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The cardiovascular consequences of fatiguing expiratory muscle work in otherwise resting healthy humans

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Running head
Expiratory muscle metaboreflex

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ABSTRACT

In 11 healthy adults (25 ± 4 years; 2 females), we investigated the effect of expiratory resisted loaded breathing [65% maximal expiratory mouth pressure (MEP), 15 breaths·min^{-1}, duty cycle 0.5; ERL_{Pm}] on mean arterial pressure (MAP), leg vascular resistance (LVR), and leg blood flow ($\dot{Q}_L$). On a separate day, a subset of 5 males performed ERL targeting 65% of maximal expiratory gastric pressure (ERL_{Pga}). ERL-induced expiratory muscle fatigue was confirmed by a 17 ± 5% reduction in MEP ($P < 0.05$) and a 16 ± 12% reduction in the gastric twitch pressure response to magnetic nerve stimulation ($P = 0.09$) from before to after ERL_{Pm} and ERL_{Pga}, respectively. From rest to task failure in ERL_{Pm} and ERL_{Pga}, MAP increased (ERL_{Pm} = 31 ± 10 mmHg, ERL_{Pga} = 18 ± 9 mmHg, *both* $P < 0.05$), but group mean LVR and $\dot{Q}_L$ were unchanged (ERL_{Pm}: LVR = 0.78 ± 0.21 vs. 0.97 ± 0.36 mmHg·ml^{-1}·min^{-1}, $\dot{Q}_L$ = 133 ± 34 vs. 152 ± 74 ml·min^{-1}; ERL_{Pga}: LVR = 0.70 ± 0.21 vs. 0.84 ± 0.33 mmHg·ml^{-1}·min^{-1}, $\dot{Q}_L$ = 160 ± 48 vs. 179 ± 110 ml·min^{-1}) (all $P \geq 0.05$). Interestingly, $\dot{Q}_L$ during ERL_{Pga} oscillated within each breath, increasing (~66%) and decreasing (~50%) relative to resting values during resisted expirations and un-resisted inspirations, respectively. In conclusion, fatiguing expiratory muscle work did not affect group mean LVR or $\dot{Q}_L$ in otherwise resting humans. We speculate that any sympathetically-mediated peripheral vasoconstriction was counteracted by transient mechanical effects of high intra-abdominal pressures during ERL.

NEW & NOTEWORTHY

Fatiguing expiratory muscle work in otherwise resting humans elicits an increase in sympathetic motor outflow; whether limb blood flow ($\dot{Q}_L$) and limb vascular resistance (LVR) are affected remains unknown. We found that fatiguing expiratory resisted loaded breathing (ERL) did not affect group mean $\dot{Q}_L$ or LVR. However, within-breath oscillations in $\dot{Q}_L$ may reflect a sympathetically-mediated vasoconstriction that was counteracted by transient increases in $\dot{Q}_L$ due to the mechanical effects of high intra-abdominal pressure during ERL.

KEYWORDS

Respiratory muscle fatigue; expiratory muscle metaboreflex; leg blood flow; leg vascular resistance; magnetic nerve stimulation.

INTRODUCTION

The respiratory and cardiovascular systems are functionally linked. The interactions between these two organ systems play a critical role in determining blood flow and oxygen delivery to the body tissues in relation to oxygen consumption ($\dot{V}O_2$), particularly during exercise. One such cardiorespiratory interaction is a fatigue-induced respiratory muscle metaboreflex (9, 40). Previously, it has been shown that fatiguing 'high-intensity' voluntary contractions of the inspiratory muscles in otherwise resting humans trigger a reflexively-mediated sympathoexcitation that is associated with a time-dependent increase in muscle sympathetic nerve activity (MSNA) and mean arterial pressure (MAP), and with vasoconstriction and a reduction in blood flow and oxygen delivery in the resting limb (41-44, 52). This inspiratory muscle metaboreflex also appears to be active during severe-intensity whole-body exercise. Indeed, relative to control conditions, attenuation of the inspiratory work of breathing via proportional assist ventilation during such exercise is associated with a decrease in noradrenaline spillover that is significantly related to a reduction in vascular resistance (LVR) and an increase in blood flow ($\dot{Q}_L$) in the exercising leg (11, 21). These findings suggest that sympathetically-mediated alterations in LVR and $\dot{Q}_L$ can be triggered by changes in inspiratory muscle work.

Our interest is in the effect of fatiguing expiratory muscle work on cardiovascular function and systemic oxygen transport. Rhythmic contractions of the expiratory muscles sustained to the point of task failure in otherwise resting humans elicit an increase in MSNA burst frequency and MAP (i.e. a sympathoexcitation) that is similar in magnitude and time-dependency to that caused by fatiguing contractions of the inspiratory muscles (10, 41). Moreover, in the resting canine, infusion of the metabolite lactic acid into the expiratory muscle circulation versus the diaphragm circulation causes a marked and comparable increase in MAP and decrease in leg vascular conductance and $\dot{Q}_L$ (39). During whole-body exercise the expiratory muscles of the rib cage and abdominal wall contribute substantially to ventilation (1, 2, 12, 23, 46) and, like the diaphragm, the expiratory muscles fatigue in response to severe-intensity exercise

performed to the limit of tolerance (46, 51). Interestingly, during submaximal exercise, augmentation of expiratory muscle work via voluntary hyperpnoea combined with an expiratory resistor is associated with greater increases in MSNA and MAP relative to the addition of voluntary hyperpnoea alone (31). In addition, it has been shown that the severity of exercise-induced quadriceps fatigue, quantified as the reduction relative to prior baseline values in magnetically evoked quadriceps twitch force (Q_{tw}), is greater after exercise of the same intensity and duration with compared to without prior induction of expiratory muscle fatigue (EMF) (47). It was suggested that this exacerbation of exercise-induced quadriceps fatigue with prior EMF was likely the consequence of attenuated $\dot{Q}_L$ and oxygen delivery to the working muscles secondary to a sympathetically-mediated vasoconstriction in the exercising limb muscles.

In combination, the aforementioned findings suggest that fatiguing expiratory muscle work elicits a sympathoexcitation (i.e. expiratory muscle metaboreflex) that is remarkably similar to that induced by very high inspiratory muscle work and/or fatigue. Although somewhat speculative, it is possible that the activation of an expiratory muscle metaboreflex could be accelerated during exercise in patients with heart failure or chronic obstructive pulmonary disease who often exhibit respiratory muscle weakness, an increased prevalence of expiratory flow limitation, and a greater work of breathing for a given ventilation compared to healthy individuals (8, 15, 19, 29). While it is well accepted that the sympathoexcitatory response to fatiguing inspiratory muscle work results in vasoconstriction and impaired blood flow and oxygen delivery to the resting and exercising limb (7, 21, 32, 41-43), the cardiovascular response to activation of an expiratory muscle metaboreflex is yet to be fully addressed. Accordingly, the aim of this study was to investigate the cardiovascular consequences of fatiguing expiratory work in healthy humans. Specifically, we determined the LVR and $\dot{Q}_L$, as well as the MAP and heart rate (HR), response to 'high-intensity' contractions of the expiratory muscles sustained to the point of task failure in otherwise resting individuals. It was

hypothesized that HR, MAP and LVR would increase and that $\dot{Q}_L$ would decrease in a time-dependent manner during fatiguing expiratory muscle work.

METHODS

Subjects

Eleven recreationally active adults participated in the study (2 females, mean ± SD: age 25 ± 4 y; stature 1.76 ± 0.06 m; body mass 73.8 ± 9.5 kg). All subjects were healthy, had no history of respiratory, cardiovascular or metabolic disease, and had pulmonary function within normal limits (forced vital capacity: 5.4 ± 0.9 L, 103 ± 9% of predicted; forced expiratory volume in 1 s: 4.5 ± 0.7 L, 104 ± 9% of predicted). The subjects abstained from food for 3 h, caffeine for 12 h, and alcohol and exercise for 48 h before each laboratory visit. Both female subjects had been using a monophasic oral contraceptive pill for >6 months prior to starting the study, and continued their oral contraceptive pill throughout the experimental period. All of the experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee and conformed to the Declaration of Helsinki (approval REF: BIOSCI 16-020). Each subject provided written informed consent prior to the commencement of any testing procedures.

Experimental Procedures

Each subject initially visited the laboratory on two separate occasions. At the first visit, the subjects were thoroughly familiarized with all of the experimental procedures and measurements, including the expiratory resistive loaded breathing (ERL) tasks and the determination of maximal expiratory mouth pressure (MEP). To ensure familiarization with the ERL tasks, each subject performed short bouts (1-2 min) of ERL until they could generate a consistent 'square-wave' in expiratory mouth pressure (Pm) for >2 min at the target pressure and in accordance with the prescribed duty cycle and respiratory frequency without coaching. On the second visit, the subjects performed ERL targeting a Pm of 2% of MEP for 5 min (*control*). Following 30 min of quiet rest, the subjects then performed ERL targeting 65% of

MEP until task failure (ERL_{Pm}). The control trial was performed first to avoid any residual effect of peripheral muscle fatigue and potential sensitisation of the metaboreflex becoming apparent during the control trial. During ERL_{Pm}, the subjects were instructed to maintain a constant Pm at the target level throughout each expiration but *were not* given any specific instructions on how to recruit the expiratory muscles. $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during ERL, and in recovery; HR and MAP were recorded continuously.

Despite the presence of ERL-induced expiratory muscle fatigue and the expected increase in MAP (*see below*: "ERL_{Pm}-induced expiratory muscle fatigue" and "Cardiovascular measurements during ERL_{Pm}"), we observed no change in group mean LVR or $\dot{Q}_L$ in response to ERL_{Pm}. At this time, we considered it possible that specifically targeting the primary expiratory muscles (*i.e. the muscles of the abdominal wall*) during ERL may increase the severity of ischemia in these muscles, hasten the onset of their fatigue, augmenting the initiation of an expiratory muscle metaboreflex and the associated cardiovascular consequences. Accordingly, a sub-sample of subjects (n = 5) attended the laboratory on a third occasion and performed ERL but this time targeted 2% (5 min, *control*) and 65% (to task failure) of maximal expiratory gastric pressure (Pga_{max}) (ERL_{Pga}). At the start of the experimental visit subjects practiced and were coached through the performance of ERL_{Pga}; the subjects were instructed to maintain a constant Pga at the target level throughout each expiration, and were explicitly instructed to 'target the abdomen' during each expiration. Once ERL_{Pga} could be performed accurately at the intended duty cycle and respiratory frequency, subjects then rested quietly for 30 min prior to performance of the ERL_{Pga} control trial. During ERL_{Pga} trials, $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during ERL, and in recovery; HR and MAP were recorded continuously.

Expiratory Resistive Loaded Breathing

All ERL trials were performed with the subjects in the semi-recumbent position and breathing through a custom-built two-way valve with a variable diameter resistor incorporated into the

143 expiratory port; inspiration was completely unimpeded. A calibrated pressure transducer
144 (DP45, Validyne Engineering, Northridge, CA, USA) was connected into the mouthpiece to
145 allow continuous measurement of Pm. During each ERL trial, the target expiratory pressure
146 (Pm or Pga) was displayed on a computer screen, and the subjects maintained a respiratory
147 frequency (f_R) of 15 breaths·min^{-1} and an expiratory duty cycle (T_E/T_{TOT}) of 0.5 by following a
148 computer generated audio signal with distinct inspiratory and expiratory tones. The subjects
149 were instructed to maintain a constant Pm or Pga at the target level throughout each
150 expiration; inspiration was unresisted. The subjects were monitored closely by the researchers
151 during each ERL trial to ensure proper timing, breathing technique, and effort. An experimenter
152 supported the subject's cheeks throughout ERL to minimize use of the buccal muscles. Airflow
153 (no. 4813, Hans Rudolph Inc.; Shawnee, KS, USA) and end-tidal partial pressure of carbon
154 dioxide ($P_{ET}CO_2$) (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA) were measured
155 throughout each ERL trial, and $P_{ET}CO_2$ was maintained within ± 3 mmHg of eucapnic control
156 values by manually adjusting the inspired fraction of CO_2. It is unlikely that such a small change
157 in $P_{ET}CO_2$ would have significant vasomotor effects (22). Doppler ultrasound (Vivid iq, GE
158 Healthcare, Milwaukee WI, USA) was used to measure $\dot{Q}_L$ during the last three minutes of
159 each period of eupnea (*rest*), every minute during all ERL trials, and during the first three
160 minutes after each ERL trial (*recovery*). MAP and HR were measured beat-by-beat using
161 finger photoplethysmography (Finapres Nova, Finapres Medical Systems, Amsterdam, The
162 Netherlands), and LVR was subsequently calculated as MAP/$\dot{Q}_L$. As per the manufacturer
163 guidelines, physiological calibration (PhysioCal) was used during ERL to maintain the
164 accuracy of Finapres recordings; measurements were averaged over 60 s and calibration
165 periods were excluded from the analysis. In addition, surface electromyography (EMG) (Trigno
166 Avanti, Delsys Inc., Natick, MA, USA) was recorded from the vastus lateralis and vastus
167 medialis of the right leg to confirm that no limb muscular contraction occurred during the ERL
168 trials.
169
170 *Expiratory resistive loaded breathing targeting expiratory mouth pressure (ERL_{Pm})*

171 The subjects rested quietly with breathing completely un-resisted for 15 min to allow accurate
172 baseline cardiovascular measurements to be made. Next, each subject expired against the
173 resistive load for 5 min whilst targeting 2% of MEP (*control*) before resting quietly with
174 breathing completely unresisted for a further 30 min. Then, each subject performed ERL whilst
175 targeting 65% of MEP until task failure (ERL_{Pm}), defined as an inability to generate the target
176 pressure for three consecutive breaths despite strong verbal encouragement. Once task
177 failure was achieved, the subjects were given strong verbal encouragement to continue
178 attempting to generate their target Pm for one additional minute (defined as the 'task failure'
179 minute). By having subjects perform this additional minute of ERL_{Pm}, we ensured that: 1) each
180 subject was truly failing the task rather than having a 'few bad breaths' and 2) we were able
181 to collect a full sample of ultrasound data without interruption during the final 'task failure'
182 minute. The presence and severity of ERL_{Pm}-induced expiratory muscle fatigue was quantified
183 as the reduction relative to prior baseline values in the MEP response to maximal Valsalva
184 maneuvers initiated from total lung capacity (35). The maximum value of 3-5 attempts that
185 varied by <10% was reported.
186
187 *Expiratory resistive loaded breathing targeting expiratory gastric pressure (ERL_{Pga})*
188 As in ERL_{Pm}, the subjects rested quietly for 15 min before expiring against the resistive load
189 for 5 min but this time targeting 2% of Pga_{max} (*control*). Following a further 30 min of quiet rest
190 each subject performed ERL targeting 65% of Pga_{max} (ERL_{Pga}) until task failure; again,
191 subjects continued to perform ERL_{Pga} for one minute after task failure was reached. The
192 presence and severity of ERL_{Pga}-induced expiratory muscle fatigue was determined as the
193 pre- to post-ERL reduction in MEP and the gastric twitch pressure (Pga_{tw}) response to
194 magnetic stimulation of the thoracic nerve roots.
195
196 **Leg Blood Flow via Doppler Ultrasound**
197 $\dot{Q}_L$ was measured using Doppler ultrasound (Vivid iq, GE Healthcare, Milwaukee, WI, USA).
198 A 10 Hz linear probe (9L-RS, GE Healthcare, Milwaukee, WI, USA) was positioned over the

superficial femoral artery of the right leg ~3-5 cm below the bifurcation of the deep and superficial femoral artery. The position of the probe was marked with indelible ink and measured from the knee to ensure accurate and consistent re-positioning across ERL trials. The Doppler sample volume was set to the full width of the artery and the angle of insonation was fixed to 60˚. Video recordings were obtained for the last 16 s of: a) each of the last three minutes of each period of eupnea (*rest*); b) every minute during each ERL trial; and c) the first three minutes after each ERL trial (*recovery*). Because of the 0.5 expiratory duty cycle during ERL, there was an equal period of inspiration and expiration for each recording. All data analysis was performed offline by the same investigator. Femoral artery diameter was determined across the entire cardiac cycle using commercially available automated edge-detecting and wall tracking software (Brachial Analyzer, Medical Imaging Applications LLC, Coralville, IA, USA), and cross-sectional area (CSA) was computed as πr^2 (43). Time-averaged mean blood velocity (V_{MEAN}) was determined for each cardiac cycle by integrating the area under curve of the entire velocity profile (EchoPAC, GE Healthcare, Milwaukee, WI, USA). Antegrade blood velocity (V_{ANT}) was calculated by integration of positive blood velocity for each cardiac cycle, and retrograde blood velocity was determined as $V_{ANT}-V_{MEAN}$. $\dot{Q}_L$ was calculated for each cardiac cycle as the product of V_{MEAN} and average CSA.

Electromyography

To confirm that the subjects avoided contraction of the non-respiratory muscles during ERL, the EMG activity of the vastus medialis (VM) and vastus lateralis (VL) of the right leg was recorded continuously (Trigno Avanti, Delsys Inc., Natick, MA, USA) according to standard guidelines (24). Subjects performed three maximal isometric contractions prior to the rested breathing phase, and all subsequent signals were normalized to the maximum EMG response. EMG signals were band-pass filtered and full-wave rectified, and the peak root mean square was calculated using a time constant of 0.1 s (VM_{RMS} and VL_{RMS}).

Expiratory Abdominal Function via Magnetic Nerve Stimulation

For ERL_{Pga}, gastric (Pga) and esophageal (Pes) pressure were measured using two balloon-tipped catheters (47-9005, Ackrad Laboratory, Berlin, Germany) that were passed via the nares and into the stomach and lower one-third of the esophagus, respectively. The esophageal balloon was filled with 1 ml of air and positioned using the occlusion technique (4). The gastric balloon was filled with 2 ml of air and positioned so that Pga was positive during eupneic breathing in the seated position. Each catheter was connected to a differential pressure transducer (DP45, Validyne Engineering, Northridge, CA, USA) that was calibrated across the physiological range using a digital pressure manometer (no. 621, Test Products International Inc., Beaverton, OR, USA).

Magnetic stimuli were delivered to the thoracic nerve roots between the 8^{th} (T8) and 11^{th} (T11) thoracic vertebrae via a 90-mm circular coil powered by a magnetic stimulator (Magstim BiStim 2, Magstim, Whitland, Wales), as described before (34, 46). The area of stimulation that evoked the greatest Pga_{tw} was located and marked for use for all subsequent stimulations, and all stimulations were delivered at a consistent relaxed end-expiratory lung volume (i.e. FRC), as judged by end-expiratory Pes. To assess whether the expiratory muscles were maximally-activated in response to thoracic nerve stimulation, three 1 Hz twitches were delivered at progressively increasing stimulator intensities (50, 60, 70, 80, 85, 90, 95 and 100%). Each stimulation was separated by ~30 s to minimize any effect of twitch potentiation. In agreement with our previous work, depolarization of the thoracic nerve roots in response to magnetic stimulation at 100% of the stimulator's power output was likely submaximal (data not shown), the technical considerations of which have been discussed in detail elsewhere (34, 46, 51).

Expiratory abdominal muscle contractility was assessed at baseline, ~5 min after *control* ERL, and 5 min after ERL_{Pga}. The potentiated twitch is a more sensitive measure of muscle fatigue relative to the non-potentiated twitch (33). Accordingly, we measured the Pga_{tw} response to a 1-Hz magnetic stimulation that was delivered at 100% of the stimulator's power output ~5 s

after a 5 s maximal expulsive maneuver that was initiated from total lung capacity. This procedure was repeated six times such that six potentiated Pga_{tw} values were obtained, with the first two measurements discarded because the degree of potentiation was slightly smaller after the first and second expulsive maneuvers. Each potentiated twitch was assessed for amplitude (baseline to peak), maximal rate of pressure development (MRPD), maximal relaxation rate (MRR), contraction time (CT) and one-half relaxation time ($RT_{0.5}$). Pga_{MAX} was calculated as the peak Pga (across 1 s) during each expulsive maneuver; Pga_{MAX} was reported as the maximum of three values that varied by ≤10%. The within-day between occasion reproducibility coefficients (coefficient of variation, CV) were 3.9, 7.7, 5.7, 1.6, 4.8 and 2.1% for Pga_{tw}, MRPD, MRR. CT, $RT_{0.5}$ and MEP, respectively.

Data capture

All pressure, airflow, hemodynamic and EMG signals were digitized at sampling rates of 150 Hz and 2 kHz (EMG only) using an analogue-to-digital converter (Micro3 1401, Cambridge Electronic Design, Cambridge, UK) and captured using commercially available software (Spike 2 version 8.0, Cambridge Electronic Design). For ERL_{Pga}, airflow and the Doppler ultrasound signal were time-aligned according to corresponding clock-times in the data acquisition software (*Spike 2*) and the ultrasound machine for each video recording. If a cardiac cycle crossed the inspiratory-expiratory cycle, it was allocated to the respiratory phase that it was initiated in.

Statistical analysis

Based on a previously reported reduction in $\dot{Q}_L$ in response to IRL of 23 ± 10% (rest vs. task failure) (43), we determined that 5 subjects would be needed to detect a significant change in $\dot{Q}_L$ at an alpha error probability of 0.05 and a statistical power of 0.90. Normality of distribution was assessed qualitatively via visual inspection of descriptive statistics, Q-Q plots and histograms, and quantitatively using the Shapiro-Wilk test and the determination of Z-scores for skewness and kurtosis. All data that violated the assumption of normality were assessed

using a Friedman's ANOVA with Bonferroni post hoc comparisons performed for significant main effects. For all normally distributed data, repeated measures ANOVA were used to compare absolute hemodynamic and cardiovascular measurements ($\dot{Q}_L$, LVR, MAP, HR), as well as EMG measurements (VM_{RMS} and VL_{RMS}) across time for ERL_{Pm} (rest vs. min 1 vs. min 3 vs. min 5 vs. task failure vs. recovery). When significant main effects were shown, post-hoc pairwise comparisons with a Bonferroni correction were made. To limit the occurrence of type 2 error for hemodynamic variables in the ERL_{Pga} trial (i.e. HR and MAP), the number of comparisons across time were limited to rest vs. min 1 vs. task failure. Repeated measures ANOVA with Bonferroni correction were also used to compare absolute MEP (ERL_{Pm} and ERL_{Pga}) and Pga_{tw} (ERL_{Pga} only) across time (baseline vs. ~5 min after control vs. 5 min after ERL). For ERL_{Pm} only, Pearson's product moment correlation coefficients (*r*) were computed to determine the relationship between the absolute change in $\dot{Q}_L$ from baseline (eupnea) to task failure and: 1) baseline expiratory muscle strength (i.e. MEP); 2) the magnitude of ERL-induced expiratory muscle fatigue (i.e. the pre- to post-ERL_{Pm} change in MEP); and 3) the time to task failure. Results are expressed as group mean ± SD and all statistical analysis was performed in SPSS Statistics 24 (SPSS Inc, Chicago, IL). The acceptable type 1 error was set at $P < 0.05$.

RESULTS

Cardiovascular measurements during eupnea

The CV and intraclass correlation coefficients for all cardiovascular parameters ($\dot{Q}_L$, LVR, MAP and HR) during resting eupneic breathing were ≤5.9% and ≥0.92, respectively. Due to the random variation in $\dot{Q}_L$ and LVR across time and the lack of external validation of absolute flow values against phantom artery preparations (49), we compared $\dot{Q}_L$ and LVR to values measured during prior eupneic control periods. However, our absolute resting values of superficial femoral artery $\dot{Q}_L$ (133 ± 34 $ml \cdot min^{-1}$; ERL_{Pm}) are well within the normal range reported for young healthy adults (70-196 $ml \cdot min^{-1}$) (26, 28, 42, 43).

Expiratory resistive loaded breathing targeting expiratory mouth pressure: ERL_{Pm}

Cardiovascular measurements during control ERL (2% of MEP)

There was no change in group mean MAP, $\dot{Q}_L$, LVR (Figure 1), HR, superficial femoral artery diameter and V_{MEAN} (Table 1) across time in response to ERL at 2% of MEP (all $P \geq 0.05$). Neither VM_{RMS} nor VL_{RMS} increased from rest to during ERL at 2% of MEP, confirming no contraction of the non-respiratory muscles ($P \geq 0.05$; Table 1).

Cardiovascular measurements during ERL_{Pm} (65% of MEP)

The ERL_{Pm} trial was performed for 9.3 ± 2.7 min. Before task failure, expiratory Pm was maintained at 97 ± 4% of the target value (Table 1). There was an immediate and sustained increase in group mean HR from rest to during ERL_{Pm} ($P < 0.05$; Table 1). Similarly, relative to resting baseline values, group mean MAP increased by 19 ± 6 mmHg ($P < 0.001$) and 31 ± 10 mmHg ($P < 0.001$) at the first minute and at task failure, respectively, during ERL_{Pm} (Figure 1) (min 1 vs. task failure, $P = 0.12$). Conversely, there was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during ERL_{Pm} ($P \geq 0.05$) (Figure 1, Table 1). The $\dot{Q}_L$ and LVR response to ERL_{Pm} was, however, highly variable between the subjects. Indeed, the change in $\dot{Q}_L$ and LVR from baseline to task failure during ERL_{Pm} ranged from –36% to +81% and from –24% to +70%, respectively (Figure 1). As in the control trial targeting 2% of MEP, there was no evidence of leg muscle contraction throughout ERL_{Pm} (Table 1).

ERL_{Pm}-induced expiratory muscle fatigue

There was no change in MEP from pre- to post-5 min of control ERL at 2% of MEP (200 ± 28 vs. 196 ± 28 cmH_2O, $P = 1.000$). There was, however, a significant reduction relative to prior baseline values in MEP following ERL_{Pm} at 65% of MEP (196 ± 28 vs. 163 ± 28 cmH_2O, $P = 0.001$) (Figure 2A).

Expiratory resistive loaded breathing targeting expiratory gastric pressure: ERL_{Pga}

Figure 3 shows breath-by-breath Pm and Pga, beat-by-beat finger arterial pressure (AP) and HR, and mean $\dot{Q}_L$ and LVR data for one individual subject during ERL targeting 2% (Figure 3A) and 65% of Pga_{MAX} (Figure 3B).

Cardiovascular measurements during control ERL (2% of Pga_{max})

Group mean MAP, $\dot{Q}_L$, LVR (Figure 4), HR, superficial femoral artery diameter and V_{MEAN} (Table 2) did not change across time during ERL at 2% of Pga_{MAX} ($P \geq 0.05$). Similarly, neither VM_{RMS} nor VL_{RMS} increased from rest to during ERL at 2% of Pga_{MAX}, confirming contraction of the non-respiratory muscles did not occur ($P \geq 0.05$) (Table 2).

Cardiovascular measurements during ERL_{Pga} (65% of Pga_{max})

ERL_{Pga} was performed for 9.5 ± 2.3 min. Prior to task failure, expiratory Pm and Pga were maintained at 99 ± 5% and 87 ± 11% of the target values, respectively (Table 2). There was a progressive increase in HR that was significantly elevated versus resting values by task failure (+31 ± 10 beats·min^{-1}, P = 0.007) (Table 2). Similarly, group mean MAP was not different to resting values at the first minute of ERL_{Pga} (+20 ± 15 mmHg; P = 0.132), but was significantly elevated by task failure (18 ± 9 mmHg, P = 0.028) (Figure 4). There was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during ERL_{Pga} (all $P \geq 0.05$) (Figure 4; Table 2). However, as in ERL_{Pm}, the $\dot{Q}_L$ and LVR response to ERL_{Pga} was highly variable between the subjects (Figure 4). There was no evidence of leg muscle contraction throughout ERL_{Pga} ($P \geq 0.05$; Table 2).

ERL_{Pga}-induced expiratory muscle fatigue

From before to after control ERL at 2% of Pga_{MAX}, there was no change in either MEP or Pga_{tw} (MEP: 190 ± 27 vs. 188 ± 27 cmH_2O, P = 1.000; Pga_{tw}: 46.1 ± 20.2 vs. 51.0 ± 26.3 cmH_2O, P = 0.554). There were no differences in twitch characteristics or M-wave responses from before to after control ERL_{Pga} (all $P > 0.05$). ERL_{Pga} at 65% of Pga_{MAX} did, however, elicit expiratory muscle fatigue as evidenced by a 16.0 ± 11.6% reduction in Pga_{tw} (P = 0.092) (Figure 2B) and

a 16.2 ± 5.8% reduction in MEP (P = 0.025) (Figure 2C). Although the group mean change in Pga_{tw} was not statistically significant, 4 of the 5 subjects exhibited a percent reduction that was > 2× greater than the CV of the measure, which is conservatively indicative of fatigue (Figure 2B). There were no changes in twitch characteristics in response to ERL_{Pga}: CT, 154 ± 23 vs 153 ± 26 ms; MRPD/Pga_{tw}, 13.5 ± 2.7 vs. 14.2 ± 2.2 s/cmH_2O; MRR/Pga_{tw}, −4.7 ± 0.8 vs. −5.2 ± 0.5 s/cmH_2O; $RT_{0.5}$, 160 ± 32 vs. 153 ± 11 ms (all P > 0.05). Similarly, M-wave characteristics were unchanged in response to ERL_{Pga}: amplitude, 2.6 ± 2.3 vs. 3.0 ± 2.9 mV; duration, 22.5 ± 4.5 vs. 24.1 ± 7.4 ms; area, 10.7 ± 8.6 vs. 13.3 ± 10.6 mV·s^{-1} (n = 3, all P > 0.05). Pre-twitch end-expiratory Pes was unchanged from baseline versus post-ERL_{Pga} control or post-ERL_{Pga} (−4.0 ± 1.5 cmH_2O vs. −4.4 ± 2.0 cmH_2O vs. −3.6 ± 1.8 cmH_2O; P = 0.476), suggesting that all stimulations were performed at a similar lung volume across time.

Within-breath cardiovascular responses to ERL

In response to ERL_{Pm} and ERL_{Pga}, group mean $\dot{Q}_L$ was not significantly different from resting eupneic values (Figure 1 and Figure 4). However, further analyses of ERL_{Pga} showed that $\dot{Q}_L$ varied cyclically in time with each respiratory phase of each breath (see Figure 5 for an individual example during ERL_{Pga} and Table 3 for group mean values). For example, when measured across an entire breath (*i.e. inspiration and expiration*), group mean $\dot{Q}_L$ over the duration of ERL_{Pga} was effectively unchanged relative to resting baseline values (+7 ± 37%; P = 0.683). However, when apportioned to each respiratory phase within a breath, $\dot{Q}_L$ during each resisted expiration increased by 66 ± 47% (P = 0.052) (range +8% to +111%) relative to $\dot{Q}_L$ measured across the entire respiratory cycle at rest. This increase was mediated predominantly by an increase in antegrade flow (mean change: +64 ± 27%; P = 0.013). By contrast, $\dot{Q}_L$ during each unresisted inspiration decreased by 50 ± 27% (P = 0.020) (range –84% to –26%) relative to $\dot{Q}_L$ measured across the entire respiratory cycle at rest. This decrease was, predominantly, the result of an increase in retrograde flow (222 ± 144%; P = 0.004). The potential mechanistic cause of such phasic swings in $\dot{Q}_L$ is discussed in section

'*Why did ERL have no effect on LVR and $\dot{Q}_L$: metabolic vs. mechanical factors*' of the discussion.

Correlations between variables

For ERL_{Pm} only, there was no significant correlation between the change in $\dot{Q}_L$ from rest to task failure and 1) baseline MEP ($r = -0.29$); 2) the change in MEP from pre to post-ERL ($r = 0.17$); or 3) time to task failure for ERL ($r = 0.27$) (all $P > 0.05$). Similarly, the change in LVR from rest to task failure during ERL_{Pm} was not associated with 1) baseline MEP ($r = 0.38$); 2) the change in MEP from pre to post-ERL ($r = -0.11$); or 3) time to task failure of ERL ($r = -0.18$) (all $P > 0.05$). These data indicate that the individual variability in cardiovascular responses to ERL_{Pm} was not associated with baseline expiratory muscle strength and/or the severity of ERL-induced expiratory muscle fatigue.

DISCUSSION

Main Findings

We investigated the cardiovascular consequences of fatiguing expiratory muscle work in healthy humans. To our knowledge, this is the first study to directly measure leg vascular resistance (LVR) and leg blood flow ($\dot{Q}_L$) in response to 'high-intensity' contractions of the expiratory muscles sustained to the point of task failure in otherwise resting individuals. The major findings were that expiratory resistive loaded breathing (ERL) targeting 65% of either expiratory mouth pressure or expiratory gastric pressure caused: 1) a substantial and sustained increase in mean arterial pressure (MAP) and heart rate (HR); and 2) fatigue of the expiratory muscles, as evidenced by a significant pre- to post-ERL reduction in maximal expiratory mouth pressure (MEP) and/or gastric twitch pressure (Pga_{tw}); but 3) no change in group mean LVR or $\dot{Q}_L$ relative to prior resting baseline values. There was, however, a large degree of between-subject variability in LVR (−33 to +104%) and $\dot{Q}_L$ (−38 to +81%) in response to ERL. Interestingly, we observed that $\dot{Q}_L$ oscillated cyclically across the respiratory phases within each breath. Indeed, relative to $\dot{Q}_L$ measured across the entire respiratory cycle

at rest (*i.e. inspiration and expiration*), $\dot{Q}_L$ increased by ~66% during each resisted expiration but decreased by ~50% during each unresisted inspiration. In combination, these data suggest that while fatiguing expiratory muscle work had no effect on group mean LVR or $\dot{Q}_L$ in otherwise resting humans, it is possible that any sympathetically-mediated peripheral vasoconstriction and reduction in $\dot{Q}_L$ was counteracted by transient increases in $\dot{Q}_L$ due to the mechanical effects of high intra-abdominal pressure during ERL (*see 'mechanical effects of expiratory resisted loading'*).

Expiratory Muscle Metaboreflex

It is well accepted that fatiguing inspiratory muscle work in otherwise resting humans is associated with neural and cardiovascular consequences. Characterized by time-dependent increases in MSNA, MAP and LVR, with a consequent decrease in $\dot{Q}_L$, this inspiratory muscle metaboreflex is thought to be triggered by fatigue-induced metabolite accumulation and stimulation of metabosentive group IV and, to a lesser extent, group III muscle afferents secondary to 'high-intensity' contractions (≥60% of maximal inspiratory mouth pressure) of the inspiratory muscles (25, 27, 39, 41, 43). Contrary to this, we found no evidence for a change in either group mean LVR or group mean $\dot{Q}_L$ in response to ERL performed to task failure. This raises two important questions: 1) were the experimental conditions required to initiate an expiratory muscle metaboreflex met in the present study; and 2) mechanistically, why did fatiguing ERL have no effect on group mean LVR and $\dot{Q}_L$ in otherwise resting humans?

Was an expiratory muscle metaboreflex initiated?

The critical tension-time index (TTI) for the diaphragm is the product of muscle tension and duty cycle (*or time*) above which blood flow to the diaphragm is limited, metabolite accumulation occurs and fatigue ensues (5, 6). The critical TTI for the expiratory abdominal muscles is unknown. However, if the expiratory TTI during loaded breathing in the present study was too low to induce expiratory muscle fatigue and metabolite accumulation, and as such an expiratory muscle metaboreflex, then it would perhaps be unsurprising that ERL did

not cause a reduction in group mean LVR and $\dot{Q}_L$. However, we are confident the 'lack' of a significant LVR and $\dot{Q}_L$ response to ERL in this study was not due to an insufficient TTI for several reasons. The expiratory TTI during ERL in the present study was 0.325, which is more than two-times greater than the critical TTI previously reported for the diaphragm (~0.15) (5, 6). Moreover, the cardiovascular consequences of inspiratory loading, including an increase in LVR and reduction in $\dot{Q}_L$, have been reported in response to inspiratory resistive loaded breathing (IRL) with a TTI as low as 0.24 (41). Given that the expiratory muscles are less fatigue-resistant compared to the inspiratory muscles (17), it is likely that our ERL protocols were above the critical TTI for the expiratory muscles and thus induced neuromuscular fatigue and metabolite accumulation. Indeed, we observed a 16-17% reduction in MEP and a 16% decrease in Pga_{tw} (ERL_{Pga} only) in response to ERL trials (Figure 2). In addition, the magnitude and temporality of the MAP response to ERL (Figure 1 & Figure 4) was remarkably comparable to that observed during similar ERL protocols. For example, during ERL_{Pm} there was an immediate and substantial increase in MAP (~19 mmHg; rest vs. min 1) that, although not significant, progressively increased over time (~12 mmHg; min 1 vs. task failure; $P = 0.12$). Similarly, in response to ERL (60% MEP, $T_E/T_{TOT} = 0.7$, $f_R = 15$ breaths/min), Derchak et al. (10) reported a sharp initial rise in MAP (~17 mmHg; rest vs. min 1) that increased progressively but non-significantly over time (~11 mmHg; rest vs. task failure); a cardiovascular response that was concomitant with a time-dependent increase in MSNA. As such, although we did not directly measure sympathetic outflow, based on the striking similarity between the magnitude and time-dependency of the MAP response to ERL in the present study compared to the study by Derchak et al. (10) who also reported a time-dependent sympathoexcitation, we are confident that we did indeed elicit an expiratory muscle metaboreflex in our subjects.

Another consideration is whether it is necessary to specifically target the expiratory abdominal muscles during ERL to elicit an expiratory muscle metaboreflex. In previous IRL studies, subjects were instructed to isolate the diaphragm during resisted inspiratory efforts (41) to

avoid the recruitment of accessory inspiratory muscles which may be heavily utilized when using 'natural' breathing techniques (37). Whether specifically targeting the diaphragm versus the inspiratory accessory muscles during IRL elicits a different cardiovascular response is currently unknown. It is possible that the diaphragm has a higher density of metaboreceptors in comparison to the accessory inspiratory muscles, eliciting a greater sympathetic response for a similar metabolic 'insult'. However, type III and IV afferent fibers have been identified in the intercostal nerve (13), and such metaboreceptors are ubiquitous among other skeletal muscles. In addition, in exercising healthy humans, the inverse relationship of sternocleidomastoid blood flow to manipulations in the inspiratory work of breathing (11) supports the idea that the accessory respiratory muscles contribute to, or are at least responsive to, a respiratory muscle metaboreflex. In the present study, performing fatiguing expiratory muscle work without specific breathing instructions (ERL_{Pm}) led to no change in LVR and $\dot{Q}_L$. Subsequently, and in line with IRL studies that specifically targeted the diaphragm, we examined the cardiovascular responses to ERL whilst subjects specifically targeted Pga (i.e. recruited the primary expiratory muscles of the abdominal wall). While it has been shown previously that the severity of expiratory muscle fatigue is increased when expiratory duty cycle (and presumably the duration of ischemia) is lengthened (48), we still found no consistent group mean change in LVR or $\dot{Q}_L$ during ERL_{Pga}. Moreover, in the five subjects who performed both ERL_{Pm} and ERL_{Pga}, Pm was similar across the two trials (127 ± 16 cmH_2O vs. 120 ± 18 cmH_2O). Therefore, we are confident that the conditions required to trigger an expiratory muscle metaboreflex were met. We speculate on why this did not translate into a reduction in $\dot{Q}_L$ in more detail, below.

Why did ERL have no effect on LVR and $\dot{Q}_L$: metabolic vs. mechanical factors

Metabolic effects of expiratory resistive loading

We hypothesized that, like for the inspiratory muscles, fatigue-induced accumulation of metabolic by-products (e.g. lactate, potassium, adenosine diphosphate) in the expiratory musculature would lead to a sympathetically-mediated time-dependent peripheral

vasoconstriction with a resultant increase in LVR and decrease in $\dot{Q}_L$ (39, 41, 44). Contrary to this hypothesis we presently found that group mean LVR and $\dot{Q}_L$ were not different compared to eupneic values in response to ERL.

Despite this, upon further analyses, we did observe a 50 ± 27% reduction in $\dot{Q}_L$ during the *unresisted inspiratory* phase of each breathing cycle, compared to resting values across the entire breath, that was largely due to an increase in retrograde flow (222 ± 144%) (Figure 5; Table 3). Although speculative, it is possible that this reduction in $\dot{Q}_L$ during the inspiratory phase of each breath, in the absence of a mechanical consequence of high intra-abdominal pressure, is indicative of an underlying peripheral vasoconstriction in the resting limb. The increase in inspiratory retrograde $\dot{Q}_L$, despite the lack of a change in superficial femoral artery diameter, is congruent with constriction of resistance arteries and arterioles downstream of the superficial femoral artery, which, rather than the larger conduit arteries, are primarily responsible for the regulation of limb blood flow (20, 30). Therefore, it is likely that peripheral vasoconstriction in the arterioles downstream of the superficial femoral artery was responsible for, or at least contributed to, the change in MAP, and the reduction in $\dot{Q}_L$ during unresisted inspirations in the present study. In further support of this assertion, a hyperemic response was displayed by the majority of subjects immediately following cessation of ERL trials (Figure 1), characteristic of arterial vasodilation following a period of vasoconstriction. So, the question becomes: if our ERL protocols did elicit a metabolite-induced and sympathetically-mediated vasoconstriction in the resting limbs, why did group mean LVR and $\dot{Q}_L$ not change across time in response to expiratory resisted breathing?

Mechanical effects of expiratory resistive loading

It is well documented that stroke volume fluctuates during the respiratory cycle due to changes in intrathoracic and intra-abdominal pressure, with consequent effects on venous return, right ventricular preload and left ventricular emptying (36). We speculate that the very-high intra-abdominal pressures during ERL in the present study may have resulted in a mechanical

cardio-pulmonary interaction that transiently influenced our recordings of $\dot{Q}_L$. Indeed, when measured across an entire breath (*i.e. inspiration and expiration*), group mean $\dot{Q}_L$ during ERL_{Pga} was effectively unchanged relative to resting baseline values (+7 ± 37%). However, when apportioned to each respiratory phase within a breath, there was a 66 ± 47% increase $\dot{Q}_L$ during each resisted expiration that was primarily mediated by an increase in antegrade flow (64 ± 27%). So, why did $\dot{Q}_L$ during each loaded expiration increase during ERL*?* While very high expiratory intrathoracic pressures may reduce transmural aortic pressure and afterload resulting in an increased stroke volume, it has also been shown that increases in abdominal pressure achieved via abdominal compression, diaphragmatic breathing, or Valsalva maneuvers attenuates venous return (16), cardiac filling, and stroke volume (36, 38, 53). Previous reports have also suggested that expulsive maneuvers generating a very-high intra-abdominal pressure with concomitant diaphragm contraction (i.e. ERL) cause a substantial blood-volume shift from the trunk to the extremities, likely originating from the splanchnic region (3). For example, a 'square-wave' increase in abdominal pressure of ~100 cmH_2O maintained for 1 s (followed by 2 s relaxation) produced a 'stroke volume' from the splanchnic bed of 350 ml and an output of 6.8 $L \cdot min^{-1}$ compared to a resting cardiac output of 5.6 $L \cdot min^{-1}$ (3). We speculate that the highly comparable Pga exhibited during ERL in the present study (~120 cmH_2O; Table 2) may have similarly transiently increased splanchnic outflow and stroke volume during the expiratory phase of each breath. Indeed, an increase in cardiac ejection and driving pressure secondary to a blood volume shift could theoretically increase antegrade $\dot{Q}_L$, even if underlying vasoconstriction is present. Because this effect was present immediately and was sustained for the duration of ERL (Figure 5 and Table 3), we predict that the splanchnic reservoir was completely refilled during each un-resisted inspiration of ERL, as found previously (3).

Individual Variability in LVR and $\dot{Q}_L$

We also observed substantial between-subject variability in LVR and $\dot{Q}_L$ in response to ERL (Figure 1 and Figure 4). Although the range of $\dot{Q}_L$ and LVR during ERL was large relative to

562 eupneic values ($\dot{Q}_L$ range: −38 to +81%), such between-subject variability is not novel. For
563 example, previous data show individual changes in $\dot{Q}_L$ ranging from approximately −40 to +5%
564 by task failure of IRL (41). So, what are the potential mechanisms for the between-subject
565 variability in cardiovascular responses to ERL? First, it is possible that subjects exhibiting a
566 greater relative increase in $\dot{Q}_L$ during ERL may have failed to incite a sufficient severity of
567 expiratory muscle fatigue to elicit the respiratory muscle metaboreflex. However, we found no
568 correlation with the change in $\dot{Q}_L$ or LVR and the magnitude of expiratory muscle fatigue,
569 baseline MEP or time to task failure. In addition, MEP decreased in every subject during ERL
570 (by > 2× CV) indicating that expiratory muscle fatigue was present in response to all trials. It
571 remains to be determined whether, for a given magnitude of respiratory muscle fatigue, the
572 activation of group III and IV muscle afferents varies among subjects. As we did not control
573 for sex or training status in the present study, it is likely that muscle morphology and substrate
574 utilization varied between subjects, which may have led to differences in fatigue-associated
575 metabolite accumulation. For example, females exhibit an attenuated inspiratory muscle
576 metaboreflex compared to males (43), and also demonstrate blunted increases in H^+ and
577 $H_2PO_4^{-1}$ in response to handgrip exercise with post-exercise forearm occlusion (14). Second,
578 differences in the magnitude of sympathetic outflow or vascular transduction between subjects
579 may affect the extent of sympathetically-mediated vasoconstriction for a given afferent
580 stimulus. Indeed, the change in MSNA in response to ERL in young healthy males is highly
581 variable, ranging from approximately -20 to +700% from baseline to task failure (10). Third,
582 there may be individual differences in the magnitude of splanchnic outflow and the consequent
583 blood-volume shift for a given increase in abdominal pressure. For example, depending on
584 the conditions of the circulatory system, the abdominal venous compartment can act as a
585 capacitor augmenting venous return (zone 3 conditions) or less commonly, as a starling
586 resistor dynamically compressing the inferior vena cava limiting venous return (zone 2
587 conditions) (45).
588
589 **Temporality of the Expiratory Muscle Metaboreflex**

It is well accepted that the autonomic and cardiovascular consequences of IRL do not become apparent until IRL has been performed for ≥2 min (41, 44). This 'time-dependency' likely reflects the time taken for fatigue-associated metabolites to accumulate and for the subsequent stimulation of type III and IV phrenic afferents to occur. However, in response to ERL we observed an abrupt initial rise in MAP that increased somewhat progressively over time (*see* Figure 1D). This poses the question; is the temporality of respiratory muscle metaboreflex initiation different for fatiguing inspiratory vs. fatiguing expiratory muscle work? It is highly likely that central expiratory motor command increased during ERL and in theory could have contributed to the observed MAP response. However, it has been shown previously that performance of ERL with a very-high expiratory Pm in the absence of expiratory muscle fatigue (95% MEP; T_E/T_{TOT} = 0.35; f_R = 12 breaths·min^{-1}) does not evoke a change in MSNA or MAP, and that central command only has a "minor, variable contribution" to the cardiovascular response to ERL (10). Perhaps a more likely mechanism underpinning the different temporality of the cardiovascular responses to ERL vs. IRL relates to differences in the fatigability of the expiratory vs. inspiratory muscles. Indeed, it is well established that the expiratory muscles are phenotypically and functionally less fatigue-resistant than the inspiratory muscles (especially the diaphragm) (17, 18, 50). As such, we speculate that the development of expiratory muscle fatigue, the subsequent accumulation of fatigue-associated metabolites, and the consequent activation of a respiratory muscle metaboreflex may occur more abruptly during ERL vs. during IRL. This rapid activation of an expiratory muscle metaboreflex would also be consistent with the substantial (although not significant) absolute rise in inspiratory retrograde flow within the first minute of ERL (*see* Table 3).

Conclusions

In response to fatiguing expiratory muscle work in otherwise resting healthy humans, we found no change in group mean leg blood flow ($\dot{Q}_L$) or leg vascular resistance, despite substantial expiratory muscle fatigue and an increase in heart rate and mean arterial pressure suggestive of an increase in sympathetic outflow. The lack of cardiovascular changes in the resting limb

may be the result of substantial cyclical increases in expiratory $\dot{Q}_L$, coincident with a mechanical effect of very-high intra-abdominal pressures, which may have masked underlying sympathetically-mediated vasoconstriction caused by an expiratory muscle metaboreflex.

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Disclosure

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

Author Contributions

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

Figure Legends

Figure 1. Mean arterial pressure (MAP, *n* = 9) (*top panels*), leg blood flow ($\dot{Q}_L$, *n* = 11) (*middle panels*), and leg vascular resistance (LVR, *n* = 9) (*bottom panels*) responses to expiratory resistive loading targeting 2% (ERL_{Pm} control) (A-C) and 65% (ERL_{Pm}) of maximal expiratory mouth pressure (D-F). Data are group means (black) and individual values (grey). Females are presented as clear open symbols (n = 2). Percent of rest is calculated as: mean value for each minute during ERL divided by the mean of the entire period of rest, multiplied by 100. For example, with a resting $\dot{Q}_L$ of 100 ml·min^{-1}, a $\dot{Q}_L$ of 130 ml·min^{-1} would equate to 130% of rest. *Significantly different to rest ($P < 0.05$).

Figure 2. Individual expiratory muscle function responses to expiratory resistive loading (ERL) targeting mouth pressure (ERL_{Pm}) (A) and gastric pressure (ERL_{Pga}) (B-C). MEP, maximal expiratory pressure; Pga_{tw}, gastric twitch pressure. Dotted lines represent 2 x CV. * $P < 0.05$ vs. pre-ERL values.

Figure 3. Cardiovascular and ventilatory responses to (A) ERL_{Pga} control and (B) ERL_{Pga} for one subject, represented by black squares in all other figures. Cardiovascular and ventilatory parameters were unchanged in response to ERL_{Pga} control. There was a time-dependent increase in HR and MAP in response to ERL_{Pga}. Pm and Pga were maintained at the target level until the final minute of ERL_{Pga}. $\dot{Q}_L$, leg blood flow; LVR, leg vascular resistance; HR, heart rate; AP, arterial pressure; Pm, mouth pressure; Pga, gastric pressure.

Figure 4. Mean arterial pressure (MAP, *n* = 5) (*top panels*), leg blood flow ($\dot{Q}_L$, *n* = 5) (*middle panels*), and leg vascular resistance (LVR, *n* = 5) (*bottom panels*) responses to expiratory resistive loading targeting 2% (ERL_{Pga} control) (A-C) and 65% (ERL_{Pga}) of maximal gastric pressure (D-F). Data are group means (black) and individual values (grey).

Figure 5. An individual example of the effect of large swings in Pm on resting $\dot{Q}_L$ during fatiguing expiratory resisted loading targeting 65% of maximal gastric pressure (ERL_{Pga}). A, raw Doppler ultrasound images of the superficial femoral artery; B, mouth pressure (Pm); C, airflow. $\dot{Q}_L$ ($ml \cdot min^{-1}$) calculated as vessel CSA * V_{MEAN}. Example periods of high antegrade and retrograde flow, during each loaded expiration and non-resisted inspiration, respectively, are highlighted. $\dot{Q}_L$, leg blood flow; CSA, cross sectional area; V_{MEAN}, mean blood velocity. The reader is directed to the online data supplement for the raw Doppler ultrasound video recording at minute 5 of ERL_{Pga} (URL, https://figshare.com/s/9d57f090c445eb5ce967; DOI, https://doi.org/10.6084/m9.figshare.13270085).

Figure 1.

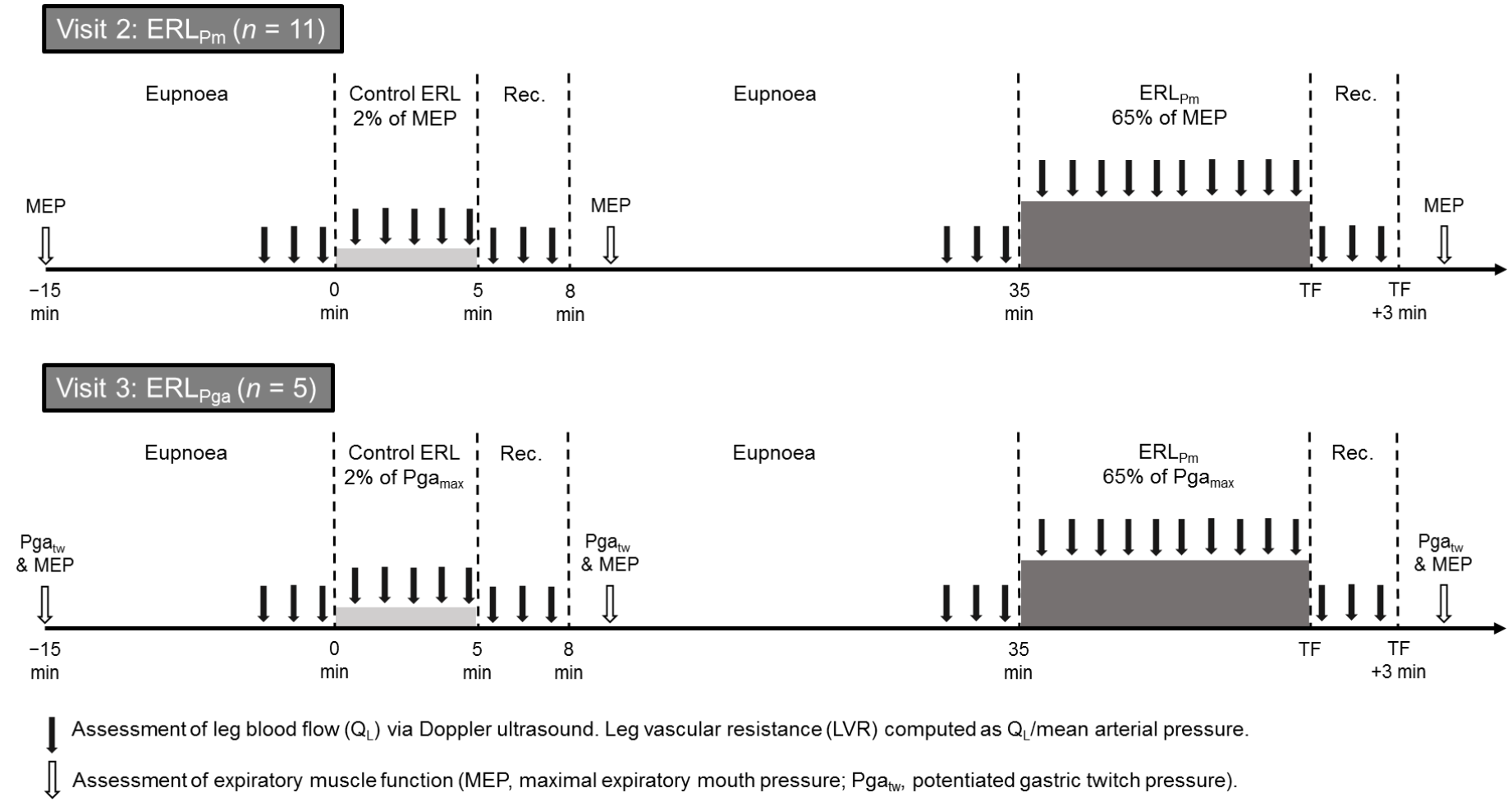

Visit 2: ERL$_{Pm}$ (n = 11)
Eupnoea
Control ERL
2% of MEP
Rec.
Eupnoea
ERL$_{Pm}$
65% of MEP
Rec.
MEP
MEP
MEP
−15 min
0 min
5 min
8 min
35 min
TF
TF +3 min
Visit 3: ERL$_{Pga}$ (n = 5)
Eupnoea
Control ERL
2% of Pga$_{max}$
Rec.
Eupnoea
ERL$_{Pm}$
65% of Pga$_{max}$
Rec.
Pga$_{tw}$ & MEP
Pga$_{tw}$ & MEP
Pga$_{tw}$ & MEP
−15 min
0 min
5 min
8 min
35 min
TF
TF +3 min
Assessment of leg blood flow (Q_L) via Doppler ultrasound. Leg vascular resistance (LVR) computed as Q_L/mean arterial pressure.
Assessment of expiratory muscle function (MEP, maximal expiratory mouth pressure; Pga$_{tw}$, potentiated gastric twitch pressure).

Figure 2.

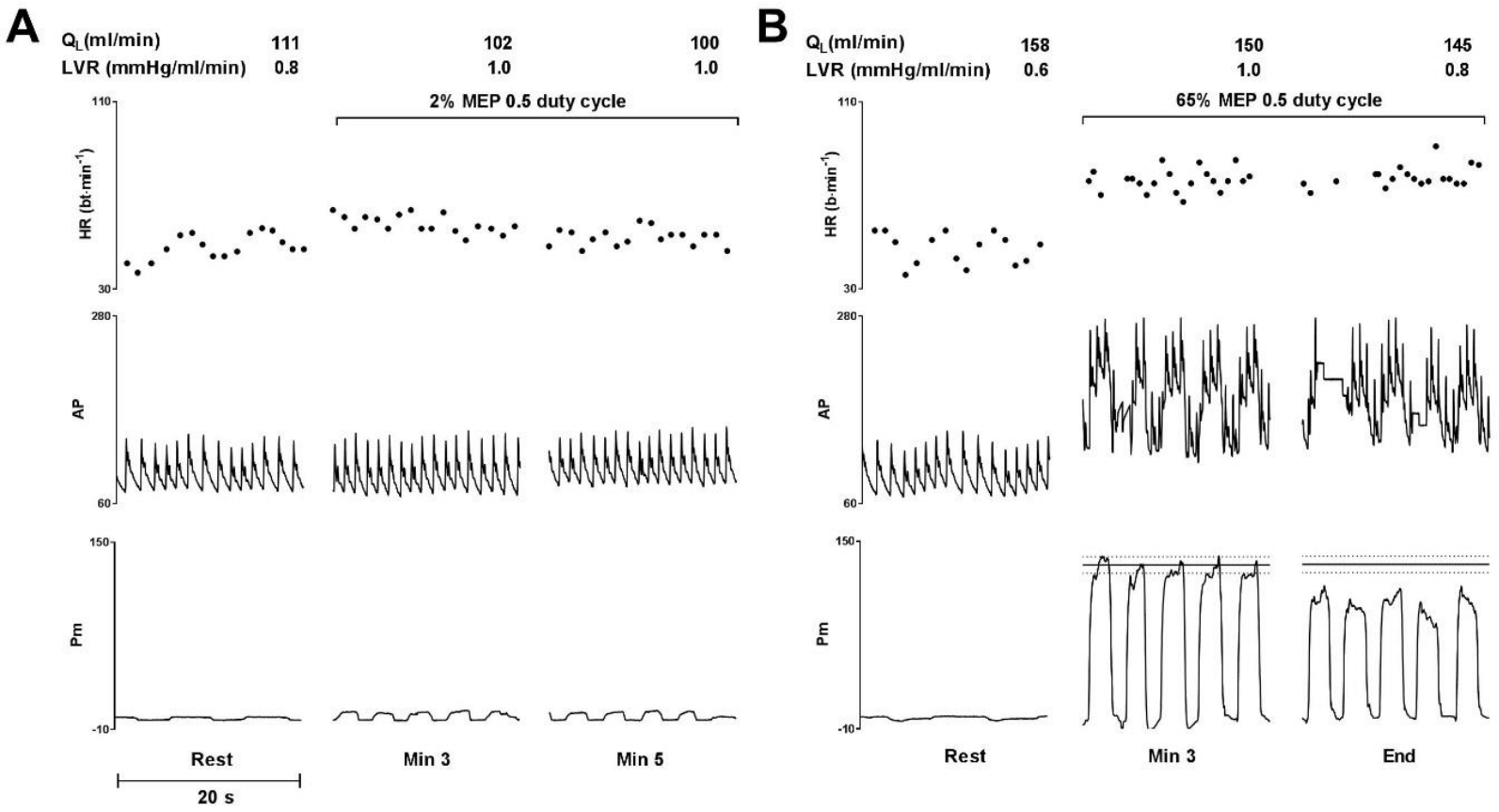

A
Q_L(ml/min) 111 102 100
LVR (mmHg/ml/min) 0.8 1.0 1.0
2% MEP 0.5 duty cycle
HR (bt·min^{-1})
AP
Pm
Rest
Min 3
Min 5
20 s
B
Q_L(ml/min) 158 150 145
LVR (mmHg/ml/min) 0.6 1.0 0.8
65% MEP 0.5 duty cycle
HR (b·min^{-1})
AP
Pm
Rest
Min 3
End

Figure 3.

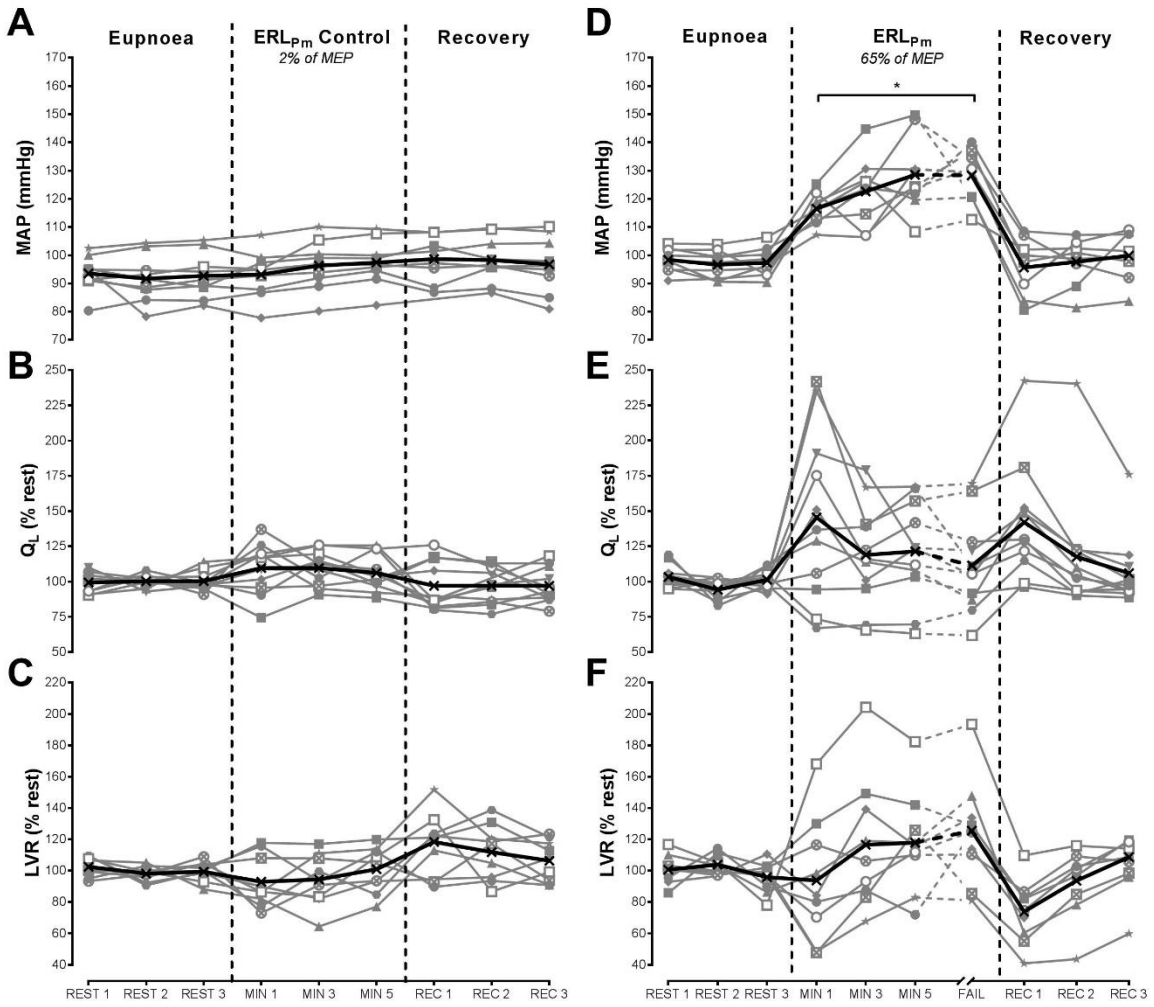

A
Eupnoea
ERLPm Control
2% of MEP
Recovery
MAP (mmHg)
B
QL (% rest)
C
LVR (% rest)
REST 1 REST 2 REST 3 MIN 1 MIN 3 MIN 5 REC 1 REC 2 REC 3
D
Eupnoea
ERLPm
65% of MEP
Recovery
*
MAP (mmHg)
E
QL (% rest)
F
LVR (% rest)
REST 1 REST 2 REST 3 MIN 1 MIN 3 MIN 5 FAIL REC 1 REC 2 REC 3

Figure 4.

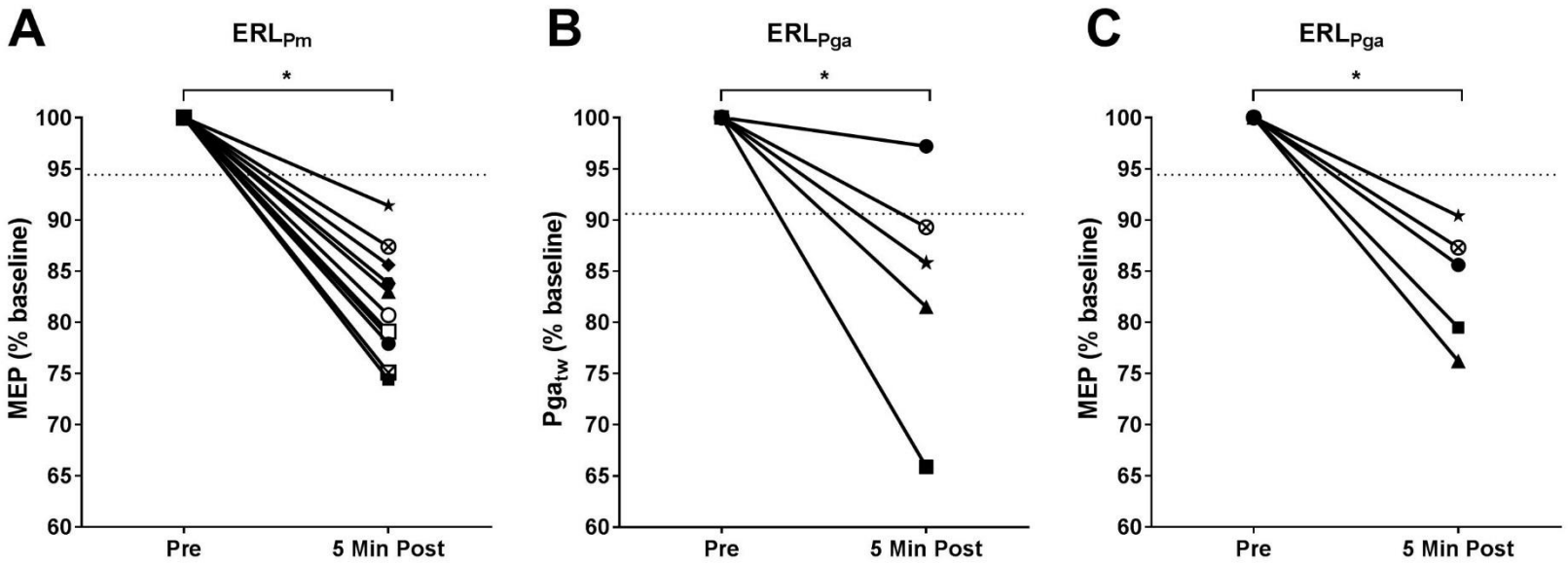
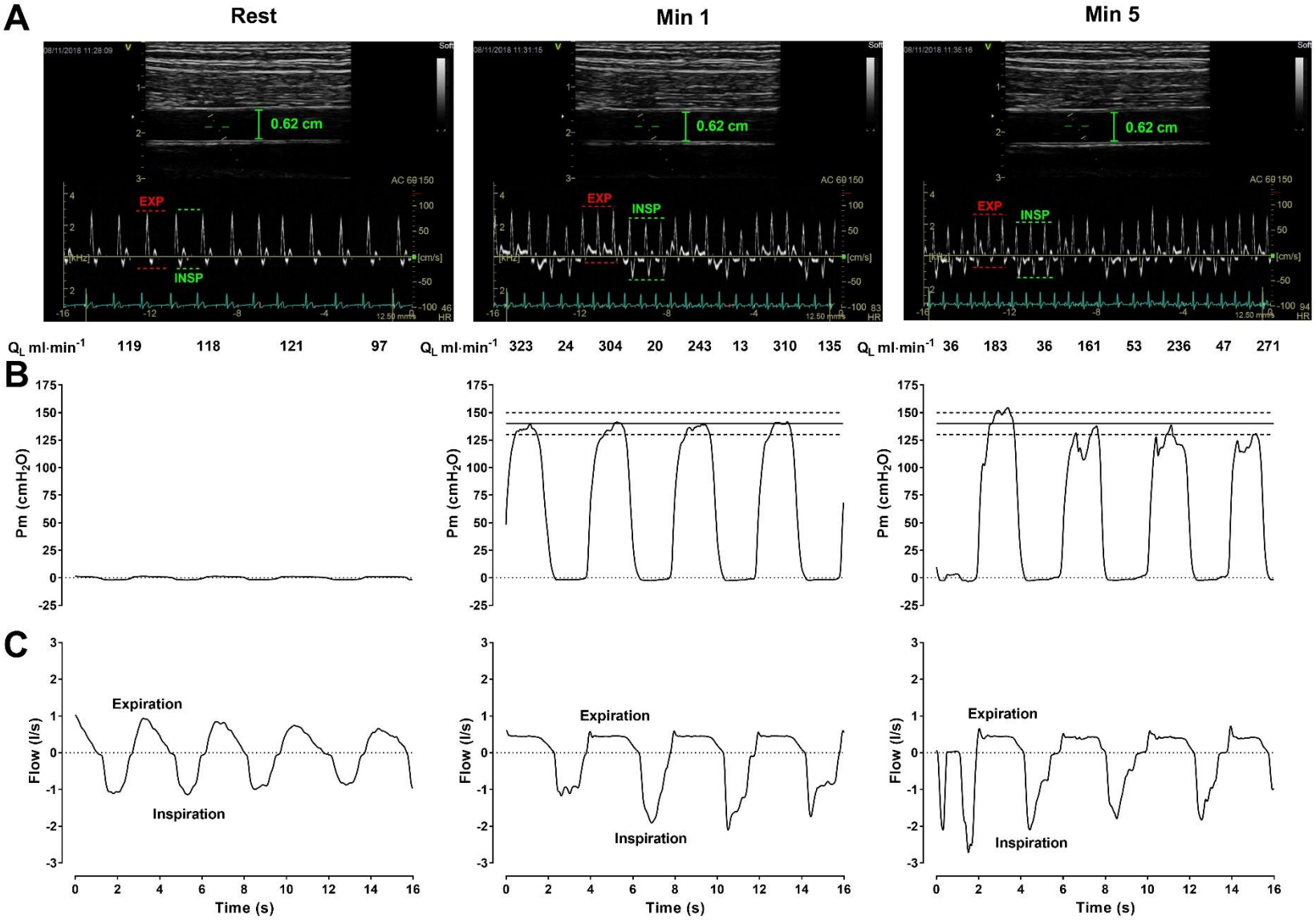

A
ERL$_{Pm}$
*
100
95
90
85
80
75
70
65
60
MEP (% baseline)
Pre
5 Min Post
B
ERL$_{Pga}$
*
100
95
90
85
80
75
70
65
60
Pga$_{tw}$ (% baseline)
Pre
5 Min Post
C
ERL$_{Pga}$
*
100
95
90
85
80
75
70
65
60
MEP (% baseline)
Pre
5 Min Post

Figure 5.


A
Rest
Min 1
Min 5
0.62 cm
EXP
INSP
Q_L ml·min^{-1} 119 118 121 97
Q_L ml·min^{-1} 323 24 304 20 243 13 310 135
Q_L ml·min^{-1} 36 183 36 161 53 236 47 271
B
Pm (cmH2O)
C
Flow (l/s)
Expiration
Inspiration
Time (s)