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ORIGINAL ARTICLE

The effect of pedalling cadence on respiratory frequency: passive vs. active exercise of different intensities

Michele Girardi[1] · Andrea Nicolò[1] · Ilenia Bazzucchi[1] · Francesco Felici[1] · Massimo Sacchetti[1]

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Abstract

Purpose Pedalling cadence influences respiratory frequency (f_R) during exercise, with group III/IV muscle afferents possibly mediating its effect. However, it is unclear how exercise intensity affects the link between cadence and f_R. We aimed to test the hypothesis that the effect of cadence on f_R is moderated by exercise intensity, with interest in the underlying mechanisms.

Methods Ten male cyclists performed a preliminary ramp incremental test and three sinusoidal experimental tests on separate visits. The experimental tests consisted of 16 min of sinusoidal variations in cadence between 115 and 55 rpm (sinusoidal period of 4 min) performed during passive exercise (PE), moderate exercise (ME) and heavy exercise (HE). The amplitude (A) and phase lag (φ) of the dependent variables were calculated.

Results During PE, f_R changed in proportion to variations in cadence ($r = 0.85$, $P < 0.001$; $A = 3.9 \pm 1.4$ breaths·min^{-1}; $\varphi = -5.3 \pm 13.9$ degrees). Conversely, the effect of cadence on f_R was reduced during ME ($r = 0.73$, $P < 0.001$; $A = 2.6 \pm 1.3$ breaths·min^{-1}; $\varphi = -25.4 \pm 26.3$ degrees) and even more reduced during HE ($r = 0.26$, $P < 0.001$; $A = 1.8 \pm 1.0$ breaths·min^{-1}; $\varphi = -70.1 \pm 44.5$ degrees). No entrainment was found in any of the sinusoidal tests.

Conclusion The effect of pedalling cadence on f_R is moderated by exercise intensity—it decreases with the increase in work rate—and seems to be mediated primarily by group III/IV muscle afferents, at least during passive exercise.

Keywords Ventilatory control · Sinusoidal exercise · Cycling · Muscle afferent feedback · Differential control

Abbreviations

A	Amplitude
ANOVA	Analysis of variance
EMG	Electromyography
f_R	Respiratory frequency
HE	Heavy exercise
HR	Heart rate
Hz	Hertz
ME	Moderate exercise
PCO_2	Pressure of carbon dioxide
PE	Passive exercise
P_{ETCO2}	End-tidal partial pressure of carbon dioxide
PPO	Peak power output
RMS	Root mean square
RPE	Rating of perceived exertion
Rpm	Revolutions per minute
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2peak}$	Peak value of oxygen uptake
V_T	Tidal volume
VT1	First ventilatory threshold
VT2	Second ventilatory threshold
φ	Phase lag

Introduction

Emerging evidence suggests the existence of a differential control of respiratory frequency (f_R) and tidal volume (V_T) during exercise (Nicolò et al. 2017b, 2018; Nicolò and Sacchetti 2019). While V_T seems to be mainly regulated by metabolic inputs (Nicolò et al. 2018), f_R appears to be substantially regulated by fast inputs, including central command (Nicolò et al. 2017b, 2018, 2020) and group III/IV muscle afferent feedback (Amann et al. 2010; Lam et al.

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✉ Andrea Nicolò
andrea.nicolo@yahoo.com

[1] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Piazza Lauro De Bosis 6, 00135 Rome, Italy

2019). A commonly proposed example of the influence of muscle afferent feedback on f_R is the effect that pedalling cadence has on f_R (Forster et al. 2012). For instance, the locomotor rhythm may influence the ventilatory rhythm via a phenomenon called entrainment, which occurs when f_R becomes 'in step' with the locomotor rhythm (Bechbache and Duffin 1977). It has been suggested that the entrainment phenomenon is mediated by group III/IV muscle afferents (Bechbache and Duffin 1977; Jasinskas et al. 1980; Bramble and Carrier 1983; Bernasconi and Kohl 1993; Potts et al. 2005), but alternative mechanisms have also been proposed (Bramble and Carrier 1983; Paterson et al. 1986). However, the occurrence of entrainment is affected by exercise modality; it is more frequent during walking and running and less frequent during cycling (Bechbache and Duffin 1977; Paterson et al. 1986; Bernasconi and Kohl 1993). Furthermore, the effect of pedalling cadence on f_R may not necessarily be mediated by entrainment (Caterini et al. 2016). Controversial findings on this issue limit our understanding of how pedalling cadence affects f_R during cycling exercise.

In humans, convincing evidence that group III/IV muscle afferent feedback mediates the effect of cadence on f_R comes from studies that used the passive exercise paradigm (Bell et al. 2003; Bell and Duffin 2003; Sato et al. 2004). Passive exercise increases the proportional contribution of muscle afferent feedback to ventilation by extensively reducing the magnitude of other major inputs driving ventilation, i.e. central command and metabolic inputs. The passive movement of the legs determines a rapid increase in f_R at the transition from rest to passive exercise, with f_R remaining relatively stable throughout several minutes and returning to resting values when passive exercise ends (Bell et al. 2003; Bell and Duffin 2003). While this response of f_R is not influenced by pressure of carbon dioxide (PCO_2) levels, V_T is affected by PCO_2 during passive exercise (Bell and Duffin 2003). The notion that afferent feedback mediates the f_R response observed during passive exercise is substantiated by direct evidence indicating that afferent feedback influences f_R both in humans (Amann et al. 2010) and animals (McCloskey and Mitchell 1972; Potts et al. 2005). However, it is not clear whether the increase in f_R observed during passive exercise in humans is mediated by entrainment.

During active exercise, the effect of pedalling cadence on f_R appears less evident. An interesting solution to quantifying this effect consists of providing sinusoidal changes in pedalling cadence (Casaburi et al. 1978; Caterini et al. 2016). Sinusoidal exercise allows dependent variables to be conveniently described in terms of amplitude (A) and phase lag (φ) from the input (i.e. cadence), thus providing quantitative information on the effect of variations in pedalling cadence on ventilatory responses. During sinusoidal exercise of moderate intensity, f_R showed a delayed response compared to variations in pedalling cadence (Caterini et al. 2016), which highlights a partial dissociation between the two variables. Using similar sinusoidal tests, Casaburi et al. (1978) found a dissociation between cadence and ventilation, but f_R data were not reported. Furthermore, during both unloaded pedalling (Jastrzębska and Kowalski 2015) and moderate exercise (Mitchell et al. 2019), an effect of cadence on f_R was observed, but the changes in the two variables were not proportional. Taken together, these data may suggest that the effect of cadence on f_R is reduced during active exercise compared to passive exercise, but direct comparison is still needed to test this notion.

High-intensity exercise is another condition showing a partial dissociation between cadence and f_R. Indeed, a variety of exercise protocols shows that f_R can change substantially despite pedalling cadence being fixed or being relatively constant (Lucía et al. 1999; Gravier et al. 2013; Nicolò et al. 2016, 2018). Hence, the effect of pedalling cadence on f_R may decrease with exercise intensity, possibly along with a decrease in the relative contribution of muscle afferent feedback to f_R regulation, as the relative contribution of other inputs (e.g. central command) may be higher during high-intensity exercise (Nicolò et al. 2018, 2020). This hypothesis is supported by direct evidence obtained under epidural anaesthesia, where the relative contribution of group III/IV muscle afferents to f_R regulation was lower at high-intensity exercise compared to moderate-intensity exercise (Amann et al. 2010). However, there is a paucity of experimental attempts aimed at evaluating whether the effect of cadence on f_R is affected by exercise intensity and to what extent. Furthermore, original experimental solutions are needed to investigate the putative mechanisms underlying the effect of cadence on f_R at different intensities.

This study aimed to systematically assess the effect of pedalling cadence on f_R across different exercise intensities. In view of the hypothesis that the effect of cadence on f_R is moderated by exercise intensity, sinusoidal variations in pedalling cadence were performed during passive exercise and active exercise of moderate and heavy intensities. We expected a decrease in the effect of cadence on f_R with the increase in exercise intensity. Furthermore, the experimental design provided was expected to shed some light on the mechanisms underlying the link between cadence and f_R, with implications for ventilatory control during exercise.

Methods

Participants

Ten well-trained male cyclists (mean ± SD: age 24 ± 3 years, stature 177 ± 8 cm, body mass 70 ± 9 kg) volunteered to participate in this study. Participants were instructed to avoid strenuous exercise and any beverages containing caffeine

or alcohol in the 24 h preceding each laboratory visit. This study was approved by the Ethics Committee of the University of Rome Sapienza and conformed to the standards set by the *Declaration of Helsinki*. Written informed consent was obtained from all of the participants.

Experimental overview

Participants visited the laboratory on 4 occasions over a two-week period, with a minimum of 48 h between visits. On the first visit, participants performed a preliminary ramp incremental test to exhaustion and a familiarization session of the experimental protocols and procedures. On the subsequent randomised visits (2–4), participants performed the same experimental protocol under three different conditions, i.e. passive exercise (PE), moderate exercise (ME) and heavy exercise (HE). The experimental protocol consisted of sinusoidal variations in pedalling cadence, which were performed on an electromagnetically braked cyclo-ergometer (Lode Excalibur Sport, Groningen, the Netherlands) during ME and HE, and on a fixed-wheel tandem bicycle during PE. For each participant, the positions of the cycle ergometer were adjusted and recorded during the first visit and were replicated during subsequent visits. Physiological, mechanical and perceptual variables were measured as described below.

Preliminary ramp incremental test and familiarisation session

Before the ramp incremental test, participants were given standard instructions for providing the rating of perceived exertion (RPE) using the 6–20 scale (Borg 1998). During the incremental test, participants were asked to rate RPE every minute as a familiarization with the scale.

The ramp incremental test was preceded by a 5-min warm-up at 100 W, 3 min of recovery and 2 min at 20 W. Subsequently, the work rate increased by 30 $W \cdot min^{-1}$ until exhaustion occurred. Preferred pedalling cadence was selected by each participant and was kept relatively constant for the entire test, which terminated when pedalling cadence fell by more than 10 rpm, despite strong verbal encouragement. The peak value of oxygen uptake ($\dot{V}O_{2peak}$) and the peak values of minute ventilation ($\dot{V}_E$), f_R and V_T were defined as the highest value of a 30-s moving average, while the peak power output (PPO) was defined as the highest value achieved at exhaustion. The first (VT1) and second (VT2) ventilatory thresholds were obtained following standard procedures as previously reported (Beaver et al. 1986).

After 30 min of recovery, participants were familiarized with the procedures and tests of the experimental tests. Special attention was devoted to guaranteeing the correct execution of the PE test, given the inherent difficulty of this task (Bell et al. 2003). While the tandem bicycle was actively driven by a well-trained cyclist (the pacemaker), the participant was asked to reduce his voluntary muscle activity as much as possible. Feedback to the participant was provided in real time by displaying the electromyographic (EMG) signal of the vastus lateralis muscle on a computer screen. By looking at the screen, the participant was instructed to minimize EMG activity until the task was performed correctly. The following section provides more details on the PE test.

Experimental tests

The same experimental protocol was performed in three different conditions (PE, ME and HE), on separate visits. The protocol consisted of 16 min of sinusoidal variations in pedalling cadence ranging from 115 to 55 rpm (zenith and nadir, respectively), with a sinusoidal period of 4 min. The 4-min sinusoidal period was set during pilot testing to limit the difficulty of performing the passive exercise, which increased substantially for shorter periods. The sinusoidal protocol was preceded by 5 min at a fixed cadence of 85 rpm and was immediately followed by 4 min at the same fixed cadence (see Figs. 1 and 2 for a graphical depiction). The first minute at 85 rpm was not included in further analysis.

During ME and HE, a computer screen was placed in front of the participants, and the cadence value required by the test was displayed every second using a custom MATLAB (R2016a Mathworks, Natick, MA) program. The participants were asked to change their pedalling cadence voluntarily and to continuously match as closely as possible the cadence value required by the test with that shown on the ergometer display. Work rate was fixed at 50% of VT1 and 60% of PPO throughout the ME and HE conditions, respectively, including the pre- and post-sinusoidal 4-min bouts at 85 rpm.

During PE, the lower limbs of the participant were moved passively using a fixed-wheel tandem bicycle, in line with previous reports (Bell et al. 2003). The shoes of the participant were strapped to the front pedals of the tandem bicycle, which were passively moved by the movement of the rear pedals. This movement was produced by a well-trained cyclist (the pacemaker) who was recruited exclusively for this task. The computer screen displaying the cadence value required by the test was placed in front of the pacemaker, who was asked to match it as closely as possible with the cadence value shown on the rpm counter of the tandem bicycle. Conversely, the participant was not allowed to see either the computer screen or the rpm counter. Participants were instructed to minimize their voluntary muscle activity throughout the PE test, and were provided with feedback from an experimenter dedicated entirely to checking their EMG activity during the test.

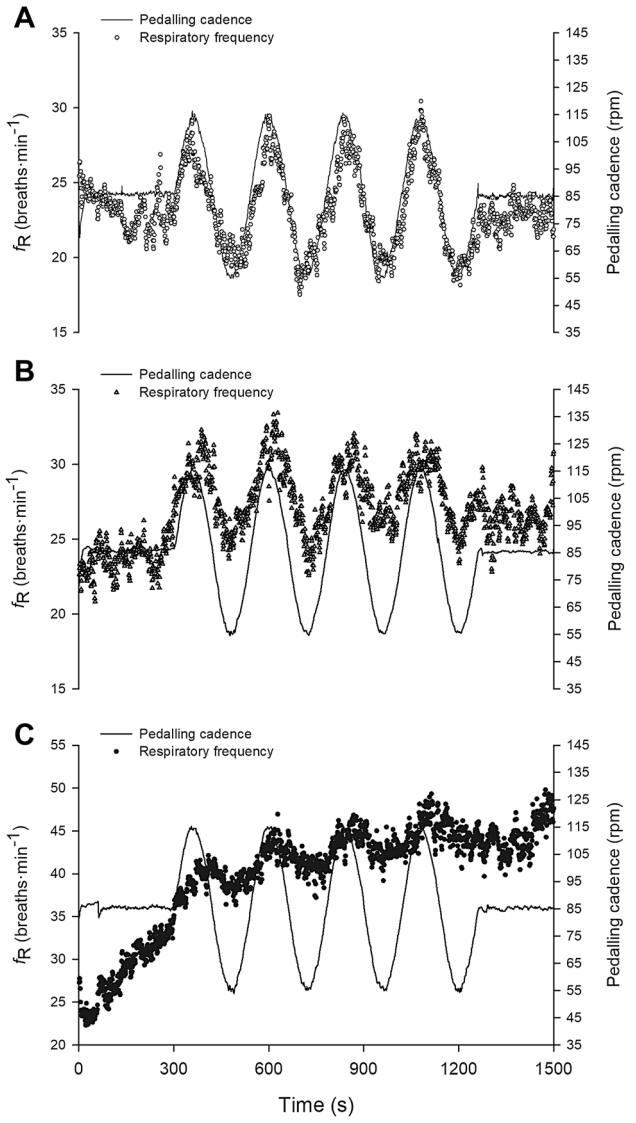

Fig. 1 Group mean response of f_R during PE (panel **a**, open circles), ME (panel **b**, open triangles) and HE (panel **c**, filled circles). The Figure depicts filtered second-by-second data. The solid line depicts the group mean response of pedalling cadence during PE (**a**), ME (**b**) and HE (**c**)

For all three conditions, participants were asked to rate their RPE at the zenith and nadir of each sinusoidal cycle, while the physiological variables were measured continuously throughout the test.

Cardiorespiratory and ventilatory variables

Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$),$\dot{V}_E$, f_R, V_T, end-tidal PCO_2 (P_{ETCO2}) and heart rate (HR) were measured breath by breath using a metabolic cart (Quark b2, Cosmed, Rome, Italy). Appropriate calibration procedures were performed following the manufacturer's instructions. Breath-by-breath data were edited for errant breaths (i.e. values resulting from sighs, swallows, coughs, etc.) by deleting values greater than 3 standard deviations from the local mean (Lamarra et al. 1987). Raw flow data (sampled at 25 Hz) were registered to compute the degree of entrainment (see "Entrainment" section).

EMG data

Surface EMG was recorded from the left vastus lateralis muscle with an 8-electrode adhesive linear array (5 mm interelectrode distance, OT Bioelettronica, Turin, Italy) via a multichannel amplifier (3 dB bandwidth, band-pass filter 10–500 Hz, Quattrocento, OT Bioelettronica, Turin, Italy). The EMG signal was acquired in a single differential configuration, sampled at 2048 Hz, and processed off-line using a custom MATLAB program. Before applying the adhesive array, the skin was shaved, slightly abraded, and cleansed with ethanol.

For the identification of each pedal cycle, a square-wave signal was obtained from a magnetic sensor placed top dead center on both the tandem bicycle used for PE and the cycle ergometer used for ME and HE. For each pedal cycle, the identification of the EMG burst was performed as described previously (Nicolò et al. 2018), and the root mean square (RMS) of the EMG signal was computed. Subsequently, RMS data were interpolated, extrapolated every second, and used for the calculation of the coefficients of the Fourier series as described in the "Sinusoidal analysis" section.

Sinusoidal analysis

Sinusoidal analysis was performed as described in a previous study (Nicolò et al. 2018). For all the variables reported in Table 1, the data were linearly interpolated, extrapolated every second, and processed using the Fourier analysis as previously reported (Casaburi et al. 1977, 1978). In accordance with classical procedures aimed at reducing the influence of random fluctuations (Casaburi et al. 1977, 1978), the first sinusoidal cycle was removed from the analysis, and the other three sinusoidal cycles were time-aligned and averaged. Subsequently, the a and b coefficients of the Fourier series were determined as follows:

$$a_k = \frac{2}{T}\sum_{t=0}^{T}\bar{x}(t)\cos\left(k\frac{2\pi}{T}t\right)\Delta t$$

$$b_k = \frac{2}{T}\sum_{t=0}^{T}\bar{x}(t)\sin\left(k\frac{2\pi}{T}t\right)\Delta t$$

where k corresponds to the number of harmonics considered ($k=1$ for the fundamental component), $\bar{x}(t)$ is the averaged

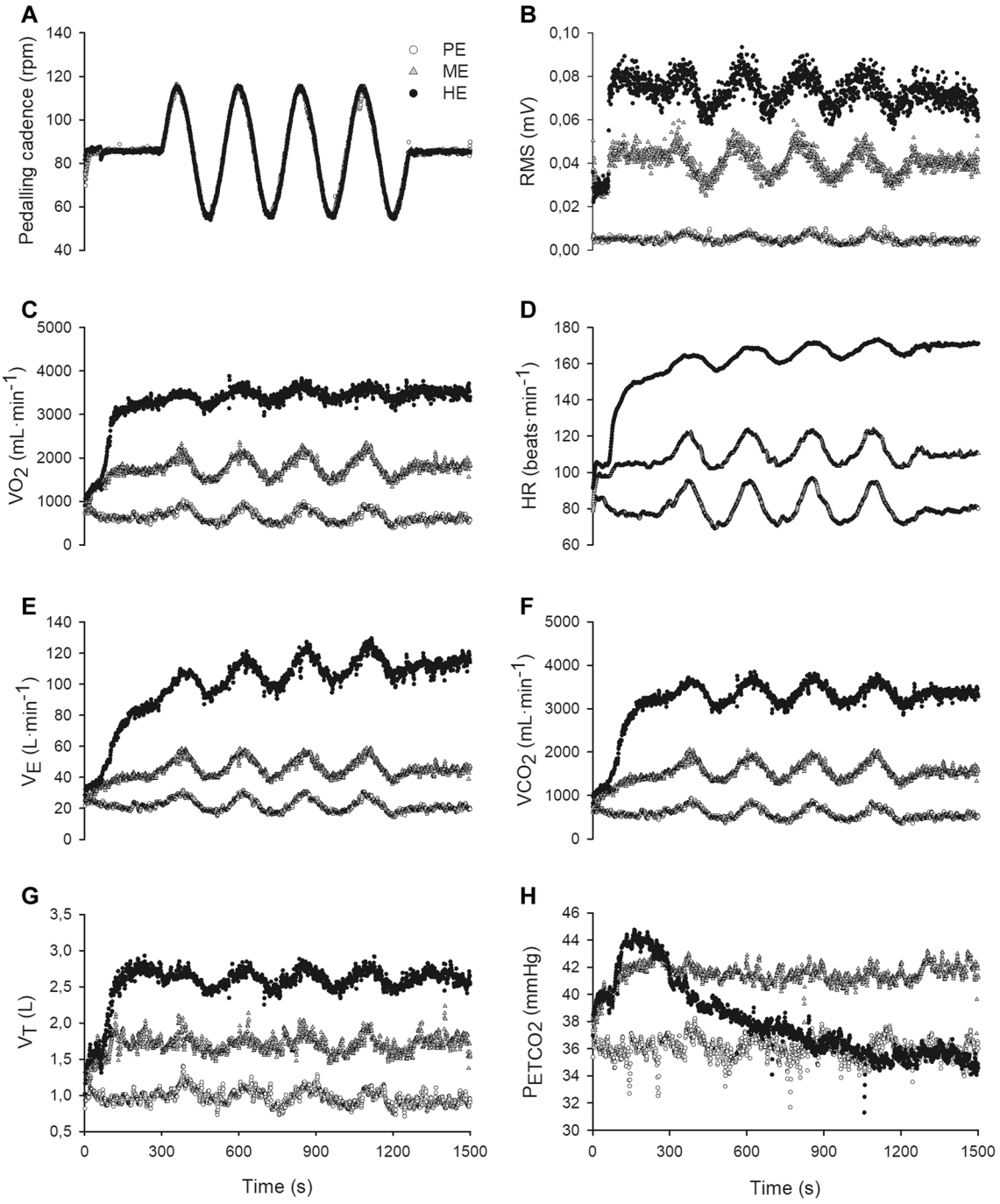

Fig. 2 Group mean response of pedalling cadence (**a**), RMS (**b**), $\dot{V}O_2$ (**c**), HR (**d**), $\dot{V}_E$ (**e**), $\dot{V}CO_2$ (**f**), V_T (**g**) and P_{ETCO2} (**h**) during PE (open circles), ME (open triangles) and HE (filled circles). The Figure depicts filtered second-by-second data

response at time t, T is the period of the sinusoid (i.e. 240 s) and Δt is the time interval between data points (i.e. 1 s). For each variable, the amplitude (A) and phase lag (φ) were computed as follows:

$$A = \sqrt{b^2 + a^2}$$

$$\varphi_{rad} = \arctan(b/a)$$

To obtain an indication of the linearity of the response of the measured variables, the A of the second and third harmonic components was computed considering k equal to 2 and 3 alternately. The magnitude of the A of the second

Table 1 Amplitude and phase lag (in degrees and seconds) of physiological variables for the three experimental conditions

	$\dot{V}O_2$ (mL·min^{-1})	$\dot{V}CO_2$ (mL·min^{-1})	P_{ETCO2} (mmHg)	$\dot{V}_E$ (L·min^{-1})	V_T (L)	f_R (breaths·min^{-1})	HR (beats·min^{-1})	RMS (m*V*)
A	#	#	#	#		#	#	#
PE	190 ± 65[a]	173 ± 52[ab]	1.0 ± 0.4	5.7 ± 1.4[ab]	0.13 ± 0.06	3.9 ± 1.4[b]	11.0 ± 3.6[b]	0.002 ± 0.003
ME	318 ± 27[b]	282 ± 32	0.6 ± 0.3	7.7 ± 1.8	0.13 ± 0.06	2.6 ± 1.3	9.5 ± 2.0[b]	0.008 ± 0.004
HE	146 ± 35	288 ± 38	0.4 ± 0.3	9.2 ± 1.7	0.15 ± 0.05	1.8 ± 1.0	4.0 ± 1.0	0.008 ± 0.007
φ (degrees)	#	#	#	#	#	#	#	#
PE	− 37.0 ± 20.2[ab]	− 36.1 ± 13.2[a]	− 61.2 ± 50.6	− 28.1 ± 11.1	− 64.7 ± 32.3[ab]	− 5.3 ± 13.9[b]	− 18.2 ± 7.2	− 8.7 ± 15.1
ME	− 11.7 ± 9.8	− 21.7 ± 10.7	− 19.7 ± 76.3	− 23.2 ± 10.6[b]	− 25.0 ± 20.7	− 25.4 ± 26.3	− 11.5 ± 15.4	52.4 ± 15.7
HE	− 12.3 ± 12.2	− 32.3 ± 9.9	34.5 ± 77.1	-38.8 ± 14.0	− 27.4 ± 20.2	− 70.1 ± 44.5	− 28.9 ± 12.9	44.8 ± 42.0
φ (seconds)								
PE	24.7 ± 13.5	24.0 ± 8.8	40.8 ± 33.8	18.7 ± 7.4	43.2 ± 21.5	3.5 ± 9.3	12.2 ± 4 .8	5.8 ± 10.0
ME	7.8 ± 6.5	14.4 ± 7.1	13.3 ± 50.9	15.4 ± 7.1	16.7 ± 13.8	16.9 ± 17.6	7.7 ± 10.3	− 34.6 ± 9.9
HE	8.2 ± 8.1	21.5 ± 6.6	− 23.0 ± 51.4	25.9 ± 9.3	18.3 ± 13.5	46.7 ± 29.6	19.2 ± 8.6	− 29.8 ± 28.0

Values are means ± SD. Statistical analysis was not performed on phase lag values in seconds, which are only provided to facilitate the physiological interpretation of the data

$\dot{V}O_2$ oxygen uptake, $\dot{V}CO_2$ carbon dioxide output, P_{ETCO2} end-tidal partial pressure of carbon dioxide, $\dot{V}_E$ minute ventilation V_T tidal volume, f_R, respiratory frequency, *HR* heart rate, *RMS* root mean square of the EMG signal, *A* amplitude, φ phase lag, *PE* passive exercise, *ME* moderate exercise, *HE* heavy exercise

[#]Effect of condition

[a]$P < 0.05$ vs. ME

[b]$P < 0.05$ vs. HE

and third harmonics was expressed as a percentage of the fundamental component (Wigertz 1970; Bakker et al. 1980); the lower the A of the second and third harmonics, the better a given dependent variable is described by a sinusoidal function.

In line with previously reported procedures (Nicolò et al. 2018), second-by-second data of the average sinusoidal cycle were averaged into 20 segments of 12 s, and correlations between variables were obtained as detailed in the "Statistical analysis" section.

Entrainment

The degree of entrainment between locomotion and ventilation was calculated as the number of expirations starting in the same phase of the pedal cycle and expressed as a percentage of the total number of breaths recorded (Bernasconi and Kohl 1993). The raw flow data and the square-wave signals used for identifying the pedal cycle were synchronized using a common trigger signal. Subsequently, the pedal cycle was subdivided into ten equal portions, and the portion of the pedal cycle in which expiration started was identified for each breath. For each of the ten portions, the distribution of the start of expiration was computed considering all the breaths. The percentage degree of entrainment was then calculated for each of the ten portions and considered significant when a value ≥ 15% ($P = 0.05$ for the chi-squared test) was observed (Bernasconi and Kohl 1993). When a significant degree of entrainment was reached for more than one portion of the pedal cycle in the same test, their percentages of entrainment were summed to obtain a single value. The degree of entrainment was calculated separately for the pre- and post-sinusoidal 4-min bouts at a fixed cadence of 85 rpm.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 23 (SPSS Inc, Chicago, Illinois, USA). A one-way repeated-measures ANOVA was used to compare the A and φ of physiological variables across the three conditions. The same analysis was used to compare the A of the second and third harmonics across conditions. When a significant difference was found, a paired Student's *t* test with Bonferroni correction was used as a follow-up analysis. A two-way repeated-measures ANOVA was used to compare the time course of RPE across conditions. The Greenhouse–Geisser adjustment was performed when the sphericity assumption was not fulfilled. Partial eta squared (η_P^2) effect sizes were calculated, an effect of $\eta_P^2 \geq 0.01$ indicating a small effect, $\eta_P^2 \geq 0.059$ a medium effect and $\eta_P^2 \geq 0.138$ a large effect (Cohen 2013) .

Using the method described by Bland and Altman (1995), within-subject correlation coefficients (*r*) were computed for evaluating the correlation between the time courses of $\dot{V}CO_2$ and V_T, $\dot{V}CO_2$ and $\dot{V}_E$, and cadence and f_R for each of the three sinusoidal tests. Pearson's correlation coefficients (*r*) were computed when correlating the A and φ of $\dot{V}CO_2$ with the *A* and φ of $\dot{V}_E$, V_T, and f_R.

A chi-squared test was used to verify whether significant degrees of entrainment occurred in the three experimental conditions.

A P value < 0.05 was considered statistically significant in all analyses. The results are expressed as means (± SD).

Results

The $\dot{V}O_{2peak}$ and the PPO measured during the ramp incremental test were 4325 ± 542 mL·min^{-1} and 410 ± 42 W, respectively. The work rate associated with VT1 and VT2 was 186 ± 27 W and 301 ± 22 W, respectively. Consequently, the work rate during the ME and HE tests was fixed at 93 ± 13 W and 246 ± 25 W, respectively. The exercise intensity set for the HE test resulted below VT2 for all the participants and corresponded to $82 \pm 10\%$ of VT2. The peak values of f_R, V_T and $\dot{V}_E$ recorded during the ramp incremental test were 62 ± 8 breaths·min^{-1}, 3.4 ± 0.5 L and 191 ± 29 L·min^{-1}, respectively.

Figure 1 shows the time course of the average response of the group for f_R during PE, ME and HE. Remarkably, f_R and cadence showed a very similar time course during PE, whereas a clear dissociation between the two variables was observed during HE. Figure 2 depicts the time course of cadence and relevant physiological variables for the three conditions, while Fig. 3 reports the response of the

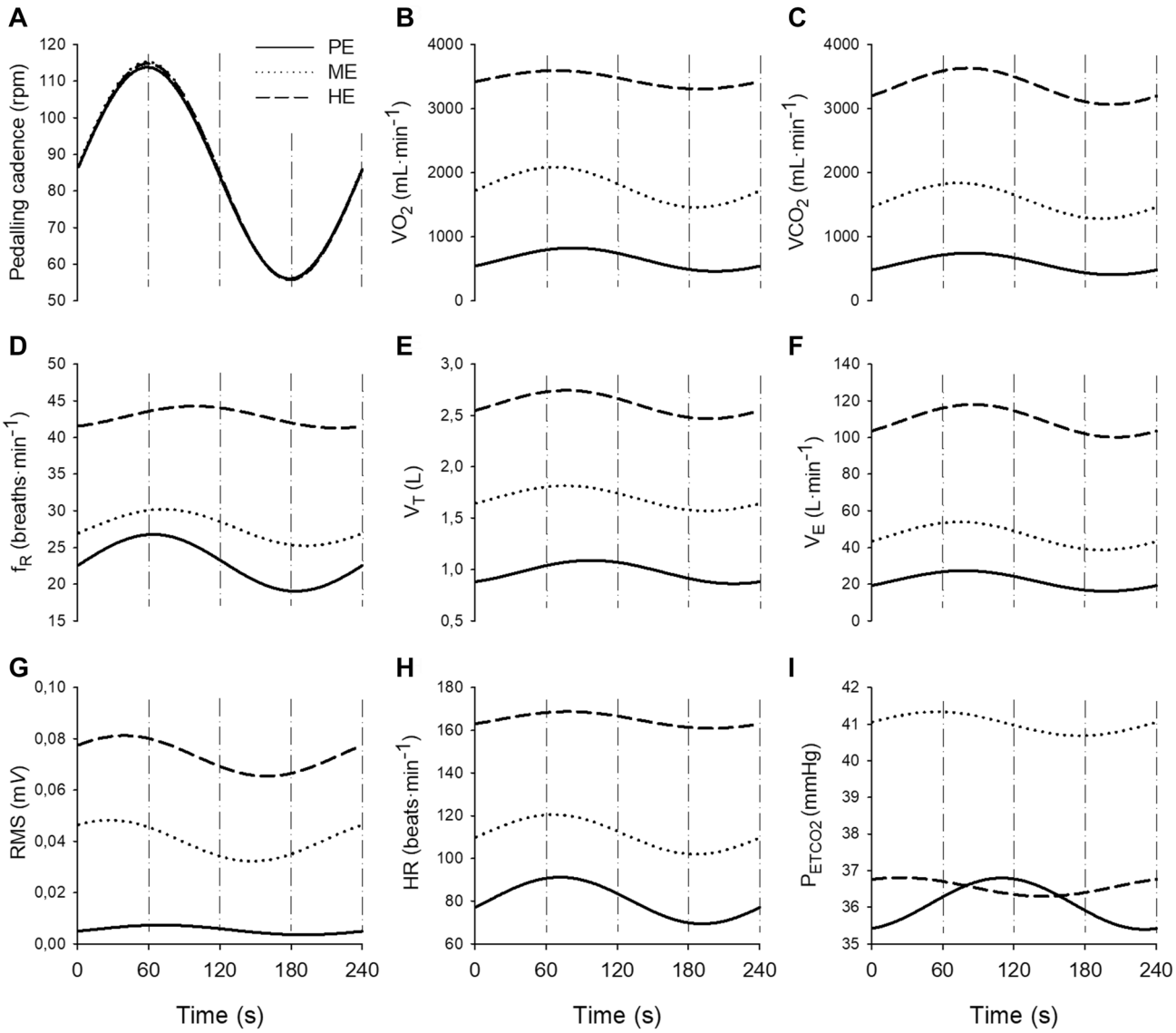

Fig. 3 Group mean fitted sinusoidal responses within the average sinusoidal cycle for pedalling cadence (**a**), $\dot{V}O_2$ (**b**), $\dot{V}CO_2$ (**c**), f_R (**d**), V_T (**e**), $\dot{V}_E$ (**f**), RMS (**g**), HR (**h**) and P_{ETCO2} (**i**) during PE (solid line), ME (dotted line) and HE (dashed line)

same variables, including f_R, within the average sinusoidal cycle. For the RMS of the EMG signal, only 9 participants were included in the analysis because of technical problems that occurred in one of the tests.

The A and φ of the physiological variables are reported in Table 1 for the three conditions. For the amplitude, a significant effect of condition ($P < 0.026$; $\eta_P^2 > 0.336$) was observed for all the variables considered, except for V_T. The Table highlights where significant differences in pairwise comparisons were observed between conditions, with the A of f_R being significantly ($P < 0.002$) higher during PE (3.9 ± 1.4 breaths·min^{-1}) compared to HE (1.8 ± 1.0 breaths·min^{-1}). For the phase lag, a significant effect of condition ($P < 0.009$; $\eta_P^2 > 0.413$) was found for all the variables considered, except for P_{ETCO2}. Table 1 highlights

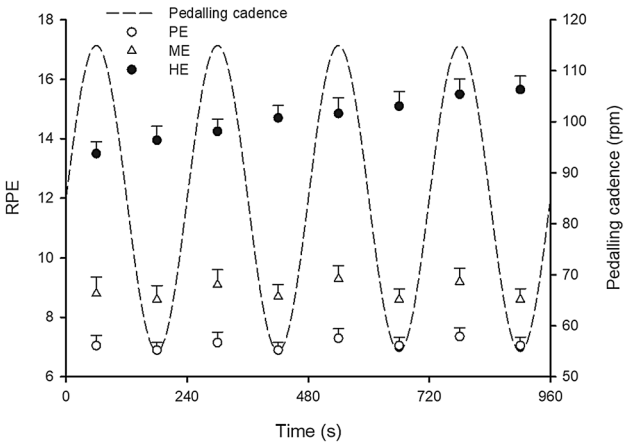

Fig. 4 Group mean response of RPE during PE (solid line), ME (dotted line) and HE (dashed line). The dashed line represents the cadence profile required by the test

where significant differences in pairwise comparisons were observed between conditions, with the φ of f_R being significantly ($P < 0.006$) shorter during PE (-5.3 ± 13.9 degrees) compared to HE (-70.1 ± 44 degrees). While statistical analysis was only performed on phase lag values in degrees, Table 1 also shows the φ in seconds to facilitate the physiological interpretation of the data.

Figure 4 shows the RPE values recorded at the zenith and nadir of each sinusoidal cycle for the three conditions. A main effect of condition ($P < 0.001$; $\eta_P^2 > 0.940$) and time ($P < 0.001$; $\eta_P^2 > 0.519$), and a significant interaction ($P < 0.05$; $\eta_P^2 > 0.448$) were found, suggesting that different levels of perceived exertion were observed across conditions.

For the same variables reported in Tables 1 and 2 shows the amplitude of the second and third harmonics expressed as a percentage of the fundamental component. During PE, relatively low A values were observed for the second and third harmonics of f_R, suggesting that f_R was well described by a sinusoidal function. Conversely, higher A values of f_R were observed for the second and third harmonics during active exercise. This was especially evident during HE where the A of the second harmonics was significantly higher ($P < 0.012$) than that of PE. This suggests that f_R did not show a linear response and was not well described by a sinusoidal function during HE.

The Bland–Altman correlation analysis showed a larger correlation between f_R and pedalling cadence during PE ($P < 0.001$; $r = 0.85$) compared to ME ($P < 0.001$; $r = 0.73$) and HE ($P < 0.001$; $r = 0.26$), suggesting that the association between the two variables decreased with the increase in work rate. A significant correlation ($P < 0.001$) with

Table 2 Amplitude of the second and third harmonic components as a percentage of the amplitude of the fundamental component for the three experimental conditions

	$\dot{V}O_2$ (mL·min^{-1})	$\dot{V}CO_2$ (mL·min^{-1})	P_{ETCO2} (mmHg)	$\dot{V}_E$ (L·min^{-1})	V_T (L)	f_R (breaths·min^{-1})	HR (beats·min^{-1})	RMS (mV)
2nd						#	#	
PE	32% ± 23	23% ± 10	49% ± 24	21% ± 10	27% ± 16	18% ± 12[b]	24% ± 10	51% ± 25
ME	15% ± 10	15% ± 9	66% ± 56	15% ± 9	34% ± 26	33% ± 24	16% ± 6	26% ± 20
HE	21% ± 11	13% ± 8	195% ± 191	24% ± 13	30% ± 14	59% ± 31	15% ± 5	41% ± 23
3rd	#	#		#		#	#	
PE	19% ± 7[a]	18% ± 9[ab]	38% ± 32	17% ± 8	22% ± 9	18% ± 9	9% ± 4	30% ± 15
ME	8% ± 5	7% ± 4	53% ± 40	9% ± 4	25% ± 22	20% ± 16	8% ± 3[b]	22% ± 16
HE	16% ± 11	8% ± 3	111% ± 120	14% ± 6	22% ± 17	40% ± 26	14% ± 5	27% ± 18

Values are means ± SD

$\dot{V}O_2$ oxygen uptake, $\dot{V}CO_2$ carbon dioxide output, P_{ETCO2} end-tidal partial pressure of carbon dioxide, $\dot{V}_E$ minute ventilation, V_T tidal volume, f_R respiratory frequency, *HR* heart rate, *RMS* root mean square of the EMG signal, *2nd* amplitude of the second harmonic component as a percentage of the amplitude of the fundamental component, *3rd* amplitude of the third harmonic component as a percentage of the amplitude of the fundamental component, *PE* passive exercise, *ME* moderate exercise, *HE* heavy exercise

[#]Effect of condition

[a]$P < 0.05$ vs. ME

[b]$P < 0.05$ vs. HE

$\dot{V}CO_2$ was found for both V_T (PE, $r=0.79$; ME, $r=0.79$; HE, $r=0.86$) and $\dot{V}_E$ (PE, $r=0.96$; ME, $r=0.98$; HE, $r=0.93$), but the extent of the correlation was higher for $\dot{V}_E$ compared to V_T in all the conditions.

Figure 5 shows the correlation of the A and φ of $\dot{V}CO_2$ with the A and φ of $\dot{V}_E$, V_T, and f_R. For the phase lag, a significant correlation was found between $\dot{V}_E$ and $\dot{V}CO_2$ ($P<0.001$; $r=0.76$) and $\dot{V}_T$ and $\dot{V}CO_2$ ($P<0.001$; $r=0.79$), but not between f_R and $\dot{V}CO_2$ ($P=0.82$; $r=0.04$). For the amplitude, a positive correlation was found between $\dot{V}CO_2$ and $\dot{V}_E$ ($P<0.001$; $r=0.73$), while a negative correlation was found between $\dot{V}CO_2$ and f_R ($P<0.05$; $r=-0.45$); no significant correlation was found between $\dot{V}CO_2$ and V_T ($P=0.11$; $r=0.29$).

Table 3 shows the results of the entrainment analysis performed separately for the sinusoidal protocol and the pre- and post-sinusoidal 4-min bouts at a fixed cadence of 85 rpm. No entrainment was found during sinusoidal exercise in any of the experimental conditions. Conversely, when pedalling cadence was kept constant at 85 rpm (pre- and post-sinusoidal exercise), a significant degree of entrainment was found for some of the participants. Table 3 reports further details on the average degree of entrainment and the number of participants who showed entrainment for each experimental condition.

Discussion

This study was designed to systematically assess the effect of pedalling cadence on the f_R response by providing sinusoidal variations in pedalling cadence during passive and active exercise of moderate and heavy intensity. The study's main finding was that the effect of cadence on f_R is moderated by

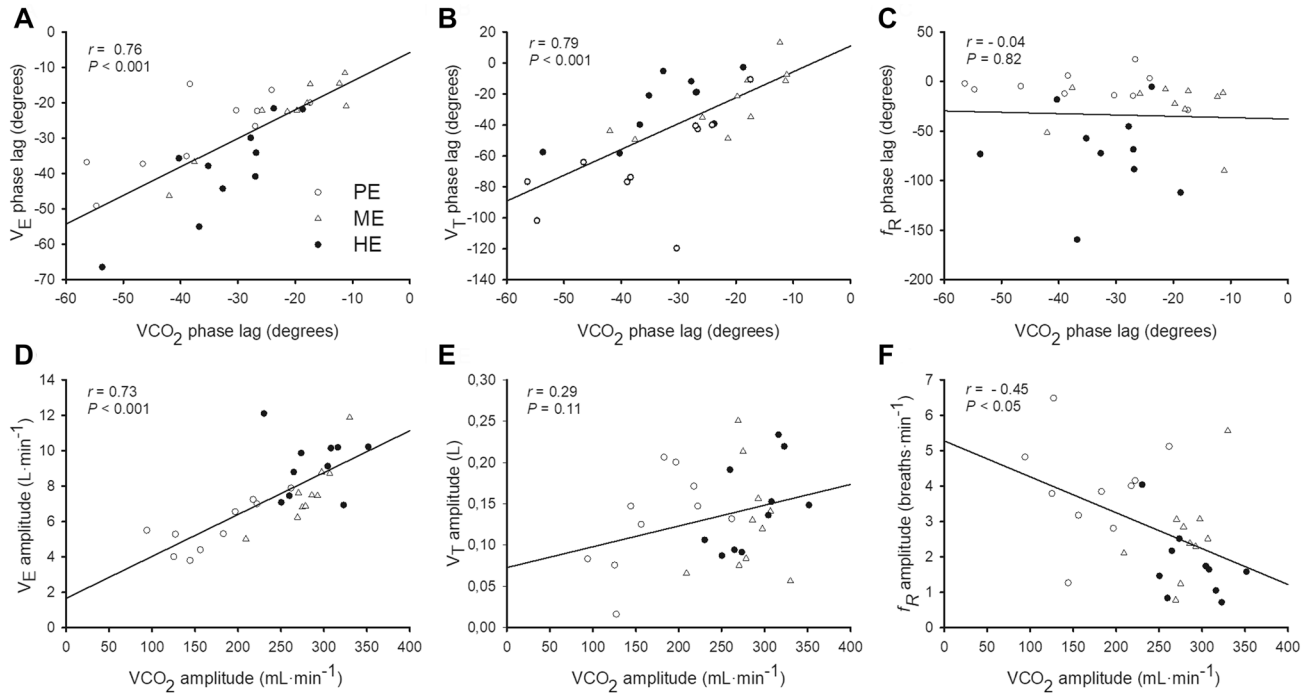

Fig. 5 Correlations between the phase lag of $\dot{V}CO_2$ and the phase lag of $\dot{V}_E$ (**a**), V_T (**b**) and f_R (**c**) for PE (open circles), ME (open triangles) and HE (filled circles). Correlations between the amplitude of $\dot{V}CO_2$ and the amplitude of $\dot{V}_E$ (**d**), V_T (**e**) and f_R (**f**) for PE (open circles), ME (open triangles) and HE (filled circles). For each panel, the correlation coefficient (r) and the related P value are reported

Table 3 Percentage degree of entrainment for the three experimental conditions in the different stages of the test

Stage of the test	PE	ME	HE
Pre-sinusoidal 85 rpm	17.01% ± 1.8 ($n=7$)	23.5% ± 6.9 ($n=3$)	20.8% ± 7.6 ($n=4$)
Sinusoidal protocol	NO	NO	NO
Post-sinusoidal 85 rpm	19.4% ± 6.2 ($n=5$)	20.1% ± 8.4 ($n=4$)	26.1% ± 15.3 ($n=2$)

Values are means ± SD

PE passive exercise, *ME* moderate exercise, *HE* heavy exercise; pre-sinusoidal 85 rpm, 4-min bout at 85 rpm preceding the sinusoidal protocol; post-sinusoidal 85 rpm, 4-min bout at 85 rpm following the sinusoidal protocol, *n* number of participants that showed entrainment, *NO* no entrainment

exercise intensity; the effect is remarkable during passive exercise, but it decreases during active exercise, especially during heavy-intensity exercise. Furthermore, this effect was not mediated by entrainment during sinusoidal exercise. The present findings provide novel insight into the link between pedalling cadence and f_R, suggesting potential mechanisms underlying this link.

To the best of our knowledge, this is the first study investigating the effect of pedalling cadence on f_R during passive sinusoidal exercise. This experimental condition revealed a strong link between pedalling cadence and f_R. First, we observed sinusoidal variations in f_R which closely resembled the variations in pedalling cadence (see Fig. 1, panel A). The accuracy in describing the f_R response by means of a sinusoidal function is corroborated by the analysis of harmonic components, showing a relatively small amplitude for the second and third harmonics compared to the fundamental component. Second, we observed a very small time delay between the fluctuations in pedalling cadence and the f_R response ($\varphi = -5.3 \pm 13.9$ degrees), while a substantial phase lag was observed for V_T ($\varphi = -64.7 \pm 32.3$ degrees). These findings corroborate previous results suggesting that f_R changes according to variations in pedalling cadence during passive exercise (Bell and Duffin 2003).

It is conceivable that the observed f_R response to PE was mainly regulated by muscle afferent feedback. The proposed experimental design was intended to decrease the relative contribution of other inputs potentially driving f_R, including the magnitude of central command, which was reduced to a great extent, as indirectly suggested by the EMG response. Furthermore, it is unlikely that f_R was driven by metabolic inputs, which were expected to show a delayed response compared to changes in pedalling cadence, as suggested by the phase lag of $\dot{V}CO_2$. Besides, metabolic inputs appear not to play a substantial role in the regulation of f_R (Nicolò et al. 2017a, 2018, 2020; Tipton et al. 2017). The precise nature of the afferent feedback mediating the observed f_R response cannot be determined by our experiments because the activity of muscle afferent fibres was not measured directly. However, previous research can help identify possible neural pathways. Unlike for afferents from groups III and IV, evidence suggests that afferent feedback from groups I and II does not influence ventilation substantially (McCloskey and Mitchell 1972). The fact that we found no entrainment during sinusoidal exercise suggests that the effect of cadence on f_R is not necessarily mediated by entrainment. This is in line with findings on animals suggesting that afferent feedback from groups III/IV can drive f_R even when entrainment is prevented by blocking the neurons contributing to this phenomenon (Potts et al. 2005). On the other hand, the passive movement of the legs may determine changes in blood flow (Radegran and Saltin 1998; Saltin et al. 1998), possibly stimulating the mechanoreceptors located in the adventitia of the postcapillary and venous network. This matters because group III/IV afferents from these receptors may influence ventilation, as proposed by the vascular distension hypothesis (Haouzi et al. 2004). The activation of metabolically sensitive III/IV afferent fibres (metaboreflex) has also been suggested as influencing f_R (Lam et al. 2019). However, the nature of our experimental intervention (passive movement of the legs) could suggest that mechanoreceptors contributed more than metaboreceptors to the observed response of f_R (Bruce et al. 2019), but further research is needed to specifically address this issue (see "Limitations" section). Collectively, the present findings reinforce the evidence that group III/IV muscle afferents play a role in regulating f_R.

Importantly, this study showed that the effect of pedalling cadence on f_R is moderated by exercise intensity. During ME, we observed a time delay of f_R ($\varphi = -25.4 \pm 26.3$) compared to the changes in cadence, which is in line with the delayed response of f_R reported by Caterini et al. (2016) during a similar sinusoidal test. Furthermore, we observed a decrease in the amplitude of f_R compared to that of PE. A reduction in the association between f_R and pedalling cadence is further suggested by the lower correlation found during ME ($r = 0.73$) compared to PE ($r = 0.85$). Furthermore, the analysis of harmonic components revealed that the f_R response was not represented so well by the fundamental component as compared to PE, thus highlighting a reduced accuracy in describing the f_R response with a sinusoidal function. Taken together, these data suggest that the effect of pedalling cadence on f_R is reduced during active exercise of moderate intensity compared to PE. This is in line with findings from previous studies showing a partial dissociation between cadence and f_R during moderate exercise (Berry et al. 1989; Jastrzębska and Kowalski 2015; Mitchell et al. 2019).

The effect of pedalling cadence on f_R was even more reduced during HE. The phase lag of f_R was both substantial ($\varphi = -70.1 \pm 44.5$ degrees) and variable across participants, and the amplitude was the lowest found in the three experimental conditions. Furthermore, harmonic analysis revealed that the f_R response during HE was poorly described by the fundamental component, suggesting that f_R did not show a clear sinusoidal response. The correlation between pedalling cadence and f_R during HE was much smaller ($r = 0.26$) than that observed during PE and ME. Moreover, f_R showed the classical drift usually observed during high-intensity exercise, indicating a dissociation between the time courses of pedalling cadence and f_R. These findings are in line with numerous observations showing a dissociation between these two variables during high-intensity exercise. During trapezoidal changes in RPE, f_R showed large fluctuations despite pedalling cadence being fixed (Nicolò et al. 2018). During incremental exercise, pedalling cadence is relatively constant while f_R increases in an exponential fashion

in different populations (Lucía et al. 1999; Gravier et al. 2013). During self-paced performance trials where pedalling cadence is relatively constant, f_R shows a linear increase over time (Nicolò et al. 2016). Collectively, the present and previous findings suggest that there is no proportionality between changes in pedalling cadence and f_R during high-intensity exercise. This does not mean that variations in cadence cannot affect f_R or $\dot{V}_E$ during high-intensity exercise, but that proportional changes between these variables may not occur (see below for discussion on the underlying mechanisms).

The comparison between the three sinusoidal tests may contribute to our understanding of the mechanisms regulating f_R during exercise. If muscle afferent feedback plays a primary role in regulating f_R during PE, the relative contribution of afferent feedback on f_R may decrease with the increase in exercise intensity during active exercise. This interpretation is in line with direct evidence indicating that the relative contribution of group III/IV muscle afferents to f_R regulation is lower during high-intensity compared to moderate-intensity exercise (Amann et al. 2010). During high-intensity exercise, other inputs may contribute to regulating f_R to a larger extent. Indirect evidence suggests that central command is a major input regulating f_R during high- but not moderate-intensity exercise (Nicolò et al. 2018; Nicolò and Sacchetti 2019). This is supported by the close association observed between f_R and RPE during a variety of high-intensity exercise protocols (Nicolò et al. 2017c), central command being a major regulator of RPE (de Morree et al. 2014; Zenon et al. 2015). The importance of central command in the control of f_R is corroborated by evidence obtained from neuroimaging studies (Thornton et al. 2001; Green et al. 2007). As such, the progressive increase in RPE during HE is in line with the possibility that central command may have at least partially determined the drift observed for f_R. Therefore, it is conceivable that the increase in the magnitude of central command with exercise intensity (i.e. from PE to HE) and time-on-task (de Morree et al. 2014; Nicolò et al. 2015, 2017b) has determined a reduction in the relative contribution of group III/IV muscle afferent feedback to the f_R response observed during HE. However, the identification of the contribution of central command and muscle afferent feedback to f_R regulation is complicated by the possible interactions occurring between these two inputs (Holwerda and Vianna 2019; Lam et al. 2019). Therefore, further research is needed to shed some light on this issue.

It is important to note that changes in pedalling cadence during active exercise do not lead only to variations in the magnitude of muscle afferent feedback. In fact, the act of voluntarily changing cadence may also determine changes in the magnitude of other inputs regulating ventilation (e.g. central command) that are not necessarily in proportion to and in phase with sinusoidal variations in cadence. Indeed, electroencephalographic data show a modulation in brain activity when pedalling cadence varies (Ludyga et al. 2016). Furthermore, neuromuscular recruitment strategies change with variations in cadence (Farina et al. 2004). These findings suggest that central command may change with pedalling cadence and, thus, affect f_R. It has also been established that cadence influences metabolic rate, as shown by the present and previous findings (Mitchell et al. 2019; Casaburi et al. 1978; Jastrzębska and Kowalski 2015), but metabolic inputs may not affect f_R substantially (Nicolò et al. 2017a, 2018, 2020; Tipton et al. 2017). Therefore, caution would be advised when attempting to explain the effect of cadence on f_R with changes in the magnitude of muscle afferent feedback during voluntary exercise. Nevertheless, the present experimental design provided an original solution to partially addressing this issue. First, we compared three different exercise-intensity domains, thus providing different levels of magnitude for some of the inputs driving ventilation (i.e. central command and metabolic inputs). Second, we used the sinusoidal exercise to quantitatively evaluate (using A and φ) whether changes in ventilatory variables are proportional to changes in pedalling cadence. In view of the substantial phase lag found for f_R during ME and especially HE—but not during PE—we suggest that the fluctuations observed for f_R during active exercise should not be entirely attributed to muscle afferent feedback. Indeed, a very short phase lag is found when muscle afferent feedback is the primary driver of f_R (i.e. during PE). While changes in the magnitude of central command may largely explain these between-condition differences observed in the f_R response, other factors also require consideration. The understanding of the contribution of muscle afferent feedback to f_R during HE is further complicated by the fact that high-intensity exercise may have led to changes in the magnitude of metabolically sensitive III/IV muscle afferent fibres that were not necessarily proportional to the sinusoidal fluctuations in pedalling cadence. Therefore, we cannot exclude that metabolically sensitive III/IV muscle afferent fibres may have contributed to the increase in the phase lag of f_R observed during HE and/or to the reduction in the f_R amplitude. It is also possible that the response of f_R during HE was affected by some intrinsic properties of the respiratory neurons, which may show a stimulus-like response with an exponential decay at the removal of the stimulus (i.e. the so-called short-term potentiation phenomenon) (Fregosi 1991; Forster et al. 2012). Hence, various factors may have contributed to the observed reduction in the effect of cadence on f_R during HE. The present study may inspire the design of future studies aiming to shed some light on this issue. For instance, further insight into the relative contribution of the inputs driving f_R is expected from the comparison of passive and active exercise when different sinusoidal

periods are imposed. Our findings support the notion that f_R and V_T are regulated, at least to some extent, by different inputs during exercise (Nicolò et al. 2017b, 2018, 2020; Nicolò and Sacchetti 2019). A time delay was found for V_T compared to changes in pedalling cadence in the three sinusoidal tests, especially in the PE test. This suggests that muscle afferent feedback may not be a primary regulator of V_T, which is in line with previous findings (Amann et al. 2010; Lam et al. 2019). Conversely, V_T is largely regulated by metabolic inputs and mediates the close association found between $\dot{V}_E$ and $\dot{V}CO_2$ during different exercise protocols (Nicolò et al. 2018; Nicolò and Sacchetti 2019). This is further evident from the present findings where a clear dissociation was found between f_R and $\dot{V}CO_2$; their phase lags showed no association while their amplitudes were even negatively correlated (see Fig. 5). Nevertheless, a close association was found between $\dot{V}_E$ and $\dot{V}CO_2$, suggesting that this link is mediated by V_T. These findings are in line with the proposition that V_T is adjusted continuously on the basis of f_R levels and the magnitude of metabolic inputs to match alveolar ventilation with metabolic requirements (Haouzi and Bell 2009; Nicolò et al. 2018; Nicolò and Sacchetti 2019).

The present findings may have important practical implications for different diseases. For instance, passive exercise has been proposed as a non-invasive solution to support paediatric patients with congenital central hypoventilation syndrome during non-REM sleep (Gozal and Simakajornboon 2000). Passive exercise managed to reduce hypercapnia via an increase in f_R and alveolar ventilation when these patients were temporarily disconnected from mechanical ventilation (Gozal and Simakajornboon 2000). Hence, the understanding of the ventilatory responses to sinusoidal passive exercise may provide useful insight to tailor passive exercise prescription in patients with congenital central hypoventilation syndrome. Likewise, it is conceivable that patients with obesity hypoventilation syndrome may benefit from the hyperventilation and hypocapnic responses induced by passive exercise, as they present with hypercapnia and hypoxemia (Olson and Zwillich 2005). Similar implications of passive exercise can be foreseen for other patients with chronic hypoventilation, including those with severe chronic obstructive pulmonary disease (Olson and Zwillich 2005). However, little is known on the acute and chronic effects of passive exercise on the ventilatory responses of diseased populations, hence requiring further investigation.

Limitations

The difficulty of investigating the control of ventilation during 'real' exercise conditions poses some challenges that need to be acknowledged. The passive exercise modality selected in this study (using a tandem bicycle) was chosen to match as closely as possible the posture of the participant and the pattern of movement across the three conditions, thus allowing for a between-condition comparison of the physiological responses. This experimental need was considered more important than the attempt to minimize the changes in $\dot{V}O_2$ and $\dot{V}CO_2$, and this is why the tandem bicycle was preferred to alternative modalities of passive exercise that are more effective in reducing such changes (Bell et al. 2003). The observed variations in $\dot{V}O_2$ and $\dot{V}CO_2$ can be attributed, at least partially, to the recruitment of postural muscles to stabilize the body (Bell et al. 2003). Hence, the PE condition was not designed to isolate the muscle afferent feedback drive to ventilation but to increase its relative contribution compared to that of other inputs driving ventilation (i.e. central command and metabolic inputs). Consequently, we cannot exclude that metabolically sensitive III/IV afferent fibres, metabolic inputs or central command may have contributed to some extent to the response of f_R observed during PE.

Conclusion

By imposing sinusoidal variations in pedalling cadence during passive and active exercise of different intensities, this study provided novel insight into the link between pedalling cadence and f_R. During passive exercise, we found a remarkably close link between pedalling cadence and f_R, with f_R changing in proportion to cadence. However, this link was moderated by exercise intensity, with a substantial reduction in the effect of cadence on f_R during high-intensity exercise. These findings suggest that group III/IV muscle afferent feedback is an important driver of f_R, but its relative contribution to f_R regulation may decrease with the increase in exercise intensity. Unlike f_R, V_T was dissociated from pedalling cadence in all the conditions tested, reinforcing the notion that f_R and V_T are regulated by different inputs. Collectively, these findings provide novel insight into the control of ventilation during exercise.

Author contribution Conception or design of the work: M.G., A.N., and M.S. Acquisition, analysis or interpretation of data for the work: M.G., A.N., I.B., F.F. and M.S. Drafting the work or revising it critically for important intellectual content: M.G., A.N., I.B., F.F. and M.S. 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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Availability of data and material The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Complaince with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee (Ethics Committee of the University of Rome Sapienza) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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