdi_{tw} vs. and Pga_{tw} within each exercise trial. *P < 0.05, significantly different to pre-exercise values.

Table 1. Physiological responses at the final min of exercise (end-exercise).