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Sensitivity of the human ventilatory response to muscle metaboreflex activation during concurrent mild hypercapnia

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Abstract

Ventilation increases during muscle metaboreflex activation when postexercise circulatory occlusion (PECO) traps metabolites in resting human muscle, but only in conditions of concurrent systemic hypercapnia. We hypothesize that a linear relationship exists between the level of hypercapnia and the magnitude of the additional hyperpnoea produced in response to a standardized level of muscle metaboreflex activation. Fifteen male subjects performed four trials, in which the end-tidal partial pressure of carbon dioxide (P_{ET,CO_2}) was elevated by 1, 3, 7 or 10 mmHg above resting values using a dynamic end-tidal forcing system. In each trial, subjects were seated in an isometric dynamometer designed to measure ankle plantar flexor force. Rest for 2 min in room air was followed by 15 min of exposure to one of the four levels of hypercapnia, at which 5 min further rest was followed by 2 min of sustained isometric calf muscle contraction at 50% of predetermined maximal voluntary strength. Immediately before cessation of exercise, a cuff around the upper leg was inflated to a suprasystolic pressure to cause PECO for 3 min, before its deflation and a further 5 min of rest, concluding exposure to hypercapnia. The PECO consistently elevated mean arterial blood pressure by ∼10 mmHg in all trials, indicating similar levels of metaboreflex activation. Increased ventilation during PECO was related to P_{ET,CO_2} as described by the following linear regression equation: Change in minute ventilation (l min^{-1}) = 0.85 × P_{ET,CO_2} (mmHg) + 0.80 (l min^{-1}). This finding supports our hypothesis and furthers the idea of a synergistic interaction between muscle metaboreflex activation and central chemoreflex stimulation.

KEYWORDS
hypercapnia, hyperpnoea, metaboreflex

1 | INTRODUCTION

The concept of central command, linking activation of descending motor pathways and the control of both cardiovascular and respiratory responses to exercise, is well developed (Eldridge, Millhorn, & Waldrop, 1981; Goodwin, McCloskey, & Mitchell, 1972; Green et al., 2007; Krogh & Lindhard, 1913). In addition, it is established that thin-fibre afferents exert feedback control and generate the exercise pressor reflex (Alam & Smirk, 1937; Coote, Hilton, & Perez-Gonzalez, 1971; McCloskey & Mitchell, 1972). Afferent fibres activated by the muscle force generated during contraction trigger the muscle mechanoreflex, and those activated by the associated metabolite accumulation engage the muscle metaboreflex, with some polymodal afferents responding to both types of stimulation (Kaufman & Forster, 1996). Typically, in human studies, the influence of the muscle mechanoreflex can be investigated by passive stretch of the muscle (Drew, McIntyre, Ring, & White, 2008; Gladwell & Coote, 2002) or by examination of responses evoked during the initial phases of electrically evoked muscle contraction (Bull, Davies, Lind, & White, 1989). The effect of muscle metaboreflex activation is commonly examined by trapping metabolites within previously exercised muscle using postexercise circulatory occlusion (PECO). This, in the absence of central command and muscle force generation, reveals the influence of muscle metaboreflex activation alone (Alam & Smirk, 1937; Bull et al., 1989; Fisher, Bell, & White, 2005; Rowell, Hermansen, & Blackmon, 1976).

The role of thin-fibre muscle afferent feedback in controlling human exercise hyperpnoea has been much debated. Historically, some were confident that receptors 'somewhere in the periphery, most probably the muscle' (Asmussen, 1967) played an important role (Dejours, 1967; Kao, 1963). However, others considered that afferent activation was unimportant, pointing out that in healthy humans the continued muscle metaboreflex activation after exercise has ceased does not sustain the hyperpnoea seen in exercise (Haouzi, Chenuel, & Chalon,

2001; Innes et al., 1989; Rowell et al., 1976; Wiley & Lind, 1971). In more recent experiments, feedback from the legs has been blocked by intrathecal administration of fentanyl (Amann et al., 2010). Unlike earlier experiments using local anaesthesia, this opioid agent does not affect the ability to activate the leg muscles voluntarily. The effects of reducing afferent input by this method are therefore not confounded by a compensatory increase in central command. This approach has consistently revealed a reduced ventilatory response during dynamic cycle exercise in both healthy subjects and patient groups (Amann et al., 2010; Gagnon et al., 2012; Olson, Joyner, Eisenach, Curry, & Johnson, 2014). These apparently contradictory findings may be reconciled if it is accepted that thin-fibre muscle afferent feedback does have an important influence on exercise hyperpnoea when combined with other synergistic inputs to respiratory control.

Recently, we have shown that a significant increase in ventilation occurs during either muscle mechanoreflex or muscle metaboreflex activation in a resting human muscle, where, by definition, there can be no central command, but only in conditions of concurrent systemic hypercapnia to activate the chemoreflex (Bruce & White, 2012, 2015, 2016; Lykidis, Kumar, Vianna, White, & Balanos, 2010). These experiments have been performed on different muscle groups, i.e. the forearm or the calf muscles, have used different exercise modes, i.e. sustained or rhythmic isometric contractions, and used simple inhalation of hypercapnic gas from a Douglas bag or more precise automated end-tidal clamping to elevate the end-tidal partial pressure of carbon dioxide (P_{ET,CO_2}). Although the results of these various approaches are consistent in supporting a ventilatory response to muscle afferent activation during mild hypercapnia, the hypercapnia varied between 7 and 10 mmHg above normal resting P_{ET,CO_2}, and the level of metaboreflex activation clearly would have varied between the muscle groups, exercise modes and durations of contraction that were studied. Therefore, a simple but important question remains unanswered, i.e. what is the relationship between the level of systemic hypercapnia and the magnitude of the additional hyperpnoea produced in response to a standardized level of muscle afferent activation? This relationship could have important implications for interpretation of studies in which muscle afferent feedback is blocked and there is a subsequent increase in P_{ET,CO_2} (Amann et al., 2010) or in conditions where CO_2 might be retained in disease states (Richerson & Boron, 2005). We aimed to examine the ventilatory response to an isolated and standardized high level of activation of the muscle metaboreflex in human calf muscles in the presence of a range of concurrent levels of hypercapnia, ranging from 1 to 10 mmHg above normal P_{ET,CO_2}.

In view of the limited data yet in existence, we hypothesized that a simple linear relationship exists between the level of hypercapnia and the magnitude of the additional hyperpnoea produced in response to a standardized level of muscle afferent activation.

New Findings

- **What is the central question of this study?**
  What is the relationship between the level of systemic hypercapnia and the magnitude of the additional hyperpnoea produced in response to a standardized level of muscle metaboreflex activation?
- **What is the main finding and its importance?**
  When a standardized activation of the muscle metaboreflex was combined with exposure to increasing levels of hypercapnia, the hyperpnoea this caused increased linearly. The concept of a synergistic interaction between the muscle metaboreflex and the central chemoreflex in humans is supported by this finding.

2 | METHODS

All participants received written and verbal information regarding the experimental procedures before giving informed written consent. They were habituated to the experimental procedures, which conformed to the *Declaration of Helsinki* and were approved by the School of Sport Exercise and Rehabilitation Sciences Research Ethics Sub-committee, reference no. ERN_11-588A. The study was not registered in a database. Participants refrained from consuming food and caffeine in the 4 h before and from performing strenuous physical activity or consuming alcohol in the 12 h before all trials. Fifteen young male subjects [mean (SD): 22.5 (4.1) years old; 175.5 (10.1) cm; 74.2 (8.8) kg] performed the four trials in a random order. Only two trials were performed on any 1 day, with a minimum of a 45 min rest period between them.

2.1 | General procedures

Participants were attached to ECG leads via electrodes placed in the lead II position and connected to a monitor (Cardiorator CR7; Cardiac Records Ltd, London, UK). A Finapres cuff (Portapress; Finapress Medical Systems, Amsterdam, The Netherlands) was wrapped around the middle finger of the left hand, which was placed on a support fixed at heart level, and a thigh cuff was wrapped around the thigh of the dominant (right) leg. This cuff was connected to a rapid inflator system (E20; Hokanson, Bellevue, WA, USA). In each trial, participants were seated in an isometric dynamometer designed to measure ankle plantar flexor force (Davies, Mecrow, & White, 1982). The leg was fixed with the thigh horizontal and the knee and ankle set at 85 deg. A shaped plate tightened down onto the leg, and this connected to a steel bar above, which was instrumented with strain gauges to transduce ankle plantar flexor force. Outputs from the dynamometer, ECG monitor and Finapres were sampled by an analog-to-digital converter (Cambridge Electronic Design 1401 plus; Cambridge Electronic Design, Cambridge, UK) at 1250 Hz and collected and displayed on a personal computer. The maximal voluntary contraction force of the plantar flexors was determined before each trial. This was taken as the highest value attained in five maximal efforts, each separated by 1 min of rest. Fifty per cent of this maximal value was then calculated and displayed as a target force on a computer screen in the visual field of the

TABLE 1 Values recorded during the 2 min of room air breathing, as the baseline period of each trial

Trial	Heart rate (beats min^{-1})	Mean arterial pressure (mmHg)	Minute ventilation (l min^{-1})	Tidal volume (l)	Breathing frequency (breaths min^{-1})
P_{ET,CO_2} +1 mmHg	81 ± 20.1	96.4 ± 18.6	19.7 ± 6.2	1.20 ± 0.34	17.5 ± 5.8
P_{ET,CO_2} +3 mmHg	88.4 ± 17.8	97 ± 13.2	18.1 ± 4.6	1.23 ± 0.50	16.5 ± 6.2
P_{ET,CO_2} +7 mmHg	88.9 ± 20.5	95.4 ± 30.6	19.6 ± 5.8	1.34 ± 0.58	15.7 ± 3.9
P_{ET,CO_2} +10 mmHg	84.2 ± 19.0	93.4 ± 10.8	19.2 ± 8.1	1.24 ± 0.50	16.2 ± 5.0

Values are given as means ± SD, n = 15. There were no significant differences between trials. Abbreviation: P_{ET,CO_2}, end-tidal partial pressure of carbon dioxide.

subject for the subsequent exercise periods. Participants were then connected to the mouthpiece of the dynamic end-tidal forcing (DEF) system and allowed to relax and establish a baseline cardiovascular and respiratory state. The DEF system has been described in detail elsewhere (Robbins, Swanson, & Howson, 1982) and was used to manipulate the partial pressures of inspired O_2 and CO_2 on a breath-by-breath basis, to induce the required level of hypercapnia whilst maintaining the end-tidal partial pressure of oxygen (P_{ET,O_2}) at the normal value. Calibrated O_2 and CO_2 analysers (MOXAR Respiratory System; AEI Technologies, Pittsburgh, PA, USA) sampled via a side-arm from the mouthpiece, which was connected in series to a bidirectional turbine (Cardiokinetics Ltd, Lancashire, UK) for measurement of respiratory air flow. Throughout the experiment, deviations of the measure values for P_{ET,O_2} and P_{ET,CO_2} from the desired values were used by the controlling computer software (Breathem; University of Oxford, UK) to adjust the composition of inspired air supplied through a fast gas mixing (MKS Instruments 1559A; Munich, Germany) and humidifying system (Fisher and Paykel Healthcare HC150; Fisher and Paykel Healthcare, Auckland, New Zealand).

2.2 | Experimental protocol

All trials began with a 2 min control period, during which participants breathed room air. This was followed by 15 min of exposure to one of the four levels of hypercapnia. These were +1, +3, +7 and +10 mmHg above the individual's baseline P_{ET,CO_2}. During the phases of hypercapnia, 5 min further rest was followed by 2 min of sustained isometric calf muscle contraction at 50% of the previously determined maximal voluntary strength. Immediately before cessation of exercise, the thigh cuff was inflated rapidly to 200 mmHg. This remained inflated for a further 3 min before deflation and the final 5 min of exposure to hypercapnia. Minute ventilation ($\dot{V}_E$), respiratory frequency (f) and tidal volume (V_T) were continuously recorded throughout the protocol using the DEF system, which clamped P_{ET,CO_2} at the chosen value. Heart rate (HR) and blood pressure (mean arterial pressure; MAP) were continuously recorded throughout the protocol.

Throughout all trials, mean average values for $\dot{V}_E$, f, V_T, P_{ET,CO_2}, HR and MAP were calculated during each minute. The cardiovascular and ventilatory responses to each phase of the trial and between the different levels of hypercapnia were compared using ANOVA with repeated measures and, where appropriate, Tukey's *post hoc* HSD comparisons of the differences between the four levels of hypercapnia. For examination of the relationship between increased ventilation during PECO and P_{ET,CO_2}, a linear mixed model with random slopes by subject was used.

Data in the table and text are expressed as means ± SD. For the figures, where effects are described, i.e. changes from baseline, the SEM is used. Statistical significance was taken as $P < 0.05$. Statistical analysis was conducted using standard statistical packages (SPSS version 22.0 and SYSTAT).

3 | RESULTS

The 50% maximal voluntary contraction values used during exercise were not significantly different between the four trials (means ± SD: +1 mmHg, 662.9 ± 161.1 N; +3 mmHg, 660 ± 175.9 N; +7 mmHg, 671 ± 175.9 N; and +10 mmHg, 654.7 ± 167.7 N; $P > 0.05$).

The baseline cardiovascular and respiratory variables measured at rest during the control period before each of the four trials are shown in Table 1. There were no significant differences between the trials for resting $\dot{V}_E$, f, V_T, MAP and HR. During the 2 min control baseline period, the mean P_{ET,CO_2} was 38.6 ± 1.1 mmHg and did not differ between trials.

3.1 | Heart rate

Mean heart rate did not change significantly from baseline levels during rest with hypercapnia at any level. During exercise, heart rate increased in all trials by 12–15 beats min^{-1}, but there were no significant differences between trials (Figure 1). Heart rate fell back to baseline within the first minute of PECO in the +1, +3 and +7 mmHg trials, but remained slightly elevated above baseline (~5 beats min^{-1}) in the +10 mmHg conditions. On deflation of the thigh cuff, there was a transient elevation in heart rate in all trials before a return to hypercapnic baseline values.

3.2 | Blood pressure

Figure 2 shows that throughout the hypercapnic rest period the mean arterial pressure gradually increased from control baseline values, and this increase reached significance by the fifth minute in all trials ($F_{4,70} = 9.59$, $P = 0.000$). The increase at this time point was significantly greater in the +10 mmHg trials than in the +1 and +3 mmHg trials ($F_{3,56} = 5.41$, $P = 0.002$), but there were no other significant differences between trials. During isometric exercise, blood pressure increased progressively and was significantly above

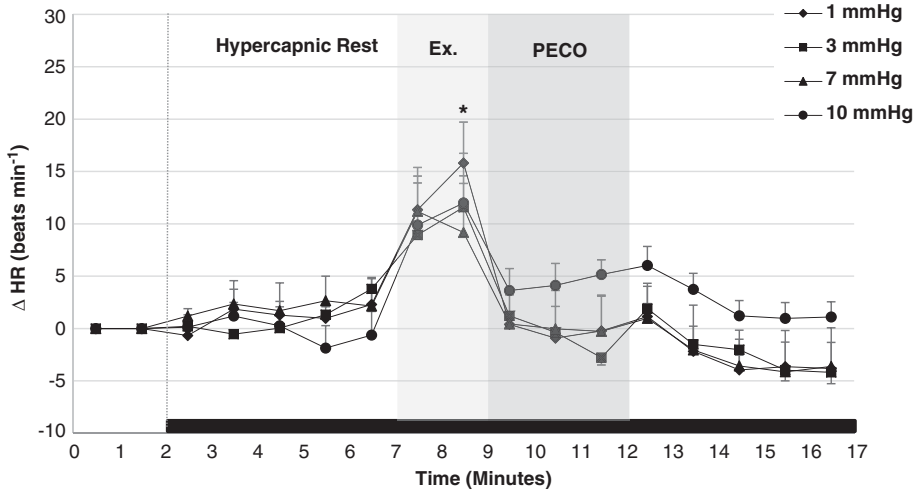

FIGURE 1 Mean ± SEM changes in heart rate (HR) from baseline during each phase of the +1, +3, +7 and +10 mmHg trials. The light shaded area indicates the exercise (Ex.) phase, and the dark shaded area indicates the postexercise circulatory occlusion (PECO) phase. Black bar indicates hypercapnia. *All conditions are significantly different from baseline values at this time point ($P < 0.05$)

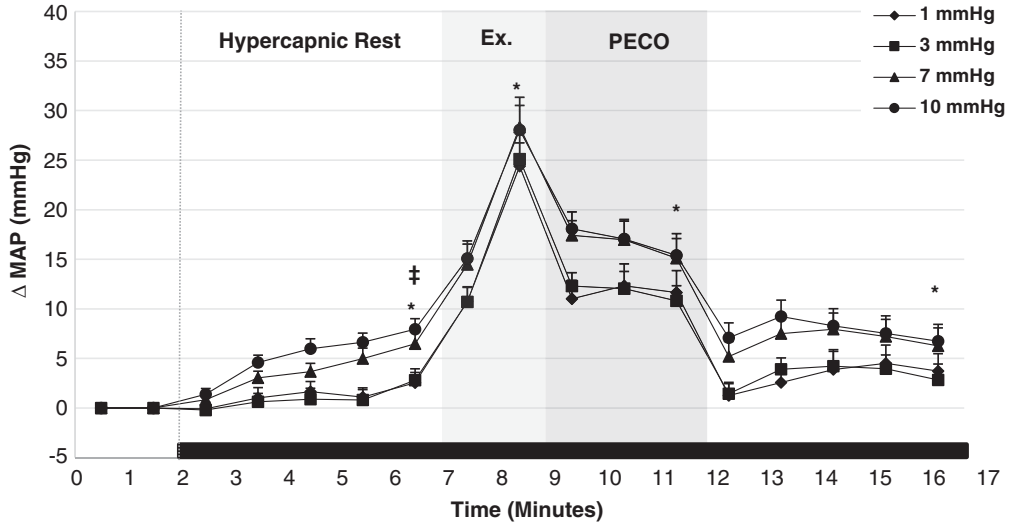

FIGURE 2 Mean ± SEM change in mean arterial pressure (MAP) from baseline during each phase of the +1, +3, +7 and +10 mmHg trials. The light shaded area indicates the exercise (Ex.) phase, and the dark shaded area indicates the postexercise circulatory occlusion (PECO) phase. Black bar indicates hypercapnia. *All conditions are significantly different from baseline values ($P < 0.05$) at this time point, and ‡the MAP value in the +10 mmHg trial is significantly higher than MAP values in +1 and +3 mmHg trials ($P < 0.05$)

the hypercapnic baseline phase in all trials. There were no significant differences between end-exercise values in the four trials. As expected, the increases during exercise were partly sustained by PECO in all trials, with MAP remaining significantly elevated above baseline ($F_{4,70} = 12.29$, $P = 0.000$). On release of the thigh cuff, MAP decreased to the hypercapnic baseline values seen in all trials.

When changes in MAP were normalized to the mean MAP values seen in the last 2 min of rest during the baseline hypercapnia phase, all differences between the trials disappeared (Figure 3).

3.3 | Ventilation

From the stable resting values given in Table 1, $\dot{V}_E$ increased significantly in all trials by the fifth minute of the baseline period of hypercapnic gas inhalation ($F_{4,70} = 53.05$, $P = 0.000$). This was by virtue of significant increases in both V_T (0.26 ± 0.12, 0.59 ± 0.12, 1.15 ± 0.15 and 1.58 ± 0.13 l; $F_{4,70} = 31.00$, $P = 0.000$) and f (0.9 ± 0.8, 2.7 ± 1.0, 5.3 ± 0.8 and 5.6 ± 0.7 breaths min^{-1}; $F_{4,70} = 12.01$, $P = 0.000$) in the +1, +3, +7 and +10 mmHg conditions, respectively.

Changes in $\dot{V}_E$ normalized to the respective resting values are shown for the four trials in Figure 4. In all trials, $\dot{V}_E$ was significantly greater than the resting values after 5 min of exposure to hypercapnia ($P < 0.05$). The responses to +7 and +10 mmHg above baseline P_{ET,CO_2} at rest were significantly higher than those to +1 and +3 mmHg ($F_{3,56} = 34.60$, $P = 0.000$). There were no other significant differences between trials.

During exercise, $\dot{V}_E$ increased further still in all trials before going on to reach a peak value in the first minute of PECO. It then fell back during the next 2 min to a level that was still significantly above the hypercapnic baseline in all trials ($F_{4,70} = 11.00$, $P = 0.000$).

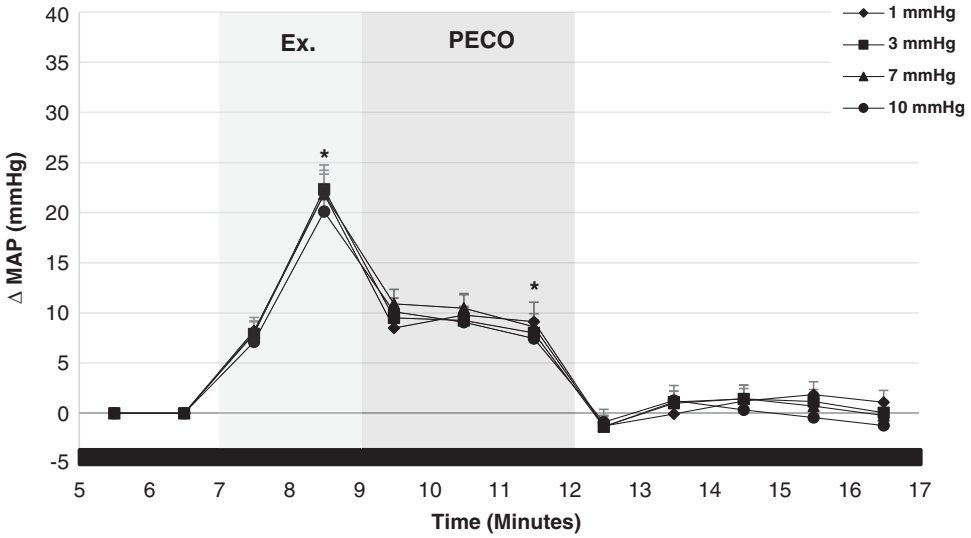

FIGURE 3 Mean ± SEM change in mean arterial pressure (MAP) from hypercapnic (baseline) rest period during each phase of the +1, +3, +7 and +10 mmHg trials. The light shaded area indicates the exercise (Ex.) phase, and the dark shaded area indicates the postexercise circulatory occlusion (PECO) phase. Black bar indicates hypercapnia. * All conditions are significantly different at this time point from hypercapnic (baseline) rest period values ($P < 0.05$)

The same pattern of statistical significance as was found at the end of the rest phases was found when examining the differences between trials during the last minute of both the exercise and the PECO phases. On release of the occluding thigh cuff, there was a small transient increase in $\dot{V}_E$ followed by a gradual decline towards baseline.

Tidal volume was not significantly altered during exercise and PECO phases from hypercapnic baseline values in any trials. Breathing frequency increased significantly ($P < 0.05$) by ~4 breaths min^{-1} during exercise in all four trials and was significantly elevated by ~2–3 breaths min^{-1} ($P < 0.05$) during PECO in the +7 and +10 mmHg trials.

It is clear that in all trials $\dot{V}_E$ did not recover to pre-exercise hypercapnic baseline values by the end of the recovery period (minute 17 of the protocol). It appears that $\dot{V}_E$ had not fully adjusted to the elevated P_{ET,CO_2} before exercise commencement and, as a result, there was a small baseline drift during the exercise, PECO and recovery phases in all trials. To correct for this, linear regression lines were constructed, for each subject in each trial, using $\dot{V}_E$ values at minutes 6, 7, 16 and 17 of the protocol (the final 2 min of the hypercapnia baseline and recovery periods). From the slope and intercept of these regression lines, $\dot{V}_E$ values in each trial and for each subject were adjusted from minutes 6 to 17. These corrected data are shown in Figure 5, which illustrates that despite the exercise intensity being fixed, $\dot{V}_E$ increased

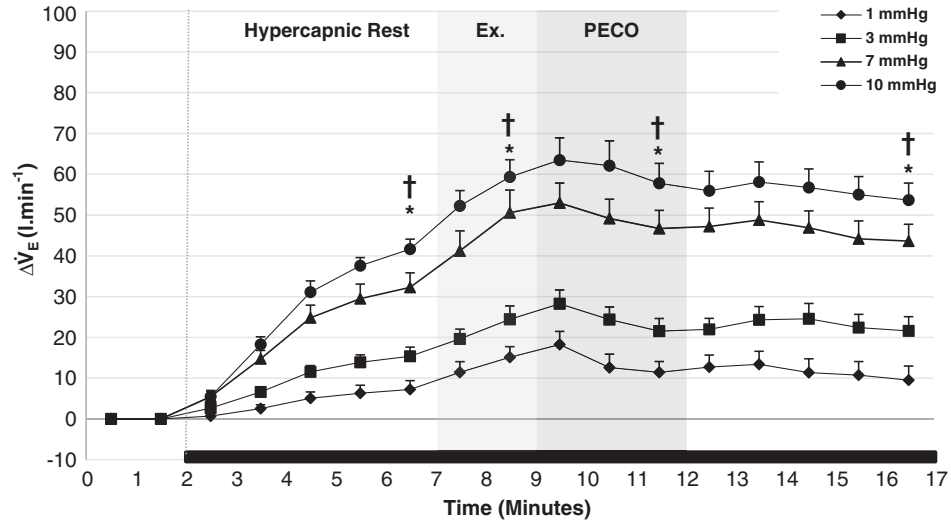

FIGURE 4 Mean ± SEM change in minute ventilation ($\dot{V}_E$) from baseline period during each phase of the +1, +3, +7 and +10 mmHg trials. The light shaded area indicates the exercise (Ex.) phase, and the dark shaded area indicates the postexercise circulatory occlusion (PECO) phase. Black bar indicates hypercapnia. * All conditions are significantly different from baseline values at this time point ($P < 0.05$). † The $\dot{V}_E$ values in both +7 and +10 mmHg trials are significantly higher than the $\dot{V}_E$ values in the +1 and +3 mmHg trials at this time point ($P < 0.05$)

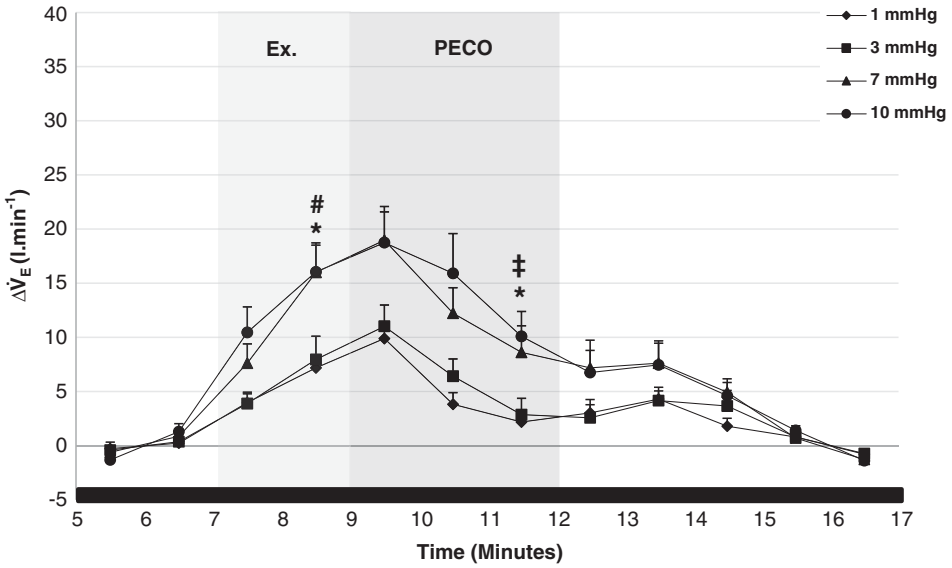

FIGURE 5 Mean ± SEM change in minute ventilation ($\dot{V}_E$) during each phase of the +1, +3, +7 and +10 mmHg trials corrected for baseline shift from minutes 6, 7, 16 and 17. The light shaded area indicates the exercise (Ex.) phase, and the dark shaded area indicates the postexercise circulatory occlusion (PECO) phase. Black bar indicates hypercapnia. *All conditions are significantly different from baseline values ($P < 0.05$). #The $\dot{V}_E$ values in both +7 and +10 mmHg trials are significantly higher than the +1 mmHg $\dot{V}_E$ value at this time point ($P < 0.05$). ‡The $\dot{V}_E$ value in the +10 mmHg trial is significantly higher than $\dot{V}_E$ values in the +1 and +3 mmHg trials at this time point ($P < 0.05$)

more during exercise with exposure to greater levels of hypercapnia. The $\dot{V}_E$ values during the last minute of the +7 and + 10 mmHg trials were significantly higher than those in the +1 mmHg trials ($F_{3,56} = 4.94$, $P = 0.004$). During PECO, ventilation fell back from its peak values in all trials but to a level that remained significantly above baseline values until removal of the thigh cuff. Again, the sustained increases in $\dot{V}_E$ during PECO were progressively greater with higher levels of hypercapnia, with the +10 mmHg values being significantly higher than the +1 and +3 mmHg values ($F_{3,56} = 4.54$, $P = 0.006$). Using the corrected $\dot{V}_E$ values shown in Figure 5, the change in ventilation during the last minute of PECO from the preceding hypercapnic baseline was calculated for each subject, in each of the four trials, as shown in Figure 6. A linear mixed model, with random slopes by subject, then revealed an effect of the increase in P_{ET,CO_2} from the control baseline level on the change in ventilation ($F_{1,57} = 9.12$, $P = 0.004$), described by the equation: $\Delta\dot{V}_E$ (l min^{-1}) = 0.85 × P_{ET,CO_2} (mmHg) + 0.80 (SEM = 0.281) (l min^{-1}). This regression line is also illustrated in Figure 6.

A follow-up ANOVA indicated that the linear component explained 94.3% of the variance over the four levels of hypercapnia, whereas the non-significant cubic, which would indicate a sigmoid function, explained only 5.2% of the variance.

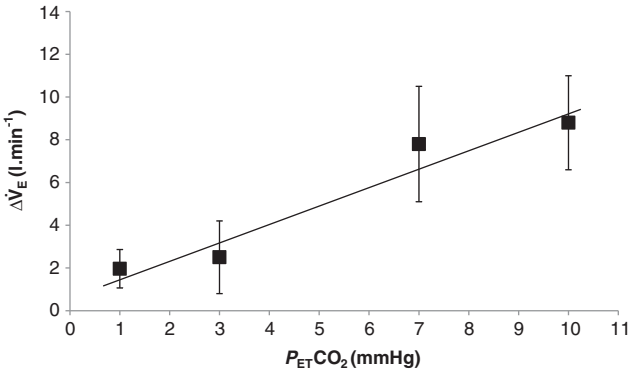

FIGURE 6 Mean ± SEM change in minute ventilation ($\dot{V}_E$) from the hypercapnic baseline level during the final minute of postexercise circulatory occlusion (PECO), when end-tidal partial pressure of carbon dioxide (P_{ET,CO_2}) is increased from control baseline levels by 1, 3, 7 and 10 mmHg. The superimposed line is described by the following equation: $\Delta\dot{V}_E$ (l min^{-1}) = 0.85 × P_{ET,CO_2} (mmHg) + 0.80 (l min^{-1})

4 | DISCUSSION

We have found that the performance of a standardized isometric exercise protocol followed by a period of PECO, at four different levels of hypercapnia, results in a consistent cardiovascular response that is independent of the immediate baseline shifts caused by the hypercapnia (Figure 4). The blood pressure response to a period of PECO is well accepted as the prime indicator of the level of activation of the muscle metaboreflex, which drives this pressor reflex (PR) (Alam & Smirk, 1937; Smith, Mitchell, & Garry, 2006).

Thus, in the present experiments this PR gives a clear indication of a consistent and equal activation of the muscle metaboreflex in all trials. During PECO combined with four different levels of hypercapnia, ventilation is elevated above levels seen with the same levels of hypercapnia alone, and the magnitude of the increase in ventilation during PECO progressively increases with the level of hypercapnia (Figure 6). Given that metaboreflex activation appears to be consistent across the four conditions, the progressive increase in the ventilatory response to PECO with increasing levels of hypercapnia supports the

idea of a synergistic interaction between the muscle metaboreflex and the chemoreflex. Furthermore, it suggests a sensitization of this interaction with increasing levels of hypercapnia.

The pressor response to isometric exercise is independent of the resting baseline blood pressure (Lind, 1983) and can be sustained until high levels of concurrent dynamic exercise (Lind & McNicol, 1967). This robust effect is also unaffected by concurrent exposure to other potent activators of the autonomic nervous system, such as the cold pressor test (Peikert & Smolander, 1991; Vianna, Sales, & Da Nóbrega, 2012). It is therefore perhaps not surprising that hypercapnia, a powerful activator of the sympathetic nervous system (Kara, Narkiewicz, & Somers, 2003; Somers, Mark, Zavala, & Abboud, 1989), which causes baseline blood pressure elevation (Figure 3), does not affect the pressor response to a standardized bout of isometric exercise and period of PECO (Figure 4). We are confident that this indicates that the level of muscle metaboreflex activation during PECO is consistent, there being no central command or muscle mechanoreflex activation at this time. The pressor responses we observed are consistent with previous values for this level of isometric exercise of the human calf muscles that have been reported by researchers in our laboratory (Bell & White, 2005). Resting heart rate does not change significantly during exposure to the low-to-moderate levels of hypercapnia used in the present study. Isometric exercise caused HR to increase by 10–15 beats min^{-1} in all trials, but unlike blood pressure there is considerable intrasubject variability in the response, and this increase from baseline reached statistical significance only in the +10 mmHg trial. Furthermore, in this trial, in contrast to the other three trials, HR did decrease during PECO, but it remained elevated above baseline until release of the thigh cuff, whereas in the other trials it decreased promptly to baseline or below during PECO and recovery. This is consistent with the idea that cardiac sympathoexcitation, owing to activation of the muscle metaboreflex, combined with sympathoexcitation, caused by this highest level of hypercapnia, provided sufficient opposition to the returning vagal tone, which occurs when the muscle mechanoreflex and central command inhibition of the vagus are removed at the end of exercise (Brack, Coote, & Ng, 2004; Fisher et al., 2010; Gladwell & Coote, 2002; Gladwell et al., 2005), to elevate HR slightly in comparison to the other trials.

It is possible that exposure to increasing levels of systemic hypercapnia could make the active muscle progressively more fatiguable owing to acidosis and also increase concentrations of metabolites produced during exercise, which then activate the exercise pressor reflex. However, the consistent pressor responses seen during PECO argue against this. In addition, in isolated mammalian muscle, even severe hypercapnia-induced acidosis, applied during intense sustained isometric exercise, has very small effects on muscle contractile characteristics. Indeed, at normal exercising muscle temperatures its effects are likely to be non-existent (Westerblad, Bruton, & Lännergren, 1997). Finally, our subjects were able to sustain the required force levels in all trials, albeit with considerable effort on each occasion.

Previous work from this laboratory showed that the augmented ventilatory response to hypercapnia was not dependent on the muscle being exposed to it; indeed, local occlusion of the circulation to the muscle before exposure of the systemic circuit to hypercapnia did not prevent the increase in ventilation seen during muscle metaboreflex activation by PECO. Importantly, brief exposure to systemic hyperoxic (95% O_2) hypercapnia (5% CO_2), intended (as a form of modified DeJours test) to suppress carotid chemoreceptor activity, had no effect on the ventilatory response, suggesting little involvement of the peripheral (carotid) chemoreflex in these conditions and a predominance of central chemoreflex activation. In contrast, exposure of the muscle to systemic hypercapnia followed by local circulatory occlusion and then a return to breathing room air (i.e. systemic normocapnia) did abolish the ventilatory response during PECO, supporting a major role for central chemoreception in helping to generate the response (Bruce & White, 2015). Edgell and Stickland (2014) report that hypoxic activation of the carotid chemoreflex combined with PECO does increase ventilation but that this increase is not greater than the sum of responses to hypoxia and PECO individually. They argue that this indicates that the metaboreflex does not sensitize the carotid chemoreflex. Thus, taken together with the MAP response data discussed earlier and our previously published experimental data it seems that in the present experiments the muscle metaboreflex is equally active during exercise and PECO in all four trials. If this drive from the muscle during PECO is constant, then the progressively larger ventilatory responses seen with increasing levels of hypercapnia must be attributable to an increasing central chemoreflex sensitivity to this input.

Based on the recommended duration of hypercapnic exposure data in the literature (Mateika & Sandhu, 2011), we expected that ventilation would have reached a new steady state after 5 min of exposure to all levels of hypercapnia. However, it is clear from examination of Figure 4 that ventilatory responses to all four levels of hypercapnia had not reached a true steady state before the start of exercise and that during the recovery phases at the end of PECO, ventilation was stable but at a slightly higher level than that seen at the end of the new baseline period. This underlying linear baseline drift during the exercise and PECO phases of the present experiments was a surprise but was confirmed by additional experimentation on a subgroup of five subjects, in whom exposure to hypercapnia for the same length of time took place, without exercise and PECO. In addition, this baseline drift was unaffected by the interpolation of the exercise bout alone at the appropriate time point. After the expected increase in ventilation attributable to the exercise, ventilation fell back to follow the drifting baseline. A period of leg circulatory occlusion alone at the appropriate time point, to mimic the effect of the PECO period but without the preceding exercise period, had a negligible effect on ventilation and was also ineffectual in altering the baseline drift. These observations are additionally useful in that they argue against an order effect of exercise *per se* and support a role for the muscle metaboreflex in causing the increased ventilation we report during PECO. Our correction of the ventilatory data for the baseline drift was made in the light of these experiments (Alghaith, 2018). The changes in ventilation from these corrected baselines, during standardized activation of the muscle metaboreflex, show a linear increase with increasing P_{ET,CO_2} (Figure 6). This finding could have important implications for

interpretation of studies where the role of the muscle metaboreflex is investigated and where arterial P_{CO_2} may be elevated as a result of the experimental intervention (Amann et al., 2010) or disease (chronic obstructive pulmonary disease; Bruce, Turner, & White, 2016). For example, Amann et al. (2010) found that ventilation during cycling exercise was reduced when muscle afferent feedback from the legs was blocked by intrathecal fentanyl infusion. Rightly, they argued that as P_{ET,CO_2} became elevated during exercise because ventilation was reduced during the block, the decrease in ventilation would, in fact, be even greater in the absence of the hypercapnia. They then 'corrected' their exercise ventilatory data for this stimulatory effect of hypercapnia based on CO_2 sensitivity data gathered on their resting subjects. The outcome of this was an estimate that muscle afferent activity during cycling exercise could account for as much as 50% of the ventilatory response. Our present data suggest that even this might be an underestimate. In conditions of muscle metaboreflex activation combined with hypercapnia, the ventilatory response is bigger than with hypercapnia alone. This sensitization effect would apply to metaboreflex feedback from any muscles that are active and, in the study by Amann et al. (2010), still able to feed back during cycling, e.g. forearm, respiratory and stabilizing torso musculature. Further work would be required to quantify exactly the magnitude of the effect of this interaction. It is of note that the decline in ventilation during block was related to a reduction in *f* in the study by Amann et al. (2010), which at first glance might seem at odds with our observation of an increase in *f* during PECO with the two higher levels of hypercapnia. However, it might be that these observations combined indicate a role for the muscle metaboreflex in the control of breathing frequency, a reduction in feedback by block lowering *f* and excitation of the metaboreflex by PECO under hypercapnia increasing it.

In conclusion, we show a linearly increasing ventilatory response to a standardized level of muscle metaboreflex stimulation when this reflex is activated in combination with increasing levels of systemic hypercapnia. This provides further support for the idea of a synergistic interaction between the central chemoreflex and the muscle metaboreflex.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

The experiments were performed in the School of Sport, Exercise and Rehabilitation, University of Birmingham. M.J.W., G.M.B. and J.M.A. contributed to the conception and design of the work. All authors contributed to the acquisition, analysis or interpretation of data for the work and to the drafting of the work and revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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REFERENCES

Alam, M., & Smirk, F. (1937). Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *The Journal of Physiology*, *89*, 372–383.

Alghaith, J. M. (2018). The role of thin fibre muscle afferent feedback, and its modification by exercise training, in human ventilatory control. Unpublished PhD thesis, University of Birmingham.

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2010). Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *Journal of Applied Physiology*, *109*, 966–976.

Asmussen, E. (1967). Exercise and regulation of ventilation. *Circulation Research*, *20*(Suppl.), I132–I145.

Bell, M. P., & White, M. J. (2005). Cardiovascular responses to external compression of human calf muscle vary during graded metaboreflex stimulation. *Experimental Physiology*, *90*, 383–391.

Brack, K. E., Coote, J. H., & Ng, G. A. (2004). Interaction between direct sympathetic and vagus nerve stimulation on heart rate in the isolated rabbit heart. *Experimental Physiology*, *89*, 128–139.

Bruce, R. M., Turner, A., & White, M. J. (2016). Ventilatory responses to muscle metaboreflex activation in chronic obstructive pulmonary disease. *The Journal of Physiology*, *594*, 6025–6035.

Bruce, R. M., & White, M. J. (2012). Muscle afferent activation causes ventilatory and cardiovascular responses during concurrent hypercapnia in humans. *Experimental Physiology*, *97*, 208–218.

Bruce, R. M., & White, M. J. (2015). The ventilatory response to muscle afferent activation during concurrent hypercapnia in humans: Central and peripheral mechanisms. *Experimental Physiology*, *100*, 896–904.

Bull, R., Davies, C., Lind, A., & White, M. (1989). The human pressor response during and following voluntary and evoked isometric contraction with occluded local blood supply. *The Journal of Physiology*, *411*, 63–70.

Coote, J., Hilton, S., & Perez-Gonzalez, J. (1971). The reflex nature of the pressor response to muscular exercise. *The Journal of Physiology*, *215*, 789–804.

Davies, C., Mecrow, I., & White, M. (1982). Contractile properties of the human triceps surae with some observations on the effects of temperature and exercise. *European Journal of Applied Physiology*, *49*, 255–269.

Dejours, P. (1967). Neurogenic factors in control of ventilation during exercise. *Circulation Research*, *20*(Suppl), I146–I153.

Drew, R. C., McIntyre, D. B., Ring, C., & White, M. J. (2008). Local metabolite accumulation augments passive muscle stretch-induced modulation of carotid–cardiac but not carotid–vasomotor baroreflex sensitivity in man. *Experimental Physiology*, *93*, 1044–1057.

Edgell, H., & Stickland, M. K. (2014). Activation of the carotid chemoreflex secondary to muscle metaboreflex stimulation in men. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *306*, R693–R700.

Eldridge, F. L., Millhorn, D. E., & Waldrop, T. G. (1981). Exercise hyperpnea and locomotion: Parallel activation from the hypothalamus. *Science*, *211*, 844–846.

Fisher, J. P., Bell, M. P., & White, M. J. (2005). Cardiovascular responses to human calf muscle stretch during varying levels of muscle metaboreflex activation. *Experimental Physiology*, *90*, 773–781.

Fisher, J. P., Seifert, T., Hartwich, D., Young, C. N., Secher, N. H., & Fadel, P. J. (2010). Autonomic control of heart rate by metabolically sensitive

skeletal muscle afferents in humans. *The Journal of Physiology*, *588*, 1117–1127.

Gagnon, P., Bussieres, J. S., Ribeiro, F., Gagnon, S. L., Saey, D., Gagne, N., … Maltais, F. O. (2012). Influences of spinal anesthesia on exercise tolerance in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *186*, 606–615.

Gladwell, V., Fletcher, J., Patel, N., Elvidge, L., Lloyd, D., Chowdhary, S., & Coote, J. H. (2005). The influence of small fibre muscle mechanoreceptors on the cardiac vagus in humans. *The Journal of Physiology*, *567*, 713–721.

Gladwell, V. F., & Coote, J. H. (2002). Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: A role for mechanoreceptors. *The Journal of Physiology*, *540*, 1095–1102.

Goodwin, G., McCloskey, D., & Mitchell, J. (1972). Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *The Journal of Physiology*, *226*, 173–190.

Green, A. L., Wang, S., Purvis, S., Owen, S. L., Bain, P. G., Stein, J. F., … Paterson, D. J. (2007). Identifying cardiorespiratory neurocircuitry involved in central command during exercise in humans. *The Journal of Physiology*, *578*, 605–612.

Haouzi, P., Chenuel, B., & Chalon, B. (2001). Control of breathing and muscle perfusion in humans. *Experimental Physiology*, *86*, 759–768.

Innes, J., Solarte, I., Huszczuk, A., Yeh, E., Whipp, B., & Wasserman, K. (1989). Respiration during recovery from exercise: Effects of trapping and release of femoral blood flow. *Journal of Applied Physiology*, *67*, 2608–2613.

Kao, F. (1963). An experimental study of the pathways involved in exercise hyperpnea employing cross-circulation techniques. In D. J. C. Cunningham & B. B. Lloyd (Eds.), *The regulation of human respiration* (pp. 461–502). Oxford: Blackwell.

Kara, T., Narkiewicz, K., & Somers, V. (2003). Chemoreflexes – physiology and clinical implications. *Acta Physiologica Scandinavica*, *177*, 377–384.

Kaufman, M. P., & Forster, H. V. (1996). Reflexes controlling circulatory, ventilatory and airway responses to exercise. In L. Rowell & J. Shepherd (Eds), *Handbook of Physiology, section 12, Exercise: Regulation and Integration of multiple Systems* (pp. 381–447). Chapter 10. Oxford, UK: Oxford University Press.

Krogh, A., & Lindhard, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *The Journal of Physiology*, *47*, 112–136.

Lind, A., & McNicol, G. (1967). Circulatory responses to sustained hand-grip contractions performed during other exercise, both rhythmic and static. *The Journal of Physiology*, *192*, 595–607.

Lind, A. R. (1983). Cardiovascular adjustments to isometric contractions: static effort. In J. T. Shepherd & F. M. Abboud (Eds), *Handbook of Physiology, section 2, The Cardiovascular System. Peripheral Circulation and Organ Blood Flow* (pp. 947–966). Chapter 26. Bethesda, MD: Am. Physiol. Soc.

Lykidis, C. K., Kumar, P., Vianna, L. C., White, M. J., & Balanos, G. M. (2010). A respiratory response to the activation of the muscle metaboreflex during concurrent hypercapnia in man. *Experimental Physiology*, *95*, 194–201.

Mateika, J. H., & Sandhu, K. S. (2011). Experimental protocols and preparations to study respiratory long term facilitation. *Respiratory Physiology & Neurobiology*, *176*, 1–11.

McCloskey, D., & Mitchell, J. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *The Journal of Physiology*, *224*, 173–186.

Olson, T. P., Joyner, M. J., Eisenach, J. H., Curry, T. B., & Johnson, B. D. (2014). Influence of locomotor muscle afferent inhibition on the ventilatory response to exercise in heart failure. *Experimental Physiology*, *99*, 414–426.

Peikert, D., & Smolander, J. (1991). The combined effect of the cold pressor test and isometric exercise on heart rate and blood pressure. *European Journal of Applied Physiology*, *62*, 445–449.

Robbins, P. A., Swanson, G. D., & Howson, M. G. (1982). A prediction-correction scheme for forcing alveolar gases along certain time courses. *Journal of Applied Physiology*, *52*, 1353–1357.

Rowell, L. B., Hermansen, L., & Blackmon, J. R. (1976). Human cardiovascular and respiratory responses to graded muscle ischemia. *Journal of Applied Physiology*, *41*, 693–701.

Richerson, G. B., & Boron, W. F. (2005). Control of ventilation. In W. F. Boron & E. L. Boulpaep (Eds.), *Medical physiology* (2nd ed., pp. 712–734). Chapter 31. Philadelphia, PA, USA: Elsevier Saunders.

Smith, S. A., Mitchell, J. H., & Garry, M. G. (2006). The mammalian exercise pressor reflex in health and disease. *Experimental Physiology*, *91*, 89–102.

Somers, V. K., Mark, A. L., Zavala, D. C., & Abboud, F. M. (1989). Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *Journal of Applied Physiology*, *67*, 2101–2106.

Vianna, L. C., Sales, A. R. K., & Da Nóbrega, A. C. L. (2012). Cerebrovascular responses to cold pressor test during static exercise in humans. *Clinical Physiology and Functional Imaging*, *32*, 59–64.

Westerblad, H., Bruton, J., & Lännergren, J. (1997). The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *The Journal of Physiology*, *500*, 193–204.

Wiley, R., & Lind, A. (1971). Respiratory responses to sustained static muscular contractions in humans. *Clinical Science*, *40*, 221–234.

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