The Journal of Physiology

Control of hyperpnoea and pulmonary gas exchange during prolonged exercise: The role of group III/IV muscle afferent feedback

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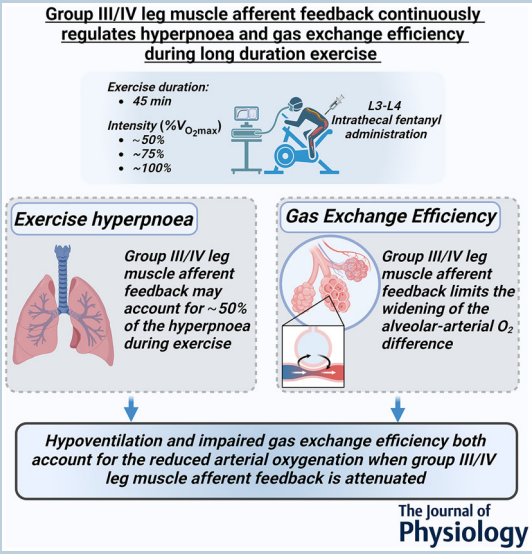
Handling Editors: Karyn Hamilton & Philip Ainslie

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP286993#support-information-section).



Abstract figure legend Group III/IV leg muscle afferent feedback has previously been recognized as an important determinant of the ventilatory response to locomotor exercise lasting <5 min. Here we demonstrate that the activity of these neurons is crucial in regulating hyperpnoea and pulmonary gas efficiency during exercise of longer duration (i.e. 45 min). Specifically, when exercise was performed with pharmacologically attenuated feedback from group III/IV leg muscle afferents (via lumbar intrathecal fentanyl), pulmonary ventilation was significantly lower and the widening of the alveolar–arterial O_2 difference substantially larger compared to the control condition performed with an intact sensory feedback system. Importantly, these differences occurred early during exercise evoking ~50%, ~75% and ~100% of $\dot{V}_{O_2max}$, and persisted until the end of each of the three bouts (20 min, 20 min and 5 min, respectively). These findings reflect the time-independent significance of feedback from group III/IV muscle afferents for regulating exercise hyperpnoea and gas exchange efficiency, and thus for optimizing arterial oxygenation and acid–base balance, during long duration human locomotion. Created with Biorender.com.

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DOI: 10.1113/JP286993

Abstract It remains unclear whether feedback from group III/IV muscle afferents is of continuous significance for regulating the pulmonary response during prolonged (>5 min), steady-state exercise. To elucidate the influence of these sensory neurons on hyperpnoea, gas exchange efficiency, arterial oxygenation and acid–base balance during prolonged locomotor exercise, 13 healthy participants (4 females; 21 (3) years, $\dot{V}_{O_2max}$: 46 (8) ml/kg/min) performed consecutive constant-load cycling bouts at ∼50% (20 min), ∼75% (20 min) and ∼100% (5 min) of $\dot{V}_{O_2max}$ with intact (CTRL) and pharmacologically attenuated (lumbar intrathecal fentanyl; FENT) group III/IV muscle afferent feedback from the legs. Pulmonary responses were continuously recorded and arterial blood (radial catheter) periodically collected throughout the exercise. Pulmonary gas exchange efficiency was evaluated using the alveolar-arterial P_{O_2} difference ($A - aD_{O_2}$). There were no differences in any of the variables of interest between conditions before the start of the exercise. Pulmonary ventilation was up to 20% lower across all intensities during FENT compared to CTRL exercise ($P < 0.001$) and this hypoventilation was accompanied by an up to 10% lower arterial P_{O_2} and a 2–4 mmHg higher P_{CO_2} (both $P < 0.001$). The exercise-induced widening of $A - aD_{O_2}$ was up to 25% larger during FENT compared to CTRL ($P < 0.001$). Importantly, the differences developed within the first minute of each stage and persisted, or further increased, throughout the remainder of each bout. These findings reflect a critical and time-independent significance of feedback from group III/IV leg muscle afferents for continuously regulating the ventilatory response, gas exchange efficiency, arterial oxygenation and acid–base balance during human locomotion.

(Received 26 May 2024; accepted after revision 5 September 2024; first published online 23 September 2024)
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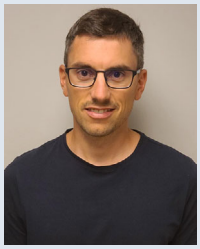
Key points

- Feedback from group III/IV leg muscle afferents reflexly contributes to hyperpnoea during short duration (i.e. <5 min) locomotor exercise.
- Whether continuous feedback from these sensory neurons is obligatory to ensure adequate pulmonary responses during steady-state exercise of longer duration remains unknown.
- Lumbar intrathecal fentanyl was used to attenuate the central projection of group III/IV leg muscle afferents during prolonged locomotor exercise (i.e. 45 min) at intensities ranging from 50% to 100% of $\dot{V}_{O_2max}$.
- Without affecting the metabolic rate, afferent blockade compromised pulmonary ventilation and gas exchange efficiency, consistently impairing arterial oxygenation and facilitating respiratory acidosis throughout exercise.
- These findings reflect the time-independent significance of feedback from group III/IV muscle afferents for regulating exercise hyperpnoea and gas exchange efficiency, and thus for optimizing arterial oxygenation and acid–base balance, during prolonged human locomotion.

Danilo Iannetta obtained his PhD in Human and Exercise Physiology at the University of Calgary (Canada). He then completed a Postdoctoral Fellowship at the University of Utah Vascular Research Lab under the supervision of Dr Markus Amann. His long-term goals are to elucidate how the cardiovascular, respiratory and neuromuscular systems integrate and influence exercise tolerance in health and disease. **Joshua C. Weavil** received his PhD from the University of Utah. His research focused on the aetiology of neuromuscular fatigue in health and disease, with special consideration to the role of group III/IV muscle afferents.




Introduction

The pulmonary response to physical exercise is tightly regulated in humans. During locomotion evoking up to 70–80% of $\dot{V}_{O_2max}$, minute ($\dot{V}_E$) and alveolar ($\dot{V}_A$) ventilation (Wasserman et al., 1966) and the transfer of O_2 and CO_2 across the alveolar–capillary barrier (Turino et al., 1963) increase in proportion to the metabolic rate to maintain arterial blood gas and acid–base homeostases (Barr et al., 1964). At higher intensities, $\dot{V}_A$ increases disproportionally relative to the metabolic demand of the exercise, which further raises alveolar P_{O_2} (P_{AO_2}), but also decreases arterial P_{CO_2} (P_{aCO_2}). Although impairments in pulmonary gas exchange, as demonstrated by a widened alveolar–arterial P_{O_2} difference ($A - aD_{O_2}$), develop during high-intensity exercise, the increased P_{AO_2} largely compensates for this impact, and arterial P_{O_2} (P_{aO_2}) remains fairly consistent in most humans, but can fall in highly trained athletes (Dempsey & Wagner, 1999). It is generally accepted that the tight regulation of exercise hyperpnoea within medullary respiratory control centres is based on the activity and interaction of three neural control mechanisms (Dempsey et al., 2022). These include a feedforward signal (i.e. 'central command') generated by cortical locomotor regions (Waldrop & Iwamoto, 2006), a feedback mechanism based on the central projection of mechano- and metabo-sensitive group III and IV muscle afferents (Dempsey et al., 2014), and humoral factors triggering carotid chemoreceptors and/or pulmonary receptors sensing changes in arterial O_2, CO_2 and pH (Forster et al., 2012).

Recent studies using lumbar intrathecal fentanyl to attenuate group III/IV leg muscle afferents provided initial human evidence for this feedback mechanism as a key determinant of the ventilatory response to locomotor exercise at various intensities (Amann et al., 2010, Amann, Blain et al., 2011; Gagnon et al., 2012; Olson et al., 2014; Sidhu et al., 2014; Sidhu et al., 2017; Smith et al., 2022). However, the duration of the exercise at each intensity in these studies was only 3–5 min and thus limited to the transient phases I and II of the ventilatory kinetics (Casaburi et al., 1989; Whipp et al., 1982). Thus, experimental evidence for or against a persistent role of these sensory neurons in regulating the steady-state hyperpnoeic response (i.e. phase III; >5–8 min) is needed as it was previously theorized that this feedback mechanism might facilitate breathing only during phase I and II, but 'resets' during phase III with no further role in regulating the hyperpnoeic response until the exercise intensity is altered again (Poon & Song, 2015).

Furthermore, previous fentanyl studies also observed that the effect of afferent blockade on $\dot{V}_E$ progressively decreases towards exhaustion during very high intensity exercise, while haemoglobin saturation continues to remain significantly lower during the exercise performed with blocked, compared to intact, group III/IV muscle afferent feedback (Amann et al., 2009, Amann, Blain et al., 2011). This observation indirectly suggests that the compromised arterial oxygenation during exercise with blocked group III/IV afferent feedback may not only stem from inadequate hyperpnoea, but also from an impairment in gas exchange efficiency. Indeed, afferent blockade-induced decreases in sympathetic outflow (Hureau et al., 2018; Wan et al., 2020*a*) may affect the transfer of O_2 and CO_2 between alveoli and capillaries, potentially by impairing lung diffusion capacity (Azzam et al., 2004; Wagner, 1992). However, it is unknown whether feedback from group III/IV muscle afferents contributes to gas exchange efficiency during exercise.

It was therefore the purpose of this study to attenuate group III/IV muscle afferent feedback during cycling exercise over a range of intensities and determine their role in regulating the hyperpnoeic response and pulmonary gas exchange efficiency during human locomotion. We hypothesized that (1) group III/IV muscle afferent feedback is required for a normal ventilatory response not only during the initial phase (i.e. 0–5 min), but also during prolonged (i.e. >5 min), constant work rate exercise, and (2) group III/IV muscle afferent feedback contributes to the maintenance of arterial oxygenation not only by regulating $\dot{V}_E$, but also by facilitating pulmonary gas exchange efficiency.

Methods

Participants and ethical approval

A total of 13 healthy recreationally active females ($n = 4$) and males (age: 21 (3) years; height: 176 (7) cm; body mass: 70 (9) kg; maximal rate of O_2 consumption ($\dot{V}_{O_2max}$): 46 (8) ml/kg/min) provided written informed consent to participate in the study. All participants were non-smokers, free of overt cardiorespiratory disease and not medicated. Before each laboratory visit, participants refrained from caffeine and alcohol for 12 h and vigorous physical activity for 24 h. Female participants were premenopausal and studied during the early follicular phase of the menstrual cycle. The study conformed to the standards set by the *Declaration of Helsinki* (except for registration in a database) and was approved by the Institutional Review Board of the University of Utah and by the Salt Lake City Veterans Affairs Medical Centre (IRB no. 62889).

Protocol

All testing sessions were completed in a temperature-controlled (∼21°C) room and performed at the same time of the day (±30 min). Participants

visited the laboratory on four separate occasions. The first visit included an incremental cycling test (20 W + 25 W/min) to exhaustion to determine peak work rate (W_{PEAK}) and $\dot{V}_{O_2max}$ (Amann et al., 2006). During the second visit, participants were familiarized with the testing procedures (i.e. neuromuscular fatigue and lung diffusion capacity assessments) and completed three consecutive bouts of cycling at intensities corresponding to 30% (78 (19) W), 50% (130 (32) W), and 80% (209 (51) W) of W_{PEAK}. These intensities were selected with the aim of eliciting metabolic rates of ∼50% (moderate intensity), ∼75% (heavy intensity) and >90% (severe intensity) of $\dot{V}_{O_2max}$, respectively. The moderate- and heavy-intensity bouts were performed for 20 min each while the severe-intensity bout for 5 min. During the third and fourth visits participants completed, in a counter-balanced order, the same exercise protocol with either intact (control condition (CTRL)) or attenuated feedback from group III/IV leg muscle afferents (FENT). During both experimental visits, the participants' radial artery was catheterized and instrumented for blood sampling. During FENT, after catheterization, fentanyl was injected intrathecally at the vertebral interspace L3–L4. Before and after these procedures, participants completed two 3-min bouts of arm cycling (at 15 W and 30 W) to evaluate potential cephalic migration of fentanyl. Participants exercised on an electromagnetically braked cycling ergometer (Velotron, Racer Mate, Seattle, WA, USA) and were instructed to maintain a cadence of 75 rpm. On day 3 and 4, neuromuscular quadriceps function was assessed before and again after the exercise to determine exercise-induced locomotor muscle fatigue. To ensure that all experimental procedures were completed before the efficacy of the afferent blockade began to wear off, the window for collecting baseline pulmonary data was minimized (i.e. 2 min). While sufficient to compare the pulmonary responses before the start of CTRL and FENT exercise, the reported pre-exercise data do not represent true resting values.

Measurements

Ventilation, pulmonary gas exchange, arterial blood gases and core temperature. An open circuit breath-by-breath metabolic cart (Innocor, Innovision, Glamsbjerg, Denmark) was used to record gas exchange and ventilatory variables during all sessions. Arterial blood was drawn at baseline before the start of the exercise as well as during exercise to measure haemoglobin saturation (H_bO_2), P_{aO_2}, P_{aCO_2} and plasma electrolytes (GEM 5000, Instrumentation Laboratories, Bedford, MA, USA). Arterial blood gases were temperature-corrected for subsequent analyses (Bradley et al., 1956). Samples were collected every minute during the first 5 min of each work rate and every 5 min thereafter. Physiological dead space (V_d) was calculated as $V_T \cdot (1 - (863 \cdot \dot{V}_{CO_2})/(\dot{V}_E \cdot P_{aCO_2})$, where V_T is tidal volume and $\dot{V}_{CO_2}$ is the rate of CO_2 excretion (Wasserman et al., 1987). Alveolar ventilation ($\dot{V}_A$) was calculated by multiplying breathing frequency (f_B) by the difference between V_T and V_d. P_{AO_2} was calculated using the equation: $P_{IO_2} - \dot{V}_{O_2}/\dot{V}_A \cdot (P_B - P_{H_2O})$ (Glenny, 2008), where P_{IO_2} is the inspired pressure of O_2, P_B is barometric pressure and P_{H_2O} is the vapour pressure of water. P_{H_2O} was corrected for changes in core temperature (Stickland et al., 2013). The $A - aD_{O_2}$ was calculated as the difference between P_{AO_2} and P_{aO_2}. Considering the influence of body temperature on blood gases and on breathing (González-Alonso et al., 2023), core temperature was continuously measured using ingestible sized capsules and a telemetry system (e-Celsius and e-Viewer, BodyCap, Caen, France). Ratings of perceived exertion and dyspnoea were obtained using the Borg modified scales for exertion and breathlessness (Borg et al., 2010).

Lung diffusion capacity. The single-breath technique using carbon monoxide (CO) was used to estimate lung diffusion capacity (D_{LCO}) and alveolar volume (Ultima PFX, MGC Diagnostics, St Paul, MN, USA). After careful familiarization during the preliminary visits, participants completed the assessments according to standard procedures (MacIntyre, 2005) while seated on the cycle ergometer. Briefly, during normal breathing, participants were instructed to exhale forcefully to residual volume at which point they immediately switched to a gas mixture containing 0.3% CO, 0.3% CH_4, 21% O_2 and balance N_2. Participants then inspired rapidly and fully to total lung capacity. After holding their breath for 4 s at total lung capacity, participants exhaled steadily to residual volume. During this exhalation, but after washout of dead space, 500 ml of expired air was automatically sampled by the system to measure the disappearance of CO. Each D_{LCO} measure was corrected for barometric pressure (Hegewald et al., 2023), CO retention (Mohsenifar & Tashkin, 1979), and for each participant's haemoglobin concentration (Cotes et al., 1972). Assessments were performed twice before the start of the exercise, and once during cycling at min 7 and 17 of the ∼50% of $\dot{V}_{O_2max}$ stage and at min 27 and 37 of the ∼75% of $\dot{V}_{O_2max}$ stage.

Intrathecal fentanyl and arm cycling. Participants sat in a flexed position, and 0.025 mg of fentanyl was delivered intrathecally at the vertebral interspace L3–L4, as previously described (Amann et al., 2009). The migration of fentanyl within the cerebrospinal fluid beyond the cervical level could depress ventilation secondary to exogenous opioids in the brainstem therein limiting the inter-

pretation of our findings (Lalley, 2003, 2008). Therefore, to evaluate whether a drug migration to the brain occurred, the ventilatory response to arm cycling (15 W and 30 W; 3 min each) was assessed before and ∼10 min after fentanyl injection (Amann et al., 2010). As fentanyl was administered in the lumbar region of the spine (to attenuate leg muscle afferent feedback), reductions of $\dot{V}_E$ in response to arm cycling would indicate cephalad migration of fentanyl. Therefore, ventilatory responses to arm cycling exercise performed before and after fentanyl administration were assessed on an individual basis. Reductions greater than two standard deviations (SD) from the breath-by-breath recording of $\dot{V}_E$ during the last minute of arm cycling under CTRL conditions were considered evidence of a direct effect of fentanyl on cerebral opioid receptors and those individuals were excluded from further analysis (Thurston et al., 2023).

Hypercapnic ventilatory responsiveness test. During the first preliminary visit, a CO_2 rebreathing test was performed to assess the participants' hypercapnic ventilatory responsiveness (HCVR) (Amann et al., 2010). The individual HCVRs were later used to quantify the extra ventilatory response (i.e. $\dot{V}_E$, in l/min) resulting from the afferent-blockade-induced increase in arterial hypercapnia (Amann et al., 2010). Briefly, upon attainment of eupnoeic steady-state ventilation (∼3 min), participants were switched to a 6-l rebreathing bag containing 3% CO_2, 27% N_2 and 70% O_2. Participants were instructed to breathe from this bag until the end-tidal pressure of CO_2 (P_{ETCO_2}) reached ∼55 mmHg. These trials were performed twice and separated by ∼5 min to allow restoration of baseline breathing. The slope of the $\dot{V}_E/P_{ETCO_2}$ relationship was computed from the best-fit regression within the linear range of the ventilatory response during the rebreathing phase.

Neuromuscular quadriceps function and electromyogram. Neuromuscular function and compound muscle action potential (M-wave) were assessed as previously described (Thurston et al., 2021). Briefly, participants performed 3-s maximal voluntary quadriceps contractions (MVC) three times, separated by ∼30 s, before and again 30–45 s after exercise. A constant current stimulator (Model DS7AH; Digitimer Ltd, Welwyn Garden City, UK), with the cathode (self-adhesive electrode) placed on the femoral triangle and the anode placed on the anterior portion of the greater trochanter, was used to deliver a square wave stimulus (200 μs). Superimposed twitches and potentiated quadriceps twitches (Q_{tw}) were evoked by a single electrical stimulation of the femoral nerve at the peak torque plateau of each MVC and ∼2 s after each MVC, respectively. Voluntary quadriceps activation (VA) was calculated as: VA (%) = (1 – SIT/Q_{tw}) × 100 (Merton, 1954). The results of the three sets of assessments, before and after exercise, were averaged and neuromuscular fatigue was quantified as a pre- to post-exercise change in neuromuscular function. Electromyogram (EMG) responses to femoral nerve stimulation, i.e. M-waves, were recorded with surface electrodes (Ag–AgCl, 10 mm in diameter) placed over the muscle belly of the vastus lateralis with a 1-cm inter-electrode distance (i.e. monopolar configuration). EMG signals were amplified (1000×; 1901, Cambridge Electronic Design, Cambridge, UK), band-pass filtered (10–1000 Hz; 1901, Cambridge Electronic Design) and converted from analog to digital at a sampling rate of 2000 Hz using a 16-bit Micro 1401 mk-II and Spike2 data collection software (Cambridge Electronic Design) via custom written program scripts. The peak-to-peak amplitude for each M-wave was measured and averaged over the three stimulation sets performed before and again after exercise.

Statistical analyses

Data are reported as means (SD). A two-way analysis of variance (ANOVA) (time × condition) was performed for each work rate to compare responses between FENT and CTRL. To compare the steady-state responses and the potential effect of exercise intensity, a two-way repeated measures ANOVA (condition × work rate) was calculated using averaged data from the last minute of exercise of each of the three work rates. Holm's sequential Bonferroni was used as post-hoc analysis. Student's t test was used to compare pre- to post-exercise change in MVC, Q_{tw}, VA and M-waves between conditions. α was set at 0.05 and significance was determined when $P \leq 0.05$. SPSS Statistics package (version 29.0, IBM Corp., Armonk, NY, USA) was used.

Results

Arm cycling exercise

The group mean ventilatory response to arm cycling at 15 W (∼26 l/min) and 30 W (∼32 l/min) was not different between pre- and post-fentanyl injection ($P = 0.187$). In all participants, the $\dot{V}_E$ response to arm cycling after fentanyl injection was within 2 SD of that recorded prior to drug injection; a direct effect of fentanyl on cerebral opioid receptors was therefore excluded in each participant.

Pulmonary ventilation, gas exchange and HCVR

Intrathecal fentanyl had no effect on the pulmonary response obtained prior to the start of exercise. The ventilatory responses to exercise are displayed in Figs 1 and 2, and averages during the last minute of each stage are shown in Table 1 and Figs 1 and 2. Starting within the first minute of each work rate, $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ and

Table 1. Pulmonary responses, acid–base, and plasma electrolytes before the start of the exercise and during the last minute of each work rate

	Pre-exercise		30% of W_{PEAK} 78 (19) W		50% W_{PEAK} 130 (32) W		80% of W_{PEAK} 209 (51) W		Work rate	Condition	Interaction
	CTRL	FENT	CTRL	FENT	CTRL	FENT	CTRL	FENT	*P*	*P*	*P*
$\dot{V}_{O_2}$ (l/min)	0.41 (0.03)	0.43 (0.05)	1.64 (0.26)	1.68 (0.27)	2.41 (0.34)	2.47 (0.38)	3.15 (0.48)	3.16 (0.55)	<0.001	0.118	0.624
$\dot{V}_{O_2}$ (% $\dot{V}_{O_2max}$)	13.3 (2.7)	13.7 (2.2)	51.5 (3.3)	52.7 (2.9)	75.8 (5.6)	77.4 (5.2)	98.8 (5.2)	98.9 (8.4)	<0.001	0.129	0.597
$\dot{V}_{CO_2}$ (l/min)	0.29 (0.05)	0.32 (0.05)	1.33 (0.19)	1.36 (0.20)	2.03 (0.25)	2.10 (0.30)	3.13 (0.52)	3.21 (0.62)	<0.001	0.119	0.867
RER	0.75 (0.10)	0.77 (0.08)	0.81 (0.06)	0.81 (0.06)	0.85 (0.05)	0.85 (0.06)	0.99 (0.07)	1.02 (0.09)	<0.001	0.249	0.498
$\dot{V}_A$ (l/min)	8 (2)	9 (2)	36 (5)	33 (5)	60 (9)	56 (8)	112 (17)	101 (20)	<0.001	<0.001	0.133
V_d (l)	0.29 (0.10)	0.26 (0.07)	0.42 (0.10)	0.38 (0.11)	0.52 (0.07)	0.46 (0.13)	0.55 (0.28)	0.54 (0.30)	0.047	0.301	0.820
V_d/V_T	0.38 (0.04)	0.36 (0.07)	0.24 (0.06)	0.21 (0.06)	0.24 (0.05)	0.21 (0.06)	0.20 (0.11)	0.20 (0.12)	0.589	0.123	0.614
P_{ETCO_2} (mmHg)	30.5 (2.6)	30.3 (3.1)	33.4 (3.2)	35.7 (4.0)	30.1 (3.8)	32.6 (3.8)	24.9 (3.1)	28.0 (4.1)	<0.001	<0.001	0.641
Lactate (mmol/l)	0.9 (0.2)	1.3 (0.3)	1.6 (0.7)	1.6 (0.6)	4.4 (1.7)	5.2 (1.6)	11.5 (2.6)	11.6 (2.9)	<0.001	0.356	0.582
Ca^{2+} (mmol/l)	0.92 (0.09)	0.92 (0.04)	0.95 (0.07)	0.96 (0.02)	0.94 (0.07)	0.96 (0.04)	0.98 (0.07)	1.01 (0.06)	0.061	0.029	0.854
Cl^- (mmol/l)	109 (2)	109 (2)	109 (2)	109 (2)	109 (2)	109 (2)	111 (2)	112 (2)	<0.001	0.664	0.376
K^+ (mmol/l)	3.5 (0.3)	3.4 (0.1)	3.8 (0.3)	3.9 (0.1)	4.1 (0.2)	4.3 (0.3)	5.0 (0.4)	5.3 (0.5)	<0.001	0.002	0.033
Na^+ (mmol/l)	139 (1)	139 (1)	140 (2)	140 (1)	141 (1)	142 (1)	145 (1)	145 (1)	<0.001	0.101	0.423
RPE	—	—	2.9 (0.9)	1.7 (0.9)	7.0 (1.6)	4.9 (1.3)	9.2 (1.0)	8.3 (1.8)	<0.001	<0.001	0.116
Dyspnoea	—	—	2.5 (0.9)	1.5 (0.8)	6.2 (1.8)	4.2 (1.4)	8.8 (1.1)	8.2 (1.8)	<0.001	<0.001	0.090

Data (n = 13) are presented as means (SD) and were analysed using two-way repeated-measure ANOVA with Bonferroni's *post hoc* test. P_{ETCO_2}, end-tidal pressure of carbon dioxide; RER, respiratory exchange ratio; RPE, rate of perceived exertion; $\dot{V}_A$, alveolar ventilation; $\dot{V}_{CO_2}$, rate of carbon dioxide excretion; V_d, dead space; V_d/V_T, dead space-tidal volume ratio; $\dot{V}_{O_2}$, rate of oxygen consumption.

$\dot{V}_E/\dot{V}_{CO_2}$ were lower during FENT compared to CTRL ($P < 0.001$). During the last minute of exercise at ∼50%, ∼75% and ∼100% of $\dot{V}_{O_2max}$, $\dot{V}_E$ was 11 (5)% ($P < 0.001$), 8 (3)% ($P < 0.001$) and 9 (9)% ($P = 0.010$) lower, respectively, during FENT compared to CTRL. Similarly, $\dot{V}_A$ was 10 (8)% ($P = 0.002$), 9 (6)% ($P = 0.026$) and 10 (14)% ($P = 0.038$) lower during FENT compared to CTRL (Table 1). As V_T was not different between conditions at any of the intensities (smallest $P = 0.176$), the lower ventilatory response resulted from the ∼13% lower breathing frequency (f_B) during FENT compared to CTRL (largest $P = 0.003$). P_{AO_2} was 1–4% lower

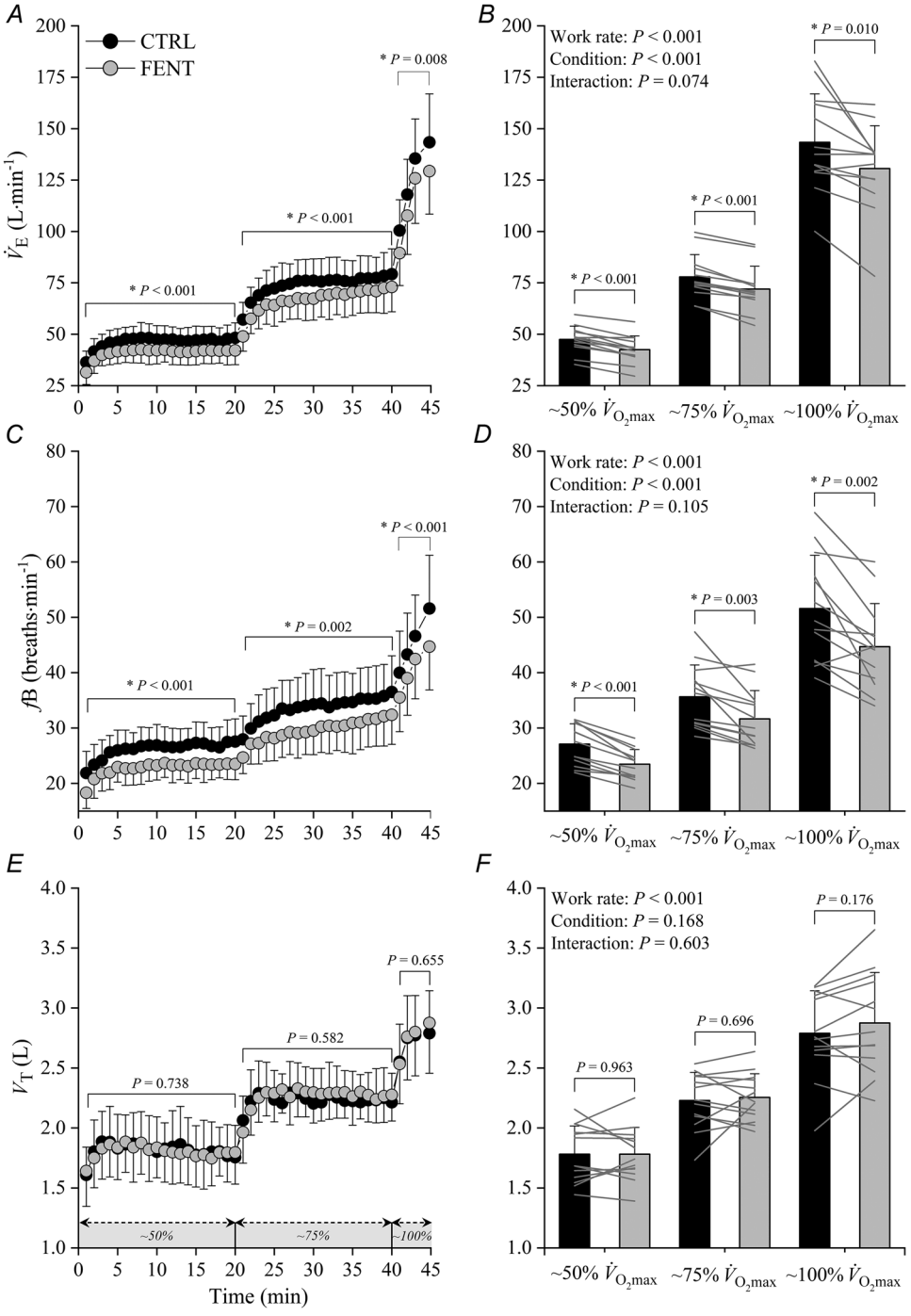


Figure 1. Ventilatory responses to exercise at ∼50%, ∼70% and ∼100% of $\dot{V}_{O_2max}$
A, *C* and *E* illustrate the responses over time; *B*, *D* and *F* reflect individual and group mean data averaged over the last minute of each work rate (n = 13). Minute ventilation, $\dot{V}_E$; breathing frequency, f_B; tidal volume, V_T.

during FENT compared to CTRL exercise at ∼50% ($P = 0.007$) and ∼75% ($P = 0.021$) of $\dot{V}_{O_2max}$, but not at ∼100% of $\dot{V}_{O_2max}$ ($P = 0.370$; Fig. 3). While the $A - aD_{O_2}$ significantly increased with each increment in work rate in both conditions, the widening of the $A - aD_{O_2}$ was 11–15% larger during FENT compared to CTRL (largest $P < 0.017$; Fig. 3). $\dot{V}_{O_2}$ ($P = 0.118$) and $\dot{V}_{CO_2}$ ($P = 0.129$) were not different between conditions across intensities, $\dot{V}_E/\dot{V}_{O_2}$ (largest $P < 0.023$) and $\dot{V}_E/\dot{V}_{CO_2}$ ($P < 0.001$) was lower during FENT compared to CTRL (Fig. 2). The group mean slope of the HCVR test was 3.22 (0.97) l/min/mmHg (range: 2.01–5.18 l/min/mmHg). Considering the surrogacy of P_{ETCO_2} for P_{aCO_2}, using the individual gain of P_{ETCO_2} of each participant's HCVR, it was estimated that the higher P_{aCO_2} during FENT (Fig. 4) prevented a further 8 (5) l/min (∼28%), 7 (5) l/min (∼18%) and 10 (8) l/min (∼16%) decreases in $\dot{V}_E$ during the last minute of exercise at ∼50, ∼75 and ∼100% of $\dot{V}_{O_2max}$, respectively.

Blood gases, acid–base balance, plasma electrolytes and core temperature

Arterial blood gases and oxygenation are shown in Fig. 4. P_{aO_2} was ∼6% lower and P_{aCO_2} ∼10% higher during FENT compared to CTRL (largest $P < 0.007$). Arterial H_bO_2 was significantly lower at each work rate during FENT compared to CTRL (largest $P = 0.021$). While pH was not different between conditions during exercise at ∼50% of $\dot{V}_{O_2max}$ ($P = 0.189$), it was lower during FENT at ∼75% ($P = 0.020$) and ∼100% of $\dot{V}_{O_2max}$ ($P = 0.008$). Plasma electrolyte and lactate concentrations are shown in Table 1. Plasma $[K^+]$ was not different between conditions at ∼50% $\dot{V}_{O_2max}$ ($P = 0.759$), but 5–6% higher during FENT at ∼75% ($P = 0.021$) and ∼100% $\dot{V}_{O_2max}$ ($P = 0.011$). Pre-exercise core body temperature was 36.8 (0.6)°C and 36.8 (0.5)°C for FENT and CTRL, respectively ($P = 0.711$). During exercise, core temperature increased progressively to similar values in FENT and CTRL (∼50% $\dot{V}_{O_2max}$: 38.3

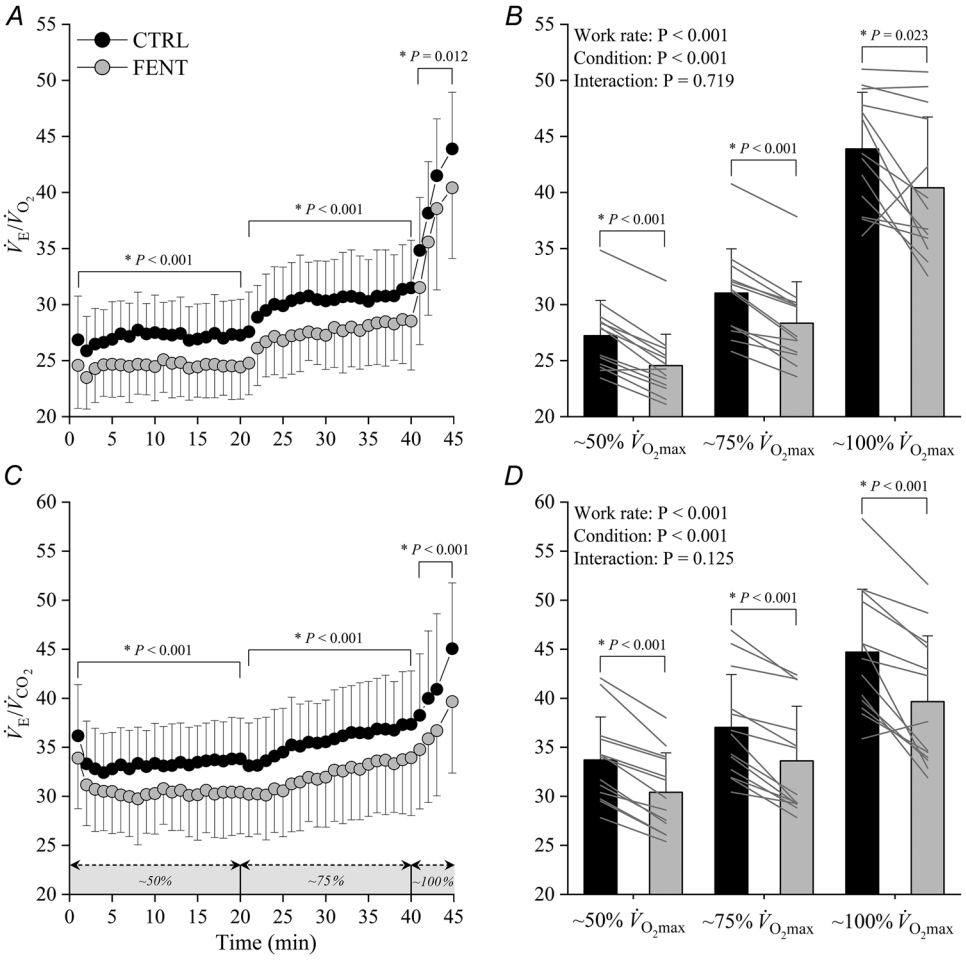


Figure 2. Ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}_{O_2}$) and carbon dioxide ($\dot{V}_E/\dot{V}_{CO_2}$) during exercise at ∼50%, ∼70% and ∼100% of $\dot{V}_{O_2max}$
A and *C* illustrate the responses over time; *B* and *D* reflect individual and group mean data averaged over the last minute of each work rate ($n = 13$).

(0.7)°C *vs.* 38.4 (0.8)°C; ∼75% $\dot{V}_{O_2max}$: 39.1 (1.2)°C *vs.* 39.1 (1.1)°C; ∼100% $\dot{V}_{O_2max}$: 39.3 (1.4)°C *vs.* 39.3 (1.3)°C.

Lung diffusion capacity

Before exercise, D_{LCO} was 42.4 (7.1) ml/mmHg/min for FENT and 42.7 (5.2) ml/mmHg/min for CTRL ($P = 0.858$). Exercise increased D_{LCO} in both conditions ($P < 0.001$). During exercise at ∼50% $\dot{V}_{O_2max}$, D_{LCO} was 48.0 (9.2) ml/mmHg/min for FENT and 48.6 (9.4) ml/mmHg/min for CTRL ($P = 0.203$); during exercise at ∼75% $\dot{V}_{O_2max}$, D_{LCO} was 52.8 (8.2) ml/mmHg/min for FENT and 51.8 (9.4) ml/mmHg/min for CTRL ($P = 0.417$). Alveolar volume was not different between FENT and CTRL before exercise (6.4 (0.5) l *vs.* 6.6 (0.5) l; $P = 0.393$) and did not change during exercise.

Neuromuscular fatigue

There were no baseline differences between FENT and CTRL for MVC (249 (67) N m *vs.* 252 (70) N m; $P = 0.460$), Q_{tw} (77 (18) N m *vs.* 74 (16) N m; $P = 0.080$), and VA (92 (5)% *vs.* 92 (5)%; $P = 0.572$). After exercise, MVC (−32 (15)% *vs.* −24 (12)%; $P = 0.022$) and Q_{tw} (−56 (16)% *vs.* −42 (14)%; $P = 0.001$) were reduced to a greater extent following FENT compared to CTRL. There was no difference in the pre- to post-exercise reduction of VA between CTRL (−2.9 (3.6)%) and FENT (−6.4 (6.2)%) ($P = 0.154$). M-waves recorded prior to exercise were not different between FENT and CTRL (16.1 (5.5) mV *vs.* 15.8 (3.9) mV; $P = 0.065$). M-waves significantly decreased from pre- to post-exercise in FENT (to 12.6 (4.2) mV) and CTRL (to 13.0 (5.0) mV), but this ∼20% reduction was not different between conditions ($P = 0.900$). This impact

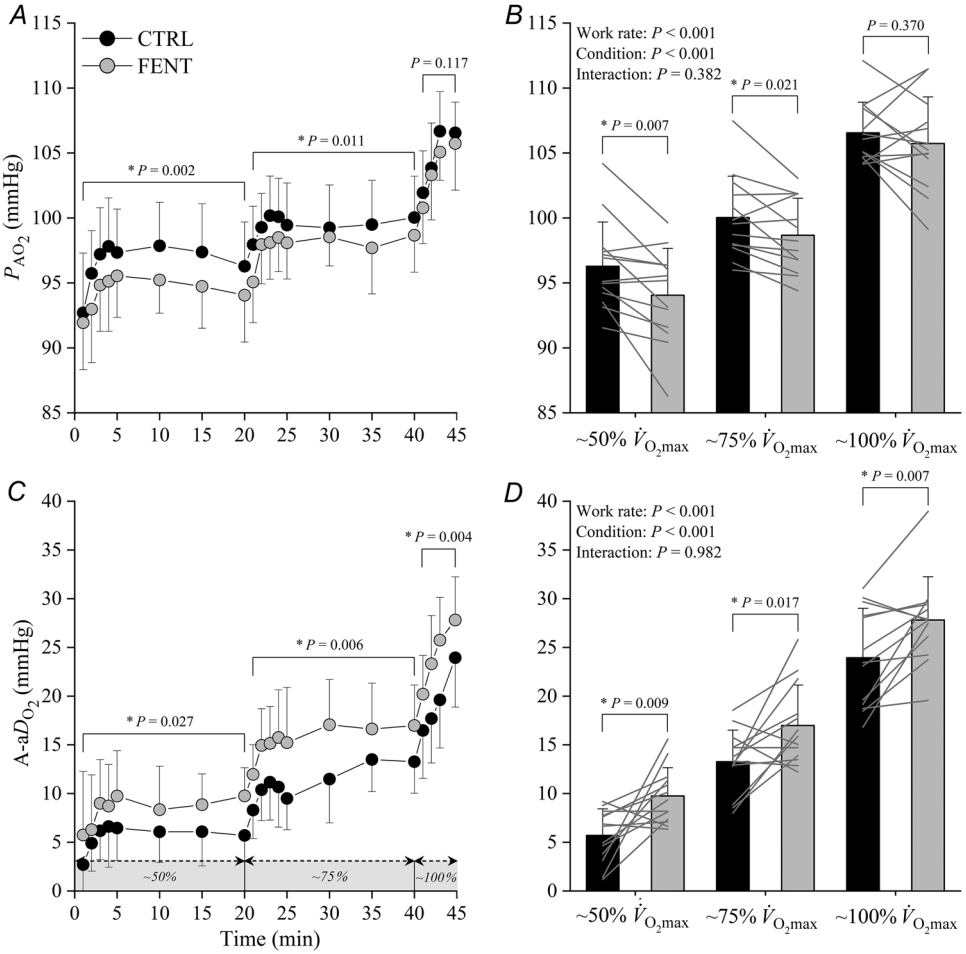


Figure 3. Alveolar pressure of oxygen (P_{AO_2}) and alveolar–arterial pressure of oxygen difference ($A - aD_{O_2}$) during exercise at ∼50%, ∼70% and ∼100% of $\dot{V}_{O_2max}$
A and *C* illustrate the responses over time; *B* and *D* depict individual data collected during the last minute of each work rate (n = 13).

on sarcolemmal membrane excitability suggests that the observed pre- to post-exercise decrease in Q_{tw} was likely an overestimation.

Discussion

By pharmacologically attenuating group III/IV leg muscle afferent feedback, this study investigated the significance of these sensory neurons for regulating the pulmonary response during long-duration locomotor exercise. In addition to contributing to the initial dynamic phase (0 to ∼5 min), the findings reflect a critical role of group III/IV muscle afferents in determining the appropriate hyperpnoeic response, arterial oxygenation, gas exchange efficiency and acid–base balance during more prolonged steady-state cycling exercise. Importantly, their contribution to various pulmonary responses remained consistent throughout the entire exercise protocol and during various intensities ranging from ∼50% to 100% of $\dot{V}_{O_2max}$. The study also revealed that, without affecting lung O_2 diffusion capacity, group III/IV muscle afferent feedback influences pulmonary gas exchange efficiency by optimizing mechanisms that prevent an excessive exercise-induced widening of the $A-aD_{O_2}$. This suggests that the compromised arterial oxygenation during exercise with attenuated muscle afferents is not only the result of alveolar hypoventilation, but also a consequence of an impairment in pulmonary gas exchange efficiency. Overall, these findings demonstrate that group III/IV leg muscle afferent feedback is obligatory for the continuous regulation of pulmonary ventilation and gas exchange efficiency and, consequently, for maintaining arterial blood gases and acid–base homeostases during locomotor exercise.

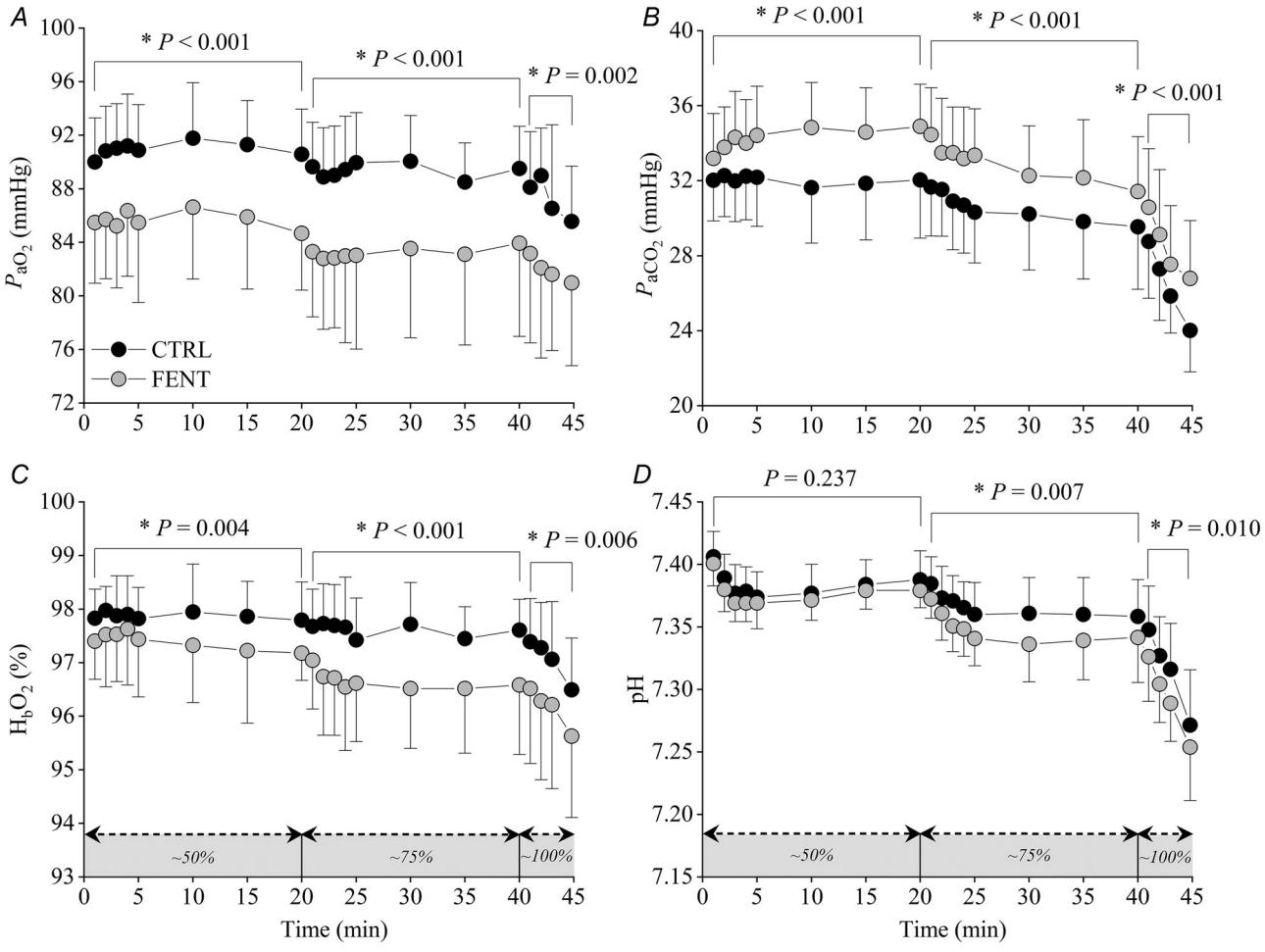


Figure 4. Arterial pressures of oxygen (P_{aO_2}) and carbon dioxide (P_{aCO_2}), haemoglobin saturation (H_bO_2), and arterial pH during exercise at ∼50%, ∼70% and ∼100% of $\dot{V}_{O_2max}$ (n = 13)
Figures illustrating individual data distribution for each variable are offered as supplementary material.

Group III/IV muscle afferent feedback and exercise hyperpnoea

The significance of group III/IV muscle afferents in regulating the hyperpnoea of exercise has long been controversial, mostly because of methodological limitations related to the manipulation of neural feedback in the exercising human (discussed in Amann et al., 2010, 2015) and evidence from animal preparations against a meaningful influence (Weissman et al., 1980). Although more recent human studies circumvented many of these issues and offered initial evidence for these sensory neurons as key determinants of the hyperpnoeic response at the onset and during the early phase of locomotor exercise (up to 5 min) (Dempsey et al., 2014, 2022), their continuous involvement and contribution to the steady-state ventilation and during prolonged exercise (>5 min) has never been elucidated. We therefore blocked feedback from group III/IV leg muscle afferents during prolonged locomotor exercise (45 min in total) and compared the ventilatory responses to those obtained during the same exercise performed with an intact feedback system. All indices of pulmonary ventilation, namely, $\dot{V}_E$, $\dot{V}_A$, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$, were significantly lower during the initial phase of each work rate in FENT compared to CTRL and this hypoventilation resulted in marked decreases in $P_{A O_2}$ and arterial oxygenation (i.e. P_{aO_2} and H_bO_2), and increases in P_{aCO_2}. Importantly, the afferent blockade-induced reduction in pulmonary ventilation and associated consequences persisted beyond the initial phase and remained significantly different from CTRL until the end of the exercise protocol (Figs 1–4). The hypoventilation was, in agreement with our previous studies, entirely mediated by reductions in breathing frequency, while tidal volume was not affected by the afferent blockade (Fig. 1*E* and *F*). This observation indirectly supports the idea that breathing frequency and tidal volume during exercise might be modulated by different control mechanisms (Nicolò & Sacchetti, 2023) and suggests an involvement of group III/IV muscle afferent feedback in regulating f_B, but not V_T, during human locomotion.

The 10–20% lower ventilatory output during FENT is even more striking considering that intrathecal fentanyl only reduces group III/IV muscle afferent feedback by ∼50–60% (Hureau et al., 2018) and that the blockade-induced hypoventilation facilitated potent ventilatory stimuli. Specifically, P_{aCO_2} and K^+ were higher and P_{aO_2} and pH lower during FENT (Fig. 4 and Table 1), likely resulting in a greater chemoreceptor-mediated ventilatory drive (Forster et al., 2012) compared to CTRL. Based on the hypercapnic ventilatory responsiveness quantified for each participant (∼3.2 l/min increase in $\dot{V}_E$ per 1 mmHg increase in P_{ETCO_2}), we estimated that the 2–4 mmHg higher P_{aCO_2} (Figure 4*B*) alone prevented another 15–30% decrease in $\dot{V}_E$ during FENT. However, given the impact of the neural interactions between different ventilatory control mechanisms (Wan et al., 2020*b*) and between central and peripheral chemoreception (Smith et al., 2010) on exercise hypernoea, this likely underestimates the exact increases in $\dot{V}_E$ resulting from the greater humoral stimuli during FENT. Furthermore, the greater development of locomotor muscle fatigue during FENT likely required higher central command (at least during the 5-min bout at 100% of $\dot{V}_{O_2max}$), a powerful ventilatory drive (Asmussen et al., 1965), in order to complete the exercise. Taken together, the greater ventilatory stimuli in FENT combined with the fact that intrathecal fentanyl only blocks about half of the afferent feedback (Hureau et al., 2018) suggests that the observed difference in pulmonary ventilation (Figs 1 and 2) underestimates the relative contribution of group III/IV muscle afferent feedback in regulating the hypernoea of exercise. While the confounding changes in ventilatory drive during FENT make quantification of the fraction of hyperpnoea provided by these sensory neurons alone difficult, it is not unreasonable to speculate that this feedback mechanism might account for at least ∼50% of the ventilatory response to locomotor exercise.

Previous studies found that the initially large blockade-induced reduction in pulmonary ventilation becomes progressively smaller during very high intensity (90–100% of $\dot{V}_{O_2max}$) cycling time trials (Amann et al., 2009) and constant-load exercise to exhaustion (Amann, Blain et al., 2011) and virtually disappears towards the end of the task. As central command and humoral stimuli rose faster and to a greater degree during the exercise with blocked, compared to intact, group III/IV muscle afferents, it was proposed that this extra ventilatory drive provided additional stimulation sufficient to mask the missing feedback contribution. The current data from the high intensity bout corroborate our earlier findings (Amann et al., 2009, Amann, Blain et al., 2011) and confirm a significant contribution of leg muscle afferent feedback to the hyperpnoea of exercise performed at $\dot{V}_{O_2max}$. However, as the high-intensity bout was limited to 5 min, we cannot confirm that the ventilatory response closer to exhaustion becomes similar during exercise with intact and blocked muscle afferent feedback.

Although the current study was not specifically designed to investigate redundant mechanism mediation of the exercise hyperpnoea (Brice et al., 1988; Forster et al., 2012; Strange et al., 1993), the findings suggest that potential compensatory increases in the activity of other ventilatory control mechanisms are simply insufficient to maintain a normal ventilatory response in the absence of group III/IV muscle afferent feedback. Based on the observation that removal of this feedback mechanism caused considerable hypoventilation and arterial blood gases and acid–base balance to deviate from their normal

levels, the current data support the hypothesis that the ventilatory response during locomotor exercise is largely the function of additive or synergistic interactions between the different ventilatory control mechanisms (Smith et al., 2010; Wan et al., 2020*b*).

Group III/IV muscle afferent feedback and pulmonary gas exchange efficiency

Earlier studies found that the afferent blockade-induced reduction in $\dot{V}_E$ during high intensity locomotor exercise gradually vanishes towards exhaustion (Amann et al., 2009, Amann, Blain et al., 2011) and that the effect of afferent blockade on $\dot{V}_E$ is relatively small during dynamic single leg knee extension exercise (Amann, Runnels et al., 2011). Despite this lack of a large effect of afferent blockade on breathing, arterial oxygenation and P_{aCO_2} remained substantially different from control exercise in these studies. These observations suggest that hypoventilation is not the only factor accounting for arterial blood gases to deviate from their normal levels during exercise with attenuated group III/IV muscle afferent feedback, but that impairments in pulmonary gas exchange may also contribute. Indeed, while the up to 25 mmHg $A-aD_{O_2}$ during CTRL is fairly normal (Dempsey & Wagner, 1999; Hopkins et al., 1998), the $A-aD_{O_2}$ was up to ∼25% greater during FENT (Fig. 3C, D). This larger impairment in gas exchange efficiency accounted for 30–40% of the difference in P_{aO_2} between FENT and CTRL. Interestingly, while the contribution of the blockade-induced hypoventilation to the fall in P_{aO_2} appears to decrease with increases in exercise intensity, the influence of the widening of the $A-aD_{O_2}$ may rise.

Prompted by these findings, we retrospectively analysed data from our earlier studies using fentanyl blockade during single leg knee extension exercise (i.e. engaging a small muscle mass) (Amann, Runnels et al., 2011) and during cycling exercise (i.e. engaging a large muscle mass) where the blockade-induced reduction in breathing was prevented by coaching the participants to maintain breathing at the level observed during control exercise (Thurston et al., 2023). In agreement with the current observations, we found that the $A-aD_{O_2}$ in these studies was also significantly greater (up to 30%) during the exercise performed with blocked, compared to intact, muscle afferents. In combination, these findings suggest that group III/IV leg muscle afferent feedback plays a significant role in optimizing pulmonary gas exchange efficiency during exercise and that this influence is independent of blockade-induced decreases in pulmonary ventilation and the muscle mass engaged in the exercise.

Although the current data are insufficient to identify the exact mechanism(s) by which group III/IV muscle afferents facilitate pulmonary gas exchange efficiency, three primary mechanisms can be discussed. First, diffusing capacity of the lungs for O_2, typically estimated by D_{LCO} and facilitated by sympathetic activity which improves pulmonary capillary permeability and fluid reabsorption in the lung interstitium (Azzam et al., 2004), is one factor influencing the magnitude of the $A-aD_{O_2}$ during exercise (Petersson & Glenny, 2014). Importantly, afferent blockade likely reduces sympathetic outflow during exercise (Hureau et al., 2018; Wan et al., 2020*a*) and this could have decreased capillary permeability and interstitial fluid reabsorption resulting in impaired pulmonary O_2 diffusion, and thus gas exchange, during FENT. However, D_{LCO} was not different between the two conditions, excluding impaired O_2 diffusion as a determinant of the compromised gas exchange efficiency during FENT.

Second, while the contribution of intra-pulmonary arteriovenous shunts to exercise-induced impairments in gas exchange has been fiercely debated (Hopkins et al., 2009; Lovering et al., 2009), it is now recognized that their role in the widening of the $A-aD_{O_2}$ is, under normal conditions, relatively small (Elliott et al., 2014) and perhaps even negligible (Vogiatzis et al., 2008). However, it cannot be excluded with certainty that group III/IV muscle afferent blockade and associated haemodynamic and autonomic consequences increased intra-pulmonary shunting and, thus, contributed to the impaired gas exchange during FENT.

Third and last, the pulmonary vasculature is under adrenergic control (Kummer, 2011) and sympatho-excitation associated with cardiovascular reflex mechanisms and exercise (Kane et al., 1994) raise pulmonary vascular resistance which, in conjunction with humoral factors, contributes to optimizing pulmonary ventilation-to-perfusion ($\dot{V}$–$\dot{Q}$) matching (Crystal et al., 1997). Because afferent blockade likely reduced sympathetic outflow during FENT (Hureau et al., 2018; Wan et al., 2020*a*), it is feasible to hypothesize that the associated impact on the pulmonary circulation resulted in a greater $\dot{V}$–$\dot{Q}$ mismatch and, subsequently, a larger impairment in gas exchange efficiency compared to CTRL. Furthermore, increases in group III/IV muscle afferent feedback relax airway smooth muscle tone and reflexly dilate the airways in anaesthetized dogs (Kaufman et al., 1985; Rybicki & Kaufman, 1985). Building upon these findings, it is possible that bronchodilatation was compromised during exercise with blocked group III/IV muscle afferents and that this impact may have affected ventilation distribution within the lungs (Hogg et al., 1972). This could have contributed to the greater $\dot{V}$–$\dot{Q}$ mismatch and, ultimately, the impairment in gas exchange efficiency. Although our interpretation suggests diminished $\dot{V}$–$\dot{Q}$ matching as the cause of worsened gas exchange efficiency when group III/IV afferents are blocked, additional work is needed to test this postulate.

Summary and conclusion

By using a pharmacological approach to attenuate group III/IV leg muscle afferent feedback during prolonged cycling exercise, we investigated the contribution of these sensory neurons to the pulmonary response to human locomotion. While afferent blockade did not affect the metabolic rate, pulmonary ventilation and gas exchange were consistently compromised, which, in combination, impaired arterial oxygenation and facilitated respiratory acidosis. These findings reflect the significance of continuous feedback from group III/IV muscle afferents for perpetually regulating exercise hyperpnoea and pulmonary gas exchange efficiency to optimize arterial oxygenation and acid–base balance during human locomotion.

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Additional information

Data availability statement

The data of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interest.

Author contributions

D.I., J.C.W. and M.A. contributed to the conception and design of the study; all authors contributed to acquisition, analysis or interpretation of data for the work; all authors contributed to drafting the manuscript or 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.

Funding

This study was supported by the National Heart, Lung, and Blood Institute (HL-162856 and HL-116579), and the U.S. Veterans Affairs Rehabilitation Research and Development (E3343-R).

Acknowledgements

The authors thank Mr Van Reese, Mr Vincent Georgescu and Mrs Jia Zhao for their help with data collection.

Keywords

arterial blood gases, exercise hyperpnoea, reflex, ventilatory control, $\dot{V}_{O_2max}$

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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