Influence of Step Frequency on the Dynamic Characteristics of Ventilation and Gas Exchange During Sinusoidal Walking in humans

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We tested the hypothesis that restricting either step frequency (SF) or stride length (SL) causes a decrease in ventilatory response with limited breath frequency during sinusoidal walking. In this study, 13 healthy male and female volunteers (mean ± SD; age: 21.5 ± 1.8 years, height: 168 ± 7 cm, weight: 61.5 ± 8.3 kg) participated. The walking speed was sinusoidally changed between 50 and 100 m·min⁻¹ with periods from 10 to 1 min. Using a customized sound system, we fixed the SF at 120 steps·min⁻¹ with SL variation (0.83–0.41 m) (SFfix) or fixed the SL at 0.7 m with SF variation (143–71 steps·min⁻¹) (SLfix) during the subjects’ sinusoidal walking. Both the subjects’ preferred locomotion pattern without a sound system (Free) and the unprompted spontaneous locomotor pattern for each subject (Free) served as the control condition. We measured breath-by-breath ventilation [tidal volume (VT) and breathing frequency (Bf)] and gas exchange [CO₂ output (˙VCO₂), O₂ uptake (˙VO₂)]. The amplitude (Amp) and the phase shift (PS) of the fundamental component of the ventilatory and gas exchange variables were calculated. The results revealed that the SFfix condition decreased the Amp of the Bf response compared with SLfix and Free conditions. Notably, the Amp of the Bf response under SFfix was reduced by less than one breath at the periods of 5 and 10 min. In contrast, the SLfix condition resulted in larger Amps of Bf and ˙V₄ responses as well as Free. We thus speculate that the steeper slope of the ˙V₄-˙VCO₂ relationship observed under the SLfix might be attributable to the central feed-forward command or upward information from afferent neural activity by sinusoidal locomotive cadence. The PSs of the ˙V₄, ˙VO₂, and ˙VCO₂ responses were unaffected by any locomotion patterns. Such a sinusoidal wave manipulation of locomotion variables may offer new insights into the dynamics of exercise hyperpnea.

Keywords: ventilation, breath frequency, step frequency, stride length, entrainment
INTRODUCTION

Humans’ sinusoidal exercising is clearly voluntary rhythmic movement in response to the stress of a varying work rate or speed. The resulting exercise-induced hyperpnea is expected to be integrated with chemical feedback from both central (Bell, 2006) and peripheral chemoreceptors (Forster et al., 2012; Ebine et al., 2018),afferent neural activity from working muscles (Eldridge et al., 1982; Eldridge, 1994; Amann et al., 2010; Forster et al., 2012), and feedforward signals from the motor cortex (Comroe and Schmidt, 1943; Dejours, 1959; Eldridge et al., 1981).

Several previous studies have used sinusoidal work forcing to define the resulting kinetics of cardiac, ventilatory, and gas exchange responses (Casaburi et al., 1977, 1978; Fukuoka et al., 2017; Ebine et al., 2018), and it is well established that these responses to a sinusoidal workload will have a consistent fundamental frequency component, which can be characterized by a mean value ($M_x$), an amplitude ($Amp$), and a phase shift ($PS$) on gas exchange kinetics (Casaburi et al., 1977). Moreover, several investigators have observed that for the same increase in metabolic rate, the hyperpnea is greater when the treadmill speed or pedaling frequency during cycling is increased, as opposed to an increase in the treadmill grade or cycling resistance (Bechbache and Duffin, 1977; Bramble and Carrier, 1983; Casey et al., 1987; Lafortuna et al., 1996). During sinusoidal exercise, greater Amps and lower Ps for pulmonary ventilation ($\dot{V}_E$) were observed when the limb-movement frequency was varied sinusoidally by alterations in cycling work rates (Casaburi et al., 1977) or in treadmill speed (Wells et al., 2007). The PS of $\dot{V}_E$ dynamics during a sinusoidal leg-cycling exercise was less for the cadence variation than for the sinusoidal pedal forcing variation (Duffin, 2014). It may also be possible that the sinusoidal step frequency (SF) of walking accelerates the ventilatory dynamics as the SF is equivalent to pedaling cadence.

Sinusoidal exercise protocols can be considered a robust method to investigate multiple factors that affect the ventilatory response (Caterini et al., 2016), and these protocols may be suitable for avoiding the entrainment of breath frequency to limb movement (Bechbache and Duffin, 1977). In this study, the SF was forced sinusoidally to minimize contributions from locomotor-respiratory entrainment even at a constrained stride length (SL) during sinusoidal walking (Caterini et al., 2016). The variation in the SF would induce sufficient exercise hyperpnea, possibly involving muscle reflex drives from type III and IV afferents (Haouzi et al., 2004; Haouzi, 2006; Bruce et al., 2019) or the central feed-forward command (i.e., feedforward mechanism from the higher central nervous system to locomotor and respiratory neurons) (Eldridge and Waldrop, 1991; Waldrop et al., 1996; Bell, 2006); and (ii) locomotion with a fixed SF may cause dynamic ventilatory depression (i.e., Amp depression in ventilation) as a secondary result of constrained Bf due to a respiratory-locomotor network (Le Gal et al., 2020). To test this hypothesis, we investigated whether the ventilatory and gas exchange responses showed different dynamics in conditions with different locomotion patterns during walking with sinusoidal speed changes.

MATERIALS AND METHODS

Subjects

The subjects were 13 healthy young male ($n = 7$) and female ($n = 6$) volunteers [age: $21.5 \pm 1.8$ years, height: $168 \pm 7$ cm, weight: $61.5 \pm 8.3$ kg; mean $\pm$ standard deviation (SD)] who were not taking any medication that could affect cardiovascular responses. The subjects were fully informed of any risks and discomforts associated with these experiments before giving their written, informed consent to participate in the study, which was approved by the ethics committees of the Institutional Review Board of Doshisha University (no. 1045).

Protocols

All experimental tests were completed in a temperature-controlled laboratory (25 $\pm$ 0.4°C with 50 ± 3% relative humidity), and all subjects wore underwear, shorts, and a T-shirt, as well as socks and shoes. The protocols used herein are based on our previous work (Fukuoka et al., 2017). The treadmill speed was changed in a sinusoidal pattern from 50 to 100 m-min$^{-1}$ at periods (T) of 10, 5, 2, and 1 min. A warm-up session consisted of steady-state walking for 4 or 6 min, which preceded each recording sinusoidal exercise session. In Protocol I, after 50, 100, and 75 m-min$^{-1}$ for 5, 3, and 4 min of warm-up walking, the sinusoidal loading was repeated for five cycles at 1-min periods, followed by three cycles at 2-min periods (Figure 1A). In Protocol II, after 75 m-min$^{-1}$ for 6 min of warm-up walking, the sinusoidal loading was repeated for three cycles at 5-min periods, followed by two cycles at 10-min periods (Figure 1E). A microcomputer transmitted the signal controlling the speed of the motor driving the treadmill (modified TMS 2200, Nihon Koden, Tokyo) through a digital-analog converter.

Using a customized personal computer sound cueing system (Arco Metronome, Ver.2.00, Arco System, Chiba, Japan), we set two locomotion patterns. In the fixed SL ($SL_{fix}$) condition, the SL was fixed at 0.7 m and the SF varied between 143 and 71 steps-min$^{-1}$ in coordination with the sinusoidal changes in treadmill speed (Figures 1B,F; Latt et al., 2008). In the fixed SF ($SF_{fix}$) condition, the SF was set at 120 steps-min$^{-1}$ with SL variation (0.83–0.41 m) during sinusoidal walking (Figures 1C,G; Casaburi et al., 1978). The subjects were instructed to synchronize their locomotion to the sound. In addition, the subjects were allowed to breathe freely, so breathing and walking were not...
FIGURE 1 | Two sinusoidal locomotion protocols at a sinusoidal speed between 50 and 100 m·min⁻¹ at the period of 1·2 min (A) and 5·10 min (E). The stride length (SL) was fixed at 0.7 m with step frequency (SF) variation (143–71 step·min⁻¹) (SLfix) (B,F) and the SF was fixed at 120 steps·min⁻¹ with stride variation (0.83–0.41 m) (SFfix) (C,G). The control (Free) condition was the subjects’ preferred locomotion pattern (D,H).

intentionally synchronized. The subject’s preferred locomotion pattern without the sound was used as the control condition (Free) (Figures 1D,H). The subjects performed each protocol on six separate occasions metricconverterProductID0.7 m (one session at a time, three sessions per week for each individual).

Measurements
A mass-flow sensor (type AB, Minato Medical Sciences, Osaka) was fit to the expiratory port of the valve of the face mask worn by the subject to continuously record the subject’s expiratory airflow, which was calibrated before each measurement with a 3-L syringe at three different flow rates. We calculated the ventilation (VE) values by integrating the tidal volume (VT; L) and breathing frequency (BF; breaths·min⁻¹). The end-tidal oxygen pressure (PETO₂; mmHg) and carbon dioxide pressure (PETCO₂; mmHg) were determined using mass spectrometry (Arco-2000, Arco System, Chiba, Japan) from a sample drawn continuously from the inside of the face mask at 1 ml·s⁻¹. This loss of gas volume was not examined in this study, because the loss of 1 ml·s⁻¹ was much smaller than the inspired and expired airflows. Three reference gases of known concentrations (O₂: 15.04%, CO₂: 2.92%, and N₂: 82.04%; O₂: 11.93%, CO₂: 6.96%, and N₂: 81.11%) and room air (O₂: 20.93%, CO₂: 0.03%, Ar: 0.94%, and N₂: 78.10%) were used to calibrate the mass spectrometer.

The volumes, flows, partial pressure of carbon dioxide (PCO₂), and partial pressure of oxygen (PO₂) at the subject’s
mouth were recorded in real time with a 50-Hz sampling frequency using a computerized online breath-by-breath system (AE-280, Minato Medical Sciences, Osaka) from the time-aligned gas volume and concentration signals. Breath-by-breath $\dot{V}_E$ (BTPS), $\dot{V}_{\text{O}_2}$ (STPD), and $\dot{V}_{\text{CO}_2}$ (STPD), $VT$, $Bf$, $P_{ET,\text{CO}_2}$, and $P_{ET,\text{O}_2}$ were determined. An electrocardiogram (ECG) was recorded using a bioamplifier (AB 621G, Nihon Kohden, Tokyo). Heart rate (HR) was measured by beat-by-beat counting from the R spike of the ECG. The signals from the treadmill were fed into a data acquisition system (PowerLab system, A/D Instruments, Castle Hill, NSW, Australia) and temporally aligned to the ventilatory and ECG data.

In all subjects, the SF and SL were measured using a switch activated by the subject stepping on a sensor on the sole of the right foot of the right leg in each protocol. The signals from the treadmill and the stepping sensor were fed into the PowerLab data acquisition system and temporally aligned with the ventilatory and ECG data.

Data Analysis
All the cardiorespiratory and locomotive data were analyzed using a Fourier analysis as previously reported (Wigertz, 1971; Haouzi et al., 1992, 2004; Fukuoka et al., 2017). The breath-by-breath ventilatory and gas exchange data were interpolated into a 1-s interval value before Fourier analysis (Supplementary Figure 1). The repeated cardiorespiratory responses to sinusoidal walking speed were superimposed in correspondence with the cycle period, and we obtained the average cardiorespiratory data at each respective cycle. The variation in the speed of the treadmill was regarded as the input function. The $Amp$ (i.e., mean to peak) and the $PS$ of the fundamental component (the same frequency as the input function) of the $\dot{V}_E$, $\dot{V}_{\text{O}_2}$, $\dot{V}_{\text{CO}_2}$, HR, and end-tidal $P_{\text{CO}_2} (P_{ET,\text{CO}_2})$ responses as well as the locomotion responses (locomotion SF and SL) were computed as follows:

$$Amp = \sqrt{Re^2 + Im^2}$$  \hspace{1cm} (1)

and

$$PS = \tan^{-1}\left(\frac{Re}{Im}\right)$$  \hspace{1cm} (2)

where the $Re$ and $Im$ are the real and imaginary components; these were calculated as follows. The larger the $PS$, the slower the response. The larger the $Amp$, the higher the responsiveness.

$$Re = \frac{2}{NT} \sum_{t=0}^{NT} \left[ (x(t) - Mx) \cos(2\pi ft) \right]$$  \hspace{1cm} (3)

and

$$Im = \frac{2}{NT} \sum_{t=0}^{NT} \left[ (x(t) - Mx) \sin(2\pi ft) \right]$$  \hspace{1cm} (4)

where $x(t)$ is the response value at time $t$ (in s), $Mx$ is the mean value of $x$ for an integer number of cycles ($N$), $T$ is the period of the input signal (in s), and $f \ (= 1/T)$ is its frequency in cycles per second.

In all subjects, the SF and SL were measured using a switch activated by stepping on a sensor on the sole of the right foot in each protocol. The signals from the treadmill and the stepping sensor were fed into the PowerLab data acquisition system and temporally aligned with the ventilatory data.

We normalized the ratios of $Amp$ of the respiratory and locomotion variables against sinusoidal change in walking speed by dividing the magnitude of variables from 50 to 100 m min$^{-1}$ during each steady-state exercise, and the results are presented as the $Amp$ ratio (%) (Fukuoka et al., 2017).

The R-R intervals during sinusoidal work were calculated beat-by-beat by the computer, and 1-s interval HR data were measured from the calculated R-R intervals (R-R) and converted as HR values (60/R-R). The subject’s locomotion SF and SL were measured with a switch activated by stepping on a sensor on the sole of the right foot in each protocol (Fukuoka et al., 2017).

Statistical Analyses
All values are presented as mean ± SD. The significance of differences in each variable ($\dot{V}_E$, $VT$, $Bf$, $\dot{V}_{\text{O}_2}$, $\dot{V}_{\text{CO}_2}$, and HR) was determined by a two-way repeated-measures analysis of variance (ANOVA) in the comparison of the three locomotion
patterns (SFfix, SLfix, and Free) × oscillation frequency period (T; 1 to 10 min). Bonferroni’s test was applied for the appropriate datasets if a significant F-value was obtained. We compared the regression coefficients of the independent variables of $V_E$ of the three locomotion patterns (SFfix, SLfix, and Free). The level of significance was set at $p < 0.05$.

RESULTS

Figure 2 shows the representative smoothing SLfix, SFfix, and Free locomotion patterns at the period of 5 min. The SLfix and SFfix were strictly controlled (Figures 2A,B). In Free locomotion, both the SL and SF responded almost synchronously with the sinusoidal walking speed (Figure 2C). The Amp, PS, and Mx under the SFfix or SLfix conditions at all periods (T: 1, 2, 5, and 10 min) are given in Figure 3. Compared with the Free locomotion, the Amp of the SF under the SLfix condition was significantly greater at all periods (pattern effect: $p < 0.001$, $\eta^2 = 0.983$) (Figure 3A). The Mx value for SF tended to be greater in the Free conditions than in the SLfix condition (pattern effect: $p = 0.065$, $\eta^2 = 0.255$) (Figure 3C). The PS for SF was not significantly different between the Free and SLfix conditions except at the 5-min period ($p < 0.01$) (Figure 3B).

The Amp of the SL under the SFfix condition was significantly larger than that under Free at all periods (pattern effect: $p < 0.001$, $\eta^2 = 0.965$) (Figure 3D). In contrast, the Mx for the SL became significantly lower under SFfix compared to Free (pattern effect: $p < 0.001$, $\eta^2 = 0.877$) (Figure 3F). There were no significant differences in the PS for the SL at any period (Figure 3E).

Dynamic Responses of Ventilatory Variables

Figure 4 illustrates the responses of ventilatory variables at the 5-min period for a representative single same subject, revealing that the VT and Bf showed markedly different responses. Specifically, under the SLfix condition, the PS for the Bf response was preceded by a delay in VT (i.e., a late appearance of the peak in VT), whereas under the SFfix condition, the PS for the VT response was preceded by a delay in the Bf (i.e., a late appearance of the peak of Bf or an almost constant).

The SFfix condition induced significantly lower Amps in $V_E$ and $P_{ETCO_2}$ at allmetricconverterProductID1 A of the periods compared with the SLfix condition, with similar values between the SLfix and Free conditions (Figures 5A,D). The Amp response for the Bf remained unchanged (<1.0 breath-min$^{-1}$) under SFfix (Figure 5B), with a pattern effect among the three patterns (pattern effect: $p = 0.006$, $\eta^2 = 0.348$). The Amp of the VT response tended to be larger under SLfix compared with SFfix and Free at all periods ($p = 0.064$, $\eta^2 = 0.204$) (Figure 5C). With respect to the PS, a significant main effect of patterns was observed in the VT (pattern effect: $p = 0.021$, $\eta^2 = 0.275$) (Figure 5G). A Bonferroni post-hoc test further revealed that the PS for the VT at the 2-min period under the SLfix condition was significantly lower than under the SFfix condition ($p < 0.05$).

However, we did not observe any effects of the patterns on the PS in $V_E$, Bf, or $P_{ETCO_2}$metricconverterProductID1 C, with period effects for $V_E$ and $P_{ETCO_2}$ (Figures 5E,F,H). In addition, a significant main effect of the pattern was observed on the Mx in $V_E$ (pattern effect: $p = 0.012$, $\eta^2 = 0.309$), with a significant difference between the Free and SLfix conditions at only the 5-min period (Supplementary Table 1, $p = 0.015$). There were no significant main effects of the locomotion patterns or frequency period, and no interaction effect in the Mx for VT, Bf, and $P_{ETCO_2}$ (all $p > 0.05$, Supplementary Table 1).

Dynamic Responses of Gas Exchange and Heart Rate Variables

The Amp values of the HR, $V_O_2$, and $V_CO_2$ variables were significantly lower under SFfix compared with SLfix and Free (pattern effect: $p = 0.001, 0.002, 0.004, 0.446, 0.408$, and 0.370, respectively, Figures 6A–C). Although the period had significant main effects on the PS of HR, $V_O_2$, and $V_CO_2$ (Figures 6D–F), those values were similar irrespective of the locomotion patterns, with the exception of a greater PS in the HR at 5 min under SFfix. We also observed no significant main effects of the locomotion patterns and frequency period, and no interaction effect in the Mx of $V_CO_2$, $V_O_2$, and HR (all $p > 0.05$, Supplementary Table 2).

Relationship Between the Amp Ratio in $V_E$ and $V_CO_2$

The Amp ratio in the $V_E$ (i.e., Amp ratio of sinusoidal forcing variations to constant) was closely related to the Amp ratio in the $V_CO_2$ when the data from all periods were pooled (SLfix: $r = 0.83$, SFfix: $r = 0.88$, and Free: $r = 0.91$, $p < 0.01$) (Figure 7). The slope of the regression lines of the $V_E$–$V_CO_2$ relationship was steeper under SLfix (s: 1.19) than under SFfix (s: 0.70) and Free conditions (s: 0.97).

DISCUSSION

The three major findings of this study are as follows: (i) the SLfix locomotion pattern increased the Amp of $V_E$ (Figure 5A) and metabolic responses ($V_O_2$ and $V_CO_2$; Figures 6B,C) compared with the SFfix (i.e., 120 steps·min$^{-1}$); (ii) the Amp of the Bf under the SFfix locomotion pattern remained unchanged (i.e., <1.0 breath·min$^{-1}$); and (iii) when the slope of the $V_E$–$V_CO_2$ relationship under the Free condition was used as the reference (1.0), the slope under SLfix was steeper than that under Free, and the slope under SFfix was lower than that under Free. These phenomena may be explained as follows: afferent feedback from the limb is important for locomotor-respiratory entrainment, whereby the discharge rhythm of sensory inputs can entrain a central respiratory pattern generator (Potts et al., 2005; Shevtsova et al., 2019; Le Gal et al., 2020).
Locomotor-Respiratory Entrainment Irrespective of the Sinusoidal Change in Speed

In human studies, locomotor-respiratory entrainment has been observed when the locomotion speed is kept constant during walking (Bramble and Carrier, 1983; Bernasconi et al., 1995; McDermott et al., 2003; O’Halloran et al., 2012). Our locomotion protocol used the sinusoidal change in speed between 50 and 100 m/min⁻¹. We thus considered the following possibilities: locomotor-respiratory entrainment forcing the synchronization of step movement and breathing rhythms is more likely to occur when the sinusoidal change in speed is synchronized with the sinusoidal change in SF (i.e., the SLfix condition). According to our hypothesis, the SFfix condition provided an unchanged Bf(i.e., <1.0 breath-min⁻¹), with the Mx for the Bf approximately 24 breaths-min⁻¹ at all periods (Supplementary Table 1 and Figure 4). Thus, the Amp of VE depended mostly on that for VT, which was characterized as the faster phasic response under the SFfix condition (Figure 5G).

Apparently, the constrained Bf under the SFfix condition induced smaller Amps of VE and PETCO2 (Figures 5A,B,D), despite the absence of differences in the Mx for VE and PETCO2 between the SFfix and SLfix conditions (Supplementary Table 1). Therefore, respiratory entrainment might be achieved by the same exercise hyperpnea without hypoventilation and lower PETCO2 even under SFfix. These phenomena suggest the physiological significance of afferent feedback from the hindlimb locomotor generators for locomotor-respiratory coupling, whereby the discharge rhythm of sensory inputs can entrain a central respiratory pattern generator (Potts et al., 2005; Shevtsova et al., 2019; Le Gal et al., 2020). It was also reported that respiratory entrainment can be achieved by lumbar and/or cervical proprioceptive input stimulations in an isolated rat or mice brain stem-spinal cord preparation (Morin and Viala, 2002; Giraudin et al., 2012; Le Gal et al., 2020). Even though the Amp of the SL under the SFfix condition was significantly (two times) larger than that under the control Free condition in all of the periods used herein, the SFfix locomotion induced the sluggish dynamics of the Amp of VE, VO2, and VCO2. Thus, the...
contribution of sinusoidal variation in SL to the adjustment in \( V_E \) would be less than that in a Free condition.

**The Physiological Implications of Sinusoidal Cadence for Ventilation**

Several research groups have compared the PS of \( V_E \) dynamics under different experimental conditions during leg cycling, namely, between sinusoidal cadence with a constant pedal force and sinusoidal pedal force with a constant pedal cadence (Casaburi et al., 1978; Duffin, 2014; Caterini et al., 2016), and they observed that the PS of \( V_E \) dynamics was much less for the cadence variation than for the sinusoidal pedal forcing variation. These studies used the fundamental concept that changing the frequency of limb movement during exercise would affect ventilation.

In contrast to those findings, our present investigation demonstrated that the \( SL_{\text{fix}} \) condition induced a significantly larger Amp of \( V_E \) with similar PS and Mx values compared with the \( SF_{\text{fix}} \) condition (Figures 4, 5A and Supplementary Table 1). The larger Amp of \( V_E \) was equal to that in the Free condition, even though the Amp of \( Bf \) and VT tended to be larger under \( SL_{\text{fix}} \) compared with Free (Figures 5B,C). The smaller PS values for \( Bf \) and the larger PS value for VT were specifically characterized...
under the SLfix condition. The increased muscle contraction of sinusoidal cadence may thus be competitively related to the faster ventilatory drive to breathe (Takano, 1988; Caterini et al., 2016; Girardi et al., 2021). Notably, this specific adaptation was to the PS rather than the magnitude (Casaburi et al., 1977; 1978; Wells et al., 2007).

In this study, the phasic responses of $\dot{V}_E$, $Bf$, and $P_{ET}CO_2$ were not significantly different among the three locomotion patterns. The discrepancies between our results and those of the studies reported by Casaburi et al. (1978), Duffin (2014), and Caterini et al. (2016) may be explained by the different study settings (e.g., leg cycling vs. walking, and/or the mode of sinusoidal changes).
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We also observed that the Amp of $\dot{V}_E$ under the SLfix condition was similar to that under the Free condition, even though the Amp of SF was sinusoidally altered (two times larger) compared with the Free condition (Figure 3A). Irrespective of the larger variation in SF during sinusoidal locomotion, the sinusoidal cadence under the present SLfix condition inherently seemed to play a dominant role in the Amp responses of the ventilatory variables.

Considering the physiological mechanisms underlying these observations, it has been shown that the groups III and IV afferents in exercising limbs are stimulated by muscle contraction (Kaufman et al., 1983; Mense and Stahnke, 1983; Bruce et al., 2019). In this study, a greater Mx of $\dot{V}_E$ under the SLfix condition was manifested compared with the Free condition, which supports the concept that the limb movement frequency is a significant factor in the greater increase of $\dot{V}_E$ during sinusoidal locomotion (Eldridge et al., 1981; Eldridge, 1994).

**Influence on $\dot{V}_E$–$\dot{V}$CO2 Linkage During Three Locomotion Patterns**

When we used the slope of the $\dot{V}_E$–$\dot{V}$CO2 relationship under the Free condition as the reference (1.0), the slope under SLfix (1.2) was steeper than that under Free, and the slope under SFfix was gentler than that in Free (Figure 7). The gait pattern for a sinusoidal variation of the SF may cause greater exercise hyperpnea, which can be interpreted as an overreaction of the additional stimulation on the respiratory center via the supraspinal locomotor center (DiMarco et al., 1983; Fukuoka et al., 2017). In other words, the greater $\dot{V}_E$ at a given $\dot{V}$CO2 under SLfix locomotion suggests that the central feed-forward command (Bell, 2006) or upward information from theafferent neural activity was stimulated by the sinusoidal locomotive cadence. Therefore, these neural drives could be partly related to the steeper slope of the $\dot{V}_E$–$\dot{V}$CO2 relationship under SLfix rather than the humoral outcome via the equivalent metabolic demand under Free (Eldridge and Waldrop, 1991; Waldrop et al., 1996; Forster et al., 2012; Fukuoka et al., 2017).

Considering Free locomotion, our subjects’ preferred SL was likely to have a lower metabolic demand (Supplementary Table 2), and a significant main effect of the locomotion patterns was observed on the Mx for $\dot{V}_E$, and there was a significant difference between the Free and SLfix conditions in the 5-min period ($p = 0.015$, Supplementary Table 1). Thus, the steeper slope of the $\dot{V}_E$–$\dot{V}$CO2 relationship under SLfix might be attributed to a greater Mx for $\dot{V}_E$. The observed differences in the ventilatory response between our SLfix and Free conditions would be attributed to the larger Amp of SF, which contributed to the neuromuscular afferent flow into the medial brain and respiratory-locomotor generation center (Eldridge et al., 1981, 1982; Eldridge, 1994).

In contrast, the slope under SFfix (0.7) was lower than that under Free even though the Mx of $\dot{V}_E$, $\dot{V}$O2, and $\dot{V}$CO2 were not significantly different between the SLfix and SFfix conditions (Figure 7 and Supplementary Table 2). The remarkable difference in the slope of the $\dot{V}_E$–$\dot{V}$CO2 relationship was due to the Amp of the SF. The lower slope was caused by the depressed $\dot{V}_E$, which we attribute to the entrained breath frequency under SFfix. The neural stimulus related to locomotion appeared to interact with the chemical stimulus in a predominantly “additive” manner (DiMarco et al., 1983). This finding may support our hypothesis that the modification of respiratory changes occurs indirectly via the gait pattern generator by maintaining the constrained SF (Le Gal et al., 2020).

We speculate that the very close association between $\dot{V}_E$ and $\dot{V}$CO2 at all locomotion patterns ($r = 0.83–0.92$, Figure 7) provides a rationale for considering mechanisms unrelated to the motor act (such as humoral factors) to explain the link between $\dot{V}_E$ and $\dot{V}$CO2 (Whipp et al., 1982). A major question in the physiological interpretation of the strong linkage between $\dot{V}_E$ and $\dot{V}$CO2 is that the CO2 amount is an adjustment factor, but the chemoreceptor does not sense the CO2 amount and respond to the partial pressure of CO2 (Forster et al., 2012). Considering that chemoreceptors are always involved, the alteration of $P_{ET}$CO2 could be involved as a derivative signal to the arterial and central chemoreceptors, which are further related to $\dot{V}_E$ (Forster et al., 2012; Ebine et al., 2018).

It has been recognized that a sinusoidal exercise protocol would indicate a lesser contribution to the ventilatory changes from the neural signals through the motor activity and/or central command (Forster et al., 2012). Wells et al. (2007) chose the shorter period of 1 or 2 min of sinusoidal change in walking speed between 3.2 and 6.4 km·h⁻¹ (approximately 53 and 107 m·min⁻¹) to emphasize the faster $\dot{V}_E$ response against locomotion. Contrary to their observations, the Amp values of $\dot{V}_E$ in this study were tightly coupled to those of $\dot{V}$CO2 during sinusoidal walking. The contribution of limb movement to exercise hyperpnea has thus been a matter of debate (Casaburi et al., 1978; Duffin, 2014; Ward, 2019). Moreover, exercise...
training can affect exercise hyperpnea through attenuation of a neural drive that is either feedforward or feedback in nature (Miyamoto et al., 2012).

**Limitations**
We set up three locomotive patterns based on the assumption of a sine wave speed, and thus we did not experiment with changing the SF sinusoidally at a fixed load (in this case, speed), which has been treated in leg cycling (Casaburi et al., 1978; Wells et al., 2007). Further studies using sinusoidal changing of the SF may be necessary.

It remains difficult to determine the relative contributions of the mechanoreflex vs. the metaboreflex to ventilatory control in humans (Olson et al., 2010, 2014). The novel findings of our present investigation possibly demonstrate that the afferent feedback from skeletal muscle triggers a marked increase in the slope of the $V_E-\dot{V}CO_2$ relationship under $SL_{fix}$ locomotion. However, we were unable to differentiate the involvements of peripheral afferent feedback from central command without the direct measures. The individual contribution of both neural factors to the ventilatory response in humans cannot be evidently stated from the experimental results in this study.

**CONCLUSION**
In summary, the $SL_{fix}$ locomotion pattern enlarged the $Amp$ of $V_E$ and metabolic responses ($\dot{V}O_2$ and $\dot{V}CO_2$, and HR) compared with the $SF_{fix}$ pattern (i.e., 120 steps-min$^{-1}$). Moreover, the $Amp$ of $Bf$ remained unchanged (<1.0 beats/min) under $SF_{fix}$. The slope of the $V_E-\dot{V}CO_2$ relationship was steeper by 1.23 times under $SL_{fix}$ and was gentler by 0.72 times under $SF_{fix}$ when the slope under the control (Free) condition was used as the reference. These results are explained as follows: afferent feedback from the limb is important in locomotor-respiratory entrainment, whereby the discharge rhythm of sensory inputs can entrain central respiratory-pattern generation. The PSs of $V_E$, $\dot{V}O_2$, and $\dot{V}CO_2$ responses were unaffected at any of the locomotion patterns. Such sinusoidal wave manipulation of locomotion variables may offer new insights into the dynamics of exercise hyperpnea.

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**DATA AVAILABILITY STATEMENT**
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**
The studies involving human participants were reviewed and approved by the Ethics Committees of the Institutional Review Board of Doshisha University (no. 1045). The patients/participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**
MF, TA, and YF conceived and design of the study. MF, TA, and KK collected the data. MF, MH, KK, and YF interpreted of the data. MF, MH, and YF wrote, reviewed, and approved the final manuscript. All authors contributed to the collection of data.

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**SUPPLEMENTARY MATERIAL**
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