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Breathing in sport and exercise: Physiology, pathophysiology and applications

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Breathing in sport and exercise: Physiology, pathophysiology and applications

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Editorial: Breathing in sport and exercise: physiology, pathophysiology and applications

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Editorial on the Research Topic
Breathing in sport and exercise: physiology, pathophysiology and applications

1 Introduction

The purpose of this Research Topic was to improve our understanding of the physiology and pathophysiology of breathing during exercise and exploit this knowledge to advance the field of breathing monitoring in applied settings. The importance of breathing monitoring is currently underappreciated in the field of sport and exercise, partially because the pulmonary system has long been considered overbuilt for exercise (Dempsey et al., 2020; Peters et al., 2023). However, the interest in breathing monitoring during exercise is growing progressively for a number of reasons: 1) the ventilatory variables [especially respiratory frequency (f_R)] are particularly sensitive to changes in exercise tolerance; 2) f_R and tidal volume (V_T) are to a large extent modulated by different inputs during exercise, and their separate measurement provides relevant information (Nicolò and Sacchetti, 2023); 3) an abundance of technological solutions is currently available to monitor breathing variables in applied settings (Massaroni et al., 2019); and 4) different breathing strategies can be implemented for research or applied purposes. Most of these points have been explored by the 16 articles published in this Research Topic. We have grouped the articles into three different categories, i.e., physiology, pathophysiology and applications, but, as intended in the conception of the Research Topic, many of them show some overlap with other categories.

2 Physiology

Two studies focused on the mechanisms underlying ventilatory control during exercise. The fact that f_R is sensitive to changes in exercise tolerance and is largely modulated by different inputs compared to V_T is corroborated by findings from Nicolò et al. They found that f_R–but not oxygen uptake and heart rate–was sensitive to the improvement in exercise tolerance observed when comparing arm + leg cycling with conventional leg cycling. This response was observed both during incremental and time-to-exhaustion tests, and a high sensitivity to improvements in exercise tolerance was also found for perceived exertion. These findings support the notion that f_R is a marker of physical effort and is substantially modulated by central command during high-intensity exercise (Nicolò and Sacchetti, 2023). A degree of interindividual variability in the breathing pattern was observed in this study, although the breathing pattern appeared to be generally consistent across tests for the same individual. To shed some light on the interindividual differences in breathing responses observed during incremental exercise, Harrison et al. evaluated whether ventilatory and anxiety responses during exercise are associated with ventilatory and anxiety responses to hypercapnia at rest. They found that this association may be moderated by the fitness level, as a positive association was found in healthy untrained individuals but not in endurance-trained athletes. Besides, higher V_T values and lower f_R values were found in athletes during hypercapnia, despite similar minute ventilation values. Future studies should further our understanding of the mechanisms underlying interindividual differences in breathing patterns during exercise.

Different studies focused on the effect of voluntarily modulating the breathing pattern, and two of them investigated the effects of breath-holding. This intervention induces a variety of physiological responses that are supposed to determine performance benefits. Yang et al. tested the effect of 8 weeks of dry dynamic breath-hold training on spleen volume and hematological parameters. Performing breath-holding during exercise was supposed to elicit a greater stimulus compared to static breath-holding. This intervention was sufficient to improve apnoea tolerance and increase spleen volume, while no increase was observed in red blood cell and hemoglobin levels. Breath-holding is also included in popular breathing techniques such as the Wim Hof Breathing Method (WHBM), which combines periods of hyperventilation followed by voluntary breath-holds at low lung volume. The acute effect of this method on repeated sprint ability was investigated by Citherlet et al. and compared with a condition where only hyperventilation was performed. While hyperventilation induces respiratory alkalosis, breath-holding counteracts the increase in blood pH and triggers the so-called diving response, leading to a series of acute physiological responses including spleen contraction. However, none of the two interventions managed to improve repeated sprint performance. Furthermore, some participants reported side effects in the hyperventilation and WHBM conditions, including dizziness, heaviness or deafness. Hence, caution is suggested when attempting to implement breath-holding strategies into training as they may not provide performance benefits or may even result in side effects.

Side effects may also be experienced when performing saturation diving, and Lian et al. investigated the effects of a single simulated 500-m saturation dive on the lung function of professional divers. They found a decrease in small airway function and diffusion function as assessed by means of pulmonary function tests performed before the dive and 3 days after. They also found that divers showing small airway dysfunction or mild obstructive lung function defects before the dive might be at greater risk of developing further post-dive abnormalities. This outlines the importance of an individualized approach to health assessment and management in this population.

3 Pathophysiology

Breathing monitoring has important implications for the identification of pathophysiological signs of cardiopulmonary and metabolic diseases. Hyrylä et al. focused on the "diabetic lung", which is an overlooked target organ of the disease (Pitocco et al., 2012). As the impairment in pulmonary function may not be evident in the first stages of diabetes, the authors attempted to identify early signs of deterioration by analyzing the morphology of the flow signal within the respiratory cycle during a cardiopulmonary exercise test (CPET). They found significant differences between healthy individuals and well-controlled type I diabetic males, the latter showing an attenuated expiratory slope (from expiratory onset to expiratory peak flow) at peak exercise but not during submaximal exercise. While the preliminary nature of the study does not allow us to speculate on the clinical relevance of such findings, a careful examination of the morphology of the breathing signal may complement more traditional assessments performed with a CPET. For instance, a CPET can be used to monitor cardiorespiratory function after recovering from surgeries or infections, as done by Lemos et al., who tested 171 overweight or obese individuals who had contracted COVID-19. They found a greater exercise tolerance in non-hospitalized patients than in patients who were hospitalized or admitted to the intensive care unit. Furthermore, non-hospitalized patients showed a higher peak oxygen uptake and an improved recovery of vital signs after the incremental test. The exploitation of the full potential of CPET is encouraged in people with cardiopulmonary and metabolic diseases.

Technological advances favor breathing monitoring outside the laboratory, with important implications for the identification of adverse events in daily life. Wang et al. monitored 578 people with respiratory disorders performing the 6-min walk test while wearing a flexible vest recording electrocardiographic, breathing and accelerometric signals, and oxygen saturation and blood pressure values. The purpose of the study was to develop models predicting adverse events occurring during the test, combining vital sign monitoring with demographic data and artificial intelligence. The models developed have the potential to provide assistive decision support for identifying patients who may require medical help during the test. This is an example of how technology may favor ubiquitous breathing monitoring in daily life and help tailor therapies to individual needs and assess their effects. Indeed, some breathing variables are sensitive to clinical deterioration, pain and infections (Nicolò et al., 2020), and the breathing signal may also be used to retrieve other useful information (e.g., number of cough events) (Otoshi et al., 2021). The impact of inhaled corticosteroids on the cough reflex was investigated by Basin et al., who used an animal model to address this issue. They found restoration of the desensitization of the cough reflex during exercise when inhaling corticosteroids, and airway inflammation appeared to be involved in the modulation of this response. These findings suggest that anti-inflammatory treatments may help manage exercise-induced cough in people with asthma.

4 Applications

We live in a time where the respiratory system has become a target of the multi-trillion-dollar health and wellness industry (Illidi et al., 2023). There is an abundance of interventions that are purported to provide benefits to the respiratory system, but commercial claims are rarely supported by scientific evidence. Some studies have attempted to shed some light on this issue, starting with the work by Harbour et al., who have reviewed the available literature on the advantages and disadvantages of adopting breathing strategies that may potentially enhance running performance. They also pointed toward the importance of using technology to help runners learn such breathing techniques. Among a variety of strategies reviewed, they focused on nasal breathing and respiratory muscle training, which have also been investigated by other articles published in this Research Topic.

Nasal breathing has received attention as it stimulates the endogenous production of nitric oxide, which being a vasodilator and a mild bronchodilator favors blood flow redistribution in the different lung regions, among other effects (Illidi et al., 2023). Nasal breathing during exercise requires awareness and training, but the feasibility of this strategy improves after some months of practice, even at relatively high exercise intensities (Harbour et al.). While the benefits of nasal breathing in exercising healthy individuals still need to be proven satisfactorily, Rappelt et al. investigated the acute effects of restricted nasal-only breathing on self-selected exercise intensity in a 60-min exercise session. They hypothesized that nasal breathing would help the participants perform low-intensity endurance training at the required intensity instead of selecting higher intensities than intended. However, no significant differences were observed in power output distribution between the nasal-only condition and the oro-nasal condition, but a lower f_R was found in the former condition. A lower f_R and a higher V_T were also found by Held et al. when comparing high-intensity exercise performed in the uphill running modality vs level running modality. While nasal-only breathing and uphill running may be useful modalities for investigating the control of f_R and V_T during exercise, the two studies were not designed with this intent, hence requiring further investigation.

When prescribed with the appropriate intensity, duration and frequency, respiratory muscle training appears to be effective in improving respiratory muscle strength/endurance and exercise capacity in healthy individuals (Illidi et al., 2023). Shei et al. outlined the importance of individualizing the prescription of inspiratory muscle training, while many of the studies conducted so far have used fixed protocols, some of which were developed for clinical populations. As it is commonly done for the locomotor muscles, the respiratory muscles could be trained by considering the principles of training, including individualization, periodization and specificity. For instance, the authors suggest that the intensity selection may take into account the breathing pattern and the demands of exercise. The reliability of the tests designed to measure important functional outcomes of the respiratory muscles (e.g., endurance) should also be considered when interpreting the efficacy of a respiratory muscle intervention (Larribaut et al., 2020). By prescribing respiratory muscle training according to the ventilatory demands of actual exercise, Chambault et al. found a greater improvement in cycling time trial performance in women than in men, and this effect was more pronounced in hypoxia. These findings are in line with evidence suggesting that the pulmonary system is challenged during exercise more in women than in men, and even more in hypoxic environments (Dempsey et al., 2020). This reinforces the suggestion by Shei et al. that respiratory muscle training should be tailored according to individual needs.

Another topic that has recently regained attention is the impact of apparatuses or protective devices used in occupational settings on breathing and related perceptions. The wide use of face masks during the COVID-19 pandemic has triggered interest in understanding their effect on exercising humans. Glänzel et al. provided a meta-analysis of the acute effects of mask-wearing during exercise on performance and psychological responses. They observed a small reduction in performance during time-to-exhaustion tests but not during most of the other exercise tests used. However, they reported increases in discomfort (large effect), dyspnea (moderate effect) and perceived exertion (small effect), although some of these effects were dependent on the type of mask used. Rives et al. simulated the inspiratory load experienced by military divers wearing a rebreather device and were able to characterize the response of the diaphragm using ultrasound. The authors found an increase in both the excursion and the thickening fraction of the right hemidiaphragm and suggested the latter being a relevant parameter to be considered in inspiratory load evaluation. While apparatuses and protective devices used in occupational settings are sometimes considered detrimental to exercise capacity or comfort, they may instead serve as facilitating tools for vital sign monitoring and work management. Indeed, face masks or other protective devices can be equipped with sensors and used for providing services to the user (Li et al., 2023).

5 Conclusion

The studies published in this Research Topic support the importance of monitoring breathing for a variety of applications. A good understanding of the physiological and pathophysiological responses to exercise should guide the monitoring of breathing in applied settings. Furthermore, special attention should be given to interindividual differences in breathing responses to tailor breathing and exercise strategies to individual needs. We hope this Research Topic may have contributed to narrowing the gap between physiology, pathophysiology and applications in the context of breathing monitoring during exercise.

Author contributions

AN: Writing–original draft, Writing–review and editing. MG: Writing–review and editing. MS: Writing–review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Sex-Specific Effects of Respiratory Muscle Endurance Training on Cycling Time Trial Performance in Normoxia and Hypoxia

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Objectives: We tested the hypotheses that respiratory muscle endurance training (RMET) improves endurance cycling performance differently in women and men and more so in hypoxia than in normoxia.

Design: A prospective pre–post cross-over study with two testing conditions.

Methods: Healthy and active women (seven, 24 ± 4 years, mean ± standard deviation [SD]) and men (seven, 27 ± 5 years) performed incremental cycling to determine maximum oxygen consumption (VO_{2peak}) and power output (W_{peak}) and on different days two 10-km cycling time trials (TTs) in normoxia and normobaric hypoxia (FiO_2, 0.135, ~3,500 m equivalent), in a balanced randomized order. Next they performed supervised RMET in normoxia (4 weeks, 5 days/week, 30 min/day eucapnic hyperpnea at ~60% predicted maximum voluntary ventilation) followed by identical post-tests. During TTs, heart rate, ear oximetry reading, and W_{peak} were recorded.

Results: The VO_{2peak} and W_{peak} values were unchanged after RMET. The TT was improved by 7 ± 6% ($p < 0.001$) in normoxia and 16 ± 6% ($p < 0.001$) in hypoxia. The difference between normoxic and hypoxic TT was smaller after RMET as compared with that before RMET (14% *vs*. 21%, respectively, $p < 0.001$). All effects were greater in women ($p < 0.001$). The RMET did not change the heart rate or ear oximetry reading during TTs.

Conclusion: We found a greater effect of RMET on cycling TT performance in women than in men, an effect more pronounced in hypoxia. These findings are congruent with the contention of a more pronounced performance-limiting role of the respiratory system during endurance exercise in hypoxia compared with normoxia and more so in women whose respiratory system is undersized compared with that of men.

Keywords: hypoxia, altitude, endurance, respiratory muscle training, sex, cycling, time trial

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INTRODUCTION

In conditions of normoxia, the capacity of the cardiorespiratory oxygen transport is the main limiting factor for maximum oxygen consumption (VO_{2max}) (Bassett and Howley, 2000; Lundby et al., 2017). Quantified with incremental testing protocols to exhaustion, VO_{2max} cannot be maintained for long. For aerobic endurance-type exercises such as running or cycling, it is the highest maintainable fraction of VO_{2max} for a given time that sets the best performance for a given distance such as the marathon, with a multitude of other interacting factors contributing to ceiling effects such as the energy cost of locomotion, development of neuromuscular fatigue, availability of energy substrate, and thermoregulation (Jones et al., 2020). In conditions of normoxia, the respiratory system is thought not to be an important limiting factor for maximum aerobic capacity in young, healthy, but not especially trained men, who possess excess respiratory system capacity (Dempsey et al., 2020). However, the respiratory system can be a limiting factor for aerobic capacity in highly trained endurance athletes, such as cross-country skiers, who have very high cardiac outputs and may experience a limitation in their expiratory flow because their high aerobic capacity leads to an "excessive" ventilatory demand outstripping their ventilatory system capacity (McKenzie, 2012; Dempsey et al., 2020) or in master athletes due to aging lungs (Burtscher et al., 2011). For endurance type of effort, fatigue of the respiratory muscle is also currently thought to be a limiting factor (Boutellier, 1998; Spengler and Boutellier, 2000; Dempsey et al., 2020).

An exposure to hypoxia leads to a ventilatory response, which increases ventilation for a given oxygen uptake. With increasing degrees of hypoxia, the respiratory system thus progressively becomes a limiting factor for cardiorespiratory oxygen transport and hence aerobic performance (Ferretti and Prampero, 1995). At extreme altitudes, the increase in oxygen uptake resulting from a further increase in ventilation is even thought to be offset by the very oxygen cost of that increase in ventilation, making the extra effort futile (Cibella et al., 1999). Apart from this limitation to oxygen uptake imposed by ventilation in conditions of low tension of inspiratory oxygen, the increase in ventilation in hypoxia for a given oxygen uptake also increases the work of breathing, thereby placing more stress on the respiratory muscles for a given level of metabolic activity of the whole organism.

For so much of exercise physiology (Ansdell et al., 2020), the form and function of the respiratory system during exercise have been studied mostly in men. In recent years, authors such as Sheel et al. (2016) began studying women and found that there are sex-based differences in the anatomy and physiology of the human respiratory system. It is currently understood that women are more susceptible to limitations in the respiratory system during exercise than men (Molgat-Seon et al., 2018; Dempsey et al., 2020). This difference is likely to be exacerbated in hypoxia. Archiza et al. (2020) compared diaphragm fatiguability from inspiratory loading in men and women and found that while men and women fatigued to the same extent in normoxia, diaphragm fatigue was greater in women compared with that in men in acute hypoxia.

In normoxia, respiratory muscle training (RMT) delays diaphragm fatigue and can improve the performance of endurance exercise (Boutellier, 1998; Spengler and Boutellier, 2000; Sheel, 2002; Illi et al., 2012; HajGhanbari et al., 2013; Segizbaeva et al., 2014; Shei, 2018). RMT can consist of inspiratory or combined inspiratory/expiratory muscle strength training (i.e., respiratory muscle strength training [RMST]) or of respiratory muscle endurance training (RMET, by means of sustained normocapnic hyperpnea) (Illi et al., 2012). In normoxia, it was reported that the metaboreflex solicited for a given level of diaphragm fatigue may be less pronounced in women compared with men (Geary et al., 2019). For exercise in hypoxia, a recent review (Álvarez-Herms et al., 2019) concluded that RMT reduced the fatigue of the respiratory muscles, delayed the metaboreflex activation of the respiratory muscles, and consequently allowed for better maintenance of SaO_2 and blood flow to the active locomotor muscles. No studies to date have reported a direct comparison of the effects of RMT in women *vs.* men on endurance performance in normoxia and hypoxia. If the effects would be greater in women as compared with those in men, this would be of relevance for training programs. Therefore, we tested the hypotheses that RMET improves the performance of endurance exercise both in normoxia and in hypoxia but more so in hypoxia and more so in women than in men.

METHODS

The study protocol was approved by the Geneva University Hospitals Research Ethics Committee and was conducted in accordance with the latest Declaration of Helsinki; all participants signed an informed consent form. Fourteen healthy recreationally active participants (seven men, 27 ± 5 years, 154 ± 47 min exercise/week; seven women, 24 ± 4 years, 80 ± 30 min exercise/week; [means ± SD]) volunteered. On day 1, they performed an incremental cardiopulmonary exercise test (CPET) on a cycle ergometer (Ergoline, Germany) till exhaustion to determine their peak aerobic power output (W_{peak}) and oxygen consumption (VO_{2peak}, Cosmed Quark b2, Italy). After instrumentation, the participants sat for 3 min on the ergometer before starting pedaling at 50 W (men) or 30 W (women) for 5 min after which the power was increased by 30 or 20 W/min for men and women, respectively, until voluntary exhaustion. The pedaling rate had to be kept between 70 and 90 rpm. The maximality was considered to be reached when at least two of the following criteria were met: VO_2 plateau, respiratory quotient > 1.1, heart rate (HR) > 90% of the theoretical peak HR, or a persisting drop in pedaling rate below 60 rpm despite a strong verbal encouragement. The post–RMET CPET data were lost for one man, and the aggregate results of the remaining six men were presented.

On day 2, the participants performed a 10-km cycling TT on a road-bike mounted on a calibrated ergometer (Spin-Trainer, Technogym, Italy) in normoxia (with local altitude 380 m) or in hypoxia (AltiTrainer, Switzerland; N_2-enriched air with an FiO_2 of 0.135, PiO_2 ∼96 mmHg, equivalent to an altitude of ∼3,500 m). On day 4, they repeated the 10-km TT in the other condition, in a balanced randomized order. The participants could see the distance covered and were instructed to complete the TTs in the shortest time possible under strong verbal encouragement all along.

The participants then completed 4 weeks of supervised RMET (5 days/week, 30 min/day eucapnic hyperpnea, in normoxia) with a partial rebreathing device (SpiroTiger, IDEAG, Switzerland) breathing at ∼60% of their individual predicted maximum voluntary ventilation (FEV1 × 40 L/min) (Quanjer et al., 2012).

Upon completion of the 4 weeks of supervised RMET, the participants then repeated the three tests (day 1: incremental exercise test; next day and 48 h later: normoxic and hypoxic TTs, randomized balanced order). During the TTs, HR (E600 wrist watch, Polar, Finland), ear oximetry reading (SpO_2, Datex Oscar 2, Finland; only in hypoxia), and W_{peak} were recorded every minute and at arrival. The participants were kept naïve concerning the specific hypothesis of the study and remained blinded to their performances and physiological parameters for all tests for the duration of the study.

The aggregate data were reported as means ± SD unless stated otherwise. The statistical analysis was performed with Prism (Version 9, Graphpad Software, San Diego, CA, USA). The normality of data distribution was verified with the Shapiro–Wilk test. For the TT performance, we used repeated measures (pre–post RMET) ANOVA with the factors woman/man and normoxia/hypoxia. For the other analyses, ANOVA or mixed-effects modeling was used, depending on any missing data. *Post-hoc* comparison of means was performed with the Sidak method. The results were considered significant when $p < 0.05$.

RESULTS

Cardiopulmonary Exercise Test

There was a significant effect of sex on absolute and relative VO_{2peak} (both $p < 0.001$) and absolute and relative W_{peak} (both $p < 0.001$). The VO_{2peak} ($p = 0.115$) and W_{peak} ($p = 0.959$) values were not changed after RMET (see **Table 1**). The peak HR was not changed after RMET (women: 183 ± 5 and 182 ± 8; men 192 ± 7 and 193 ± 7 bpm).

Time Trial Performance

For both men and women, RMET significantly improved the TT performance more in hypoxia (by 16 ± 6%, $p < 0.001$) than in normoxia (by 7 ± 6%, $P < 0.001$) (see **Figure 1**). The difference between normoxic and hypoxic TT was smaller after RMET as compared with that before RMET (14% *vs.* 21%, respectively, $p < 0.001$). The effects of RMET on TT performance were greater in women (pre–post: $p < 0.001$; normoxia–hypoxia: $p < 0.001$). Women had higher average saturations during TTs in hypoxia than men (women pre-RMET, 85 ± 2, and post-RMET, 85 ± 4%; men pre-RMET, 81 ± 3, and post-RMET, 80 ± 2%, $p < 0.001$, no effect of RMET *per se*). RMET did not affect the mean nor the final levels of HR or ear oximetry reading during TT in normoxia or hypoxia (**Table 2**).

DISCUSSION

Based on our results, the hypotheses that (i) RMET improves performance of endurance exercise both in normoxia and in hypoxia, (ii) but more so in hypoxia, and (iii) more so in women than in men are not refuted. These findings are in agreement with earlier studies on the effects of RMET on the performance of cycling TT and contribute to the novel findings of a greater effect of RMET on the performance in 10-km TT in hypoxia than in normoxia, and an effect of sex on the extent of RMET-induced improvement of the performance in 10-km TT in both normoxia and hypoxia.

Respiratory Muscle Endurance Training

Earlier studies remained somewhat inconclusive on the effects of RMT on high-intensity endurance-type exercise (Morgan et al., 1987; Fairbarn et al., 1991). But since the pioneering work by Boutellier and Piwko (1992), although initially received with some reserve (Sheel, 2002), numerous studies have confirmed the positive effects of RMST and RMET on respiratory muscle force and endurance and also on the performance of large muscle group–type exercises such as running or cycling (Shei, 2018). Several systematic quantitative meta-analyses now have consolidated the collective evidence in favor of RMT (Illi et al., 2012; HajGhanbari et al., 2013; Sales et al., 2016; Karsten et al., 2018; Lorca-Santiago et al., 2020).

Various devices and methods for RMT exist, such as inspiratory loaded breathing or eucapnic hyperpnea (Menzes et al., 2018). Conform the systematic review by Illi et al. (2012), we chose to use RMET consisting of 30 min/day eucapnic hyperpnea at ∼60% of predicted maximum voluntary ventilation, 5 days/week, for 4 weeks, expecting improvements in 10-km TT cycling in normoxia by at least 5%. This method allows training the respiratory muscles with a hyperpnea similar to that developed during actual exercise, loading both the inspiratory and expiratory muscles, thus inducing global fatigue of the respiratory muscles (Verges et al., 2010). We found an average TT improvement of 7% in our normoxic conditions, in the expected range for healthy but not especially trained young adults (Illi et al., 2012). We deliberately chose to recruit recreationally active but not especially trained participants, since the meta-analysis by Illi et al. (2012) suggested that less-fit subjects benefit more from RMET than highly trained athletes. The latter already have more trained respiratory muscles because of their regular exposure to sustained exercise hyperpnea (Dempsey et al., 2020) and, therefore, benefit less from further RMET (Illi et al., 2012).

Maximum Oxygen Consumption

We did not find effects of RMET on VO_{2peak} nor on W_{peak}. In their meta-analysis, Illi et al. (2012) reported that out of the 22 studies that assessed VO_{2peak} before and after RMT, only two reported that VO_{2peak} was changed. Illi et al. (2012) contended that this is because the time spent exercising at high fractions of VO_{2peak}, toward the end of a CPET when the respiratory muscles are most likely to fatigue, is too short to elicit sufficient fatigue of the respiratory muscle to prematurely limit the test. This reasoning is supported by their meta-regression showing

TABLE 1 | Results of cardiopulmonary exercise testing (CPET).

	Pre-RMET				Post-RMET			
	Wpeak (watts)	Wpeak/kg (watts/kg)	VO_2peak (ml/min)	VO_2peak/kg (ml/min/kg)	Wpeak (watts)	Wpeak/kg (watts/kg)	VO_2peak (ml/min)	VO_2peak/kg (ml/min/kg)
Women (N = 7)	148 ± 33	2.7 ± 0.6	2,435 ± 406	44.0 ± 7.6	159 ± 26	2.9 ± 0.5	2,663 ± 361	47.9 ± 5.6
Men (N = 6)	282 ± 53	3.6 ± 0.5	4,572 ± 568	57.8 ± 2.8	271 ± 36	3.4 ± 0.4	4,578 ± 648	57.9 ± 4.4

Mean ± SD pre vs. post values of peak power output (W_{max}) and peak oxygen consumption (VO_{2peak}). There were no significant effects of respiratory muscle endurance training (RMET). Men: means of N = 6; one post-RMET cardiopulmonary exercise test (CPET) measurement was lost due to technical problems.

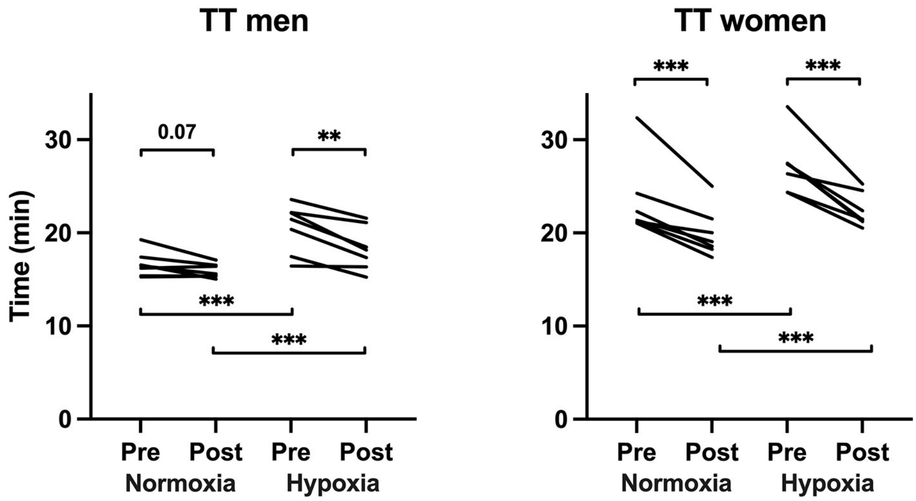

FIGURE 1 | Individual times of the 10-km time trials before and after respiratory muscle endurance training (RMET). $^{**}p < 0.01$ and $^{***}p < 0.001$. Pre–post RMET normoxia and hypoxia means were different between men and women: normoxia before RMET, $p < 0.001$, hypoxia before RMET, $p < 0.001$, normoxia after RMET, $p < 0.001$, and hypoxia after RMET, $p < 0.05$ (Sidak multiple comparison adjusted).

TABLE 2 | Parameters monitored during the 10-km cycling time trials.

	Pre-RMET				Post-RMET			
	Men		Women		Men		Women	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
Wmean (watt)	240 ± 45	185 ± 34	130 ± 18*	105 ± 12*	231 ± 25	177 ± 16	150 ± 60*	113 ± 9*
Wpeak (watt)	310 ± 103	281 ± 111	157 ± 22*	138 ± 18*	267 ± 35	225 ± 42	196 ± 108*	138 ± 20*
HRmean (bpm)	169 ± 13	158 ± 11	164 ± 7	157 ± 12	171 ± 8	161 ± 10	160 ± 13	161 ± 10
HRpeak (bpm)	188 ± 14	183 ± 10	180 ± 5	177 ± 7	190 ± 7	181 ± 8	182 ± 7	177 ± 7
SpO_2mean (%)	-	81 ± 3	-	85 ± 2*	-	80 ± 2	-	85 ± 4*
SpO_2min (%)	-	73 ± 8	-	75 ± 8	-	75 ± 3	-	75 ± 9

*SpO_2 was not monitored in normoxia. *Significantly different from the corresponding variable for men.*

that the improvement in performance after RMT was greater for longer test durations.

Endurance Performance

Esposito et al. (2010) assessed the effects of an RMET protocol similar to ours on VO_{2max} in normoxia and hypoxia. They found that RMET improved the respiratory function but did not have any effect on VO_{2max}, neither under normoxic nor under hypoxic conditions. However, they did not test if RMET improved prolonged aerobic endurance exercise in those conditions.

The effects of RMET seem to be greatest for sustained endurance efforts at intensities of 65–85% of peak aerobic power (Morgan et al., 1987; Spengler and Boutellier, 2000; Illi et al., 2012; Shei, 2018). During the normoxic TTs, our participants developed 89% of normoxic peak aerobic power on average, while in hypoxia, they were only able to maintain 69% of their normoxic peak aerobic power on average. Given that hypoxia exacerbates fatigue of the respiratory muscles for matched ventilatory patterns (Verges et al., 2010) and hypoxia leads to an increase in ventilation, these intensities were likely high enough to elicit fatigue of the respiratory muscles during the TTs. The increased TT performance after RMET thus probably was the result of an improved training status and fatigue resistance of the respiratory muscles of our participants (Segizbaeva et al., 2014).

Given that exercise in hypoxia exacerbates fatigue of the respiratory muscles (Verges et al., 2010), we surmised that the effect of RMET would be greater in hypoxia as compared with that in normoxia. In agreement with our study, the increase of average performance in normoxia after RMET was 7%, while in hypoxia it reached 16%. While numerous studies have reported positive effects of RMT on endurance performance in normoxia (Illi et al., 2012; HajGhanbari et al., 2013; Sales et al., 2016; Karsten et al., 2018; Shei, 2018; Lorca-Santiago et al., 2020), only few studies have looked at the effects of RMT in normoxia on performance in hypoxia.

Hursh et al. (2019) reported that inspiratory muscle training (daily repeated full inspirations against 80% of maximal inspiratory pressure [MIP] for 6 weeks) in well-trained cyclists increased VE and VO_2 during a 20-km cycling TT test, improving it by 1.4% while breathing 16% O_2 (equivalent to an altitude of 2,500 m). We tentatively explained our greater effect from our RMET protocol, the lower FiO_2, and the less trained participants. Salazar-Martínez et al. (2017) found that 6 weeks of inspiratory pressure-threshold RMST improved ventilatory efficiency in both normoxia and hypoxia (VE/VCO_{2slope}) from the beginning of exercise until the second ventilatory threshold. The performance of cycling TT (10-min all out) was improved in both normoxia and hypoxia after RMST, and TT performance was correlated with the slope of oxygen uptake efficiency but not with VE/VCO_{2slope}. Lomax et al. (2017) found that ventilatory efficiency, estimated by the ratio of peripheral capillary oxygen saturation (SpO_2) to VE (SpO_2/VE), was improved after hypoxic inspiratory RMT but not after normoxic inspiratory RMT. Held and Pendergast (2014), in conditions of hyperbaria, also reported an increased ventilatory efficiency following RMST, possibly linked to altered pulmonary mechanics and breathing patterns.

Keramidas et al. (2010) found, in healthy physically active but untrained men, that 4 weeks of daily cycling training at 50% of aerobic power combined with an RMET protocol similar to ours improved time to exhaustion in normoxia at 80% of peak aerobic power more compared with the group that only performed cycling training and no RMET, but not in hypoxia. They explained this somewhat paradoxical finding mentioning the greater intensity of relative exercise used in their hypoxic constant load test to exhaustion. Their design used the same absolute workload equivalent to 80% of normoxic pre-RMET peak aerobic power, leading to very high relative intensities in hypoxia (92% of pre-RMET peak aerobic power), too high probably for a discernable effect of RMET.

Katayama et al. (2019), in male intercollegiate competitive runners, compared 6 weeks of eucapnic hyperpnea RMET similar to ours performed either in normoxia or in hypoxia (first at 90% and then at 80% SaO_2) for 6 weeks. Time to exhaustion at 95% of VO_{2peak} in normoxia was increased after RMET to a similar extent in both groups (9–12%). No performance test was conducted in hypoxia. However, the response of exercise blood pressure was attenuated after RMET similarly in both groups, suggesting a decrease of the metaboreflex of the respiratory muscles.

Hypoxia comprises aerobic exercise capacity, and the respiratory system likely plays a bigger role in limiting performance at high altitude than at low altitude (Kayser, 2005). A compensatory hyperventilation aiming to offset the effect of a reduction in inspired oxygen tension leads to a greater load on the respiratory muscles for a given metabolic rate. The combination of more respiratory work and hypoxemia together causes early fatigue of the respiratory muscles, contributing to the limitation of endurance-type performance at high altitude (Helfer et al., 2016). RMET may thus be a valuable strategy to prevent or delay these mechanisms. Downey et al. (2007) tested this hypothesis with an inspiratory load RMST protocol but found no effect on time to exhaustion at 85% of peak normoxic aerobic power, either in normoxia or in hypoxia (14% O_2, equivalent of 3,200 m). The absence of effects in their study may be explained by the use of an inspiratory muscle training protocol, while expiratory muscles also likely play a role (Verges et al., 2010), a respiratory pattern that differs from that experienced during hyperpnea, and the very high intensity of their test in hypoxia (85% peak normoxic aerobic power) leading to a time to exhaustion 50% less than in normoxia. By contrast, Helfer et al. (2016) used an RMET protocol similar to ours, which produces a breathing pattern resembling that of exercise-induced hyperpnea, and found that it improved exercise time to exhaustion at 75% of peak aerobic power in hypoxia by 44%. Our finding of a relatively greater effect of RMET on the performance in 10-km cycling TT in hypoxia as compared with that in normoxia suggests that RMET may be of a particular interest for those who want to prepare for endurance-type efforts at high altitudes, such as trekkers, climbers, trail-runners, and cyclists.

Sex Difference

Women and men differ with regard to exercise physiology (Ansdell et al., 2020) and, in specific, also with regard to respiratory physiology (Sheel et al., 2016). Women are more susceptible to limitations of the respiratory system during exercise than men (Molgat-Seon et al., 2018; Dempsey et al., 2020), and this difference is likely to be exacerbated in hypoxia. We found that the effect of RMET on the performance in 10-km cycling TT was greater in women than in men. Why would women have profited more from RMET in hypoxia in our study as compared with men? Geary et al. (2019) found that, in normoxia, matching men and women for absolute diaphragmatic work resulted in an equal degree of diaphragm fatigue, despite women performing significantly greater work relative to body mass. Archiza et al. (2020) compared diaphragm muscular strength-matched healthy women and men upon 5 min of inspiratory loading at equal absolute muscle work both in normoxia and in hypoxia. While in normoxia, diaphragm fatigue was similar in both men and women, when acutely exposed to severe hypoxia (SpO_2 ∼80% and CaO_2 ∼16 ml/dl), the diaphragm fatigue was worsened only in women, suggesting that contrary to normoxic condition, the healthy female diaphragm would be more susceptible to fatigue under hypoxic condition. Perhaps, the effect of RMET in women was therefore proportionally greater in hypoxia than normoxia in comparison with men in our study.

The study of Salazar-Martínez et al. (2017) included 16 participants of which seven were women. Effects of sex were not a primary outcome of the study, but the authors stated that there were no significant differences between men and women for the change in the 10-min all-out TT in hypoxia and normoxia. At least two explanations may be advanced for their negative findings. First, they used an inspiratory loading protocol and not a more ecological valid RMET protocol such as ours, and second, their endurance test was probably performed at a too high intensity to reveal any effects of such training on the endurance of the respiratory muscles. Similarly, Guenette et al. (2006) found no difference between men and women using an inspiratory loading training protocol (5 days/week, two sets of 30 inspirations against 50% MIP) and a different endurance test, (time to exhaustion at 80% peak aerobic power). We contended that such designs do not adequately allow testing the hypothesis that global RMET, including both inspiratory and expiratory muscles and using breathing patterns that resemble those experienced during exercise-induced hyperpnea, can improve the performance of prolonged aerobic endurance.

LIMITATIONS

Our results should be interpreted taking into account several important methodological limitations. First, placebo as well as nocebo effects can occur, also in exercise studies (Hurst et al., 2019). The difficulty is to design adequate placebo or sham intervention arms, which for the eucapnic hyperpnea that we used as *verum* is complicated. Since in their quantitative meta-analysis, only 43% of the included RMT studies included a sham-training group to account for a possible placebo effect of RMT, Illi et al. (2012) could specifically look if it made any difference to have a sham or placebo group in RMT studies. Their analysis suggested that the presence and type of such a control group did not make a difference. Also, our finding of unchanged VO_{2peak} after RMET corroborates this contention. Overall, we deemed our choice to use a pre–post hypoxia–normoxia cross-over design without a sham condition to be a reasonable trade-off but cannot fully exclude that placebo and/or training effects could have influenced our results. Second, we did not specifically quantify the effects of RMET on respiratory muscle force and performance with maximum inspiratory pressure or respiratory endurance test to see if RMET had greater effects in women as compared with men. Third, even though our participants were young active people who were not engaged in structured athletic training, the men were somewhat more physically active as compared with the women and, therefore, relatively better trained, in part reflected in their respective VO_{2peak} values, which may have led to a different effect of the RMET in the two groups. Fourth, our sample size was rather small, and our study needs replication beyond our pilot approach. Fifth, we did not quantify locomotor nor respiratory muscle fatigue after the TT to see if it was changed, nor did we quantify any other metaboreflex-caused cardiovascular changes after RMET. Sixth, for technical reasons, we could not collect respiratory data during the TTs and, therefore, could not compare breathing patterns during the TTs, before and after RMET. Seventh, we chose a 10-km cycling TT for assessing the effects of RMT. The relatively greater effects of RMET on the TT performance in hypoxia may in part also have been due to the fact that the duration of exercise for the same distance was greater in hypoxia than in normoxia. Also, since we did not implement TT familiarization sessions, we could not exclude that some learning effects may have influenced our results. Finally, we did not collect any data allowing to look into the potential mechanistic explanations for our findings. Follow-up studies are needed to look into the possible mechanistic explanations underlying the differing effects of RMET on endurance performance in women compared with men.

CONCLUSION

Women are more prone to limitation by the respiratory system of endurance-type exercise performance. Apart from limitation of expiratory flow, fatiguability of the respiratory muscles may also play a role. We found that eucapnic hyperpnea training 5×30 min/week for 4 weeks significantly improved the performance in the 10-km TT, more in women than in men, and more so in hypoxia than in normoxia. This greater effect of RMT on TT performance in hypoxia than in normoxia is compatible with the contention of a more pronounced performance-limiting role for the respiratory system during endurance exercise in hypoxia compared with normoxia, and more so in women whose respiratory system is undersized compared with that of men. Due to the limitations of our pilot study design, these results need to be confirmed in trained athletes and complemented with additional exploration of explanatory mechanisms. These would include quantifying the training-induced respiratory muscle strength and endurance, and any attenuation of the respiratory muscle metaboreflex and of the decreased rating of perceived breathlessness or rating of perceived exertion (Shei, 2018). Our results suggest a further potential for RMET for improving the endurance performance, specifically for performance at high altitudes and especially for women.

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 Comité d'éthique de la recherche du Canton de Genève. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JC and GG collected the experimental data. All authors analyzed the data. BK wrote the first draft of the manuscript. All authors contributed to the design of the protocol, final article, and approved the submitted version.

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Acute Effects of the Wim Hof Breathing Method on Repeated Sprint Ability: A Pilot Study

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The Wim Hof breathing method (WHBM) combines periods of hyperventilation (HV) followed by voluntary breath-holds (BH) at low lung volume. It has been increasingly adopted by coaches and their athletes to improve performance, but there was no published research on its effects. We determined the feasibility of implementing a single WHBM session before repeated sprinting performance and evaluated any acute ergogenic effects. Fifteen amateur runners performed a single WHBM session prior to a Repeated Ability Sprint Test (RAST) in comparison to voluntary HV or spontaneous breathing (SB) (control) in a randomized cross-over design. Gas exchange, heart rate, and finger pulse oxygen saturation (SpO_2) were monitored. Despite large physiological effects in the SpO_2 and expired carbon dioxide (VCO_2) levels of both HV and WHBM, no significant positive or negative condition effects were found on RAST peak power, average power, or fatigue index. Finger SpO_2 dropped to 60 ± 12% at the end of the BHs. Upon the last HV in the WHBM and HV conditions, end-tidal CO_2 partial pressure ($PETCO_2$) values were 19 ± 3 and 17 ± 3 mmHg, indicative of respiratory alkalosis with estimated arterial pH increases of +0.171 and of +0.181, respectively. Upon completion of RAST, 8 min cumulated expired carbon dioxide volumes in the WHBM and HV were greater than in SB, suggesting lingering carbon dioxide stores depletion. These findings indicate that despite large physiological effects, a single WHBM session does not improve anaerobic performance in repeated sprinting exercise.

Keywords: Wim Hof breathing method, hyperventilation, apnea, RAST, anaerobic performance

INTRODUCTION

Wim Hof is a Dutch athlete, nicknamed "Iceman" for his ability to withstand freezing temperatures. He has accumulated 20 "world records" for feats such as standing in a container while covered with ice cubes for 2 h, climbing Mount Kilimanjaro in shorts, swimming 60 m underneath ice, and running a half marathon barefoot on snow and ice north of the Arctic Circle (Hof, 2020c). He attributes these feats to training with his Wim Hof Method (WHM). This is a combination of breathing exercises [Wim Hof breathing method (WHBM): periods of hyperventilation (HV) followed by voluntary breath-holds (BH) at low lung volume (Hof, 2020b)], repeated exposure to cold, and mental commitment (Hof, 2020e). The WHM allegedly provides benefits such as stress reduction, enhanced creativity, more focus and mental clarity, better sleep, improved cardiovascular health, and improved exercise performance (Hof, 2020b). The latter would include faster recovery from physical exertion, heightened focus and mental composure, and increased muscular endurance (Hof, 2020d).

Many athletes have adopted the WHM, such as the tennis player Novak Djokovic (Novak Djokovic on Instagram: @iceman_hof how did we do?…, 2021), the surfer Kelly Slater (Kelly Slater's Bizarre, Daredevil-Inspired Breathing Technique, 2021), the American football punter Steve Weatherford (Hof, 2020a), the rower Janneke van der Meulen (Hof, 2020d), the UFC fighter Alistair Overeem, and the big wave surfer Laird Hamilton (Hof, 2021).

While the WHM seems to present interesting benefits, there is virtually no published research on its effects on sport performance. However, studies have shown that HV, which is part of the WHBM, can improve anaerobic performance (Ziegler, 2002; Sakamoto et al., 2014; Jacob et al., 2015). HV induces hypocapnia and drives the reaction sequence $H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$ more to the right, elevating blood pH (Saladin and Miller, 2004). This respiratory alkalosis may improve anaerobic performance by compensating exercise-induced metabolic acidosis (Jacob et al., 2008). The effects on the performance of HV, combined with BH, as done in the WHBM, have not been investigated yet.

By itself, BH drives the reaction sequence $H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$ more to the left, inducing a respiratory acidosis (Pflanzer, 2004). Thus, in the WHBM, the BH-induced CO_2 retention would counter the HV-induced respiratory alkalosis. BH also triggers the so-called "diving response" (Foster and Sheel, 2005), which includes bradycardia, peripherical vasoconstriction, increased blood pressure, and contraction of the spleen (Dujic et al., 2011). The latter releases ∼100 ml of concentrated red cells into the circulation, which may influence performance, even though most investigations did not find improvement in performance following apneas (Du Bois et al., 2014; Sperlich et al., 2015; Yildiz, 2018).

Any effects of HV or the WHBM are expected to be short-lasting and most likely to occur in short duration anaerobic lactic type performance such as the Wingate test, which is a cycle ergometer test more specific to cycling-based sports. The development of the Repeated Ability Sprint Test (RAST) provides a reliable, valid (Zagatto et al., 2008), and practicable field test to determine running anaerobic power (Nick and Whyte, 1997). With 6 × 35 m repeated sprints, the total running time is close to 30 s, making the test comparable with the Wingate test. Times and body weight can be used to calculate maximal and average power outputs along with a fatigue index. Repeated high-intensity sprints cause substantial metabolic acidosis, contributing to muscular fatigue and metabolic output decline (Kairouz et al., 2013).

Therefore, the aims of this pilot study were to determine the feasibility of implementing the WHBM before sport performance and evaluate whether a single WHBM session provides any acute ergogenic effects during repeated-sprint bouts. In addition to performance, physiological and psychological data were collected to allow a better global understanding of the WHBM.

Abbreviations: BH, breath-hold; FI, fatigue index; HV, hyperventilation; RAST, repeated ability sprint test; RPE, rate of perceived exertion; RV, residual volume; SB, spontaneous breathing; TLC, total lung capacity; WHBM, Wim Hof breathing method; WHM, Wim Hof method.

METHODS

Experimental Approach to the Problem

We did a randomized, controlled three-way crossover pilot study to (1) assess the feasibility of pre-performance WHBM and (2) compare the acute effects of single sessions of the WHBM, HV, and SB on performance. Feasibility concerned recruitment, execution of the WHBM, and data collection. Performance was assessed with the RAST. Body mass and running times were used to calculate peak power, average power, and fatigue index (FI) (Nick and Whyte, 1997) to compare performance between conditions. Gas exchange, oxygen saturation (finger pulse oximetry), heart rate (HR), rate of perceived exertion (RPE), and responses to three questionnaires were collected to evaluate whether there were any differences in RAST performance between the breathing methods that would correlate with physiological changes.

Participants

Inclusion criteria included being a healthy regular runner (in base training for more than 3 years with 2 or more running training sessions per week) in order to limit variability in sprinting performance, being familiar with maximal sprinting, being an adult (18 years or older), and being male (to avoid influence from hormone level changes and to limit the total number of participants to be recruited). The participants were recruited through word of mouth and social media. Sixteen physical education students volunteered with one participant excluded because of an injury, resulting in a final sample size of 15 (72.4 ± 6.3 kg, 24.5 ± 2.3 years, 10.3 ± 4.7 training years, 7.6 ± 2.5 h/week training, 7.3 ± 1.1 peak sprint power in watt/kg; mean values ± standard deviations).

Procedures

The experiments were performed in late summer 2020 on a covered 50 m tartan track ("La Pontaise," Lausanne, Switzerland, elevation 597 m). Weather influenced ambient temperature, which was systematically measured. Once a week (same day, same time) for 4 weeks; each participant was seen by the same experimenter. The first meeting was used to answer questions and obtain written consent, to record demographic data, to collect a baseline running time with the control condition, and to familiarize the participants with the testing and the two breathing maneuvers to maximize reliability. The next three sessions were used to assess each method (WHBM, HV, and SB) in a balanced randomized order (computer-generated sequence). The participants were kept blinded to the results of the sprints until the end of the last session. They were instructed to maintain their usual diet and lifestyle during the study, to abstain from tobacco, coffee, alcohol, and ergogenic drugs the day of testing, and to abstain from any important exercise the day leading up to the test.

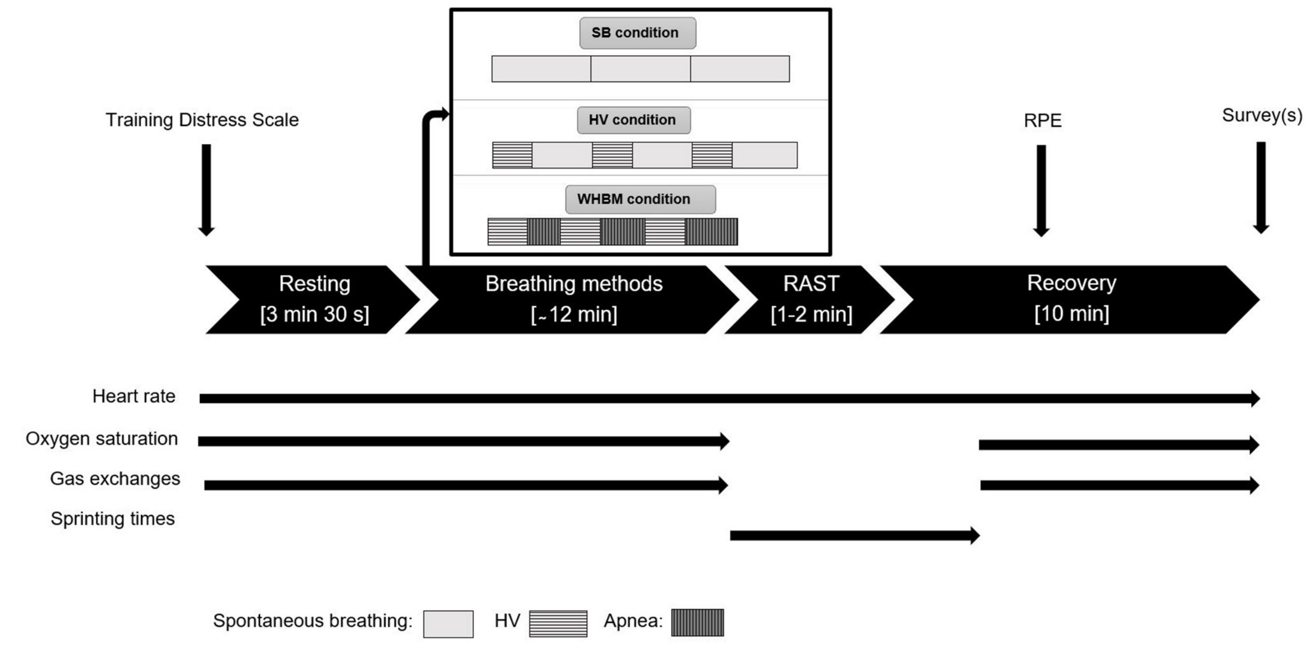

FIGURE 1 | Overview of the protocol. Thin straight black arrows indicate when different data collection occurred. Apnea rectangles length increases because the breath-holds (BHs) durations spontaneously increased.

Participants started each session with the same self-chosen warm-up. Then, they took a supine position on a floor mat and rested for 3 min 30 s for the acquisition of resting values before executing the specific breathing method (WHBM, HV, and SB) while conserving the supine position. Then, after 10 s, the participants prepared for the start of the RAST. The test involved performing 6 × 35 m sprints with 10 s rests between sprints. The participants took a sprint 3-point stance start position 3 s before each sprint, waited for the "go" signal, and then performed all-out sprints under strong verbal encouragement. The participants were instructed to perform each sprint as fast as possible and not use a pacing strategy. The timing was started manually and automatically stopped with photocells using an electronic timer (Witty, Microgate, Bolzano, Italy). Upon arrival, the participants immediately took their supine position again for 10 min of post-sprint measurements.

Breathing Methods

The WHBM was performed using the audio guide on the WHM mobile app. The participants performed three cycles of the WHBM as prescribed by the official website (Hof, 2020b). One cycle consisted of hyperventilating for 30 breaths, defined as respiratory movements of maximum amplitude at the frequency given by the audio guide (~0.32 Hz). This period lasted ~1 min 30 s. The participants then fully exhaled to residual volume (RV) and held their breath as long as possible (BH). At breaking point, they inhaled to total lung capacity (TLC) and kept their breath for 15 s before starting the next cycle. HV was performed as in the WHBM, except that the BH times were substituted with ~2 min 30 s of spontaneous breathing (the prior estimated breath hold duration), for a total duration of 12 min for the breathing maneuvers. The control condition consisted of spontaneous breathing (SB) for 12 min. Upon completion of the last cycle, the participants took off the face mask and the finger oximeter and prepared for RAST (see **Figure 1**).

Repeated Sprint Ability

Performance was assessed with the RAST. Peak power and average power were obtained using the equation $Power = \left(bodymass \times distance^2\right)/time^3$ and the FI was calculated using the equation $FI = \left(Peak\ Power - Min\ Power\right)/Total\ Sprint\ Time$ (Nick and Whyte, 1997).

Physiological Monitoring

Except during RAST, gas exchange was monitored breath-by-breath throughout the experiment with a portable metabolic device (K5, COSMED, Rome, Italy) and a face mask. The device was calibrated before each test using a 3 L syringe and gas mixtures of known concentration. Delay calibration and scrubber testing were done regularly. Oxygen uptake (VO_2), expired carbon dioxide (VCO_2), and minute ventilation (VE) were averaged for 1 min during rest, for the entire HVs durations, for 5 breaths immediately after RAST, and for 1 min after 8 min 30 s of post-RAST recovery. End-tidal O_2 partial pressure ($PETO_2$) and end-tidal CO_2 partial pressure ($PETCO_2$) were averaged for 1 min during rest, for the 5 last breaths during HV, and for the 5 last breaths pre-sprint. During the spontaneous breathing periods between HV, values were averaged for the entire duration. To obtain post-RAST cumulated oxygen uptake (VO_2-OFF), cumulated expired carbon dioxide (VCO_2-OFF), and cumulated ventilation (VE-OFF), breath-by-breath data were cumulated for 9 min post-sprint and the volumes equivalent to 9 min at rest (pre-RAST) were subtracted. $PETCO_2$ values were used to estimate pH levels according to the algorithm of Siggaard-Andersen (Siggaard-Andersen and Siggaard-Andersen, 1990) and to estimate pH variations according to Dubose's equation (Dubose, 1983): pH variation = 0.08 × (40 – $PETCO_2$ measured) / 10.

Apart from during RAST, middle finger pulse oxygen saturation (SpO_2) was recorded throughout with an oxygen saturation monitor (Pulsox PO-400, Contech Medical Systems, Qinhuangdao, China). Data were averaged for 2 min during pre-RAST resting, for 5 s at approximately the highest value during hyperventilation, for 5 s at approximately the lowest value during breath holds, for the entire duration during the spontaneous breathing intervals in the HV condition, and for the 10 s pre-RAST.

HR was collected throughout with a thoracic belt and wristwatch (H10 belt and V800 watch, Polar Electro Oy, Kempele, Finland). HR data were filtered using custom routines (MATLAB, the Mathworks, Nattick, MA, USA) with detection and compensation of ectopic beats, median filtering to remove isolated outliers, detection of block errors, and replacement by interpolated values. Data were then averaged over 1 min during rest, over 5 beats around the lowest value during breath holds in WHBM; over 5 beats around the lowest value during spontaneous breathing periods between hyperventilation periods; over 5 beats around the highest value during hyperventilation; for 750 s, 30 s, 5 beats, for the SB, HV, and WHBM conditions, respectively, before RAST; over 5 beats for HR recovery after 1 min; and over 5 beats for HR recovery after 2 min. During RAST, the highest HR was retained as HRmax. HR reserve percentage was calculated using the Karvonen Formula: $HR\ reserve\ percentage = (HRmax\ measured\ during\ sprints - Resting\ HR)/(HRmax\ predicted - Resting\ HR)$, where HRmax predicted was calculated as 220 - age.

Questionnaires

Prior to each session, the participants completed the Training Distress Scale as a performance readiness assessment (Grove et al., 2014). RPE was recorded on a Borg CR10 scale 1 min after the RAST. A custom questionnaire was used for the subjective assessment of the three sessions. The items were ≪ *I felt negative effect(s) of this way of breathing used before the repeated sprint test* ≫ : yes/no, and if yes, which one(s); ≪ *I felt positive effect(s) of this way of breathing before the repeated sprint test* ≫: yes/no, and if yes, which one(s); ≪ *To perform the test, this way of breathing made me feel overall* ≫ *:* visual analog scale going from strongly disadvantaged to strongly advantaged; and ≪ *I plan to reuse this way of breathing in the future in my personal practice* ≫ *:* yes/no.

Upon completion of the last session, we added the following additional items: ≪ *Rank from 1 to 3 the methods you felt the best in preparation for sprinting (1 being the best and 3 being the worst)* ≫ and ≪ *Rank from 1 to 3 the method you think you performed the best with (1 being the best and 3 being the worst)* ≫. Study data were collected and managed using REDCap electronic data capture tools hosted at UniSanté, Lausanne, Switzerland. An overview of the data collection is shown in **Figure 1**.

Statistical Analysis

As no data were available for power calculations since this research was a pilot study, none were performed. For the resting physiological measurements, breathing method physiological measurements, RAST and recovery physiological measurements, and questionnaire results, Shapiro-Wilk's test was used to ensure variable normality. If normality was ensured, condition effects were analyzed using linear mixed models (participants as the random effect and condition as a fixed effect) in two steps. First, to exclude period and carry-over effects of the cross-over trial, we included the effect of time or sequence (fixed effects) as an addition to the model. When time and sequence had no effect, they were removed in the second step. There was a sequence effect for HR at pre-sprint and for the respiratory frequency at rest, and a time effect for VE at HV1. Thus, they were considered in the second step. Normality was not found for Fatigue Index (WHBM condition), for resting $PETCO_2$ (WHBM condition), for resting VCO_2 (SB and HV condition), for VCO_2 at HV1 (SB condition), for VCO_2 at HV2 (SB condition), for VCO_2 at HV3 (SB and HV condition), for VCO_2 post recovery (SB condition), for VCO_2-OFF (SB condition), and for visual analogic scale ≪ *To perform the test, this way of breathing made me feel overall* ≫ (SB condition). A non-parametric repeated measures analysis (Friedman test) was performed for these variables. Statistical tests were corrected for multiple comparisons using Bonferroni correction. While these tests were performed using SPSS, version 26 (IBM Corp., Armonk, NY, USA), repeated measures correlation tests were performed between VCO_2-OFF and subjective variables with rmcorr library of R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). For all tests, significance was set at $p \leq 0.05$.

RESULTS

Checking of respect for the protocol, the temperature records, and the performance readiness survey results assured that the experiments occurred in reliable and valid conditions. Due to data recording errors, SpO_2 was not saved for two participants in the WHBM condition and respiratory parameters were not saved for one participant in the SB condition.

Feasibility and Performance

Out of 16 participants, 1 participant was excluded because of a muscular injury (in the lower limb during the first sprint), resulting in a final sample size of 15. The participants were able to perform the WHBM and HV methods without difficulties. Only minor malfunctions or technical problems occurred during data collection. As described in the section Surveys, 47% of the participants asserted to use the WHBM in the future while 53% did not. The values of RAST peak power, average power, and FI are shown in **Table 1**. There were no significant differences between conditions.

TABLE 1 | Performances determined during the repeated ability sprint test (RAST) realized with SB, HV, and WHBM conditions.

	SB	HV	WHBM	*p*
Peak power (watts)	501.9 ± 104.5	492.3 ± 112.7	501.3 ± 115.6	0.720
Average power (watts)	418.2 ± 84.3	407.6 ± 85.8	413.6 ± 86.7	0.360
Fatigue index	4.6 ± 2.2	4.7 ± 2.2	4.9 ± 1.7	0.819

SB, spontaneous breathing; HV, hyperventilation; WHBM, Wim Hof Breathing Method.
Mean values ± standard deviations.

TABLE 2 | Resting values in SB, HV, and WHBM conditions.

	SB	HV	WHBM	*p*
HR (bpm)	75 ± 10	77 ± 10	79 ± 12	0.122
SpO_2 (%)	96 ± 1	96 ± 1	96 ± 1	0.298
$PETO_2$ (mmHg)	102 ± 3	102 ± 5	103 ± 5	0.570
$PETCO_2$ (mmHg)	42 ± 7	40 ± 3	40 ± 4	0.167
VO_2 (ml/min)	366 ± 36	364 ± 70	400 ± 63	0.119
VCO_2 (ml/min)	414 ± 54	403 ± 105	449 ± 90‡	**0.017**
VE (L/min)	11.2 ± 1.4	11.4 ± 2.8	12.7 ± 2.0†	**0.025**
Tidal volume (mL)	1068 ± 288	1123 ± 383	1174 ± 287	0.595
Respiratory frequency (/min)	12 ± 4	11 ± 3	12 ± 3	0.881

SB, Spontaneous Breathing; HV, Hyperventilation; WHBM, Wim Hof breathing method.
Mean values ± standard deviations.
†Significantly greater than SB.
‡Significantly greater than HV. Bold values indicate significant differences.

Resting Physiological Measurements

Resting values are presented in **Table 2**. A significant difference was found in VE between SB and WHBM conditions ($p = 0.039$) and in VCO_2 between HV and WHBM conditions. According to the algorithm of Siggaard-Andersen (Siggaard-Andersen and Siggaard-Andersen, 1990), $PETCO_2$ values indicated resting pH values of 7.380, 7.397, 7.397 for the SB, HV, and WHBM conditions, respectively.

Breathing Method Physiological Measurements

Table 3 presents HR max, SpO_2 max, $PETO_2$ max, $PETCO_2$ min, average VO_2, average VCO_2, average VE, average tidal volume, and average respiratory frequency during the hyperventilation periods for the WHBM condition. It further lists HR min, SpO_2 min, and BH duration during the BH periods. For the HV condition, the same parameters during the hyperventilation periods are shown; HR min and averaged values for the other parameters during the spontaneous breathing periods between the hyperventilation periods are also shown. Pre-sprint values are presented for both conditions.

The single inhalation to TLC following breath holding at RV to breaking point generally induced a sharp increase of HR. HR then decreased again during the 15 s additional BH at TLC. An example of a HR pattern is presented in **Figure 2**. A strong correlation ($r = -0.731$) between BH duration and SpO_2 was found (**Figure 3**). In the WHBM condition, VO_2 during HV2 and HV3 was −288 ± 182 ml and −437 ± 207 ml, respectively, inferior to assumed VO_2 consumption during the respective previous BH (resting VO_2 × BH duration). Assuming no net change in blood buffer status, according to the algorithm of Siggaard-Andersen (Siggaard-Andersen and Siggaard-Andersen, 1990), the minimal $PETCO_2$ values reached at the end of HV3 corresponded to a pH of 7.651 in the WHBM condition and 7.688 in the HV condition, confirming a state of respiratory alkalosis. The pre-RAST value for the HV condition corresponded to a pH 7.559, suggesting partial correction of the respiratory alkalosis. Similarly, according to Dubose's equation (Dubose, 1983), the minimal $PETCO_2$ reached at the end of HV3 corresponded to a pH variation of +0.171 units pH in the WHBM condition and +0.181 in the HV condition. The pre-RAST value for the HV condition corresponded to an increase of +0.123 units pH.

The average values of VE, VO_2, and VCO_2 measured at rest, during HV1, HV2, and HV3, immediately upon arrival after RAST, and during recovery in all three conditions are shown in **Figure 4**.

RAST and Recovery Physiological Measurements

Table 4 presents RAST and recovery heart rate. No significant differences were found between the conditions for any of these measurements.

The values of VE, VO_2, and VCO_2 measured post-RAST and post-recovery are shown in **Figure 4** with VE-OFF, VO_2-OFF, and VCO_2-OFF.

TABLE 3 | Physiological measurements during WHBM and HV conditions.

		HR (bpm)	SpO_2 (%)	$PETO_2$ (mmHg)	$PETCO_2$ (mmHg)	VO_2 (ml/min)	VCO_2 (ml/min]	VE (L/min)	Tidal volume (ml)	Respiratory frequency (1/)	BH duration (s)
WHBM condition	HV1	110 ± 13.2	99 ± 0	128 ± 2	22 ± 3	501 ± 79	1344 ± 213	61.9 ± 9.2	3148 ± 537	25 ± 19	–
	BH1	67 ± 15	77 ± 9	–	–	–	–	–	–	–	74 ± 16
	HV2	108 ± 14	99 ± 0	128 ± 2	20 ± 3	673 ± 128	1179 ± 181	61.2 ± 10.9	3135 ± 553	20 ± 2	–
	BH2	67 ± 1	68 ± 10	–	–	–	–	–	–	–	101 ± 19
	HV3	108 ± 16	99 ± 1	128 ± 2	19 ± 3	730 ± 129	1112 ± 201	61.4 ± 13.3	3112 ± 773	22 ± 8	–
	BH3	66 ± 14	60 ± 12	–	–	–	–	–	–	–	119 + 26
	Pre-sprint	80 ± 17	62 ± 12	–	–	–	–	–	–	–	–
		Ø	¥		‡						¥
HV condition	HV1	104 ± 12	99 ± 1	128 ± 2	21 ± 3	488 ± 89	1308 ± 208	59.5 ± 12.8	2979 ± 672	22 ± 7	–
	SB1	71 ± 12	97 ± 1	108 ± 6	30 ± 2	299 ± 68	310 ± 83	11.6 ± 3.3	1065 ± 368	12 ± 3	–
	HV2	104 ± 13	99 ± 1	129 ± 2	18 ± 2	511 ± 124	1136 ± 201	61.6 ±14.0	3072 ± 859	23 ± 13	–
	SB2	68 ± 9	98 ± 1	107 ± 7	26 ± 3	292 ± 75	249 ± 87	10.8 ± 4.2	988 ± 381	12 ± 3	–
	HV3	102 ± 12	99 ± 0	129 ± 2	17 ± 3	542 ± 120	1030 ± 161	60.9 ± 13.0	3130 ± 696	20 ± 4	–
	SB3	66 ± 11	98 ± 1	109 ± 8	24 ± 3	329 ± 82	266 ± 95	12.8 ± 5.4	1144 ± 480	13 ± 4	–
	Pre-sprint	72 ± 13	97 ± 2	105 ± 10	25 ± 4	411 ± 111	294 ± 116	13.5 ± 5.7	1076 ± 559	15 ± 6	–
				#	†						

SB, spontaneous breathing; HV, hyperventilation; WHBM, Wim Hof breathing method.
Mean values ± standard deviations.
–, Non available data.
[Ø]Indicates that, in average, HR significantly increased with the HVs and significantly decreased with the BHs compared to resting values in WHBM condition.
[¥] Indicates that values were significantly different between BH1, BH2 and BH3 in WHBM condition.
[#]Indicates that values were significantly greater of resting value in HV1, HV2 and HV3 in HV condition.
[†]Indicates that pre-sprint value was significantly greater than HV3 in HV condition.
[‡]Indicates that values were significantly different between HV1 and HV2, and between HV1 and HV3 in WHBM condition.

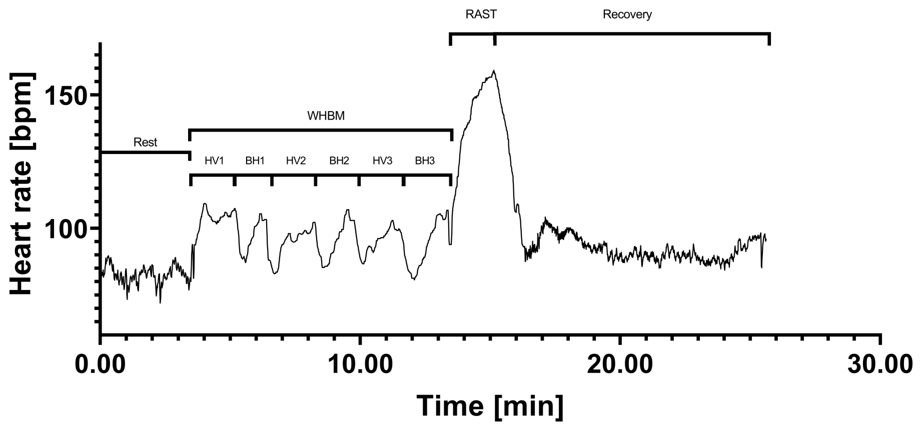

FIGURE 2 | Participant 7 heart rate (HR) data in the Wim Hof breathing method (WHBM) condition.

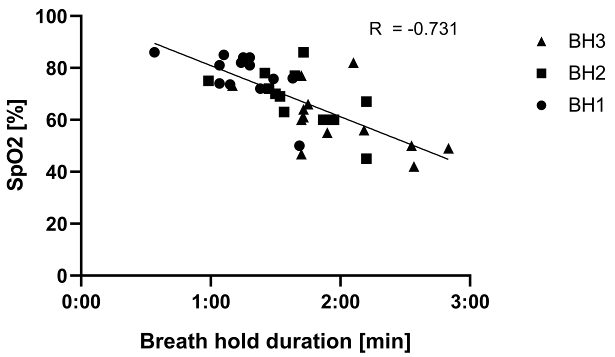

FIGURE 3 | Relation between breath hold durations following hyperventilations (HVs) and oxygen saturation (SpO2) in the Wim Hof breathing method (WHBM) condition.

Surveys

RAST Borg CR10 scores were 7.5 ± 1.2, 7.6 ± 1.1, and 6.9 ± 1.4 in SB, HV, and WHBM conditions, respectively. Statistical differences were found for WHBM condition versus HV ($p = 0.008$) and versus SB condition ($p = 0.017$).

Negative effects of tingling, numbness, dizziness, and heaviness were reported by 60% of the participants for the HV condition. Negative effects of heaviness and deafness were reported by 33% of participants for the WHBM. Positive effects of improved breathing and less fatigue were reported by 73% of the participants for the HV condition. Positive effects of improved breathing, less fatigue, and increased energy were reported by 87% of the participants for the WHBM condition. Finally, 47% reported planning to reuse the WHBM in their personal training and competitive practice in the future while 53% declared not to.

Participants felt the most advantaged when performing the test in the WHBM condition, followed by the HV condition (**Figure 5**). A significant difference was found between WHBM and SB conditions.

Overall assessment of the breathing methods provided the results shown in **Table 5**. A majority of participants assessed WHBM as the best and SB as the worst.

DISCUSSION

Feasibility and Performance

This study reports the feasibility and effects of practicing the WHBM before repeated sprinting performance. The acute effects of a single session of WHBM were assessed on repeated-sprint bouts and on various physiological and psychological variables. After receiving anecdotal information from early adopters, we expected that the use of WHBM might give an edge for sprinting performance. Participants consented to comply and adequately performed the WHBM and procedures, contributing to a valid assessment. Despite some lightheadedness and tingling, they were able to perform the RAST as required. However, in spite of large physiological effects of both HV and WHBM, no significant condition effect was found regarding performance, peak power, average power, or fatigue index. Apart from the CO_2 stores depletion that persisted through the RAST (VCO_2-OFF), the observed physiological effects were specific and immediate to the respiratory maneuvers, and they did not translate into global physiological repercussions that could change performance. It follows that, despite subjective preference for the two breathing methods in comparison with spontaneous breathing, in the present experimental conditions, the acute application of two specific breathing methods did not convey any advantage for repeated sprinting performance as assessed with the RAST. However, the lower post-sprint CO_2 levels in HV and WHBM conditions could be a factor that explains the subjective preference. Indeed, VCO_2 levels are linked to respiratory distress in dyspnea and apnea studies. Although weak, a correlation between VCO_2-OFF and the survey item ≪ *Rank from 1 to 3 the method you think you performed the best with* ≫ was found ($r = -0.445$, $p = 0.014$). However, the subjective preference could also reflect some placebo mechanism.

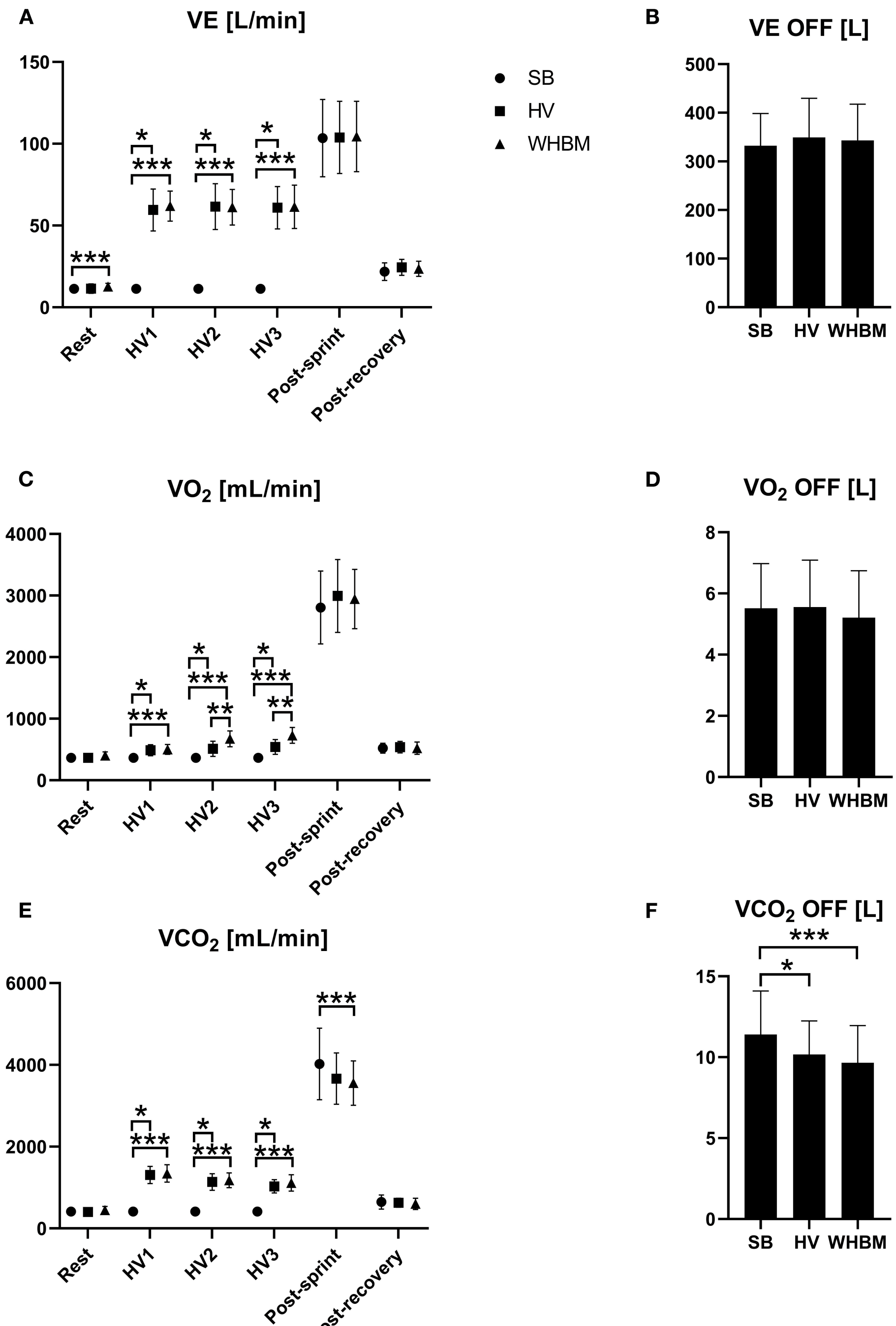

FIGURE 4 | (A) Changes in minute ventilation (VE), **(B)** cumulated ventilation (VE-OFF), **(C)** oxygen uptake (VO_2), **(D)** cumulated oxygen uptake (VO_2-OFF), **(E)** expired carbon dioxide (VCO_2), and **(F)** cumulated expired carbon dioxide (VCO_2-OFF) for SB, HV and WHBM conditions. SB, spontaneous breathing; HV, hyperventilation; WHBM, Wim Hof Breathing Method. *indicates a significant difference between SB and HV condition, **indicates significant difference between HV and WHBM condition, ***indicates significant difference between SB and WHBM condition.

Respiratory Alkalosis

$PETCO_2$ values indicated a blood pH increase of +0.171 (to reach an estimated pH of 7.651) upon the last HV in the WHBM condition, which could have had ergogenic effects on anaerobic performance by preventing and/or compensating exercise-induced metabolic acidosis (Jacob et al., 2008). However, in the WHBM condition, the HV-induced respiratory alkalosis was attenuated by the ensuing BH-induced CO_2 retention.

TABLE 4 | Repeated ability sprint test (RAST) and recovery heart rate during WHBM and HV conditions.

	SB	HV	WHBM	*p*
Pre-sprint (bpm)	76 ± 8	72 ± 13	80 ± 17	0.083
Highest HR during sprint (bpm)	173 ± 8	172 ± 7	173 ± 8	0.122
Highest percentage of HR reserve during sprints (%)	81 ± 7	80 ± 7	80 ± 7	0.063
HR recovery after 1 min (bpm)	–49 ± 14	–48 ± 10	–48 ± 13	0.755
HR recovery after 2 min (bpm)	–62 ± 9	–62 ± 9	–61 ± 10	0.477

SB, spontaneous breathing; HV, hyperventilation; WHBM, Wim Hof breathing method.
Mean values ± standard deviations. HR recovery values indicate post exercise drops in HR from highest HR during sprint, in bpm.

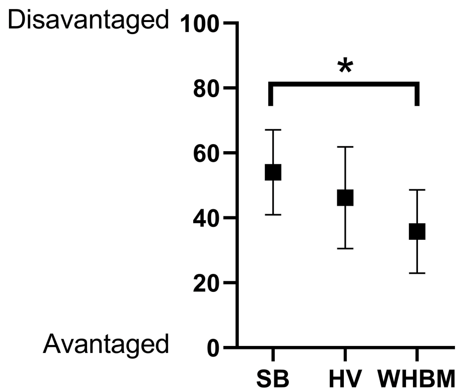

FIGURE 5 | Results to visual analogic scale "To perform the test, this way of breathing made me feel overall" (mean ± standard deviation). *$p < 0.05$.

Indeed, $PETCO_2$ values decreased along the HVs to a lower level in HV compared to the WHBM condition (down to 17 ± 3 versus 19 ± 3 mmHg, respectively). This difference was not significant, but it can be hypothesized that it increased slightly with the last BH. Additionally, HVs effects were probably also attenuated during the delay before the RAST. In the HV condition, $PETCO_2$ rose pre-sprint to values that corresponded to a +0.123 pH change (and an estimated pH of 7.564) instead of +0.181 (and a pH of 7.682)] at the end of the last HV. Thus, we speculate that the WHBM pH change was slightly inferior to +0.123 before beginning the RAST. In agreement, another study on WHBM reported that a representative subject ended the last BH with a pH of 7.50, a value 0.10 higher than at the start (Kox et al., 2014). In comparison, other studies reported improved performance using HV with comparable or smaller pH changes (Sakamoto et al., 2014; Jacob et al., 2015), greater pH changes (Ziegler, 2002), and smaller pH changes using bicarbonate supplementation (Costill et al., 1984; Bishop et al., 2004). However, other studies did not find improved performance even though comparable or smaller pH changes were reported (Jacob et al., 2008; Kairouz et al., 2013; Sakamoto et al., 2018). While the literature is not unanimous, HV, by inducing respiratory alkalosis, may have positive effects on anaerobic type performance.

Diving Response

BH by itself triggers the so-called diving response, and when coupled with stimulation of facial cold receptors, a greater response is seen (Foster and Sheel, 2005). The diving response includes bradycardia, peripherical vasoconstriction, increased blood pressure, and contraction of the spleen (Dujic et al., 2011). Breath-holding is known to induce spleen contraction leading to an increase of blood hemoglobin concentration (Schagatay et al., 2001). Spleen contraction releases stored erythrocytes into the circulation. A single contraction causes a hemoglobin increase that corresponds to a 3–10% increase in blood oxygen carrying capacity (Stewart and McKenzie, 2002). The increased hemoglobin levels may have potentially beneficial effects on performance for both increased blood O_2 carrying capacity and increased CO_2 buffering capacity (Schagatay et al., 2012). However, the increased blood pressure and peripheral vasoconstriction might impair performance. Studies evaluating the effect of apneas on performance found no improvement (Du Bois et al., 2014; Sperlich et al., 2015; Yildiz, 2018). In consistence with these studies, the WHBM did not enhance performance in this investigation.

Blood Oxygen Saturation

Oxygen saturation dropped to very low levels at the end of the BHs (60%). The 10 s delay before the start of RAST might not have allowed full recovery of saturation, which could have impaired performance. In another study assessing an apnea test following a forced expiration, oximetry recovery times were between 20 and 40 s (Plas and Bourdinaud, 1953).

Catecholamine and Cortisol Levels

In another investigation (Kox et al., 2014), the authors reported that, while WHBM led to no increase in norepinephrine, dopamine, and cortisol levels, significantly higher plasma epinephrine levels were found. The latter has a powerful vasodilator effect on blood vessels in skeletal muscle (Davis et al., 2008) and stimulates glycogenolysis (Kenney, 2012), which could lead to ergogenic effects in the WHBM condition.

Gas Exchange

Comparable VE was observed during HVs in the HV and WHBM conditions. Compared to spontaneous breathing, VO_2 showed significantly higher values for the HV condition, which is possibly linked to greater work performed by the respiratory muscles (Coast et al., 1993). VE was further increased in the WHBM condition, likely due to the BH-induced oxygen desaturation leading to a hypoxic ventilatory response (**Figure 4C**). Intriguingly, the difference in VO_2 consumption between the WHBM and HV conditions during HV2 and HV3 was inferior to assumed VO_2 consumption during the previous BH. VCO_2 showed significantly higher values in the HV condition as a result of larger CO_2 elimination, and it

TABLE 5 | Overall assessment of breathing methods in percentage of participants.

		SB	HV	WHBM
The best / the worst method in term of sprint test	The best	13.3%	13.3%	73.3%
	The worst	53.3%	46.7%	0.0%
The best / the worst method in term of perceived performance	The best	13.3%	20.0%	66.7%
	The worst	53.3%	46.7%	0.0%

rose to an even greater extent in the WHBM condition given the CO_2 accumulation during the post-HV BHs. However, this difference was not significant (**Figure 4E**). VCO_2-post after RAST in the WHBM condition was significantly smaller than in the SB condition, while in the HV condition it was not, which is counter-intuitive given the CO_2 accumulation during BHs. In addition, it is interesting to note that VCO_2-OFF was significantly smaller in both the WHBM and HV conditions than in the SB condition, which suggests that some of the HV-induced CO_2 stores depletion had persisted through the RAST.

Breath-Hold Duration

BH durations were comparable to the durations most people can achieve with no prior HV and at full lung volume. We speculate that several factors counterbalanced each other, resulting in these durations. The most potent regulator of ventilatory drive is pH, followed by partial pressure of carbon dioxide (PCO_2), and, to a lesser extent, partial pressure of oxygen (PO_2) (Saladin and Miller, 2004). There is also a substantial prolongation of BH time that is independent of chemical stimuli, which has been attributed to neural input from pulmonary stretch receptors (Mithoefer et al., 1953). Thus, apnea at low lung volume as performed in WHBM leads to shorter BHs because of the decreased stimulus to the pulmonary stretch receptors and accelerated onset of hypoxia and hypercapnia (decreased O_2 pulmonary volume and decreased capability to dilute the rise in metabolically-derived CO_2 levels) (Skow et al., 2015). However, considering that HV reduces arterial CO_2 content and increases arterial pH, the subsequent BH duration should be longer, as it will take longer to reach the threshold of chemoreceptor activation (Skow et al., 2015). Consistent with previous findings (Schagatay et al., 1999), the latter is also the more likely reason as to why BH duration significantly increased between sets: the successive HVs progressively reduced arterial PCO_2 as suggested by the decreasing $PETCO_2$ values from one to the other HV. Under such conditions, arterial PO_2 can decrease to a greater extent (Djarova et al., 1986) and trigger spleen contraction as described above. In this study, oxygen saturation dropped progressively through the BH sets and reached severe hypoxemia levels (60%) at the end of the last BH, which is consistent with another WHBM investigation where values reportedly even decreased to about 50% (Kox et al., 2014). The correlation between SpO_2 and BH duration (**Figure 2**) further illustrates the above explanations. In BHs following HV, activation of the peripheral chemoreceptors from sensing increased CO_2 is delayed, and their activation from sensing decreased O_2 is increased. If SpO_2 decreased to a great extent during BHs, it resumed normal values during the HVs, i.e., almost fully saturated (Kenney et al., 2012). Consistent with these studies, $PETO_2$ significantly increased above resting value during HV1, HV2, and HV3 in this study, indicating the effect of hyperventilation on alveolar gas composition.

Heart Rate

In the WHBM condition, HR significantly increased (by 31 bpm on average) during the HVs and significantly decreased (by 13 bpm on average) during the BHs compared to resting values. The HR increases during HVs were probably due to increased motor drive for increased respiratory muscle activity (Cummin et al., 1986). The HR drops during the BHs are generally attributed to an increase in cardiac parasympathetic drive triggered in response to breathing cessation (Cherouveim et al., 2013). However, the HR drops were also observed in the HV condition; thus, HV cessation may be the main explanation. The sharp HR increase following the first inhalation after BHs observed in this study has been previously described. Upon resumption of breathing after an apnea test following forced expiration, pulses give way sometimes to an irregular acceleration, which tends to stabilize quickly (10–15 s) at approximately its initial rate (Plas and Bourdinaud, 1953). Because of these mechanisms, pre-sprint HR was not significantly different between conditions. During the sprints, HR reserve percentage reached testified to the high intensity of the effort.

Surveys

Psychological assessment showed rather positive results in the WHBM condition; RPE was significantly the lowest, participants felt significantly advantaged the most to perform the test, and felt the best for doing the sprint test and thought they performed the best. Around three-quarters of them reported positive effects such as less fatigue, increased energy, and improved breathing. Speculatively, the latter could potentially be due to reduced work of breathing resulting from pre-activation of sympathetic drive and catecholamine secretion, leading to bronchodilation and decreased airflow resistance (Sakamoto et al., 2014). On the other hand, negative effects such as deafness or dizziness were reported, potentially caused by the HV induced hypocapnia causing cerebral vasoconstriction (Skow et al., 2015). In the end, around half of our participants declared that they would consider the WHBM in their personal practice in the future. The participants reported significantly lower RPE with the WHBM. This may be due to a lesser "maximality" of the sprinting effort or reflect some placebo mechanism. The participants were necessarily aware that the experiment aimed to assess the effects of two breathing methods on repeated sprinting performance. Their belief that the WHBM might be beneficial could have led to such results or even worse, it may even have negated a decreased performance from WHBM-induced changes in physiological variables such as SpO_2.

Limitations

Several limitations should be considered when interpreting the results of this research. First, the relatively small sample size affected the reliability of the study. Second, because one is supposed to do a deep inspiration from RV to TLC 15 s before the end of the BH in WHBM and arterial blood gas analysis was not feasible, it was not possible to document pulmonary or blood gas exchanges during and at the end of the WHBM BHs. Also, there are several studies in the literature expressing reservations about the reliability of SpO_2 in situations leading to deep hypoxemia (Pottecher et al., 2003), indicating that the SpO_2 results during the BHs should be interpreted with caution. Third, fatigue index values were rather low compared to other studies: 7.1 in students of physical education and sport exercise (Paradisis et al., 2005), 5.4 after a power endurance training in basketball players (Balčiunas et al., 2006), 8.1–10.5 in elite basketball players (Pojskić et al., 2015), 4.2 in football players, 4.2 in sprinters, 4.2 in takrawer, 2 in volleyballers, and 4.6 in Pencak Silater athletes (Nasuka et al., 2019). The results could have been different if the participants had reached higher FI. A sensitivity analysis in this study on the four participants with a FI superior to five did not show statistical differences either. Finally, the participants described starting the RAST after resting motionless for ∼12 min in a supine position as difficult. Vigorous respiratory muscle contraction in the WHBM and HV conditions and maximal apnea in the WHBM condition could have mitigated these negative effects.

Another investigation could evaluate using the WHBM sitting on a chair or standing to limit the difficulty previously mentioned even if it would involve a less optimal breathing position. It would also be interesting to assess effects of apnea followed by HV, as potential ergogenic apnea-induced spleen contraction could last long enough and potential ergogenic HV-induced effects would be optimized if exercise was performed without delay. Moreover, while this study focused on acute effects of the WHBM, it would be of interest to assess its regular use in combination with training. Repeated-sprint training in hypoxia has been shown to induce greater improvement of repeated-sprint performance than in normoxia (Brocherie et al., 2017). Also, repeated sprint-induced arterial desaturation through voluntary hypoventilation at low lung volume induced greater enhancement in competitive swimmers than in normoxia (Trincat et al., 2017), and the magnitude of the improvement (+35%) was comparable to that obtained with repeated sprinting in hypoxia in cycling (+38%) (Faiss et al., 2013) and in double poling cross-country skiing (+58%) (Faiss et al., 2015). Similar or even greater improvements might be expected with a regular use of the WHBM.

PRACTICAL APPLICATIONS

This investigation is the first to evaluate the WHBM in view of improving repeated sprinting performance. While this pilot study underlined the possibility of practicing an acute session of WHBM before sport performance, it also presented side effects and did not enhance any performance parameter (peak power, average power, and FI) in later sprint sets. It appears unworthy to carry this method out as the improved physiological parameters did not translate into a performance increase. Based on the results found in this study, we do not recommend applying this method with the view of improving performance, at least not for repeated sprinting.

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 Research Ethics Committee of the Canton Vaud. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KG, BK, FC, and TC: conception and design, analysis and interpretation of data, drafting the article, critically revising the article, and final approval of the article. TC and KG: acquisition of data. All authors contributed to the article and approved the submitted version.

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Changes in Diaphragmatic Function Induced by an Increased Inspiratory Load Experienced by Military Divers: An Ultrasound Study

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Background: Inspiratory loading is experienced by military divers when they use rebreather device. Our objective was to assess the changes in diaphragm function induced by an increase in inspiratory load at values similar to those experienced by divers in real life.

Methods: We recorded the excursion and the thickness of the right hemidiaphragm in 22 healthy male volunteers under inspiratory load conditions, using ultrasound in B- and M-mode. The measurements were performed at tidal volume and during breathing at 50% of inspiratory capacity. The breathing rate was regulated and similar in the various sessions with and without load.

Results: The rebreather device used by French military divers leads to an increase in inspiratory load of close to 30 cmH_2O. Consequently, the session under load was performed using a device set to this threshold. Significant increases in the excursion and the thickening fraction of the diaphragm were observed between the sessions at tidal volume and at high volume. With addition of the inspiratory load, the excursion of the right hemidiaphragm increased significantly from 2.3 to 3.4 cm at tidal volume and from 3.9 to 4.7 cm at high volume. The thickening fraction increased significantly from 30.4 to 76.6% at tidal volume and from 70 to 123% at high volume. The statistical analysis demonstrated that assessment of the changes of the thickening fraction during breathing at tidal volume was the most relevant marker to assess the impact of the inspiratory load on the diaphragm.

Conclusion: Diaphragm ultrasound can be used to assess the changes in the diaphragm contraction pattern secondary to an increase in the respiratory load that can be generated by use a diving apparatus. The recording of the changes of the motion, and more importantly of the thickness of the diaphragm, during the breathing cycle is able to provide relevant information regarding the inspiratory load.

Keywords: respiratory muscle, diving, diaphragm, rebreather diving, chest ultrasonography

INTRODUCTION

SCUBA diving leads to alterations in the work of breathing through the use of a gas mixture delivered by a regulator under hyperbaric conditions (Clarke and Flook, 1999). Ventilatory stressors are further increased during strenuous swimming. The changes in ventilatory load differ according to the position of the diver and the apparatus used. During a SCUBA diving ascent, when the diver is in an erect position, the hydrostatic pressure gradient between the regulator in the mouth and the lung centroid (i.e., the point of confluence of the forces exerted by the respiratory system) is negative (Lundgren, 1999; Krauza and Lundgren, 2007). This results in an increase in inspiratory load and a decrease in alveolar pressure (Castagna et al., 2018). Some divers are less able to tolerate an increased ventilatory workload, thus resulting in a risk of dyspnea and hyperventilation that exposes the diver to pulmonary barotrauma. Furthermore, through heart-lung interaction, the decrease in thoracic pressure induces an increase in the cardiac preload. The blood mass transfer toward the lungs and the heart can contribute to certain injuries such as water immersion pulmonary edema, particularly during strenuous exercise (Boussuges et al., 2017).

The increase in inspiratory load is all the more acute in military divers using a closed-circuit apparatus with a rebreathing bag on their back (the pressure gradient then becomes the pressure difference between the rebreather and the lung centroid) because the imbalance is present during the entire ventral kicking dive.

As the diaphragm supports the main part of the inspiratory workload, the diaphragmatic function can be modified during SCUBA diving. Assessing diaphragmatic function is difficult in healthy volunteers. The use of tools available for this purpose is limited due to either the risks associated with ionizing radiation and the need for transport (fluoroscopy) or the complex nature and the invasive characteristics of the test (measurement of transdiaphragmatic pressure; Minami et al., 2018). Over the past 25 years, several studies have indicated that ultrasound is useful for morphological and functional assessment of the diaphragm (Ueki et al., 1985; Ayoub et al., 1997; Cohn et al., 1997; Boussuges et al., 2009). Various methods such as recording of the motion using M-mode ultrasonography or measurement of the changes of the diaphragmatic thickness during the breathing cycle have been proposed (for a review see Boussuges et al., 2020).

To assess the inspiratory stressors induced by a SCUBA breathing apparatus, analysis of the diaphragmatic function in healthy volunteers submitted to an increase in inspiratory resistance similar to that in a real SCUBA dive are likely to be informative. It has been reported, based on M-mode sonography, that a major increase in an inspiratory flow-resistive load can induce significant changes in the diaphragm contraction pattern, such as an increase in the inspiratory time and the diaphragm excursion (Soilemezi et al., 2013). However, in the work of Soilemezi et al. (2013), the volunteers were submitted to an inspiratory flow-resistive load equal to 50 $cmH_2O/l/s$, which is higher than the stressors that may be experienced by divers in real life.

The first objective of this study was to assess the changes in diaphragmatic function induced by an increase in inspiratory load at values similar to those encountered by divers using a rebreather. The second aim of the study was to determine the most appropriate ultrasound parameters to analyze the impact of an increase in inspiratory load on the diaphragm.

MATERIALS AND METHODS

Preliminary Study

To estimate the hydrostatic loading induced by the rebreathers used by the divers of the French Navy (Standard Complete Range Autonomous Breathing Equipment-STD CRABE, Aqualung, France), pressure-volume loops were recorded on a ventilator simulator (ANSTI LSTF 100 m, JFD, United Kingdom). The operating principle of the simulator is depicted in **Figure 1**. The hydrostatic imbalance was measured using a differential pressure sensor, which provides a readout of the difference between the pressure measured at the lung centroid of the mannequin (location and normalized dimensions, measurement in water) and the pressure at the mouth of the mannequin (measurement in the ventilatory circuit).

Main Study

In a previous study, Soilemezi et al. (2013) reported that when healthy volunteers were equipped with a mouthpiece and a nose clip, which leads to an increase in inspiratory resistance, the motion of the right hemidiaphragm increased from 1.7 ± 0.5 cm to 2.3 ± 0.9 cm (mean ± SD) compared to no instrumentation. In our work, the calculation of the sample was based on this previous study. For an alpha risk of 0.05% and a power of 80%, we determined that at least 18 healthy volunteers would have to be included in the study.

The study was approved by the Regional Ethics Committee (Aix Marseille University, CPP-1, NoA01299-32) and all of the volunteers provided their written informed consent to participate in the experiment. The research was conducted according to the Helsinki Declaration regarding medical research involving humans.

Prior to the ultrasound measurements, a pulmonary function test was carried out in all of the healthy volunteers in order to determine their inspiratory capacity with spirometry maneuvers (Spirobank II Smart, MIR, Langlade, France).

Protocol

Two sessions of the experiment were conducted: the volunteers were either submitted or were not submitted to a high inspiratory load, applied in a random order. The volunteers and the investigators were blinded to the inspiratory load used.

The volunteers were assessed in a seated position while breathing in a circuit equipped with a single-use mouthpiece, a disposable filter, and a commercially available threshold loading device (Philips Threshold IMT, Suresnes, Philips, France). For measurements without load, a tube of the same diameter and the same dead space (63 ml) was used instead of the

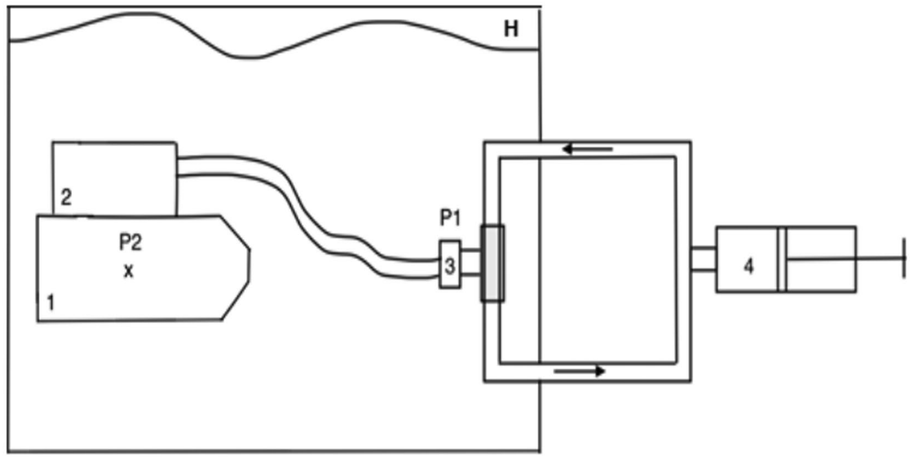

FIGURE 1 | Principle of the measurements on the ventilator simulator. The mannequin and rebreather are immersed in water of a sufficient depth to preclude surface effects, but no deeper than 2 m. 1 = mannequin, x = lung centroid, 2 = rebreather, 3 = rebreather mouthpiece, 4 = pump with valve for unidirectional flow in the circuit, P1 and P2 = pressure sensors (differential pressure sensor), H = hyperbaric chamber.

threshold loading device. Furthermore, a spirometer designed in our laboratory (Schmid and Boussuges, 2019) was placed at the end of the line. The spirometer was connected to a computer interface, which allowed the breathing rate and the gas volume to be measured continuously (breath-by-breath). The volunteers had real-time monitoring of inspired volume and breathing rate using a bar-graph. In order to ensure that the measured volumes were accurate, the spirometer was calibrated between each study phase, with the entire chain. Furthermore, this device was connected to the ultrasound machine *via* the electrocardiogram cables to record the signals of the beginning of both inspiration and expiration on the ultrasound images (Schmid and Boussuges, 2019).

The ultrasound measurements were performed during quiet breathing at tidal volume and during breathing at 50% of inspiratory capacity (deep breathing). The gas volumes were determined individually before the beginning of the study and a bar graph was used to guide the volunteers and to limit the variability of the breathing volume during the ultrasound examinations. The breathing rate and the volume were, therefore, similar between the two conditions involving load and no load.

Ultrasound Examination

The study of diaphragmatic function was performed using an Esaote portable ultrasound system (Mylab 25CV, Genoa, Italy). Excursions and thickening of the right hemidiaphragm were studied.

Measurement of the Excursion of the Right Hemidiaphragm

The method used to record the diaphragmatic motion has been published (Boussuges et al., 2021). Briefly, the liver was used as an acoustic window to visualize the right hemidiaphragm. A 2–3.5 MHz frequency probe (PA 230E Esaote, Genoa, Italy) was placed between the midclavicular and the middle axillary lines, below the right costal margin, and directed medially, cephalically, and dorsally so that the ultrasound beam was perpendicular to the posterior part of the vault. When the line of the M-mode could not be perpendicular to the cranial-caudal motion, M-anatomical mode was used. After correct viewing of the hemidiaphragm in two-dimensional mode (B-mode), M-mode was used to display the movement of the diaphragm along the selected line. The inspiratory and the expiratory cranio-caudal displacements of the diaphragm lead to a shortening and a lengthening, respectively, of the distance between the probe and the diaphragm. The diaphragmatic motion was assessed by M-mode while the patient breathed on tidal volume (quiet breathing) and at deep breathing.

Measurement of the Thickness of the Right Hemidiaphragm

The thickness of the diaphragm was measured at the area of apposition of the right hemidiaphragm to the rib cage under the costo-phrenic angle. A 7.5–12 MHz frequency probe (LA 523 probe, Esaote, Genoa, Italy) was placed at the level of the 8th and 9th intercostal spaces on the anterior or the middle axillary line, which allows the diaphragm to be visualized between the pleural and peritoneal membranes. In accordance with current recommendations, measurement of the diaphragm thickness was performed from the middle of the pleural line to the middle of the peritoneal line (Carrillo-Esper et al., 2016) at the end of expiration and at the end of inspiration during rest ventilation, as well as during deep breathing.

Measured Variables

The ultrasound parameters measured using M-mode included the excursion (cm), the duration (sec), and the velocity (cm sec^{-1}) of the inspiratory motion. The parameters measured by B-mode included the thickness at end-inspiration, the thickness at end-expiration, and the thickening fraction (i.e., the ratio of the thickness at end-inspiration - the thickness at end-expiration divided by the thickness at end-expiration). All of the ultrasound parameters were recorded on the computer of the ultrasound machine for subsequent blind analysis. Measurements were obtained from the average of at least three different breathing cycles.

Statistics

The data are expressed as mean ± SD. The statistical tests were performed with R statistical software. The cohorts for comparison consisted of the healthy volunteers during two sessions, with and without inspiratory load, at two respiratory regimens, i.e., tidal volume and 50% inspiratory capacity.

Comparison between the continuous variables was carried out with the analysis of variance. If the distribution of the residuals was not normal, a log10 transformation was performed on response variable Y and then a two-way ANOVA was computed with the transformed response variable. *Post hoc* analyzes were performed when needed with Holm correction. The interaction effect (breathing volume: load) was studied to determine the ultrasound parameters related to the changes in breathing conditions. Differences between groups were considered significant at $p < 0.05$.

RESULTS

Preliminary Study

The pressure-volume loops recorded on the ventilator simulator are presented in **Figure 2**. The loops are in principle centered on the pressure 0 when measuring the work of breathing. We shifted them by the value of the hydrostatic imbalance measured on the rebreather in a horizontal position (the devices are carried on the diver's back). This value was measured at −27 mbar (cmH_2O). The maximal inspiratory pressure varied according to the ventilation output: −30 cmH_2O for 15 l/min, −31 cmH_2O for 22.5 l/min and − 35 cmH_2O for 40 l/min. According to the ventilator regimen of the volunteers (quiet breathing and increase in the breathing volume at 50% of inspiratory capacity) the inspiratory load, chosen for the experiment, was −30 cmH_2O.

Main Study

In total, 22 healthy male adult volunteers were included in this study. The mean age, weight and height were 34 ± 9 years, 73 ± 6 kg, 175 ± 5 cm, respectively.

Images of the right hemidiaphragm were successfully obtained from the 22 healthy volunteers. Their average inspiratory capacity was determined to be 3.7 ± 0.6 l.

During the entire experiment, the breathing rate was similar and individually regulated depending on the respiratory parameters recorded during quiet breathing. It comprised 12 to 16 breaths per minute. The mean tidal volume was 0.7 ± 0.1 l leading to a ventilation output of 8.5 ± 1.2 l/min. During the session at 50% of inspiratory capacity, the breathing volume and the ventilation output were 1.9 ± 0.3 l and 24 ± 6 l/min, respectively.

During the session at tidal volume, the inspiratory resistance induced by the threshold set at 30 cmH_2O was calculated to be 31 ± 10 $cmH_2O/l/s$ using the formula delta P/dV/dt according to the Poiseuille's law with delta p = 30 cmH_2O, DV = tidal volume and dt = inspiratory time.

This calculation was a simple estimate. Indeed, the increase in inspiratory load is known to alter the flow, especially at high volume, leading to a turbulent flow. To calculate airway resistances using Poiseuille's law, the flow should be laminar. Therefore, we have chosen to report the estimate of inspiratory resistance at tidal volume, only.

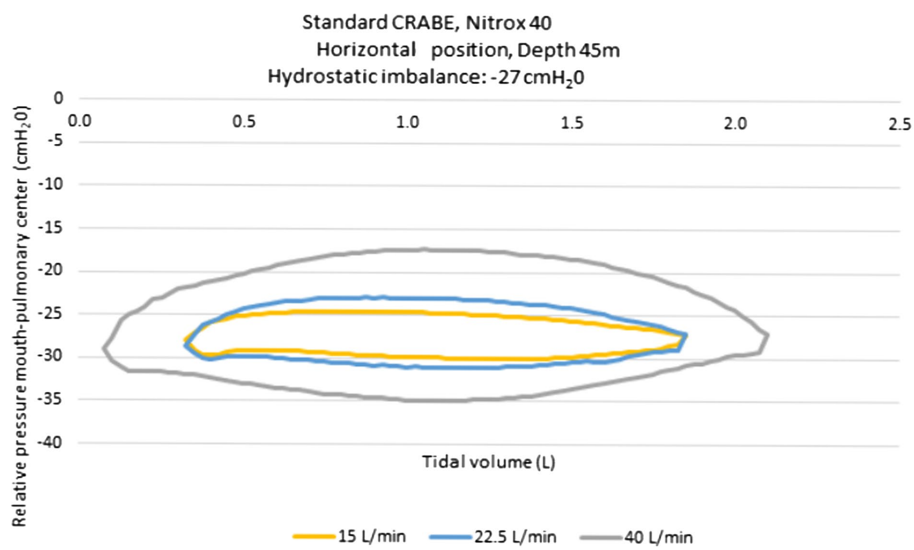

FIGURE 2 | Pressure-volume loops obtained with the standard CRABE rebreather on a ventilatory simulator, at a 45-meter depth under three ventilatory flow conditions.

Statistical Study

The end expiratory thickness did not vary significantly, at any of the examination time points. Irrespective of the session (without load and with a load of 30 cmH_2O), significant increases in both the excursion (**Figures 3, 4**) and the thickening fraction (**Figures 5, 6**) were recorded in the measurements performed when the volunteers breathed at 50% of inspiratory capacity compared to tidal volume.

Tables 1, 2 and **Figure 7** report the changes induced by the addition of the inspiratory load in volunteers breathing at tidal volume and at 50% inspiratory capacity, respectively.

The interaction effect (breathing volume: load) was significant on the thickening fraction (%) [$F(1,84) = 0.11$, p value $= 0.029$] only (**Tables 3, 4**). This means that the thickening fraction was the better parameter to assess the impact of the changes in breathing volume and inspiratory load.

DISCUSSION

The main finding of this study is that an increase in the inspiratory load that can be experienced by military divers leads to an increase in the excursion and the thickening fraction of the right hemidiaphragm. Our statistical analysis retained assessment of the thickening fraction during quiet breathing as the best parameter for accurate assessment of the consequences of an increase in inspiratory resistance on diaphragmatic function.

Previous studies have shown that various parameters such as the physical effect of water immersion (Moon et al., 2009), the increase in the breathing gas density (Held and Pendergast, 2013), and the pressure difference between the regulator and the lung centroid (the negative static lung load; Lundgren, 1999) lead to an increase in the work of breathing during SCUBA diving. The inspiratory static lung loading has been estimated to be approximately −25 cmH_2O in a diver in a prone position under water using a closed-circuit apparatus with a rebreathing bag on their back (at a lower hydrostatic pressure than the lung centroid; Lundgren, 1999). The increase in inspiratory load led to a more negative pleural pressure that was variably estimated as a function of the diver, the ventilatory regimen, the depth, and the diving apparatus. In our study, the level of the inspiratory load was based on the recording of the pressure-volume loops of the rebreather used by French military divers on a respiratory simulator. The inspiratory load chosen for the experiment was −30 cmH_2O according to the ventilation output of the volunteers. We have studied two ventilatory regimens, i.e., breathing at tidal volume and at 50% of inspiratory capacity, to simulate an increase in ventilation output induced by swimming. As expected, we observed increased excursions at high volume compared to tidal volume. This has been reported previously in studies performed in healthy volunteers (Cohen et al., 1994; Houston et al., 1994). For example, Houston et al. (1994) recorded cranio-caudal excursions of the posterior part of each hemidiaphragm on successive respiratory cycles in 14 healthy subjects. Spirometric measurements were recorded simultaneously on a spirometer. The authors observed a linear relationship between the diaphragmatic excursion and the inspired volumes.

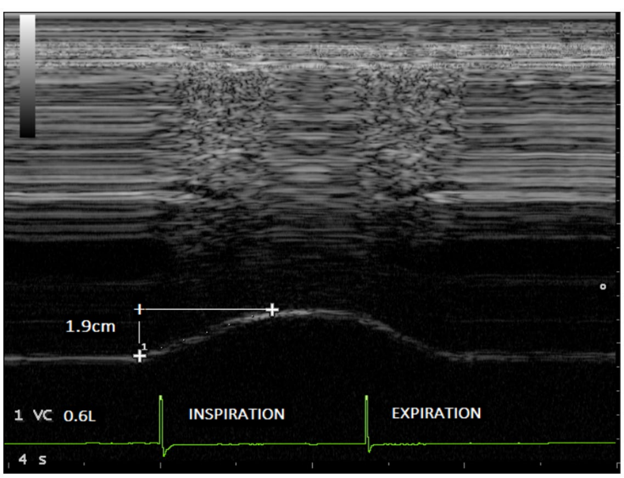

FIGURE 3 | Diaphragmatic excursion recorded in one volunteer during breathing at tidal volume (0.6 l) without load (1.9 cm).

During breathing against an elevated inspiratory load, an increase in the right hemidiaphragm excursion was found compared to breathing without resistance. This difference was significant at tidal volume and at high volume. Furthermore, at tidal volume, the velocity of the diaphragm was increased. These findings were suggested by previous works. Ayoub et al. (1997) found an increase in the duration and the amplitude of the diaphragmatic motion during an increase in inspiratory resistance induced by the addition of a spirometric chain in 8 subjects. Furthermore, Soilemezi et al. (2013) reported an increase in diaphragm excursion with the addition of low resistance (mouthpiece) or high resistance (inspiratory resistance at 50 cm H_2O/l/s) compared to breathing without a device. In contrast to our findings, at high resistance, they found a decrease in the breathing rate that led to an increase in the inspiratory time and a decrease in the inspiratory velocity.

The discrepancies between studies can be explained by a number of differences between the protocols. On the one hand, the inspiratory resistance was lower in our work (estimated mean of 31 ± 10 cm H_2O/l/s). On the other hand, we had set a fixed breathing rate for all phases of the experiment. The regulation of the breathing rate was chosen to make it easier to record the differences between the ultrasound markers of diaphragm contractility in the two conditions. However, this led to a degree of deviation from the conditions encountered in real life. This could have affected the overall respiratory adaptation, in particular the changes in the inspiratory time and the velocity.

Our statistical analysis revealed that the thickening fraction was increased at high volume compared to tidal volume. This increase was the result of an increase in the diaphragm thickness at the end of inspiration. Furthermore, as expected and according to previous studies that reported a good reproducibility of this measurement (Cohn et al., 1997; Matamis et al., 2013; Goligher et al., 2015), the same thickness was recorded at the end of

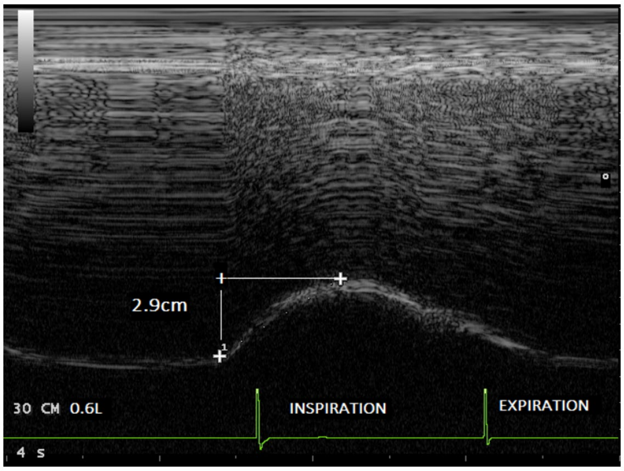

FIGURE 4 | Diaphragmatic excursion recorded in the same volunteer during breathing at tidal volume (0.6l) with an inspiratory load of 30 cmH_2O (2.9cm).

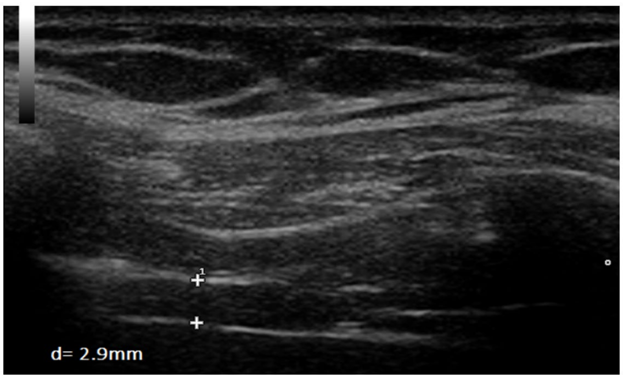

FIGURE 5 | Measurement of diaphragm thickness in one volunteer at end-inspiration without load (d=2.9mm).

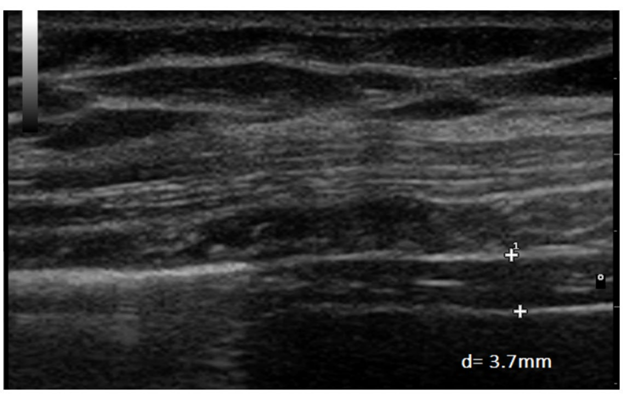

FIGURE 6 | Measurement of diaphragm thickness in the same volunteer at end-inspiration with an inspiratory load of 30 cmH_2O (d=3.7mm).

TABLE 1 | Changes in the diaphragmatic parameters induced by a high inspiratory load in volunteers breathing at tidal volume.

	Without load	Load 30 cmH_2O	p
	Mean ± SD		
Excursion (cm)	2.3 ± 0.5	3.3 ± 0.9	<0.001
Inspiratory time (sec)	1.5 ± 0.5	1.6 ± 0.7	NS
Inspiratory velocity ($cm\,sec^{-1}$)	1.6 ± 0.5	2.3 ± 0.8	<0.002
Thickness at end-expiration (mm)	2.1 ± 0.5	2.1 ± 0.4	NS
Thickness at end-inspiration (mm)	2.7 ± 0.6	3.6 ± 0.8	<0.001
Thickening fraction (%)	31 ± 12	73 ± 23	<0.001

expiration. The increase in the thickening fraction at high volume was not surprising since a relationship was previously found between the thickening fraction and the inspiratory volume measured by spirometry (Goligher et al., 2015).

The thickening fraction was increased under inspiratory load both at tidal and at high volume.

During breathing at a high inspiratory load, the increase in diaphragmatic work has been demonstrated using a number of different procedures. Transdiaphragmatic pressure requires simultaneous recordings of esophageal pressure and gastric pressure, and it is calculated as the differential pressure between these two signals (Minami et al., 2018). Abdominal pressure usually increases during inspiration whereas esophageal pressure decreases as this reflect the lowering of the pleural pressure. It has been reported that transdiaphragmatic pressure increased progressively in COPD patients from a low to a high intensity of threshold load training (from 30 to 80% of maximal inspiratory pressure), based on use of a Philips Threshold IMT (Wu et al., 2017). In such circumstances, the increase in diaphragm activity has been also demonstrated by the neural respiratory drive measured from the diaphragm electromyogram (Jolley et al., 2008; Wu et al., 2017). Previous studies have reported that some ultrasound parameters correlated with physiological measurements of diaphragm strength. Ueki et al. (1985) reported a strong correlation between the maximum inspiratory pressure and the thickening fraction. Cardenas et al. (2018) found that the diaphragm mobility and the thickening fraction were related to the inspiratory muscle strength assessed by maximum inspiratory pressure or transdiaphragmatic pressure. Goligher et al. (2015) have shown that the thickening fraction correlated with the electrical activity recorded by diaphragm electromyography and transdiaphragmatic pressure, although the correlation coefficient was low. With a high inspiratory load, it is thought that the extra diaphragmatic inspiratory muscles including the external intercostal, sternocleidomastoid and scalene muscles are solicited in addition to the increase in the activity of the diaphragm (de Troyer et al., 2005; Hudson et al., 2007). This can explain the discrepancies between studies and the fact that some authors have reported a lack of or a low correlation between the ultrasound marker of diaphragm activity and inspiratory load (Goligher et al., 2015; Oppersma et al., 2017).

TABLE 2 | Changes in the diaphragmatic parameters induced by a high inspiratory load in volunteers breathing at high volume.

	Without load	load 30 cmH$_2$O	p
	Mean ± SD		
Excursion (cm)	4.1 ± 1	4.8 ± 0.9	0.01
Inspiratory time (sec)	1.8 ± 0.5	2 ± 1.1	NS
Inspiratory velocity (cm sec^{-1})	2.6 ± 1.3	2.9 ± 1.4	NS
Thickness at end-expiration (mm)	2.1 ± 0.5	2 ± 0.4	NS
Thickness at end-inspiration (mm)	3.7 ± 1.1	4.6 ± 1	<0.005
Thickening fraction (%)	76 ± 26	128 ± 39	<0.001

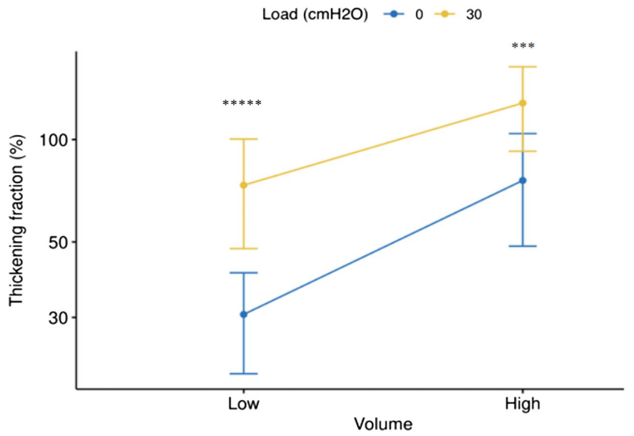

FIGURE 7 | Error bar (Mean ± SD) of changes of thickening fraction of right hemidiaphragm (Log10) in the sessions with and without load at low (tidal volume) and high volume (50% of inspiratory capacity). *****$p < 10^{-11}$, ***$p < 0.001$.

TABLE 3 | Study of the interaction effect (breathing volume: load).

	F	value of p
Excursion (cm)	F (1.84) = 1.03	NS
Inspiratory time (sec)	F(1.84) = 0.14	NS
Inspiratory velocity (cm.sec-1)	F (1.84) = 0.07	NS
Thickness at end-expiration (mm)	F (1.84) = 0	NS
Thickness at end-inspiration (mm)	F (1.84) = 0	NS
Thickening fraction (%)	F (1.84) = 0.11	<0.05

To assess the changes in diaphragm function induced by an increase in inspiratory load that can be experienced by military divers, our statistical analysis demonstrated that the more sensitive parameter should be the measurement of the thickening fraction. This result is in keeping with the study of Umbrello et al. (2015) performed in intensive care unit. These authors reported that diaphragm thickening was a better indicator of respiratory effort than diaphragm excursion.

The purported relevance of the use of a high ventilation output to detect the changes in the thickening fraction in volunteers submitted to inspiratory loading is not supported by our results. Indeed, although the breathing rate was the same, a change in the respiratory pattern including an increase in inspiratory velocity was observed at tidal volume, during the session with load in comparison with the session without load (see **Table 1**). This difference was not observed when volunteers breathed at high volume (see **Table 2**). Furthermore, the difference in the thickening fraction between the two sessions was more significant at tidal volume than at high volume (see **Figure 7**). Consequently, the study of high volumes does not appear to bring any additional interest. Therefore, to assess the respiratory stressors induced by the use of a diving apparatus, it can be recommended to study the changes in the diaphragm thickening fraction during quiet breathing, which is easier to perform and better tolerated by the volunteers.

Study Limits and Perspectives

Our study used a model that reproduced an inspiratory load close to the resistance experienced by SCUBA divers, on the basis of measurements made by a ventilator simulator on a rebreather used by French Navy. It would be relevant to repeat the study in divers using their rebreather with the common stressors experienced during open-water diving, i.e., water immersion and a hyperbaric environment. Thanks to the progress in technologies, such a study is a realistic prospect. Indeed, it was recently demonstrated that it is possible to assess the cardiac function of a diver using underwater Doppler-echocardiography at a 10-meter depth in a swimming pool (Marabotti et al., 2013). In further works, to assess the contribution of the changes in the respiratory function on the risk of pulmonary edema, it would be useful to record hemodynamic data by ultrasound in combination with assessment of the respiratory function. Using these methods, it would be possible to compare the cardio-respiratory impact of the various apparatuses used by divers.

In contrast to previous studies, in our work, the inspiratory resistances were not calculated individually according to the maximal inspiratory pressure. Consequently, during inspiratory load, the work of breathing, as a percentage of the maximal capacity, could differ between individuals according to their respiratory capacity. This method was chosen because the level of inspiratory resistance was determined based on the measurement of a ventilator simulator. This inspiratory load is experienced by military divers irrespective of their level of fitness and their respiratory capacity.

In the present study, the thickening fraction appeared to be a better marker of an increase in diaphragm activity under inspiratory load compared to diaphragm excursion. In a recent study, Oppersma et al. (2017) suggested that the assessment of the diaphragm using speckle-tracking imaging should be more informative than conventional ultrasound. They reported that speckle-tracking parameters such as the strain and the strain rate correlated better with transdiaphragmatic pressure and electric activity of the diaphragm than the thickening fraction. Consequently, in

TABLE 4 | Type III ANOVA results for the thickness fraction.

Parameter	Sumsq	df	Statistic	Value of p
(Intercept)	75.464	1	3.326	<0.001
Breathing volume (low-high)	1.691	1	74.557	<0.001
Inspiratory load (without-30 cmH_2O)	0.622	1	27.425	<0.001
Volume:load	0.112	1	4.936	<0.05
Residuals	1.905	84		

further studies, it would be interesting to use speckle-tracking imaging to assess the changes in diaphragm function induced by dive apparatuses such as rebreathers.

CONCLUSION

An inspiratory load similar to the level experienced by military divers using a rebreather led to an increase in both the excursion and the thickening fraction of the right hemidiaphragm.

Diaphragm ultrasound is a promising tool for assessment of the impact of dive apparatuses on diaphragmatic function. In this context, the more relevant parameter appeared to be assessment of the changes in the thickening fraction during breathing at tidal volume.

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 Aix Marseille University, CPP-1, NoA01299-32. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB and FB conceived and designed the study. SR and BS assisted with the technical aspects of the protocol, recruited all the participants, and were involved in the acquisition of the data. AB and SR performed the ultrasound examinations. AB and GC analyzed the data and performed the statistical analysis. AB, GC, and SR have drafted the article while FB and BS revised it critically for important intellectual content. All authors have given final approval of the version to be published.

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Time to Move Beyond a "One-Size Fits All" Approach to Inspiratory Muscle Training

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Inspiratory muscle training (IMT) has been studied as a rehabilitation tool and ergogenic aid in clinical, athletic, and healthy populations. This technique aims to improve respiratory muscle strength and endurance, which has been seen to enhance respiratory pressure generation, respiratory muscle weakness, exercise capacity, and quality of life. However, the effects of IMT have been discrepant between populations, with some studies showing improvements with IMT and others not. This may be due to the use of standardized IMT protocols which are uniformly applied to all study participants without considering individual characteristics and training needs. As such, we suggest that research on IMT veer away from a standardized, one-size-fits-all intervention, and instead utilize specific IMT training protocols. In particular, a more personalized approach to an individual's training prescription based upon goals, needs, and desired outcomes of the patient or athlete. In order for the coach or practitioner to adjust and personalize a given IMT prescription for an individual, factors, such as frequency, duration, and modality will be influenced, thus inevitably affecting overall training load and adaptations for a projected outcome. Therefore, by integrating specific methods based on optimization, periodization, and personalization, further studies may overcome previous discrepancies within IMT research.

Keywords: training prescription, performance, respiratory muscle strength, respiratory muscle endurance, pulmonary function

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INTRODUCTION

Inspiratory muscle training (IMT) has been thoroughly investigated over several decades as a rehabilitation tool (Gosselink et al., 2011; Smart et al., 2013; Cahalin and Arena, 2015; Menezes et al., 2016; Shei et al., 2016b; Charususin et al., 2018a; Shei and Mickleborough, 2019) and ergogenic aid (Sheel, 2002; Illi et al., 2012; HajGhanbari et al., 2013; Karsten et al., 2018; Shei, 2018) in healthy, clinical, and athletic populations, with generally positive findings. IMT is an intervention aimed to strengthen the inspiratory muscles, primarily the diaphragm and other inspiratory muscles such as the external intercostals, scalenes, and sternocleidomastoid (Celli, 1986; Sheel, 2002; Ratnovsky and Elad, 2005; Dominelli and Sheel, 2012; Illi et al., 2012; HajGhanbari et al., 2013; Shei et al., 2016b; Reid et al., 2018; Walterspacher et al., 2018; Ando et al., 2020; Derbakova et al., 2020). In clinical populations, IMT may aid in overcoming disease-associated pathologies related to the pulmonary system, such as respiratory muscle weakness, altered operating lung volumes, and expiratory flow limitation, thus improving clinical status, exercise capacity, and quality of life (Aaron et al., 1992a,b; Lisboa et al., 1994, 1997; Johnson et al., 1995; Kosmas et al., 2004; Calverley and Koulouris, 2005; Dhand, 2005; McConnell, 2005; Turner et al., 2012; Laviolette et al., 2014; Price et al., 2014; Weatherald et al., 2017; Moore et al., 2018; Chung et al., 2021). Conversely, in healthy and athletic populations, IMT can enhance respiratory muscle function, translating into a potential ergogenic benefit even in the absence of pulmonary system abnormalities. Several seminal studies have documented significant respiratory muscle fatigue during exercise (Johnson et al., 1992; Harms et al., 1997, 1998; St Croix et al., 2000) and observed a respiratory muscle metaboreflex (Harms et al., 1997, 1998; Witt et al., 2007). The respiratory muscle metaboreflex is a phenomenon where blood is shunted away from locomotor muscles and toward respiratory muscles in response to a large increase in the work of breathing (Dominelli et al., 2017; Sheel et al., 2018). More recently, the role of the respiratory muscles during exercise and the occurrence of respiratory muscle fatigue during and after exercise has been an area of focus (Dempsey et al., 2006, 2012; Aliverti, 2016; Oueslati et al., 2018). Thus, even in non-clinical populations, IMT may enhance respiratory muscle function.

Many different forms of IMT have been developed, including pressure-based and volume-based loading protocols. Typically, these protocols require subjects to inspire against a resistance or maintain a prescribed level of minute ventilation to load the respiratory muscles and produce a training adaptation. IMT (1) promotes diaphragm hypertrophy (Enright et al., 2006; Downey et al., 2007; Shei, 2018; Shei et al., 2018); (2) increases the proportion of type I fibers and the size of type II fibers in the external intercostal muscles (Huang et al., 2003); (3) attenuates the respiratory muscle metaboreflex (Sheel, 2002; Gething et al., 2004; Enright et al., 2006; Witt et al., 2007; McConnell, 2009; Turner et al., 2012, 2016; Raux et al., 2013, 2016; Price et al., 2014; Lomax et al., 2017); (4) decreases inspiratory muscle motor drive with preserved pressure generation (Huang et al., 2003; Price et al., 2014; Ramsook et al., 2017); (5) improves respiratory muscle economy (Enright et al., 2006; Turner et al., 2012; Held and Pendergast, 2014; Shei et al., 2016a,b; Shei, 2018); 6) decreases the rating of perceived breathlessness or rating of perceived exertion (Sheel, 2002; Gething et al., 2004; Downey et al., 2007; McConnell, 2009; Price et al., 2014; Lomax et al., 2017; Ramsook et al., 2017); (7) reduces the work of breathing (Gething et al., 2004; McConnell, 2009; Turner et al., 2012; Price et al., 2014; Shei et al., 2016a,b); (8) improves respiratory muscle endurance (Gething et al., 2004; Enright et al., 2006; McConnell, 2009; Price et al., 2014; Sales et al., 2016; Shei et al., 2016a,b); (9) improves ventilatory efficiency (Sheel, 2002; Gething et al., 2004; Enright et al., 2006; Turner et al., 2012; Bernardi et al., 2014; Price et al., 2014; Lomax et al., 2017; Salazar-Martínez et al., 2017); (10) reorganizes respiratory muscle recruitment pattern (Enright et al., 2006; Walterspacher et al., 2018); (11), improves breathing pattern during exercise hyperpnea (Charususin et al., 2016); and (12) reduces cytokine release (Mills et al., 2013, 2014). While some of these adaptations are well-characterized, others are postulated to occur with published studies showing conflicting results. For example, while Ray et al. (2010) observed a reduction in work of breathing following IMT, Langer et al. (2018) did not. Putatively, not all these proposed adaptations may be observed in all populations who undertake IMT, and population-specific and individual-specific variations could reasonably be expected. Collectively, these adaptations may underpin exercise enhancement or functional improvement in athletic and clinical populations. More recently, IMT has been studied in occupational settings, such as military and emergency services and recreational settings, which require personnel and participants to exercise while carrying a load on the thoracic cavity (e.g., protective equipment, backpacks to transport gear and provisions, etc.) (Sperlich et al., 2009; Faghy et al., 2016; Shei et al., 2017, 2018; Shei, 2018; Hinde et al., 2020). In this application, IMT appears to be effective in improving work and exercise capacity, and is likely due, in part, to the higher ventilatory demand and workload due to load carriage. Thus, enhancements in respiratory muscle function here again optimize performance. A summary of physiological adaptation to IMT and applications of IMT is given in **Figure 1**.

INCONSISTENCIES IN INSPIRATORY MUSCLE TRAINING

In spite of these findings, translation of physiological adaptations into clinically or competitively meaningful improvements for clinical and athletic populations has not been uniformly observed (McConnell, 2012; Patel et al., 2012). Contributing factors to these heterogeneous findings likely include variations in the study population, study sample size, training protocol (intensity, duration, frequency, rest intervals, etc.), whether training was completed at rest or during concurrent exercise, training type (pressure-threshold, flow-resistive, normocapneic hyperpnea, etc.), among other factors (Illi et al., 2012; Patel et al., 2012; HajGhanbari et al., 2013; Formiga et al., 2018; Shei, 2018; Larribaut et al., 2020). A separate consideration is the ability to reliably evaluate respiratory muscle functional outcomes, such as strength, endurance, activation pattern, etc., to appreciate the potential benefits associated with respiratory muscle training. Most commonly, maximal inspiratory and expiratory mouth pressures (PI_{max}, PE_{max}, respectively) are used to assess respiratory muscle strength. Other measures, including but not limited to transdiaphragmatic pressure, sustained maximal inspiratory pressure (SMIP), respiratory muscle power output, fatigue index, inspiratory duty cycle, minute ventilation, breathing frequency, and tidal volume, have also been used to assess respiratory muscle function. Presently however, there is no consensus as to which measures are most appropriate in the context of evaluating responses to IMT. While it is reasonable to tailor outcome measures to the stated goals of each study, developing a core set of common measures would be a prudent step forward in enhancing the rigor of future IMT studies.

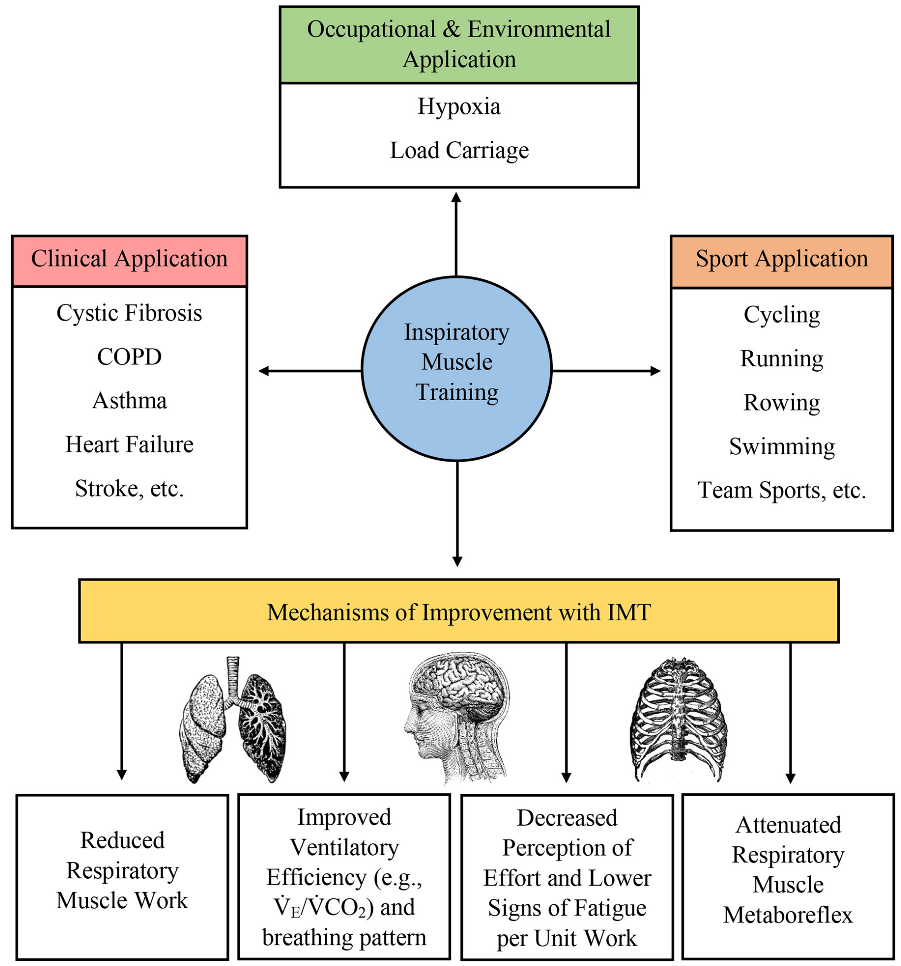

FIGURE 1 | Summary of clinical, occupational, environmental, and sport application of IMT as well as a brief overview of physiological adaptations induced by IMT.

Several recent reviews concluded that matching the IMT training prescription to the ventilatory demands of the exercise task likely optimize the ergogenic effect of IMT (HajGhanbari et al., 2013; Shei, 2018). This recommendation for IMT training specificity highlights the need to critically examine the training protocols which have been tested and to evaluate whether the training load imposed by these protocols sufficiently overloads the respiratory muscle to induce meaningful training adaptations (Formiga et al., 2018; Larribaut et al., 2020). Indeed, the concept of training specificity, in which the training stimulus is matched as closely as possible to the criterion task, has long been understood and adopted in the field of sport science (Hawley, 2008). Yet, the majority of IMT research has used fixed protocols, some of which were developed for clinical populations (Johnson et al., 1998; Villafranca et al., 1998; Weiner et al., 1999, 2000) but broadly applied to healthy and athletic populations. One of the most common pressure-threshold IMT protocols involves completing 30 breaths, twice daily at 50% of a subject's PI_{max}, five times a week for 6 weeks (Romer et al., 2002a,b; Kilding et al., 2010; Turner et al., 2012). While many variations exist and prescribe varying numbers of repetitions, frequencies, and durations, IMT training "intensities" are still largely based on a percentage of PI_{max} or SMIP. Acknowledging that for research purposes, standardizing intervention protocols is necessary for experimental control and uniformity between subjects, from a practitioner's standpoint, this approach may not be optimal for producing *training* adaptations in real-world settings. As such, we propose that further consideration be given to the *training* aspect of IMT.

TRAINING PRESCRIPTION CONSIDERATIONS

Coaches and practitioners routinely tailor training programs to suit the goals and needs of individual athletes and constantly make adjustments based on numerous factors, including the athlete's response to training, injuries, illnesses, and environmental factors, to name a few. The training prescription is seldom uniform and typically contains varied workouts, commonly organized into micro-, meso-, and mega-cycles in a periodized fashion (Issurin, 2010; Kiely, 2018;

Bompa and Buzzichelli, 2019). In this light, perhaps it is time to investigate IMT as a training tool, rather than a research intervention, and consider periodization of IMT and varying individual training sessions to achieve a specific training goal (aerobic, threshold, neuromuscular, anaerobic, etc.) much like sports training varies workout prescription to achieve training adaptations. In order to achieve this, particular consideration will need to be given to the context or application of IMT, i.e., rehabilitation vs. ergogenic aid for sport performance. Within these contexts, factors, such as frequency, duration, and modality (which affect overall training load) will influence how the coach or practitioner adjusts the given IMT prescription.

Training load is perhaps the most important factor in optimizing IMT prescription. While historic protocols are somewhat individualized in that the "intensity" prescribed is based on an individual's PI_{max} or SMIP, the percentage of PI_{max} or SMIP is often arbitrarily selected and then fixed at that level. Careful consideration of the training goals, such as improving respiratory muscle strength vs. endurance, might lead one to prescribe a higher "intensity" for a strength-oriented training session or a lower "intensity" for endurance-oriented sessions, to allow a subject to complete more repetitions or a longer training session. Within training load, the number of repetitions could also be adjusted according to the goals of an individual training session. Special consideration should be given for training toward a pre-defined "task failure," as some studies have shown that in limb locomotor resistance training, a larger training effect is produced when training is conducted to task failure (Burd et al., 2010; Schoenfeld et al., 2015, 2016). As previously suggested, in the context of IMT, task failure may be defined by using a pre-specified threshold of inspiratory pressure, volume, or minute ventilation, or failure to achieve a prescribed breathing frequency, tidal volume, or both (Shei, 2020). Using a pre-defined task failure threshold could help ensure that each training session provides a sufficient training stimulus to induce adaptations which in turn may enhance the efficacy of IMT. This is particularly important for populations requiring a higher training load to produce training adaptations in the respiratory muscles, such as swimmers and other aquatic-based athletes, including SCUBA divers (Lindholm et al., 2007; Mickleborough et al., 2008; Shei et al., 2016a,b; Vašíčková et al., 2017; Lomax et al., 2019; Yañez-Sepulveda et al., 2021). Moreover, this approach could help match the IMT training prescription to the ventilatory demands of the athlete or patient's goals, whether that is sustained hyperpnea during prolonged exercise or improving the ability to complete activities of daily living.

Aside from the broader considerations of training frequency, intensity, and periodization, critically evaluating the specific training mode will also be crucial to optimizing future IMT prescription and research. Several recent areas of investigation have garnered growing interest, which merits further discussion. First, there is increasing interest in concurrent exercise and IMT, i.e., rather than completing IMT at rest, the athlete or patient uses the IMT device while simultaneously completing another exercise, such as running or cycling (Hellyer et al., 2015; Granados et al., 2016; McEntire et al., 2016; Porcari et al., 2016; Shei, 2018; Shei et al., 2018). To date, only a small number of studies have investigated concurrent IMT and exercise. However, early findings suggest that IMT performed during concurrent cycling exercise results in greater diaphragm activation, as demonstrated by electromyography (EMG), and that concurrent training improves both ventilatory threshold and respiratory compensation threshold, and power output at these thresholds (Hellyer et al., 2015; Porcari et al., 2016). Using an IMT device during exercise may cause mild hypoxemia, possibly due to inadequate hyperventilation (Granados et al., 2016). Factors, such as the breathing pattern that can be sustained against the external resistance may influence whether exercise hyperpnea with concurrent IMT may be adequate. It is also possible that a relative hypoventilation relative to work rate could lead to a decrease in work rate intensity to preserve arterial blood gases, rather than causing hypoxemia. Relative hypoventilation in this context may also induce hypercapnia, which could limit performance but also represent an additional training stimulus. Considering these factors, it is certainly possible that concurrent IMT and exercise may compromise endurance exercise performance and workload during a given training session. Thus, any compromise in the ability to sustain a given workload resulting from simultaneously using an IMT device should be weighed against the potential benefits of loading the respiratory muscles with the IMT device. As previously discussed, even in this context, consideration should be given to the selected load and duration of training for concurrent IMT and exercise. A recent study employing an inspiratory load of 15% of PI_{max} concurrently during exercise training found no difference between concurrent IMT plus exercise training and exercise training alone after 3 weeks of training. After 6 weeks of training, however, the concurrent training produced an ~8% improvement 5-mile cycling time trial performance (McEntire et al., 2016). Therefore, even given a relatively low resistive load, concurrent IMT and exercise training over a longer period may still induce appreciable respiratory muscle adaptations and subsequent performance benefits.

Next, the lung volume(s) at which IMT is completed are also important to consider, as highlighted by a recent publication by Van Hollebeke et al. (2020). In this study, 48 healthy volunteers were randomly assigned to perform either pressure-threshold IMT initiated from residual volume (RV) or functional residual capacity (FRC), or tapered flow resistive loading initiated from RV. The authors found that only training initiated from FRC resulted in consistent improvements in respiratory muscle function at higher lung volumes, whereas improvements after the standard protocol initiated from RV were restricted to PI_{max} gains at lower lung volumes. Thus, considering the operating lung volumes of the athlete or patient's activities may be an important factor when deciding the lung volumes at which IMT should be initiated.

Finally, work led by Dominelli et al. (2015a,b), Molgat-Seon et al. (2018a,b), Welch et al. (2018a,b), Geary et al. (2019), and Archiza et al. (2021) has shown apparent sex differences in respiratory muscle fatigability and workload, which is important to consider in the context of IMT. Due in part to anatomical differences, such as smaller airway diameter and smaller thoracic volume compared to males, females generally have a higher work of breathing when minute ventilation, operating lung volume, breathing frequency, and tidal volume are matched (Dominelli et al., 2019). More recent evidence suggests that females are more resistant to respiratory muscle fatigue compared with males (Welch et al., 2018a; Geary et al., 2019), although this difference in respiratory muscle fatigability does not appear to influence exercise performance (Welch et al., 2018b). Despite this, because of the comparatively higher respiratory muscle fatigue resistance in females, it may be that females require a higher prescribed IMT training load compared with males in order to induce training adaptations. Interestingly, however, a recent investigation of respiratory muscle endurance training (RMET) in healthy active men and women found a greater ergogenic effect of RMET on cycling time trial performance in women compared to men, an effect which was even more pronounced in hypoxia (Chambault et al., 2021). It is possible then, that females may not in fact require a higher prescribed training workload, and that there may in fact be a sex-specific differential response to RMET. Regardless, further investigation on sex differences in both IMT prescription and response is warranted.

Most respiratory muscle training paradigms have focused on IMT in patients with pulmonary disease [e.g., asthma, chronic obstructive pulmonary disease (COPD)] or respiratory muscle weakness (e.g., multiple sclerosis, Parkinson's disease) with expectations to improve ventilatory capacity. While expiration during resting is passively mediated by the recoil of the lung and thorax, forced expiration and expiration during exercise requires expiratory muscle activation, which requires muscles of the abdominal wall, in particular the transverse abdominis and the internal and external oblique muscles, as well as internal intercostals. Expiratory muscles, especially the upper airway musculature, play an essential role during phonation, airway clearance and expectoration. Therefore, interest in expiratory muscle strength training (EMT) has developed, particularly for improving non-ventilatory functions, such as coughing, speaking, and swallowing. A number of studies have shown that EMT is effective in increasing the strength of the expiratory muscles resulting in augmenting the expiratory driving pressure used for cough, speech, or swallow (Kim and Sapienza, 2005). EMT elicits similar responses to IMT in the expiratory muscle system, and similar to IMT, improvement of the maximal expiratory pressure is the hallmark parameter of effective EMT. Interestingly, EMT leads to improved maximal inspiratory pressure, indicating involvement of the inspiratory muscles in the process of expiration, whereas IMT does not improve maximal expiratory pressure (McConnell, 2013).

While the ergogenic benefits of either IMT or EMT alone have clearly been established, combined IMT/EMT have not been widely reported. However, a number of studies have highlighted the possibly overlooked ergogenic potential of combined IMT/EMT in patients with Duchenne muscular dystrophy or spinal cord muscular atrophy (Gozal and Thiriet, 1999), multiple sclerosis (Ray et al., 2013) and COPD (Weiner et al., 2003). These, and other studies, indicate that combined IMT/EMT may at least be equally effective to either method alone, and might be the preferred method of RMT in respiratory muscle disorders in which training of both muscle groups is of greater importance, such as in COPD and neuromuscular disorders.

A few studies have investigated the effect of combined IMT/EMT on respiratory muscle function and exercise performance in healthy individuals. Griffiths and McConnell (2007) showed that 4 weeks of IMT and EMT increased inspiratory and expiratory mouth pressures, respectively. However, only IMT improved rowing performance, while EMT and combined IMT/EMT did not improve rowing performance (Griffiths and McConnell, 2007). Amonette and Dupler (2002) using combined IMT/EMT for 4 weeks showed that this type of training, although increasing strength of the respiratory muscles as seen by increases in expiratory mouth pressures, produced no changes in pulmonary function or VO_{2max} (Amonette and Dupler, 2002). However, this study was limited by a low sample size (eight subjects in the treatments group and four subjects in the control group).

FUTURE DIRECTIONS AND CHALLENGES

Future studies in IMT should aim to address the considerations discussed here, and in particular, consider a more personalized or "precision" approach to tailoring training prescription to the individual needs and goals of the patient or athlete (**Figure 2**). Inherent in specifying treatment objectives is distinguishing between athletes and clinical populations. Whereas, athletes may seek to improve ventilatory efficiency or lessen diaphragmatic fatigue, the aim for COPD patients may be to lessen dyspnea or improve strength of the inspiratory muscles. While it will certainly be challenging to achieve an individualized approach while retaining adequate experimental control, developing and validating innovative methodologies better suited to individualized training will aid in determining whether such an approach is feasible and effective. Such approaches might use targeted training intensities for a fixed or variable period of time, and the timing of progression to different intensities could be dictated by a pre-defined, but uniformly applied protocol. These "checkpoints" for progression could be based on changes in PI_{max}, the ability to complete a given set of training breaths, or the ability to complete a progressive test, such as the test of incremental respiratory endurance (TIRE) regimen. The TIRE regimen is a common protocol in flow-resistive IMT, such as with the RT2 and PrO_2 devices (Mickleborough et al., 2008; Shei et al., 2016a, 2018; Hursh et al., 2019). Similarly, training groups could be enrolled at similar points in their training cycles to adapt a periodized approach to IMT into research studies and real-world application. These novel approaches will require testing and validation, but should they bear fruit, these innovations can help usher in a new era of IMT research that addresses fundamental questions regarding how to optimize training prescription and the consequent adaptations and benefits.

Aside from adjusting training protocols, future studies also need to consider what endpoints are most relevant, how to adequately power and control studies (for example, achieving a proper placebo control and double-blinding studies), how

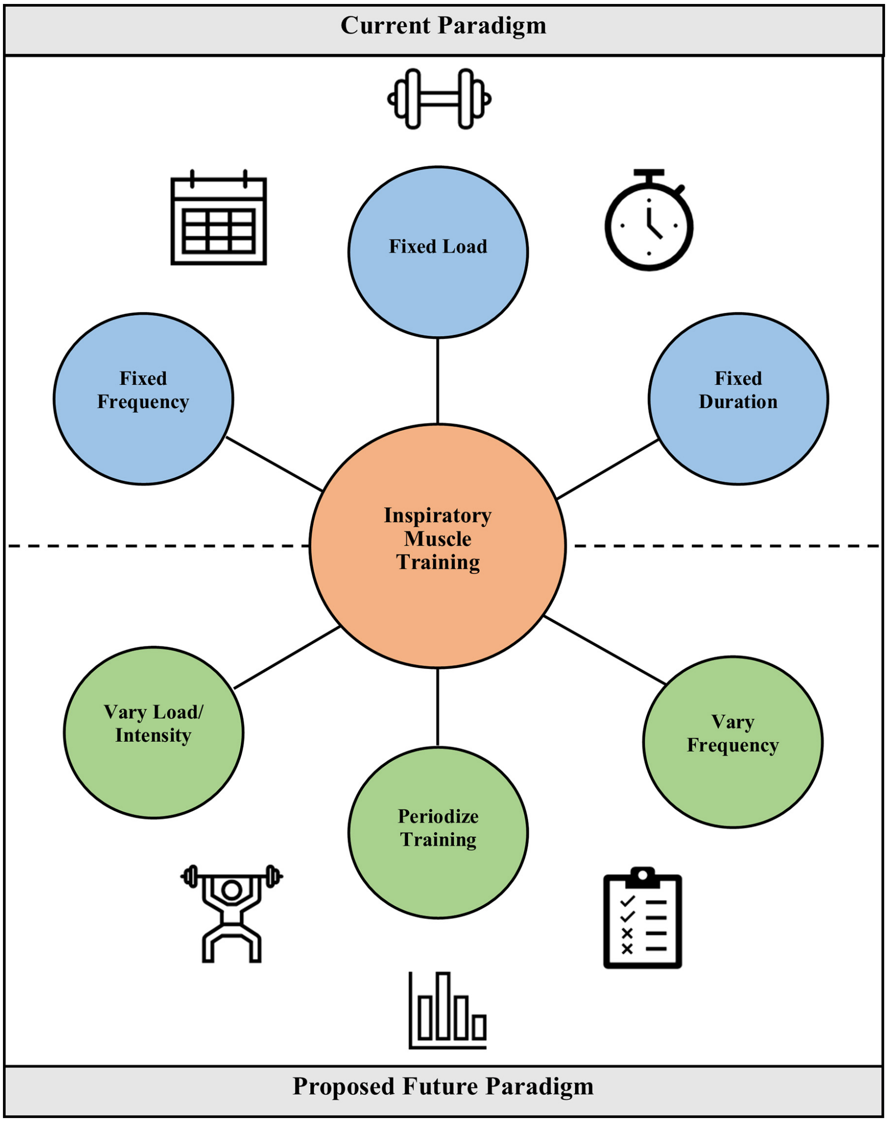

FIGURE 2 | Comparison of the current paradigm for IMT prescription vs. a proposed future paradigm in which load and intensity are varied, training is periodized, and frequency is varied. The latter constitutes a more individualized training prescription akin to how sport coaches prescribe training to their athletes.

to balance baseline participant characteristics, what sample size is needed, and how to design and execute multicenter studies. As highlighted by Patel and colleagues (Patel et al., 2012), a large, randomized, placebo-controlled, double-blinded, multicenter, akin to what a Phase 3 pivotal trial would be for pharmacological interventions, would be a significant step forward in elucidating and validating any potential efficacy resulting from IMT. Recognizing that even within homogenous groups responders and non-responders appear, large-scale projects should also be paired with methodological designs equipped to analyze individual responses—which assumes individual variability will be both present and relevant—as has been demonstrated recently (Hecksteden et al., 2018). While such studies are costly, complex, and require a robust network of qualified investigators, the data from such trials have become the standard for determining the safety and efficacy of medical interventions.

To the best of the authors' knowledge, only three large randomized controlled trials studying IMT have been completed, all in the COPD patient population (Beaumont et al., 2018; Charususin et al., 2018b; Schultz et al., 2018). However, while these studies enrolled 611 (Schultz et al., 2018), 219 (Charususin et al., 2018b), and 150 patients (Beaumont et al., 2018) patients respectively, they were all single-center studies. All three studies found that IMT enhanced respiratory muscle function, and while one study found reductions in dyspnea symptom scores during endurance cycling (Charususin et al., 2018b), the other two found no benefit of IMT on quality of life or dyspnea (Beaumont et al., 2018; Schultz et al., 2018), and none of the studies observed improvements in 6-min walk distance. These studies suggest that there may not be a positive benefit from IMT. In contrast, however, a smaller study by some of the same authors of the large, multicenter randomized controlled trials found that IMT in COPD patients with low maximal inspiratory pressures did improve dyspnea and exercise endurance, which was associated with a reduced diaphragm activation relative to maximum (Langer et al., 2018). Discrepant findings between these three studies may be due to a number of factors including the baseline characteristics of the patients, whether physiological and perceptual improvements (respiratory muscle function, dyspnea, etc.) translate into functional improvements, whether the training protocol provided an adequate training stimulus, and whether training was sustained for a sufficient time. A key difference between the three large studies (Beaumont et al., 2018; Charususin et al., 2018b; Schultz et al., 2018) and the smaller study (Langer et al., 2018) is how the intervention and control interventions were provided. In the larger studies the intervention was general exercise training plus IMT, and control was general exercise training plus sham IMT, whereas the smaller study investigated the effect of IMT as a standalone intervention vs. sham IMT. The second difference is that the constant work rate endurance test was used as an outcome in smaller study. Another factor that is frequently overlooked is that "sham" IMT against relatively low resistances could have effects on respiratory muscle function especially in frail populations, such as many older patients with chronic diseases or patients admitted to the ICU. Thus, questions remain regarding whether more nuanced and progressive individualized training prescriptions may produce different outcomes given the physiological plausibility behind putative IMT benefits. Perhaps then, it is time to consider adopting new approaches to IMT research to tailor the right intervention to the right population and optimize treatment/training effects, and determine whether there is, or is not, a true benefit of IMT.

CONCLUSION

In summary, despite decades of research on IMT, with some studies showing clear benefit and others showing no benefit, it is uncertain why some populations respond to IMT, and some do not. While there are certainly inherent differences in study populations and how IMT is being applied (i.e., as a rehabilitative tool, or for endurance exercise, or team sport exercise), questions regarding how training prescription has historically been done and whether that approach truly optimizes the response to IMT remain. It is time to consider new approaches to IMT that better match how practitioners in sport and exercise training design and apply training plans for athletes. By integrating these methods, such as periodization, better optimization of training load, and considering other factors, such as concurrent IMT and exercise training or the lung volumes at which IMT is completed, future studies may overcome previous shortcomings by providing a tailored, personalized approach that addresses the needs of the individual athlete or patient.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

R-JS conceived of the idea. R-JS, HP, AS, and TM drafted and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript prior to submission.

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Impact of Inhaled Corticosteroids on the Modulation of Respiratory Defensive Reflexes During Artificial Limb Exercise in Ovalbumin-Sensitized Rabbits

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Introduction: Cough is a major lower airway defense mechanism that can be triggered by exercise in asthma patients. Studies on cough reflex in experimental animal models revealed a decrease of cough reflex sensitivity during exercise in healthy animals, but a lack of desensitization in ovalbumin-sensitized rabbits. The aim of our study is to evaluate the impact of inhaled corticosteroids on cough reflex during artificial limb exercise in an animal model of eosinophilic airway inflammation.

Materials and Methods: Sixteen adult ovalbumin-sensitized rabbits were randomly divided into two groups. The "OVA-Corticoid" group (n = 8) received inhaled corticosteroids (budesonide; 1 mg/day during 2 consecutive days) while the "OVA-Control" (n = 8) group was exposed to saline nebulization. The sensitivity of defensive reflexes induced by direct mechanical stimulation of the trachea was studied in anesthetized animals, at rest and during artificial limb exercise. Cell count was performed on bronchoalveolar lavage fluid and middle lobe tissue sections to assess the level of eosinophilic inflammation.

Results: All rabbits were significantly sensitized but there was no difference in eosinophilic inflammation on bronchoalveolar lavage or tissue sections between the two groups. Artificial limb exercise resulted in a significant (p = 0.002) increase in minute ventilation by 30% (+ 209 mL.min^{-1}, ± 102 mL/min^{-1}), with no difference between the two groups. 322 mechanical tracheal stimulations were performed, 131 during exercise (40.7%) and 191 at rest (59.3%). Cough reflex was the main response encountered (46.9%), with a significant increase in cough reflex threshold during artificial limb exercise in the “OVA-Corticoid” group ($p = 0.039$). Cough reflex threshold remained unchanged in the “OVA-Control” group ($p = 0.109$).

Conclusion: Inhaled corticosteroids are able to restore desensitization of the cough reflex during artificial limb exercise in an animal model of airway eosinophilic inflammation. Airway inflammation thus appears to be involved in the physiopathology of exercise-induced cough in this ovalbumin sensitized rabbit model. Inhaled anti-inflammatory treatments could have potential benefit for the management of exercise-induced cough in asthma patients.

Keywords: asthma, cough, exercise, corticosteroids (CS), defensive reflex

INTRODUCTION

Cough is a physiological respiratory defense mechanism regulated by a complex reflex arc to protect the lower airways. However, when cough becomes chronic (lasting more than 8 weeks), it may directly impact patient’s quality of life (Chamberlain et al., 2015). Chronic cough can be associated with various underlying disorders, including chronic obstructive pulmonary disease or gastro-esophageal reflux, but diseases involving eosinophilic airway inflammation such as asthma appear to be predominant (Diver et al., 2019). Exercise is a common trigger of cough in asthma patients, prevalence studies have shown that up to 80% of untreated asthma patients may experience an exercise-induced exacerbation of their symptoms, including coughing (Del Giacco et al., 2015). The pathophysiological mechanisms involved in exercise-induced cough are multiple and complex, and its management is not currently standardized. Recent international recommendations from the Global Initiative for Asthma (*GINA*) suggest rescue therapy in the event of symptom onset or before exercise, combining a low-dose inhaled corticosteroid and a long-acting bronchodilator (GINA, 2020 report). These recommendations have not yet been adopted by French Society of Respiratory Disease, which recommends the use of a short-acting bronchodilator in these situations (Recommandations pour la prise en charge et le suivi des patients asthmatiques, 2020).

However, there is evidence to suggest that when cough stimulation is imposed during exercise, the respiratory centers focus on adapting to the metabolic needs imposed by exercise, with an increase in ventilation and a decrease in cough sensitivity (Kondo et al., 1998; Lavorini et al., 2010). Previous study conducted on anaesthetized rabbits supports this hypothesis, with an increase in the cough reflex threshold during exercise (Poussel et al., 2014). On the opposite, in an animal model of eosinophilic airway inflammation (ovalbumin-sensitized rabbits), there was an absence of desensitization of the cough reflex during artificial limb exercise suggesting the key role of inflammation in the occurrence of exercise-induced cough (Tiotiu et al., 2017). Intravenous corticosteroids appear to be able to restore the desensitization of the cough reflex during artificial limb exercise in the same experimental animal model (Valentin et al., 2020). The main goal of this study was to investigate the impact of inhaled corticosteroid therapy on exercise-induced respiratory defense reflexes in ovalbumin-sensitized rabbits.

MATERIALS AND METHODS

Ethical Approval

The study involved sixteen New Zealand adult rabbits (8 females, weight = 3.09 kg ± 0.12) housed in a conventional facility at Animal House of the Campus Biology-Health, University of Lorraine, from February to April 2021. The experimental procedures were developed in accordance with the local ethics committee on animal testing. The study protocol was approved by the Comité d’Éthique Lorrain en Matière d’Expérimentation Animale (CELMEA) and the *Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation* (APAFIS #26735). Animal housing and experiments were performed according to the directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. Principle of replacement, reduction and refinement were ensured in order to reduce to the minimum not only the number of animals used but also any possible pain, suffering, distress or lasting harm to the animals. Animal suppliers were authorized by, and registered with the competent authority. All animals were provided with appropriate accommodation, environment, food, water and care to their health and well-being. Rabbits were randomized in two groups: “OVA-Corticoid” group ($n = 8$) and “OVA-Control” group ($n = 8$).

Sensitization Protocol

All rabbits were sensitized to ovalbumin by two peritoneal injections on day 0 and day 13 of a solution containing 1 mL of saline (0.9% NaCl) in which 0.1 mg of ovalbumin and 10 mg of aluminum hydroxide $Al(OH)^{3-}$ were dissolved. Ten days later, rabbits were exposed daily for 3 days to aerosols of ovalbumin at a concentration of 2.5 mg/L (50 mg of ovalbumin dissolved in 20 mL of 0.9% NaCl). Each aerosol was administered in a Plexiglas box using an ultrasonic nebulizer (SYST’AM, LS290) producing droplets with an aerodynamic diameter between 1 and 5 μm for 20 min per nebulization. The last OVA aerosol was performed 48 h before the mechanical cough challenge (**Figure 1**). Sensitization was verified by performing intradermal skin tests at day 21 with subcutaneous injection of 0.1 mL of a 200 μg/L ovalbumin solution into the skin of the dorsal region of each rabbit. A saline injection was performed following the same protocol (0.1 mL in the dorsal region). The reaction was assessed at 24 h by measuring the induration at the

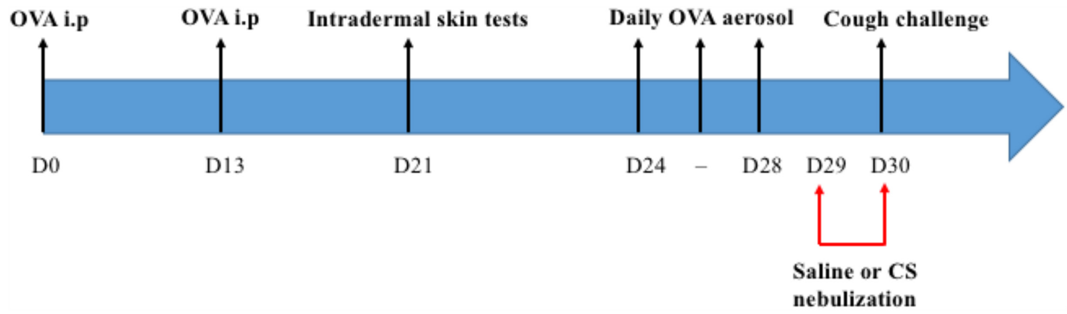

FIGURE 1 | Pre-experimental interventions. Each rabbit received intraperitoneal (i.p) injections of ovalbumin (OVA), an intradermal skin test and a daily aerosol of OVA for 3 consecutive days between D24 and D28. They were challenged with saline or corticosteroid (CS) nebulizations at D29 and D30.

injection site using an electronic caliper (RS Pro Electronic Digital Caliper).

Corticosteroid Nebulization

Rabbits randomized to the "OVA-Corticoid" group received two corticosteroid nebulization (one mg of budesonide dissolved in 20 mL of saline) the day before and 1 h before the cough challenge. Each aerosol was administered for 20 min, using the device described above (Plexiglas box with an ultrasonic nebulizer). Rabbits randomized to the "OVA-Control" group were exposed to saline aerosol, following the same experimental design (i.e., 24 and 1 h before the experiment) (**Figure 1**).

Experimentation

Analgesia premedication was performed by an intramuscular injection of buprenorphine 20 μg/kg (Bupaq multidose 0.3 mg/mL) in hind limb muscle. Induction of anesthesia was conducted 15 min after premedication by a slow intravenous injection over 1 min, in the marginal vein of the ear, of a solution of propofol (Propovet) 3 mg/kg and ketamine 3 mg/kg. As soon as induction was complete, maintenance of anesthesia was initiated with propofol 0.8 mg/kg/min and ketamine 0.2 mg/kg/min, administered *via* the catheter left in place (i.e., marginal vein of the ear). The depth of anesthesia was monitored throughout the experiment by respiratory rate, the presence of a corneal reflex but the absence of ear retraction and ear pinch response. At the end of the experiment, euthanasia was performed by an intravenous injection of pentobarbital (Exagon) 1 mL/kg (i.e., 400 mg/kg).

A sagittal midline incision of the skin tissues of the neck was made on anesthetized rabbit, followed by careful dissection of the subcutaneous and muscular tissues. The trachea was partially transected transversely and each rabbit was tracheostomized (at the caudal part of the trachea) and intubated using a steel tracheostomy cannula, allowing spontaneous breathing. Body temperature was recorded continuously with an electronic thermometer (Physiotemp Instruments, YSI 402 Clifton, NJ, United States) and maintained at 38 °C using circulating warm water pad. Heart rate was monitored by electrocardiogram electrodes placed on the chest. Tidal volume (VT), respiratory frequency (RF) and ventilated flow were recorded throughout the entire experiment by a pneumotachograph (No. 0 Fleisch pneumotachograph with linear range ± 250 mL/s) connected to the tracheal cannula. As described previously, airway resistance (Rrs) was measured by adaptation of the forced oscillation technique to assess exercise-induced bronchodilation (Poussel et al., 2014).

The exercise simulation protocol on anesthetized rabbits consisted of electrical stimulation of hind limb muscles by electrodes (Dura-Stick Premium, REF 42205, DJO, United States) connected to an electrical stimulator (Neuro Trac Rehab, Verity Medical LTD, United Kingdom). Muscle contractions were triggered by a 2 s electrical stimulation with an intensity between 10 and 30 mA. Each stimulation was separated by a free interval of 4 s. The protocol was maintained for 4 min, in order to increase ventilation by at least 30% compared to resting ventilation. Respiratory variables were recorded at rest and during muscle contractions induced by muscle electrostimulation.

A semi-rigid Silastic® catheter (0.7 mm, OD Metric) connected to a rotary electric motor (low voltage DC motors 719RE280, MFA/Comodrills, United Kingdom) was inserted into the tracheostomy cannula to stimulate the airway mucosa and trigger a respiratory defensive reflex. The electric motor has a rotating speed of about 60 revolutions per minute allowing mechanical tracheal stimulations of 50–1,000 ms. All stimulations were delivered during inspiratory phase in order to promote the cough reflex over the expiratory reflex (Varechova et al., 2010). Three sequences of four stimulations were performed at rest and during artificial limb exercise on each rabbit with 10 min of recovery without any stimulation between each sequence.

The electromyographic activity of abdominal muscles was measured by insertion of bipolar stainless steel wire electrodes (A-M Systems INC, Sequim, WA 98,382) in either the transverse abdominal muscles or the external oblique muscles in order to confirm active respiratory movement associated with the response to tracheal stimulation.

Bronchoalveolar Lavage

After tracheal stimulation, a polyethylene-190 catheter was positioned *via* the endotracheal cannula at the carina and lavage was performed by slow injection followed by aspiration of 5 mL of saline 3 times. Bronchoalveolar Lavage (BAL) was collected and filtered with a nylon tissue perforated with 60 μm mesh to remove mucus. The solution was then diluted (1/10), placed in a centrifuge for 10 min at a speed of 600 rpm (Cytospin AutoSmear OF-120E) and stained by the May-Grunwald-Giemsa technique to identify cell populations. On each slide, at least 100 cells were counted using light microscopy (Olympus CDD camera in 1,360 × 1,024 pixel, ×40 objective) and differentiated according to their morphological characteristics between: macrophages, lymphocytes, neutrophils, eosinophils, basophils and monocytes.

Histology

Following bronchoalveolar lavage and animal euthanasia, a median sternotomy was performed to collect the middle lobe of the right lung of each rabbit and a sample of trachea, at distance from the tracheotomy orifice. All specimens were 4% formalin fixed and paraffin embedded. Sections of 2 to 4 μm thickness were carried out with a microtome and then stained with a trichromatic hematoxylin eosin saffron stain. For each rabbit, a pathologist selected the area that appeared to contain the greatest number of inflammatory elements in the trachea and lung. A cell count of eosinophils under light microscopy was performed on the selected area and on 10 consecutive fields at ×400 magnification. The results were expressed as the mean number of eosinophilic cells per field for each rabbit, at the pulmonary and tracheal level.

Data Analysis

Analogic signals were acquired using a PowerLab 30 series system (ADInstruments, ML880 PowerLab 16/30) with an acquisition frequency of 200 Hz and a sampling resolution of 16 bits. The digitized data was analyzed using LabChart8-Pro software (ADInstruments, v 7.1). Before cough challenge, baseline values (at rest and during artificial limb exercise) were recorded by averaging the respiratory variables (VT, RF, V̇Emax: peak expiratory flow) over three consecutive respiratory cycles. The response to tracheal stimulation was assessed by changes in VT and V̇Emax compared to baseline values. For each of the parameters, standard deviation (SD) was also calculated, providing information on the variability of these parameters during spontaneous ventilation. A significant defensive response to mechanical tracheal stimulation was defined by a response in which the ventilatory parameters considered were outside the 99th percentile (i.e., above the mean + 3 times SD). Cough reflex was defined by a significant increase in VT, peak expiratory flow (V̇Emax) and EMG. The cough threshold (CT) was defined as the shortest stimulation duration necessary to provoke at least one cough reflex. Expiratory reflex was retained in case of an isolated increase in V̇ Emax.

Statistical analysis was performed with JMP 9.0.0 software (2010 SAS Institute Inc.) allowing comparisons of qualitative and quantitative data. The incidence and analysis of responses to mechanical tracheal stimulation in both conditions (rest and artificial limb exercise) were analyzed by the Chi-square (χ^2) or Fisher test. Paired *t*-test was used for the comparison of respiratory variables (VT, V̇E, Rsr) and cell counts in the lung and trachea and Mann-Whitney *U*-test was used for the comparison of cell counts in BAL. Wilcoxon non-parametric test was applied to compare rest and exercise cough threshold. Results are expressed as mean ± SD and a statistical significance was retained for a $p < 0.05$. The primary endpoint was the cough reflex threshold (in milliseconds).

RESULTS

Population

All 16 rabbits completed the ovalbumin sensitization protocol. There was no significant difference between the two groups in terms of sex and weight ("OVA-Control" group: 3.11 ± 0.10 kg; "OVA-Corticoid" group: 3.07 ± 0.13 kg, *p* = NS). A death occurred during induction of anesthesia in the "OVA-Control" group, the rabbit was excluded from the study as no data regarding respiratory defense reflexes could be obtained.

Ventilation and Reflexes

The exercise simulation protocol resulted in an increase in minute ventilation in both groups, from 680 $mL.min^{-1}$ ± 128 mL/min^{-1} at rest to 889 mL/min^{-1} ± 128 mL/min^{-1} during exercise (+ 30.7%, $p = 0.002$; paired *t*-test). Overall, Rrs decreased significantly from 20.4 ± 4.39 $hPa.s.L^{-1}$ to 17.2 ± 3.32 $hPa.s.L^{-1}$ ($p = 0.011$; paired *t*-test) during muscle contractions. There was no significant difference in minute ventilation and respiratory resistance at rest and during artificial limb exercise between the two groups (*p* = NS; paired *t*-test).

During the experiment, 322 mechanical tracheal stimulations were performed, 131 during artificial limb exercise (40.7%) and 191 at rest (59.3%). The responses obtained after tracheal stimulation were an expiratory reflex in 17.1% of cases, a cough reflex in 46.9% of cases (**Figure 2**) and no response in 36% of cases. The cough reflex threshold in milliseconds was measured for each rabbit (**Table 1**). There was a significant increase in the exercise cough reflex threshold in the "OVA-Corticoid" group ($p = 0.039$; Wilcoxon) representing a restoration of cough reflex desensitization during artificial limb exercise in these sensitized rabbits treated with inhaled corticosteroids. No change in cough reflex threshold during muscular electrostimulation was found in the "OVA-Control" group ($p = 0.109$; Wilcoxon).

Bronchoalveolar Lavage

Eosinophils were the predominant inflammatory cells found in BAL, representing 13.7% of the cells in the "OVA-Control" group compared to 12.3% in the "OVA-Corticoid" group (*p* = NS; Mann-Whitney *U*-test). There was no difference in eosinophilic or neutrophilic inflammation in the bronchoalveolar lavage fluid between the two groups (**Table 2**).

Histology

Eosinophilic inflammation was significantly more important in parenchymal than in tracheal areas in the "OVA-Control" group ($p < 0.01$, *p* = NS in "OVA-Corticoid" group; paired *t*-test). There was no difference in tracheal eosinophilic infiltration between the two groups (*p* = NS; paired *t*-test; **Table 3**). A trend toward a

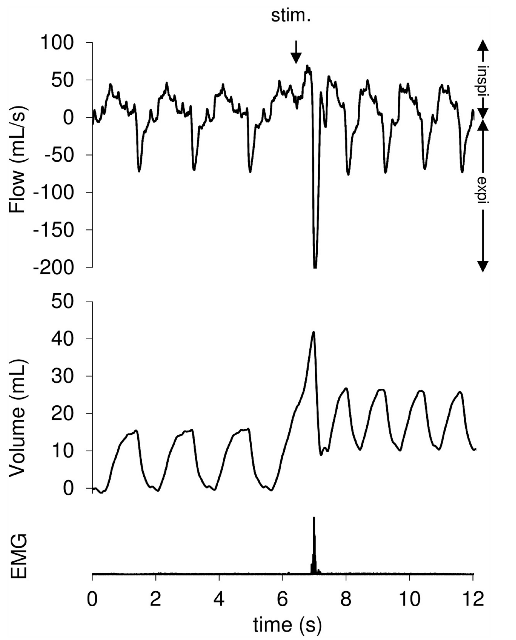

FIGURE 2 | Cough reflex following a tracheal mechanical stimulation. The cough reflex is characterized by an increase in tidal volume (V_T) associated with a concomitant increase of peak expiratory flow ($\dot{V}$ Emax). The downward arrow indicates tracheal stimulation (Stim.). Positive and negative airflow rates indicate inspiration (insp.) and expiration (exp.), respectively. Abdominal muscles electromyogram (EMG) also showed activity on the stimulation breath.

decrease in eosinophilic inflammation in the lung was found in the "OVA-Corticoid" group (p = 0.07; paired t-test).

DISCUSSION

This study is the first to evaluate the impact of inhaled corticoids on cough reflex sensitivity during artificial limb exercise in a model of allergic airway inflammation. Corticosteroids appear to be able to increase cough reflex threshold during exercise. Despite the lack of some more control groups such as non-exercise animals with or without ovalbumin, our finding highlights the key role of eosinophilic inflammation in the modulation of cough.

Our animal model (*Oryctolagus cuniculus*) is of particular interest to study the impact of inhaled cortisteroids on cough reflex *in vivo*. Indeed, the airway innervation of lagomorphs is close to humans with a similar organization of the cough reflex, including A δ and C fibers (Spina et al., 1998). Besides, the OVA-sensitized rabbit is a well-established model of asthmatic disease with a similar therapeutic response to corticoids to that observed in humans (Keir and Page, 2008). This model allowed us to perform a mechanical cough challenge in spontaneous ventilation under anesthesia unlike smaller animal models such as the guinea pig. All tracheal mechanical stimulations were carried out during the inspiratory phase to favor the cough reflex over the expiratory reflex (46.9% of CR against 17.1% of ER) (Varechova et al., 2010). However, general anesthesia results in decreased conductivity of neural pathways and inhibition of the central nervous system. Our model did not allow us to investigate

TABLE 2 | Mean cell counts of eosinophils and neutrophils in bronchoalveolar lavage (expressed as% of total cell count) in both groups ("OVA-Corticoids" group and "OVA-Control" group).

	« OVA-Corticoid » group	« OVA-Control » group	*p*
Eosinophils (%)	12.3	13.7	NS
Neutrophils (%)	0.12	0.71	NS

Mann-Whitney U-test for comparison between groups.

TABLE 3 | Tracheal and parenchymal eosinophil cell counts expressed as number of cells per field (mean ± standard deviation) in both groups ("OVA-Corticoids" group and "OVA-Control" group).

	« OVA-Corticoid » group	« OVA-Control » group	*P*
Trachea	9.79 ± 6.5	11.1 ± 8.6	NS
Lung	13.1 ± 5.3	15.8 ± 8.4	NS (0,07)

Paired t-test for comparison between groups.

TABLE 1 | Distribution of cough reflex (CR) threshold (expressed in milliseconds) at rest and during exercise in both groups ("OVA-Corticoid" group and "OVA-Control" group).

« OVA-Corticoid » group			« OVA-Control » group		
Rabbit	**CR threshold at rest**	**CR threshold during exercise**	**Rabbit**	**CR threshold at rest**	**CR threshold during exercise**
5	150	600	1	1,000	1,000
6	300	300	2	50	50
7	150	150	3	150	150
8	50	50	11	600	300
9	50	300	12	300	150
10	50	150	13	300	50
15	50	150	14	150	150
16	50	150			

CR threshold increased during exercise in the "OVA-Corticoid" group (p = 0.039; Wilcoxon test) whereas it was unchanged in the "OVA-Control" group (p = 0.109; Wilcoxon test).

the cortical modulation of the cough reflex nor its behavioral component (Plevkova et al., 2021).

Our original protocol of electrical muscle stimulation lead to a significant increase in minute ventilation in all rabbits, simulating a moderate effort. Exercise is a well-known cough trigger, especially in dry and cold environments (Turmel et al., 2012). Indeed, the hyperventilation imposed by exercise induces a significant heat and water loss responsible for mucosal edema and changes in bronchial caliber. In addition, it can cause epithelial damages and chronic airway inflammation (Kippelen and Anderson, 2012), with the release of inflammatory mediators such as prostaglandins able to stimulate cough receptors (Coleridge et al., 1976). In fact, airway inflammation appears to have a major impact on cough reflex modulation during exercise, involving medullary structures and higher brain areas, even if brainstem mechanisms underlying the cough reflex and its regulation are only partially understood (Mutolo, 2017; Cinelli et al., 2018). Indeed, an important degree of modulation of the CR has already been shown, involving inflammatory agents at various levels (peripheral and central) (Mutolo, 2017; Cinelli et al., 2018) either enhancing or decreasing CR. In healthy subjects, when stimulation is imposed during exercise, the respiratory centers appear to favor increased ventilation over cough reflex (Lavorini et al., 2010). In contrast, in an animal model of allergic airway inflammation, there appears to be a lack of desensitization of cough reflex during artificial limb exercise (Tiotiu et al., 2017). More precisely, eosinophilic inflammation seems to be particularly involved in the modulation of cough. Eosinophils can influence sensory pathways involved in defensive reflexes in numerous ways, including the release of extracellular ATP (Turner and Birring, 2019). Indeed, in OVA-sensitized mice exposed to allergen challenge, an increase ATP level was found in BAL fluid as in asthma patients (Idzko et al., 2007). Besides, the implication of the P2 × 3 receptors located on the terminal of C fibers in cough is now well-established (Bonvini and Belvisi, 2017). Inhalation of ATP is able to induce a chemical cough by stimulating these ligand-gated ion channels, but endogenous ATP released by inflammatory cells in the airways can also trigger these receptors (Turner and Birring, 2019). The efficiency of P2 × 3 receptor antagonists developed in refractory chronic cough demonstrates the major role of extracellular ATP in cough hypersensitivity syndrome (CHS) (Morice et al., 2019, 2021). Dedicated studies are needed to assess their relevance in exercise-induced cough.

In our study, we did not find a lower eosinophilic cell count in the parenchyma or BAL fluid in the "OVA-Corticoid" group compared to the "OVA-Control" group. This result could reflect an insufficient posology of budesonide although it corresponds to a high daily dose for an asthma patient (1,000 mg). In addition, the exact dosage of budesonide that reached the lower airways is uncertain due to the inhaled route. However, a functional impact of corticosteroids on eosinophilic cells with decreased release of inflammatory mediators cannot be excluded.

Despite the use of a well-established model of cough, our study has limitations. Allergic inflammation induced by sensitization to ovalbumin and aluminum hydroxide is not the most representative of asthma. Although this protocol allowed us an important eosinophilic airway inflammation, its impact on other key elements of the Th2 response potentially involved in cough such as mast cells or IL-4 receptors is less clear. For example, a sensitization to House Dust Mite, using only the inhalation route for sensitization and exposure to allergens, would be more in line with clinical reality (Buday et al., 2016). However, the superiority of this protocol over ovalbumin sensitization remains to be demonstrated. It is important to note that our exercise protocol was not able to significantly modify the airway resistances in either the "OVA-Corticoid" group or the "OVA-Control" group. Exercise-induced cough seems to be frequently related to bronchoconstriction, particularly in asthma patients, as smooth muscle contraction leads to SAR receptors stimulation (Widdicombe, 2001; Boulet and Turmel, 2019). However, according to other studies, the association between bronchomotricity and cough reflex sensitivity seems less clear (Lavorini et al., 2010). Finally, the use of buprenorphine, an opioid agonist-antagonist, may have an impact on cough sensitivity. Indeed, neuromodulators have proven antitussive properties and a daily low dose of morphine is currently recommended for the treatment of chronic refractory cough (Morice et al., 2007, 2020). Still, a single administration before the cough challenge should not have influenced our results.

CONCLUSION

In conclusion, our study is the first to demonstrate the restoration of desensitization of cough reflex during artificial limb exercise by inhaled corticoids. Despite the lack of difference in inflammatory cell count, airway inflammation appears to be the key factor in the modulation of cough during exercise. Our results are thus in line with the recent *GINA* recommendations to use an inhaled corticoid before exercise in asthma patients. A better understanding of the complex mechanisms underlying exercise-induced cough might allow the development of an effective and much-needed therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Comité d'Éthique Lorrain en Matière d'Expérimentation Animale and Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (APAFIS #26735).

AUTHOR CONTRIBUTIONS

SB, SV, SD-A, BC, and MP: study design and development, data analysis and interpretation, writing the manuscript, and final approval of the submitted version. BD and LF: study design and development, data analysis and interpretation, and final approval of the submitted version. DG, CP, and EA: study design and development, and final approval of the submitted version. All authors contributed to the article and approved the submitted version.

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Altered Expiratory Flow Dynamics at Peak Exercise in Adult Men With Well-Controlled Type 1 Diabetes

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Type 1 diabetes may, in time, cause lung dysfunction including airflow limitation. We hypothesized that ventilatory flow morphology during a cardiopulmonary exercise test (CPET) would be altered in adult men with well-controlled type 1 diabetes. Thirteen men with type 1 diabetes [glycated hemoglobin A_{1C} 59 (9) mmol/mol or 7.5 (0.8)%, duration of diabetes 12 (9) years, and age 33.9 (6.6) years] without diagnosed diabetes-related complications and 13 healthy male controls [age 37.2 (8.6) years] underwent CPET on a cycle ergometer (40 W increments every 3 min until volitional fatigue). We used a principal component analysis based method to quantify ventilatory flow dynamics throughout the CPET protocol. Last minute of each increment, peak exercise, and recovery were examined using linear mixed models, which accounted for relative peak oxygen uptake and minute ventilation. The type 1 diabetes participants had lower expiratory peak flow ($P = 0.008$) and attenuated slope from expiration onset to expiratory peak flow ($P = 0.012$) at peak exercise when compared with the healthy controls. Instead, during submaximal exercise and recovery, the type 1 diabetes participants possessed similar ventilatory flow dynamics to that of the healthy controls. In conclusion, men with relatively well-controlled type 1 diabetes and without clinical evidence of diabetes-related complications exhibited attenuated expiratory flow at peak exercise independently of peak oxygen uptake and minute ventilation. This study demonstrates that acute exercise reveals alterations in ventilatory function in men with type 1 diabetes but not until peak exercise.

Keywords: cardiopulmonary exercise test, elastic recoil, principal component analysis—PCA, pulmonary function, ventilatory flow

INTRODUCTION

Type 1 diabetes has been linked to pulmonary dysfunction (Kolahian et al., 2019), which is typically absent at the time diabetes is diagnosed but develops over time. Understanding pathophysiological mechanisms underlying diabetes-related pulmonary manifestations is important when outlining their potential clinical significance and implications (Kolahian et al., 2019; Kopf et al., 2021).

Reported signs of type 1 diabetes-related pulmonary dysfunction include restricted lung capacity (Niranjan et al., 1997), reduced lung elastic recoil (Schuyler et al., 1976), reduced dynamic lung compliance (Strojek et al., 1992), peripheral airway obstruction (Mancini et al., 1999), and diminished lung diffusion capacity (Wheatley et al., 2011). In addition, lower respiratory muscle strength has been linked to diabetes (Fuso et al., 2012) and reduced maximal minute ventilation to type 1 diabetes (Komatsu et al., 2005; Koponen et al., 2013). Nevertheless, lung dysfunction in type 1 diabetes has tended to be overlooked, as it does not seem to hinder normal daily activities (Goldman, 2003). Patients with type 1 diabetes have also exhibited reduced peak pulmonary oxygen uptake ($\dot{V}O_{2peak}$) (Komatsu et al., 2005; Peltonen et al., 2012; Rissanen et al., 2015), which is an integrated measure of pulmonary, cardiovascular, and skeletal muscle function (Wagner, 1996) and thus a strong predictor of respiratory and cardiovascular morbidity and mortality (Steell et al., 2019).

The aim of this study was to assess ventilatory function during a cardiopulmonary exercise test (CPET) to reveal early diabetes-related pulmonary complications in men with well-controlled type 1 diabetes. Specifically, we used a principal component analysis based method to determine ventilatory dynamics throughout the exercise protocol. While diabetes may affect the lung by several mechanisms over time, it is unknown if ventilatory flow adjustments to different intensities of acute dynamic exercise are affected already at early stages of the disease.

MATERIALS AND METHODS

This retrospective and cross-sectional study was a part of an EDGE (Exercise, Diet and Genes in Type 1 Diabetes Mellitus) study belonging to a Canadian-Finnish research collaboration entitled "ARTEMIS—Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection of Discovery, Prevention and Knowledge Exchange" (Noble et al., 2013). Twenty-six non-smoking, normally physically active males, including 13 type 1 diabetes participants (DM) and 13 healthy controls matched for peak minute ventilation (CON), underwent a CPET until volitional exhaustion in a previously described clinical laboratory setting (see Rissanen et al., 2018 for further details). The DM group had no diagnosed or evidence of diabetes-related microvascular complications (neuropathy, nephropathy, and retinopathy), hypertension, or any chronic diseases expect for their type 1 diabetes. Furthermore, the participants were under no medication apart from insulin therapy of type 1 diabetes participants. The CON participants had no long-term or chronic diseases and were overall healthy. Although all the subjects were non-smoking during the time of study, three control participants had a smoking background or had been casually smoking years before the study. The Ethics Committee of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland approved the study (308/13/03/00/2008, September 2, 2008). The participants gave their written informed consent prior to participating the study.

Physical activity was determined from a questionnaire. The participants were asked to record how many times per week and for how long per time they usually exercise with moderate or vigorous intensity. Total exercise time per week was then summarized and regarded as individual's self-reported physical activity. Participants underwent flow-volume spirometry (Medikro Spiro 2000, Medikro, Kuopio, Finland and z-scores according to Kainu et al., 2016) measurements at rest and a CPET under laboratory conditions on a cycle ergometer (Monark Ergomedic 839E; Monark Exercise AB, Vansbro, Sweden). A 12-lead electrocardiogram was recorded using Powerlab system (ADInstruments, Oxford, United Kingdom). Ventilatory flow was measured throughout the CPET with a low resistance turbine (Triple V; Jaegar Mijnhardt, Bunnik, Netherlands), which was calibrated with a syringe (3.00 L, Hans Rudolph Inc., Kansas City, MO, United States). The sampling frequency of the ventilatory flow was 33.33 Hz, which is adequate for capturing ventilatory flow characteristics as the meaningful frequency content is below 10 Hz. Later on, the flow data were upsampled to 100 Hz to achieve 0.01 s point precision for the determination of ventilatory flow cycles. Pulmonary oxygen uptake was measured continuously (AMIS 2000; Innovision A/S, Odense, Denmark).

The CPET measurement protocol consisted of a 5-min rest on the ergometer followed by a 5-min warm-up (unloaded pedaling). After the warm-up, the exercise protocol started with a 40 W load and the work rate was increased by 40 W every 3 min until volitional fatigue (peak exercise). Finally, a recovery period (sitting on the ergometer) of 5 min followed the peak exercise. $\dot{V}O_{2peak}$ and the highest point of end-tidal P_{CO_2} and estimated arterial P_{CO_2} (Jones et al., 1979) were determined as the maximum value of moving average filtered data (30-s window width). In addition, end-tidal P_{CO_2} and estimated arterial P_{CO_2} were assessed from the last 30 s of exercise (peak exercise). $\dot{V}O_2$/work rate slope was determined from the onset of incremental exercise until volitional exhaustion. Ventilatory threshold 1 was primarily determined by the V-slope method (i.e., identifying a consistent increase in the CO_2 output/$\dot{V}O_2$ relationship) (Beaver et al., 1986). Minute ventilation/CO_2 output slope ($\dot{V}E/\dot{V}CO_2$ slope) was determined from the onset of incremental exercise until a respiratory compensation point (Neder et al., 2017); the respiratory compensation point was primarily determined by identifying a consistent increase in the $\dot{V}E/\dot{V}CO_2$ relationship (Beaver et al., 1986). $\dot{V}O_{2peak}$ of predicted (%) was determined based on Hansen et al. (1984) as recommended (Guazzi et al., 2012). Breathing rate was determined from the continuous airflow using Welch's periodogram with three 50% overlapping Hanning windows, and minute ventilation was calculated using breathing rate and expiratory tidal volume.

The CPET data analysis was conducted in separate windows from the beginning of the exercise to the recovery. One-minute analysis windows were placed to the end of every fully completed exercise step. In addition, 1-min windows were placed at subjective peak exercise (last minute before fatigue) and to the last minute of the recovery period. These choices allow comprehensive evaluation of the ventilatory flow from the beginning of exercise to the recovery while ensuring robustness.

Ventilatory Flow Analysis

In this section, we propose a robust analysis method for the ventilatory flow as flow characteristics can vary substantially at lower exercise intensities and is subject to gasping, coughing, and swallowing, for example. To capture subjective ventilatory dynamics, inspiratory and expiratory phases were identified with an in-house zero-crossing algorithm. A consecutive inspiratory phase followed by an expiratory phase constituted a single flow cycle. Flow cycles within an analysis window formed an ensemble, in which flow cycles were aligned according to expiration onset time. As breathing tends to vary, 25% of flow cycles were excluded by computing L1-norm of z-score standardized parameters. These standardized parameters were inspiration onset, inspiratory peak flow, time of the inspiratory peak flow, end of expiration, expiratory peak flow, and time of the expiratory peak flow. The top 25% of cycles, which received the highest norm values, were excluded as divergent cycles. An example of excluded flow cycles within one ensemble is presented in **Figure 1A**.

As time-alignment of flow cycles is crucial in an ensemble analysis, we improved alignment of the cycles by using a genetic algorithm. Our genetic algorithm searches for optimally aligned ensemble using a fitness function that is based on the principal component analysis. This method decomposes a set of correlated flow cycles into a set of uncorrelated principal components and their eigenvalues, which describe the amount of variation a single principal component can explain. The fitness function used in the genetic algorithm calculates the ratio of the first (largest) eigenvalue with respect to the rest of the eigenvalues, which is maximized when the first principal component fits the ensemble data optimally. This ratio has been proposed as an option for time-alignment previously by Garde et al. (2017).

The genetic algorithm provides a sub-optimal solution, which improves alignment by allowing cycles to be shifted not more than half a second forward or backward. Once sub-optimal alignment has been achieved (**Figure 1B**), flow cycles are denoised by estimating each cycle with two or more principal components that explain at least 95% of the flow cycle ensemble variance (**Figure 1C**). Principal components corresponding to the smallest eigenvalues are treated as noise, and thus, their contribution to flow cycles is filtered out. Principal component analysis has previously been used with flow cycles to distinguish abnormal respiratory morphologies in patients with chronic heart failure (Garde et al., 2011).

Ventilatory flow parameters were first computed for every flow cycle separately and then averaged over the ensemble to obtain mean values reflecting properties of an average cycle. Determined parameters were ventilatory tidal volume, peak flow, and slope for both inspiratory and expiratory phases of the ventilatory cycle (see **Figure 1D**). Tidal volume is the volume of air moved into or out of the lungs during inspiration or expiration during a single respiratory flow cycle. Peak flow captures the maximal airflow produced inward or outward, which depends on elastic properties of the lung, resistance of the airways, and respiratory muscle activation. Finally, pulmonary capacity to accelerate airflow is quantified by the slope, which connects start of each ventilatory phase to the peak flow point.

Statistics

Variables are presented as mean (SD) or as mean (95% CI). Demographics, gas exchange, and spirometry parameters were compared with Welch two sample *t*-test. Longitudinal analysis setting is prone to missing data points and induces correlation due to repeated measurements. To account for these circumstances, we used linear random intercept models with fixed effects of group, load, minute ventilation, interaction between load and group as well as interaction between load and minute ventilation. In addition, we included relative $\dot{V}O_{2peak}$ as a covariate and treated load as a categorical variable. We used restricted maximum likelihood estimation for fitting and estimated marginal means for stepwise group comparison with Kenward-Roger degrees of freedom approximation. Statistical analysis was only considered at the points where both of the groups had seven or more subjects. Expiratory peak flow, inspiratory slope, and expiratory slope were log-transformed to meet multivariate normality assumptions. Lastly, we fitted a linear model to compare the associations between minute ventilation and expiratory peak flow and expiratory slope at peak exercise between groups. Predictors of the linear model were: group, peak ventilation, and interaction between group and peak ventilation. Peak ventilation was zero-centered before the fit; thus, the intercept describes the within-group value of an expiratory flow parameter at mean peak ventilation. Statistical analyses were conducted with R 4.0.2 (R Core Team, 2020).

RESULTS

DM and CON groups were comparable in terms of age, physical activity, anthropometrics, and resting blood pressure, as presented in **Table 1**. Moreover, forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and FEV1/FVC were similar between the groups.

Table 2 summarizes CPET data. The participants' efforts during CPET were similarly maximal in the DM and CON groups based on respiratory exchange ratio, rating of perceived exertion, and % of age-predicted maximal heart rate at peak exercise. The DM group was unable to reach as high maximal work rate as the CON group. $\dot{V}O_2$/work rate slope was normal and equal in DM and CON (9.9 ± 1.0 mL/min/W vs. 9.9 ± 0.8 mL/min/W, respectively, P = 0.948). Due to observing moderate, albeit statistically insignificant, between-group differences in $\dot{V}O_{2peak}$ (absolute L/min: P = 0.060; relative mL/kg/min P = 0.107), we included the relative $\dot{V}O_{2peak}$ as a covariate in the linear mixed models to account for this difference. When interpreted as % of predicted, $\dot{V}O_{2peak}$ was lower in DM than in CON. At peak exercise, minute ventilation, breathing rate, end-tidal P_{CO_2}, and estimated arterial P_{CO_2} did not differ between DM and CON. Instead, arterial O_2 saturation at peak exercise was statistically lower in CON than in DM but within or very close to normal levels. $\dot{V}O_2$ at ventilatory threshold 1 did not differ between DM and CON. In addition, $\dot{V}E/\dot{V}CO_2$ slopes reflected ventilatory efficiency to be both normal and comparable between the groups, and also the highest values of end-tidal and estimated arterial P_{CO_2} were similar between the groups.

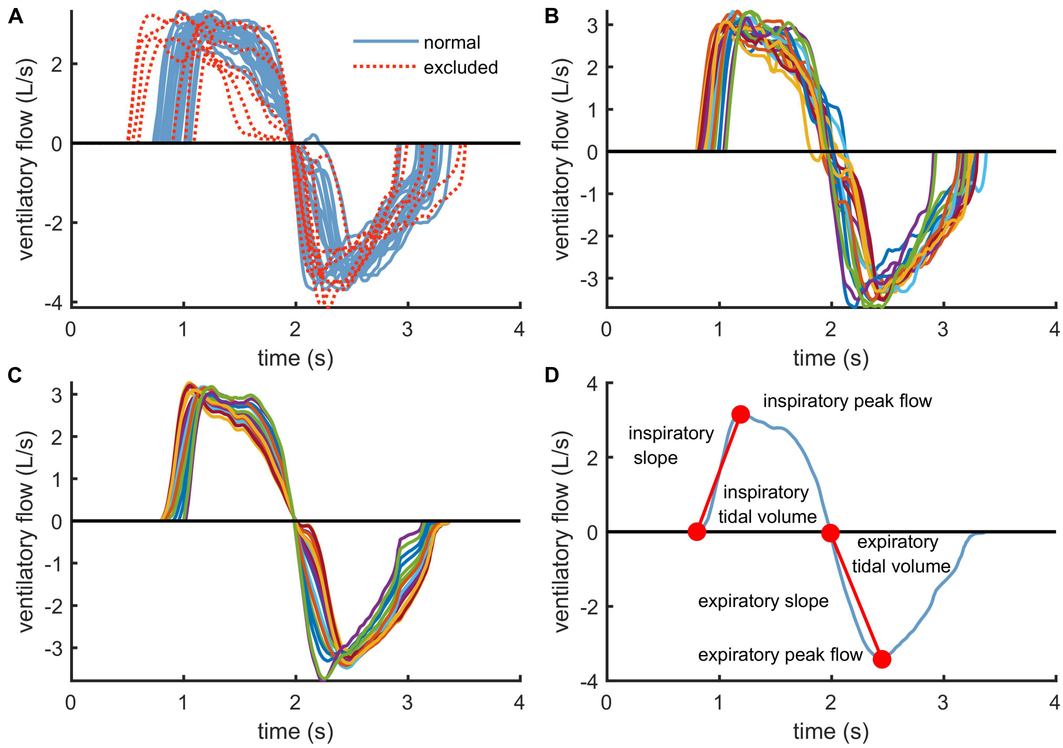


FIGURE 1 | Clarification of the ventilatory flow analysis workflow: **(A)** exclusion of extreme flow cycles within an ensemble using L1-norm, **(B)** time-aligned ensemble using the genetic algorithm, **(C)** principal component transformed flow cycles and **(D)** derivation of the dynamic ventilatory flow parameters from each flow cycle.

Parameters related to ventilatory flow dynamics are presented for the expiratory phase in **Figure 2** and for the inspiratory phase in **Figure 3**. At peak exercise, the DM group had lower expiratory peak flow ($P = 0.008$) and attenuated expiratory slope ($P = 0.012$) when compared with the CON group (**Figure 2**). In addition, mean expiratory flow at peak exercise was lower in the DM group as compared to the CON group (**Table 2**). Furthermore, the DM group had higher expiratory tidal volume at 40 W and 80 W loads ($P = 0.046$ and $P = 0.043$, respectively, **Figure 2**), and declined inspiratory slope at 40 W load ($P = 0.026$, **Figure 3**) compared to the CON group. During recovery, the groups were comparable for both inspiratory and expiratory phases.

Finally, the associations between minute ventilation and expiratory peak flow and expiratory slope at peak exercise are presented in **Figure 4**. Linear dependency between peak minute ventilation and both expiratory parameters is clearly observed. However, at the mean peak minute ventilation, the intercepts of the DM group were lower for expiratory peak flow ($P < 0.001$) and expiratory slope ($P = 0.005$) compared to the CON group.

TABLE 1 | Descriptive statistics.

	DM (n = 13)	CON (n = 13)	P-value
Age (y)	33.9 (6.6)	37.2 (8.6)	0.283
Self-reported physical activity (h:min/wk)	2:32 (2:05)	3:24 (2:00)	0.299
Body mass (kg)	78.4 (11.2)	79.8 (6.7)	0.700
BMI (kg/m^2)	24.8 (3.0)	24.8 (2.1)	0.995
Height (cm)	178 (8)	179 (3)	0.483
FEV1 (L)	4.47 (0.50)	4.43 (0.60)	0.837
FEV1 (z-score)	0.10 (1.10)	0.00 (1.16)	0.830
FVC (L)	5.36 (0.55)	5.43 (0.71)	0.774
FVC (z-score)	-0.28 (0.92)	-0.21 (1.20)	0.869
FEV1/FVC (%)	84.24 (6.71)	81.35 (5.56)	0.243
FEV1/FVC (z-score)	0.65 (1.07)	0.29 (1.06)	0.401
Systolic blood pressure at rest (mmHg)	131 (14)	132 (22)†	0.899
Diastolic blood pressure at rest (mmHg)	83 (8)	81 (16)†	0.674
HbA_{1c} (%)	7.5 (0.8)†		
HbA_{1c} (mmol/mol)	59 (9)†		
DM duration (y)	12 (7)		

†*Stands for n = 12; variables are presented as mean (SD); DM, diabetes group; CON, control group; HbA_{1c}, glycated hemoglobin A_{1c}.*

DISCUSSION

The results of this study indicate altered ventilatory flow dynamics in men with relatively well-controlled type 1 diabetes at peak exercise but not during submaximal exercise. In particular, the DM group had lower expiratory peak flow and declined expiratory slope at peak exercise when compared with the CON group. Importantly, we accounted for minute ventilation and relative $\dot{V}O_{2peak}$ in the linear mixed models. Thus, the observed alterations in expiratory flow dynamics are rather linked with

TABLE 2 | Cardiopulmonary exercise test.

	DM (n = 13)	CON (n = 13)	P-value
Peak exercise			
Work rate (W)	228 (34)	260 (37)	0.028
$\dot{V}O_{2peak}$ (L/min)	2.78 (0.46)	3.13 (0.45)	0.060
$\dot{V}O_{2peak}$ (mL/kg/min)	35.6 (4.5)	39.8 (7.6)	0.107
$\dot{V}O_{2peak}$ of predicted (%)	94 (14)	107 (15)	0.029
Heart rate (bpm)	182 (11)	177 (12)	0.255
Heart rate of age-predicted maximum (%)	98 (5)	97 (6)	0.623
Respiratory exchange ratio	1.22 (0.07)	1.18 (0.04)	0.101
Rating of perceived exertion	19 (1)	19 (1)	0.547
SpO_2 (%)	97 (2)	95 (3)	0.011
End-tidal P_{CO_2} (mmHg)	36 (6)	36 (5)	0.715
Estimated arterial P_{CO_2} (mmHg)	38 (5)	38 (5)†	0.852
Minute ventilation (L/min)	113 (24)	121 (14)	0.321
Breathing rate (breaths/min)	44 (7)	44 (8)	0.899
Inspiratory time (s)	0.71 (0.10)	0.71 (0.12)	0.938
Expiratory time (s)	0.80 (0.13)	0.72 (0.11)	0.114
Mean inspiratory flow (L/s)	3.4 (0.7)	3.7 (0.4)	0.251
Mean expiratory flow (L/s)	3.4 (0.8)	4.0 (0.5)	0.047
Ventilatory threshold 1			
$\dot{V}O_2$ (L/min)	1.83 (0.41)	1.88 (0.32)	0.714
$\dot{V}O_2$ (mL/kg/min)	23.4 (4.7)	23.9 (5.5)	0.781
Ventilatory efficiency			
$\dot{V}E/\dot{V}CO_2$ slope	26 (3)	28 (4)	0.186
Highest end-tidal P_{CO_2} (mmHg)	49 (3)	49 (5)	0.852
Highest estimated arterial P_{CO_2} (mmHg)	50 (3)	50 (4)†	0.778

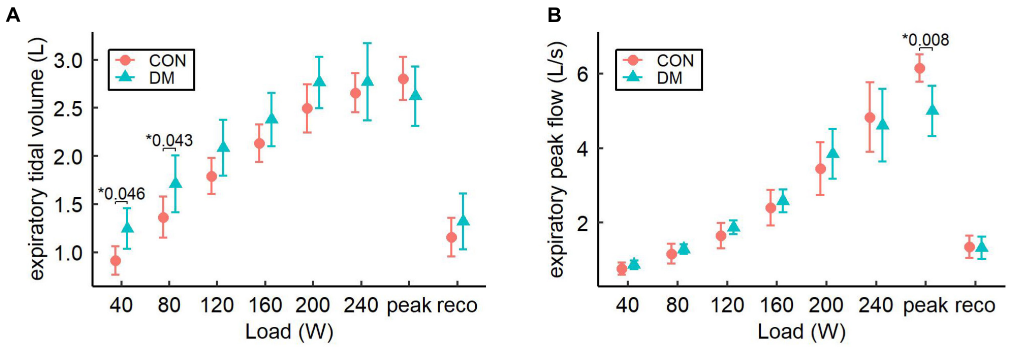
†*Stands for n = 12; variables are presented as mean (SD); DM, diabetes group; CON, control group.*

type 1 diabetes than due to any between-group differences in minute ventilation or $\dot{V}O_{2peak}$. This is also supported by **Figure 4**, which shows that expiratory peak flow and expiratory slope at peak exercise were lower in the DM group than in the CON group at a given level of minute ventilation at peak exercise. Based on the findings, at peak exercise, individuals with well-controlled type 1 diabetes require more time to produce their expiratory peak flow and eventually also attain lower expiratory peak flow compared to healthy individuals. These findings suggest that type 1 diabetes, even when it is relatively well-controlled, targets the lung, but the observed diabetes-related pulmonary manifestations are only observable at peak ventilatory demands and remain subclinical during normal daily activities.

Mechanisms behind the observed alterations in expiratory flow dynamics in type 1 diabetes may involve diabetes-related reduction of lung elastic recoil (Schuyler et al., 1976), which mimics accelerated aging (Hsia and Raskin, 2007). Reduced elastic recoil reduces the alveolar-intrapleural driving pressure of expiratory airflow and may thus exaggerate dynamic airway compression, which is magnified further if other mechanisms limiting airflow also exist (West, 2012). In terms of other coexisting mechanisms limiting expiratory airflow, signs of peripheral airway obstruction, not necessarily detected by routine pulmonary function tests at rest (e.g., flow-volume spirometry), have been reported in adults with type 1 diabetes (Mancini et al., 1999). In addition to reduced elastic recoil, reduced dynamic lung compliance has been observed in patients with type 1 diabetes and hypothesized to be another sign of diminished lung elasticity (Strojek et al., 1992). Such diabetes-related signs of pulmonary dysfunction are associated with hyperglycemia-induced pathological processes targeting the lung structure: experiments in lungs of rats with streptozotocin-induced diabetes have revealed accumulation of elastin and collagen with reduced breakdown of connective tissue protein structures (Ofulue and Thurlbeck, 1988) and recently also substantial impairments in respiratory mechanics (e.g., increased lung tissue viscance and elastance) linked to morphological and biochemical hyperglycemia-related alterations (e.g., alveolar septal thickening, alveolar collapse, inflammatory cellular infiltration) (Machado et al., 2021). In humans, reduced total lung capacity as an indicator of lung volume restriction has been found in hyperglycemic but not in normoglycemic adults with type 1 diabetes (Niranjan et al., 1997), which further suggests glycemic status is linked with lung structure and function. In addition, studies utilizing non-invasive methodology have suggested that diabetes might limit the functional reserves of the pulmonary vasculature (Chance et al., 2008; Roberts et al., 2018), which has the largest microvascular bed in the body. However, potential effects of diabetes-related pulmonary microvascular disease on lung structure and function are unclear.

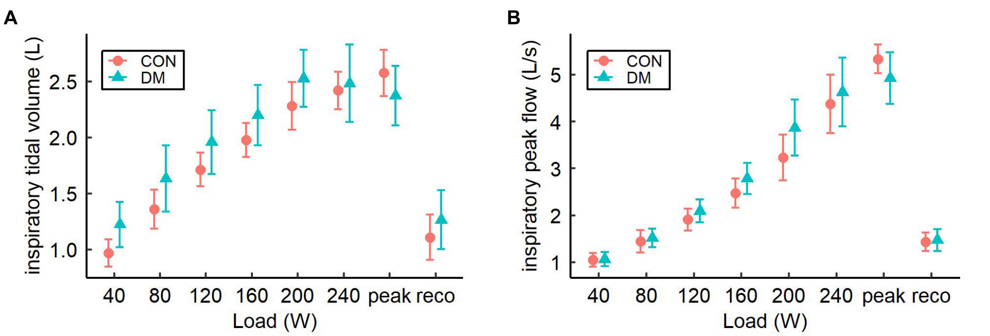
One further mechanism potentially explaining the diabetes-related alterations in expiratory flow dynamics is linked to autonomic nervous system. Existing diabetic autonomic neuropathy has been shown to affect bronchomotor tone and airway caliber at rest and during tilt testing (Santos e Fonseca et al., 1992), and it has been hypothesized that dysregulated bronchomotor tone might exaggerate airway stiffness during exercise in patients with diabetes (Nesti et al., 2020). If existing, such exaggerated airway stiffness during exercise would magnify exertional dynamic airway compression and expiratory airflow limitation (West, 2012). However, the patients belonging to the DM group in our study had no history or clinically overt symptoms or signs of autonomic neuropathy. In addition, CPET unmasked no obvious signs of autonomic dysfunction in the DM group, when it comes to heart rate responses, ventilatory efficiency (reflected by $\dot{V}E/\dot{V}CO_2$ slope), or the highest values (i.e., the setpoints) of both end-tidal and estimated arterial P_{CO_2}, for instance. Thus, our data do not support autonomic dysfunction to be a likely mechanism behind the diabetes-related alterations in expiratory flow dynamics in this study.

The observed alterations in expiratory flow dynamics in the DM group have potential to affect tolerance of acute stress such as acute dynamic exercise. The reduced expiratory parameters in the DM group at peak exercise reflect expiratory flow limitation and may lead to excessive work of breathing at least near-maximal exercise intensities. This might have widespread implications for respiratory and skeletal muscle fatigue and thereby exercise tolerance as increased work of breathing has potential to affect systemic blood flow distribution along with increasing exercise intensity as extensively reviewed by Sheel et al. (2018). Niranjan et al. (1997) previously measured ventilatory power requirement, linked to work of breathing, and found it to be exaggerated



	CON (n)	DM (n)
40 W	13	13
80 W	13	13
120 W	13	13
160 W	13	13
200 W	13	11
240 W	11	7
280 W	(3)	(1)
320 W	(2)	(0)
relative peak	13	13
recovery	13	12

FIGURE 2 | Pairwise comparison of estimated expiratory flow parameters (mean and 95% CI): **(A)** expiratory tidal volume, **(B)** expiratory peak flow, and **(C)** expiratory slope for the control (CON) and the diabetes (DM) groups. Table describes the number of participants for both groups and for each increment. Increments with less than seven subjects are not shown in the graphs and the sample sizes for these increments are presented in parenthesis. * Only *P*-values less than 0.05 are shown.



	CON (n)	DM (n)
40 W	13	13
80 W	13	13
120 W	13	13
160 W	13	13
200 W	13	11
240 W	11	7
280 W	(3)	(1)
320 W	(2)	(0)
relative peak	13	13
recovery	13	12

FIGURE 3 | Pairwise comparison of estimated inspiratory flow parameters (mean and 95% CI): **(A)** inspiratory tidal volume, **(B)** inspiratory peak flow, and **(C)** inspiratory slope for the control (CON) and the diabetes (DM) groups. Table describes the number of participants for both groups and for each increment. Increments with less than seven subjects are not shown in the graphs and the sample sizes for these increments are presented in parenthesis. *Only *P*-values less than 0.05 are shown.

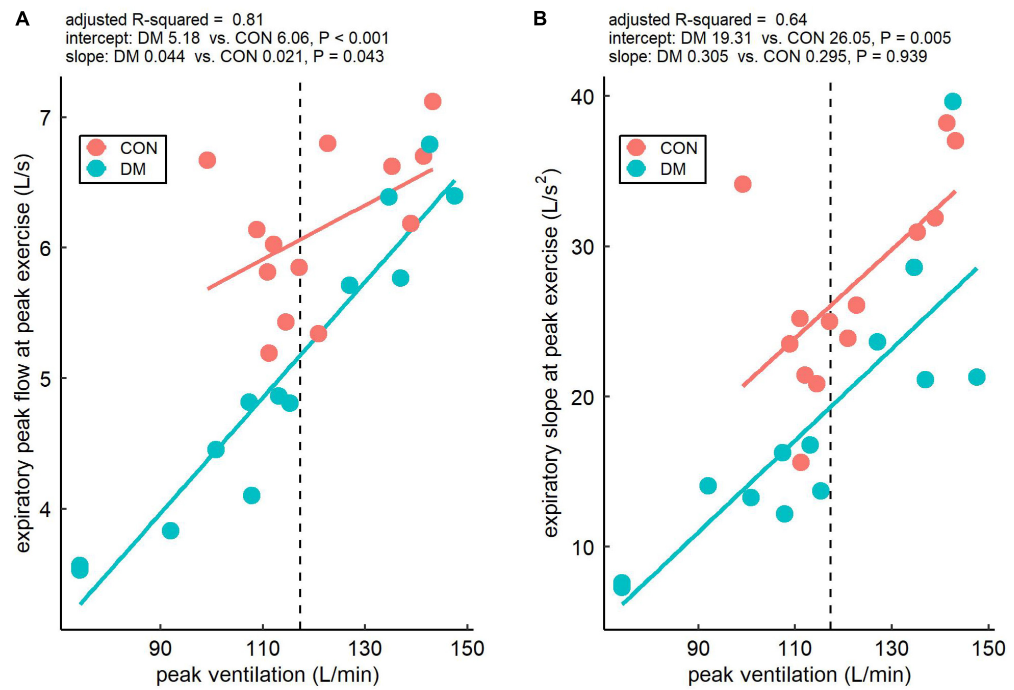


FIGURE 4 | Relationship of expiratory peak flow **(A)** and expiratory slope **(B)** with minute ventilation at peak exercise for the control (CON) and the diabetes (DM) groups. The dashed vertical lines represent the mean of peak ventilation within the study participants, and a linear model was fitted to the data to compare intercepts and slopes between the groups. The intercepts describe the absolute within-group values of the two expiratory flow parameters at the mean peak ventilation (117 L/min) and the slopes describe the effect of ventilation on the two expiratory flow parameters within the groups.

in adults with type 1 diabetes from low-to-maximal exercise intensities. A greater demand for ventilatory power together with diabetes-related reductions in respiratory muscle strength and endurance (Fuso et al., 2012) might promote exercise intolerance in type 1 diabetes. We did observe elevated expiratory tidal volume at 40 W and 80 W and declined inspiratory slope at 40 W load in the DM group, probably suggesting a greater ventilatory demand at low-to-moderate loads. However, this is not likely due to pathological effects of diabetes but rather a consequence of higher relative work rate at fixed loads as the DM group had lower maximal work rate. Moreover, we measured neither work of breathing nor respiratory muscle characteristics in our current study; thus, this study provides no evidence of potential diabetes-related increase in work of breathing or its detrimental effects on exercise tolerance.

Although they may have only minor implications for tolerance of everyday activities and stress in relatively young adults with well-controlled type 1 diabetes, our findings of the diabetes-related alterations in expiratory flow dynamics may on a large scale be regarded as an early and subclinical sign of diminished respiratory reserves. Indeed, patients with diabetes often have diminished respiratory and/or cardiovascular reserves, which may be unmasked by acute exercise provocations (e.g., CPET possibly combined with multimodal imaging data) and develop via several multi-organ mechanisms as comprehensively reviewed elsewhere (Poitras et al., 2018; Nesti et al., 2020; Pugliese et al., 2021). From a perspective beyond exercise provocations, diminished respiratory reserves of patients with diabetes may become unmasked also when they are challenged by acute illness, which has been proposed to be the case during the ongoing SARS-CoV-2 pandemic (Caruso and Giorgino, 2020). Physical activity and exercise are an important part of overall management of diabetes (Colberg et al., 2016) and also associated with reduced mortality in type 1 diabetes (Tikkanen-Dolenc et al., 2017). As regards lung structure and function, a recent study on rats with streptozotocin-induced diabetes demonstrated how performing moderate-intensity physical exercise efficiently protected against diabetes-induced alterations in lung histology and mechanics (Machado et al., 2021). However, further basic, translational, and clinical research on the mechanisms, predictors, modifiers, and natural course of diabetes-related pulmonary manifestations are needed to identify the clinical significance of and optimal ways to prevent and treat the "diabetic lung" (Kolahian et al., 2019; Kopf et al., 2021).

Strengths and Limitations

The strengths of this study include the data collection itself, where high-quality ventilatory flow was recorded throughout the CPET protocol. Furthermore, a new approach using principal component analysis together with the genetic algorithm was proposed for the analysis of ventilatory flow dynamics. In contrast, this study is limited by a relatively small sample size, in addition to which the results apply only to male sex. It should also be noted that timing of the volitional fatigue with respect to the exercise protocol work rate increments varied between participants, but the timing was similar between the DM and CON groups. In addition, the methodology used for flow cycle estimation effectively excluded divergent flow cycles, thus providing representative flow cycle estimates, regardless of the timing of the volitional fatigue.

CONCLUSION

In conclusion, our results indicate altered expiratory flow dynamics in men with relatively well-controlled type 1 diabetes. This was observed as reduced expiratory peak flow and attenuated expiratory slope from expiration onset to the expiratory peak flow at peak exercise near $\dot{V}O_{2peak}$ in the DM group. However, such diabetes-related pulmonary manifestations were not observed at low-to-moderate ventilatory demands. Overall, these novel findings emphasize the lung as a target organ in diabetes mellitus but suggest that diabetes-related pulmonary complications at early stage of type 1 diabetes are subclinical and hardly affect ventilatory function during most of the daily activities.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to privacy statements. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

VH analyzed the data and drafted the manuscript. A-PR, AK, and JP collected the data. HT and JP devised the study and acquired funding for the study. MT devised analyses and acquired funding. All authors revised manuscript critically for important intellectual content, approved the final version of the manuscript, and agreed to be accountable for all the aspects of work.

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BRIEF RESEARCH REPORT
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Perceptual and Ventilatory Responses to Hypercapnia in Athletes and Sedentary Individuals

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Purpose: Hypercapnic chemosensitivity traditionally captures the ventilatory response to elevated pressures of carbon dioxide in the blood. However, hypercapnia also contributes to subjective breathing perceptions, and previously we demonstrated a closer matching of perception to changes in ventilation in athletes compared to controls. Here we investigated any potential underlying hypercapnic chemosensitivity differences between groups, and explored whether these measures relate to ventilatory and perceptual responses during exercise as well as trait levels of affect.

Methods: A hypercapnic challenge, incremental maximal exercise test and affective questionnaires were completed by 20 endurance athletes and 20 age-/sex-matched sedentary controls. The hypercapnic challenge involved elevating end-tidal PCO_2 by 0.8% (6.1 mmHg) and 1.5% (11.2 mmHg) for 3 min each (randomised), with constant end-tidal oxygen. Ventilatory and perceptual responses to hypercapnia were compared between groups, and within each group the relationships between hypercapnic chemosensitivity (slope analyses) and exercising ventilation and perceptions were calculated using Spearman's non-parametric correlations.

Results: While absolute ventilation differences during hypercapnia and exercise were observed, no group differences were found across hypercapnic chemosensitivity (slope) measures. Correlation analyses revealed the anxiety hypercapnic response was related to maximal exercise anxiety, but only in sedentary individuals.

Conclusion: Ventilatory and perceptual hypercapnic chemosensitivity do not differ between athletes and sedentary individuals. However, ventilatory and anxiety hypercapnic chemosensitivities were related to ventilatory and anxiety responses during exercise in untrained individuals only. Athletes may employ additional strategies during exercise to reduce the influence of chemosensitivity on ventilatory and perceptual responses.

Keywords: ventilation, perception, breathlessness, anxiety, athletes, hypercapnic chemosensitivity

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INTRODUCTION

Hypercapnia occurs when there is elevated pressure of carbon dioxide in the blood (PCO_2). Increases in metabolic rate due to physical activity or exercise will increase the cellular production of CO_2, and resulting PCO_2. To mitigate the acidic nature of this elevated PCO_2, chemoreceptors in the brainstem and periphery (carotid and aortic bodies) tightly control cerebral blood flow and drive ventilation (hyperpnea) to exhale excess CO_2 (Feldman et al., 2003; Ainslie and Duffin, 2009; Ogoh et al., 2009). However, there is a broad range of variability in the magnitude of an individual's ventilatory response to hypercapnia (Griez et al., 1990; Houtveen et al., 2003; Li et al., 2006; Peebles et al., 2007; Ogoh et al., 2008; Faull et al., 2016, 2019; Sackett et al., 2018). Furthermore, while hypercapnic chemosensitivity is a large contributing factor to ventilatory control during exercise, additional drivers such as central command output, muscle afferent feedback (Turner et al., 1997; Dempsey and Smith, 2014) and even associative conditioning (Turner and Sumners, 2002; Turner and Stewart, 2004) can influence ventilatory patterns.

Alongside hypercapnia-induced changes in ventilation, elevated PCO_2 can also drive perceptions of both breathlessness (Banzett et al., 1990, 2008; Lane and Adams, 1993; Society, 1999; Lansing et al., 2009) and anxiety (Griez et al., 1990; Smoller et al., 1996; Houtveen et al., 2003; Johnson et al., 2012; Goossens et al., 2014). Importantly, increased ventilation due to hypercapnia does not directly translate to increased perceptions of breathlessness and anxiety (Banzett et al., 1990; Li et al., 2006), and previously we demonstrated a stronger relationship between hypercapnia-induced changes in ventilation and breathing perceptions (breathlessness and anxiety) in athletes compared to sedentary controls at rest (Faull et al., 2016). This raises the question as to whether there is an inherent difference in hypercapnic chemosensitivity in the ventilatory and/or perceptual domains in athletes, and how these responses at rest may translate to differences in ventilation and perceptions during exercise. Understanding these relationships will help shed light on the contribution of baseline ventilatory and perceptual hypercapnic chemosensitivities to our responses during incremental exercise.

Finally, exercise has been associated with reduced levels of affective traits such as anxiety and depression (Herring et al., 2011, 2014), while enhanced hypercapnic perceptions have been reported in individuals with greater trait anxiety (Li et al., 2006), panic disorder (Griez et al., 1990) and those with increased somatic symptoms (Houtveen et al., 2003). Therefore, one mechanism underlying the reduction in negative affect with regular exercise may be *via* decreasing subjective perceptual sensitivity to hypercapnia, possibly due to repeated interoceptive exposure to elevated PCO_2 during exercise (Meuret et al., 2018). Exploring the relationship between perceptual sensitivity to hypercapnia, exercise exposure and measures such as anxiety and depression may help shed light on this effect.

Here, we utilised the athlete and sedentary groups from Faull et al. (2016) to investigate any differences in hypercapnic chemosensitivity for both ventilation and subjective perceptions. Additionally, we explored how chemosensitivity measures relate to ventilatory and perceptual responses during exercise, as well as trait measures of anxiety, depression and anxiety sensitivity (anxiety toward bodily symptoms).

METHODS

The data used for these analyses were collected as part of a wider study that considered both the physiological and functional brain response to breathlessness (Faull et al., 2016, 2018). Data pertaining to the hypercapnic challenge, incremental exercise test and questionnaires were utilised here.

Participants

Two groups of individuals were recruited into this study, with 20 endurance athletes and 20 age- and sex-matched sedentary controls (10 males and 10 females in each group; mean age ± SD, 26 ± 7 years). Endurance athletes completed five or more training sessions per week in either running, cycling or rowing, while sedentary individuals were not involved in any organised sport and minimal commuting activity. One athlete did not complete the maximal exercise test due to injury. The Oxfordshire Clinical Research Ethics Committee approved the study and volunteers gave written, informed consent prior to participation. Participant anthropometrics are reported in **Table 1**.

TABLE 1 | Participant anthropometrics.

	Athletes	Sedentary	*p*-value
Females/Males	10/10	10/10	NA
Training volume (hours/week)	11.5 ± 0.2	0.0 ± 0.0	NA
Age (years)	25.8 ± 1.7	25.7 ± 1.7	0.95
Height (m)	1.8 ± 0.2	1.7 ± 0.0	0.01*
Weight (kg)	75.2 ± 2.3	68.7 ± 3.0	0.09
BMI (kg/m^2)	23.1 ± 0.6	23.3 ± 0.8	0.87
FVC (L)	5.7 ± 0.2	4.2 ± 0.3	<0.01*
FVC predicted (L)	5.2 ± 0.2	4.7 ± 0.2	0.10
FVC (% predicted)	108.3 ± 2.0	90.9 ± 4.3	<0.01*
FEV1 (L)	4.4 ± 0.2	3.4 ± 0.2	<0.01*
FEV1 predicted (L)	4.4 ± 0.2	4.0 ± 0.2	0.10
FEV1 (% predicted)	100.5 ± 2.1	90.9 ± 4.3	<0.01*
FEV1/FVC (%)	78.2 ± 1.6	81.3 ± 1.0	0.10
MVV (L/min)	150.9 ± 9.6	113.0 ± 8.8	0.01*
MVV predicted (L/min)	182.0 ± 6.3	144.8 ± 8.0	<0.01*
MVV (% predicted)	82.3 ± 3.5	77.7 ± 3.6	0.36
Trait anxiety	29.6 ± 1.3	30.8 ± 1.5	0.54
Anxiety sensitivity index	13.5 ± 1.4	16.1 ± 1.7	0.24
Depression	6.4 ± 0.9	7.6 ± 1.1	0.40

*Data adapted from Faull et al. (2016). Mean ± SE reported for each group. BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1s; FEV1/FVC, forced expiratory volume in 1s as a fraction of forced vital capacity; MVV, maximal voluntary ventilation. Predicted values for FVC and FEV1 were calculated using Global Lung Index reference values (Global Lung Function Initiative, 2021; Hall et al., 2021), and predicted values for MVV were calculated with reference to FEV1 (Neder et al., 1999). *Significantly different ($p < 0.05$) between groups.*

Questionnaires

Participants completed questionnaires to measure anxiety (Spielberger State-Trait Anxiety Inventory; STAI) (Spielberger, 2010; Vitasari et al., 2011; Thomas and Cassady, 2021), depression (Centre for Epidemiologic Studies Depression Scale; CES-D) (Radloff, 1977; Weissman et al., 1977) and anxiety sensitivity, which measures anxiety toward anxiety symptoms (Anxiety Sensitivity Index; ASI) (Reiss et al., 1986; Maller and Reiss, 1987). Questionnaires were completed on paper and were scored according to their respective manuals.

Spirometry

Participants additionally completed baseline spirometry measures as part of the wider study protocol. Participants breathed through a mouth-piece (Hans Rudolf, Kansas City, MO, United States) and turbine connected to gas and flow analyser (Cortex Metalyser 3B, Cranlea Human Performance Ltd., Birmingham, United Kingdom) while wearing a nose clip. Metasoft studio software (Cortex, Versions 3.9.9 and 4.9.0, Cranlea Human Performance Ltd., Birmingham, United Kingdom) was used to calculate all spirometry measurements. Forced vital capacity (FVC) and Fraction of Expired Volume in 1 s (FEV1) were measured using a full inspiration and expiration, repeated three times in accordance to established guidelines (Levy et al., 2009). Spirometry protocols matched those defined in the American Thoracic Society and European Respiratory Society 2019 update for usable tests (Graham et al., 2019), although FVC measures were not followed by a full inspiration. The best of two repeats of maximal voluntary ventilation (MVV) were recorded, where participants were asked to maximally ventilate through the mouthpiece for 10 s.

Hypercapnic Challenge

Participants were positioned supine and asked to breathe through a custom-built gas mixing circuit *via* a mouthpiece (Scubapro United Kingdom Ltd., Mitcham, United Kingdom) connected to a bacterial and viral filter (GVS, Lancashire, United Kingdom) whilst wearing a nose clip. Participants were given prism glasses such that they could see and respond to questions presented on a computer screen *via* a button box throughout the task. The breathing circuit allowed for measures of end-tidal pressure of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) *via* polyethylene extension tubing (Vygon SA, Ecouen, France) connected to a gas analyser (ADInstruments Ltd., Oxford, United Kingdom). A spirometer (ADInstruments Ltd., Oxford, United Kingdom) simultaneously measured ventilatory flow and volume, and all devices were connected to a data acquisition device (Powerlab; ADInstruments Ltd., Oxford, United Kingdom) with measures recorded using physiological monitoring software (Labchart 7; ADInstruments Ltd., Oxford, United Kingdom).

Following 8 min of rest where participants breathed humidified medical air, two three-minute hypercapnic periods of elevated $P_{ET}CO_2$, either 0.8% (6.1 mm Hg) or 1.5% (11.2 mm Hg) above baseline were administered (randomised order), separated and followed by 4 min of rest breathing medical air. $P_{ET}CO_2$ values were chosen to induce two distinguishable levels of hypercapnia within a tolerable range for all participants, and hypercapnia was achieved by titrating a CO_2 mixture (25% CO_2; 21% O_2; balance N_2; supplied by BOC Gas, Oxford, United Kingdom) into a custom-built mixing chamber and breathing circuit (see Faull et al., 2016 for details). $P_{ET}O_2$ was maintained at resting levels throughout the task by simultaneously titrating a hypoxic gas (7% O_2; balance nitrogen; supplied by BOC Gas, Oxford, United Kingdom) into the inspiratory mixture. Every 4 min participants were asked to rate their breathlessness by answering the question "How breathless are you" *via* the button box between "Not at all breathless" (0%) and "Most intense breathlessness imaginable" (100%) using a visual analogue scale (VAS). Participants additionally answered the question "How anxious are you about your breathing?" using a VAS between "Not at all anxious" (0%) and "Extremely anxious" (100%) following the breathlessness rating.

Incremental Maximal Exercise Test

Participants completed an incremental exercise test to exhaustion on a stationary bicycle ergometer (Ergoline 500, Lindenstrasse, Germany). Participants were fitted with a facemask (Hans Rudolph Inc., Kansas, United States) and turbine connected to a gas and flow analyser (Cortex Metalyser 3B, Cranlea Human Performance Ltd., Birmingham, United Kingdom) for breath-by-breath measures of expired gases and ventilatory flow. Heart rate was measured by a Polar heart rate monitor (Polar, Kempele, Finland) connected *via* Bluetooth. Exercise was initiated between 50 and 150 W according to predicted maximal effort, and cadence was self-selected cadence with an aim of 90 rpm. Three-minute stages at 50 W increments were completed until volitional exhaustion. Breathlessness and breathing-related anxiety were additionally rated on a 0–100% VAS scale verbally in the last 30 s of each stage and at exhaustion. Physiological measures were averaged across the final 30 s at each stage, and the anaerobic threshold for each participant was determined by visual inspection using the V-slope method (Wasserman et al., 1973; Beaver et al., 1986).

Statistical Analyses

To measure both the ventilatory and perceptual responses to hypercapnia, chemosensitivity metrics for ventilation, tidal volume, breathing rate, breathlessness and anxiety of breathing were calculated. A separate linear model was fit for each of these measures against $P_{ET}CO_2$ during the hypercapnic challenge, and the slope coefficient (representing the rate of change in each of the metrics according to the mm Hg increase in $P_{ET}CO_2$) was used as the subsequent hypercapnic chemosensitivity metric. Following tests for data normality (Anderson-Darling test, with an alpha value of $p < 0.05$ rejecting the null hypothesis of normally distributed data), the slope parameter for each of the measures was compared between athlete and sedentary groups using two-tailed independent t-tests. If the data were not normally distributed, significant group differences were tested using non-parametric Wilcoxon rank sum tests. To account for the multiple group comparison tests, we utilised False Discovery Rate (FDR)-corrected significance values at $p < 0.05$, with values surviving $p < 0.05$ uncorrected reported as exploratory results.

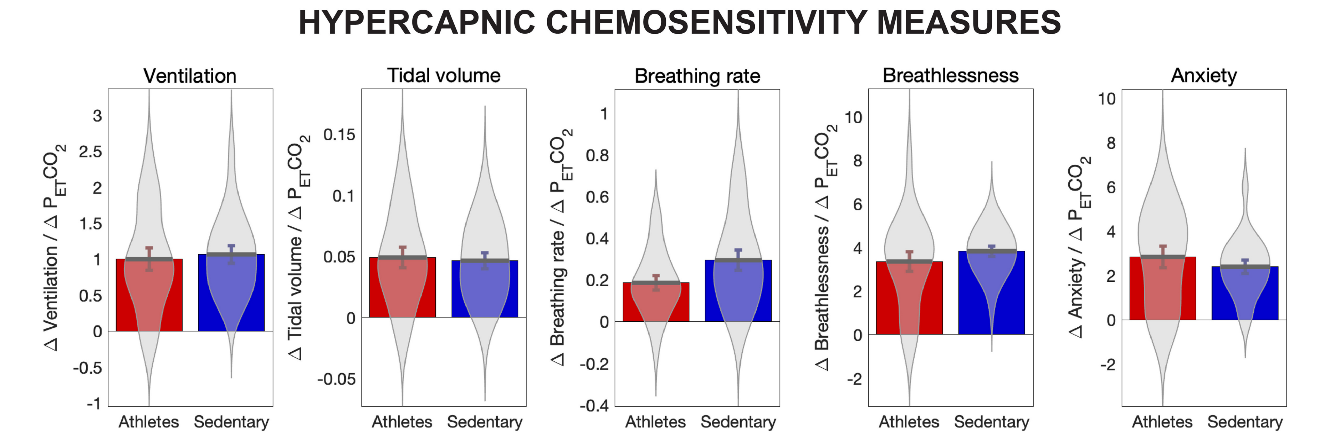

FIGURE 1 | Hypercapnic chemosensitivity measures for athletes and sedentary controls. Hypercapnic chemosensitivity for ventilation, tidal volume, breathing rate, perception of breathlessness and perception of breathing-related anxiety are the rate of change in each value for each mm Hg increase in $P_{ET}CO_2$, calculated using the slope parameter from a fitted linear model. Ventilation, tidal volume and breathing rate were normalised to height. No significant differences were observed between groups for any chemosensitivity measures.

Tidal volume and breathing rate during both the hypercapnic challenge and exercise were also compared between the groups, in addition to the comparisons previously reported by Faull et al. (2016).

To compare each of the hypercapnic chemosensitivity measures with both exercising variables (ventilation, breathlessness and anxiety scores at anaerobic threshold and maximal exercise) and questionnaire scores relating to affect (anxiety, depression, anxiety sensitivity), we constructed a full correlation matrix of these variables for each of the athlete and sedentary groups. To reduce the impact of outliers with only 20 participants in each group, we employed non-parametric Spearman correlations, with significance taken as correlation coefficients having a $p < 0.05$ with FDR correction for multiple comparisons across the correlation matrix. Values surviving $p < 0.05$ uncorrected are reported as exploratory results.

RESULTS

Hypercapnic Chemosensitivity

Hypercapnic chemosensitivity values (slope parameter for the change in each metric in response to increases in $P_{ET}CO_2$) for ventilation, tidal volume, breathing rate, breathlessness and anxiety of breathing are provided in **Figure 1**. Ventilation, tidal volume and breathing rate were normalised to height. No significant differences were observed between athletes and sedentary groups.

A summary of the ventilatory and perceptual responses to the hypercapnic challenge for athletes and sedentary groups can be seen in **Table 2**. As reported previously (Faull et al., 2016), athletes and sedentary individuals were found to differ at rest for ventilation. Additionally, here we have found differences in tidal volume at rest (mean ± SE: athletes 1.37 ± 0.14 L vs. sedentary 0.83 ± 0.07 L; $z = 3.19$; $p < 0.01$; Wilcoxon rank sum) and both mild hypercapnia (mean ± SE: athletes 1.99 ± 0.18 L vs. sedentary 1.26 ± 0.10 L; $z = 3.02$; $p < 0.01$; Wilcoxon rank sum) and moderate hypercapnia (mean ± SE: athletes 2.39 ± 0.18 L vs. sedentary 1.74 ± 0.17 L; $t = 2.62$; $p = 0.01$; t-test), as well as differences in breathing rate at rest (mean ± SE: athletes 10.47 ± 0.70 bpm vs. sedentary 13.79 ± 0.90 bpm; $t = -2.91$; $p = 0.01$; t-test) and both mild hypercapnia (mean ± SE: athletes 12.30 ± 0.95 bpm vs. sedentary 17.71 ± 1.29 bpm; $z = -3.15$; $p < 0.01$; Wilcoxon rank sum) and moderate hypercapnia (mean ± SE: athletes 14.42 ± 0.96 bpm vs. sedentary 19.20 ± 1.30 bpm; $t = -2.94$; $p = 0.01$; t-test).

Ventilation and Perception During Exercise

A summary of the ventilatory and perceptual responses at both anaerobic threshold and maximal exercise for athletes and sedentary groups can be seen in **Table 2**. As reported previously (Faull et al., 2016), athletes and sedentary individuals were found to differ at both anaerobic threshold and maximal exercise for ventilation, and anxiety of breathing was greater in athletes at maximal exercise. Additionally, here we found differences in tidal volume at anaerobic threshold (mean ± SE: athletes 2.56 ± 0.14 L vs. sedentary 1.47 ± 0.13 L; $t = 5.76$; $p < 0.01$; t-test) and maximal exercise (mean ± SE: athletes 2.82 ± 0.10 L vs. sedentary 1.92 ± 0.15 L; $z = 3.82$; $p < 0.01$; Wilcoxon rank sum), and also for breathing rate at maximal exercise (mean ± SE: athletes 52.46 ± 2.57 bpm vs. sedentary 41.38 ± 1.38 bpm; $z = 2.74$; $p = 0.01$; Wilcoxon rank sum) but not anaerobic threshold (mean ± SE: athletes 30.45 ± 1.13 bpm vs. sedentary 28.63 ± 1.79 bpm; $t = 0.85$; $p = 0.40$; t-test).

Correlations Between Hypercapnic Chemosensitivity, Exercise and Affect

A full correlation matrix was calculated between hypercapnic chemosensitivity measures, exercise and affect values for each of the athlete and sedentary groups (**Figure 2**). As seen in **Figure 2**,

TABLE 2 | Ventilatory and perceptual responses to a hypercapnic challenge for athletes and sedentary controls.

Rest	**Athletes**	**Sedentary**	**t/z statistic**	***p*-value**	**Test**
$P_{ET}CO_2$ (mm Hg)	38.9 ± 1.1	40.1 ± 0.8	−0.45	0.66	Wxn
$P_{ET}O_2$ (mm Hg)	116.3 ± 1.7	115.7 ± 1.5	0.27	0.79	Ttest
Ventilation (L/min)	13.0 ± 0.8	10.5 ± 0.6	2.34	0.02*	Wxn
Ventilation/height (L/min/m)	7.2 ± 0.4	6.1 ± 0.3	1.99	0.05	Ttest
Tidal volume (L)	1.4 ± 0.1	0.8 ± 0.1	3.19	<0.01*	Wxn
Tidal volume/height (L/m)	0.8 ± 0.1	0.5 ± 0.0	3.02	<0.01*	Wxn
Breathing rate (bpm)	10.5 ± 0.7	13.8 ± 0.9	−2.91	0.01*	Ttest
Breathing rate/height (bpm/m)	5.8 ± 0.4	8.2 ± 0.6	−3.16	<0.01*	Ttest
Breathlessness rating (%)	3.1 ± 1.0	4.6 ± 0.9	−1.66	0.10	Wxn
Breathing anxiety rating (%)	3.1 ± 0.9	5.1 ± 1.1	−1.56	0.12	Wxn
Hypercapnia: Mild	**Athletes**	**Sedentary**	**t/z statistic**	***p*-value**	**Test**
$P_{ET}CO_2$ (mm Hg)	46.0 ± 1.0	46.5 ± 0.7	0.15	0.88	Wxn
$P_{ET}O_2$ (mm Hg)	114.7 ± 1.2	115.8 ± 1.0	−0.70	0.49	Ttest
Ventilation (L/min)	23.3 ± 2.3	20.6 ± 1.1	0.45	0.66	Ttest
Ventilation/height (L/min/m)	12.9 ± 1.3	12.0 ± 0.6	−0.07	0.95	Wxn
Tidal volume (L)	2.0 ± 0.2	1.3 ± 0.1	3.02	<0.01*	Wxn
Tidal volume/height (L/m)	1.1 ± 0.1	0.7 ± 0.1	2.77	0.01*	Wxn
Breathing rate (bpm)	12.3 ± 0.9	17.7 ± 1.3	−3.15	<0.01*	Wxn
Breathing rate/height (bpm/m)	6.9 ± 0.6	10.5 ± 0.9	−3.12	<0.01*	Wxn
Breathlessness rating (%)	26.0 ± 4.5	21.9 ± 3.5	0.72	0.48	Ttest
Breathing anxiety rating (%)	18.9 ± 4.0	17.2 ± 3.1	−0.23	0.82	Wxn
Hypercapnia: moderate	**Athletes**	**Sedentary**	**t/z statistic**	***p*-value**	**Test**
$P_{ET}CO_2$ (mm Hg)	50.6 ± 1.1	51.2 ± 0.7	−0.49	0.63	Ttest
$P_{ET}O_2$ (mm Hg)	115.9 ± 1.0	117.6 ± 1.3	−1.00	0.32	Ttest
Ventilation (L/min)	34.0 ± 3.3	31.1 ± 2.6	0.70	0.49	Ttest
Ventilation/height (L/min/m)	18.8 ± 7.9	18.1 ± 1.4	0.33	0.74	Ttest
Tidal volume (L)	2.4 ± 0.2	1.7 ± 0.2	2.62	0.01*	Ttest
Tidal volume/height (L/m)	1.3 ± 0.1	1.0 ± 0.1	2.47	0.02*	Ttest
Breathing rate (bpm)	14.4 ± 1.0	19.2 ± 1.3	−2.94	0.01*	Ttest
Breathing rate/height (bpm/m)	8.0 ± 0.6	11.4 ± 0.9	−2.77	0.01*	Wxn
Breathlessness rating (%)	41.9 ± 5.4	47.8 ± 2.8	−0.69	0.49	Wxn
Breathing anxiety rating (%)	36.5 ± 5.8	32.3 ± 3.6	0.62	0.54	Ttest
Exercise: Anaerobic threshold	**Athletes**	**Sedentary**	**t/z statistic**	***p*-value**	**Test**
Work rate (W)	219.7 ± 10.5	101.3 ± 5.6	5.26	<0.01*	Wxn
VO_2 (mL/min/kg)	36.5 ± 2.5	20.3 ± 1.0	5.91	<0.01*	Ttest
$P_{ET}CO_2$ (mm Hg)	41.6 ± 0.8	40.7 ± 0.8	0.77	0.45	Ttest
$P_{ET}O_2$ (mm Hg)	108.4 ± 1.0	109.2 ± 0.9	−0.59	0.56	Ttest
Ventilation (L/min)	79.7 ± 3.8	38.6 ± 2.2	4.96	<0.01*	Wxn
Ventilation/height (L/min/m)	42.1 ± 1.9	22.4 ± 1.2	4.99	<0.01*	Wxn
Tidal volume (L)	2.6 ± 0.1	1.5 ± 0.1	5.76	<0.01*	Ttest
Tidal volume/height (L/m)	1.4 ± 0.1	0.8 ± 0.1	6.11	<0.01*	Ttest
Breathing rate (bpm)	30.5 ± 1.1	28.6 ± 1.8	0.85	0.40	Ttest
Breathing rate/height (bpm/m)	16.8 ± 0.6	17.0 ± 1.2	−0.09	0.93	Ttest
Heart rate (bpm)	152.7 ± 3.2	135.2 ± 3.8	3.47	<0.01*	Ttest
Breathlessness rating (%)	22.9 ± 3.8	16.1 ± 3.0	1.30	0.19	Wxn
Breathing anxiety rating (%)	5.9 ± 1.8	5.1 ± 2.1	0.69	0.49	Wxn
Exercise: Maximum	**Athletes**	**Sedentary**	**t/z statistic**	***p*-value**	**Test**
Work rate (W)	325.0 ± 13.3	173.8 ± 10.2	8.94	<0.01*	Ttest
VO_2 (mL/min/kg)	50.8 ± 1.6	31.6 ± 1.6	8.22	<0.01*	Ttest

(Continued)

TABLE 2 | (Continued)

Exercise: Maximum	Athletes	Sedentary	t/z statistic	*p*-value	Test
$P_{ET}CO_2$ (mm Hg)	33.2 ± 1.0	35.3 ± 0.8	−1.69	0.10	Ttest
$P_{ET}O_2$ (mm Hg)	120.1 ± 1.2	117.9 ± 0.8	1.52	0.14	Ttest
Ventilation (L/min)	146.4 ± 8.2	77.8 ± 6.0	6.63	<0.01*	Ttest
Ventilation/height (L/min/m)	80.5 ± 4.2	44.7 ± 3.0	6.86	<0.01*	Ttest
Tidal volume (L)	2.8 ± 0.1	1.9 ± 0.2	3.82	<0.01*	Wxn
Tidal volume/height (L/m)	1.6 ± 0.1	1.1 ± 0.1	3.78	<0.01*	Wxn
Breathing rate (bpm)	52.5 ± 2.6	41.4 ± 1.4	2.74	0.01*	Wxn
Breathing rate/height (bpm/m)	28.9 ± 1.4	24.3 ± 0.9	2.77	0.01*	Ttest
Heart rate (bpm)	180.2 ± 1.7	172.9 ± 2.9	2.04	0.05*	Ttest
Breathlessness rating (%)	80.7 ± 5.1	72.5 ± 3.8	1.91	0.06	Wxn
Breathing anxiety rating (%)	45.3 ± 8.1	22.3 ± 4.5	1.89	0.06	Wxn

*Values were taken at rest and two levels of hypercapnia (0.8% and 1.5% increases in $P_{ET}CO_2$) while $P_{ET}O_2$ was held constant (iso-oxia). Mean ± SE reported for each group. *Significant differences between groups, compared using either an unpaired T-test (Ttest) if data were normally distributed or a Wilcoxon rank sum test (Wxn) if data were not normally distributed. Data adapted from (Faull et al., 2016).*

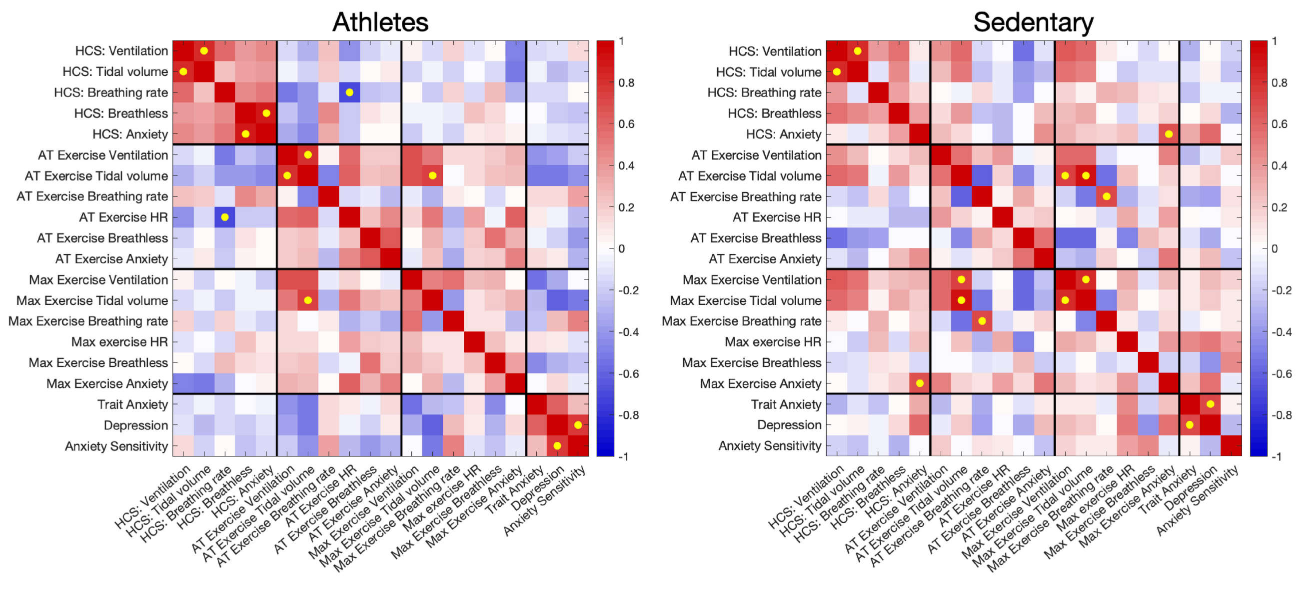

FIGURE 2 | Correlations between ventilatory and perceptual hypercapnic chemosensitivity measures, exercising ventilation, heart rate and perceptions, and affective measures of anxiety, depression and anxiety sensitivity. Correlations were performed separately for athletes and sedentary groups. Yellow dots denote correlations that have $p < 0.05$ and are FDR-corrected for multiple comparisons. HCS = Hypercapnic chemosensitivity; AT = Anaerobic threshold, determined using the V-slope method (Wasserman et al., 1973; Beaver et al., 1986).

athletes demonstrated stronger and more consistent correlations between hypercapnic chemosensitivity metrics than sedentary individuals. Both athletes and sedentary groups showed strong correlations between anaerobic threshold and maximal exercising ventilations and tidal volumes, with breathlessness and anxiety ratings correlated at anaerobic threshold for both groups.

A significant relationship was also seen between hypercapnic chemosensitivity of breathing anxiety ratings and maximal exercise breathing anxiety in sedentary participants. Additionally, breathlessness ratings at anaerobic threshold were inversely correlated with maximal exercise ventilation in the sedentary group. For the athletes, a significant inverse relationship was found between hypercapnic reactivity for tidal volume and heart rate at anaerobic threshold.

For both groups, anxiety and depression scores were closely correlated. Additionally, a relationship was observed between depression and ASI affective scores in the athlete group, which was not apparent in the sedentary group. The affective scores did not correlate strongly with any other exercise or hypercapnic measures, although several weaker relationships were observed with these measures (see **Figure 2**).

DISCUSSION

Main Findings

Hypercapnic chemosensitivity has typically been measured as the change in ventilation in response to a hypercapnic challenge (Peebles et al., 2007; Ogoh et al., 2008; Faull et al., 2016; Sackett et al., 2018). Here, we extended this to include the perceptual responsivity to hypercapnia using ratings of breathlessness and anxiety toward breathing. As only weak relationships were observed between ventilatory and perceptual responsivity parameters, these measures appeared to be largely independent. There were no differences in any of the measured hypercapnic chemosensitivity responses (ventilation, tidal volume, breathing rate, breathlessness and anxiety of breathing) between athletes and sedentary controls. However, different ventilatory strategies were found during the hypercapnic challenge, with athletes utilising larger tidal volumes and lower breathing rates during hypercapnia (adjusted for height differences between groups), despite no differences in overall ventilation. Athletes also recorded greater work rate and volume of oxygen consumption (VO_2) as expected, and correspondingly greater ventilation during sub-maximal and maximal exercise.

Additionally, the relationship between hypercapnic chemosensitivity and exercising ventilation and perceptions differed between groups. Sedentary individuals demonstrated a strong relationship between hypercapnic chemosensitivity of anxiety at rest and breathing anxiety during maximal exercise, while athletes demonstrated a strong inverse relationship where greater hypercapnic chemosensitivity of breathing rate was related to lower heart rate during exercise at anaerobic threshold. Finally, no hypercapnic nor exercising ventilatory parameters or perceptions were related to affective traits of anxiety, depression and anxiety sensitivity.

Hypercapnic Chemosensitivity and Exercise

Here we have shown that hypercapnic chemosensitivity appears to be related to exercising perceptions of anxiety in sedentary individuals. This means that those who have a greater anxiety response to hypercapnia also report greater perceptions of breathing anxiety when exercising at maximal intensity. As this relationship was not observed in athletes, it is possible that training may allow factors such as increased motor drive and conditioned responses (Turner et al., 1997; Turner and Sumners, 2002; Turner and Stewart, 2004; Dempsey and Smith, 2014) to override some of the effects of perceptual hypercapnic chemosensitivity during exercise. Conversely, in athletes, greater hypercapnic chemosensitivity of breathing rate was strongly related to lower heart rate during sub-maximal exercise. This possibly reflects a compensatory mechanism whereby smaller hypercapnia-stimulated changes in breathing rate can be accounted for by larger increases in heart rate during exercise, both of which can act to maintain arterial blood gas homeostasis (Meersman et al., 1995; Convertino, 2019).

Despite no differences in hypercapnic chemosensitivity measures between the groups, we did observe marked discrepancies in the ventilatory strategies employed during the hypercapnic challenge. During the ventilatory response to hypercapnia, tidal volume was greater and breathing rate lower in athletes, although ventilatory responses overall were similar between groups. These differences in ventilatory patterns remained after standardisation against height, and percentage of predicted values for FVC and FEV1 were also lower in sedentary individuals. While exercise training typically results in limited changes in lung capacity measures but improvements in measures of lung function (Dunham and Harms, 2012; Khosravi et al., 2013), these differences may be due to a combination of training and a self-selection bias, where individuals with better ventilatory capacity choose to participate in endurance sports (which improves lung function), resulting in greater tidal volumes and lower breathing rates during ventilation.

Finally, there were no relationships between hypercapnic chemosensitivity nor exercising parameters with trait measures of anxiety, depression or anxiety sensitivity. Notably, athletes demonstrated a strong relationship between depression and anxiety sensitivity (fear of anxiety symptoms) that was not present in sedentary individuals, while overall scores for both measures were similar between groups. This may be related to a greater awareness and anticipation of body symptoms in athletes (Faull et al., 2018), although further research is required to understand the effects of exercise training on perception of anxiety symptoms in the body.

Limitations

This study is a supplementary analysis of previously published work and is exploratory in nature. A number of limitations must be addressed in further work in this area, beginning with testing participants in consistent postures across exercise and hypercapnic chemosensitivity measures. Here, participants underwent the hypercapnic challenge while supine (for ease of for ease of use of the custom-built breathing system designed to deliver hypercapnic stimuli), while exercise was undertaken seated on a bicycle ergometer. Postural differences are known to affect lung function measures (Allen et al., 1985), and thus these differences may have confounded the results in the current study.

Secondly, limited physiological data were available in this study. Future work may look to incorporate measures of blood lactate, blood pressure and/or oxygen saturation measures to better understand the physiological and perceptual responses to hypercapnic stimuli in athletes and sedentary controls. Additionally, female participants were not tested in the same part of their menstrual phase, likely adding variability to the physiological and perceptual responses recorded in this dataset.

Finally, the correlations reported here cannot be assumed to infer causation. The results of this study can only provide us with an overview as to the possible relationships between hypercapnic chemosensitivity and exercising physiology and perceptions. Further research is required using perturbations (such as hypercapnic and hypoxic stimuli *during* exercise), such that the influence of hypercapnic chemosensitivity directly on exercising parameters can be inferred.

CONCLUSION

Hypercapnic chemosensitivity does not appear to be altered in athletes compared to sedentary individuals, either in the ventilatory or perceptual domains. Multiple relationships exist between hypercapnic chemosensitivity and exercising ventilation/perceptions in sedentary individuals but not athletes, which may be due to exercise training or self-selection biases. Sedentary individuals may use both ventilatory and perceptual responses to hypercapnia to constrain their exercising performance, while athletes may override these signals using factors such as goal-directed increases in motor output.

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 Oxfordshire Clinical Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

OH and KP contributed to conception and design of the study. OH collected the data and performed the statistical analyses. OH wrote the first draft of the manuscript, with advice from KP and BR. All authors contributed to manuscript revision, read, and approved the submitted version.

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Breath Tools: A Synthesis of Evidence-Based Breathing Strategies to Enhance Human Running

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Running is among the most popular sporting hobbies and often chosen specifically for intrinsic psychological benefits. However, up to 40% of runners may experience exercise-induced dyspnoea as a result of cascading physiological phenomena, possibly causing negative psychological states or barriers to participation. Breathing techniques such as slow, deep breathing have proven benefits at rest, but it is unclear if they can be used during exercise to address respiratory limitations or improve performance. While direct experimental evidence is limited, diverse findings from exercise physiology and sports science combined with anecdotal knowledge from Yoga, meditation, and breathwork suggest that many aspects of breathing could be improved *via* purposeful strategies. Hence, we sought to synthesize these disparate sources to create a new theoretical framework called "Breath Tools" proposing breathing strategies for use during running to improve tolerance, performance, and lower barriers to long-term enjoyment.

Keywords: breathing pattern, coupling, running, techniques, strategies, respiration, ventilation

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INTRODUCTION

Breathing is natural and automatic, sustaining life by the simple movement of air. Despite the apparent simplicity of this process, the understanding of breathing has recently been advanced extensively through investigations in medicine, sports science, and psychophysiology. The recent SARS-COVID-19 global epidemic has reminded many of the significance of breathing and the consequences of respiratory distress.

Several recent studies have brought renewed attention to the anthropological roots of breathing and its effect upon overall well-being. Yogic techniques have for millenia utilized breath awareness and exercises to cultivate "prana" (meaning both "breath" and "life force" in Sanskrit), while meditation, breathwork practices, and freediving also take advantage of breathing techniques for calm, focus, and performance. Resonant frequency breathing performed in heart-rate variability (HRV) biofeedback has significant positive effects upon HRV itself, overall autonomic nervous system regulation, and related emotional states such as anxiety and depression (Lehrer et al., 2020). Performing these breathing techniques at rest has additive effects upon cognitive function, decision-making, and concentration in sport (Jimenez Morgan and Molina Mora, 2017; De Couck et al., 2019). These effects are extremely valuable in sports contexts where both mental and physical performance affect positive psychological states such as perceived efficacy and enjoyment (Ogles et al., 1995). Although slow breathing is demonstrably efficacious at rest, the utility of slow breathing during exercise is understudied.

Recent reviews have explored the various mechanisms that may cause breathing to limit physical performance during exercise (Amann, 2012; Dempsey et al., 2020), but little work has attempted to address these mechanisms or improve breathing directly during exercise. Although running is both one of the most popular (Statista, 2018) and well-studied physical activities, very few studies have directly investigated the use of breathing techniques during running as done during Yoga, meditation, and cycling (Vickery, 2008; Saoji et al., 2019). Running deserves special attention not only for its immense global popularity but also because runners are driven by a complex mix of psychological and emotional motives (Ogles et al., 1995; Pereira et al., 2021). Since breathing can heavily affect the psychological perception of exercise (Laviolette and Laveneziana, 2014), improving breathing during running may influence tolerance or psychological state during activity. Considering the popularity of running and the diverse benefits of breathing strategies in other contexts, we sought to synthesize the available evidence to demonstrate how breathing could be used to ease respiratory distress and improve running performance and psychological states.

This narrative synthesis has three main goals:

1. Provide an updated overview of exercise breathing pattern, and identify respiratory limitations to running
2. Define and describe breathing strategies that provide physical and mental benefits for runners
3. Discuss practical applications and recommendations for future studies of breathing techniques during running.

RESPIRATION DURING EXERCISE

The onset of exercise stimulates rapid, characteristic changes in ventilation (V_E) over 20 times greater than that at rest (hyperpnoea). The increase from an average 6 L/min up to 150 L/min occurs in response to various metabolic, homeostatic, and peripheral stimuli (**Figure 1**). While not completely understood, humans' precise control of exercise hyperpnoea is driven by multiple redundant control mechanisms, such as biochemical feedback loops [especially by the partial pressure

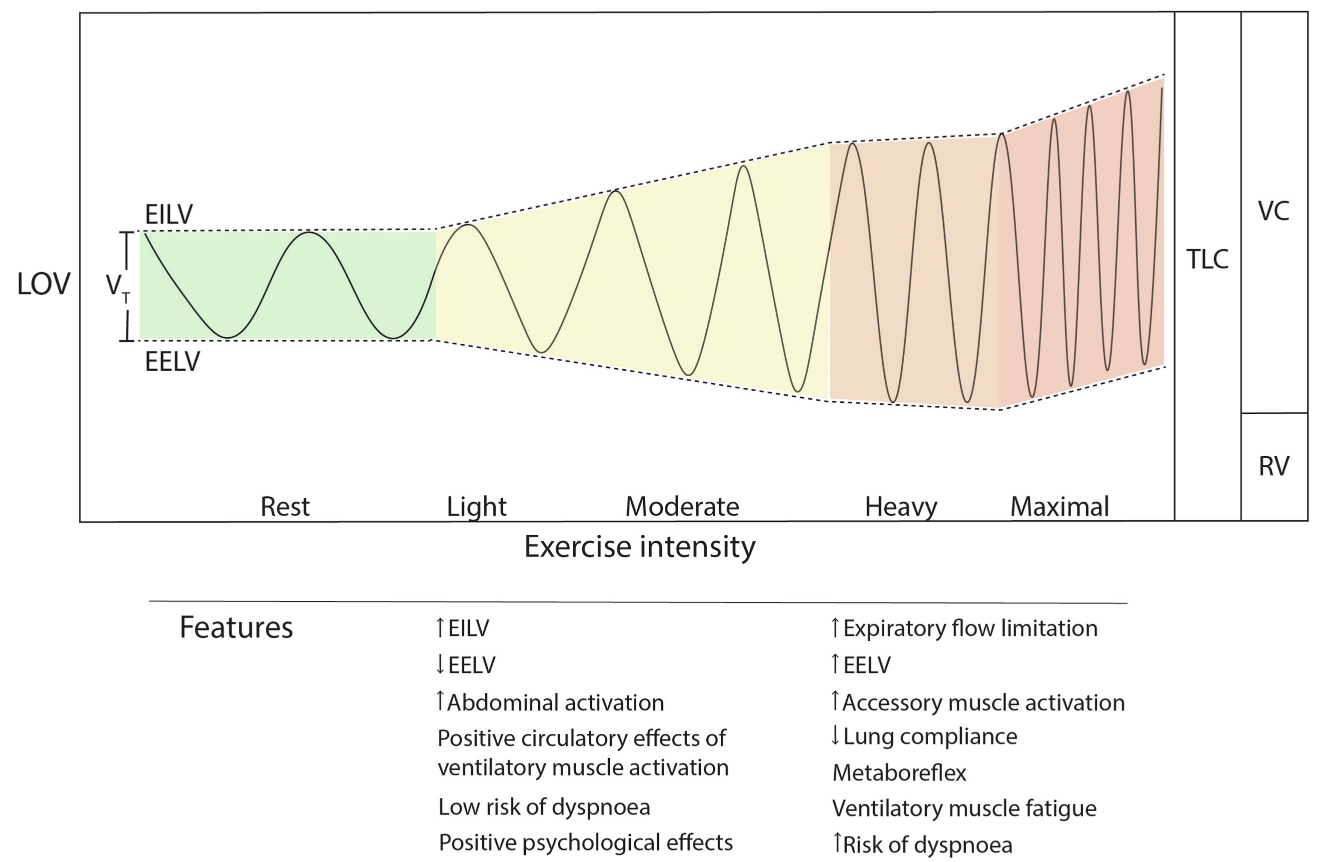

FIGURE 1 | Exercise breathing pattern changes during increasing exercise intensity. Note the nonlinear increase in breathing rate and unequal partitioning of EILV and EELV as intensity increases. TLC, total lung capacity; LOV, lung operating volume; VC, vital capacity; RV, residual volume; EILV, end-inspiratory lung volume; EELV, end-expiratory lung volume; and V_T, tidal volume.

of carbon dioxide (pCO_2) and blood pH], central command (neural feed-forward), and peripheral afferent feedback from the working limbs (Forster et al., 2012). During steady-state exercise, the healthy respiratory system precisely tunes V_E to match metabolic rate and maintain equal O_2 and CO_2 balance at every level of the system (Ferretti et al., 2017). Above the respiratory compensation point (RCP; a.k.a., second ventilatory threshold), V_E increases nonlinearly beyond the increase in CO_2 consumption (VCO_2). Ventilatory change points are trait- and state-dependent, continuously adjusting to factors like anaerobic energy utilization, blood buffering, and metabolic acidosis. Indeed, the respiratory system is remarkable in responding "just right" to exercise in most scenarios, efficiently managing V_E proportional to CO_2 production (VCO_2). Exercise V_E increases linearly ($r^2 = 0.99$) with inspiratory drive (V_T/T_I; measured as mean inspiratory flow), reflecting increased neural drive to the inspiratory musculature (Naranjo et al., 2005). This is achieved *via* patterns of breathing rate (BR), depth, and coordinated muscle activity that maximize O_2 perfusion and minimize the metabolic work of breathing (WOB; Dempsey et al., 2020). Nevertheless, there is considerable individual and situational variance in breathing pattern (BP) response to V_E demands (Naranjo et al., 2005; Gravier et al., 2013).

Exercise Breathing Pattern

During exercise, it is thought that individuals intuitively select the BP that minimizes the metabolic cost of V_E (Mead, 1960; Benchetrit, 2000; Welch et al., 2019). This is termed the "principle of minimal effort." The popular definition of BP includes an inhale, an exhale, and a pause (**Table 1**). It is primarily determined by four principal variables: inspiratory flow profile (rate of airflow during inhale), inspiratory duration (T_I), expiratory flow profile, and expiratory duration (T_E; Tipton et al., 2017). These variables determine the typical BP descriptors of BR ($60{*}T_B^{-1}$, where $T_B = T_E + T_I$) and depth (tidal volume; V_T). Duty cycle (dc or breath ratio; measured as T_I/T_B) constrains flow to determine BP timing, depth, and airway (nose vs. mouth; Naranjo et al., 2005). These components are regulated by multiple overlapping control mechanisms, leading to a variety of coordinated patterns to achieve respiratory homeostasis. Unlike other physiological processes, BP can be consciously altered, for example to be faster or slower. Although sometime conscious, it is largely unconscious, with bidirectionality between physiological and psychological mechanisms; this qualifies BP as a "psychophysiological" construct. While V_E is ultimately the product of BR and V_T (Equation 1), these determinants adjust differently to various regulatory mechanisms, as do related subcomponents of BP such as timing, coordination, and coupling, discussed below (**Table 1**).

$$\dot{V}_E = BR * V_T$$

Exercise BP is modulated by central and peripheral neural mechanisms, chemoreflex stimulation, attention, and emotions, and biomechanical rhythms, among other factors. While V_E increase necessitates elevated breathing rate (BR, a.k.a. respiratory frequency) and/or V_T, their increases are independently regulated. Recent work indicates that during exercise BR is more "behavioral" and primarily driven by central command (activity in motor and premotor areas of the brain) and muscle afferents (Amann et al., 2010; Nicolo et al., 2016, 2017a, 2018). BR and effort are closely correlated across many different exercise intensities and experimental conditions (Nicolò et al., 2020b) because perceived exertion is likely signaled by the magnitude of central command outflow (Marcora, 2009). At submaximal intensities, BR is also affected by cognitive load, emotions, environmental stress, and exercise rhythm (more on this below; Homma and Masaoka, 2008; Grassmann et al., 2016; Tipton et al., 2017). BR is acutely responsive, adjusting almost immediately to abrupt changes in exercise intensity and stress, such as anticipatory anxiety, pain, and cold exposure (Masaoka and Homma, 2001; Tipton et al., 2017). At high relative intensities above the RCP, continued increases in V_E are accomplished primarily *via* BR (tachypnoea; fast BR above ~80% peak BR); this point is termed the "tachypnoeic shift" (Sheel and Romer, 2011). The correlation between BR and perceived effort is particularly strong at these intensities as BR adjusts independent of absolute workload, metabolism, or muscular activation (Nicolò et al., 2018; Cochrane-Snyman et al., 2019). This may be due to increased levels of central command activity compared with low or moderate-intensity exercise (Nicolò et al., 2020a). At maximal exertion, peak BR varies substantially between individuals from 35 to 70 breaths per minute (bpm; Blackie et al., 1991; Naranjo et al., 2005).

While BR responds quickly to "fast" inputs, evidence suggests that during exercise V_T adjusts slowly to optimally match alveolvar V_E to VCO_2 (Nicolò et al., 2017a, 2018). In their "unbalanced interdependence" model, Nicolo and Sacchetti (2019) propose that V_T is secondarily regulated on the basis of BR to maintain biochemical homeostasis. This differential control likely extends across most exercise intensities (Nicolo et al., 2020). Studies report diverse responses of V_T to increasing exercise intensity; in untrained exercisers, V_T tends to increase until either the first or second ventilatory threshold, after which it either plateaus or declines (Gravier et al., 2013). While this plateau generally coincides with the tachypnoeic shift, some elite athletes appear to increase V_T beyond the RCP and up to total exhaustion (Lucía et al., 1999). Generally, V_T peaks around 50%–60% (1.9–2.7 L) of vital capacity (VC; total amount of air exhaled after maximal inspiration; Blackie et al., 1991), although in some untrained persons as low as 35% (Gravier et al., 2013) and elite athletes as high as 70% VC (Lucía et al., 1999).

The tachypnoeic shift typical of the RCP cannot entirely explain V_T plateau during normal exercise conditions, since the plateau occurs before the RCP in some individuals (Gravier et al., 2013), and not at all in others (Lucía et al., 1999). Lung mechanoreceptor feedback may explain some of these disparities. V_T limits are likely governed by the principle of minimal effort, as vagally-mediated afferent feedback from pulmonary stretch receptors regulates lung operating volumes [LOV; relative to end-inspiratory (EILV) and end-expiratory volume (EELV)] to minimize the WOB (Breuer, 1868; Hering, 1868; Clark and von Euler, 1972; Sheel and Romer, 2011).

TABLE 1 | List of breathing pattern components and common abbreviations.

Abbreviation	Variable (units)	Definition
BP	Breathing pattern	Differential trait and state-dependent control of breathing rhythm and mechanics
BR	Breathing rate (bpm)	Respiratory frequency; number of breaths taken per minute
Dc	Duty cycle, breath ratio (%)	Breath timing; relative percentage of inhale time to the complete breath cycle (T_I/T_B in %)
EELV	End-expiratory lung volume	Volume of the lungs at the end of an expiration
EID	Exercise-induced dyspnoea	Excessive perceived breathlessness during activity
EILV	End-inspiratory lung volume	Volume of the lungs at the end of an inspiration
FR	Flow reversal	Instant of breath switching; e.g., from inhale to exhale or exhale to inhale
LOV	Lung operating volume (%)	Mean diaphragm position at a given tidal volume (mean of EELV + EILV as % of TLC)
LRC	Locomotor-respiratory coupling (steps:breath)	Synchronization between flow reversal and movement; e.g., running footstrike
RV	Reserve volume (l)	Amount of air that remaining in airway and lungs after maximal expiration
T_B	Breath cycle time (s)	Total breath time from inspiration to next inspiration ($T_I + T_E$)
T_E	Exhale time (s)	Exhale duration during one breath cycle
T_I	Inhale time (s)	Inhale duration during one breath cycle
TLC	Total lung capacity (l)	Total amount of air present in lungs after maximal inspiration
TLD	Thoraco-lumbar depth (%)	Ratio of thorax to abdominal expansion contributing to total tidal volume
VC	Vital capacity (l)	Total amount of air exhaled after maximal inspiration (TLC-RV)
V_T	Tidal volume (l), depth	Breathing depth; total amount of air inspired during one breath cycle
V_D	Ventilatory dead space (l)	Sum of airway volumes which do not contribute to gas exchange
V_E	Minute ventilation (L/min)	Quantity of air breathed per minute
VO_2	Oxygen consumption (L/min)	Oxygen consumption; difference between oxygen inspired and oxygen expired in a unit of time
WOB	Work of breathing	Metabolic energy demand of ventilation
-	Thoraco-lumbar coordination (s)	Breathing coordination; time lag between thoracic and abdominal flow reversal
-	Ventilatory drive (l/s)	Total output of ventilatory pump; mean inspiratory flow rate (V_T/T_I)
-	Ventilatory efficiency	Ventilatory pump response to increasing demands, frequently measured as V_E/VCO_2 slope

These mechanical limitations may interact with pCO_2, which is known to suppress pulmonary stretch receptor outflow (Schelegle and Green, 2001). Clark et al. (1980) observed this phenomenon with progressive levels of hypercapnia during incremental exercise increasing V_T peak. Nevertheless, despite higher relative V_T peak, CO_2 levels are similar or reduced in elite athletes vs. untrained persons at equivalent absolute work rates (Lucía et al., 1999). Hence, some of the mechanisms that affect the V_T plateau and tachypnoeic shift during exercise are not yet entirely clear. Despite large inter-individual differences in relative V_T peak, it is unclear if this is a fixed characteristic of BP; fitness level and training appear to have no effect on the V_T plateau or the VE/VCO_2 relationship (Salazar-Martinez et al., 2016, 2018). It is believed that the attainment of V_T peak is the only circumstance at which V_T substantially affects BR (Sheel and Romer, 2011; Nicolò et al., 2018).

While the tachypnoeic shift is an adaptive, essential response to maintain respiratory homeostasis at high relative exercise intensities, it coincides with increased WOB, decreased ventilatory efficiency, and peripheral fatigue (Naranjo et al., 2005; Ward, 2007; Gravier et al., 2013). Although exercise below the RCP triggers near-universal positive affect, there are homogenously negative psychological changes above the RCP (Ekkekakis et al., 2011). This may be explained by the close correlation ($r = 0.71$) between tachypnoea and dyspnoea during incremental exercise (Tsukada et al., 2017). While the mechanisms causing dyspnoea are complex and varied (Sheel et al., 2011), recent studies suggest that the psychological "unpleasantness" dimension of dypsnoea at its onset may contribute substantially to the near-simultaneous presentation of tachypnoea (Izumizaki et al., 2011; Tsukada et al., 2017).

Humans generally switch airway from the nose to mouth as V_E increases above 40 L/min (Saibene et al., 1978). Duty cycle (dc; T_I/T_B) increases from resting values from about 40% (slightly longer exhale than inhale) to 50% (equal inhale to exhale) or greater at maximal intensity (Naranjo et al., 2005; Kift and Williams, 2007; Salazar-Martinez et al., 2018). Shorter T_E vs. T_I implies that mean expiratory flow rate must exceed mean inspiratory flow rate (rate of airflow during breath phase) in order to maintain constant LOV.

Exercise-induced V_E and drive increases trigger altered ventilatory pump musculature activity and coordination. From rest to 70% max workload, diaphragmatic pressure increases more than twofold, accompanied by an increased velocity of shortening, which contributes 70%–80% of the total inspiratory force (Wallden, 2017). As exercise intensity increases, active exhales (expiratory muscle activation) lower the inspiratory WOB by reducing end-expiratory lung volume, modulating lung compliance, and storing elastic energy in the ventilatory pump musculature (Aliverti, 2016). The primary expiratory muscles are the internal obliques, which may reach 50% maximum voluntary contraction at maximal intensity (Ito et al., 2016). The intercostals, parasternals, scalenes, and neck muscles contribute to ventilation at high intensities by moderating EILV and airway caliber (e.g., dilation and inflammation). Altogether, the diaphragm and associated ventilatory pump musculature are remarkably efficient [~3%–5% total O_2 consumption (VO_2)] and fatigue-resistant at submaximal intensities (Welch et al., 2019; Sheel et al., 2020).

Locomotor-Respiratory Coupling

Humans are among a large proportion of animals that entrain BR to movement. The synchronization of locomotion to breath is termed "locomotor-respiratory coupling" (LRC), and involves a dual-synchronization not only of frequency [e.g., BR = step rate (SR)] but also event phase (e.g., footstrike synchronized with breath onset; O'Halloran et al., 2012). While most quadrupedal mammals utilize a 1:1 phase-locked locomotion-to-breath ratio while running due to mechanical constraints of the thorax, humans' upright gait permits BR adjustment independent of locomotion (Bramble and Carrier, 1983). Although humans lack this mechnical constraint on breathing, they have been observed performing LRC during several rhythmic activities, such as walking, running, cycling, rowing, cross-country skiing, and even finger-tapping (Bechbache and Duffin, 1977; Persegol et al., 1991; Fabre et al., 2007; Bjorklund et al., 2015; Mathias et al., 2020). Swimming is a prime example of phase-locked breathing, as swimmers inhale during specific phases of the stroke when the face is not underwater.

Despite an apparent freedom from quadrupedal thorax constraints on breathing, LRC in humans is likely affected by various biomechanical phenomena specific to running. The "visceral piston" (three-dimensional displacement of the abdominal mass during locomotion) affects diaphragmatic contraction *via* direct ligamentous connections (Daley et al., 2013). Axial-appendicular dynamics have the potential to positively or negatively affect V_T depending on the phasic relationship to inhale and exhale (Bramble, 1989). The effect of footstrike timing and impact forces upon V_T is termed "step-driven flows," and may affect V_E up to 10%–12% (Daley et al., 2013). This could be detrimental when the timing of footstrike is out of phase (unsynchronized) with breath onset (flow reversal; FR) but additive when in-phase (synchronized). When the inhale is synchronized with peak visceral downward velocity, it pulls on the diaphragm, increasing the velocity of shortening. Daley et al. (2013) found that runners naturally prefer LRC with phase synchronziation at additive (flow-enhancing) phases, and that ventilatory transitions (change from inspiration to expiration) were quicker in these conditions of LRC. They concluded that the visceral piston and rhythmic arm movement substantially affect step-driven flows and LRC has a physiologically significant mechanical effect on breathing dynamics. These findings suggest that LRC is a result of the "minimal effort" hypothesis of breathing. If LRC reduces the WOB, it may contribute to a delayed onset of ventilatory muscle fatigue, especially at high exercise intensities, long exercise durations, or in special populations predisposed to respiratory distress (discussed below; Daley et al., 2013).

Locomotor-respiratory coupling is likely modulated by an interaction of mechanical, neurological, and metabolic interactions during running. Recent work indicates that LRC in humans is probably neurophysiological in origin, as there is a direct neurological link in humans between the respiratory and locomotor central pattern generators in the spinal cord (Le Gal et al., 2014; Del Negro et al., 2018). Group III and IV afferent feedback from the working limbs appears to affect LRC, since activities with higher-frequency limb movement produce higher levels of entrainment (Bechbache and Duffin, 1977; Caterini et al., 2016). However, close associations between limb movement, BR, and pCO_2 suggest that chemoreflexive feedback affects the strength of entrainment (Forster et al., 2012). Cyclical, high-frequency activities such as running are more likely to induce entrainment vs., for example, walking, and LRC is most likely to occur at higher intensities near VO_{2max} (maximal oxygen uptake; Bechbache and Duffin, 1977; Bernasconi and Kohl, 1993). Notably, these studies reported that increases in velocity of movement affected the strength of LRC more than intensity increase *via* load or gradient. There appears to be an influence of training history upon entrainment, where task preference and experience are positively associated with LRC onset and strength (Kohl et al., 1981; Bramble and Carrier, 1983; Stickford et al., 2020). These relationships were independent of overall fitness, so sport-specific experience may coincide with LRC as a learned skill (perhaps unconsciously). Finally, studies utilizing metronomes to instruct movement appeared to quickly and strongly influence LRC (Bechbache and Duffin, 1977; Bernasconi et al., 1995). Entrainment is likely to occur spontaneously and consistently in the presence of some or all of the above conditions.

Respiration as a Limiting Factor

The respiratory system in healthy individuals is considered to be generally well-adapted for the demands of exercise (Amann, 2012; Dempsey et al., 2020). Nevertheless, accumulating evidence strongly suggests that the respiratory system is "underbuilt" for the demands of intense exercise. At exercise around or above 80%–85% VO_{2max}, three primary mechanisms cause the respiratory system to limit performance: exercise-induced arterial oxyhemoglobin desaturation, excessive ventilatory muscle work, and intrathoracic pressure effects on cardiac output (Amann, 2012). Specific scenarios (e.g., hypoxia and cold/dry climates) expose respiratory system vulnerabilities at submaximal intensities, and certain populations (e.g., elite athletes, females, and elderly) are especially susceptible; these phenomena have been recently detailed in extensive reviews (Dempsey et al., 2020; Archiza et al., 2021). While the exact limiting mechanisms differ (structural or functional), these situations and individuals bring the respiratory system close to its physiological limits. However, physiological limits do not fully explain the prevalence of exercise-induced breathlessness (a.k.a dyspnoea; EID).

An estimated 20%–40% of otherwise healthy runners experience EID even at low absolute exercise intensities (Johansson et al., 2015; Smoliga et al., 2016; Ersson et al., 2020). This could be because unfit or deconditioned individuals may approach high levels of exertion and experience limb fatigue at low absolute workloads (Abu-Hasan et al., 2005). It could also be related to mouth breathing, since mouth-only breathing at submaximal intensities causes airway irritation, and possibly subsequent exercise-induced laryngeal obstruction (EILO; Mangla and Menon, 1981; Johansson et al., 2015). While the majority of EID prevalence may be explained by physiological limitations and deconditioning, the other most likely cause is dysfunctional breathing (Depiazzi and Everard, 2016). Distinct from pathology, dysfunctional breathing (DB; suboptimal BP) can cause otherwise healthy runners to experience premature onset of fatigue and subsequent EID (Boulding et al., 2016). Depiazzi and Everard (2016) submit that any BP deviating

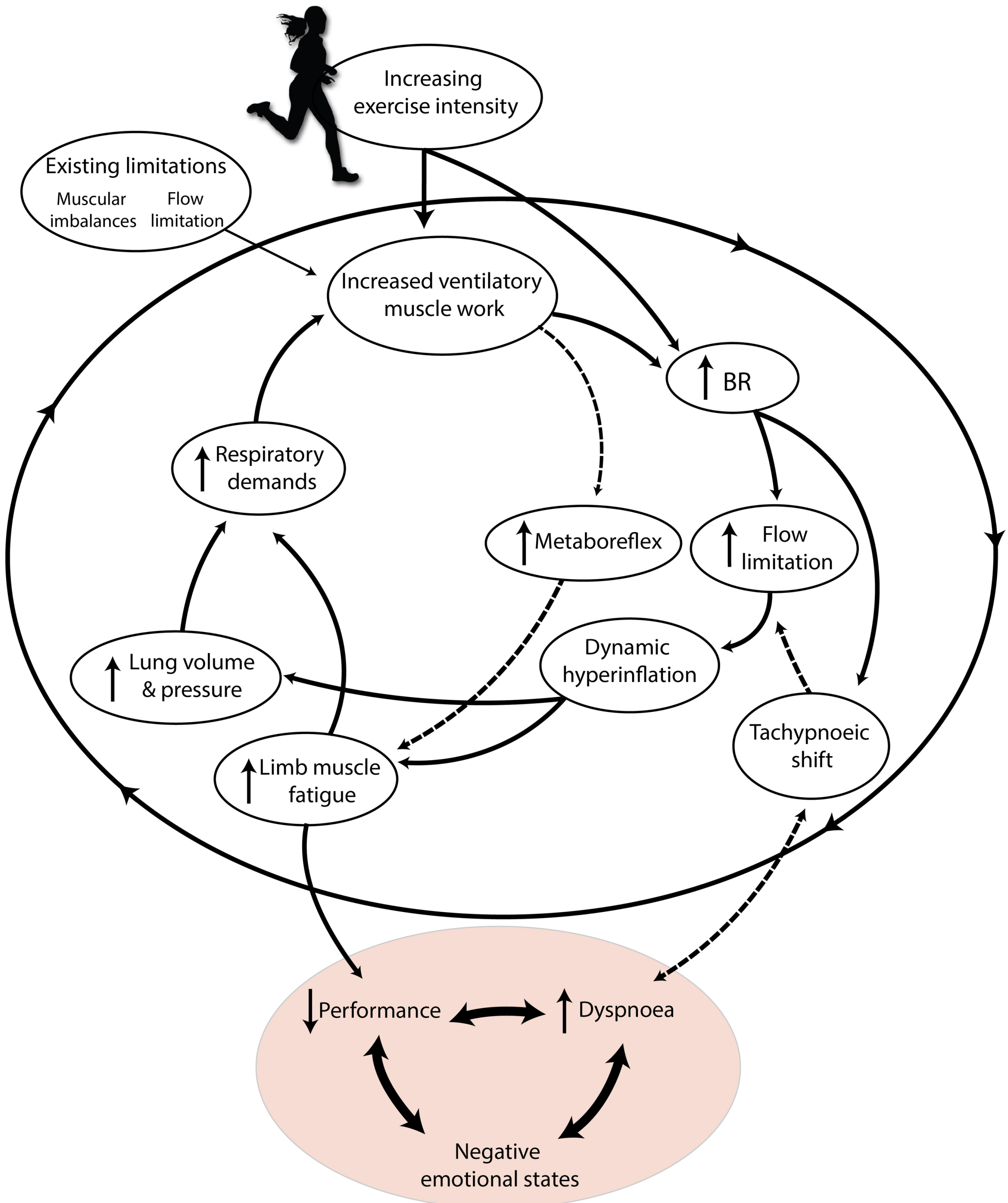

FIGURE 2 | The "respiratory limiting cycle" cascade of phenomena leading to respiration limiting exercise performance and enjoyment. Increasing exercise intensity interacts with pre-existing individual constraints, causing an accumulation of respiratory phenomena that ultimately harm performance and cause dyspnoea. Dashed arrows indicate mechanisms specific to high relative exercise intensities. Adapted with permission from BradCliff® and Bradley and Clifton-Smith (2009).

from slow, coordinated, diaphragmatic breathing has the potential to be "dysfunctional." Chronic stress (internal or external) or negative emotional states could cause habitual DB during exercise (Homma and Masaoka, 2008; Tipton et al., 2017). Whether caused by physiological or psychological limits, fatigue and EID could contribute to cessation of exercise, increased rating of perceived exertion (RPE), or negative emotional states (**Figure 2**; Bradley and Clifton-Smith, 2009; Weinberger and Abu-Hasan, 2009). Hence, here we aim to identify three important shared phenomena that lead to respiration limiting exercise performance, tolerance, and enjoyment: dynamic hyperinflation, blood stealing, and hyperventilation.

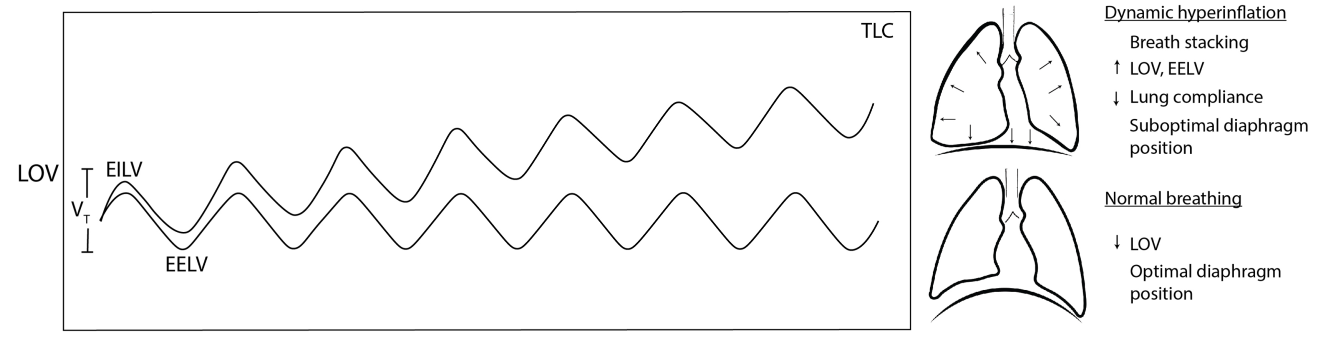

FIGURE 3 | Dynamic hyperinflation occurs when accumulated breath stacking progressively increases lung operating volume. When lung operating volume approaches total lung capacity, lung stiffness, and suboptimal diaphragm position increase the work of breathing (WOB) and dyspnoea. TLC, total lung capacity; LOV, lung operating volume; EILV, end-inspiratory lung volume; EELV, end-expiratory lung volume; and V_T, tidal volume.

Exercise BP may fail to provide the "just right" response in the presence of flow limitation. During high intensity exercise, large increases in ventilatory flow may cause narrowing of the airway related to the Venturi effect and Bernoulli principle, among other constraints. This is termed "exercise-induced largyngeal obstruction," and it is particularly common in elite athletes who generate large V_E at high intensities (Smoliga et al., 2016). Up to 20% of elite athletes, females, adolescents, and overweight individuals may experience this during low-intensity activity (Smith et al., 2017; Dempsey et al., 2020; Ersson et al., 2020). DB phenotypes including upper-thoracic-dominant breathing and core muscle hypertonicity (such as in low back pain compensation) are also risk factors (Chaitow et al., 2014). Flow limitation could lead to "breath stacking," a negative consequence when subsequent breaths have slightly larger inspiratory than expiratory flow (Ward, 2007). Breath stacking causes EILV and EELV to progressively increase, leading to dynamic hyperinflation (**Figure 3**; Sheel et al., 2020). At these higher LOV, the lungs are stiffer, less compliant, and require more muscle work to expand (Sheel et al., 2020). Unfortunately, dynamic hyperinflation places the diaphragm in a suboptimal length for expanding the lungs and managing intrathoracic pressures, further fatiguing the ventilatory musculature (Aliverti, 2016).

Diaphragmatic breathing positively affects blood shifting between the trunk and the extremities during exercise (Aliverti et al., 2010). However, during heavy exercise above ~80% peak work rate, increasing intra-thoracic pressure acts like a Valsalva maneuver, decreasing stroke volume and cardiac output (Aliverti, 2016). Furthermore, at sustained high intensities the diaphragm fatigues; it demands up to 14%–20% of cardiac output and 10%–16% of VO_2 on top of concurrent accessory and expiratory muscle fatigue (Welch et al., 2019). Ventilatory muscle fatigue at high intensities triggers the metaboreflex, which ensures that the ventilatory pump maintains adequate perfusion by shunting blood from the working muscles *via* sympathetically-mediated vasocontriction. This competition for oxygen-rich blood is termed "blood stealing"; a detailed review is available elsewhere (Sheel et al., 2018). Although its negative haemodynamic effects generally only occur above 85% VO_{2max}, its relationship to BP is unclear. Since the tachypnoeic shift and dynamic inflation associated with heavy exercise also elevate the WOB, it is likely that they contribute to blood stealing (Amann, 2012).

Exercise hyperpnoea is usually a "just right" respiratory response to maintaining biochemical homeostasis with increasing intensity (Dempsey et al., 2020). However, high BR may be psychologically disadvantageous, since the tachypnoeic shift onset is closely associated with EID (Izumizaki et al., 2011; Tsukada et al., 2017). Some runners may experience tachypnoea and associated dyspnoea prematurely. Healthy adult females, for instance, have hormonally-determined lung and airway limitations that predispose them to higher average BR, lower V_T, and increased risk of EID (Itoh et al., 2007; Dempsey et al., 2020). During running, V_T is constrained more than in other activities, and the tachypnoeic shift occurs relatively earlier (Elliott and Grace, 2010; Marko, 2020). This limitation is partially attributed to competing demands for postural and ventilatory function upon the diaphragm as well as step-driven flows (Chaitow et al., 2014; Stickford and Stickford, 2014). Another factor could be surface inclination; Bernardi et al. (2017) demonstrated that gradients above 20%–30% decrease thoraco-lumbar coordination ($r = 0.99$) and subsequent ventilatory efficiency ($r = -0.265$). Subsequently, this harmed BP (lower V_T, increased BR), oxygen saturation, and performance. Since these effects were independent of absolute altitude and fatigue, they concluded that this was due to trunk inclination limiting ribcage expansion.

If the tachypnoeic response is early, or inappropriately dramatic, such as in hyperventilation DB, hypocapnia could reduce peripheral muscle perfusion *via* the Bohr effect (Depiazzi and Everard, 2016). This may accelerate blood stealing and limb muscle fatigue. Additionally, lower pCO_2 is associated with earlier V_T peak (Clark et al., 1980), which could lead to accelerated increases in BR to increase V_E. Hyperventilation is accompanied by increased flow rates, which could lead to airway narrowing and flow limitation (Dempsey et al., 2020).

Hyperventilation, hyperinflation, and blood stealing might together form a negative feedback loop if unchecked. If this cycle is not addressed, it could lead to EID, impaired performance, or negative emotional affect (Bradley and Clifton-Smith, 2009; Chaitow et al., 2014). If these phenomena could be avoided, we theorize that individuals could benefit from enhanced performance, reduced perception of fatigue, or prevention of negative psychological states (**Figure 2**).

A related, but intensity-independent aspect of respiratory limitations almost unique to running is exercise-related transient abdominal pain (ETAP), also known as "side stitch." First mentioned by Pliny the Elder, there is still no consensus on the exact etiology of this unpleasant phenomenon (Morton and Callister, 2015). Unfortunately, this unpleasant, painful experience affects up to 70% of runners per year, which is at best frustrating and at worst a reason for exercise cessation. Some experts believe that phrenic nerve irritation related to repeated right footstrike and exhalation synchronization might be the cause (Coates and Kowalchik, 2013). Specifically, irritation of the parietal peritoneum is the most likely cause of ETAP, especially during running and in the right lower quadrant (Morton and Callister, 2015). It could be that LRC at even ratios (such as 2:1 strides per breath), leading to ipsilateral footstrike on expiration, is actually a risk factor for developing side stitch in runners.

Breathing Patterns Can Be Modified and Improved

While breathing usually provides the "just right" response to the physiological demands of exercise, respiratory limitations can lead to negative performance or psychological outcomes. If BP can be "improved" to prevent or delay the onset of dyspnoea, or to increase ventilatory efficiency, then it can benefit not only exercise performance but also the psychological effects of exercise. Although acute BP modification and longer-term breathing "retraining" have well-established benefits for human health (Zaccaro et al., 2018; Lehrer et al., 2020), this field has only recently gained attention in exercise science. A recent review addressed this disparity by exploring the utility of breath retraining for respiratory-limited athletes (Allado et al., 2021); they found that several targeted techniques (e.g., Olin EILOBI breathing) can improve symptoms of flow limitation. Several studies have utilized principles of breath retraining at rest (e.g., slow diaphragmatic breathing) to demonstrate increased exercise performance (Jimenez Morgan and Molina Mora, 2017; Bahensky et al., 2021), but it is unclear if these can be implemented during exercise, and what psychological benefits result. Whether modifying BP is possible without compromising the "minimal effort" homeostasis of the respiratory system requires discussion and more direct study. In fact, one study examining the effects of internal attentional focus upon breathing reported no overall benefit for movement economy, despite positive effects upon V_E, respiratory quotient, and heart rate (Schucker et al., 2014). Nonetheless, it is known that breathing techniques improve positive emotion (Zaccaro et al., 2018), and that positive emotions can increase running economy (Brick et al., 2018), so some accommodation might unlock such performance benefits. In fact, doing so during running might be the most specific application to maximize adaptations. Despite a lack of direct evidence, theoretical and experimental findings from fields, such as cycling, respiratory medicine, and Yoga indicate various limiting mechanisms that can be addressed with breathing techniques. We hypothesize that breathing strategies employed during running could improve performance, attenuate EID, or enhance psychological states.

BREATH TOOLS

Renewed attention to breathing techniques has inspired substantial scientific scrutiny and interest among practioners, but to our knowledge, no one has yet attempted to summarize breathing strategies for exercise in an evidence-based, organized manner. Thus, the following section is a description of techniques and "breath tools" with potential benefits to the runner. Each tool is described with an acknowledgement of some historical and anecdotal perspectives as well as a synthesis of its benefits for running biomechanics, biochemistry, and psychophysiology. The "advanced" tools are slightly different, as they increase respiratory stress to catalyze positive adaptations *via* training. We summarize these strategies in roughly ascending order of benefit, complexity, and risk.

Rate

Humans have long known the value of slower BR. While religious ceremonies, Yoga, and meditation rituals have explored the practice for thousands of years, recent work has confirmed the value of slow breathing for biochemical and psychophysiological benefits at rest (Russo et al., 2017; Zaccaro et al., 2018). Although evidence suggests that sustained high BR during activity could lead to respiratory limitations (see "Respiration as a Limiting Factor"), reduced BR has been understudied as a standalone breathing strategy during running.

Slow BR may reduce the work of accessory respiratory muscles and subsequent WOB during exercise (Chaitow et al., 2014; Welch et al., 2019). Perhaps, the most simple advantage is improved gas exchange. Since BR is inversely proportional to V_T at a given V_E (Equation 1), slower breathing implicity induces greater depth of breathing (**Figure 4**). Since there is a fixed anatomic dead space (V_D; average 150 ml of airway segments that do not participate in gas exchange), increases in V_E *via* V_T (instead of BR) beneficially manipulate relative V_D (V_D:V_T ratio). For example, in conditions of isoventilation (Equation 1), a BR increase from 20 to 40 bpm at 10 L/min V_E causes an increase of 30% V_D relative to V_T, and a reduction of 300 ml in alveolar ventilation (**Table 2**). In contrast, increasing V_E to 20 L/min by only increasing V_T (to 1.0 L) has the effect of halved relative V_D (15%) and a 21% increase in alveolar ventilation. Simplistically, increasing V_E *via* V_T (instead of BR) allows for more oxygen-rich breaths and greater alveolar ventilation.

Several studies have demonstrated remarkable plasticity of V_T in healthy individuals at submaximal intensities (Vickery, 2008; Bahensky et al., 2019; Cleary, 2019; Bahensky et al., 2020).

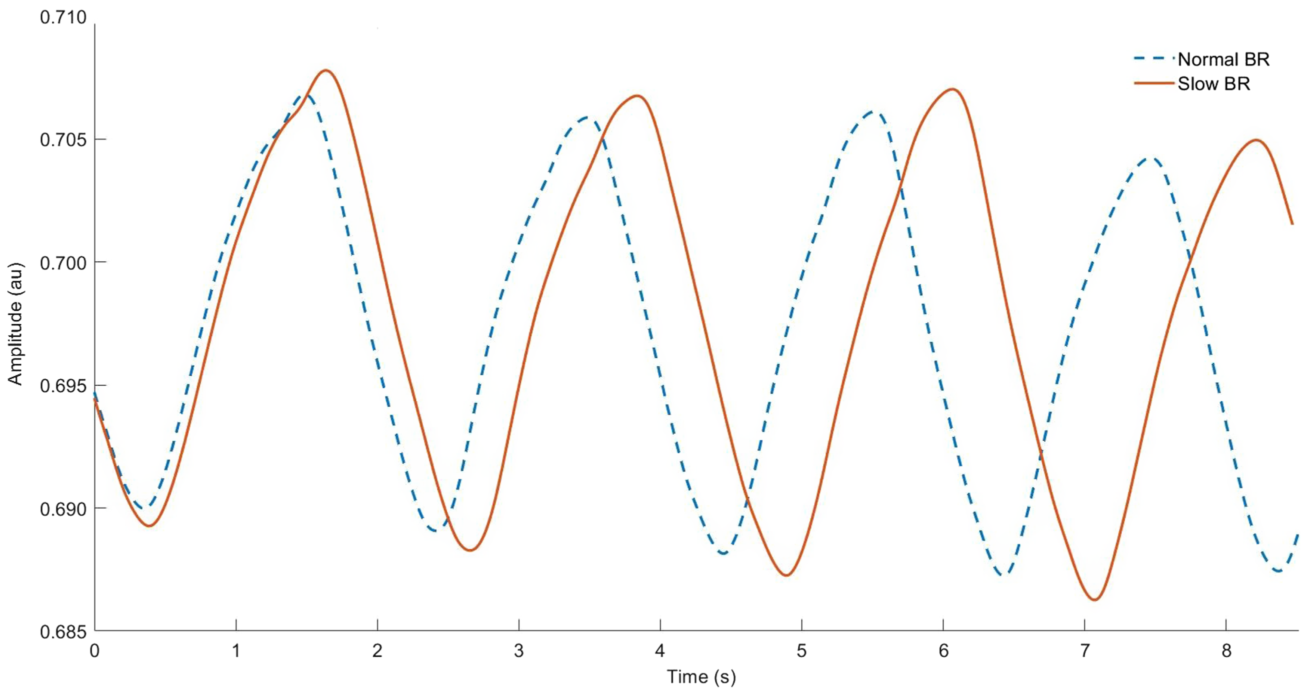

FIGURE 4 | Respiratory inductance plethysmography data from our lab showing normal breathing (dashed line) vs. "rate" breathing strategy (solid line). Note longer breath duration (horizontal) and related larger tidal volume (vertical) for each breath cycle.

TABLE 2 | Effect of breathing pattern on alveolar ventilation and dead space in three scenarios. Adapted from Braun (1990).

Breathing pattern	Minute ventilation (V_E, L/min)	Breathing rate (BR, bpm)	Tidal volume (V_T, L)	Dead space volume (V_D, ml)	Alveolar ventilation rate (L/min)	Relative V_D (V_D/V_T, %)
Normal	10	20	0.50	150	7.0	30%
Slow, deep	10	10	1.0	150	8.5	15%
Fast, shallow	10	40	0.25	150	4.0	60%

These findings may stand in contrast to the "minimal effort" hypothesis. We suspect that since an already-small percentage of VC is used for normal V_T during exercise, an incrementally larger V_T does not undermine the lung volume/pressure relationship, and the BR/V_T relationship may be more "flexible" than previously thought. This is likely not the case above the RCP, where BR increases are driven substantially by central command (Nicolò et al., 2018) and most exercisers reach V_T peak (Blackie et al., 1991). We suspect that this strategy is most appropriate at low relative exercise intensities, and perhaps not helpful or even harmful at high exercise intensities; future studies should investigate this difference.

Given the close association between BR and RPE (Nicolò et al., 2018; Cochrane-Snyman et al., 2019), we speculate that slower BR may decrease perceived feelings of effort at a given exercise intensity. Hypothetically, slower BR may "trick" the brain into feeling exercise to be easier. Hence, lower perceived effort might be reflected in improved performance or positive psychological states (Noakes, 2012). Moreover, since BR reflects the physiological response to cognitive and environmental stress at rest (Grassmann et al., 2016; Tipton et al., 2017), slowing BR during exercise may improve mental performance and calmness. As slow BR is known to positively impact autonomic nervous system balance and vagal tone at rest (Lehrer et al., 2020), it is possible that there is a similarly "optimal" BR during running that enhances the pleasant feelings of exercise (Homma and Masaoka, 2008). One study that manipulated BR during cycling found lower RPE, suppressed sympathetic and increased parasympathetic activity when breathing at very low BR of 10 bpm vs. unconstrained BR (Matsumoto et al., 2011). More studies are needed to evaluate such findings in running.

Another potential application of the "rate" strategy is to regulate exercise intensity. As BR is closely correlated with physical effort, we speculate that constant BR may limit physical output. Since mechanical limitations partially determine the comfortable limit of V_T, constant "paced" BR therefore has a theoretical upper limit of V_E. Paced BR therefore deterministically limits overexertion since V_E cannot easily increase. For example, given a typical VC of 4 L and assumed V_T peak of 60% VC (Naranjo et al., 2005), running with paced BR at 20 bpm would limit comfortable V_T to 2.4 L and V_E to 48 L/m. If the runner speeds up, increasing metabolic demands but not V_E, there might be a dissociation of the VCO_2/V_E relationship. Increased pCO_2 could trigger dyspnoea and air hunger (Sheel et al., 2011); this is a strong cue to "slow down." In this way, breathing could be used to deliberately impose a limit on exercise intensity, potentially aiding in sustainable pacing of exercise. This could be especially helpful for unfit beginner runners to prevent overexertion. Considering the complex "minimal effort" regulation of BP, paced BR during exercise could cause adverse effects such as respiratory discomfort or EID; this requires more study. Nonetheless, elite athletes express lower levels of BP variability during exercise vs. healthy sedentary individuals (Castro et al., 2017), suggesting that decreased BR variability could be advantageous. Experimental investigations could address this topic by including subjective assessment of dyspnoea intensity and discomfort on top of objective measurement of physiological performance (Lewthwaite and Jensen, 2021).

Practical application of paced breathing during running invites scientific exploration. In biofeedback studies, visual feedback has been used to successfully fix BR at specific rates (Davis et al., 1999; Blum et al., 2019). Auditive feedback may be especially appropriate for field running, since over 60% of runners listen to audio, on average, during their run (Nolan, 2016). We have demonstrated in our lab that runners can easily follow continuous and periodic auditive BR instruction during running (van Rheden et al., 2021). The specific parameters for the "rate" strategy need further definition: there is likely not an absolute "best" BR for all runners, but rather a relative decrease that optimizes the benefits outlined above. Nicolò et al. (2017b) suggest monitoring BR as a percentage of an individual's peak BR (BR/BR_{Peak}), and this could be used similarly for the "Rate" strategy. A decrease of 10%–20% is perhaps prudent as used in previous studies with breathing retraining (Bahensky et al., 2021).

Deep

Given the interdependence of BR and V_T, depth of breathing is largely dependent upon the former. However, equivalent V_T can be achieved with more or less diaphragmatic engagement, and at variable LOV. Pranayama Yoga, Zen, and Transcedental meditation practices include conscious diaphragmatic breathing exercises shown to be effective for improving exercise capacity, stress reduction, and reducing symptoms of respiratory disease (Hamasaki, 2020). Thus, breathing depth ought to be considered distinctly modifiable.

Although V_T must adjust proportionally with BR to match V_E demands, it can perhaps be altered independently. Elite athletes have demonstrated ventilatory compensation strategies favoring V_T increases relatively greater than non-elites, especially in acute hypoxia (Lucía et al., 2001; Tipton et al., 2017). This may be an adaptive mechanism to aid in the elevated V_E demands of high performance, as lung structure is remarkably intractable even with training (Dempsey et al., 2020). Not only is increasing V_E *via* V_T preferable and possible (section "rate"), but this "depth" should come from the abdominal ribcage (**Figure 5**).

Upper-thoracic dominant breathing is associated with increased flow limitation, WOB, hyperventilation, and postural instability (Nelson, 2012; Chaitow et al., 2014; Depiazzi and Everard, 2016; Wallden, 2017). Conversely, diaphragmatic breathing is correlated with various positive health benefits, including reduced resting heart rate, post-exercise oxidative stress, increased postural control, and baroreflex sensitivity (Hazlett-Stevens and Craske, 2009; Martarelli et al., 2011; Nelson, 2012; Hamasaki, 2020). The effect of diaphragmatic breathing during exercise on these parameters is unknown. We suspect that this is due to methodological difficulties in measuring diaphragmatic contribution to breathing noninvasively. Nonetheless, diaphragmatic deep breathing might help to attenuate respiratory limitations in vulnerable individuals and situations. We suspect that this could result in reduced risk for EID and negative psychophysiological consequences.

Greater depth of breathing can be achieved through exercises to improve diaphragmatic function, and thoraco-lumbar coordination. Several publications have provided summaries of such exercises, which include paced breathing, biofeedback, and manual therapy (Hazlett-Stevens and Craske, 2009; Saoji et al., 2019; Hamasaki, 2020). On the other hand, examples of manipulating breathing depth during exercise are limited. One simple method is implementing the "rate" strategy to force V_T to increase in response to slow BR (section "rate"). However, this does not necessarily cue diaphragmatic breathing as we have described. Some products such as the Buteyko belt® (Buteyko Clinic International, Galway, Ireland) may bring awareness to the diagphragm and abdominal ribcage *via* external tactile stimulation, but it is unclear if these are suitable during exercise. Sensors such as the Hexoskin garments (Carre Technologies, Canada) are capable of measuring abdominal ribcage expansion, but they experience significant signal contamination as a result of soft tissue artifact when used during running (Harbour et al., 2021). Visual and auditive methods from the field of biofeedback could be particularly suitable for cueing this strategy during running.

Nose

The nose is the primary point of entry and exit of the airway during healthy breathing at rest. It is functionally equipped to humidify, warm, and filter inspired air (Walker et al., 2016). It also increases nitric oxide production in the airway, and has positive effects upon pulmonary perfusion (Sanchez Crespo et al., 2010), head posture (Sabatucci et al., 2015), and cognitive function (Zelano et al., 2016). Mouth breathing is more common during exercise and for those with nasal breathing difficulties (Niinimaa, 1983). However, unlike nose breathing, habitual mouth breathing is linked with DB and numerous pathologies, including upper respiratory tract infections, rhinitis, and asthma (Chaitow et al., 2014; Walker et al., 2016). Despite these concrete advantages at rest, nasal breathing during exercise has seen

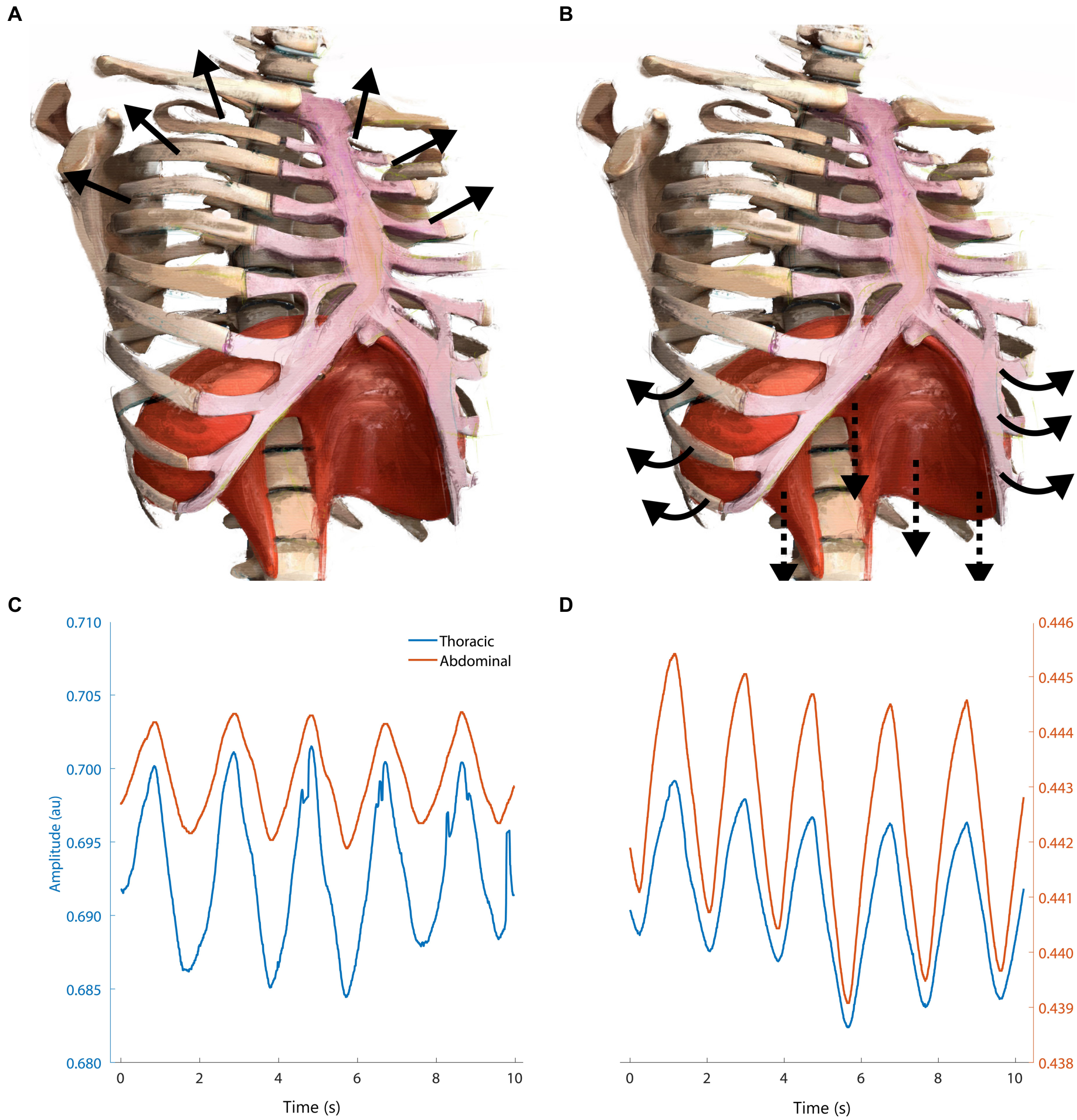

FIGURE 5 | Schematic showing the difference between upper-thoracic dominant breathing **(A,C)** vs. "deep" diaphragmatic breathing **(B,D)**. **(A)** Upper-thoracic breathing elevates and expands the upper ribcage, visible in **(C)** respiratory inductance plethysmography measurements from our lab showing increased amplitude in thoracic vs. abdominal bands. **(B)** Deep breathing flattens the diaphragm against the inferior abdominal viscera, expanding the abdominal ribcage *via* pump- and bucket-handle mechanisms. Adapted from Isometric angle of diaphragm and ribcage by Chest Heart & Stroke Scotland and Stuart Brett, The University of Edinburgh 2018 CC BY-NC-SA; arrows added for emphasis.

mixed attention and enthusiam as a standalone breathing strategy. Nose breathing is encouraged during Pranayama Yoga exercises, and also popularized by authors/bloggers Brian McKenzie (Sh//ft.®) and Patrick McKeown (Oxygen Advantage®) as a psychophysiological state modulator. Humans usually switch to mouth breathing at $V_E = 40$ L/min, leading to the assumption that mouth breathing is a requirement during exercise (Saibene et al., 1978). Despite this assumption, studies have demonstrated that humans have surprising flexibility in airway choice during exercise (Morton et al., 1995; Dallam and Kies, 2020).

Thomas et al. (2009) reported that subjects were able to maintain nasal breathing up to 85% VO_{2max} during exercise when instructed with a familiarization but no other accommodation. With an adaptation period, nasal breathing during exercise may cause reduced BR, reduced hypocapnia, and increased nitric oxide production (Dallam et al., 2018). Nitric oxide production is itself beneficial as a vasodilator and bronchodilator (Sanchez Crespo et al., 2010; Thornadtsson et al., 2017), perhaps reducing the risk for flow limitation. While nasal breathing utilizes a smaller airway, which is a limitation at higher exercise intensities, it appears to increase diaphragmatic function (Trevisan et al., 2015), which could be a long-term advantage (see section "Deep"). Some studies have reported favorable performance effects, such as decreased respiratory exchange ratio, VO_2, and increased running economy and time to exhaustion (Morton et al., 1995; Recinto et al., 2017). We estimate that these effects might benefically decrease RPE or dyspnoeic sensations, although direct study is required. Conversely, nasal breathing during heavy exercise leads to higher exercise HR and no difference in power output or anaerobic performance, perhaps as a result of greater inspiratory muscle load (Recinto et al., 2017).

The filtration and humidification functions of the nose may help at any exercise intensity to prevent EID and pathogen or particulate inhalation (Mangla and Menon, 1981; Aydın et al., 2014). The risk for Rhinitis and upper respiratory tract infections is substantially reduced with nasal breathing during exercise (Walker et al., 2016). Airway choice also impacts head posture and glossopharyngeal mechanics (Okuro et al., 2011; Sabatucci et al., 2015), suggesting that nasal breathing could be a long-term strategy to prevent EILO. Although there is limited evidence on the psychophysiological correlates of nasal breathing during exercise, studies suggest that nasal breathing at rest leads to improved cognitive function, emotional appraisal, memory, and lower perception of fear (Zelano et al., 2016). Hence, we suggest that nasal breathing is beneficial for its positive effects on performance, airway quality, and cognitive function during low-intensity exercise.

Implementing nasal breathing during exercise requires awareness and accommodation. Anecdotal evidence suggests that 10–12 weeks are required for meaningful changes in nasal breathing comfort and relief of airway restriction to occur, while intervention studies have examined learning periods of up to 6 months (Dallam et al., 2018). Conversely, several reports indicate that nasally-restricted breathing causes nasal airway resistance to drop in days and even minutes as a result of nitric oxide production and shifting nasal mucosa (Shturman-Ellstein et al., 1978; McCaffrey and Kern, 1979; Mertz et al., 1984). Rather, nasal breathing is self-manifesting: performing it encourages subsequent ease. In fact, nasal airway resistance falls during exercise, regardless of the airway used (Olson and Strohl, 1987). This is why the "nose" strategy is indeed an accessible choice for most runners; barriers to uptake are most likely related to habituation alone. Nasal breathing requires some adaptation, but the ideal protocols and individual differences need more investigation. An understudied aspect is whether diaphragm fatigue is improved or harmed with nose breathing, given its active resistance as a smaller airway. Finally, we recommend exercising caution when performing studies on nasal breathing in an exercise physiology setting specifically related to spiroergometry masks and their adverse effects on BP (Gilbert et al., 1972; Laveneziana et al., 2019). Future studies should explore nasal breathing in natural running settings with minimally invasive equipment, and also the details of nasal breathing accommodation.

Active Exhale

The benefits of long, slow exhales have been long promoted in Yoga and meditation fields to enhance health and well-being (Saoji et al., 2019). Some running coaches and experts have also touted this strategy, suggesting it enhances breathing depth and aerobic endurance (Jackson, 2002; Coates and Kowalchik, 2013). Although there are few studies directly examining manipulation of the exhale phase during exercise, combined evidence from other domains supports several advantages of "active" exhales.

Longer exhales may exploit respiratory sinus arrythmia to improve HRV and subjective well-being at rest (Matsumoto et al., 2008; Van Diest et al., 2014). While inspiration enhances sympathetic and suppresses parasympathetic activation, during expiration, the opposite occurs, triggering vagal afferents (Seals et al., 1993; Hayano et al., 1994). This phenomenon has been observed during incremental exercise (Blain et al., 2005). Indeed, breathing may be the main mechanism responsible for short-term HR fluctuations, especially at higher intensities (Bernardi et al., 1990; Prigent et al., 2021). Matsumoto et al. (2011) tested this effect during exercise: longer exhales (33 vs. 50% dc) caused improved HRV, ventilatory efficiency (V_E/VCO_2 19.1 ± 2.9 vs. 22.1 ± 4.4), and VO_2 during incremental cycling. These profound results have yet to be replicated in the literature.

A separate but related approach to exhale manipulation is conscious recruitment of the expiratory musculature. Although expiration becomes active by default during exercise, additional contraction of the abdominals may confer additional benefits. In other words, stronger, forced exhales in combination with a lower duty cycle make the "active exhale." Active exhales cause a fuller upward excursion of the diaphragm, which generates passive elastic forces, lowers diaphragmatic work, and assists in postural stabilization (Wuthrich et al., 2014; Wallden, 2017). Greater abdominal recruitment might help to partition the WOB and delay the onset of ventilatory muscle fatigue at high intensities, but this aspect is apparently unstudied. While active exhales are automatic in most situations, the respiratory limitations outlined above can cause this pattern to become dysregulated. Hence, in the presence of these limitations, it is possible that purposeful active exhales could assist in maintaining optimal LOV (**Figure 6**). Lower duty cycle limits inspiratory flow, and allows more time for expiratory flow; this may reduce expiratory flow limitation. In addition, the assymetric flow profile accompanying long exhales implies that peak negative inspiratory pressure exceeds negative expiratory pressure. This could have net positive effects on intrathoracic pressure as a limiter to cardiac output during high-intensity exercise (Amann, 2012). No studies have explored this aspect yet in the literature.

The mountaineering community has long-touted a version of active exhales ("rescue breath" or "pressure breath") for managing respiratory distress at altitude (Expeditions, 2014). Ian Jackson's Breathplay technique highlights this strategy, claiming a number of benefits that were examined by Wojta et al. (1987) after a 3-day training period. The study found a delayed onset of fatigue, lower HR (1.9%), and longer time to exhaustion (7.2%) during an incremental cycling test to

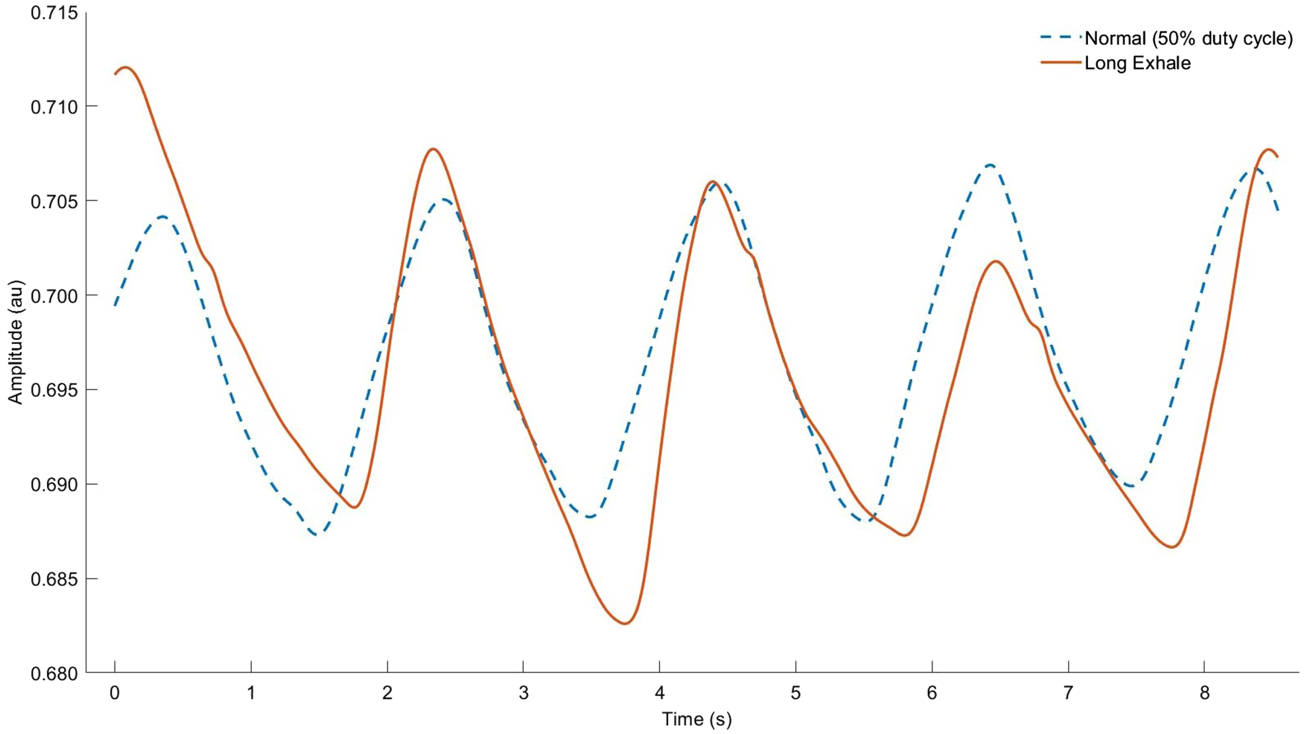

FIGURE 6 | Respiratory inductance plethysmography (RIP) data from our lab showing normal breathing (dashed line) vs. "active exhale" breathing strategy (solid line). Note that raw RIP data depict inductance, where signal increases (upward slope) correspond to the exhale phase. Observe the identical breath cycle time, but shorter relative inhale and longer exhale (smaller breath ratio) as well as lower average lung operating volume throughout the breath cycle (higher signal units indicating decreased sensor stretch).

exhaustion. In addition, they reported a substantial delay in the onset of peak CO_2 (40%), presentation of RQ = 1 (60 s later), and anaerobic threshold (120 s). Replication studies are needed to confirm these distinct results. Positive expiratory pressure may contribute to these ergogenic effects; Rupp et al. (2019) reported that forced exhales benefit peripheral and central circulation and oxygenation, especially in hypoxia, that is probably caused by increased alveolar pressure and resultant hyperperfusion. When used intermittently, this may allow marginal increases in pCO_2, which enhance the Bohr Effect. This may be especially relevant for individuals and situations predisposed to hypocapnia, such as intense exercise and hyperventilation DB.

A valuable addition to the active exhale is phonation. For example, the yogic technique Bhramari Pranayama (humming during the exhale) may be effective in cueing active exhales since it not only adds additional airway resistance on the out-breath, but it profoundly increases free nitric oxide (up to 15-fold at rest; Weitzberg and Lundberg, 2002; Pramanik et al., 2009). This may enable nasal breathing at higher intensities, or ease flow limitation. This unconventional aspect has the additional benefit of amusement for the runner (or those around them). Future work should clarify whether this technique can reduce respiratory limitations or if it might adversely irritate laryngeal structures, especially whether there is an ideal frequency to perform it.

Performing active exhales during running probably requires attention, instruction, and habituation. Visual modes of biofeedback may be especially effective if displaying real-time LOV. A valuable cue may be to "squeeze all the air out" (Jackson, 2002) or fully "empty" the lungs before the inhale (Johnston et al., 2018). Other techniques such as pursed lips breathing could be combined to exploit the ergogenic effects of positive expiratory pressure, which is particularly relevant when exercising at high intensity or altitude (Rupp et al., 2019). A major limitation to studying or performing the active exhale is its deviation from the "minimal effort" BP; duty cycle is remarkably constant in most healthy exercisers (Naranjo et al., 2005), and it may require substantial cognitive focus to maintain this technique for long periods of time.

Sync

Locomotor-respiratory coupling, once penned "rhythmic breathing" by medical doctor Irwin Hance in 1919, has been the object of much scientific investigation for at least 50 years, and has wide cultural influence (Hey et al., 1966; Coates and Kowalchik, 2013). While bipedalism gives humans flexibility to perform it or not during locomotion, LRC has been observed at many ratios during running (commonly reported 4:1, 6:1, 8:1, 5:1, and 3:1 steps per breath; Bramble and Lieberman, 2004; Stickford and Stickford, 2014).

The passive assistance of step-driven flows may assist V_E increases without elevating the WOB (Daley et al., 2013; Stickford and Stickford, 2014). Several studies report that LRC decreases VO_2, increases running economy, and reduces dyspnoea (Garlando et al., 1985; Bernasconi et al., 1995; Takano and Deguchi, 1997; Hoffmann et al., 2012). Some have speculated that active exhales may further enhance the exhale phase in combination with LRC, as concentric contraction of the abdominal and pelvic floor musculature may optimize visceral compressive forces when synchronized with step-driven flows (Daley et al., 2013; Wallden, 2017). The "free" work granted by step-driven flows may realize some of the benefits of other strategies, since greater V_T enables slower BR and reduced flow velocity at a given V_E. If this eases flow limitation or EID, then it can also lead to associated positive psychological outcomes.

As noted in section "respiration as a limiting factor," LRC at even ratios (e.g., 4:1 or 6:1) could be a risk factor for side stitch. However, it might also be used to prevent it. Some experts recommend exhalation on alternate steps specifically to avoid side stitch (Jackson, 2002; Coates and Kowalchik, 2013). Using an odd-numbered LRC ratio (e.g., 5:1 or 7:1; **Figure 7**) causes exhales to occur on opposite footstrikes, potentially limiting parietal peritoneum irritation. This might avoid such unpleasant pain and discomfort during running.

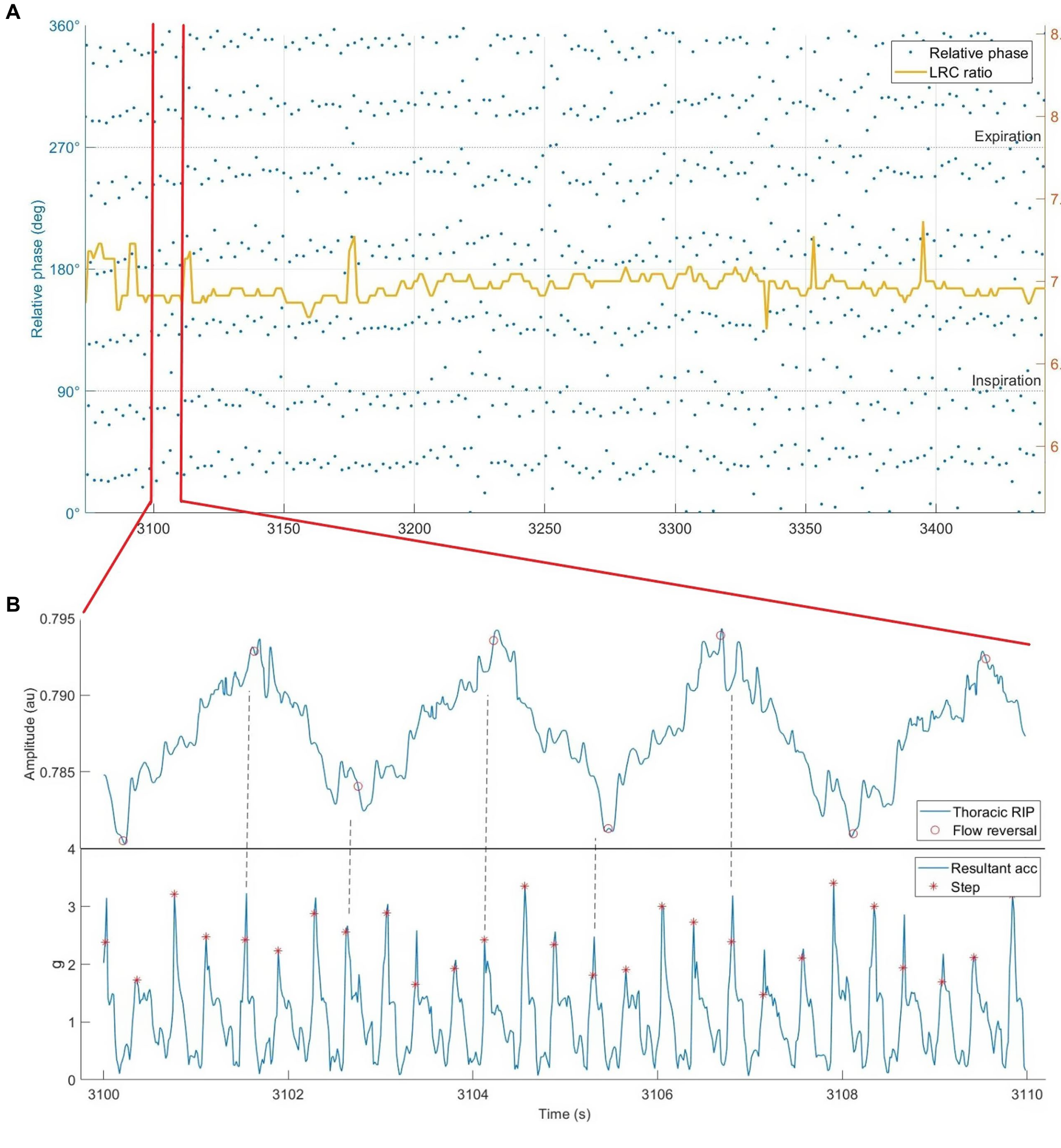

FIGURE 7 | Respiratory inductance plethysmography data from our lab showing locomotor-respiratory coupling (LRC). **(A)** Phase synchrogram and LRC ratio plotted during 8 min of running at an instructed LRC ratio 3:4 (steps per inhale:steps per exhale). Note the quantity of steps synchronized with inspiration vs. expiration. All relative phase shifted 90° for visibility. **(B)** Subsection of 10 s of raw RIP and hip-mounted accelerometer data while running at 3:4 LRC. Dotted lines added to emphasize step & flow reversal synchronization.

Locomotor-respiratory coupling may be energetically advantageous not only for its synergistic respiratory advantages, but also as a mediator of BR and running pace. As described in the "rate" strategy, given the close correlation between BR and RPE, and also the known stability SR during running (Van Oeveren et al., 2019), LRC may be an effective way to stabilize running pace. LRC ratios of 4:1 and 5:1 for a runner with a preferred SR around 180 (right and left, steps per minute) would thus correlate to BR of 45 and 36, respectively. Different LRC ratios could thus be utilized as a "gears" system corresponding to different perceptual and physiological levels of effort.

Since a primary mechanism of LRC during running appears to be neurological, purposeful performance of LRC may have additive psychological benefits. The immersive psychophysiological experience of flow is suggested to occur with the presence of three conditions: an activity with clear goals and progress, immediate feedback, and balance between perceived challenges and competence (Snyder et al., 2020). Entrainment, such as that of breath to step during LRC, is likely to enhance flow experience in runners (Bood et al., 2013; Nijs et al., 2020). Indeed, LRC during running satisfies the primary conditions for inducing a trance state: physical exertion, rhythm, and concentration (Damm et al., 2020). Such rhythmicity is comforting, sedating, and hypnotic, and rhythmic stability may lower stress on the nervous system by reducing cognitive fatigue (Ross et al., 2013). Future studies should explore LRC and flow phenomenology especially as it pertains to runners.

The practical application of LRC is not trivial. It is possible that the additional concentration required to execute LRC during running negates any physiological benefit. Nevertheless, past studies that found no benefit for LRC examined it in untrained individuals or during unfamiliar tasks (Yonge, 1983; Maclennan et al., 1994). Perhaps some learning and accommodation is required to realize ergogenic advantages. Although many elite runners perform it unconsciously (Bonsignore et al., 1998; McDermott et al., 2003), limited evidence is available regarding learning this as a deliberate breathing strategy, especially in sub-elite runners. A notable exception instructed LRC *via* haptic feedback (vibration) timed with footstrikes on either the exhale or inhale (Valsted et al., 2017). They found comparable success when the feedback was periodic (1 min of instruction followed by 2 min or no instruction) or self-selected vs. continuous. Nonetheless, some runners found LRC difficult or the instruction annoying. Attention is needed in this field to develop intelligent systems for LRC instruction and feedback, with auditive modes being understudied. Runners should probably synchronize breath to step, instead of step to breath, since deviating from individually preferred SR might be energetically disadvantageous or increase injury risk (Adams et al., 2018; De Ruiter et al., 2019). Smart feedback systems should consider adapting breath instruction to the current SR to avoid such effects and to maximize entrainment (Bood et al., 2013; Van Dyck et al., 2015). Practically, runners should use an odd ratio (such as 5:1 or 7:1) to capture the benefits of longer exhales and side stitch prevention.

Advanced Breath Tools

These breathing strategies are labeled "advanced" because they require either special equipment or are especially difficult to perform. They also carry some risk, which should be considered in context vs. the potential benefits and population of interest. Nevertheless, they are included here because they have demonstrated ergogenic benefits and are suitable for application during running.

Strength

Respiratory muscle training (RMT) has been extensively studied as an alternative strategy to improve breathing during exercise. The use of resistive breathing devices such as the Training Mask® and POWERbreathe® stress the respiratory system, resulting in positive changes in ventilatory efficiency, muscle recruitment patterns, oxygen delivery, and reduced WOB and dyspnoea (Karsten et al., 2018; Shei, 2018; Lorca-Santiago et al., 2020). Readers are directed to these three recent reviews for a detailed explanation of these mechanisms. While the majority of studies leverage these methods at rest, several studies have examined the effects of concurrent resistive breathing during exercise (Hellyer et al., 2015; Porcari et al., 2016; Barbieri et al., 2020). Experts in this field have suggested that concurrent RMT is underexplored and may, in fact, be the most effective means of transferring the benefits of RMT to sport performance (Karsten et al., 2019). Unfortunately, high-quality studies examining these scenarios are lacking.

The Olin EILOBI techniques were developed by J. Tod Olin and colleagues as a variant of inspiratory resistance breathing specifically to address EILO (Johnston et al., 2018). They were conceived to be used specifically during exercise when EILO occurs to maximize specificity. Although primarily developed for clinical applications, the self-resisted nature of this technique may qualify as RMT and be suitable for other settings. Johnston et al. (2018) report alleviation of EILO symptoms in 66% of their participants, and we suspect that this could be valuable for other runners to prevent flow limitation. While this technique is complex to learn, some components (emptying, abdominal ribcage focus) may be helpful for improving breathing mechanics.

We found exactly one study that specifically tested RMT methods during running. Granados et al. (2016) reported that wearing the Training Mask® (Training Mask LLC; Cadillac, MI, United States) during running at 60% VO_{2max} induced hypoxaemia without substantial increases in RPE or anxiety. They concluded that incorporation of RMT methods part-time in a training routine is a convenient, time-efficient approach to benefit from RMT. Nevertheless, more studies are needed to explore long-term use of such methods. If the muscle recruitment pattern triggered by resisted breathing is not deep & diaphragmatic, it may not accumulate adequate stimulus to induce diaphragmatic hypertrophy, or it may habituate DB (Karsten et al., 2018, 2019). While many respiratory-limited individuals could benefit substantially from RMT's subsequent reduction in EID (Bernardi et al., 2015), females appear less receptive to its benefits (Schaer et al., 2019). Finally, while it could be dangerous to induce additional respiratory distress during running, the potential ergogenic and psychological benefits suggest that careful protocol development is a key to making this a viable breathing "strategy" among runners.

Hold

Breath holding (BH, also known as hypoventilation, CO_2 tolerance, or air hunger training) garnered recent popularity due to large performance benefits reported in swimming and techniques popularized by freediving (Holfelder and Becker, 2019). The various effects of hypoxia and hypercapnia have been rigorously studied (Millet et al., 2016; Girard et al., 2020), and BH is an accessible method for runners to replicate such benefits. In short, BH is a strong metabolic stressor similar to hypoxic training that causes accelerated muscle deoxygenation, hypercapnia, and increased muscle activity during exercise (Kume et al., 2016; Toubekis et al., 2017). BH protocols lasting 3–5 weeks reported performance gains of 3%–4% related to two acute mechanisms: increased stroke volume (up to 30%) and haemoglobin concentration (up to 10%; Woorons et al., 2016; Lapointe et al., 2020; Woorons et al., 2020). These ergogenic benefits are likely due to increased left ventricular stroke volume (Woorons et al., 2021b) and post-BH spleen contraction (Inoue et al., 2013). Only one study was found that examined the acute effects of BH during running (Woorons et al., 2021a). They reported dramatic central and peripheral deoxygenation when performing maximal end-expiratory BH at 60%–100% of maximal aerobic velocity, which could provide adequate stimulus for the aforementioned training effects if performed systematically.

Characteristics of elite free-divers suggest that long-term adaptations to BH include reduced CO_2 chemosensitivity and increased lung volume (Bain et al., 2018; Elia et al., 2019). Repeated hypercapnia (Bloch-Salisbury et al., 1996) and endurance training (Katayama et al., 1999) cause long-term adaptations to lower chemosensitivity (measured as the ventilatory response to a given absolute workload). Moreover, reduced chemosensitivity during exercise is a characteristic of trained athletes vs. healthy sedentary individuals (McConnell and Semple, 1996). While increased pCO_2 is responsible for the sensation of "air hunger" (Banzett et al., 1990), it also allows for enhanced O_2 transport *via* the Bohr Effect. Decreased CO_2 sensitivity, therefore, may allow for enhanced ventilatory efficiency and reduced BR. No studies could be found directly investigating this mechanism in exercise.

Performing BH during running is best in a safe, supervised environment with prior familiarization with BH techniques at rest. The cited studies suggest a work:rest ratio of 1:1.5 or 1:2 (e.g., 10 s hold followed by 20 s running) for 10–12 repetitions. Notably, participant instructions often include counting cycles per breath to "pace" BH duration; this is could facilitate use of the "hold" tool in the field. Most protocols recommend end-expiratory BH since it accelerates hypoxaemia and hypercapnia; this is done by performing a long exhale, and then another, down to residual volume (**Figure 8**). End-inspiratory BH and very slow BR trigger similar levels of hypercapnia,

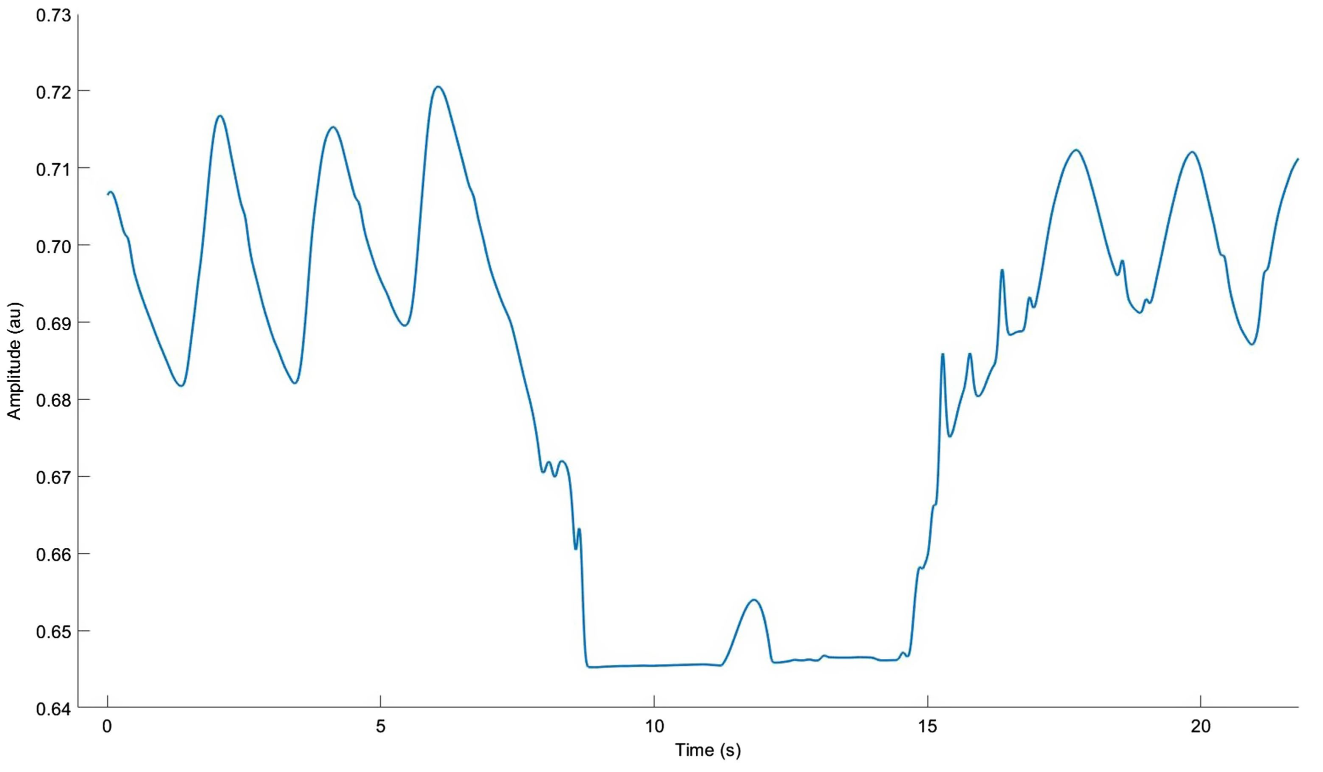

FIGURE 8 | Respiratory inductance plethysmography data from our lab showing normal breathing with one approximate 10 s end-expiratory breath hold. Note the very long double exhale and brief diaphragm twitch.

but not of hypoxaemia required to maximize training effects (Yamamoto et al., 1988; Holfelder and Becker, 2019). Adverse effects include hypercapnia-induced headaches, lung injury, syncope, and neurological harm if performed too often or aggressively (Matsuo et al., 2014). "Hold" is likely to be intensely difficult and psychologically unpleasant since it induces large feelings of air hunger. This may increase the risk for anxiety and emotional distress in many individuals (von Leupoldt et al., 2008). Conversely, if it can be tolerated long enough to realize its dramatic performance benefits, it may cause beneficial reductions in EID, especially at high intensities. Important work is being done to assist the execution and safety of BH with wearable devices (Vinetti et al., 2020), but this has not extended to BH during running.

PRACTICAL APPLICATIONS

Breathing is Quantifiable

The control and expression of breathing pattern is tractibly understood and every step can be reliably and specifically measured (Del Negro et al., 2018). We suggest measuring and reporting dyspnoea intensity and discomfort in exercise studies since these data points assist substantially in identifying respiratory limitations (Lewthwaite and Jensen, 2021). If a runner has access to qualified exercise physiology personnel, cardiopulmonary exercise testing procedures can be especially useful; we recommend measuring ventilatory thresholds, tachypnoeic shift onset, VO_{2max}, and approximate representations of ventilatory efficiency (including, but not limited to: running economy, V_E/VCO_2 slope, and V_T/BR quotient). Voluntary spirometry measures such as forced expiratory volume and maximum voluntary ventilation may provide additional insights. For a detailed description of these laboratory-based procedures, the reader is directed to recent reviews (Janssens et al., 2013; Barnes and Kilding, 2015; Laveneziana et al., 2019; Segizbaeva and Aleksandrova, 2019; Ionescu et al., 2020).

Recent developments in wearable sensors have expanded the capabilities of respiratory system monitoring outside of the lab. Non-contact methods such as respiratory inductance plethysmography and capacitive sensors can provide estimations of BR, thoraco-lumbar coordination, and the ratio of thoracic-to-abdominal ribcage breathing (thoraco-lumbar depth) and V_T in the field (Bernardi et al., 2017; Leutheuser et al., 2017; Massaroni et al., 2019). Combined FR and step detection in garments such as the Hexoskin® (Carre Technologies, Canada) could enable LRC estimation in the field (Harbour et al., 2021), although such applications are scarce. When viewed in combination with performance measures, monitoring BP may therefore reveal deep individual constraints and context-specific insights that could be modified or improved with these proposed breath tools. Alternatively, these BP measurement methods and protocols could also be used to scientifically evaluate the effectiveness of these strategies during breath retraining interventions.

Using Breath Tools

Although the value and quantity of resources related to breath retraining at rest is substantial, less is known regarding implementing breathing strategies during running. While we have provided some specific recommendations for each strategy, some general recommendations can be made. Simple awareness of BP encourages slow BR and greater depth during running (Schucker et al., 2014; Schucker and Parrington, 2019). Breathing exercises at rest can develop awareness of thoraco-lumbar coordination that carries over into exercise performance (Hagman et al., 2011; Kiesel et al., 2020). Runners can easily access such exercises in many Yoga and meditation practices (Ma et al., 2017; Saoji et al., 2019).

A unified theory of breathing strategy prescription would carefully choose the techniques above specific to the needs of the runner and scenario (**Table 3**). We propose the "Sync"

TABLE 3 | Overview of breath tools strategies description and application.

Breath tool	Description	Primary mechanisms	Advantages	Disadvantages	Applications
Rate	↓ and/or paced BR	↓ relative V_D; ANS regulation	↑ perfusion; ↓ dyspnoea; and pacing assistance	↑ V_T at less-compliant lung volumes, initial air hunger	Novice runners; low-intensity exercise
Deep	↑ V_T *via* diaphragmatic engagement	↓ BR; ↑ abdominal ribcage contribution to V_E	↓ WOB, LOV; ↑ postural control	Difficult to cue	Biofeedback; thoracic-dominant breathers
Nose	Constant or intermittent nasal breathing	↑ NO; ↑ air humidification, warming, and filtration	↓ airway constriction; ↑ diaphragmatic activation	Difficult at high intensities; time required for habituation	Low intensity exercise; extreme climates
Active exhale	Longer, forceful exhale phase with/without phonation	↓ expiratory flow velocity: ↑ abdominal engagement, expiratory pressure, and NO	↓ flow limitation, LOV; ↑ perfusion; and ANS regulation	↓ relative T_I; difficult to cue	Constant for calming effects; intermittent during high intensity or at altitude
Sync	Step & breath synchronization at whole-integer ratios	Step-driven flows; rhythmic entrainment	↓ WOB; pacing assistance; hypnotic	Difficult to learn; even ratios ↑ side stitch	Odd ratios for ↓ side stitch; ↑ breath awareness
Strength	Respiratory muscle resistance training	↑ ventilatory muscle activation, metabolic stress	↓ WOB, dyspnoea; ↑ diaphragmatic activation	Special equipment needed; unclear protocols	Low intensity exercise; training for competition
Hold	Intermittent brief end-expiratory breath holds	↑ biochemical stress, spleen contraction	↓ chemosensitivity; cardiovascular performance	Risk of syncope, intense air hunger unpleasant	Pre-competition; elite sport

Mechanisms, advantages, and disadvantages are based on a mixture of theoretical and empirical evidence as described in the main text. Applications are based on preliminary subjective findings of the authors, and do not constitute absolute recommendations. ANS, autonomic nervous system; BR, breathing rate; LOV, lung operating volume; NO, nitric oxide; T_I, inhale time; WOB, work of breathing; V_T, tidal volume; and V_D, dead space.

tool as a near-universal practical recommendation, since it can be leveraged to manipulate BR, depth, and timing. However, there is limited knowledge available on how to learn this skill. Simply counting steps per breath is likely only suitable for skilled runners with experience in rhythm (e.g., musicians or dancers). Coates and Kowalchik (2013) describe a multi-step learning process that may be suitable for coaches and athletes in field running. This could be combined with the "gears" system suggested by McKenzie (2020) to adjust BP to running intensity, or the postural and verbal cues of Jackson (2002) to maintain active exhales and proper abdominal engagement. Preliminary data from our lab suggest that even novice runners can perform this skill within one session given step-synchronous audio guidance, although with variable cognitive load.

The field of Human-Computer Interaction shows immense promise in learning breathing strategies during running, with demonstrators such as Strive (Valsted et al., 2017) and Counterpace® (Constantini et al., 2018) exploiting step-synchronized feedback modes. Core principles such as multi-sensory experience, user-centered design, and embodied interaction can guide the design of future systems to teach runners how to breathe during running (Wiehr et al., 2017; Mencarini et al., 2019; van Rheden et al., 2020). We propose auditive and haptic-based feedback systems that are field-ready with real-time learning possibilities regarding the runner's current BP and adherence to the desired strategy.

Evidence suggests that breathing strategies such as LRC might be more effective at relative intensities lesser or greater than self-selected speeds (Stickford and Stickford, 2014). Variations away from preferred gait speed tend to increase the energy cost of transport (Hunter and Smith, 2007); thus, at metabolically "suboptimal" speeds, breathing strategies have a greater theoretical benefit. At lower intensities, we hypothesize that most runners could benefit from slower, deeper, nose breathing, which reduces the risk for respiratory limitations. These benefits may be especially helpful in extreme environments (hypoxic, dry, and cold) when the risk for EID is higher (Weiler et al., 2016). At higher intensities, strategies such as the active exhale and sync might be more helpful, as they might improve gas exchange, optimize trunk kinematics, lower the WOB, and maintain sustaintable BR. We speculate that the greatest benefits would be realized in respiratory-limited runners; this requires more study.

LIMITATIONS

Although breathing during running is measurable, modifiable, and improvable, several limitations must be addressed before further study and application. Our review includes a mix of experimental and theoretical evidence which requires more direct investigation. Notably, there is a strong hypothesis that BP manipulation is likely to be ineffective or even harmful when initially employed. If indeed healthy human ventilatory response is "just right," and lung structure is not plastic (Dempsey et al., 2020), then perhaps changing BP will not result in improved respiratory performance. Ventilatory efficiency and overall BP are considered the result of complexity in a well-adjusted system (Benchetrit, 2000); any pertubations could be not only cognitively demanding, but also energetically costly. On the other hand, studies have shown that positive BP changes can be habituated over time periods spanning 2–6 months (Vickery, 2008; Dallam et al., 2018; Bahensky et al., 2019, 2021). It is unknown when exactly BP changes occur and under what conditions. Some studies have questioned the benefit of internal focus during running, suggesting it leads to technique breakdown and performance disruption (Beilock et al., 2002; Hill et al., 2020). Thus, there may be a switching point when the mental effort to change BP decreases, perhaps unlocking resultant benefits. Nevertheless, changing BP requires a suppression of natural reflexes and ingrained habits. More work is needed to clarify if, how, and when BP changes and which conditions facilitate this.

CONCLUSION

We have synthesized the evidence to demonstrate how purposeful breathing strategies might improve running *via* specific biochemical, biomechanical, and, ultimately, psychophysiological mechanisms. Breathing strategies have the potential to significantly improve ventilatory efficiency and exercise performance but estimates of effect size are scarce and variable. It is likely that breathing strategies do not acutely improve exercise performance but have the potential to increase it 1%–5% over a longer learning period. Respiratory-limited individuals have the most to gain by using these techniques. We theorize that the greatest benefits are psychological; increased exercise tolerance or positive psychological states might increase runners' exercise habits and long-term training adherence. Intervention studies are needed to study these likely transformative benefits *in vivo*, especially over longer durations and with populations predisposed to respiratory limitations.

AUTHOR CONTRIBUTIONS

EH and TF: conceptualization. EH: writing—original draft preparation and visualization. EH, TF, and TS: writing—review and editing. TF and HS: supervision and funding acquisition. HS: project administration. All authors contributed to the article and approved the submitted version.

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Effect of a Single Simulated 500 m Saturation Dive on Lung Function

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Background: Whether deep saturation diving causes injury to lung function remains controversial and the mechanism is unclear. The present study aimed to evaluate the effects of a 500 m simulated single saturation dive on lung function.

Methods: A retrospective study was performed in nine professional divers who spent 176 h in a high-pressure environment simulating a depth of 500-m saturation dive (51 atm, 5.02 Mpa). Pulmonary function parameters were investigated and compared before and on 3 days after the dive.

Results: Nine professional divers aged (36 ± 7) years were enrolled. Three days after the dive, the parameters related to expiratory flow (forced expiratory volume in 1 s (FEV_1)/ forced vital capacity (FVC)) were decreased; the parameters related to small airway function (forced expiratory flow at 50%, 75% of FVC exhaled and forced mid-expiratory flow) were decreased compared with those before the dive (both $p < 0.05$). Additionally, after the dive, the parameters related to pulmonary diffusion function were decreased compared with those before the dive (both $p < 0.05$). The parameters related to lung volume (residual volume, vital capacity and total lung volume) and those related to respiratory exertion (peak expiratory flow and forced expiratory flow at 75% of FVC exhaled) were not significantly different between after and before the dive. Two divers with small airway dysfunction before the dive had obstructive ventilatory dysfunction after the dive. Additionally, mild obstructive ventilatory dysfunction in three divers before the dive became severe after the dive. After a bronchial dilation test, five divers showed improvement of FEV_1, which ranged from 0.10 to 0.55 L. Chest radiographs and echocardiography of all divers were normal after diving.

Conclusion: 500 m simulated saturation diving induces a decrease in small airway function and diffusion function. This injury may be associated with small airway and diffusion membrane lesions.

Keywords: saturation diving, lung function, pulmonary diffusion function, forced vital capacity, forced expiratory volume in 1 s

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INTRODUCTION

Saturation diving is a diving method that allows people to be directly exposed to high-pressure environments to achieve long-term and large-depth work. It is also a cutting-edge technology for human beings to challenge ocean space and physiological limits. The characteristic of saturation dives are high density of the breathing gas and high ambient pressure. In the traditional view, a high density of the breathing gas and a high ambient pressure are the leading cause of lung function defects in divers (Skogstad et al., 1996; Tetzlaff and Thomas, 2017). Lung function defects recover soon after the end of the diving. A previous study showed no significant changes in lung function after 1–2 days and 6–8 weeks after a helium–oxygen mixed gas saturation dive to 2.5 MPa (Thorsen et al., 2006). However, in other studies, the divers still had lung function defects a long time after they finished diving. Lehnigk et al. found that a single saturation dive of 360 or 450 m impaired gas exchange at 0, 24, and 48 h after the dive (Lehnigk et al., 1997). Thorsen et al. showed that 5–450 m of sea water saturation diving decreased mid-expiratory flow and pulmonary diffusion function (Thorsen et al., 1994). However, the sample size of these studies was limited. Previous reported saturation diving depths of 5–450 m did not reflect the effects of deeper saturation diving on lung function. Some data were also not systematic and complete, which affected the research conclusions. Therefore, whether deep saturation diving causes injury to lung function remains controversial and the mechanism is unclear.

The 500 m saturation diving land-based manned experiment is the deepest simulated saturated diving test ever reported in China. During the experiment, the divers entered a pressurized chamber on land to simulate the deep ocean. The ambient pressure at depths of 500 m of sea water, corresponding to absolute pressures of 5.02 MPa. The present study aimed to evaluate the effects of a 500 m simulated single saturation dive on lung function.

METHODS

This retrospective study was conducted in the Pulmonary Function Room of Zhongshan Hospital, Fudan University. Nine divers who completed a 500 m simulated saturation dive in June 2021 were enrolled in the study.

The divers began to exercise 2 months before the diving test, and the amount of exercise was set according to the tolerance required in the diving process. The training predive included: 1) Physical training:jogging and other morning exercises, swimming pool training in the afternoon and strength training in the gym in the evening. 2) Specific training: Each diver underwent 20 times progressive compression and decompression exercises. After that, the diver completed a fitness test in a high-pressure environment. The schedule of diving included compression time 175 h, bottom time 176 h and decompression time 21 days. The pressurization rate slowed down as the simulated depth of the chamber increased. Each of the nine divers spent 176 h in a high-pressure environment simulating a depth of 500 m (51 atm, 5.02 Mpa) to complete various experiments. The breathing atmosphere was a mixture of oxygen and helium with the PO_2 at 0.4 bar.

Lung function tests were performed 1 day before diving and 3 days after the dive. Chest radiographs and Doppler echocardiography were performed 3 days after dive. The local ethics committee approved the study protocol.

The weight and height of the divers were measured while wearing indoor clothing. Lung function were investigated with an MS-PFT Spirometer (Jaeger, Germany). A trained operator demonstrated the required maneuvers to the divers before the tests. We recorded and analyzed the following pulmonary function parameters: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1/FVC, peak expiratory flow (PEF), forced expiratory flow at 25, 50, and 75% of exhaled FVC (FEF_{25}, FEF_{50}, and FEF_{75}), mid-expiratory flow (FEF25-75%), diffusion capacity of the lungs for carbon monoxide (DLCO), DLCO/unit of alveolar volume (DLCO/VA), residual volume (RV), vital capacity (VC), and total lung capacity (TLC). DLCO and TLC were determined by one breath dispersion method.

The detection of pulmonary function parameters and interpretation were followed in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) standardization (Culver et al., 2017; Graham et al., 2019). Obstructive ventilatory defect was defined as reduced FEV_1/FVC lower than lower limit of normal. The severity were defined by FEV_1%pred (FEV_1 measured value/FEV_1 predicted value); mild (FEV_1%pred > 70%), moderate (60% ≤ FEV_1%pred < 69%), moderately severe (50% ≤ FEV_1%pred < 59%), severe (35% ≤ FEV_1%pred < 49%), and very severe (FEV_1% < 35%pred)

TABLE 1 | Lung function test results from nine divers before dive.

Divers	Ages (ys)	Height (cm)	Weight (kg	FEV_1	FVC	FEV_1/ FVC	PEF	FEF_{25}	FEF_{50}	FEF_{75}	RV	TLC	RV/ TLC	DLCO	DLCO/ VA
1	41	171	80	98.4	116.8	69.28	93.00	65.40	58.50	52.60	133.53	113.70	133.53	132.53	106.35
2	34	177	91	103.7	122.8	69.97	97.50	73.10	64.50	56.40	152.49	123.80	152.49	118.59	84.42
3	33	174	74	103.4	117.8	74.03	103.10	96.30	63.50	49.70	118.81	112.14	118.81	106.06	86.63
4	28	182	95	85.0	91.0	77.44	89.01	78.89	63.53	56.89	102.46	89.02	102.46	107.64	105.68
5	53	189	90	111.1	122.3	74.52	125.36	113.68	85.05	53.57	131.38	119.91	131.38	129.12	93.26
6	31	179	67	124.1	119.8	89.18	126.43	124.69	140.84	109.18	108.53	111.53	108.53	138.82	114.38
7	34	175	83	115.8	116.2	83.35	122.25	135.83	111.04	89.05	120.33	113.65	120.33	107.85	84.97
8	34	170	63	108.6	112.4	82.48	130.14	107.78	108.54	77.04	101.72	103.88	101.72	98.27	90.35
9	35	167	78	103.9	101.6	85.06	113.86	135.70	134.88	83.29	83.46	93.91	83.46	94.48	94.51

[a]Lung function test parameters are presented as percentage of measured/predicted values, except for FEV1/FVC; Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; FEF_{25}, EF_{50}, and FEF_{75}, forced expiratory flow at 25, 50, and 75% of exhaled FVC; DLCO, diffusion capacity of the lungs for carbon monoxide; DLCO/VA, DLCO/unit of alveolar volume; RV, residual volume; VC, vital capacity; TLC, total lung volume.

(Pellegrino et al., 2005). If the subject did not meet the criteria of obstructive ventilatory defect, FEF25-75%, FEF 50% and FEF75% were used to evaluate the small airways function. when two of the above three indicators are below 65% prediction values, small airway dysfunction was defined (Graham et al., 2019).

Bronchodilation tests were performed on divers with obstructive airway dysfunction. At 15–20 min after inhaling salbutamol, a percentage change ≥12% and an absolute change ≥200 ml in FEV_1 compared with pre-bronchodilator values were considered as a positive bronchodilation test.

Statistical analysis and graphing were performed using GraphPad Prism software, version 7.0 (GraphPad Software, United States). The Kolmogorov–Smirnov test was used to analyze the normality of data. The paired *t*-test was used to compare the parameters. $p < 0.05$ was considered statistically significant.

RESULTS

Nine experienced saturation divers were included in this study. The mean age of the divers was 36 ± 7 years (range: 31–5 years), and all the divers were men. The mean height was 176 ± 7 cm and the mean weight was 80 ± 11 kg.

Pre-Dive Lung Function

The ventilatory function of all divers was appropriate before the dive. The mean values of FVC and FEV_1 were (5.33 ± 0.61) L and (4.16 ± 0.51) L, respectively. The mean percentages of the predicted values of FVC and FEV_1 were 113 ± 10.58% and 106 ± 11.03%, respectively. Among the divers, three (33.3%) had mild obstructive ventilatory function defect and two (22.2%) had small airway dysfunction. These three divers with mild obstructive ventilatory function defect had bronchodilation test. Absolute change in FEV_1 ranged from 0.15 to 0.33 L, and percentage change in FEV_1 varied from 3.54 to 9.30%. All had a negative bronchial dilation test.

All (100.0%) divers had a normal DLCO and DLCO/unit of alveolar volume before the dive (**Table 1**).

TABLE 2 | Changes in pulmonary function parameters between 3 days after the simulated dive and before the dive.

	Predive	3 days after Dive	*p* Value
FEV1 (L)	4.16 ± 0.51	4.04 ± 0.54	0.039
FVC (L)	5.33 ± 0.61	5.34 ± 0.65	0.479
FEV1/FVC	78.4 ± 7.0	75.6 ± 8.2	0.009
PEF (%)	111 ± 16	108 ± 19	0.273
FEF_{25} (%)	103 ± 27	95 ± 32	0.114
FEF_{50} (%)	92 ± 32	77 ± 27	0.010
FEF_{75} (%)	70 ± 21	63 ± 24	0.023
$FEF_{25\text{-}75}$ (%)	89 ± 27	77 ± 27	0.001
FET(s)	5.9 ± 1.4	5.5 ± 1.4	0.421
RV (%)	117 ± 21	115 ± 19	0.776
TLC (%)	109 ± 11	108 ± 9	0.902
RV/TLC (%)	114 ± 15	112 ± 17	0.758
DLCO(%)	115 ± 16	103 ± 12	0.020
DLCO/VA (%)	96 ± 11	87 ± 10	0.001
VC(%)	113 ± 11	114 ± 10	0.351

[a]*Lung function test parameters are presented as percentage of measured/predicted values, except for FEV_1/FVC; Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; FEF_{25}, FEF_{50}, and FEF_{75}, forced expiratory flow at 25, 50, and 75% of exhaled FVC; DLCO, diffusion capacity of the lungs for carbon monoxide; DLCO/VA, DLCO/unit of alveolar volume; RV, residual volume; VC, vital capacity; TLC, total lung capacity.*

Lung function Before and After the Dive

Three days after the simulated 500 m saturation dive, the nine divers performed a lung function test again. FEF_{50}, FEF_{75}, $FEF_{25–75\%}$, FEV_1/FVC, FEV_1, DLCO, and DLCO/VA were significantly decreased 3 days after the dive compared with those before the dive (all $p < 0.05$, **Table 2**). There were no significant differences in volume parameters (VC, RV, TLC, and RV/TLC) between after and before dive (**Figure 1**). FVC, PEF, and FEF_{25} were also not different between after and before the dive. A diver showed a large decrease in TLC (from 120 to 90% of the predicted value), while two divers showed a rather large increase (from 89 to 108%, from 94 to 105% of the predicted value) (**Figure 1**). Chest radiographs and Doppler echocardiography of all divers were normal after diving.

Three days after the dive, two divers with small airway dysfunction before the dive developed into mild obstructive ventilation defect; three divers with mild obstructive lung function defect before the dive still had mild obstructive lung function defect, the FEV_1%pred average of three divers decreased from (86.7 ± 1.9)% to (82.1 ± 3.7)%. After a bronchial dilation test, divers with obstructive ventilatory dysfunction showed an improvement in FEV_1. Absolute change in FEV_1 ranged from 0.10 to 0.55 L, and percentage change in FEV_1 varied from 2.53 to 17.46%. Two (40%) divers had a positive bronchial dilation test. After bronchodilation test, 3 (60%)divers returned to pre-dive FEV_1 levels.

DISCUSSION

We report the effect of simulating a 500 m saturation dive on lung function. We found that the divers showed decreased small airway function and decreased diffusion function.

In this study, the divers still had impaired lung function 3 days after diving, which is in contrast to previous studies (Thorsen et al., 2006). This finding suggests that, even though the divers had a normal density of the breathing gas and a normal ambient pressure, the injury to lung function was not fully recovered by 3 days after the dive. Therefore, the injury cannot be entirely attributed to a high density of the breathing gas and high ambient pressure.

revious studies have shown decreased FEV_1/FVC in divers who work at depths up to 340 m (Crosbie et al., 1979). In our study, a 500 m simulated saturation dive also resulted in a decrease in FEV_1/FVC. One explanation for the decreased FEV_1/FVC was associated with the increase in FVC. Several studies have shown that diving training contributes to a large VC (Crosbie et al., 1979; Thorsen et al., 1993; Thorsen et al., 1994). VC and FVC did not significantly change between before and after diving in our study, which indicated that the divers in our study had fully simulated pre-dive training, and that pre-dive breathing work had been increased to the same level as that during the simulated dive. Therefore, the decrease in the FEV_1/FVC

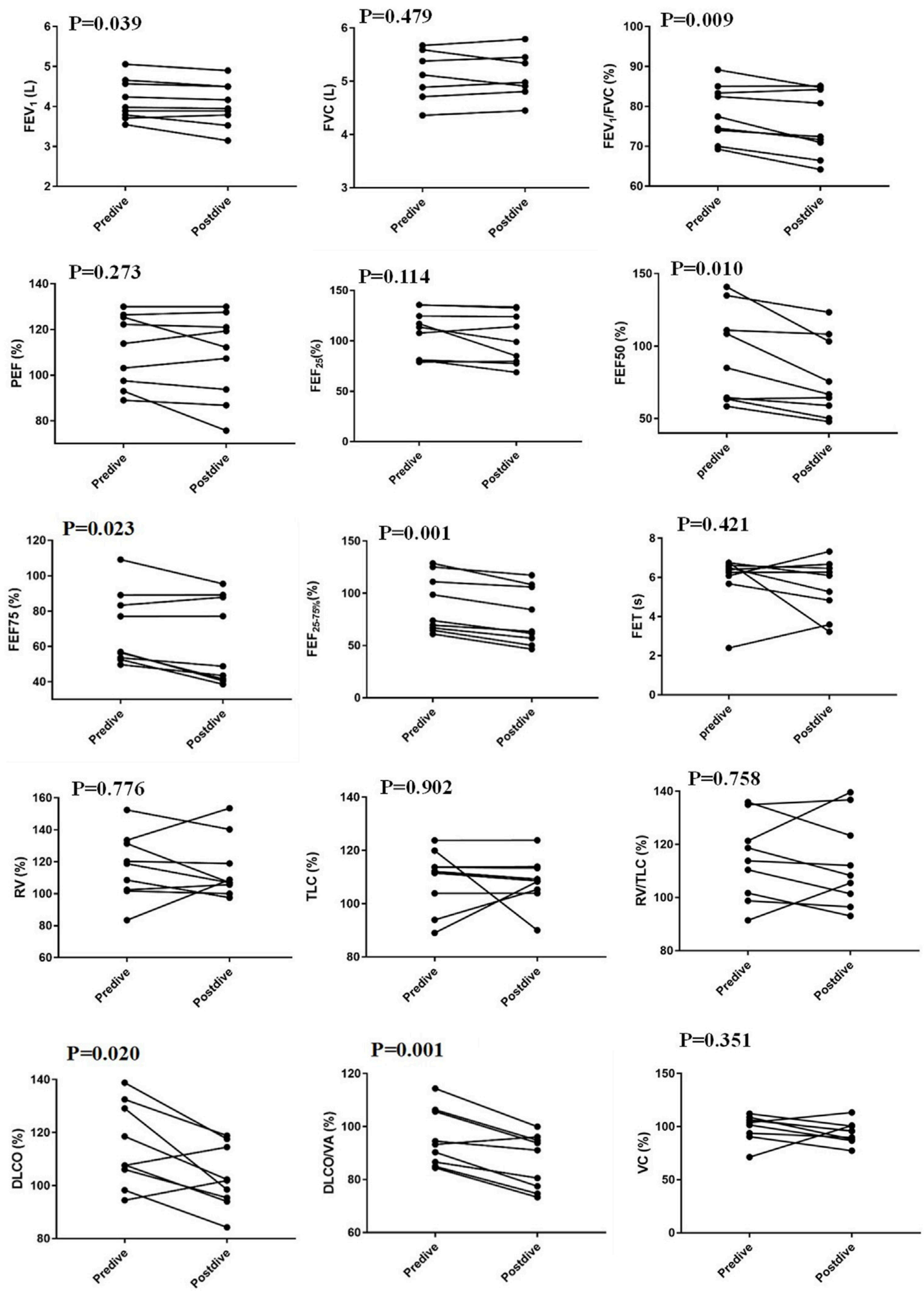

FIGURE 1 | Changes in pulmonary function parameters between 3 days after a simulated dive and before the dive. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; PEF, peak expiratory flow; FEF25, FEF50, and FEF75, forced expiratory flow at 25%, 50%, and 75% of exhaled FVC; DLCO, diffusion capacity of the lungs for carbon monoxide; DLCO/VA, DLCO/unit of alveolar volume; RV, residual volume; VC vital capacity; TLC, total lung capacity; FET, forced expiratory time.

ratio in this study was not related to the changes in FVC. Additionally, in our study, force-dependent parameters (PEF and FEF_{25}) did not significantly change before and after diving, which reflect that the lung function parameters were comparable before and after diving. Previous study found the decrease of FVC after deep sea dives using a closed-circuit Rebreather (CCR) (Dugrenot et al., 2021), which was different from our result. There were important differences between these two study. The diving depths of the two studies varied widely, at 500 m and around 100 m respectively. The 100-m dive is relatively easy to complete; while 500 m saturated diving is very difficult because of the huge variation in pressure of alveolar gas and tissue dissolved gas. The decompression was slower and performed in a dry chamber in our study, while CCR dives decompression was in water and relatively rapid. The partial pressure of oxygen was 0.4 bar (40 kpa) during saturation dives in our study, against 120–160 kPa in CCR dive. Different partial pressure of oxygen produced different concentrations of reactive oxygen species, which may be the reason for the increase of extravascular lung water during CCR diving. Therefore, hyperbaric oxygen during CCR diving may induce a decrease in FVC, but have little effect on saturated diving.

In this study, the pulmonary function parameters reflecting small airway function (FEF_{50}, FEF_{75}, and forced mid-expiratory flow) were decreased 3 days after the dive compared with those before the dive. The simultaneous reduction in FEV_1/FVC and end-expiratory flow were an indication of obstructive of changes in the airway. In our study, the divers with a small airway dysfunction before the dive developed obstructive ventilatory dysfunction after the dive. Additionally, the divers with mild obstructive ventilatory dysfunction before the dive showed decreased of FEV1%pred after the dive, which support the obstructive airway changes either. Excluding the effect of a change in lung volume and perceived exertion, we speculate that the small airway dysfunction may have been related to small airway lesions during the deep dive.

In previous study, saturation diving reduced lung diffusion capacity. Lehnigk found normal DLCO, elevated VA, and decreased DLCO/VA after a single saturation dive of 360 m or 450 m[4]. The reduction in DLCO/VA after the dive may have been due to an elevation in VA (Sames et al., 2018). In our study, the 500 m simulated saturation dive decreased both DLCO and DLCO/VA. The divers in this study had normal lung function or only a mild obstructive lung function defect before the dive. None of the divers had any significant differences in lung volume before and after diving. Additionally, no pulmonary vascular diseases were found in any of the divers by echocardiography. The decrease in diffusion function could have been due to a lesion of diffusion membranes.

Usually, the small airway lesions and the diffusion membranes lesion was synchronous with lung parenchyma injury. In this study, the pressure of oxygen in breathing atmosphere was dynamically set at about 0.4 bar during the diving. In this situation, the incidence of oxygen poisoning was rare. At high ambient pressure, the amount of oxygen free radicals increased. It was not clear whether the increase of oxygen free radicals induced lung parenchymal injury, which can be clarified in future studies. Pulmonary oxygen toxicity (POT) index in our study we calculated using Equation ($t^2 \times PO_2^{4.57}$, where t is the time in h, and PO_2 is the partial pressure of oxygen in bar) was 470 (Arieli, 2019). The levels of POT was associated with the duration of lung function impairment after saturated diving. The higher level of POT may be the reason for the small airway lesions and the diffusion membranes lesion 3 days after diving in our study.

A bronchial dilation test in five divers with obstructive ventilator dysfunction 3 days after the simulated dive showed varying degrees of improvement in FEV_1. This finding suggested that peripheral airway lesions may include spasm of airway smooth muscle, but this is not completely reversible. Bronchodilators may be an effective method of ameliorating lung function defects induced by deep saturation diving.

TLC was indirectly detected by one breath dispersion method, which was influenced by many factors. In subjects with no or mild obstructive lung function defect, the trend of FVC, VC and TLC was consistent. VC and FVC were determined directly with good reliability. So, the reliability of research conclusions is not affected.

There are some limitations to this study. First, because only nine divers participated in the 500 m saturation dive, the limited sample size may have affected the reliability of the conclusions. Second, because of the limitations of research ethics and research conditions, a pulmonary function test was not performed underwater, which would directly reflect the effect of saturation diving on lung function. Finally, we hypothesized that deep saturation diving induced small airway and diffusion membrane lesions, which is just based on lung function tests. However, because of ethical limitations, no pathological examination was conducted.

In conclusion, deep saturation diving induced a decrease in small airway function and diffusion function. The injury maybe associated with small airway and diffusion membrane organic lesion.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion 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 committee of Fudan Medical university, No.B-2019-248. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LZ design study and revised the manuscript, NL analysis data and prepare the manuscript, LH, SW, LX, LL, YY, and YG collect data, SW and LX search the literature and analysis data, LZ reviewed the results and made critical comments on the manuscript.

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Predicting Adverse Events During Six-Minute Walk Test Using Continuous Physiological Signals

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Background and Objective: The 6-min walk test (6MWT) is a common functional assessment test, but adverse events during the test can be potentially dangerous and can lead to serious consequences and low quality of life. This study aimed to predict the occurrence of adverse events during 6MWT, using continuous physiological parameters combined with demographic variables.

Methods: 578 patients with respiratory disease who had performed standardized 6MWT with wearable devices from three hospitals were included in this study. Adverse events occurred in 73 patients (12.6%). ECG, respiratory signal, tri-axial acceleration signals, oxygen saturation, demographic variables and scales assessment were obtained. Feature extraction and selection of physiological signals were performed during 2-min resting and 1-min movement phases. 5-fold cross-validation was used to assess the machine learning models. The predictive ability of different models and scales was compared.

Results: Of the 16 features selected by the recursive feature elimination method, those related to blood oxygen were the most important and those related to heart rate were the most numerous. Light Gradient Boosting Machine (LightGBM) had the highest AUC of 0.874 ± 0.063 and the AUC of Logistic Regression was AUC of 0.869 ± 0.067. The mMRC (Modified Medical Research Council) scale and Borg scale had the lowest performance, with an AUC of 0.733 and 0.656 respectively.

Conclusion: It is feasible to predict the occurrence of adverse event during 6MWT using continuous physiological parameters combined with demographic variables. Wearable sensors/systems can be used for continuous physiological monitoring and provide additional tools for patient safety during 6MWT.

Keywords: 6-min walk test, adverse events, machine learning, wearable devices, physiological signals

INTRODUCTION

The 6-min walk test (6MWT) is a submaximal, simple, low-cost, and effective exercise test used to obtain the functional capacity of patients with moderate to severe cardiopulmonary disease (Solway et al., 2001; American Thoracic Society, 2002). The 6-min walk distance (6MWD) is one of the key observations in clinical trials, and can be used as a predictor of diagnosis, prognosis and survival of patients with cardiopulmonary diseases, such as chronic obstructive pulmonary disease (COPD) (Casanova et al., 2008; Dajczman et al., 2015; Meena et al., 2020), interstitial lung diseases (Brown and Nathan, 2018), pulmonary hypertension (Farber et al., 2015; Gadre et al., 2017) and lung transplant (Castleberry et al., 2017).

6MWT has been used as a part of the standard procedure for cardiopulmonary function assessment and is considered to be safe for most patients. However, given the complexity of respiratory disease and the severity of the disease, adverse events during the 6MWT are still potentially dangerous.

Jenkins and Cecins (2011) suggested to revise the American Thoracic Society (ATS) 6MWT guidelines to monitor SpO_2 continuously during 6MWT, because oxygen desaturation may increase the possibility of cardiac or other complications. 3.9% of patients with acute myocardial infarction (AMI) suffered angina, drop in blood pressure, or ventricular tachycardia (Diniz et al., 2017). Roberts et al. (2015) reported that there were lower quality of life and mood scores among patients who experienced adverse events compared with patients without adverse events. Serious adverse events may lead to the death of inpatients, the occurrence of life-threatening events, or the extension of the current hospital stay (Morris et al., 2015). Therefore, it is particularly important to be able to predict adverse events simply and quickly. In addition, since 6MWT is closer to daily life than lung function tests to assess the patient's overall performance, the prediction of adverse events in 6MWT is also instructive for early warning in routine daily monitoring.

However, traditionally, the occurrence of adverse events is mainly judged by medical staff's observation on patient's performance and the patient's complaint. Moreover, ATS guidelines (2002) and some studies (Roberts et al., 2015; Douwes et al., 2016) only used oximeters to monitoring oxygen saturation intermittently. This type of subjective judgments and short-term data monitoring of physiological parameters are not very useful for rapid identification of adverse events. Variation in physiology parameters can reflect changes in the state of the human body, even hours before an adverse event occurs (Brekke et al., 2019; Leenen et al., 2020; Yilmaz et al., 2020). Rodríguez et al. (2017) reported that heart rate recovery at 1 min (HRR1) after 6MWT is an independent predictor factor for acute exacerbation of COPD (AECOPD). Morita et al. (2018) reported that HRR1 may reflect the patient's exercise capacity, lifestyle and functional status. Mazzuco et al. (2017) reported that heart rate variability (HRV) can be useful in the functional assessment of COPD. The emergence of wearable physiological parameter monitoring systems provides the technical means for continuous and non-invasive dynamic monitoring of physiological parameters during 6MWT.

TABLE 1 | Baseline demographic data.

Variable	Value
Demographics	
Number	578
Gender (male), *n* (%)	435 (75.3)
Age (years), mean (SD)	62.2 (11.1)
Height (m), mean (SD)	163 (6.94)
Weight (kg), mean (SD)	61.7 (12.4)
BMI, mean (SD)	23.1 (3.9)
6MWD (m), mean (SD)	413.3 (101.3)
Principal Diagnosis, *n* (%)	
COPD	228 (39.4)
Pneumonia	48 (8.3)
Bronchiectasis	77 (13.3)
Asthma	76 (13.1)
Lung cancer	59 (10.2)
Pulmonary fibrosis	52 (9.0)
Pulmonary arterial hypertension	24 (4.2)
Pulmonary embolism	14 (2.4)
Scales, mean (SD)	
Borg	0.6 (1.0)
mMRC	1.2 (1.3)
Adverse events, *n* (%)	
Intolerable dyspnea	66 (11.4)
Chest pain or tightness	17 (2.9)
Lack of physical strength	14 (2.9)
Palpitation	10 (1.7)
Dizziness	5 (0.9)
Oxygen inhalation	5 (0.9)

BMI, body mass index; 6MWD, 6-min walk distance; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; mMRC, modified medical research council.

In order to reduce the risk of patients during 6MWT and remind nurses to pay attention to high-risk groups, this study aimed to predict the occurrence of adverse events during 6MWT through continuous dynamic physiological parameters monitored by wearable devices.

METHODS

Patients

The multi-center study was conducted at Chinese PLA General Hospital, Hainan Hospital of Chinese PLA General Hospital and the Second Xiangya Hospital of Central South University. Patients with respiratory disease who undertook 6MWT with wearable devices from June 2019 to September 2020 were included. Exclusion criteria: 1) patients with comorbid neurological and muscular diseases or limited daily activities. 2) Patients with missing or poor-quality signals and those who did not complete 6MWT as required by the protocol. 3) Patients with unstable angina or myocardial infarction within 1 month. The study was approved by the Medical Ethics Committee (Ethics No.: S2018-095-01, Clinical Trail No.: ChiCTR-POC-17010431).

578 patients were included in this study. Patients' characteristics are shown in **Table 1**. The mean age was 62.2 ± 11.1 years old. The mean BMI was 23.1 ± 3.9. There were 39.4% of the patients who had COPD, the most common disease among the patients. 81 patients were combined with

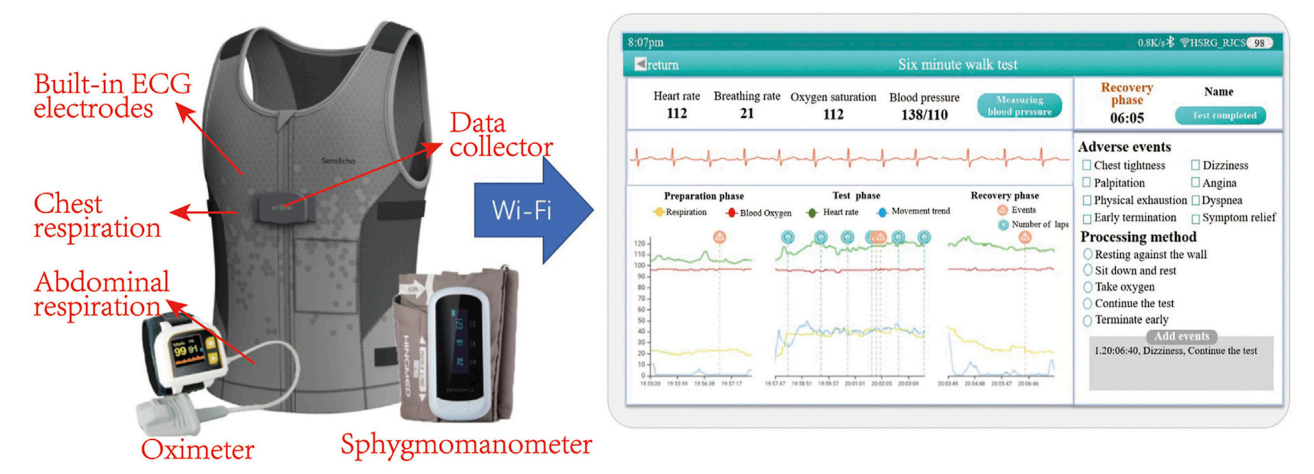

FIGURE 1 | Picture of multi-functional 6MWT system, including wearable devices and an intelligence terminal device (a hand-held PAD). Wearable devices include a flexible vest, an oximeter and a blood pressure monitor. The system is able to monitor ECG, respiratory waves, triaxial acceleration, oxygen saturation and blood pressure. The physiological signal can be transmitted to the PAD *via* Wi-Fi and displayed in real time.

hypertension. 38 patients were combined with coronary heart disease. 31 patients were combined with diabetes. The mean score on Borg fatigue score was 0.6 ± 1.0. The mean score on modified Medical Research Council (mMRC) dyspnea score was 1.2 ± 1.3.

Multi-Functional 6-Min Walk Test System

In this study, data were collected by using a sensor-based system, which achieves physiological signal monitoring and recording during the whole process of 6MWT. The system consists of two key components as shown in **Figure 1**: the flexible vest and the intelligence terminal device (i.e., a hand-held PAD). The flexible vest is our self-developed wearable device, SensEcho, which is able to provide ECG, respiratory waves and triaxial acceleration signals, and to communicate with third-party devices such as blood pressure monitor and oximeter. The flexible vest is connected with ECG electrodes, which can collect single-lead ECG signal. Two elastic bands are embedded in the chest and abdomen positions respectively using respiratory induction plethysmography (RIP) technology, which allow accurate recording of chest and abdominal breathing movement. The triaxial acceleration sensor is built into the data collector. Oxygen saturation and blood pressure were measured by oximeter and sphygmomanometer respectively.

The real-time physiological signals can be displayed on the terminal device, and the system provides the functions of early warning based on physiological signal monitoring and manually recording the adverse events, such as hypoxia or dyspnea. Moreover, the system is able to generate a report which summarizes the performance of the patient in the 6MWT by comprehensive analysis of physiological parameters and upload the overall data of 6MWT process to the cloud sever.

6-Min Walk Test Protocol

All hospitals followed the same 6MWT protocol. About 10 min before the 6MWT, the patient put on the SensEcho device and had a rest. Medical staff opened the application on the PAD and connected the terminal to measure basic physiological parameters such as heart rate, breath rate, blood pressure and oxygen saturation remotely. Meanwhile, patients were instructed to complete Borg fatigue score, mMRC dyspnea scale on the PAD.

The 6MWT was carried out in a 30-m-long flat corridor in accordance with the recommended ATS scheme. Once the patient started walking on the start line, a timer was activated and patients should walk as far as possible for 6 min. At 1-min intervals, the app on the pad encouraged the patient in a standardized way. Medical staff observed the patient's ECG, respiration, and oxygen saturation on the PAD in real time and recorded the laps. If an adverse event occurred during the 6MWT, the event and the treatment would be recorded on the PAD in time. Patients can have a rest or stop the test at any time. At the end of 6MWT, patients would be asked to stop and rest against the wall for 2 min before taking off the device and 6MWD was recorded.

Adverse Events

According to ATS guidelines and previous studies, symptoms such as intolerable dyspnea, palpitation, dizziness, chest pain or chest tightness, fatigue and hypoxia during 6MWT were considered as adverse events (American Thoracic Society, 2002; Jenkins and Cecins, 2011; Roberts et al., 2015).

73 patients (12.6% of total cohort) experienced adverse events. 10 patients have prematurely terminated the test due to intolerable dyspnea or oxygen therapy. As shown in **Table 1**, the most common adverse event was intolerable dyspnea (66 patients). The mean distance of all patients was 413.3 ± 101.3 m. The mean distance walked by patients who developed adverse event was 278.1 ± 132.1 m. The mean distance walked by patients without adverse events was 444.0 ± 90.9 m. When a patient had more than one adverse event during 6MWT, we adopted the time of the first occurrence. The time distribution of adverse events among patients was shown in **Figure 2**. It could be seen that

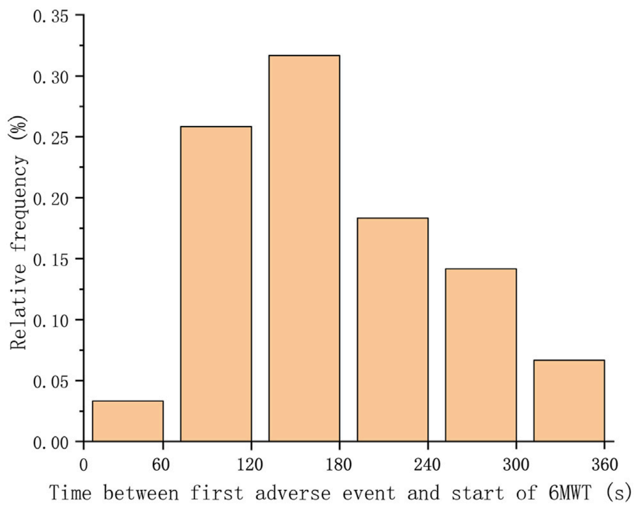

FIGURE 2 | Distribution of the time between the occurrence of the first adverse event and the beginning of the 6MWT. Adverse events of over 95% of patients occurred after 1 min.

approximately 95% adverse events occurred 1 min after the beginning of 6MWT. Only four patients had an adverse event within 1 min and the adverse event they had was intolerable dyspnea.

Data Pre-Processing and Feature Extraction

Firstly, the raw ECG and respiratory signals were smoothed and filtered and outliers were removed. Then, Hamilton's method (Hamiltom and Tompkins, 1986) was used to detect R peaks of ECG signal. Khodadad's method (Khodadad et al., 2018) was used to detect peaks and valley value of respiratory signal.

55 features were divided into five categories, including ECG signal features, respiratory signal features, SpO_2 features, motion amplitude features and demographic features. We selected resting segment data of the 2 min before 6MWT to calculated the average resting heart rate (HR_{rest}), respiratory rate (RR_{rest}), SpO_2 ($SpO_{2\ rest}$) and HRV parameters (time-domain parameters, frequency domain parameters and nonlinear-domain parameters). Few adverse events occurred in the first minute, so the first minute data during 6MWT was included to calculate features. The peak heart rate (HR_{peak}), peak respiratory rate (RR_{peak}), minimum SpO_2 ($SpO_{2\ min}$) and mean heart rate (HR_{mean}), respiratory rate (RR_{mean}) and SpO_2 ($SpO_{2\ mean}$) during the first minute of 6MWT were calculated. Then, the difference between corresponding features in resting phase and 6MWT process was calculated.

In addition, HR slope and HR intercept were respectively defined as the slope and intercept of line that fits the beat-by-beat HR of the first minute 6MWT process by the least square method. The oxygen desaturation area was defined as the sum of the difference between the baseline of oxygen saturation and the oxygen saturation per second during the 6MWT process (Flaherty et al., 2006). Demographic features included age, gender and BMI. Motion amplitude feature, as shown in following, was calculated by the triaxial acceleration signal, transforming the raw x, y, and z acceleration data into signal vector magnitude (SVM) data (Bidargaddi et al., 2007).

$$SVM = \sqrt{\sum_{k=0}^{n} \left(X_k^2 + Y_k^2 + Z_k^2\right)}$$

In the above formula, n is the product of the sampling rate and the recording time. X_k is a vector representing the acceleration along X axis, while the other axes are represented by vectors of Y_k, and Z_k, respectively.

Models

First, the recursive feature elimination method based on Logistic Regression with L1 and L2 regularization was used to select the best features to prevent overfitting. Then, 5-fold cross validation was used to train and test the models including Logistic Regression, Support Vector Classifications (SVCs), Random Forest, eXtreme Gradient Boosting (XGBoost) and Light Gradient Boosting Machine (LightGBM) with the selected features. For comparison, we also calculated scales' ability to predict adverse events, including mMRC, Borg before 6MWT. The predictive ability of the models and scales were evaluated through ROC curve and area under the curve (AUC). In addition, sensitivity, specificity, positive likelihood ratio and negative likelihood were also calculated. Comparisons among machine learning models were provided as well.

RESULTS

Adverse Events

Figure 3 showed the trend of physiological signals and the occurrence of adverse events in a patient during the 6MWT process. When the patient started walking, the heart rate and breath rate increased, while oxygen saturation dropped rapidly. There were two adverse events during the 6MWT, the second of which was severe intolerable dyspnea and the patient received oxygen therapy after that.

Model Performance

Combining the characteristics of the resting phase and the movement phase, the AUC reached the maximum value when 16 features were selected.

The ROC curves comparing machine learning models using Logistic Regression, SVCs, Random Forest, LightGBM and XGBoost and scales using mMRC and Borg were presented in **Figure 4**. The ROC curve was the average result of 5-fold cross-validation. As shown in **Figure 4**, machine learning models performed better than traditional scales. Among models, the machine learning model with the best performance was LightGBM, with a mean AUC of 0.874, while the mMRC scale and Borg scale had the lowest performance, with an AUC of 0.733 and 0.656 respectively.

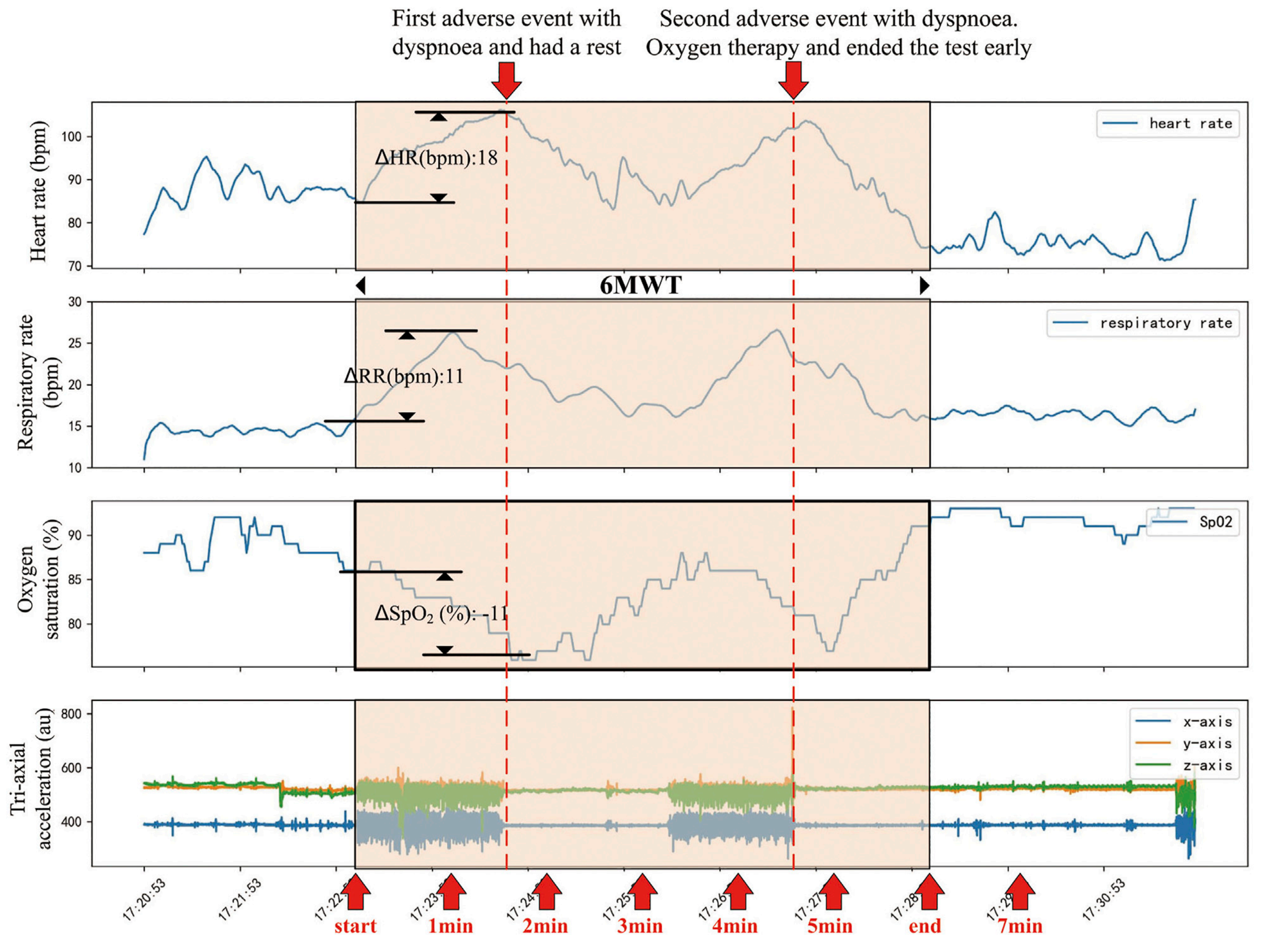

FIGURE 3 | An example of changes in physiological parameters of a patient before, during and after the 6MWT process. This patient had a rapid increase in heart rate and respiratory rate while a rapid drop in oxygen saturation following the beginning of 6MWT. About 90 s after the beginning of 6MWT, the patient had a dyspnea and had a rest. During the rest period, the patient's heart rate, respiratory rate and oxygen saturation recovered. About 280 s after the beginning of 6MWT, the patient developed a more severe intolerable dyspnea and was treated with oxygen. The 6MWT was terminated about 80 s early.

The classification results comparing different machine learning models using 5-fold cross-validation were presented in **Table 2**. The performance of LightGBM was stable and the best among machine learning models, with the highest AUC and the smallest standard deviation. The model with the highest positive likelihood ratio was SVC (linear kernel), and the model with the lowest negative likelihood ratio was LightGBM. Feature importance ranking in lightGBM model was shown in **Figure 5**.

DISCUSSION

The multifactorial complexity of diseases and the human body makes it challenging to predict adverse events during 6MWT. As far as we know, many studies have focused on the prediction of 6MWD and the relationship between 6MWD and diseases. However, there are few studies predicting adverse events with continuous physiological parameters combined with easily measurable demographic variables, possibly due to a lack of powerful tools. The combination of wearable physiological parameter monitoring devices and interpretable artificial intelligence can help solve these problems. Machine learning algorithms are able to recognize patterns within data. A data model built on the learned patterns from the training dataset predicts the results of the unseen test dataset. Similar to a human learning process, machine learning algorithms become more powerful with more increased data and experience. This study provides an assistive decision support tool for whether patients need additional medical help during 6MWT. At the same time, this study also provides new ideas and methods assessing the safety and security of patients during 6MWT.

High percentage of adverse events occurrence from the first minute is an objective fact found in the large sample of this study. As for the reasons, the 6MWT is a sub-maximal exercise test that assesses functional capacity by measuring the total distance walked in 6 min. The activity level in the first minute may not be enough to cause adverse events. On the other hand, changes in

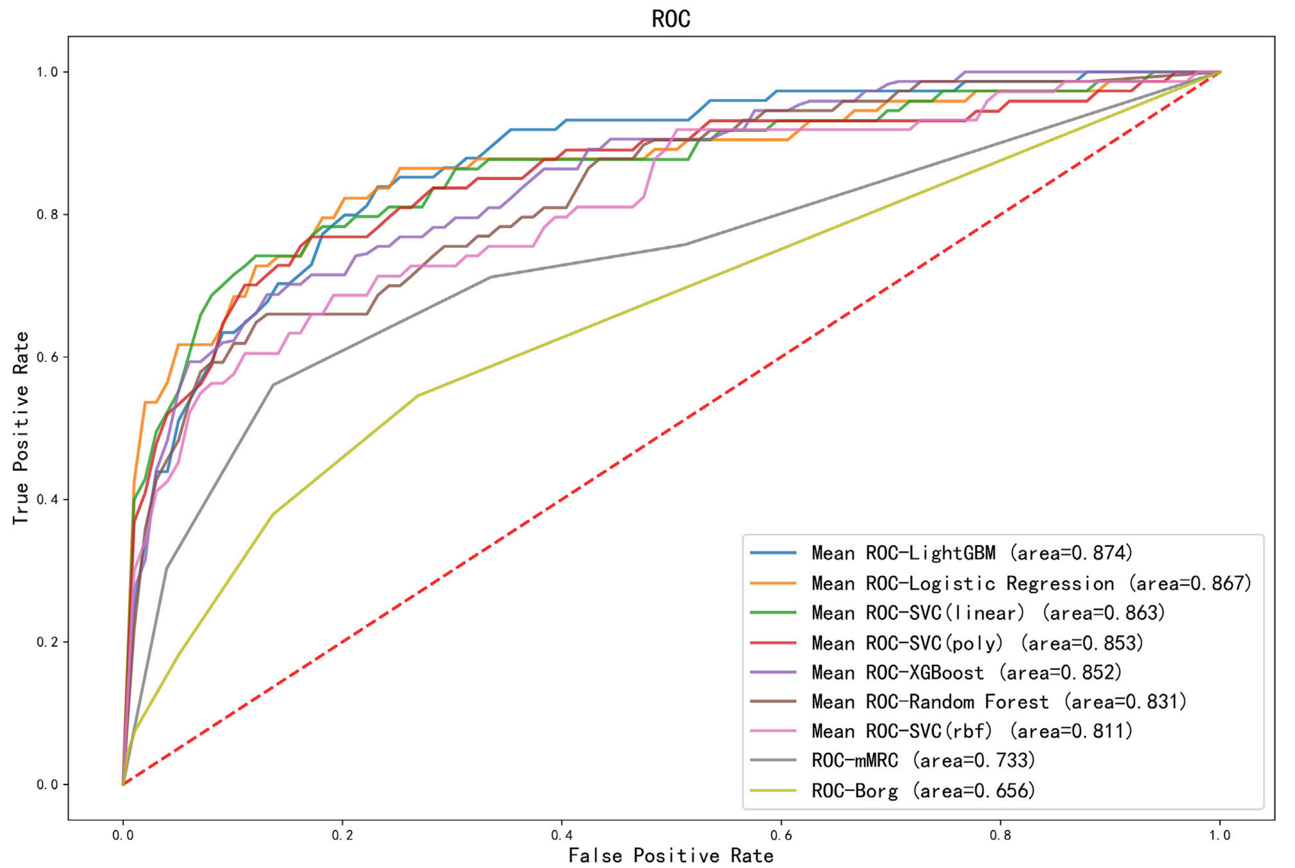

FIGURE 4 | ROC curves for different machine learning models and scoring scales. The ROC curves for machine learning models were the average score of the results of a five-fold cross-validation. AUC results of all machine learning models outperformed the scales. SVC, support vector classification; LightGBM, light gradient boosting machine; XGBoost, extreme gradient boosting; mMRC, modified medical research council dyspnea scale.

physiological parameters may precede the onset of symptoms (Brekke et al., 2019).

Scales are the most frequently used method to assess the patients' physical condition. However, the scale method is easily affected by individual subjectivity. Physiological parameters can be used for objective and quantitative assessment of physical state. As shown in **Figure 3**, physiological parameters changed significantly before and after 6MWT and adverse events. As shown in **Figure 4**, the predictive ability of the combination of machine learning and physiological parameters was significantly better than the predictive ability of scales. To further improve the 6MWT assessment, a physiological parameters-based assessment scale similar to the NEWS score (national early warning score, to identify patients with high risk of an acute and unstable disease) could be established as a later research direction.

Among the selected features, heart rate related features and HRV features are the most common ones, which are often ignored in clinical researches. Heart rate and HRV are regulated by sympathetic nerves and parasympathetic nerves, which is a reliable reflection of the many physiological factors modulating the normal rhythm of the heart. Therefore, heart rate and HRV can reflect changes in the body's state more accurately (Acharya et al., 2006). Moreover, the resting heart rate is relatively low in people with better cardiopulmonary function, so HR_{rest} is also an important indicator for predicting adverse events. The increase in heart rate within 1 min represents the heart's ability to respond to exercise (Cannière et al., 2020). HR intercept, another expression of HR_{rest}, has the advantage of being less affected by abnormal values. As for HRV, total power density spectral is an important predictor. High-frequency HRV reflects rapid changes in beat-to-beat variability caused by the activity of parasympathetic nerve (vagal nerve) (Ziemssen and Siepmann, 2019). Patients with chronic respiratory disease, especially those with COPD and idiopathic pulmonary fibrosis (IPF), have an increased respiratory rate (Schertel et al., 2017; McKinstry et al., 2018). Therefore, RR_{rest} is also a reflection of cardiopulmonary function. To our surprise, the change in respiratory rate during walking is not a significant predictor. Although studies have shown that asymptomatic exertional hypoxemia is not associated with an increase in the incidence of complications or adverse events during the 6MWT (Roberts et al., 2015; Afzal et al., 2018), in our study, the decreased oxygen saturation values and the oxygen desaturation area are still important for predicting adverse events. The SVM is an indicator to measure the intensity of physical activity, and it reflects the exercise

TABLE 2 | Comparison between different machine learning models validated by 5-fold validation.

	AUC	SN	SP	+LR	−LR
LightGBM	0.874 ± 0.063	0.859	0.768	3.616	0.184
Logistic Regression	0.868 ± 0.067	0.837	0.778	3.770	0.210
SVC (linear)	0.863 ± 0.070	0.742	0.879	6.132	0.294
SVC (poly)	0.853 ± 0.085	0.769	0.829	4.497	0.279
XGBoost	0.852 ± 0.071	0.688	0.869	5.252	0.359
Random forest	0.831 ± 0.070	0.66	0.869	5.038	0.391
SVC (rbf)	0.811 ± 0.098	0.687	0.808	3.578	0.387

AUC, area under the curve; SN, sensitivity; SP, specificity; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

performance during 6MWT. Patients with poor exercise capacity are more likely to have adverse events.

Currently, 6MWT is widely used in clinical practice. However, the entire 6MWT process presents the following risks and shortcomings. First, 6MWT is mostly performed in patients who are post-surgical or have severe cardiopulmonary disease, which poses the test a certain risk. This risk will increase due to the lack of objective monitoring throughout the test (Morris et al., 2015). Second, with the highly developed information technology, evaluation of a patient's cardiopulmonary function by a single indicator (6MWD) will inevitably leave out a large amount of high-value information. Moreover, the 6MWD is extremely sensitive to methodology (Agarwala and Salzman, 2020) and the same 6MWD evaluation approach and grading criteria may not be applicable in the different test site and population (Dourado, 2011). It is reported that differences in the performance of individuals on the 6MWT within Brazil and abroad still existed, and it is necessary to provide specific calculations and evaluation methods for each population and ethnic group (Dourado, 2011). The sensor-based 6MWT is therefore particularly important. The appearance of wearable physiological monitoring systems has greatly facilitated the application of 6MWT, ensuring the safety of the test and making full use of the data. In addition, the sensor-based 6MWT offers patients a personalized approach to cardiopulmonary function assessment.

An advantage of wearable devices is that they can provide mobile and ubiquitous health monitoring in daily life. The model proposed in this paper can be applied to daily life monitoring, especially to identify people at high risk of adverse events because their quality of life and mood scores are worse (Roberts et al., 2015). In addition to adverse events during 6MWT, there are few studies on the association of physiological parameters during 6MWT with adverse events in other scenarios. For example,

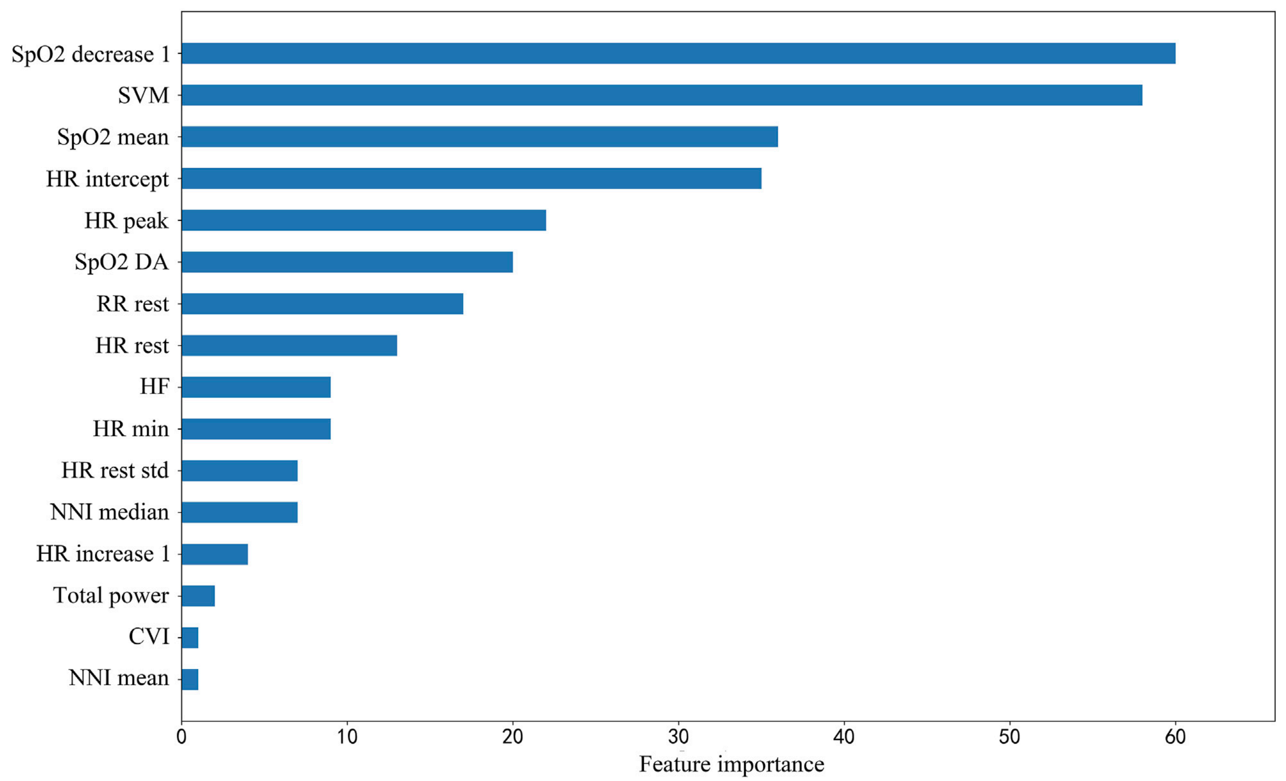

FIGURE 5 | Feature importance ranking in lightGBM model. SpO2_decrease1, Spo2 decrease value in 1 min; SVM, signal vector magnitude; SpO2 mean 1, mean value of SpO2 in first 1 min; HR intercept, heart rate intercept; HR peak, peak heart rate; SpO2 DA, SpO2 desaturation area; RR rest, breath rate during rest segment; HR rest, heart rate during rest segment; HF, high frequency power, one of HRV parameters; HR min, minimum heart rate; HR rest std, standard deviation of beat-to-beat heart rate during rest segment; HR increase 1, heart rate increase value in 1 min; Total power, one of HRV parameters; CVI, cardiac vagal index, one of HRV parameters; NNI mean, mean value of normal-to-normal intervals.

postoperative pulmonary complications (PPCs) are significant causes of increased length of stay and medical costs, poor prognosis, and death in patients undergoing heart valve surgery. Although 6MWT results have been shown to be associated with adverse prognostic events and risk of death (Casanova et al., 2008), its application to predict PPCs has not been reported. This study has a high reference value for this direction.

There are still some limitations in this study. Firstly, in order to simplify the models, improve the practicality and reduce the burden on healthcare professionals, the patient's spirometry values, comorbidities, lab tests and medication were not included in the model and it can be expected that the inclusion of the above parameters would help to further improve the model performance. Secondly, the diseases of the patients included in this study were complex and the probability of adverse events varied from patients to patients with different diseases. However, from another perspective, the complexity of the disease in multi-center patients made the models had a certain degree of extrapolation. Thirdly, due to the sample size, we treated adverse events as an overall outcome. In the follow-up study, we will expand the sample size and focus on each outcome independently. Meanwhile, with the increased sample size, the performance of the model will also be improved and robust.

Adverse events during 6MWT can be dangerous. Our study predicted the occurrence of adverse events using continuous physiological parameters collected by the sensor-based 6MWT system from multi-center patients during the 6MWT. This study provides additional safety for the patients and offers new methods and ideas for patient monitoring during 6MWT.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because patient privacy needs to be protected. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Chinese PLA General Hospital S2018-095-01. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: JW, HX, and ZZ, data curation: JW, YZ, QW, and YS, formal analysis: JW and YZ, funding acquisition: ZZ, investigation: YZ, QW, and YS, methodology: JW, JZ, YL, and ZZ, resourse: YL, SC, and ZZ, writing—original draft: JW and YZ, writing—review and editing: JW, YZ, and ZZ.

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Body composition and cardiorespiratory fitness in overweight or obese people post COVID-19: A comparative study

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The present study aimed to evaluate the body composition and cardiorespiratory fitness of overweight or obese people after COVID-19. 171 volunteers of both sexes (men, $n = 93$ and women, $n = 78$) between 19 and 65 years old were allocated into three groups according to the severity of their symptoms of COVID-19: non-hospitalized people/mild symptoms ($n = 61$), hospitalized ($n = 58$), and hospitalized in an intensive care unit-ICU ($n = 52$). Two laboratory visits were carried out 24 h apart. First, a medical consultation was carried out, with subsequent measurement of body weight and height (calculation of body mass index) and body composition assessment via electrical bioimpedance. After 24 h, a cardiorespiratory test was performed using the Bruce protocol, with a direct gas exchange analysis. Hospitalized individuals had significantly higher values for fat mass and body fat percentage than non-hospitalized individuals ($p < 0.05$). Significantly higher values were found for heart rate (HR) and peak oxygen consumption (VO_2peak) for individuals who were not hospitalized when compared to those hospitalized in the ICU ($p < 0.05$). Significantly higher values for distance, ventilation, and the relationship between respiratory quotient were found for non-hospitalized individuals compared to hospitalized individuals and those in the ICU ($p < 0.05$). After the cardiorespiratory test, higher values for peripheral oxygen saturation (SpO_2) were observed for non-hospitalized individuals than for all hospitalized individuals ($p < 0.05$). Diastolic blood pressure was significantly higher at the tenth and fifteenth minute post-Bruce test in hospitalized than in non-hospitalized participants ($p < 0.05$). Based on these results, proposals for cardiopulmonary rehabilitation are indispensable for hospitalized groups considering the responses of blood pressure. Monitoring HR, SpO_2, and blood pressure are necessary during rehabilitation to avoid possible physical complications. Volume and intensity of exercise prescription should respect the physiologic adaptation. Given lower physical conditioning among all the groups, proposals for recovering from health conditions are urgent and indispensable for COVID-19 survivors.

KEYWORDS

exercise test, outcome and process assessment, health care, delivery of health care, physical fitness, COVID-19, obesity

Introduction

Over the past 2 years, the COVID-19 pandemic has been a cause of significant global morbidity and mortality (Hopkins, 2022). The short- and long-term impacts of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection have become a challenge for promoting health recovery actions for COVID-19 survivors. Symptoms of the post-COVID syndrome (or long-term COVID) include cardiovascular, pulmonary, and neuromuscular changes, among other organic and psychosocial manifestations (Higgins et al., 2021), and persistent symptoms after medical release for the SARS-CoV-2 (Elkrief et al., 2022). These changes are also present in patients with obesity, which could potentially make them more susceptible to the development of post-COVID syndrome (Maffetone and Laursen, 2020; Higgins et al., 2021; Elkrief et al., 2022). Early diagnosis and targeted interventions for patient recovery can provide better outcomes for disease sequelae and complications of long-term post-COVID or COVID-19 syndrome (Al-Aly et al., 2021), especially in patients with risk factors for respiratory and cardiovascular alterations. However, multiple cases have presented after the least severe forms of COVID-19, with short-term (~1 month) and long-term (≥6 months) sequelae impacting between 34.8% to 65.5% and 31.0%–67%, respectively, of COVID-19 survivors (Groff et al., 2021).

The World Health Organization (WHO) has published the "Clinical management of COVID-19: living guidance" (World Health Organization, 2021), which establishes four classifications of COVID-19 symptoms (mild, moderate, severe, and critical), which are linked to the impacts caused by SARS-CoV-2. A recent systematic review with a meta-analysis by Dessie and Zewotir (2021) pointed out that age, male sex, smoking, chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), cancer, acute renal failure, systemic arterial hypertension, diabetes mellitus, and obesity increase the risks associated with the more severe symptoms of COVID-19. Given this, based on preventive and health promotion actions, public policies that aim to provide a healthy diet, physical activity, and tobacco control can contribute to improving the health and quality of life of the population and, consequently, reduce the costs related to the treatment of chronic diseases and the risk of developing complications from COVID-19 (Brazil, 2010).

Science has discussed better outcomes given modifiable aspects for people who contracted COVID-19, such as reduced fat mass (Maffetone and Laursen, 2020), increased cardiorespiratory fitness (Af Geijerstam et al., 2021; Brandenburg et al., 2021), increased muscle strength (Af Geijerstam et al., 2021), and a higher level of physical activity (Pitanga et al., 2021). Supporting this perspective, Sallis et al. (2021) reported that physically inactive people who had COVID-19 had a higher rate of hospitalization and ICU admission than physically active people. Pitanga et al. (2021) identified in an ecological analysis, in 26 Brazilian capitals and the federal district, an inverse correlation between the practice of physical activity in leisure time and deaths from COVID-19 (r = -0.44; p = 0.03) and lethality (r = −0.51; p = 0.01). Similarly, Barbagelata et al. (2021) found that asymptomatic patients had a significantly higher peak oxygen consumption (VO_2peak) in the Bruce test than those with long-term COVID conditions. However, the authors did not control their findings by body mass index (BMI) and the severity of cases, i.e., whether there was a need for in-patient or ICU admission for treatment of COVID-19. This is a relevant factor since the need for intubation or oxygen support may be correlated with greater deficits in respiratory capacity, which are more frequent in patients with obesity (Freire et al., 2022).

Because of this, people with obesity could present intense symptoms associated with COVID-19, linked to greater inflammation, impaired immune response, worse respiratory function, and pro-coagulant profile (Stefan et al., 2020). The possible association between anthropometric body composition and cardiorespiratory

TABLE 1 General characteristics of participants of both sexes by the severity of COVID-19.

Variables	ICU (*n* = 52)	Hospital (*n* = 58)	Non-hospitalized (*n* = 61)
Age	50.3 ± 11.0	48.1 ± 13.3	48.0 ± 13.0
Gender			
Male	30 (57.7%)	30 (51.7%)	33 (54.1%)
Female	22 (42.3%)	28 (48.3%)	28 (45.9%)
Medical history			
Hypertension	18 (34.6%)	13 (22.0%)	14 (23.3%)
Diabetes	11 (21.2%)	4 (6.8%)	6 (10.0%)
Dyslipidemia	15 (28.8%)	11 (18.6%)	9 (15.0%)
COPD	0 (0%)	0 (0%)	1 (1.7%)
Asthma	0 (0%)	1 (1.7%)	0 (0%)
CAD/revascularization	2 (3.8%)	0 (0%)	4 (6.7%)
Smoking			
No	40 (76.9%)	48 (82.8%)	51 (83.6%)
Past or today	12 (23.1%)	10 (17.2%)	10 (16.4%)
Length of stay			
Hospital (d)	8.0 (4.0–13.0)	8.0 (6.0–11.0)	—
Intensive care (d)	14.0 (4.7–14.0)	—	—
Total (d)	22.0 (12.0–35.0)	8.0 (6.0–11.0)	—
Type of respiratory support			
None	1 (1.9%)	11 (19.3%)	59 (96.7%)
Catheter	35 (67.3%)	40 (69.0%)	2 (3.3%)
High flow mask	33 (63.5%)	24 (41.4%)	0 (0%)
Non-invasive mechanical ventilation	33 (63.5%)	11 (19.0%)	0 (0%)
Invasive mechanical ventilation	35 (67.3%)	0 (0%)	0 (0%)
Physical activity ≥150 min/week before COVID-19	23 (44.2%)	28 (48.3%)	43 (70.5%)*

Note: data are expressed as the mean and (±) standard deviation, minimum, maximum, and relative frequency (%); *, higher values when compared to ICU, and hospitalized groups ($p < 0.05$); COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; ICU, intensive care unit; NIMV, non-invasive mechanical ventilation; IMV, invasive mechanical ventilation. Analyze One-way ANOVA.

parameters and the severity of the symptoms of COVID-19 (mild, moderate, and severe/critical) has not yet been verified. Therefore, the objective of the present study was to analyze the body composition and cardiorespiratory fitness of overweight or obese people according to the severity of their symptoms of COVID-19. As a hypothesis, it is believed that higher fat mass and lower cardiorespiratory fitness are related to the severity of symptoms of SARS-CoV-2 survivors.

Materials and methods

Experimental design

This cross-sectional, experimental, and comparative study was conducted between August and December 2021 in Maringá, Paraná, Brazil. The present study recruited people with COVID-19 who were not hospitalized (no admission), hospitalized, or admitted to the

TABLE 2 Physiological variables in each stage of the Bruce test for COVID-19 survivors.

Stage	HR (bpm)			VE (L/min)			VO_2 peak ($ml.kg^{-1}.min^{-1}$)			VO_2/HR		
	NH	Hospital	ICU	NH	Hospital	ICU	NH	Hospital	ICU	NH	Hospital	ICU
S1	121.2 ± 20.42	119.8 ± 22.9	124.73 ± 21.6	23.9 ± 8.0	23.9 ± 8.3	25.9 ± 9.4	13.9 ± 3.7	14.6 ± 4.6	14.7 ± 4.3	10.6 ± 4.0	11.4 ± 4.2	10.6 ± 3.3
S2	135.2 ± 18.9	135.9 ± 23.3	137.7 ± 22.6	34.0 ± 10.9	33.5 ± 11.8	34.5 ± 11.8	18.3 ± 4.5	18.6 ± 5.1	17.9 ± 4.6	12.5 ± 4.2	12.6 ± 4.2	11.9 ± 3.4
S3	155.8 ± 16.9	151.7 ± 23.5	149.0 ± 22.3	48.8 ± 16.8	42.7 ± 15.9	45.5 ± 19.0	23.0 ± 6.3	21.6 ± 6.3	21.2 ± 6.4	13.6 ± 5.1	13.3 ± 4.4	13.0 ± 4.2
S4	172.5 ± 15.6**	155.7 ± 22.1	155.6 ± 21.5	66.3 ± 21.7**	52.9 ± 21.9	49.2 ± 14.5	28.3 ± 7.6‡	25.4 ± 9.1	22.8 ± 5.4	14.7 ± 5.1	15.1 ± 4.7	13.5 ± 4.1
S5	172.9 ± 20.7	165.3 ± 16.0	151.8 ± 20.1	61.5 ± 25.3	55.6 ± 17.7	57.6 ± 10.6	29.6 ± 9.9	26.9 ± 9.8	25.3 ± 3.8	14.0 ± 6.6	17.2 ± 4.4	17.8 ± 3.2
S6[a]	185.0 ± 4.24	174.5 ± 0.7	163.0	98.0 ± 5.3	68.9 ± 13.4	72.5	33.7 ± 7.0	29.2 ± 0.3	25.5	14.8 ± 0.4	17.7 ± 5.6	19.2

Note: Numerical data is described as mean and standard deviation (SD). Categorical data is described as absolute and relative (%) frequencies. NH, non-hospitalized; ICU, intensive care unit; S1 = Stage 1; S2 = Stage 2; S3 = Stage 3; S4 = Stage 4; S5 = Stage 5; S6 = Stage 6; HR, heart rate; VE, ventilation; VO_2peak = peak oxygen consumption; VO_2/HR, relationship between peak oxygen consumption and heart rate. * = Statistically significant difference between NH, and ICU, groups ($p < 0.05$); ** = Statistically significant difference between NA, and Hospital/ICU, groups ($p < 0.05$); ‡ = Statistically significant difference between NH, and Hospital groups ($p < 0.05$); † = Statistically significant difference between Hospital and ICU, groups ($p < 0.05$); [a]=ICU, group values have no SD, reported since only one individual from this group performed S6.

TABLE 3 Physiological, subjective variables and frequencies of participants in each stage of the Bruce Test for COVID-19 survivors.

Stage	RQ			RPE (a.u.)			min.SpO_2 (%)			n (%)		
	NH	Hospital	ICU	NH	Hospital	ICU	NH	Hospital	ICU	NH	Hospital	ICU
S1	1.2 ± 0.3**	1.0 ± 0.1	1.0 ± 0.2	8.9 ± 2.3	9.7 ± 3.2	9.5 ± 2.9	97.1 ± 1.4**	96.0 ± 2.0†	94.9 ± 2.2	61 (100.0)	58 (100.0)	52 (100.0)
S2	1.1 ± 0.1**	1.0 ± 0.1	1.0 ± 0.1	11.5 ± 3.4	12.3 ± 3.5	13.1 ± 3.6	96.8 ± 1.5*	95.7 ± 2.3†	94.0 ± 3.2	59 (96.7)	57 (98.3)	51 (98.1)
S3	1.2 ± 0.2**	1.0 ± 0.1	1.1 ± 0.1	14.2 ± 3.5	15.2 ± 4.0	15.3 ± 4.3	96.5 ± 1.9*	95.5 ± 2.6†	93.5 ± 3.2	56 (91.8)	51 (87.9)	41 (78.8)
S4	1.3 ± 0.2**	1.1 ± 0.1	1.1 ± 0.1	16.0 ± 3.1	15.8 ± 3.7	15.5 ± 4.9	96.1 ± 2.0*	94.9 ± 2.0†	92.3 ± 4.1	35 (57.4)	29 (50.0)	21 (40.4)
S5	1.4 ± 0.2	1.1 ± 0.0	1.1 ± 0.1	16.4 ± 2.1	14.8 ± 2.3	11.4 ± 3.0	95.1 ± 3.2	93.9 ± 3.5	91.0 ± 7.0	17 (27.9)	8 (13.8)	5 (9.6)
S6[a]	1.8 ± 0.4	1.1 ± 0.0	1.0	19.0 ± 1.4	15.5 ± 3.5	18.0	89.5 ± 2.1	95.0 ± 2.8	93.0	2 (3.3)	2 (3.4)	1 (1.9)

Note: Numerical data is described as mean and standard deviation (SD). Categorical data is described as absolute and relative (%) frequencies. NH, non-hospitalized; ICU, intensive care unit; S1 = Stage 1; S2 = Stage 2; S3 = Stage 3; S4 = Stage 4; S5 = Stage 5; S6 = Stage 6; RQ, respiratory quotient; RPE, rating of perceived exertion; SpO_2 = minimum peripheral oxygen saturation; n = number of participants. * = Statistically significant difference between NH, and ICU, groups ($p < 0.05$); ** = Statistically significant difference between NA, and Hospital/ICU, groups ($p < 0.05$); ‡ = Statistically significant difference between NH, and Hospital groups ($p < 0.05$); † = Statistically significant difference between Hospital and ICU, groups ($p < 0.05$); [a]=ICU, group values have no SD, reported since only one individual from this group performed S6.

intensive care unit (ICU) via referrals from the Municipal Hospital of Maringá through TV, radio, and social media dissemination. Interested parties contacted the Interdisciplinary Laboratory for Intervention in Health Promotion (LIIPS) multidisciplinary team at Cesumar University. Participants came to the laboratory and performed two assessments at a 24-h interval. First, the participants had a consultation with an intensive care physician. Participants answered a detailed anamnesis on personal and family history, previous illnesses, symptoms of COVID-19, hospitalization (if the patient was hospitalized), and self-reported level of physical activity (means by a validated questionnaire—presented in sections above); in addition to an anthropometric and body composition assessment (protocol previously sent to the patients), as detailed in subsequent sessions. Finally, 24 h after the first assessment, the participants completed the cardiorespiratory fitness test (see the protocol below). All participants were informed about the study's objectives and signed informed consent forms (ICF). This study followed the recommendations proposed by resolution 466/12 of the Ministry of Health of the Brazilian Government and the Declaration of Helsinki, approved by the Research Ethics Committee from Cesumar University, under number 4,546,726.

Participants

One hundred seventy-one volunteers of both sexes participated in this study and were allocated into three experimental groups according to the clinical picture of their acute COVID-19 infection: ICU (n = 52), hospital (n = 58), and non-hospitalized (n = 61). More information about the volunteers is presented in Table 1. The severity of COVID-19 was classified according to the guide "Clinical management of COVID-19: living guidance" (World Health Organization, 2021). The inclusion criteria were as follows: 1) being between 19 and 65 years old; 2) being overweight or obese; 3) having a positive diagnosis for COVID-19 via qualitative molecular testing (RT–PCR); 4) having contracted COVID-19 between 03 January/2021 and 01 July/2021; 5) having received the first dose of COVID-19 vaccine; and 6) having received medical clearance for the cardiorespiratory fitness test. As exclusion criteria, the following participants were not accepted: 1) patients with debilitating neurological diseases; 2) people with limited mobility (use of a cane or wheelchair); 3) people with a body mass index below or within normal limits; 4) people without medical clearance to perform the Bruce test; and 5) non-agreement to sign the ICF.

Clinical evaluation

Data collection was performed in the following order: 1) blood pressure measurement after 5 min of rest in a calm and quiet place, following the VIII Brazilian Guidelines on Arterial Hypertension (Barroso et al., 2020) with the evaluators' reproducibility for measuring blood pressure was 0.99 for the intraclass coefficient (ICC); 2) heart rate (HR) mensuration and peripheral oxygen saturation (%SpO_2), both at rest, using a finger oximeter (Alfamed®, model sense 10, Lagoa Santa, Minas Gerais, Brazil) positioned on the index finger; 3) height and body mass mensuration (using a stadiometer and a scale) and body composition with electrical bioimpedance (device information is presented below); 4) a questionnaire for patient identification and initial screening regarding lifestyle, clinical history [history of surgeries, noncommunicable chronic diseases, continuous use medications and physical activity, means by the Short International Physical Activity Questionnaire/IPAQ—version validated in Brazil by Matsudo et al. (2001)—with retrospective information referring to prior SARS-CoV-2 infection was applied] and information on the clinical picture of acute COVID-19 (main signs and symptoms presented, severity of COVID-19, as well as the possible need for ventilation invasive or noninvasive mechanics and central reported sequelae); and 5) a cardiorespiratory fitness test (with a treadmill and a direct analysis of gas exchange). The patients' information about physical activity means by IPAQ was collected before the contraction of COVID-19 for all patients, i.e., there was a retrospective completion considering the period before COVID-19 infection (there were no more collections after the COVID-19 discharge).

Medical consultation, anthropometry, and body composition assessment were performed on the first visit to the Interdisciplinary Laboratory for Intervention in Health Promotion. On the second visit, 24 h later, a cardiorespiratory fitness test was conducted using the Bruce protocol according to the Brazilian Society of Cardiology (Barroso et al., 2020).

Anthropometry and body composition

The participants' height was measured using a Sanny® stadiometer measuring 2.20 m with a precision of 0.1 cm (Standard model, ES 2030, São Bernardo do Campo, São Paulo, Brazil); body mass was measured on a mechanical scale (Welmy® mechanics with a capacity of 300 kg and precision of 100 g, Model 104A, Santa Bárbara do Oeste, São Paulo, Brazil), according to the protocol established by Freitas Junior (2018), and the body mass index (BMI) was subsequently calculated. Body composition assessment was performed using a tetrapolar electrical bioimpedance (BIA) (InBody 570®, Biospace Co. Ltd., Seoul, Korea) with eight tactile points and a capacity of 250 kg with an accuracy of 100 g. The volunteers were instructed to 1) fast for 4 h; 2) not to ingest liquids, including caffeine and water; 3) to abstain from the use of alcoholic beverages 2 days before the evaluations; 4) to stop exercising the day before the test; 5) to urinate 30 min before the evaluation and 6) to not wear metals on the body (Branco et al., 2019). In line with previous studies, the laboratory temperature was maintained at 24°C (Heyward, 1996; de Souza Marques et al., 2021).

Ergospirometric test

The Bruce submaximal test was used to assess the participants' cardiopulmonary fitness (Bruce and Kusumihosmer., 1973). This protocol was chosen based on Itagi et al. (2020) for people with obesity. The Bruce protocol consisted of seven stages lasting 3 min each. The Bruce test was performed on an Inbramed treadmill (model ATL 24, Porto Alegre, Rio Grande do Sul), with progressive increases in speed and slope. The first stage started with a speed of 2.7 km/h and a slope of 10%. At the end of each stage, the speed increased progressively and nonlinearly, increasing the slope by 2%.

Gas exchange analyses

The VO 2000® metabolic gas analyzer (Medgraphics Corp., Saint Paul, United States of America) associated with the pneumotach, which connects the silicone mask to the equipment, was used to measure the lung capacity of patients based on the following parameters: expired air (VE/min), peak oxygen consumption (VO_2peak—mL.kg^{-1}. min^{-1}), oxygen pulse (O_2/HR), respiratory exchange ratio (VCO_2/VO_2 - RQ), HR (bpm), total distance covered (km/h) and application of the

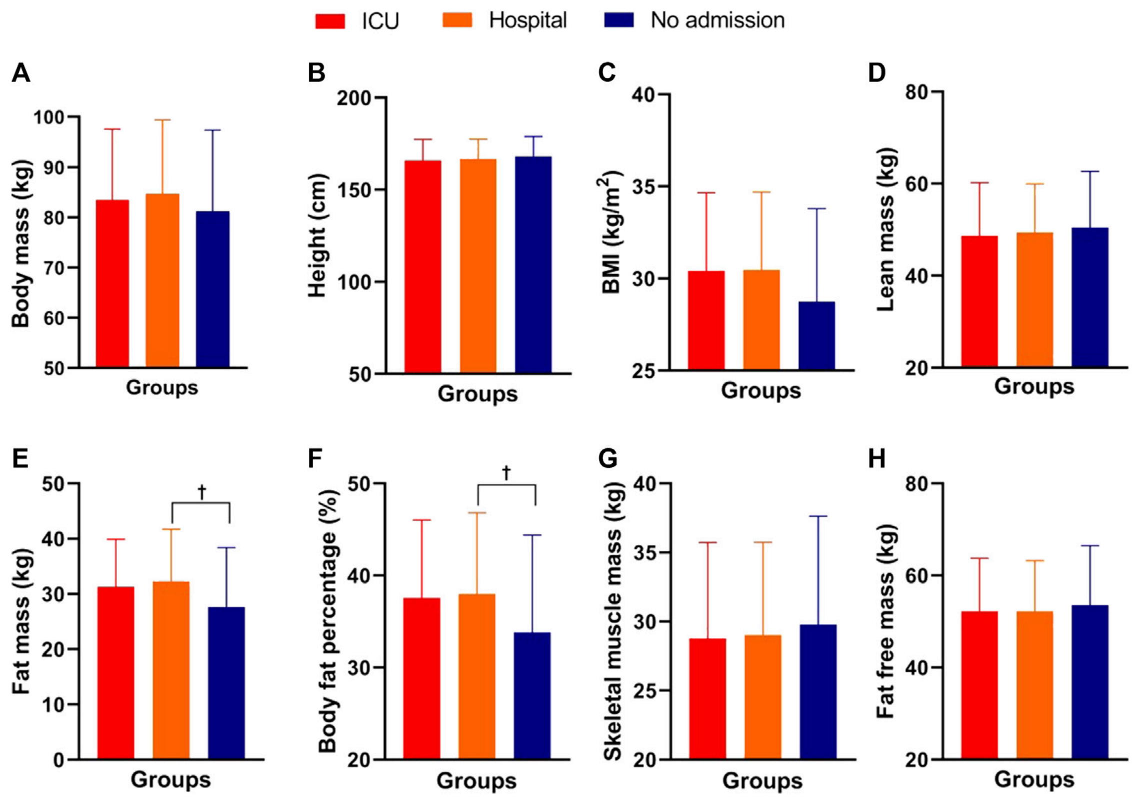

FIGURE 1
Morphological parameters of male and female COVID-19 survivors in the ICU, hospitalized, and non-hospitalized groups. Note: data are presented as the mean and (±) standard deviation; Panel **(A)** = body mass; Panel **(B)** = height; Panel **(C)** = body mass index; Panel **(D)** = lean mass; Panel **(E)** = fat mass; Panel **(F)** = body fat percentage; Panel **(G)** = musculoskeletal mass; Panel **(H)** = fat-free mass; † = COVID-19 hospitalized group had significantly higher values than the non-hospitalized COVID-19 group. One-way ANOVA with Bonferroni post hoc test. Level of significance established = $p < 0.05$.

rating of perceived exertion (RPE) at the end of the test means by the Borg scale (6–20) (Borg, 1982). As suggested by the manufacturers, the equipment was automatically calibrated at the beginning of each cardiorespiratory fitness test. The intraclass correlation coefficient reported in a previous study was 0.98 (Crouter et al., 2006). The tests were conducted by a medical team consisting of an intensive care physician, a nurse, and two exercise physiologists.

Test monitoring

The multi-professional team constantly monitored the cardiorespiratory fitness test and was attentive to extreme tiredness, $\%SPO_2$, and HR responses. As mentioned earlier, physiological and RPE were monitored at each stage. All participants were instructed to respond to RPE on the Borg (Borg, 1982) 6–20 scale. The cardiopulmonary fitness test was terminated on the following occasions: 1) voluntary withdrawal of the participants; 2) RPE ≥19 a.u.; 3) RQ ≥ 1.15; 4) lower limb fatigue and/or 5) physical impossibility of maintaining intensity during the test. After the end of the Bruce test, HR and $\%SpO_2$ were monitored minute by minute for 15 min. SBP and DBP were measured immediately after the end of the physical assessment and every 5 min during the next 15 min.

Statistical analysis

Based on the study by Barbagelata et al. (2021), the sample size calculation in G*Power (version 3.1, University of Dusseldorf, Germany) showed that 137 volunteers would be enough for an $\alpha = 0.05$ and a $\beta = 0.80$. Statistical analyses were performed using GraphPad Prism software version 8.1.0. Data are presented as the mean ± standard deviation (SD). First, data normality was tested using the skewness-kurtosis test, considering values from 2 to −2 to indicate a need to perform parametric statistical analyses. One-way analysis of variance (one-way ANOVA) was used to locate possible differences between the groups. Data with a nonparametric distribution were analyzed using the Kruskal–Wallis test. Comparisons between pre- and post-cardiorespiratory fitness tests were performed via a two-way mixed-measures ANOVA (for repeated measures). Bonferroni's post-hoc test was used when a significant difference was found. The significance level established for all tests was $p < 0.05$. The partial eta square (η^2) was calculated according to the classification by Richardson (2011) using the following interpretation scale: 0.0099 [*small*], 0.0588 [*moderate*], and 0.1379 [*large*]. Cohen´s *d* was also calculated using the following rating: 0.20 [*small*], 0.80 [*moderate*], and >0.80 [*large*] (Cohen, 1988).

Results

Table 1 presents the general characteristics of the present study participants stratified by the severity of their symptoms of COVID-19.

No significant differences were observed for age, systemic arterial hypertension, dyslipidemia, or smoking among the three experimental groups ($p > 0.05$). Significant differences were detected between the groups for self-reported physical activity ($F_{2,168} = 4.89$; $p = 0.008$) with superior values for the non-hospitalized group when compared to ICU ($p = 0.01$ days = −0.54—*moderate*) and hospitalized ($p = 0.04$; $d = -0.46$—*moderate*). Figure 1 shows the morphological parameters of the COVID-19 survivors in the three experimental groups of the present study.

Table 2 presents physiological variables in each stage of the Bruce test, and Table 3 presents subjective variables and frequencies of participants in each stage of the Bruce test for COVID-19 survivors. For the first stage (S1), significant differences were found among the groups for SpO_2 ($F_{2,165} = 18.92$; $p < 0.001$; $\eta^2 = 0.19$ - *large*), with higher values in non-hospitalized group when compared to hospital ($p = 0.004$; $d = 0.61$ - *moderate*) and ICU ($p < 0.001$; $d = 1.17$—*large*), and higher values were observed in hospital than ICU group ($p = 0.010$; $d = 0.57$—*moderate*). Also, a significant difference was identified for RQ ($F_{2,163} = 14.71$; $p < 0.001$; $\eta^2 = 0.15$—*large*), with higher values in non-hospitalized group when compared to hospital ($p < 0.001$; $d = 0.99$—*large*) and ICU ($p = 0.001$; $d = 0.70$—*moderate*) groups. No significant differences were detected for HR, VE, VO_2peak, VO_2/HR, and RPE ($p > 0.05$). 100% of the patients performed the S1 stage.

For the second stage (S2), significant differences were found between the groups for SpO_2 ($F_{2,162} = 18.34$; $p < 0.001$; $\eta^2 = 0.18$ - *large*), with higher values in non-hospitalized group when compared to ICU group ($p < 0.001$; $d = 1.16$—*large*), and higher values were identified for hospital when compared to ICU group ($p < 0.001$; $d = 0.72$—*moderate*). Besides, a significant difference was identified for RQ ($F_{2,163} = 15.09$; $p < 0.001$; $\eta^2 = 0.16$—*large*), with higher values in non-hospitalized group when compared to hospital ($p < 0.001$; $d = 0.98$—*large*) and ICU ($p = 0.001$; $d = 0.74$—*moderate*) groups. No significant differences were observed for HR, VE, VO_2peak, VO_2/HR, and RPE ($p > 0.05$). 59 (96.7%) of non-hospitalized, 57 (98.3%), hospitalized and 51 (98.1%) of ICU patients completed this stage (S2).

For the third stage (S3), significant differences were found between the groups for SpO_2 ($F_{2,141} = 15.63$; $p < 0.001$; $\eta^2 = 0.18$—*large*), with higher values in non-hospitalized group when compared ICU group ($p < 0.001$; $d = 1.16$—*large*), and higher values were identified for hospital when compared to ICU group ($p = 0.001$; $d = 0.77$—*moderate*). Moreover, a significant difference was detected for RQ ($F_{2,144} = 23.96$; $p < 0.001$; $\eta^2 = 0.25$—*large*), with higher values for in non-hospitalized group when compared to hospital ($p < 0.001$; $d = 1.24$—*large*) and ICU ($p = 0.001$; $d = 1.10$—*large*) groups. No significant differences were observed for HR, VE, VO_2peak, VO_2/HR, and RPE ($p > 0.05$). 56 (91.8%) of non-hospitalized, 51 (87.9%), hospitalized and 41 (78.8%) of ICU patients completed this stage (S3).

For the fourth stage (S4), significant differences were observed for HR ($F_{2,82} = 7.73$; $p < 0.001$; $\eta^2 = 0.16$—*large*), with higher values in non-hospitalized group when compared to hospital ($p = 0.003$; $d = 0.87$—*large*) and ICU ($p = 0.006$; $d = 0.87$—*large*) groups. Significant differences were detected for VE ($F_{2,81} = 5.71$; $p = 0.005$; $\eta^2 = 0.12$—*moderate*), with higher values in non-hospitalized group when compared to hospital ($p = 0.028$; $d = 0.66$—*moderate*) and ICU ($p = 0.009$; $d = 0.84$—*large*) groups. Besides, significant differences were detected for VO_2peak ($F_{2,82} = 3.48$; $p = 0.036$; $\eta^2 = 0.08$—moderate), with higher values in non-hospitalized group when compared to ICU group ($p = 0.03$; $d = 0.72$—*moderate*). Significant differences were observed for RQ ($F_{2,81} = 30.86$; $p < 0.001$; $\eta^2 = 0.43$—large), with higher values in non-hospitalized group when compared to hospital ($p < 0.001$; $d = 1.82$—*large*) and ICU ($p < 0.001$; $d = 1.62$—*large*) groups. Also, significant differences were observed for SpO_2 ($F_{2,78} = 13.37$; $p < 0.001$; $\eta^2 = 0.26$—*large*), with higher values in non-hospitalized group than compared to ICU group ($p < 0.001$; $d = 1.43$—*large*), and higher values in hospital group when compared to ICU group ($p = 0.003$; $d = 0.08$—*small*). No significant differences were detected for VO_2/HR and RPE ($p > 0.05$). 35 (57.4%) of non-hospitalized, 29 (50.0%), hospitalized and 21 (40.4%) of ICU patients completed this stage (S4).

For the fifth stage (S5) and For the sixth stage (S6), was not possible to perform statistical analysis - in order to not to make a type 1 error, since the number of participants in these stages was low [S5: 17 (29.7%) of non-hospitalized, 8 (13.8%), hospitalized and 5 (9.6%) of ICU patients completed this stage, and S6: 2 (3.3%) of non-hospitalized, 2 (3.4%), hospitalized and 1 (1.9%) of ICU patients completed this stage].

Significant differences were observed between the groups for fat mass ($F_{2,168} = 3.82$; $p = 0.024$; $\eta^2 = 0.04$—*small*) and body fat

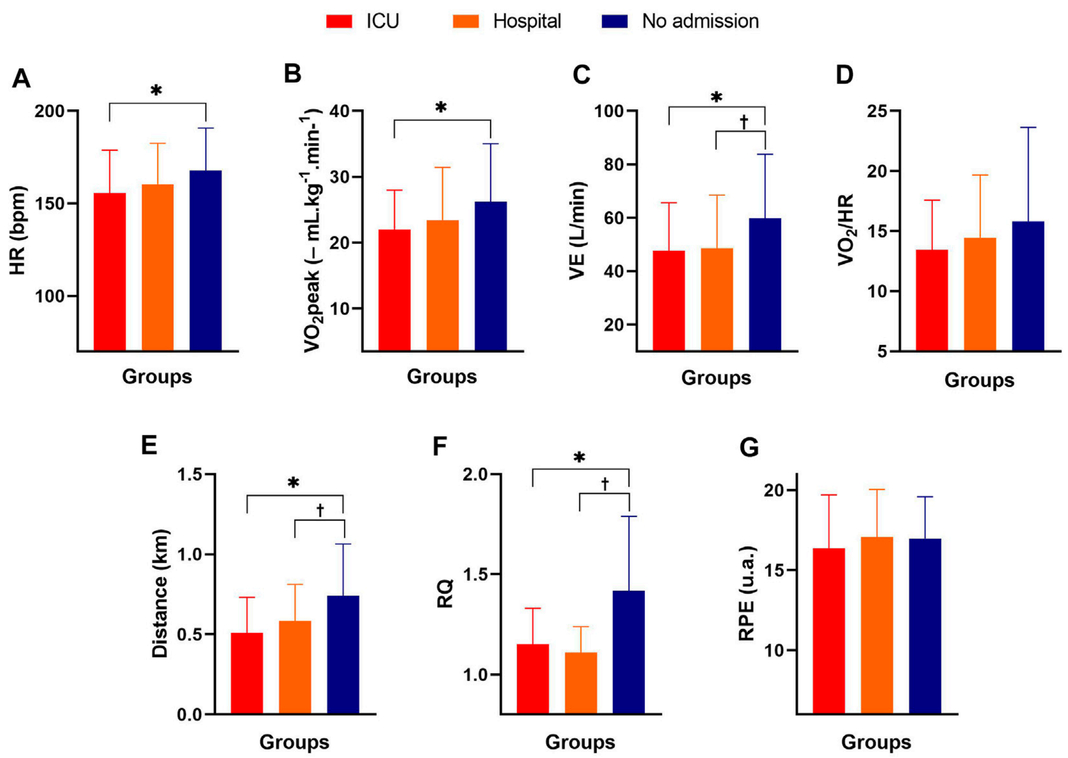

FIGURE 2
Cardiopulmonary parameters of male and female COVID-19 survivors in the ICU, hospitalized, and non-hospitalized groups. Note: data are expressed as the mean and standard deviation; Panel **(A)** = HR (heart rate); Panel **(B)** = VO_2 peak (peak oxygen consumption); Panel **(C)** = VE (ventilation); Panel **(D)** = VO_2/HR (relationship between oxygen consumption and heart rate); Panel **(E)** = distance; Panel **(F)** = VCO_2/VO_2 (ratio between respiratory exchanges); Panel **(G)** = RPE (rating of perceived exertion); * = significant difference between the non-hospitalized group and the ICU group; † = significant difference between the non-hospitalized and hospitalized groups. One-way ANOVA with Bonferroni post hoc test. Level of significance established = $p < 0.05$.

percentage ($F_{2,168} = 3.55$; $p = 0.031$; $\eta^2 = 0.04$—*small*), with higher values in the hospitalized group compared to the non-hospitalized group ($p = 0.028$, $d = 0.48$—*moderate*; $p = 0.049$, $d = 0.44$—*moderate*, respectively). No significant differences were found among the groups for total body mass, height, BMI, lean mass, musculoskeletal mass, or fat-free mass ($p > 0.05$). Figure 2 shows the cardiopulmonary parameters of the men and women who survived COVID-19 in the ICU, hospitalized, and non-hospitalized groups.

Significant differences were found among the groups for HR ($F_{2,168} = 4.17$; $p = 0.017$; $\eta^2 = 0.05$—*small*), with higher values for the non-hospitalized group than for the ICU group ($p = 0.015$; $d = -0.54$—*moderate*). For VE, significant differences were also observed among the groups ($F_{2,168} = 6.15$; $p = 0.003$; $\eta^2 = 0.07$ - *moderate*), with higher values for the non-hospitalized group compared to the ICU group ($p = 0.007$; $d = -0.58$—*moderate*) and hospital ($p = 0.012$; $d = -0.54$—*moderate*). For VO_2peak, a significant difference was also observed among the groups ($F_{2,168} = 4.41$; $p = 0.014$; $\eta^2 = 0.05$—*small*), with higher values for the non-hospitalized group than for the ICU group ($p = 0.013$; $d = -0.55$—*moderate*). Significant differences were also identified between the groups for the distance covered in the test ($F_{2,168} = 11.02$; $p < 0.001$; $\eta^2 = 0.12$ - *moderate*), with higher values for the non-hospitalized group when compared to the ICU group ($p < 0.001$; $d = -0.86$—*large*) and hospitalized group ($p = 0.006$; d = −0.58—*moderate*). For the RQ, significant differences were observed between the groups ($F_{2,168} = 25.65$; $p < 0.0001$; $\eta^2 = 0.23$—*large*), with higher values for the non-hospitalized group than for the ICU ($p < 0.0001$; d = 0.91—*large*) and hospitalized ($p < 0.0001$; $d = 1.10$—*large*) groups. No significant differences were found between the groups for VO_2/HR or the RPE post-Bruce test ($p > 0.05$). Figure 3 shows the $\%SPO_2$, HR, SBP, and DBP behavior before, during, and after the Bruce test at different measurement times in the three groups.

For the post Bruce test $\%SpO_2$, significant differences were identified among the groups ($F_{2,165} = 10.12$; $p < 0.001$; $\eta^2 = 0.11$—*moderate*), with higher values for the non-hospitalized group when compared to the ICU group ($p < 0.0001$; $d = 0.79$—*moderate*) and for the hospitalized group when compared to the ICU group ($p < 0.0001$; $d = 0.62$—*moderate*); there were no

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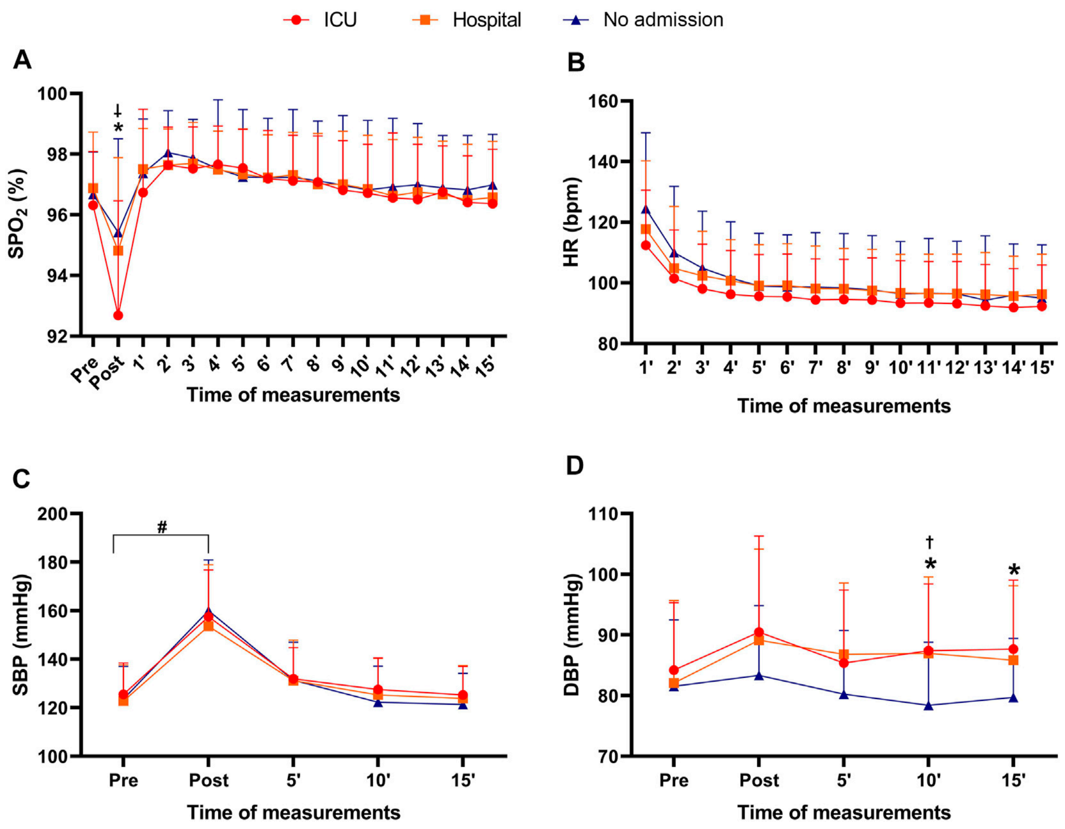

FIGURE 3
Peripheral oxygen saturation, heart rate, systolic and diastolic blood pressure before, during, and after Bruce testing at different times in COVID-19 survivors in the ICU, hospitalized, and non-hospitalized groups. Note: data are presented as the mean and (±) standard deviation; Panel **(A)** = peripheral oxygen saturation before the Bruce test, subsequently after, and for 15 min after the test; Panel **(B)** = heart rate at the end of the Bruce test and for 15 min after the test; Panel **(C)** = pre-test systolic blood pressure, subsequently after the test, 5 min after the test, 10 min after the test and 15 min after the test; Panel **(D)** = pre-test diastolic blood pressure, subsequently after the test, 5 min after the test, 10 min after the test and 15 min after the test; $\%SPO_2$ = peripheral oxygen saturation; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; * = significant difference between non-hospitalized and ICU; † = significant difference between the non-hospitalized and hospitalized; ‡ = significant difference between ICU and hospitalized; # = significant difference between pre and post times for the three experimental groups. Two-way ANOVA with Bonferroni post hoc test. Level of significance established = $p < 0.05$.

other significant differences ($p > 0.05$). Additionally, it was observed that the $\%SpO_2$ of the ICU ($p < 0.0001$; $d = 1.22$—*large*) and hospitalized ($p < 0.0001$; $d = 0.08$—*small*) post Bruce test groups showed lower values when compared to the pre-test time. For HR, no significant differences were observed among the groups during the 15 min after the Bruce test ($p > 0.05$). For SBP, higher values were found post-test when compared to pretest for the ICU ($p < 0.0001$; $d = 1.94$—*large*), hospitalized ($p < 0.0001$; $d = 1.48$—*large*) and non-hospitalized groups ($p < 0.0001$; $d = 2.08$—*large*). No significant differences were observed for SBP in subsequent measurements in the three experimental groups ($p > 0.05$). For DBP, significant differences were observed (Bruce's post-test) ($F_{2,168} = 4.17$; $p = 0.017$; $\eta^2 = 0.05$—*moderate*), with higher values for the ICU group than for the non-hospitalized group ($p = 0.025$; $d = 0.29$—*moderate*). Furthermore, the DBP of the non-hospitalized group 10 minutes after the exercise test was significantly lower than the values presented by the ICU ($p < 0.008$; $d = 0.83$—large) and hospitalized groups ($p < 0.01$; $d = 0.74$—*moderate*) at the same measurement point. Finally, 15 minutes after the Bruce test, the blood pressure of the non-hospitalized group remained lower than the DBP of the ICU group ($p < 0.04$; $d = 0.75$—*moderate*). The three experimental groups identified no significant differences between pre-test $\%SpO_2$, pre-test SBP, pre-test DBP, or final SBP ($p > 0.05$).

Discussion

The main results of this study indicated 1) a higher level of self-reported physical activity for the non-hospitalized group when compared to the hospital and ICU groups; 2) fat mass and body fat percentage were significantly higher in the hospitalized group when compared to the non-hospitalized group; 3) the final HR and VO_2peak after the Bruce test was significantly higher in the non-hospitalized group when compared to the ICU group; 4) the distance covered in the Bruce test was significantly greater in the non-hospitalized group when compared to the hospitalized and ICU groups; 5) %SPO_2 was lower after the Bruce test in the ICU group when compared to the non-hospitalized group; and 6) the final post-Bruce test DBP was significantly higher in the ICU and hospitalized groups.

On the other hand, no significant differences were identified among the three experimental groups for comorbidities associated with obesity, smoking, lean mass, musculoskeletal mass, fat-free mass, VO_2/HR, RPE, or HR after the Bruce test over 15 min of measurement. The study hypothesis was confirmed, considering the differences in body composition and cardiorespiratory fitness in non-hospitalized and hospitalized individuals.

There was no significant difference in the body mass index among the different groups, i.e., ICU, hospitalized, and non-hospitalized, although there was a significant difference in body fat percentage, with lower values for the non-hospitalized group than the hospitalized group. Thus, it is conjectured that not just BMI but the higher body fat percentage may contribute to influencing the outcome of COVID-19. Excess body fat promotes hyper inflammation at a systemic level, with the secretion of proinflammatory mediators, such as cytokines, adipokines, and chemokines, with a consequent reduction in the immune response (Maffetone and Laursen, 2020). It is possible to affirm that being overweight and obese promoted a significant worsening of the symptoms of COVID-19, but prior assessments were not carried out before the pandemic.

However, actions that encourage the regular and systematic practice of physical activity, i.e., with emphasis on resistance exercises to promote changes in body composition, with a reduction in fat mass and an increase in musculoskeletal mass (with a consequent change in body fat percentage, in addition to aerobic exercise, to improve cardiorespiratory conditioning (Garber et al., 2011), have become essential for improving physical fitness and thus the health of the population. The literature has already pointed out an inverse correlation between the level of physical activity and accumulated deaths from COVID-19 (Pitanga et al., 2021).

The cardiorespiratory fitness of the hospitalized and ICU groups was significantly lower than that of the non-hospitalized group. Cardiorespiratory assessment mainly aims to verify the patients' physical capacity, effort tolerance, and possible cardiopulmonary abnormalities (Fletcher et al., 2013). Silva et al. (2021) found that hospitalization rates for COVID-19 among endurance athletes were significantly lower than expected. Brandenburg et al. (2021) pointed out that healthy individuals with better cardiorespiratory fitness (able to walk 4.8 km without feeling extremely tired and able to perform slow and fast walking and running) had a lower hospitalization rate than individuals with lower cardiorespiratory fitness. Because of this, the two pieces of evidence mentioned earlier (Brandenburg et al., 2021; Silva et al., 2021) suggest that cardiorespiratory fitness has a cardioprotective effect, reducing hospitalization rates, like that observed in the present study, i.e., individuals with lower cardiorespiratory fitness had more severe symptoms of COVID-19, although we cannot confirm such differences, given the experimental design of the present study. Besides that, it was essential to emphasize that low levels of cardiorespiratory fitness could impact obese adolescents (Alemayehu et al., 2018; Salvadego et al., 2018). Thus, public policies to promote physical activity, healthy nutrition and safe mental health are indispensable in the early stages of life (Branco et al., 2019; Branco et al., 2020; Branco et al., 2021).

Additionally, Sallis et al. (2021) suggested that regular and systematic physical activity following established guidelines of 150 min/week reduced hospitalization rates to 3.2% in the hospital, 1% in the intensive care unit, and 0.4% for deaths among 3,118 patients who had COVID-19. Therefore, it can be inferred that the hospitalization rate, both in the ward and in the intensive care unit, is higher among individuals with low cardiorespiratory fitness and with a lower level of physical activity when compared to individuals with higher cardiorespiratory fitness and a higher level of physical fitness (Af Geijerstam et al., 2021; Christensen et al., 2021). The present study indicated a significant difference in physical activity among the groups, i.e., the non-hospitalized group showed higher physical activity levels than both hospitalized groups (before COVID-19 contraction). In Brazil, between January and July of 2021, several places, including gyms, squares, and parks, in addition to other places that were possible to practice physical activity, were closed due to COVID-19. Many decrees authorized and disallowed access to public places. Thus, the physical activity level declined a lot during this period. Given this, it is not possible to establish a relationship between cause and effect between physical activity and COVID-19, although the level of physical activity values was higher in the non-hospitalized group when compared to the hospitalized ones.

VE during the Bruce test was higher in the non-hospitalized group than in the hospitalized and ICU groups. The increased intensity can explain these differences in the test differed between the participants (Herdy et al., 2016). Concerning VO_2peak, Barbagelata et al. (2021) found significantly lower values for patients with post-COVID-19 syndrome than for asymptomatic subjects (25.8 ± 8.1 ml kg^{-1}. min^{-1} vs. 28.8 ± 9.6 ml kg^{-1}. min^{-1}; $p = 0.017$). These responses may be related to the long period of physical inactivity that resulted in cardiorespiratory deconditioning, residual inflammation (convalescent phase), possible systemic and/or organ damage, prolonged invasive or noninvasive ventilation, poor health conditions, or even the sum of the conditions (Carfì and Bernabei, 2020). In addition, long-term functional impairment after hospital discharge for COVID-19, particularly for those admitted to the ICU, stands out as a possible sequela (Clavario et al., 2021). Consequently, the reduced performance on the cardiorespiratory fitness test of the hospitalized and ICU groups would be justified by the deleterious effects of the sum of the symptoms of COVID-19, i.e., a lower VO_2 peak and shorter distance covered in the test.

It is also worth mentioning that the RPE did not present a significant difference among the groups, indicating that the perceived intensity was similar. Furthermore, it was found that the distance covered during the Bruce test was significantly longer in the non-hospitalized group than in the two hospitalized groups. The worse performance on the Bruce test of patients in the hospitalized groups can be explained by possible sequelae of COVID-19 related to myositis and myalgia (associated with the severity of symptoms of COVID-19) (Paliwal et al., 2020), physical deconditioning promoted by hospitalization and a lack of neuromuscular stimuli (Solverson et al., 2016), and a reduced cardiorespiratory capacity (Af Geijerstam et al., 2021; Brandenburg et al., 2021; Silva et al., 2021). For this reason, carrying out health-related physical fitness tests (tests to measure muscle strength, muscle endurance, flexibility, and cardiorespiratory conditioning, in addition to assessing body composition to verify lean mass, fat, and body fat percentage) are essential to more assertively direct rehabilitation/training and nutrition programs.

Physical inactivity is not solely responsible for the worsening symptoms of COVID-19. Petrovic et al. (2020) reported that the cytokine storm resulted from an increase in low-grade inflammation due to obesity and associated comorbidities (Maffetone and Laursen, 2020), and even the use of immunosuppressants was related to the worsening symptoms of COVID-19, additionally to heart disease, dysregulation of the renin-angiotensin-aldosterone system, plaque destabilization (causing acute coronary syndrome), and the promotion of a prothrombotic state and clotting disorders (Silva et al., 2022). In congruence with the American College of Sports Medicine (Pescatello, 2005), DBP elevation is not typical during a cardiorespiratory test. Potential mechanisms associated with the hypertensive response of post-Bruce DBP can be explained by the excessive elevation of the double product that can result in global subendocardial ischemia due to an inability to maintain myocardial oxygen supply and demand (Ha et al., 2002). On the other hand, Sydó et al. (2018) point out that the etiology of an increase in DBP after physical exercise is not fully elucidated in the literature; possible risk factors or cardiovascular disease may not be associated with increased DBP. The same authors suggest that the central focus of analysis should be on cardiorespiratory capacity and recovery heart rate, although the present study did not identify a difference in HR during the 15 min of measurement. Recently, Freire et al. (2022) pointed out that people with post-COVID-19 obesity had an increase in the stress index and a reduction in parasympathetic activity compared to people without obesity who were discharged after COVID-19. However, the present study did not measure heart rate variability in the fast and slow recovery phases after physical effort, which can be considered a limitation. Another limitation of this study was a lack of control of respiratory frequency after the Bruce test; thus, transposing to practice, respiratory rate monitoring before, during and post-exercise could be investigated in post-COVID-19 patients with different symptoms.

Another point that deserves attention is related to post-COVID-19 systemic arterial hypertension. Chen et al. (2021) pointed out that 93% of critically ill patients (hospitalized in an intensive care unit) had cardiac lesions, and systemic arterial hypertension may be a sequela of COVID-19. Therefore, troponin I and angiotensin-2 monitoring can monitor hemodynamic parameters in serial assessments, as Chen et al. (2021) described. Considering that the responses of the cardiopulmonary system can be dysfunctional, the practice of recreational or high-performance physical activity should be carefully evaluated by a multidisciplinary team (Colombo et al., 2021). The type, volume, and intensity of physical exercise should be analyzed and monitored before, during, and after the sessions via % SpO_2, HR, respiratory frequency, and blood pressure and recorded to compare with the subsequent sessions to analyze the impact of the physiological stress caused by exercise rehabilitation/adaptation on post-COVID-19 patients.

Post-COVID-19 patients will need post-hospital care to minimize possible biopsychosocial sequelae so that the patient does not become “invisible” to society. However, early mobilization strategies can be adopted to reduce possible sequelae resulting from COVID-19, following the guidelines for early mobilization in an intensive care unit (Aquim et al., 2019). An additional possible limitation of this study is the absence of measurement of heart rate variability to monitor the rapid and slow rate phases of heart rate variability, as well as to the study design, i.e., being cross-sectional, which does not allow for a cause and effect relationship to be delineated. As a strong point, morphological and cardiorespiratory aspects that need rehabilitation and are linked to physical fitness related to health were verified. Therefore, strategies for recovering from health conditions through physical activity and incorporating a healthy diet become essential for COVID-19 survivors, especially those who are symptomatic. Public policies for stimulating physical activity, healthy nutrition, and reducing tobacco are indispensable independently of COVID-19. Finally, it is suggested to periodically monitor body composition and cardiorespiratory variables to verify possible sequelae related to COVID-19 and organic behavior in the face of physical stress.

Conclusion

Based on the present study’s findings, it is concluded that fat mass and body fat percentage were significantly higher in hospitalized post-COVID-19 participants. The cardiorespiratory fitness of the hospitalized and ICU groups was significantly lower than the non-hospitalized group, especially the ICU group, although there was no significant difference among the groups for RPE in the post-Bruce test. Vital signs were significantly different in hospitalized participants compared to non-hospitalized participants (after the Bruce test: lower %SPO_2 and higher DBP in hospitalized participants), suggesting specific actions based on responses to rehabilitate the survivors (especially those who were hospitalized). Another point that should be considered is the vital signs before, during, and after cardiopulmonary rehabilitation; the multidisciplinary team must monitor HR, SpO_2, and blood pressure are necessary during rehabilitation to avoid possible physical complications. The volume and intensity of physical exercises should be adjusted, conforming to the physiological adaptation of the patients. Although the experimental design does not allow the relationship between a healthy lifestyle and cause and effect, the scientific literature already points out pieces of information more than necessary to improve physical activity, promote healthy nutrition, and reduce tobacco to improve health. Thus, behavioral changes and public policies are indispensable to promoting health and reducing hospitalization costs. Finally, it is considered essential and urgent to improve the body composition and cardiorespiratory fitness of overweight and obese COVID-19 survivors independently of hospitalization.

Data availability statement

The raw data supporting the conclusion 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 This study followed the recommendations proposed by resolution 466/12 of the Ministry of Health of the Brazilian Government and the Declaration of Helsinki, approved by the Research Ethics Committee from Cesumar University, under number 4,546,726. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceived and designed the experiments: ML, SM, SR, PB, and BB. Performed the experiments: ML, GC, CH, VP, GM, LM, AS, and BB. Analyzed the data: ML, VP, LM, AS, JM, and BB. Contributed reagents/materials/analysis tools: ML, AS, SM, SR, PB, JM, and BB. Wrote the paper: ML, GC, CH, VP, GM, LM, AS, SM, SR, PB, JM, and BB.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Eight weeks of dry dynamic breath-hold training results in larger spleen volume but does not increase haemoglobin concentration

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Purpose: It has previously been reported that repeated exposure to hypoxia increases spleen size and haemoglobin (HGB) level and recent reports on the effect apnoea has on spleen size and haematological parameters are contradictory. Therefore, this study aims to evaluate the effect apnoea training has on spleen size and haematological parameters.

Methods: The breath-holding (BH) group was comprised of 12 local student-athletes with no BH exercise experience who performed BH jogging and BH jumping rope dynamic apnoea protocols, five times weekly for 8 weeks. The BH event duration was progressively increased as the apnoea tolerance of the athletes improved (20 to 35 s). The same training task was performed by the control group ($n = 10$) without BH. Spleen sizes were measured with an ultrasound system and a complete blood cell analysis was performed on the median cubital venous blood.

Results: Spleen volume in the BH group increased from 109 ± 13 ml to 136 ± 13 ml ($p < 0.001$), and bulky platelets decreased from 70.50 ± 5.83 to 65.17 ± 5.87 ($p = 0.034$), but no changes were recorded for erythrocytes ($p = 0.914$), HGB ($p = 0.637$), PLTs ($p = 0.346$) and WBC ($p = 0.532$). No changes were recorded for the control group regarding spleen size or haematological parameters.

Conclusion: Eight weeks of dry dynamic apnoea training increased spleen size and decreased the number of circulating bulky platelets in the athletes who were assessed in this study. However, the baseline RBC counts and HGB levels of the athletes were not altered by the training programme.

KEYWORDS

apnoea, breath-holding, spleen size, haemoglobin, red blood cells, immunity

1 Introduction

Mammalian oxygen conservation mechanisms are activated rapidly during apnoea or diving (Kooyman et al., 1981; Schagatay et al., 2007), inducing a cardiovascular diving reflex that is characterised by heart rate deceleration, vasoconstriction and blood redistribution (Elsner and Gooden. 1983; Qvist et al., 1986), effectively slowing oxygen consumption and extending apnoea duration (Gooden 1994; Schagatay et al., 2001).

Another effective mechanism through which mammals respond to hypoxia is splenic contraction (Hurford et al., 1996; Cabanac et al., 1997). The spleen is a major blood reservoir in mammals (Anderson and Rogers., 1957; Guntheroth and Mullins., 1963; Espersen et al., 2002) and during strenuous exercise or apnoea, splenic contraction pumps stored concentrated red blood cells (RBCs) into circulation (Koga, 1979; Laub et al., 1993; Stewart and McKenzie. 2002; Bakovic et al., 2005; Richardson et al., 2009; Bakovic et al., 2013). This increases blood oxygen storage and oxygen-uptake kinetics (Thomas and Fregin, 1981; Longhurst et al., 1986; Wagneft et al., 1995; Schagatay et al., 2001; Brijs et al., 2020; Holmström et al., 2021). For example, hooded seals release oxygenated haemoglobin (HGB) from the spleen into circulation, which can increase dive duration by approximately 105 s, and harp seals can increase drive duration by 80 s (Cabanac et al., 1997). Splenectomy results in the loss of this ability (Persson et al., 1973; Thomas and Fregin, 1981; Baković et al., 2005; Bakovic et al., 2013; Brijs et al., 2020; Joyce and Axelsson. 2021).

Researchers have discovered that repeated low-oxygen exposure and long-term hypoxia cause splenic expansion (Bouten et al., 2019; Holmström et al., 2019; Lodin-Sundström et al., 2021). Under hypoxia, hypoxia-inducible factor (HIF-2) activates the erythropoietin (EPO) transcription gene while promoting renal cortex EPO-expressing cells as a means of producing EPO, thereby stimulating the production of erythrocytes and HGB (Tsuchiya et al., 1997; Warnecke et al., 2004; Jelkmann 2011).

Extensive studies of splenic and haematological reactions after apnoea have already been performed in the literature (Shephard et al., 2016; Elia et al., 2021; Pernett et al., 2021; Nordine et al., 2022). It has been proven that long-term static apnoea training increases both spleen volume and HGB (Bouten et al., 2019). As far as we are aware, no previous studies have reported the longitudinal effects of dry dynamic apnoea training on splenic size and haematological parameters. Dry dynamic apnoea has physiological demands that are significantly different to static apnoea (Bergman et al., 1972; Elia et al., 2021c; Nordine et al., 2022). Under dry dynamic apnoea conditions, myocardial and skeletal muscle oxygen consumption increases, heart rate becomes faster and acute conflict occurs between the oxygen conservation mechanism of the body and skeletal muscle oxygen demand (Elia et al., 2019; Elia et al., 2021c; Nordine et al., 2022). It can be reasonably assumed that dry dynamic apnoea training protocols provide greater hypoxic stimulation than static apnoea (Elia et al., 2019; Elia et al., 2021c; Nordine et al., 2022), but further research is required to confirm this.

Therefore, the aim of this study is to investigate the effect 8 weeks of dry dynamic breath-holding (BH) training has on spleen size and haematological parameters among student-athletes. As the spleen is a significant reservoir for immune cells, relevant parameters are included as indicators for assessing whether BH training induces immune function changes that have not been previously performed. It is hypothesised that 8 weeks of dry dynamic BH training increases spleen volume, RBC counts and HGB levels, but has no impact on immune function.

2 Materials and methods

2.1 Ethical aspect

This study is in full compliance with the World Medical Association Declaration of Helsinki and received ethical approval from the Guizhou University subcommittee of Human Medicine Experimental Ethics (No: HMEE-GZU-2022-T002). All volunteers were fully informed of any potential risks presented by this study and all provided written informed consent before their participation. The parents or guardians of subjects under 18 years of age signed informed consent forms on the behalf of their children.

2.2 Study design

The study was performed using a 2-arm randomised controlled experimental design for 8 weeks and involved five consecutive training sessions each week. The training consisted of two phases and lasted a total time of approximately 60 min. The first phase involved a 15-min warm-up, including a 10-min run and 5 minutes of stretching exercises. The second phase was dynamic apnoea training that lasted for approximately 45 min. Participants were subjected to baseline spleen volume measurement and venous blood sampling prior to the study. This was followed by a familiarisation session. The spleen size and venous blood samples of all volunteers were collected within 48 h of completion of the training. The same researchers were responsible for the management of the entire study. The experimental procedure can be seen in Figure 1.

2.3 Participants

Twenty six local student-athletes from Guizhou University were recruited to participate in this study. The volunteers were stratified according to gender prior to commencement of the

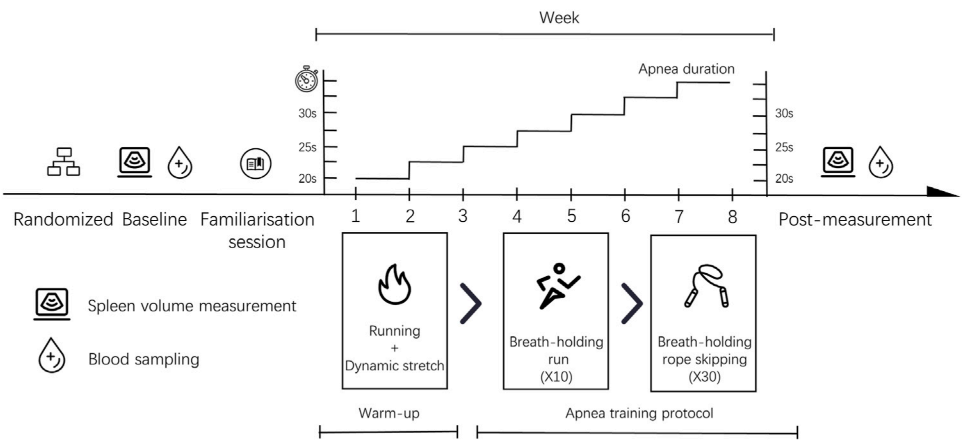

FIGURE 1
Study design.

study and then completely randomised into groups within each stratum using a computer-generated random number sequence. They were assigned to either the BH group or the control (Con) group.

Four volunteers (BH group, two men; Con group, one man and one woman) were excluded from the study as a result of withdrawal or low attendance. Other volunteers fully completed the training plans.

Inclusion criteria included members of the local population, aged 17–24 years, physically and mentally healthy and a willingness to attend long-term training sessions. Exclusion criteria included suffering from recent sports injuries, regular drug-taking, genetic disease or family disease history, having donated blood or smoked in the last 3 months, participation in BH-related sports (including free diving or synchronised swimming) and suffering from obstructive sleep apnoea syndrome. All volunteers were asked to maintain the same training level as before the study and not to donate blood, travel at high altitude or participate in any other studies. They were also advised to strictly adhere to the prescribed routine and informed that they could withdraw from the study at any time.

3 Experimental procedures

3.1 Preliminary measures

Anthropometrics, spleen quantification and blood sampling were conducted at Guiyang Huaxi District People's Hospital. Weight and height measurements were taken using the X-Scan Plus II (JAWON, South Korea). Blood pressure and heart rate were measured using an electronic blood pressure monitor (YE-680, Yuwell, Suzhou, China). It was requested that all subjects avoid any vigorous activity and refrain from the consumption of caffeinated and alcoholic beverages 24 h prior to the test. In addition, they were required to abstain from food and water for 8 h before the measurement.

3.2 Spleen imaging

Tests were conducted in the morning. All volunteers were asked to rest on their backs for 20 min upon arrival at the imaging centre (~25°C) and this was followed by a spleen scan. Subjects were positioned in the right lateral recumbent position, exposing the left lumbar and abdomen. At this time point, they were able to breathe freely. Maximum spleen length (L), maximum width (W) and maximum thickness (T) were all measured by an experienced physician (Wang*) using a colour ultrasound diagnostic system (Philips Affiniti 70W, Amsterdam Dutch). The scan lasted for 8–10 min and the results were documented and evaluated by another physician.

Figure 2A shows the maximum spleen length and thickness. Length was measured as the greatest overall dimension and thickness was measured as the shortest distance between the hilum and the outer convex surface of the spleen. Measurements were taken as perpendicular to each other as was possible. Figure 2B shows the maximum spleen width. Maximum width was measured as the greatest overall dimension. Spleen volume was calculated using the long ellipsoid formula: Spleen volume (ml) = 0.523 × (L × T × W).

3.3 Blood sample and analysis

A 2–3 ml blood sample was collected from the median cubital vein in an ethylenediaminetetraacetic acid (EDTA) vacuum blood collection tube. It was stored at 2–8°C, protected from

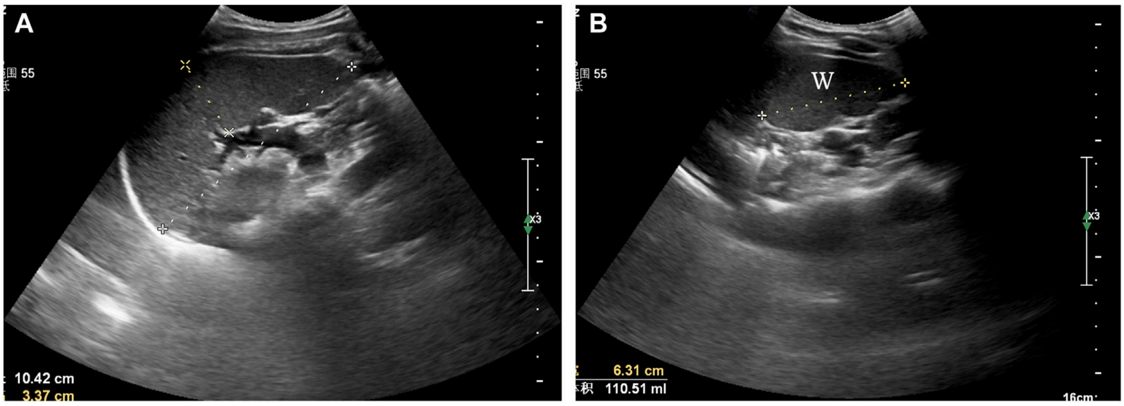

FIGURE 2
Ultrasonographic assessment of spleen volume. **(A)** maximum spleen length (L) and thickness (T), **(B)** maximum spleen width (W).

shock and examined within 2 h of collection. The collected blood samples were analysed using the Mindray BC-6800 Plus, BC-5180 CRP (Shenzhen, China) fully automated haematology analyser.

3.4 Familiarisation session

The researchers conducted a familiarisation session within 48 h of completion of the baseline measurements. All volunteers were provided with a detailed guide to the precautions during BH. They were informed to avoid hyperventilation and excessive air inhalation before holding their breath, concentrate on the BH task and not to swallow or exhale any gas during the BH. They were also guided to familiarise themselves with the entire experiment procedure and practice it until they were able to complete all training tasks correctly and successfully.

3.5 Apnoea training protocol

The training is conducted on a plastic track with cushioning technology, which can effectively reduce the risk of injury caused by falls. The dynamic apnoea training protocol involved 10 BH sessions of jogging and 30 repeats with a jump rope. A fixed BH duration and an escalating load model were used in the study. Volunteers were asked to achieve the 20-s BH goal during the first week. The average increase was 2.5 s per week and this peaked at 35 s in week 8. Exercise intensity was 60%–70% of the maximum heart rate load. Volunteers inhaled approximately 70%–85% of their maximum lung capacity before holding their breath. During BH sessions, the volunteers wear nose clips. In addition, finger clip pulse oximetry (Wellday MD300C23; Jiang Xi, China) was used for monitoring the heart rate and blood oxygen of participants during the training period. The device dynamically generates real-time SPO2 values within 8 s, which are read and recorded by the researcher.

3.5.1 Warm-up

In order to minimise the impact warm-up would have on the results, all volunteers performed the same warm-up routine in this study, involving 10 min of jogging and 5 min of dynamic stretching exercises. Exercise intensity was approximately 65%–75% of maximum heart rate. The dynamic stretching protocol was specifically designed to enable volunteers to adequately activate critical regions, including shoulders, elbows and knees. Injury potential was minimised as much as possible during the training.

3.5.2 Apnoea protocol

3.5.2.1 Breath-holding run

A researcher drove a small electric vehicle with a fixed speed function and asked participants to run anticlockwise around a track at a constant speed of ~2.35 m/s. When all volunteers were ready and wore nose clips, the researchers signalled them to hold their breath using a verbal command and timed them using a stopwatch. During the final 10 s of apnoea duration, the researchers gave an oral countdown and offered verbal encouragement. Once the apnoea was completed, the researchers told the volunteers to stop holding their breath and to continue their run. At this point, volunteers were allowed to remove the nose clip breathe freely and continue to the next training after 30 s. This process was repeated a total of 10 times. The Con group performed the same training task without BH and the researchers monitored them during the training period. After completing the training, volunteers were allowed a relaxation break of 2 min.

TABLE 1 Anthropometric characteristics of participants.

	BH group (n = 12, M = 6)	Control group (n = 10, M = 5)	p
	Mean ± SE	Mean ± SE	
Age (years)	21 ± 0	20 ± 0	0.327
Height (cm)	170 ± 3	174 ± 3	0.389
Body mass (kg)	59 ± 3	59 ± 3	0.999
BMI	20.3 ± 0.5	19.5 ± 0.5	0.254
BPM	63 ± 1	65 ± 1	0.215
sBP (mmHg)	109 ± 2	105 ± 3	0.418
dBP (mmHg)	66 ± 1	67 ± 2	0.582

Note: *denotes significance (p < 0.05).

3.5.2.2 Breath-holding jump rope

When all volunteers were ready and wore nose clips, the researchers signalled them to hold their breath using a verbal command and timed them using a stopwatch. During the final 10 s of apnoea duration, the researchers gave an oral countdown and offered verbal encouragement. At the end of the apnoea duration, the volunteers were told to stop holding their breath and were allowed to breathe freely and relax. After 30 s of rest, the next session was performed immediately. This process was repeated a total of 30 times. The control volunteers performed the same training task without BH and researchers monitored them during the training period.

3.5.3 Termination of experimental criteria

Training was terminated temporarily if any volunteer experienced dizziness, blurred vision, tinnitus, or nausea or had a pulse oximetry (SpO2) level of <60. In addition, an emergency response team of two trained researchers was in place to address any accidents that may occur during the training. The training site was near a hospital, meaning that volunteers could receive timely medical assistance in case of accident.

3.6 Statistical analysis

All data analysis and graph construction were performed using IBM SPSS Statistics 26 (Armonk, NY: IBM Corp, United States) and Origin9.1 (OriginLab, Northampton MA, United States) software. Shapiro-Wilk test was applied for assessing the normal data distribution. Levene's test was employed to test for homogeneity of variances. Repeated two-way ANOVA tests were applied as a means of assessing the differences between the resting baseline measurements and other data collection time points. Bonferroni was employed to perform multiple comparisons. Pearson's correlation coefficients were applied for determining inter-variable relationships. Mann-Whitney U-test and Wilcoxon signed-rank test were used as non-parametric tests in cases where the data variance was not homogeneous or non-normally distributed. p < 0.05 was considered as an indicator of statistical significance. All data is presented as the mean ± standard error (SE) or 95% confidence interval (CI).

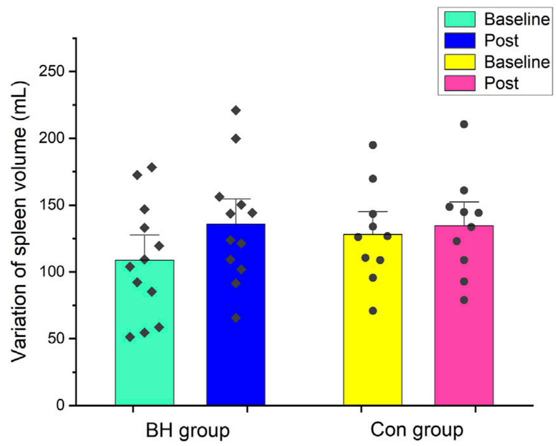

FIGURE 3
Mean (±SE) spleen volume (ml) variation from baseline to after 8 weeks in BH group and Con group.

4 Results

4.1 Subjects

Table 1 shows there to be no significant differences between the groups of volunteers regarding age, height, weight, heart rate or blood pressure level. No blurred vision, tinnitus or nausea were detected during training and relatively few volunteers exhibited signs of mild dizziness at the start of the training period.

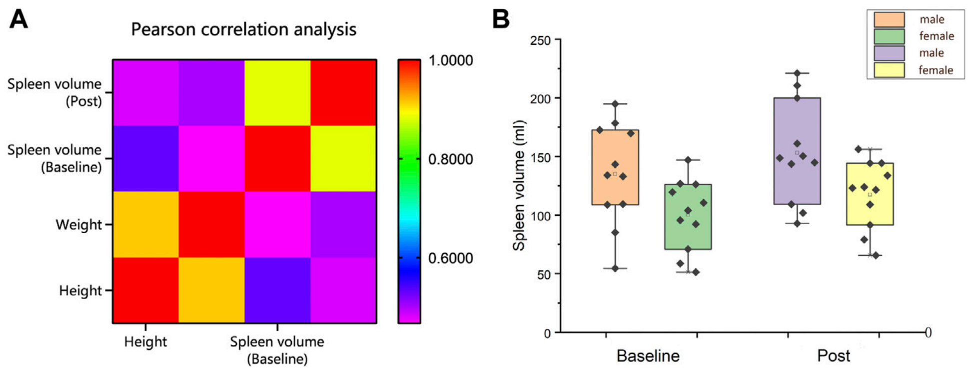

FIGURE 4
(A) Correlation analysis of height and weight with spleen size; **(B)** Spleen volume (ml) comparison between male and female before and after training (Mean ± SE).

TABLE 2 Variations in red blood cells, haemoglobin and related derivatives.

Item (unit)	BH group		p	Con group		p
	Baseline	8 weeks post		Baseline	8 weeks post	
RBC	4.88 ± 0.14	4.87 ± 0.12	0.914	5.07 ± 0.12	5.06 ± 0.11	0.813
HGB	147.7 ± 4.3	148.4 ± 4.9	0.637	149.5 ± 5.3	149.7 ± 6.2	0.908
HCT	45 ± 1	44 ± 1	0.511	45 ± 1	45 ± 2	0.830
MCV	92 ± 1	91 ± 1*	0.048	89 ± 2	89 ± 2	0.629
MCH	30 ± 1	30 ± 1	0.501	30 ± 1	30 ± 1	0.832
MCHC	330 ± 2	333 ± 3*	0.026	330 ± 3	331 ± 3	0.392
RDW-CV	12.8 ± 0.2#	12.9 ± 0.2	0.595	13.8 ± 0.4#	13.4 ± 0.2	0.064

Notes: RBC denotes red blood cell count (10^{12}/L); HGB denotes haemoglobin (g/L); HCT denotes haematocrit (%); MCV denotes mean corpuscular volume (fL); MCH denotes mean corpuscular haemoglobin (pg); MCHC denotes mean corpuscular haemoglobin concentration (g/L); RDW-CV denotes coefficient variation of red blood cell volume distribution width (%). *A significant difference in baseline values ($p < 0.05$); #a significant difference between groups ($p < 0.05$).

4.2 Spleen volume

Figure 3 shows spleen size changes in both groups after training. After 8 weeks interventional training, spleen size increased from 109 ± 13 ml (95% CI: 81–136 ml) to 136 ± 13 ml (95% CI: 108–164 ml) in the BH group (+24.72%, $p < 0.001$). No difference was observed before (128 ± 11 ml, 95% CI: 102–154 ml) or after training (135 ± 12 ml, 95% CI: 108–161 ml) in the Con group ($p = 0.375$).

Pearson's correlational analysis found there to be a moderate correlation between spleen size and height (Baseline: $r = 0.530$, $p = 0.011$; Post $r = 0.483$; $p = 0.023$) and weight (Baseline: $r = 0.467$, $p = 0.029$; Post: $r = 0.502$; $p = 0.017$) among 22 volunteers (Figure 4A). In this study, female spleen sizes were found to be significantly smaller than male spleen sizes (Baseline: 100 ± 9 ml vs. 135 ± 13 ml, $p = 0.041$; Post: 111 ± 9 ml vs. 15 ± 13 ml, $p = 0.033$; Interaction effect: gender * time, $p = 0.762$) (Figure 4B).

4.3 Erythrocytes and haemoglobin

Table 2 shows RBCs, HGB level and related derived index changes following training. No changes in RBCs ($p = 0.914$), HGB ($p = 0.637$), HCT ($p = 0.511$), MCH ($p = 0.501$) or RDW-CV ($p = 0.595$) were recorded in the BH group following training. MCV decreased from 91.93 ± 1.27 to 91.27 ± 1.48 (−0.72%, $p = 0.048$) and mean cell haemoglobin concentrations (MCHC) increased from 329.92 ± 2.05 to 334.42 ± 2.76 (+1.36%, $p = 0.026$) in the BH group following training. No significant changes were observed in the Con group.

4.4 Blood PLTs

Table 3 shows the changes in PLT-related indicators following training. No changes in PLTs ($p = 0.346$), mean

TABLE 3 Variation of platelet-related indicators.

Item (unit)	BH group		p	Con group		p
	Baseline	8 weeks post		Baseline	8 weeks post	
PLT	252.83 ± 12.39	246.67 ± 12.18	0.346	245.40 ± 11.27	244.90 ± 15.36	0.944
MPV	10.4 ± 0.4	10.1 ± 0.4	0.052	9.9 ± 0.3	9.9 ± 0.2	0.441
PDW	16.3 ± 0.1	16.3 ± 0.1	0.999	16.1 ± 0.1	16.2 ± 0.1	0.310
PCT	0.26 ± 0.01	0.25 ± 0.01	0.100	0.24 ± 0.01	0.24 ± 0.01	0.999
P-LCC	70.50 ± 5.83	65.17 ± 5.87*	0.034	62.00 ± 4.70	62.50 ± 4.75	0.847

Notes: PLT denotes platelet count (10^9/L); MPV denotes mean platelet volume (fL); PDW denotes platelet distribution width (%); PCT denotes plateletcrit (%); P-LCC denotes platelet large cell counts (bulky platelets) (10^9/L). *A significant difference in baseline values ($p < 0.05$); #A significant difference between groups ($p < 0.05$).

TABLE 4 Variations of immune cell parameter.

Item (unit)	BH group		p	Con group		p
	Baseline	8 weeks post		Baseline	8 weeks post	
WBC	5.55 ± 0.42	5.96 ± 0.37	0.532	6.09 ± 0.59	6.42 ± 0.77	0.649
NEU	3.21 ± 0.40	3.27 ± 0.34	0.933	3.69 ± 0.58	3.99 ± 0.90	0.708
LYM	1.89 ± 0.11	2.20 ± 0.13*	0.014	1.91 ± 0.67	1.95 ± 0.18	0.761
MON	0.34 ± 0.04	0.39 ± 0.02	0.097	0.33 ± 0.02	0.35 ± 0.02	0.627
EOS	0.09 ± 0.01	0.07 ± 0.01	0.250	0.14 ± 0.03	0.11 ± 0.03	0.294
BAS	0.02 ± 0.00	0.02 ± 0.00	0.999	0.03 ± 0.00	0.03 ± 0.00	0.999

Notes: WBC denotes white blood cell count (10^9/L); NEU denotes neutrophil counts (10^9/L); LYM denotes lymphocyte counts (10^9/L); MON denotes monocyte counts (10^9/L); EOS denotes eosinophil counts (10^9/L); BAS denotes basophile counts (10^9/L). *A significant difference with baseline values ($p < 0.05$); #A significant difference between groups ($p < 0.05$).

PLT volume (MPV) (p = 0.052), platelet distribution width (PDW) (p = 0.511) or plateletcrit (PCT) (p = 0.100) were recorded in the BH group following training. The number of bulky platelets (P-LCC) decreased from 70.50 ± 5.83 to 65.17 ± 5.87 (−7.57%, p = 0.034) in the BH group following training, while no significant changes were observed in the Con group.

Pearson's correlation analysis found the decrease in PLTs (6.17) and P-LCC (6.60) to have a significant correlation in the BH group following training (r = 0.721, p = 0.008).

4.5 Immune cells

Table 4 presents the changes in the immune-cell parameters after training. No changes in WBC (p = 0.532), neutrophil counts (NEU) (p = 0.933), monocyte counts (MON) (p = 0.097), eosinophil counts (EOS) (p = 0.250), and basophile counts (BAS) (p > 0.999) were recorded after training in the BH group. Lymphocyte counts (LYM) increased from 1.89 ± 0.11 to 2.20 ± 0.13 (+16.40%, p = 0.014) in the BH group after training. No significant changes were noted in the Con group.

5 Discussion

This study evaluated the effects dry dynamic apnoea training has on spleen size and haematological parameters among athletes. The primary findings showed that 8 weeks of dynamic apnoea training increased spleen size, reduced the number of bulky PLTs in circulation and showed no significant changes in HGB, RBC, PLT or WBC.

The spleen volumes of volunteers were evaluated using sonography. The techniques and standards that are used for the determination of spleen volume by ultrasonography have been presented in previous studies (Koga and Morikawa. 1975; Rezai et al., 2011). Sonography is a quick, easy, inexpensive and relatively accurate examination method and it presents no risk of radiation exposure (Yetter et al., 2003; Natsume et al., 2011). The efficacy and reliability of sonography have been determined by many studies that have compared measurements with CT scan results or autopsy results (Koga and Morikawa, 1975; Schlesinger et al., 1993; Loftuset al. 1999; Lamb et al., 2002; Yetter et al., 2003). The standard clinical ellipsoid equation (L × T × W × 0.523) was used in this study for evaluating spleen volume as it has good efficacy and is regularly used for evaluating the volume of irregularly-shaped organs including the spleen and uterus (Sauerbrei et al., 1986; Sonmez et al., 2007).

The results of this study are in accordance with those from the report by Bouten on increased spleen size in volunteers following apnoea training (Bouten et al., 2019), which reports that repeated exposure to hypoxia affects spleen size. However, no spleen volume increase was observed by Elia or Engan in their respective studies (Engan et al., 2013; Elia et al., 2021c). In comparison to the two-week training programme of Engan, the training periods of both Bouten and this study were significantly longer. Rodriguez et al. also reported a spleen volume increase of 40% after 6 weeks of travelling at high altitude (Rodríguez-Zamora et al., 2015). Therefore, it was speculated that some time-dose response may have potentially contributed to the result. More extended intervention periods and more potent stimuli are required for increasing spleen size. In a six-week study by Elia, no spleen volume increase was observed. A possible reason is that although the study extended the training periods, the training sessions (24 sessions) were fewer than those in the study by Bouten et al. (2019). Furthermore, the longer interval between weeks (3 days) may also have attenuated the training effect. Similarly, Elia et al. (2021c) noted that splenic expansion may require more prolonged periods in their study. In addition, four volunteers (BH group, two men; Con group, one man and one woman) were excluded from this study due to withdrawal or low attendance. Therefore, the BH group had a slightly smaller baseline spleen volume than the Con group. Before this, the male spleen volume is generally larger than the female spleen volume has been confirmed (Spielmann et al., 2005; Chow et al., 2016). We corrected the baseline spleen volume differences by employing baseline spleen volume as a covariate and found a significant difference in the intervention effect between the two groups ($p = 0.023$).

The mechanism for determining spleen size still remains unknown (Elia et al., 2021b). Several studies have reported spleen size to be affected by age, sex, height, weight, and ethnicity (Kaneko et al., 2002; de Bruijn et al., 2008; Hiraiwa et al., 2022). This study determined a correlation between spleen size and height ($r = 0.530$; $p = 0.011$) and weight ($r = 0.467$; $p = 0.029$). In contrast, Elia et al. reported there to be no direct correlation between spleen size and height ($r = -0.0001$, $p = 0.995$) or weight ($r = -0.040$, $p = 0.863$) in their study on a group of elite BH divers and non-divers (Elia et al., 2019). Similar results were reported in a study by Schagatay et al. (2012). This difference in results can potentially be explained by spleen size being significantly different between individuals (Elia et al., 2021b). In addition, spleen size may be affected by acquired factors including hypoxia or training (Rodríguez-Zamora et al., 2015; Bouten et al., 2019; Kaiser et al., 2022). Therefore, the occupations of subjects and the activities they perform must be adequately considered.

Ilardo et al. (2018) conducted a comparative genetic study on the outstanding Bajau divers in Southeast Asia. They found spleen size to be genetically determined without any phenotypic plasticity (Ilardo et al., 2018). However, some recent studies have suggested that spleen size may be influenced by complex interaction between genetic susceptibility and environmental exposure (Schagatay et al., 2020; Holmström et al., 2020; Bouten et al., 2019; Rodríguez-Zamora et al., 2015; Schagatay et al., 2015). For example, Sherpas who live at high altitude tend to have larger spleen volumes than those who live at lower altitude and the average spleen volume of Sherpas is larger than that of Nepalese people who live at lower altitudes (Holmström et al., 2020). In addition, it has been confirmed that the spleen has regenerative abilities (Pabst et al., 1984; Ando et al., 2004; Ibrahim et al., 2005; Petrovai et al., 2013). Researchers found that among 207 athletes in the Lanzhou region of China who engaged in different sports, 71.2% of males and 66.7% of females had enlarged spleens, while 56% of males and 34% of females had enlarged livers in comparison to those of the local population. The authors of the paper suggested that blood flow, oxygen consumption and metabolism due to high-intensity exercise can result in the spleen making changes as a means of adapting to the demands of the body. However, the study made no further distinctions based on the sports that were undertaken by the athletes (MingLi et al., 1992).

In this study, no significant changes were detected following training in both RBC and HGB, which is in accordance with the study results of Engan et al. (2013) and Elia et al. (2021c). EPO plays a crucial role in the regulation of RBC and the production of HGB (Adamson 1996; Jelkmann 2003). EPO production increases in the context of systemic hypoxia or hypoxemia. In 2008, de Bruijn et al. (2008) reported that circulating EPO concentrations increased by 16% over 3 h following a series of repeated BH events, which indicates that the erythropoietic process is enhanced (Banfi 2008). Similar observations were reported by Kjeld et al. (2015) and Elia et al. (2019). However, some researchers have conservative opinions regarding whether the increase in EPO concentration induced by BH is sufficient for stimulating an increase in erythrocyte and HGB volume (Elia et al., 2021b). For example, Engan et al. (2013) reported reticulocyte counts to increase by 15% ($p < 0.05$) following BH training, while no significant change was detected in the baseline HGB. Similar results were also reported by Elia et al. (2021c). Conversely, Bouten et al. (2020) reported a 3.3% increase in baseline HB following 8 weeks of BH training. However, it was also noted that the effect of plasma and blood volume changes in HB could not be ruled out completely. In this study, no HGB changes could be attributed for the following reasons: 1) volunteers lived at medium or high altitude for a long time (1,130 m above sea level), making them resistant to low-oxygen level; 2) baseline erythrocyte (male: 5.18, female: 4.58) and HGB levels (male: 159.33, female: 136.00) of the volunteers were already incredibly close to the upper limit of the range of normal values prior to the intervention. Therefore, the training may have been insufficient for the generation of more powerful stimuli for erythrocytes and HGB production in organisms.

It is believed that this is the first study that has noted a decrease in baseline P-LCC following apnoea training. PLT levels were not altered and the spleen is a dynamic reservoir of bulky PLTs (Bakovic et al., 2013). Exchangeable splenic PLT pool size increases as spleen size increases ($r = 0.76$, $p < 0.001$) (Wadenvik et al., 1987). When the spleen becomes enlarged, splenic blood PLT density is higher than circulating PLT density (Aster 1966). In addition, Ando et al. (2004) found there to be a distinctly negative correlation between an increase in spleen volume and circulating PLT count ($r = -0.411$, $p = 0.045$). Therefore, a potential explanation for the decrease in bulky PLTs in this study is that enlarged spleens increase the aggregation effect on bulky PLTs. While the alteration in PLT count following training was not statistically significant, it was observed that the decrease in number (6.16) approximated the reduction in the number of bulky PLTs (5.33). An association between reduced PLTs and reduced bulky PLTs was also determined by correlation analysis and a significant correlation was found ($r = 0.721$; $p = 0.008$). In addition, Aster suggested a theory that the possibility of not excluding splenomegaly (causing hypersplenism) inhibits the bone marrow *via* humoral control. Splenomegaly results in an increased aggregation of PLTs while the regenerative capacity of PLTs is inhibited (Aster 1966).

This study found that lymphocytes proliferated in the BH group after 8 weeks of dynamic training and the other immunological parameters remained unaltered. Hypoxia is an environmental stressor that causes the induction of neuroendocrine responses and changes in specific components in the immune system (Facco et al., 2005), including the redistribution of T lymphocytes, a significant reduction in $CD4^+$ T-cells and impaired T-cell activation and proliferation (Meehan et al., 1988; Klokker et al., 1993), thereby affecting the human immune function. A combination of exercise and hypoxia results in the effects on immune function being more pronounced than those of exercise or hypoxia independently (Mazzeo, 2005). For example, the combined effect of exercise and hypoxia has a more significant effect on natural killer cells than those that are under normoxia (Klokker et al., 1993). Evidence from cross-sectional studies has shown that a group of Olympic-level athletes noted a decrease in whole blood leukocyte count following 21 days of altitude training ($p < 0.05$) (Pyne et al., 2000). Kitaev and Tokhtabaev, (1981) reported B cells and active immunoglobulin counts to increase following 25 days at high altitude (3,200 m). In another study, no effect on B-cell counts was noted at different altitudes (Facco et al., 2005). Conversely, no change in WBC was observed in this study ($p = 0.532$). However, an increase in lymphocytes from 1.89 ± 0.11 to 2.20 ± 0.13 was observed in the BH group. This parameter remained at the normal range and had no significant effect on the immune function of the athlete, unless the change crossed a critical threshold.

6 Conclusion

This is the first study that has investigated the efficacy of 8 weeks of dry dynamic apnoea training on splenic volumes and haematological indices. The study results found dry dynamic breath-hold training to increase spleen volume mong athletes without any alteration to their RBC or HGB levels.

7 Limitations

The current study design has several limitations, which should be acknowledged. First, The sample size of our study was small, thus may make the Statistical results underrepresentation. The second criticism concerns the validity of the ultrasonographic assessment of spleen volume. We observed larger spleen volume variation in the breath hold group individuals and reduced spleen volume in control group 2 volunteers (9 ml and 16 ml). Although individual splenic volume and contractility are highly variable in humans (Elia et al., 2021b). It is significant to note that measurement errors associated with ultrasound measurements could have affected these variations (Holmström et al., 2021). Finally, limited by the conditions, only 2-time points were measured, which failed to further eliminate experimental errors.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Guizhou University subcommittee of human medicine experimental ethics. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

KY, W-BW, Z-HY and X-LC made equal contributions to this work. Z-BY contributed to study conception, laboratory organisation and critical review. YJ, J-FG and M-MD contributed to organisation and implementation. All authors contributed to the article and have approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.925539/full#supplementary-material

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Facial mask acute effects on affective/psychological and exercise performance responses during exercise: A meta-analytical review

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Background: Face masks are widely used during the COVID-19 pandemic as one of the protective measures against the viral infection risk. Some evidence suggests that face mask prolonged use can be uncomfortable, and discomfort can be exacerbated during exercise. However, the acute responses of mask-wearing during exercise on affective/psychological and exercise performance responses is still a topic of debate.

Purpose: To perform a systematic review with meta-analysis of the acute effects of mask-wearing during exercise on affective/psychological and exercise performance responses in healthy adults of different/diverse training status.

Methods: This review (CRD42021249569) was performed according to Cochrane's recommendations, with searches performed in electronic (PubMed, Web of Science, Embase, SportDiscus, and PsychInfo) and pre-print databases (MedRxiv, SportRxiv, PsyArXiv, and Preprint.Org). Syntheses of included studies' data were performed, and the RoB-2 tool was used to assess the studies' methodological quality. Assessed outcomes were affective/psychological (discomfort, stress and affective responses, fatigue, anxiety, dyspnea, and perceived exertion) and exercise performance time-to-exhaustion (TTE), maximal power output (PO_{MAX}), and muscle force production] parameters. Available data were pooled through meta-analyses.

Results: Initially 4,587 studies were identified, 36 clinical trials (all crossover designs) were included. A total of 749 (39% women) healthy adults were evaluated across all studies. The face mask types found were clothing (CM), surgical (SM), FFP2/N95, and exhalation valved FFP2/N95, while the most common exercises were treadmill and cycle ergometer incremental tests, beyond outdoor running, resistance exercises and functional tests. Mask-wearing during exercise lead to increased overall discomfort (SMD: 0.87; 95% CI 0.25–1.5; p = 0.01; I^2 = 0%), dyspnea (SMD: 0.40; 95% CI 0.09–0.71; p = 0.01; I^2 = 68%), and perceived exertion (SMD: 0.38; 95% CI 0.18–0.58; $p < 0.001$; I^2 = 46%); decreases on the TTE (SMD: −0.29; 95% CI −0.10 to −0.48; $p < 0.001$; I^2 = 0%); without effects on PO_{MAX} and walking/running distance traveled ($p > 0.05$).

Conclusion: Face mask wearing during exercise increases discomfort (large effect), dyspnea (moderate effect), and perceived exertion (small effect), and reduces the TTE (small effect), without effects on cycle ergometer PO_{MAX} and distance traveled in walking and running functional tests. However, some aspects may be dependent on the face mask type, such as dyspnea and perceived exertion.

Systematic Review Registration: [https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021249569], identifier [CRD42021249569].

KEYWORDS

mask-wearing, physical exercise (EX), pandemic (COVID-19), respirator, face mask

1 Introduction

The world witnessed the emergence of a new virus in China. The virus was later termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and defined as the causal agent of the disease known as coronavirus disease 2019—COVID-19 (Ludwig and Zarbock, 2020). From this, health control agencies describe that social distancing is one of the main measures to prevent the spread of the disease, and personal and environmental protection measures and surface hygiene would be essential to avoid contagion (Aquino et al., 2020). Among the protective means, face mask wearing was considered one of the main resources capable of slowing down the advance of the pandemic (Asadi-Pooya and Cross, 2020; Chu et al., 2020). Face mask use is widely discussed during the COVID-19 pandemic and its used is defended by experts as it is a simple, inexpensive, and potentially effective measure to reduce the transmission of respiratory diseases (Asadi et al., 2020; Asadi-Pooya and Cross, 2020). However, the protection offered by the face mask wearing seems to depend on the type of mask, with models that are more or less effective in containing the emission of aerosol particles (Asadi et al., 2020; Fischer et al., 2020; Ramirez-Moreno et al., 2020), such as FFP2/N95 respirators, that provide greater protection but also have greater resistance than surgical masks (SM) (Hopkins et al., 2021).

Beyond the discussions about its effectiveness against coronavirus infection (Asadi et al., 2020; Liang et al., 2020), there are reports of prolonged use of the face mask causing skin lesions, headaches, discomfort and malaise, signs of stress, anxiety, and claustrophobia (Ramirez-Moreno et al., 2020). It may occur because the face mask creates a closed-circuit environment of inspired and expired air (Tornero-Aguilera et al., 2021), increasing ventilation due to carbon dioxide (CO_2) re-inhalation (Hopkins et al., 2021). Inspiration of CO_2 appears to be the driving force behind the increased ventilation when breathing through a face mask, since a 1-mm Hg increase in alveolar CO_2 partial pressure appears to be sufficient to increase ventilation (Hopkins et al., 2021). Face mask use lead to increased subjective stress responses and discomfort levels (Andre et al., 2018; Morris et al., 2020; Tornero-Aguilera et al., 2021), and the adverse effects caused by wearing a face mask seem to be potentiated during exercise due to a reduction in the ability to breathe comfortably (Hopkins et al., 2021; Reychler et al., 2021), for these reasons, its use in closed environments (e.g., gyms and training centers) is still much discussed (Hopkins et al., 2021).

Recent systematic reviews (Engeroff et al., 2021; Shaw et al., 2021) investigated the face mask using during exercise on some psychophysiological responses. Face mask wearing during exercise seems to increase the perceived exertion and dyspnea (Shaw et al., 2021), impair cardiorespiratory parameters (e.g., oxygen uptake and ventilation) (Engeroff et al., 2021), without affecting oxygen saturation, and heart rate (Engeroff et al., 2021; Shaw et al., 2021). Although there is already evidence of the face mask using effects on physiological outcomes, no systematic review or meta-analysis focused on quantifying the magnitude of the effects of face mask wearing during exercise exclusively on affective/psychological outcomes. Furthermore, the effects stratified by type of face mask need to be further explored.

Considering that mask wearing may cause discomfort and this could compromise the exercise performance parameters (Motoyama et al., 2016; Andre et al., 2018; Fikenzer et al., 2020; López-Pérez et al., 2020; Tornero-Aguilera et al., 2021; Boyle et al., 2022), the acute effects of face mask use were investigated through different exercise performance aspects. Previous systematic reviews (Engeroff et al., 2021; Shaw et al., 2021) showed no effects of face mask wearing on exercise performance. However, exercise performance was pooled through different outcomes. The literature shows different physical performance protocols tested comparing with and without face mask use. Decreases in the time-to-exhaustion (TTE) (Öncen and Pinar, 2018; Driver et al., 2021; Boyle et al., 2022), maximal power output (PO_{MAX}) in cycle ergometer (Fikenzer et al., 2020; Egger et al., 2021; Zhang et al., 2021), total volume and the maximum number of repetitions during resistance exercises (Rosa et al., 2022), and performance in sprint tests (Dantas et al., 2021; Modena et al., 2021; Tornero-Aguilera et al., 2021) were reported. However, there seems to be no consensus in the literature on the occurrence of these effects. In addition, we have not found systematic reviews so far that have investigated the effects of face mask use exclusively on exercise performance parameters.

Knowledge about the acute effects of face mask wearing during exercise on affective/psychological aspects and their potential adverse effects on exercise performance outcomes is still unclear, especially when considering the different types of face mask. This gap in the literature shows that this systematic review is relevant and can be useful to help physical therapists and trainers understand and make decisions regarding the acute effects of the face mask wearing during exercise. Therefore, we aimed to perform a systematic review with meta-analysis of trials that tested the face mask acute effects on affective/psychological and exercise performance responses during exercise in healthy adults of different/diverse training status.

2 Methods

2.1 Study reporting and protocol registration

A systematic review with meta-analysis was performed following the recommendations from the Cochrane Collaboration (Higgins et al., 2019) and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes) reporting guidelines (Page et al., 2021). The review protocol was registered on PROSPERO under the number CRD42021249569. The study selection, data extraction, and the methodological quality assessment of the included studies were conducted by two independent investigators (M.H.G. and E.M.) and when there was some disagreement between the results of the two reviewers, a third reviewer (S.K.P.) was consulted to reach a consensus.

2.2 Data sources and searches

In March 2022, searches were performed in five electronic databases (ISI Web Knowledge, MEDLINE/Pubmed, Embase, SportDiscus, and PsychInfo) and preprint databases (MedRxiv, SportRxiv, PsyArXiv, Preprint.Org) without time and language restrictions. Furthermore, reference lists of relevant reviews (Feye and Magallanes, 2019; Andreu, 2021; Haraf et al., 2021; Shaw et al., 2021) and included trials were manually searched. Combinations were used with the descriptors and keywords adapted for each database (adult OR athletes) AND (mask OR "respiratory protection device") AND (exercise OR sport OR "physical activity" OR athletic performance" OR "aerobic exercise" OR "resistance training"). The searches were combined using the terms MeSH or EMTREE, and using Boolean operators "AND" and "OR." Full details of the search strategy are presented in the Supplementary Table S2.

2.3 Study selection and screening criteria

The present review was composed of a three-stage screening process, which was conducted by two independent reviewers (M.H.G. and I.M.B.). When there was between-reviewers disagreement, a third reviewer (S.K.P.) was consulted to reach a consensus. Initially, were screened titles and excluded irrelevant papers (e.g., *in vitro* studies). In the second stage, the reviewers screened studies by abstracts. In the last stage, the full text of the studies was assessed according to the eligibility criteria.

The inclusion criteria adopted were: 1) randomized and non-randomized controlled trial; 2) healthy adults (described as ages between 19 and 44 years by Medical Subject Headings); 3) face mask wearing during sports practices, aerobic and/or resistance exercise intervention; 4) control condition with a no-wearing face mask during exercise; and 5) assessment of affective/psychological and/or exercise performance parameters. Studies that did not meet all the inclusion criteria or that presented the following exclusion criteria were excluded: 1) measurements of chronic effects; 2) other facial mask types (e.g., elevation training mask; air-laine breathing apparatus; self-contained breathing apparatus, sport protective helmets); and 3) no accessible full-text.

Randomized and non-randomized controlled trials (crossovers or parallel-group studies) with the full and accessible text were included in this review. Primary outcomes considered were as acute effects of facial mask in: 1) affective/psychological parameters (discomfort, stress and affective responses, fatigue, anxiety, dyspnea, and perceived exertion); 2) exercise performance parameters [TTE, PO, bar propulsive velocity (BPV), number of repetitions, muscle force production, and walk/running acceleration, speed, and time]. The secondary outcomes were: 1) face mask type [i.e., clothing (CM), SM, FFP2/N95, and FFP2/N95 with exhalation valve (FFP2/N95 + EV)]; 2) exercise type, volume, and intensity.

2.4 Data extraction

Searches on databases were completed by 7 March 2022. The data from each study were extracted individually and exported to a spreadsheet. Study design, sample size, participant characteristics, face mask type, exercise (type, volume, and intensity), measured outcomes (main outcomes and assessments), and results [intervention (face mask wearing) and control (no-face mask wearing) pre-post changes by mean and standard deviation values] were extracted.

Data from both crossover and parallel-group studies were included. When studies did not provide enough data (i.e., incomplete reporting), the corresponding author of the study was contacted by email and asked to provide additional information. Case the database was provided by the authors, the mean and standard deviation values were calculated for quantitative analysis (Epstein et al., 2021; Fukushi et al., 2021; Modena et al., 2021). When authors did not respond or could not provide the required data, the mean and standard deviation values were obtained manually from the plots using the ImageJ tool (version 1.48v, National Institutes of Health, Bethesda, MA, United States), whenever possible. When access to the data was not possible, or in case of incompatible data (e.g., non-groupable exercise), the study was not included in the quantitative analysis.

2.5 Data synthesis and statistics

Common outcomes among three or more studies were considered for the meta-analyses using the standardized mean difference (SMD), standard error (SE), and 95% confidence interval (95% CI) as measures of effect, dispersion, and range, respectively. The effect size was classified according to Cohen's d-values (Higgins et al., 2019), where SMD: <0.40 = small effect; 0.40–0.70 = moderate effect; >0.70 = large effect (Cohen, 1988). Therefore, seven meta-analyses (discomfort, dyspnea, perceived exertion, TTE, absolute and relative PO_{MAX}, and distance traveled in walking and running functional tests) were performed based on the face mask wearing acute effects during exercise. We have included over 10 studies for dyspnea and perceived exertion, allowing us to perform subgroup analysis on the different face mask types (i.e., CM, SM, FFP2/N95 + EV, and FFP2/N95). When the study presents more than one mask type, for the meta-analysis, the chosen mask was determined from the criterion of the least restrictive face mask to the most restrictive (ascending order: clothing, SM, FFP2/N95 + EV, and FFP2/N95), considering the least restrictive face masks (i.e., those made from cloth) as it is the most used by the general population. When the study performed tests at different intensities, the intensity that generated the greatest metabolic load was adopted for analysis. In studies that used both face masks with and without an exhalation valve, the face mask without a valve was considered for analysis, since it is more common in the general population and during the COVID-19 pandemic.

In the crossover trials, when a study did not present the correlation coefficient (r) values between the pre-post changes, a sensitivity analysis using different estimate values ($r = 0.5$; $r = 0.7$; and $r = 0.8$) was performed. As none of the values directly affected the results of the meta-analyses, we adopted a conservative estimate of "$r = 0.7$." For all the meta-analyses, we used the random-effects model. This model was adopted *a piori* due to the expected heterogeneity in the studies' intervention types, and the evaluation of the common measures, and confirmed by the I^2 test, which was interpreted according to Higgins et al. (2003) considering that the values above 25% and 50% were classified as moderate and high heterogeneity, respectively. When moderate or high heterogeneity was found (values > 25%), sensitivity analysis was performed and the heterogeneity was explored. All statistical analyses were performed using the Comprehensive Meta-Analysis (version 3.0; Biostat, Englewood, NJ, United States). The level of statistical significance was determined as $\alpha \leq 0.05$.

2.6 Studies' quality appraisal

The methodological quality of the included studies was examined by two independent reviewers (E.M. and A.R.B.), with a third reviewer consulted in cases of disagreement (M.H.G.). The assessment tool was the Revised Cochrane Risk-of-Bias Tool for Randomized Trials (ROB 2) for parallel-group and crossover trials (Sterne et al., 2019). This tool was used assignment to intervention (the "intention-to-treat" effect) and has five domains for judgment of risk of bias: randomization process; deviations from intended intervention, missing outcome data; result measurement; selection of the reported result. Each domain was rated as "low risk of bias," "high risk of bias," and "some concerns" for the reported outcomes, and the overall risk of bias judgment: low risk of bias—all domains showed a low risk of prejudice; some concerns: in at least one domain for this outcome but not being at high risk of bias for any domain, and high risk of bias—when in at least one domain for this outcome or have some concerns for multiple domains in some way that substantially reduces confidence in the result.

3 Results

3.1 Search results

A flow diagram of the literature search and screening is displayed below (Figure 1). The initial search identified 4,587 studies in all combined databases. In the full-text stage, 69 studies were eligible, and in the end, 34 studies (Zimmerman et al., 1991; Roberge et al., 2010; Roberge et al., 2012a; Roberge et al., 2012b; Chen et al., 2016; Kim et al., 2016; Person et al., 2018; Fikenzer et al., 2020; Lässing et al., 2020; Morris et al., 2020; Wong et al., 2020; Ade et al., 2021; Bar-On et al., 2021; Dantas et al., 2021; Doherty et al., 2021; Driver et al., 2021; Egger et al., 2021; Epstein et al., 2021; Fukushi et al., 2021; Kampert et al., 2021; Mapelli et al., 2021; Modena et al., 2021; Reychler et al.,

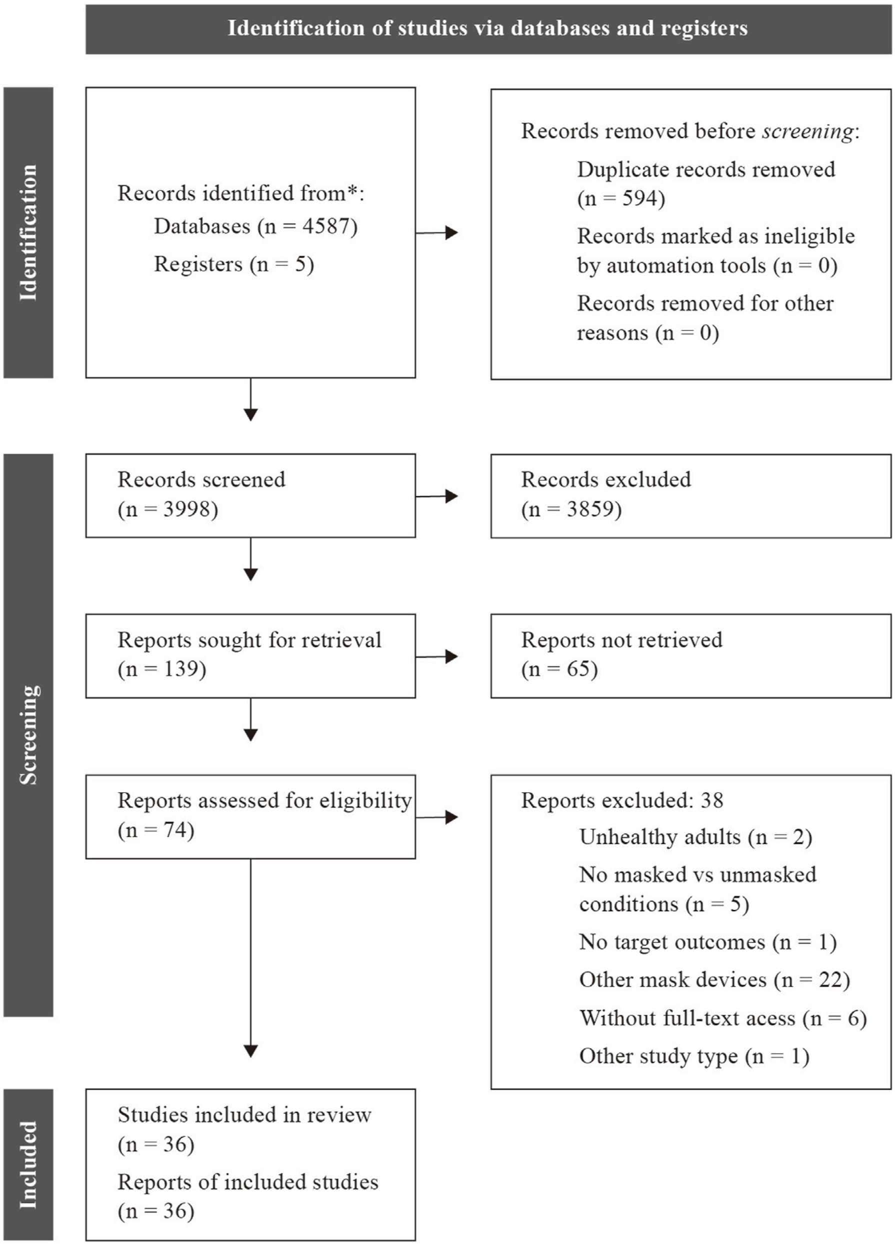

FIGURE 1
PRISMA chart of the study flow.

2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Slimani et al., 2021; Tornero-Aguilera et al., 2021; Yoshihara et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Jesus et al., 2022; Ng et al., 2022; Rosa et al., 2022; Steinhilber et al., 2022) met all the eligibility criteria and were included in the review. Two additional studies (Shaw et al., 2020; Otsuka et al., 2022) were found from other sources (e.g., references from included studies, and publications founded in other reviews). Therefore, 36 eligible studies were included in the review.

3.2 Characteristics of the included studies

Table 1 summarizes the main information of the included studies. Most studies were randomized controlled trials (n = 34), except for two studies (Roberge et al., 2012a; Roberge et al., 2012b) that did not perform randomization. The crossover design was adopted by all the studies. All studies presented a control condition (i.e., no face mask wearing). The samples were composed mostly by healthy (Zimmerman et al., 1991; Roberge

TABLE 1 Summary of studies on the acute effects of comparison between use of mask versus no-mask wearing during exercise.

Study	Design/ Mask type	Participants	Exercise protocol	Outcomes
Ade et al. (2021)	RCT Crossover design 1: CON 2: CM 3: SM 4: FFP2/N95	Healthy and physically active adults. (n = 11; M = 5; W = 6) 30 ± 11 years	Incremental cycle ergometer exercise to exhaustion (increases of 20 W/min; until the participant could not maintain the pedal cadence of 60 rpm for 5 consecutive revolutions).	-Affective/psychological responses -Dyspnea: ↑9% RPE: ns -Exercise performance responses PO_{MAX}: ns
Bar-On et al. (2021)	RCT Crossover design 1: CON 2: SM	Healthy adults. (n = 10; M = 5; W = 5) 28 ± 5 years	Constant load treadmill walking for 5 min: a) slow walk (4 km/h at 0° inclination); b) brisk walk (7 km/h at 0° inclination).	-Affective/psychological responses RPE: [ab]↑39%
Cabanillas-Barea et al. (2022)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Healthy adults., (n = 50; M = 26; W = 24), 21 ± 5 years	6 MWT	-Affective/psychological responses, Dyspnea: ↑61%–129%, -Exercise performance responses, Distance traveled: ns
Chen et al. (2016)	RCT Crossover design 1: CON 2: FFP2/N95 3: FFP2/N95 + EV	Healthy adults., (n = 15; M = 15; W = 0), 28 ± 2 years	Constant load treadmill walking for 5 min (treadmill speed of 1.6 m/s and 0° inclination).	-Affective/psychological responses, Dyspnea: ↑369%–431%
Dantas et al. (2021)	RCT Crossover design 1: CON 2: CM	Track and field athletes., (n = 10; M = 7; W = 3), 23 ± 4 years	Outdoor track field running test (five maximal 30 m sprints, with 4 min rest between runs); and vertical jump (countermovement jump).	-Affective/psychological responses, Affect: ↓114%–148%, RPE: ↑46%, -Exercise performance responses, Sprint time: ns, Sprint acceleration: ns, Jump height: ns
Doherty et al. (2021)	RCT Crossover design 1: CON (MP) 2: CON 3: CM 4: SM	Healthy adults., (n = 12; M = 7; W = 5), 26 ± 3 years	Constant load cycle ergometer submaximal exercise for 8 min (submaximal exercise at 70% HR_{MAX}).	-Affective/psychological responses, Dyspnea: ↑42%
Driver et al. (2021)	RCT Crossover design 1: CON 2: CM	Healthy adults., (n = 31; M = 17; W = 14), 23 ± 3 years	Incremental treadmill cardiopulmonary exercise test (Bruce's standard protocol).	-Affective/psychological responses, RPE: ns, Dyspnea: ↑31%, -Exercise performance responses, TTE: ↓14%
Egger et al. (2021)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Well-trained healthy athletes (2 road cyclists; 8 mountain bikers, and 6 triathletes), (n = 16; M = 16; W = 0), 27 ± 7 years	Incremental cycle ergometer exercise to exhaustion (starting at 100–150 W; increases of 50 W every 3 min until voluntary exhaustion or when subjects were unable to maintain a pedaling cadence of 50 rpm for more than 10 s).	-Affective/psychological responses, RPE: ns, -Exercise performance responses, PO_{MAX}: ↓4%–6%

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TABLE 1 (*Continued*) Summary of studies on the acute effects of comparison between use of mask versus no-mask wearing during exercise.

Study	Design/ Mask type	Participants	Exercise protocol	Outcomes
Epstein et al. (2021)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Healthy and physically active adults., (*n* = 10; M = 5; W = 5), 28 ± 5 years	Incremental cycle ergometer exercise to exhaustion (starting at 25 W; increases of 25 W every 3 min until voluntary exhaustion; cadence of 55–65 rpm).	-Affective/psychological responses, RPE: ns, -Exercise performance responses, TTE: ns
Fikenzer et al. (2020)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Healthy and physically active adults., (*n* = 12; M = 12; W = 0), 38 ± 6 years	Incremental cycle ergometer exercise to exhaustion (starting at 50 W; increases of 50 W every 3 min until voluntary exhaustion; cadence of 60–70 rpm).	-Affective/psychological responses, Overall discomfort: ↑86%–150%, -Exercise performance responses, PO_{MAX}: ↓5%
Fukushi et al. (2021)	RCT Crossover design 1: CON 2: CM 3: SM	Healthy adults., (*n* = 24; M = 15; W = 9), 21 ± 1 years	Incremental treadmill cardiopulmonary exercise test (Modified Bruce's standard protocol).	-Affective/psychological responses, RPE: ↑25%–44%
Jesus et al. (2022)	RCT Crossover design 1: CON 2: SM	Healthy adults., (*n* = 32; M = 16; W = 16), 24 ± 3 years	Constant load cycle ergometer exercise [two different intensities: a) moderate exercise at 25% below VT; and b) severe exercise at 25% above VT].	-Exercise performance responses, TTE: [a]ns [b]↓10%
Kampert et al. (2021)	RCT Crossover design 1: CON 2: CM 3: FFP2/N95	Healthy and physically active adults., (*n* = 20; M = 11; W = 9), M: 39 ± 11 years, W: 35 ± 11 years	Incremental treadmill cardiopulmonary exercise test (constant speed; treadmill grade increased from 0% to 2% at 2 min; and continuous increases by 1% every minute until voluntary exhaustion).	-Affective/psychological responses, RPE: ns, Overall discomfort: ↑60%, -Exercise performance responses, TTE: ns
Kim et al. (2016)	RCT Crossover design 1: CON 2: FFP2/N95 3: FFP2/N95 + EV	Healthy adults., (*n* = 12; M = 12; W = 0), 24 ± 2 years	Constant load treadmill walking for 1 h (low-moderate work rate at 5.6 km/h and 0° inclination).	-Affective/psychological responses, RPE: ns, Dyspnea: ↑23%–41%, Thermal sensation: ns
Lässing et al. (2020)	RCT Crossover design 1: CON 2: SM	Healthy adults., (*n* = 14; M = 14; W = 0), 26 ± 4 years	Constant load cycle ergometer exercise for 30 min (50% of $PO_{MAX;}$ cadence of 60–70 rpm).	-Affective/psychological responses, RPE: ns
Mapelli et al. (2021)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Healthy adults., (*n* = 12; M = 6; W = 6), 41 ± 12 years	Incremental cycle ergometer exercise (aimed to achieving peak exercise in ~10 min).	-Affective/psychological responses, Dyspnea: ud, -Exercise performance responses, PO_{MAX}: ↓4%–5%
Modena et al. (2021)	RCT Crossover design	Amateur soccer players., (*n* = 21; M = 21; W = 0), 25 ± 5 years	Running protocol [4 min running at: a) 8 km/h; and b) 10 km/h; 8 bouts of 90 m intermittent running; and YoYo-Intermittent Recovery Test Level-1].	-Affective/psychological responses, RPE: ns, Dyspnea: ns, -Exercise performance responses, Distance traveled: ↓11%–13%

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TABLE 1 (*Continued*) Summary of studies on the acute effects of comparison between use of mask versus no-mask wearing during exercise.

Study	Design/ Mask type	Participants	Exercise protocol	Outcomes
	1: CON 2: CM 3: SM			
Morris et al. (2020)	RCT Crossover design 1: CON 2: FFP2/N95	Healthy adults., (n = 8; M = 8; W = 0), 35 ± 7 years	Light exercise simulating work in healthcare and related settings for 45 min (100 W; equivalent to ~5 METs).	-Affective/psychological responses, Dyspnea: ↑140%, Facial thermal discomfort: ↑22%
Ng et al. (2022)	RCT Crossover design 1: CON 2: SM	Healthy and trained adults., (n = 8; M = 4; W = 4), 25 ± 3 years	Incremental cycle ergometer exercise to exhaustion (starting at 50 W; increases of 25 W every 3 min until voluntary exhaustion).	-Affective/psychological responses, Dyspnea: ns, -Exercise performance responses, TTE: ↓6%, PO_{MAX}: ns
Otsuka et al. (2022)	RCT Crossover design 1: CON 2: SM	Healthy and sedentary adults., (n = 6; M = 6; W = 0), 24 ± 2 years	Incremental cycle ergometer exercise to exhaustion (increases of 20 W every minute until voluntary exhaustion).	-Affective/psychological responses, RPE: ns, Dyspnea: ↑30%
Person et al. (2018)	RCT Crossover design 1: CON 2: SM	Healthy adults., (n = 44; M = 18; W = 26), 22 ± 3 years	6 MWT	-Affective/psychological responses, Dyspnea: ↑22%, -Exercise performance responses, Distance traveled: ns
Reychler et al. (2021)	RCT Crossover design 1: CON 2: CM 3: SM	Healthy adults., (n = 20; M = 11; W = 9), 22 ± 2 years	STS^{1min}	-Affective/psychological responses, Dyspnea: ↑100%, Overall discomfort: ↑3%–4%, -Exercise performance responses, T-REPS: ns
Roberge et al. (2010)	RCT Crossover design 1: CON 2: FFP2/N95 3: FFP2/N95 + EV	Healthy adults., (n = 10; M = 3; W = 7), 25 (20–45) years	Constant load treadmill walking for 1 h: a) 1.7 mph; b) 2.5 mph.	-Affective/psychological responses, Overall discomfort: ^{ab}ns, RPE: ^{ab}ns
Roberge et al. (2012b)	CT 1: CON 2: SM	Healthy adults., (n = 20; M = 13; W = 7), 23 ± 3 years	Constant load treadmill walking for 1 h (low-moderate work rate at 5.6 km/h and 0° inclination)	-Affective/psychological responses, RPE: ns
Roberge et al. (2012a)	CT 1: CON 2: FFP2/N95 3: FFP2/N95 + EV 4: FFP2/N95 5: FFP2/N95 + EV	Healthy adults., (n = 20; M = 13; W = 7), 23 ± 3 years	Constant load treadmill walking for 1 h (low-moderate work rate at 5.6 km/h and 0° inclination)	-Affective/psychological responses, RPE: ↑8%
Rojo-Tirado et al. (2021)	RCT Crossover design	Healthy sportswomen., (n = 13; M = 0; W = 13), 22 ± 2 years	Incremental treadmill cardiopulmonary exercise test [treadmill grade of 1%; warm-up of 6 km/h for 3 min; starting the incremental phase with	-Affective/psychological responses, RPE: ns, Dyspnea: ns, -Exercise performance responses, TTE: ns

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TABLE 1 (*Continued*) Summary of studies on the acute effects of comparison between use of mask versus no-mask wearing during exercise.

Study	Design/ Mask type	Participants	Exercise protocol	Outcomes
	1: CON 2: CM 3: FFP2/N95		8 km/h of running, with increases of 0.2 km/h every 12 s (1 km/h/min) until it was possible to maintain the treadmill speed].	
Rosa et al. (2022)	RCT Crossover design 1: CON[a] 2: CON[b] 3: FFP2/N95[a] 4: FFP2/N95[b]	Healthy and resistance-trained adults., (n = 17; M = 17; W = 0), 28 ± 4 years	Resistance training with BP exercise [4 sets to failure with resting interval of 2 min between sets, performed at different intensities: a) moderate—50% 1RM; and b) high—70% 1RM; with a wash-out of 72 h between sessions].	-Affective/psychological responses, RPE[a]: ns, RPE[b]: ns, -Exercise performance responses, Maximal BPV_{BP}: [a]ns [b]↓10%, Mean BPV_{BP}: [a]ns [b]↓14%, T-$REPS_{BP}$: [ab]ns, T-VOL_{BP}: [ab]ns
Ryu and Jong-Geun , (2021)	RCT Crossover design 1: CON 2: SM 3: FFP2/N95	Healthy adults., (n = 11; M = 11; W = 0), 23 ± 3 years	Incremental treadmill cardiopulmonary exercise test (Bruce's standard protocol).	-Exercise performance responses, TTE: ns
Shaw et al. (2020)	RCT Crossover design 1: CON 2: CM 3: SM	Healthy and physically active adults., (n = 14; M = 7; W = 7), 28 ± 9 years	Incremental cycle ergometer exercise to exhaustion (starting at 35–100 W; increases of 35 W every 2 min until voluntary exhaustion; cadence of 70–75 rpm).	-Affective/psychological responses, RPE: ns, -Exercise performance responses, TTE: ns, PO_{MAX}: ns
Slimani et al. (2021)	RCT Crossover design 1: CON 2: CM	Healthy adults., (n = 17; M = 9; W = 8), 18 years	Warm-up exercises for 15 min [light runs (4 min); arm circles, jumping jacks, high knees jog, and back kicking (2.5 min); stretching exercises (1.5 min); and 4 sets of each exercise—push-up, sit-up, and squat (30 s of work per 30 s of rest)].	-Affective/psychological responses, RPE: ↑27%
Steinhilber et al. (2022)	RCT Crossover design 1: CON 2: CM 3: SM 4: FFP2/N95 + EV	Healthy adults., (n = 39; M = 20; W = 19), 38 ± 14 years	Constant load cycle ergometer exercise until exhaustion [two different intensities according to HR: a) 130 bpm; and b) 150 bpm].	-Affective/psychological responses, RPE: [ab]ns, Dyspnea: [a]↑22%–35% [b]↑29–35%, -Exercise performance responses, PO_{MAX}: [a]↓9% [b]ns
Tornero-Aguilera et al. (2021)	RCT Crossover design 1: CON 2: SM	Recreational athletes., (n = 72; M = 45; W = 27), 28 ± 6 years	Outdoor track field running tests: a) 50 m; b) 400 m.	-Affective/psychological responses, RPE: [a]ns [b]↑7%, Subjective stress responses: [a]ns [b]↑18%, -Exercise performance responses, Sprint time: [a]↑13% [b]↑19%
Wong et al. (2020)	RCT Crossover design 1: CON 2: SM	Healthy and physically active adults., (n = 23; M = 10; W = 13), 34 ± 11 years	Constant load treadmill walking for 6 min (4 km/h at 10° incline).	-Affective/psychological responses, RPE: ↑13%–20%
Yoshihara et al. (2021)	RCT Crossover design 1: CON	Healthy and physically active adults., (n = 12; M = 8; W = 4), 24 ± 3 years	Constant load treadmill walking/jogging for 1 h (speed increases of 0.5–1.0 mph every 2 min until voluntary exhaustion).	-Affective/psychological responses, RPE: ns, Dyspnea: ↑379%–761%, Thermal sensation: ns, Fatigue level: ns

(Continued on following page)

TABLE 1 (*Continued*) Summary of studies on the acute effects of comparison between use of mask versus no-mask wearing during exercise.

Study	Design/ Mask type	Participants	Exercise protocol	Outcomes
	2: CM 3: SM 4: FFP2/N95			
Zhang et al. (2021)	RCT Crossover design 1: CON 2: SM	Healthy adults., (*n* = 71; M = 35; W = 36), 28 ± 8 years	Incremental cycle ergometer exercise to exhaustion (incremental power of 15–25 W/min until exhaustion calculated to let subjects finish the exercise load test between 8 and 12 min; cadence of 60 rpm).	-Affective/psychological responses, RPE: ↑19%, Dyspnea: ↑19, -Exercise performance responses, TTE: ns, PO_{MAX}: ↓5%
Zimmerman et al. (1991)	RCT Crossover design 1: CON 2: SM	Healthy adults., (*n* = 12; M = 12; W = 0), 21 (18–24) years	Constant load cycle ergometer exercise (280–350 W; constant rate).	-Functional responses, Hand grip strength: ns

RCT, randomized controlled trial; CON, control condition (non-fascial mask wearing); CM, clothing mask; SM, surgical mask; FFP2/N95, facepiece respirator type 2; MP, mouthpiece; EV, exhalation valve; a: lower exercise intensity condition; b: higher exercise intensity condition; n: sample size; M, men; W, women; ± standard deviation values; min, minute; rpm, revolutions per minute; mph, miles per hour; h, hour; km/h, kilometers per hour; m, meters; RM, maximum repetitions; sec, seconds; BP, bench press exercise; VO_2max, maximal oxygen uptake; METs, metabolic equivalent of task; PO_{MAX}, maximal power output; HR, heart rate; HR_{MAX}, predicted maximum heart rate; VT, ventilatory threshold; 6 MWT, six-minute walk test; STS^{1min}, one-min sit-to-stand test; ns, statistically non-significant; ud, unavailable data; ↓ statistically significant lesser compared to the condition without mask; ↑ statistically significant greater compared to the condition without mask; RPE, rate of perceived exertion; T-REP, total number of repetitions; T-VOL, total volume of repetitions; TTE, time-to-exhaustion; BPV, bar propulsive velocity.

et al., 2010; Roberge et al., 2012a; Roberge et al., 2012b; Chen et al., 2016; Kim et al., 2016; Person et al., 2018; Lässing et al., 2020; Morris et al., 2020; Bar-On et al., 2021; Doherty et al., 2021; Driver et al., 2021; Fukushi et al., 2021; Mapelli et al., 2021; Reychler et al., 2021; Ryu and Jong-Geun, 2021; Slimani et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Jesus et al., 2022; Steinhilber et al., 2022), healthy physically active (Fikenzer et al., 2020; Shaw et al., 2020; Wong et al., 2020; Ade et al., 2021; Epstein et al., 2021; Kampert et al., 2021; Yoshihara et al., 2021), healthy trained (Rojo-Tirado et al., 2021; Ng et al., 2022) resistance-trained (Rosa et al., 2022), and healthy sedentary (Otsuka et al., 2022) adults; athletes (Dantas et al., 2021; Egger et al., 2021; Tornero-Aguilera et al., 2021) and amateur soccer players (Modena et al., 2021). The sample size varied from six (Otsuka et al., 2022) to 72 (Tornero-Aguilera et al., 2021) participants per condition. Some studies (*n* = 10) (Person et al., 2018; Shaw et al., 2020; Ade et al., 2021; Bar-On et al., 2021; Driver et al., 2021; Reychler et al., 2021; Rojo-Tirado et al., 2021; Zhang et al., 2021; Ng et al., 2022; Steinhilber et al., 2022) determined the sample size based on sample calculation. A total of 749 participants: 460 men and 289 women; with a mean age of 27 years old, were evaluated across all studies.

Among the types of exercise, the participants performed on cycle ergometer (*n* = 14) (Zimmerman et al., 1991; Fikenzer et al., 2020; Lässing et al., 2020; Shaw et al., 2020; Ade et al., 2021; Doherty et al., 2021; Egger et al., 2021; Epstein et al., 2021; Mapelli et al., 2021; Zhang et al., 2021; Jesus et al., 2022; Ng et al., 2022; Otsuka et al., 2022; Steinhilber et al., 2022), walking in treadmill (*n* = 13) (Roberge et al., 2010; Roberge et al., 2012a; Roberge et al., 2012b; Chen et al., 2016; Kim et al., 2016; Wong et al., 2020; Bar-On et al., 2021; Driver et al., 2021; Fukushi et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Yoshihara et al., 2021), outdoor track field running (*n* = 2) (Dantas et al., 2021; Tornero-Aguilera et al., 2021), high intensity interval training (*n* = 2) (Modena et al., 2021; Slimani et al., 2021), resistance exercise (*n* = 1) (Rosa et al., 2022), and other functional tasks (*n* = 5), such as vertical jump (*n* = 1) (Dantas et al., 2021), six-minute walk test (6 MWT) (Person et al., 2018; Cabanillas-Barea et al., 2022) and one-min sit-to-stand test (STS^{1min}) (Reychler et al., 2021), and healthcare work tasks (Morris et al., 2020). Regarding the types of face mask, studies used CM (*n* = 13) (Shaw et al., 2020; Ade et al., 2021; Dantas et al., 2021; Doherty et al., 2021; Driver et al., 2021; Fukushi et al., 2021; Kampert et al., 2021; Modena et al., 2021; Reychler et al., 2021; Rojo-Tirado et al., 2021; Slimani et al., 2021; Yoshihara et al., 2021; Steinhilber et al., 2022), SM (*n* = 27) (Zimmerman et al., 1991; Roberge et al., 2012a; Roberge et al., 2012b; Person et al., 2018; Fikenzer et al., 2020; Lässing et al., 2020; Shaw et al., 2020; Wong et al., 2020; Ade et al., 2021; Bar-On et al., 2021; Doherty et al., 2021; Egger et al., 2021; Epstein et al., 2021; Fukushi et al., 2021; Mapelli et al., 2021; Modena et al., 2021; Reychler et al., 2021; Ryu and Jong-Geun, 2021; Tornero-Aguilera et al., 2021; Yoshihara et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Jesus et al., 2022; Ng et al., 2022; Otsuka et al., 2022; Steinhilber et al., 2022), FFP2/N95 (*n* = 14) (Roberge et al., 2010; Chen et al., 2016; Kim et al., 2016; Fikenzer et al., 2020; Morris et al., 2020; Ade et al., 2021; Egger et al., 2021; Epstein et al., 2021; Kampert et al., 2021; Mapelli

Study name	Sample size: Mask	Sample size: Control	SMD	SE	Lower limit	Upper limit	p	Relative weight
Roberge et al. (2010)	10	10	0.61	0.46	-0.28	1.51	0.18	47.91
Fikenzer et al. (2020)	12	12	1.12	0.44	0.26	1.98	0.01	52.09
			0.87	0.32	0.25	1.50	0.01	

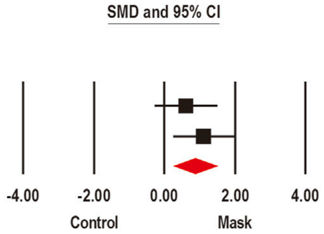


FIGURE 2
Acute effects of face mask vs. control (unmasked condition) on discomfort. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: (SMD: 0.87; 95% CI 0.25–1.5; p = 0.01; I^2 = 0%).

et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Yoshihara et al., 2021; Cabanillas-Barea et al., 2022; Rosa et al., 2022), and FFP2/N95 + EV (n = 5) (Roberge et al., 2010; Roberge et al., 2012a; Chen et al., 2016; Kim et al., 2016; Steinhilber et al., 2022). The effects of face mask wearing during exercise on affective/psychological parameters were assessed by some studies (n = 33) (Roberge et al., 2010; Roberge et al., 2012a; Roberge et al., 2012b; Chen et al., 2016; Kim et al., 2016; Person et al., 2018; Fikenzer et al., 2020; Lässing et al., 2020; Morris et al., 2020; Shaw et al., 2020; Wong et al., 2020; Ade et al., 2021; Bar-On et al., 2021; Dantas et al., 2021; Doherty et al., 2021; Driver et al., 2021; Egger et al., 2021; Epstein et al., 2021; Fukushi et al., 2021; Kampert et al., 2021; Mapelli et al., 2021; Modena et al., 2021; Reychler et al., 2021; Rojo-Tirado et al., 2021; Slimani et al., 2021; Tornero-Aguilera et al., 2021; Yoshihara et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Ng et al., 2022; Otsuka et al., 2022; Rosa et al., 2022; Steinhilber et al., 2022), while others (n = 21) (Zimmerman et al., 1991; Person et al., 2018; Fikenzer et al., 2020; Ade et al., 2021; Dantas et al., 2021; Driver et al., 2021; Egger et al., 2021; Epstein et al., 2021; Kampert et al., 2021; Mapelli et al., 2021; Modena et al., 2021; Reychler et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Tornero-Aguilera et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Jesus et al., 2022; Ng et al., 2022; Rosa et al., 2022; Steinhilber et al., 2022) assessed its effects on exercise performance responses.

3.3 Face mask wearing effects on affective/psychological responses

3.3.1 Discomfort

Overall discomfort was assessed by four studies (Roberge et al., 2010; Fikenzer et al., 2020; Kampert et al., 2021; Reychler et al., 2021), during incremental cycle ergometer (Fikenzer et al., 2020) and treadmill (Kampert et al., 2021) exercises, constant walking on the treadmill (Roberge et al., 2010), and STS^{1min} (Reychler et al., 2021), using CM (Kampert et al., 2021; Reychler et al., 2021), SM (Fikenzer et al., 2020; Reychler et al., 2021), FFP2/N95 (Roberge et al., 2010; Fikenzer et al., 2020; Kampert et al., 2021), and FFP2/N95 + EV (Roberge et al., 2010) mask types. A meta-analysis was performed to estimate the effects for mask overall discomfort (n = 2) (Roberge et al., 2010; Fikenzer et al., 2020) (Figure 2). A large effect was observed for increased discomfort (SMD: 0.87; 95% CI 0.25 to 1.5; p = 0.01; I^2 = 0%) with the use of face mask in exercise.

Thermal sensations and facial thermal discomfort during exercise were investigated by two (Kim et al., 2016; Yoshihara et al., 2021) and a single study (Morris et al., 2020), respectively. Thermal sensations were assessed during constant load treadmill walking/jogging for 1 h, using CM and SM (Yoshihara et al., 2021), FFP2/N95 (Kim et al., 2016; Yoshihara et al., 2021), and FFP2/N95 + EV (Kim et al., 2016). No effect was identified by the use of the face mask on thermal sensations. On the other hand, Morris et al. (2020) observed increased (22%) facial thermal discomfort with the use of FFP2/N95 during 45 min of light exercise simulating work in healthcare and related settings.

3.3.2 Subjective stress responses

Subjective stress responses were investigated by a single study (Tornero-Aguilera et al., 2021) through a subjective perceived stress scale of 0–100 points to assess the degree to which situations in individual life are perceived as stressful (Cohen and Janicki-Deverts, 2012), measured after 50 m and 400 m outdoor track field running tests using SM. While the use of the face mask produced no stress responses in the 50 m test, the perceived stress was 18% higher compared to the control condition during the 400 m test. Therefore, although the evidence is limited, it is possible that the face mask may increase stress responses, although this effect may be dependent on the duration and intensity of the exercise.

3.3.3 Affective responses

Affective responses (i.e., psychological manifestations selected for their ability to promote health, well-being and to solve recurrent adaptive problems) (Ekkekakis et al., 2005) were also investigated during exercise wearing a face mask by a single study (Dantas et al., 2021). Dantas et al. (2021) assessed affective responses by a feeling scale (11-point bipolar scale, composed of

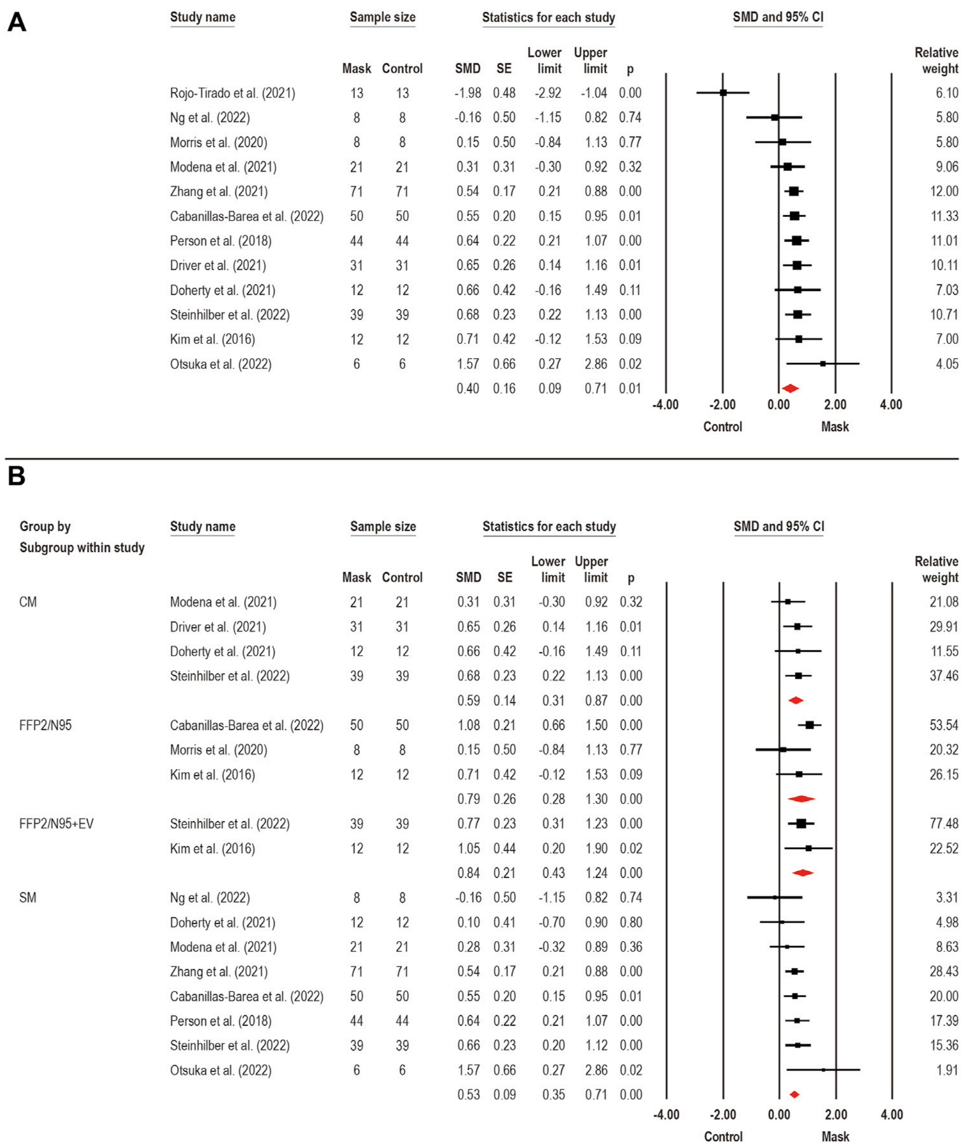


FIGURE 3
Acute effects of face mask vs. control (unmasked condition) on dyspnea. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: **(A)** analysis of face mask wearing effects on dyspnea (SMD: 0.40; 95% CI 0.09–0.71; p = 0.01; I^2 = 68%); **(B)** subgroup analysis of face mask wearing effects by face mask type [CM: clothing mask (SMD: 0.59; 95% CI 0.31–0.87; $p < 0.001$; I^2 = 0%); SM: surgical mask (SMD: 0.53; 95% CI 0.35–0.71; $p < 0.001$; I^2 = 0%); FFP2/N95: facepiece respirator type 2 (SMD: 0.79; 95% CI 0.28–1.30; $p < 0.001$; I^2 = 37%); and FFP2/N95 + EV: facepiece respirator type 2 with exhalation valve (SMD: 0.84; 95% CI 0.43–1.24; $p < 0.001$; I^2 = 0%)].

negative valences including: −5 = very bad, −3 = bad, −1 = reasonably bad; positive valences including: +5 = very good; +3 = good and +1 = reasonably good; and 0 being neutral) and showed lower affective responses (114%–148%) during outdoor track field running tests and countermovement jump using a CM by track and field athletes in comparison to non-masked condition. Although a single study reported lower affective responses with face mask wearing than a non-masked condition, the low number of studies limits further conclusion.

3.3.4 Fatigue

Fatigue was investigated during exercise wearing a face mask by a single study (Yoshihara et al., 2021). Yoshihara et al. (2021) used CM, SM, and FFP2 masks during constant load treadmill walking or jogging for 1 h, and fatigue was measured through 10-point fatigue level scale (ranging from no fatigue at all to completely fatigued). Despite the lack of studies, no effect of face mask wearing was reported, suggesting that face mask use does not increase fatigue levels during exercise.

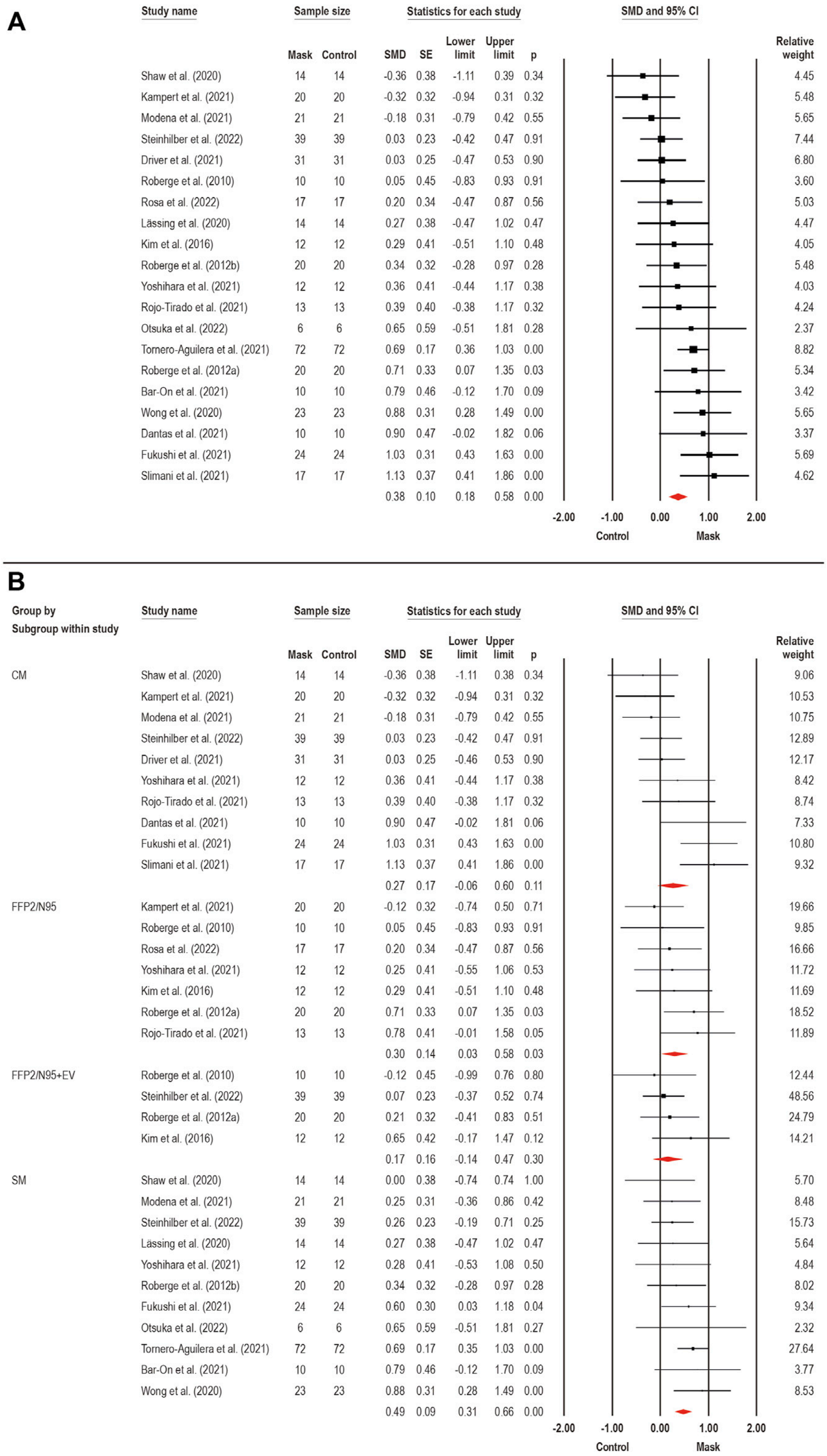


FIGURE 4
Acute effects of face mask vs. control (unmasked condition) on perceived exertion. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: **(A)** analysis of face mask wearing effects on perceived exertion (SMD: 0.38; 95% CI 0.18–0.58; p < 0.001; I^2 = 46%); **(B)** subgroup analysis of face mask wearing effects by face mask type [CM: clothing mask (SMD: 0.27; 95% CI −0.06 to 0.60; p = 0.11; I^2 = 62%); SM: surgical mask (SMD: 0.49; 95% CI 0.31–0.66; p < 0.001; I^2 = 0%); FFP2/N95: facepiece respirator type 2 (SMD: 0.30; 95% CI 0.03–0.58; p = 0.03; I^2 = 0%); and FFP2/N95 + EV: facepiece respirator type 2 with exhalation valve (SMD: 0.17; 95% CI −0.14 to 0.47; p = 0.30; I^2 = 0%)].

3.3.5 Dyspnea

Dyspnea (i.e., subjective experience of breathing discomfort and shortness of breath) (Reychler et al., 2021) was assessed by 16 studies. Twelve studies (Kim et al., 2016; Person et al., 2018; Morris et al., 2020; Doherty et al., 2021; Driver et al., 2021; Modena et al., 2021; Rojo-Tirado et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Ng et al., 2022; Otsuka et al., 2022; Steinhilber et al., 2022) were pooled in a meta-analysis to estimate face mask wearing effects on dyspnea (Figure 3A). A moderate overall effect was detected by face mask on the dyspnea (SMD: 0.40; 95% CI 0.09 to 0.71; $p = 0.01$; $I^2 = 68\%$). Considering the existence of high heterogeneity, subgroup analyzes were performed for face mask type effects on dyspnea (Figure 3B): SM ($n = 8$) (Person et al., 2018; Doherty et al., 2021; Modena et al., 2021; Zhang et al., 2021; Cabanillas-Barea et al., 2022; Ng et al., 2022; Otsuka et al., 2022; Steinhilber et al., 2022), CM ($n = 4$) (Doherty et al., 2021; Driver et al., 2021; Modena et al., 2021; Steinhilber et al., 2022), FFP2/N95 ($n = 3$) (Kim et al., 2016; Morris et al., 2020; Cabanillas-Barea et al., 2022), and FFP2/N95 + EV ($n = 2$) (Kim et al., 2016; Steinhilber et al., 2022). The subgroup analysis showed a large effect for FFP2/N95 + EV (SMD: 0.84; 95% CI 0.43–1.24; $p < 0.001$; $I^2 = 0\%$) and FFP2/N95 (SMD: 0.79; 95% CI 0.28–1.30; $p < 0.001$; $I^2 = 37\%$), and moderate effect for CM (SMD: 0.59; 95% CI 0.31–0.87; $p < 0.001$; $I^2 = 0\%$) and SM (SMD: 0.53; 95% CI 0.35–0.71; $p < 0.001$; $I^2 = 0\%$). These findings suggest that face mask wearing can increase dyspnea sensations during exercise and the magnitude of this effect seems to depend on the type of face mask. In summary, mask type FFP2/N95 have a large effect size, while the CM and SM masks have a moderate effect size.

3.3.6 Perceived exertion

Perceived exertion (i.e., an appropriate measure of internal training load) (Dantas et al., 2021) was assessed by 25 studies. A meta-analysis was performed to estimate the face mask wearing effects on perceived exertion (Figure 4A). A total of 20 studies pooled for an overall analysis, in which a small effect was observed for mask wearing on perceived exertion (SMD: 0.38; 95% CI 0.18–0.58; $p < 0.001$; $I^2 = 46\%$). Considering the existence of moderate heterogeneity, subgroup analyzes were performed for face mask type effects on perceived exertion (Figure 4B): SM ($n = 11$) (Roberge et al., 2012b; Lässing et al., 2020; Shaw et al., 2020; Wong et al., 2020; Bar-On et al., 2021; Fukushi et al., 2021; Modena et al., 2021; Tornero-Aguilera et al., 2021; Yoshihara et al., 2021; Otsuka et al., 2022; Steinhilber et al., 2022), CM ($n = 10$) (Shaw et al., 2020; Dantas et al., 2021; Driver et al., 2021; Fukushi et al., 2021; Kampert et al., 2021; Modena et al., 2021; Rojo-Tirado et al., 2021; Slimani et al., 2021; Yoshihara et al., 2021; Steinhilber et al., 2022), FFP2/N95 ($n = 7$) (Roberge et al., 2010; Roberge et al., 2012a; Kim et al., 2016; Kampert et al., 2021; Rojo-Tirado et al., 2021; Yoshihara et al., 2021; Rosa et al., 2022), and FFP2/N95 + EV ($n = 4$) (Roberge et al., 2010; Roberge et al., 2012a; Kim et al., 2016; Steinhilber et al., 2022). When analyzing the effects by the subgroups, we observed a moderate effect for SM (SMD: 0.49; 95% CI 0.31–0.66; $p < 0.001$; $I^2 = 0\%$), and small effect for FFP2/N95 (SMD: 0.30; 95% CI 0.03–0.58; $p = 0.03$; $I^2 = 0\%$), while no effects were found for CM (SMD: 0.27; 95% CI −0.06 to 0.60; $p = 0.11$; $I^2 = 62\%$) and FFP2/N95 + EV (SMD: 0.17; 95% CI −0.14 to 0.47; $p = 0.30$; $I^2 = 0\%$). Therefore, the face mask wearing effects on perceived exertion seems to depend on the type of face mask used, in which only the SM and FFP2/N95 mask types seem to increase perceived exertion during exercise.

3.4 Face mask wearing effects on exercise performance responses

3.4.1 Time-to-exhaustion performance

The TTE were assessed by nine trials (Shaw et al., 2020; Driver et al., 2021; Epstein et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Zhang et al., 2021; Jesus et al., 2022; Ng et al., 2022) through submaximal ($n = 1$) (Jesus et al., 2022) and incremental ($n = 4$) (Shaw et al., 2020; Epstein et al., 2021; Zhang et al., 2021; Ng et al., 2022) exercise on cycle ergometer, and incremental exercise on treadmill ($n = 4$) (Driver et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021), with CM ($n = 4$) (Shaw et al., 2020; Driver et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021), SM ($n = 6$) (Shaw et al., 2020; Epstein et al., 2021; Ryu and Jong-Geun, 2021; Zhang et al., 2021; Jesus et al., 2022; Ng et al., 2022), and FFP2/N95 ($n = 4$) (Epstein et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021) face masks. A meta-analysis pooled nine studies (Shaw et al., 2020; Driver et al., 2021; Epstein et al., 2021; Kampert et al., 2021; Rojo-Tirado et al., 2021; Ryu and Jong-Geun, 2021; Zhang et al., 2021; Jesus et al., 2022; Ng et al., 2022) to estimate the overall effect of face mask wearing on TTE, which is displayed in Figure 5. We found a harmful effect of small magnitude on TTE with face mask use (SMD: −0.29; 95% CI −0.10 to −0.48; $p < 0.001$; $I^2 = 0\%$), suggesting that wear a face mask may reduce the exercise duration due to a shorter time to reach exhaustion.

3.4.2 Power output performance

The PO_{MAX} (i.e., highest power output achieved during the cycle ergometric test) were assessed by eight trials (Fikenzer et al., 2020; Shaw et al., 2020; Ade et al., 2021; Egger et al., 2021; Mapelli et al., 2021; Zhang et al., 2021; Ng et al., 2022; Steinhilber et al., 2022), respectively, through submaximal ($n = 1$) (Steinhilber et al., 2022) and incremental ($n = 7$) (Fikenzer et al., 2020; Shaw et al., 2020; Ade et al., 2021; Egger et al., 2021; Mapelli et al., 2021; Zhang et al., 2021; Ng et al., 2022) exercise on cycle ergometer, with CM ($n = 3$) (Shaw et al., 2020; Ade et al., 2021; Steinhilber et al., 2022), SM ($n = 8$) (Fikenzer et al., 2020; Shaw et al., 2020; Ade et al., 2021; Egger et al., 2021; Mapelli et al., 2021; Zhang et al., 2021; Ng et al., 2022; Steinhilber et al., 2022), and FFP2/N95

Study name	Sample size: Mask	Sample size: Control	SMD	SE	Lower limit	Upper limit	p	Relative weight
Ryu et al. (2021)	11	11	-1.05	0.45	-1.94	-0.16	0.02	4.70
Driver et al. (2021)	31	31	-0.70	0.26	-1.21	-0.18	0.01	14.20
Kampert et al. (2021)	20	20	-0.33	0.32	-0.95	0.30	0.31	9.59
Jesus et al. (2022)	32	32	-0.27	0.25	-0.77	0.22	0.28	15.40
Ng et al. (2022)	8	8	-0.26	0.50	-1.25	0.72	0.60	3.85
Rojo-Tirado et al. (2021)	13	13	-0.20	0.39	-0.97	0.57	0.61	6.28
Epstein et al. (2021)	10	10	-0.16	0.45	-1.04	0.72	0.72	4.84
Zhang et al. (2021)	71	71	-0.16	0.17	-0.49	0.17	0.34	34.38
Shaw et al. (2020)	14	14	0.24	0.38	-0.50	0.98	0.53	6.75
			-0.29	0.10	-0.48	-0.10	0.00	

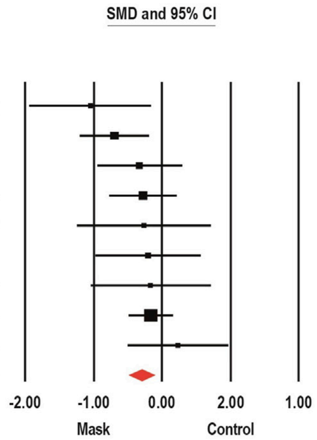
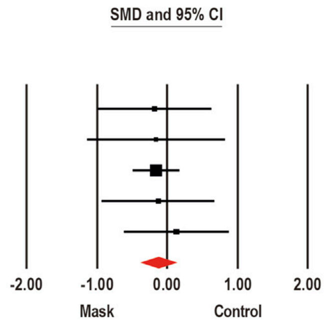


FIGURE 5
Acute effects of face mask vs. control (unmasked condition) on time-to-exhaustion performance. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: (SMD: −0.29; 95% CI −0.10 to −0.48; $p < 0.001$; $I^2 = 0\%$).

A

Study name	Sample size: Mask	Sample size: Control	SMD	SE	Lower limit	Upper limit	p	Relative weight
Fikenzer et al. (2020)	12	12	-0.18	0.41	-0.98	0.63	0.67	10.25
Ng et al. (2022)	8	8	-0.16	0.50	-1.14	0.82	0.75	6.84
Zhang et al. (2021)	71	71	-0.15	0.17	-0.48	0.18	0.36	60.68
Mapelli et al. (2021)	12	12	-0.13	0.41	-0.93	0.67	0.75	10.26
Shaw et al. (2020)	14	14	0.13	0.38	-0.61	0.87	0.73	11.97
			-0.12	0.13	-0.38	0.14	0.36	



B

Study name	Sample size: Mask	Sample size: Control	SMD	SE	Lower limit	Upper limit	p	Relative weight
Egger et al. (2021)	16	16	-0.66	0.36	-1.37	0.05	0.07	25.50
Fikenzer et al. (2020)	12	12	-0.20	0.41	-1.00	0.60	0.63	20.68
Steinhilber et al. (2022)	39	39	0.00	0.23	-0.44	0.44	1.00	53.82
			-0.21	0.20	-0.60	0.18	0.29	

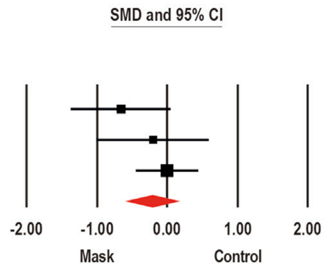


FIGURE 6
Acute effects of face mask vs. control (unmasked condition) on maximal power output (PO_{MAX}) performance. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: **(A)** analysis of face mask wearing effects on PO_{MAX} by absolute values (W) (SMD: −0.12; 95% CI −0.38 to 0.14; $p = 0.36$; $I^2 = 0\%$); **(B)** analysis of face mask wearing effects on PO_{MAX} by body mass relative values (W/kg) (SMD: −0.21; 95% CI −0.60 to 0.18; $p = 0.29$; $I^2 = 17\%$).

($n = 5$) (Fikenzer et al., 2020; Ade et al., 2021; Egger et al., 2021; Mapelli et al., 2021; Steinhilber et al., 2022) face masks. Two meta-analyses estimate the face mask wearing effects on absolute ($n = 5$) (Fikenzer et al., 2020; Shaw et al., 2020; Mapelli et al., 2021; Zhang et al., 2021; Ng et al., 2022) and relative ($n = 3$) (Fikenzer et al., 2020; Egger et al., 2021; Steinhilber et al., 2022)

PO_{MAX} (Figures 6A,B, respectively). No effects of face mask wearing were observed on both absolute (SMD: −0.12; 95% CI −0.38 to 0.14; p = 0.36; I^2 = 0%) and relative (SMD: −0.21; 95% CI −0.60 to 0.18; p = 0.29; I^2 = 17%) PO_{MAX}.

Maximum and mean BPV were measured during the bench press exercise during face mask wearing (Rosa et al., 2022). After four sets of bench press at 50% and 70% of one-maximum repetition (RM) wearing an FFP2/N95, Rosa et al. (2022) observed lower maximum (−10%) and mean (−14%) BPV only during high-intensity exercise (i.e., 70% 1RM). Although limited to a single study, the negative effects of face mask on maximum and mean BPV appear to be intensity-dependent, suggesting that harmful effects caused by the use of the face mask only occur at higher intensities.

A single study (Dantas et al., 2021) evaluated jump performance through the maximum height reached in the countermovement jump using a CM. However, no effects were found on jump performance with the use of face mask.

3.4.3 Muscle force and exercise performance of the total volume and maximum number of repetitions

The effects of face mask use on muscle force were also investigated (n = 1) (Zimmerman et al., 1991). Zimmerman et al. (1991) assessed muscle force through hand grip strength after constant load cycle ergometer exercise wearing SM, and no effects of face mask use were detected.

The exercise total volume (n = 1) and the maximum number of repetitions (n = 2) were also assessed. Rosa et al. (2022) assessed total volume and the maximum number of repetitions during four sets of bench press exercise, at intensities of 50% and 70% of 1RM, wearing FFP2/N95, and found no effects of face mask use. Reychler et al. (2021) assessed the performance of the STS^{1min} with the use of CM and SM and reported no effects of face mask use. Thus, the use of a face mask does not seem to influence the performance of the total volume and the maximum number of repetitions.

3.4.4 Walking and running tests' performance

Three studies (Person et al., 2018; Modena et al., 2021; Cabanillas-Barea et al., 2022) investigated the face mask wearing effects on distance traveled (i.e., total distance achieved during testing) in walking and running tests, such as 6 MWT (Person et al., 2018; Cabanillas-Barea et al., 2022) and YoYo-Intermittent Recovery Test (YYIRT) (Modena et al., 2021), using CM (Modena et al., 2021), SM (Person et al., 2018; Modena et al., 2021; Cabanillas-Barea et al., 2022), and FFP2/N95 (Cabanillas-Barea et al., 2022). A meta-analysis pooling three studies (Person et al., 2018; Modena et al., 2021; Cabanillas-Barea et al., 2022) to estimate the face mask wearing effects on distance traveled during walking and running tests (SMD: −0.09; 95% CI −0.35 to 0.17; p = 0.51; I^2 = 0%), as can be seen in Figure 7. No significant adverse effects were detected for face mask wearing on distance traveled assessed during walking and running tests.

Two studies (Dantas et al., 2021; Tornero-Aguilera et al., 2021) investigated the impact of face mask use on sprint time at 30 m (Dantas et al., 2021), 50 m (Tornero-Aguilera et al., 2021), and 400 m (Tornero-Aguilera et al., 2021), with the use of CM (Dantas et al., 2021) and SM (Tornero-Aguilera et al., 2021) mask types. While Dantas et al. (2021) found no effects caused by the face mask wearing, Tornero-Aguilera et al. (2021) observed greater sprint times in 50 m (13%) and 400 m (18%) compared to unmasked condition.

Acceleration was also measured during the sprint. A single study (Dantas et al., 2021) evaluated sprint acceleration during five maximum 30 m sprints using a CM. However, no negative effects on sprint acceleration were found with the use of a face mask.

3.5 Studies' quality appraisal

In most studies (n = 36), a visual analysis showed an overall risk of "some concerns." Most studies did not describe the randomization process (e.g., randomization, software, and others), allocation concealment (use of envelopes), and there was no pre-specified analysis plan available, so it was unclear whether the reported analyzes were pre-specified. Only few studies (n = 6) had a protocol record in clinical trials, however, it did not describe the pre-analyses. In general, a single study was judged as "low risk of bias" (Shaw et al., 2020). For the affective/psychological outcomes such as overall discomfort (n = 4), thermal sensations (n = 2), facial thermal discomfort (n = 1), stress (n = 1) and affective responses (n = 1), fatigue (n = 1), dyspnea (n = 16), and perceived exertion (n = 24) the overall risk was judged as "some concerns." In exercise performance outcomes, TTE (n = 9), PO_{MAX} (n = 8), BPV (n = 1), jump height (n = 1), the maximum number of repetitions (n = 2), muscle strength (n = 1), distance traveled (n = 3), sprint time (n = 2) and acceleration (n = 1) were assessed and all the studies' overall risk were judged as "some concern." Full details of the risk of bias assessment are presented in the Supplementary Material.

4 Discussion

To the best of our knowledge, this study presents the first synthesis of available evidence on the acute effects of face mask use during exercise on affective/psychological and exercise performance responses exclusively in healthy adults of different/diverse training status. We found that the face mask wearing during exercise increases discomfort, dyspnea, and perceived exertion (especially wearing SM and FFP2/N95 mask types). Furthermore, face mask wearing can reduce

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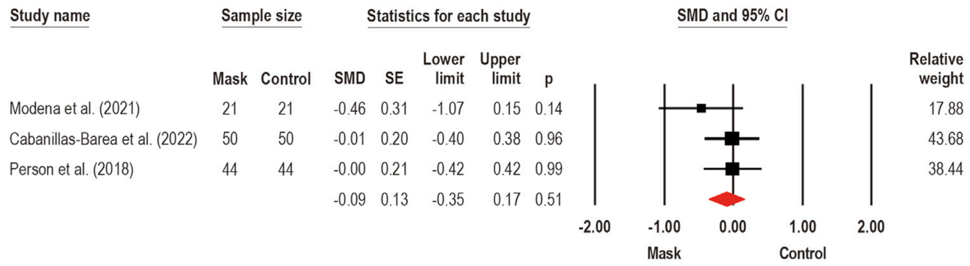

FIGURE 7
Acute effects of face mask vs. control (unmasked condition) on the distance traveled performance during walking and running functional tests. Forest plots with pooled standardized mean difference (SMD), standard errors (SE), and 95% confidence intervals (CI) are displayed: (SMD: −0.09; 95% CI −0.35 to 0.17; p = 0.51; I^2 = 0%).

the TTE during treadmill and cycle ergometer tests, without affecting PO_{MAX} and distance traveled in functional tests.

Previously, studies have found adverse effects of face mask wearing on overall (3%–150%) (Fikenzer et al., 2020; Kampert et al., 2021; Reychler et al., 2021) and facial thermal (22%) (Morris et al., 2020) discomforts during exercise. Our results showed a large negative effect of face mask use on discomfort levels, compared to no-mask condition. Adverse effects were also observed on subjective stress responses (18%) (Tornero-Aguilera et al., 2021) and affective responses (114%–148%) (Dantas et al., 2021). Regarding the stress and affective responses, although there seems to be an effect of the mask on these parameters, the number of studies is still small. Discomfort caused by face mask wearing can be explained by some factors such as inadequate respiratory gas exchange (Tornero-Aguilera et al., 2021), reduced ventilatory capacity (Engeroff et al., 2021), and changes in the mask's microenvironment caused by sweat and water vapor retention, which causes even more breathing resistance and discomfort sensations (Yoshihara et al., 2021), and can lead to a stressful event in individuals (Andre et al., 2018) by symptoms such as tightness, suffocation, and claustrophobia in those who use it (Driver et al., 2021).

Dyspnea represents a subjective experience of breathing discomfort which consists of qualitatively distinct sensations that vary according to the exercise intensity (Reychler et al., 2021). Through our meta-analysis, we found a moderate adverse effect of exercise with a face mask on dyspnea. However, high heterogeneity values were observed and explored. A subgroup analysis showed that effects induced by the face mask use during exercise seems to depend on the type of mask used, since FFP2/N95 type (with and without exhalation valve) have greater negative effects on dyspnea than CM and SM types. Our findings confirm and strengthen the existing evidence and the reported moderate effect by a previous meta-analytical review (Shaw et al., 2021), indicating that face mask wearing during exercise causes an increase in dyspnea. The respiratory resistance caused by the mask seems to cause increased dyspnea level at the clinically relevant threshold (Reychler et al., 2021).

The perceived exertion has been constantly assessed during face mask wearing on different exercises (Ade et al., 2021; Driver et al., 2021; Slimani et al., 2021; Tornero-Aguilera et al., 2021; Rosa et al., 2022). Although the literature shows contradictory results, a recent meta-analysis (Shaw et al., 2021) showed increased perceived exertion of moderate and small effects for SM and FFP2/N95 face masks, respectively. In our study, we showed a small effect of face mask use on perceived exertion. However, a moderate magnitude heterogeneity was identified and explored. A subgroup analysis indicated that the increased perceived exertion only occurs during SM and FFP2/N95 mask-wearing, which demonstrate increases with moderate and small effect sizes, respectively. Airflow resistance is a key element of the face mask function (Hopkins et al., 2021), and although face masks offer different levels of breathing restriction, the observed effect on perceived exertion may be more related to the covered no-mask condition, as several studies (Lässing et al., 2020; Driver et al., 2021; Egger et al., 2021) found no effects of face mask wearing during cardiopulmonary exercise testing associated with spirometry equipment (i.e., mouthpiece and tubing), which can promote increased breathing resistance similar to FFP2/N95 respirators (Hopkins et al., 2021). Therefore, the simple fact of using the cardiopulmonary test equipment may have been enough to cause discomfort and perceived exertion in both masked and unmasked conditions in a similar response, and so it is possible that the face mask exclusive effects can be mitigated. Many studies (Motoyama et al., 2016; Andre et al., 2018; Dantas et al., 2021; Slimani et al., 2021; Tornero-Aguilera et al., 2021) that found greater perceived exertion wearing a face mask have been performed exercises at higher intensity levels associated with activities that involved multiple muscle groups, and in its control condition, the face was fully uncovered, as in non-laboratory tests. Therefore, the non-spirometry tests, such as outdoor track field running tests (Dantas et al., 2021; Tornero-

Aguilera et al., 2021), may be more likely to show the exclusive effects of face mask use on perceived exertion.

As the exercise perceived exertion seems to increase when using a face mask, it would be expected that the TTE could be reduced with its use. Although the literature shows contradictory results, one of the studies (Jesus et al., 2022) that showed a decreased (-10%) TTE with the face mask use observed this effect during severe exercise intensity (i.e., 25% above ventilatory threshold) but not at moderate exercise intensity (i.e., 25% below ventilatory threshold), which suggests that the face mask use effect on TTE seem to be dependent on the exertion intensity, once this effect may be perceptible only during high-intensity exercise. In our study, we reveal decreased TTE with face mask use, showing that face mask wearing can produce a small negative effect on the performance of TTE. Psychophysiological factors may explain these detrimental effects on TTE. As mentioned previously, discomfort, stress responses, and perceived exertion are some of the main reasons that justify the observed impairments (Tornero-Aguilera et al., 2021). Previously, a large inverse correlation ($r = -0.73$, $p = 0.020$) between dyspnea and TTE (Boyle et al., 2022), added to that, studies have already reported lesser ventilation with face mask wearing (Fikenzer et al., 2020; Egger et al., 2021; Umutlu et al., 2021), once higher-intensity exercise (e.g., TTE) necessitates higher ventilation (Hopkins et al., 2021), and therefore, the greater the exertion intensity, greater are the impact of the face mask wearing (Engeroff et al., 2021). Face mask wearing negative effects were also observed in other physiological parameters, such as arterial oxygen saturation (Kampert et al., 2021; Romero-Arenas et al., 2021; Tornero-Aguilera et al., 2021) and oxygen uptake (Fikenzer et al., 2020; Driver et al., 2021; Egger et al., 2021), that also been reported and confirmed by recent meta-analyses (Engeroff et al., 2021). Thus, together, the negative responses of psychophysiological parameters could justify the reduction of TTE performance with face mask wearing.

Exercise performance parameters such as PO could also be affected by face mask wearing. The PO_{MAX} depends on energy consumption and the maximum oxygen uptake (Fikenzer et al., 2020), and as face mask wearing appears to decrease oxygen uptake (Engeroff et al., 2021), the reduction in PO_{MAX} seems to be mainly related to negative effects on respiratory function (Fikenzer et al., 2020). Increases in the respiratory muscles' work and competition for blood supply between these respiratory muscles and exercising muscles can also help to explain the observed decreases (Romero-Arenas et al., 2021). However, the decreased PO_{MAX} (4%–9%) (Fikenzer et al., 2020; Egger et al., 2021; Zhang et al., 2021; Steinhilber et al., 2022) may be minimal effects that may be negligible, once our meta-analyses have shown that these detrimental effects are not significant when considering both absolute (Watts) and relative (Watts/kg) PO_{MAX}. Nevertheless, these findings should be analyzed with caution due to the number of studies that are still small and do not allow analysis by different mask types.

Maximum and mean BPV collected during the ascending portion of a given movement are widely used to assess sports performance due to the force-velocity relationship (Loturco et al., 2015). A single study found detrimental effects on maximum and mean (Rosa et al., 2022) BPV during high-intensity bench press exercise using a face mask. However, authors observed no effects during low-intensity bench press exercise. The lower BPV observed only during the high-intensity exercise suggests greater fatigue induced by the face mask compared to low-intensity exercise (Rosa et al., 2022). Previously, Jagim et al. (2018) reported decreased BVP (2%–4%) during bench press exercise with the use of elevation training mask. Deleterious effects such as the fatigue (Rosa et al., 2022) associated to the reduced use of fast-twitch muscle fibers (Jagim et al., 2018) could explain the lower BPV. Fast-twitch muscle fibers produce the greatest response to lactate due to their greater dependence on the anaerobic glycolytic system (Jagim et al., 2018). Therefore, decreased blood lactate levels as observed by Jagim et al. (2018) and Motoyama et al. (2016), could indicate the reduced levels of recruitment of fast-twitch fibers, causing slower movements. Although some evidence points to a possible reduction in BPV with the use of the face mask, these effects must be interpreted with caution due to the low number of studies. High methodological quality clinical trials should be developed to better understand the acute effects of face mask use on BPV, which is an important parameter of muscle performance (Loturco et al., 2015).

The vertical jump, considered as another muscle performance parameter, was assessed by a single study (Dantas et al., 2021), which found no effects of face mask use on countermovement jump maximum height. Although there seem to be no negative effects of face mask use on jumping performance, the lack of studies does not allow us to fully understand the effects of face mask use on jumping performance. Future studies should investigate the effects of facemask use in consecutive jump protocols, which may be more susceptible to showing possible face mask harmful effects.

Beyond the force-velocity relationship, face mask use could also impair the muscle force production. Previously, studies showed impaired knee extensors isometric strength (da Silva et al., 2022) and the maximum number of repetitions in the squat (Andre et al., 2018), leg press (Andre et al., 2018), and bench press (Motoyama et al., 2016) exercises, when using another kind of face mask (i.e., elevation training mask). Even though the literature shows small evidence of adverse effects produced by the use of elevation training mask, limited evidence suggests that face mask use does not affect these parameters. A single study (Zimmerman et al., 1991) tested the handgrip strength after a constant load cycle ergometer exercise wearing SM and found no effects. However, it is possible that the face mask wearing effects on muscle force are not perceptible due to the lack of specificity between the way in which the muscle force was assessed and the type of performed exercise. Furthermore, we observed no harmful effects of face mask use on the maximum number of repetitions in the bench press exercise (Rosa et al., 2022) and STS^{1min} (Reychler et al., 2021), using CM and SM (Reychler et al., 2021) and FFP2/N95 (Rosa et al., 2022).

Despite this, the available evidence does not allow us to state that the use of a face mask does not affect the muscle force and the maximum number of repetitions, and thus, more studies are needed to understand the face mask wearing effects on muscle force production performance.

The performance of the walking and running tests such as 6 MWT (Person et al., 2018; Cabanillas-Barea et al., 2022), YYIRT (Modena et al., 2021), and outdoor track field running (Dantas et al., 2021; Tornero-Aguilera et al., 2021) were also investigated. The distance traveled was assessed during the 6 MWT (Person et al., 2018; Cabanillas-Barea et al., 2022) and YYIRT (Modena et al., 2021). While no effects of face mask use were identified during the 6 MWT (Person et al., 2018; Cabanillas-Barea et al., 2022), shorter distances (11%–13%) were achieved in YYIRT (Modena et al., 2021). However, our meta-analysis showed that these effects are not significant, suggesting that face mask use does not negatively affect the performance of both tests. Despite the absence of negative effects, our findings must be interpreted with caution, since the distance covered was evaluated by tests with different characteristics, which could respond differently to mask use due to different exercise intensities. The 6 MWT is a test in which participants had to walk as fast as possible without running for 6 min (Cabanillas-Barea et al., 2022), on the other hand, YYIRT is a running incremental exercise used both to simulate high-intensity exercise and to assess specific aerobic fitness related to team sports performance (Modena et al., 2021).

For the effects of the face mask on sprint performance, studies investigated the face mask wearing effects on sprint time (Dantas et al., 2021; Tornero-Aguilera et al., 2021) and acceleration (Dantas et al., 2021). Sprint time was assessed during 30 m (Dantas et al., 2021), 50 m (Tornero-Aguilera et al., 2021), and 400 m (Tornero-Aguilera et al., 2021), using CM (Dantas et al., 2021) and SM (Tornero-Aguilera et al., 2021). While Dantas et al. (2021) found no face mask wearing negative effects in 30 m sprints, Tornero-Aguilera et al. (2021) observed greater sprint times (13%–18%) at distances from 50 m compared to control condition. Increased sprint time could be explained by potential metabolic changes and decrease in muscle efficiency (consequence of impaired autonomic stability), lower cardiac fitness, and decreased muscle blood supplies (Tornero-Aguilera et al., 2021). Although the available evidence is limited to two studies, the use of the face mask seems to be able to produce negative effects on the sprint time at distances from 50 m, however, the evidence does not allow us to understand if these effects are caused by the different sprint distances or by the different face mask used between the studies. Regarding acceleration, (Dantas et al., 2021), found no effects of face mask use during five maximum 30 m sprints wearing a CM. Nonetheless, this evidence is insufficient to state that face mask use does not affect sprint acceleration. Therefore, high methodological quality clinical trials are needed to understand the real effects of face mask use on sprint performance.

Our study has some limitations. First, we focused on investigating the effects of a face mask wearing on apparently healthy adults. Therefore, we cannot assert that our findings can be applied to other populations, such as children and the elderly or people with clinical conditions. Studies showed some variability in face mask type, exercise modality, and exertion intensity. However, the low number of studies did not allow us to explore these data further to better understand the real effects of face mask use in each condition. Some studies (Lässing et al., 2020; Driver et al., 2021; Egger et al., 2021) have assessed psychophysiological responses from cardiopulmonary tests using spirometry equipment over the face mask. Covering the face even without the combination with the face mask can promote adverse effects and cause discomfort and greater perceived exertion. Therefore, one way to avoid this bias would be to perform high-intensity exercises (e.g., outdoor track field running tests) with a control condition without any kind of device that covers the face. In this same perspective, most studies that assessed the impact of the face mask are based on laboratory protocols, whose practical applicability is questionable (Dantas et al., 2021). Nonetheless, functional tests can provide clearer information about the real effects of the face mask during exercise. Fourth, most studies (n = 26) did not determine the sample size through a sample size calculation and our analysis is on the risk of type I error. Further high methodological quality studies should explore the influence of each mask type in different intensities and types of exercise, in addition to exploring the long-term effects of face mask use. Studies found in pre-print databases have not usually been peer-reviewed. Although we included preprint databases in our search strategy, no non-peer-reviewed studies were found and included in our review. Lastly, due to the small number of studies, we could not perform publication bias tests.

Our study has several implications for exercise practice. In summary, we indicate that face mask using negatively affects affective/psychological responses such as discomfort, dyspnea, and perceived exertion, and can reduce exercise time at high intensities, but does not produce harmful effects on distance traveled in walking and running functional tests. Nevertheless, it is well documented that exercise works against several chronic diseases, and is strongly associated with reduced risk for severe COVID-19 outcomes (Sallis et al., 2021), and thus, although face mask causes impairments in some affective/psychological and exercise performance aspects during exercise, they are well-established protective measures against airborne infectious diseases (e.g., COVID-19), especially when combined with other preventive measures such as ventilation and distancing, which together can reduce viral concentrations in the environment and increase the protective effectiveness of face masks to contain viral transmission (Liang et al., 2020; Cheng et al., 2021). Apparently, the face masks that offer the greater protection levels (e.g., SM or FFP2/N95) may be the same ones that produce the greatest negative impacts on exercise (Engeroff et al., 2021). However, they may be recommended for indoor and/or group activities, especially when the environment is poorly ventilated (Cheng et al., 2021) or adequate physical distancing cannot be maintained, such as in gyms or training centers (Shurlock et al., 2021). Based on the study data, we do not recommend the abandonment of masks when exercising, but we recommend that affective/psychological and exercise performance responses may be influenced by the face mask use and some adaptations to the intensity of exercise may be required for those exercising for health.

5 Conclusion

Based on the available evidence, face mask wearing during exercise increases discomfort (large effect), dyspnea (moderate effect), and perceived exertion (small effect). Moreover, face mask use can reduce the TTE performance (small effect), without effects on cycle ergometer PO_{MAX} and distance traveled in walking and running functional tests. However, some aspects may be dependent on the face mask type, as the increased dyspnea (large effect for FFP2/N95 + EV and FFP2/N95; moderate effect for CM and SM) and perceived exertion (moderate and small effects for SM and FFP2/N95, respectively).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

All authors contributed to the study conception and design. The idea for the article came from the authors MHG, IMB, and FJL. The study selection from the literature, data extraction, and assessment of the methodological quality of the studies were performed by MHG, EM, SKP, and ARB. The data analyses were performed by MHG, IMB, and FBS. The first draft of the manuscript was written by MHG and all authors commented on previous versions of the manuscript. The critical review of the intellectual content was performed by MHG, FBS and FJL. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.994454/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Risk of bias rating for discomfort, stress and affective responses, and fatigue outcomes, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S2
Risk of bias rating for dyspnea outcome, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S3
Risk of bias rating for perceived exertion outcome, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S4
Risk of bias rating for time-to-exhaustion (TTE) performance outcome, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S5
Risk of bias rating for maximal power output (POMAX) performance outcome, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S6
Risk of bias rating for performance of the maximum number of repetitions, muscle strength, bar propulsive velocity, and jump height outcomes, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

SUPPLEMENTARY FIGURE S7
Risk of bias rating for distance traveled performance, sprint acceleration, and sprint time outcomes, displayed as traffic light plots. The colours indicate high (red), unclear (yellow) or low (green) risk for the respective bias domain/item. D1: randomization process; DS: bias arising from period and carryover effects; D2: deviations from the intended interventions; D3: missing outcome data; D4: measurement of the outcome; D5: selection of the reported result.

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Increased oxygen uptake in well-trained runners during uphill high intensity running intervals: A randomized crossover testing

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The time spent above 90% of maximal oxygen uptake ($\dot{V}O_2max$) during high-intensity interval training (HIIT) sessions is intended to be maximized to improve $\dot{V}O_2max$. Since uphill running serves as a promising means to increase metabolic cost, we compared even and moderately inclined running in terms of time ≥90% $\dot{V}O_2max$ and its corresponding physiological surrogates. Seventeen well-trained runners (8 females & 9 males; 25.8 ± 6.8yrs; 1.75 ± 0.08m; 63.2 ± 8.4kg; $\dot{V}O_2max$: 63.3 ± 4.2 ml/min/kg) randomly completed both a horizontal (1% incline) and uphill (8% incline) HIIT protocol (4-times 5min, with 90s rest). Mean oxygen uptake ($\dot{V}O_2mean$), peak oxygen uptake ($\dot{V}O_2peak$), lactate, heart rate (HR), and perceived exertion (RPE) were measured. Uphill HIIT revealed higher ($p \leq 0.012$; partial eta-squared (pes) ≥ 0.351) $\dot{V}O_2mean$ (uphill: 3.3 ± 0.6 vs. horizontal: 3.2 ± 0.5 L/min; standardized mean difference (SMD) = 0.15), $\dot{V}O_2peak$ (uphill: 4.0 ± 0.7 vs. horizontal: 3.8 ± 0.7 L/min; SMD = 0.19), and accumulated time ≥90% $\dot{V}O_2max$ (uphill: 9.1 ± 4.6 vs. horizontal: 6.4 ± 4.0 min; SMD = 0.62) compared to even HIIT. Lactate, HR, and RPE responses did not show mode*time rANOVA interaction effects ($p \geq 0.097$; pes ≤0.14). Compared to horizontal HIIT, moderate uphill HIIT revealed higher fractions of $\dot{V}O_2max$ at comparable perceived efforts, heartrate and lactate response. Therefore, moderate uphill HiiT notably increased time spent above 90% $\dot{V}O_2max$.

KEYWORDS

incline, intervals, performance, injury, running

1 Introduction

High level endurance training requires large training volumes (Seiler, 2010). In elite athletes, commonly, a high proportion of this training volume is performed at low training intensities (Seiler, 2010). However, to achieve an optimal metabolic training stimulus on maximal oxygen uptake ($\dot{V}O_2max$), it has been recommended to perform a certain amount of high-intensity interval training (HIIT). This recommendation is especially relevant for well-trained endurance athletes (Laursen and Jenkins, 2002). Thereby, HIIT involves repeated bouts of high-intensity exercise interspersed with recovery periods (Laursen and Jenkins, 2002; Buchheit and Laursen, 2013). This training method mainly focuses on $\dot{V}O_2$max improvements (Midgley et al., 2006; Buchheit and Laursen, 2013), as the upper limit to the aerobic metabolism and a key determinant of endurance performance (Joyner and Coyle, 2008). In order to improve $\dot{V}O_2$max in highly trained endurance athletes, it has been suggested that a prolonged time at intensities corresponding to a high percentage of maximal oxygen uptake is important (Wenger and Bell, 1986; Midgley et al., 2006). Therefore, the quality of a HIIT session can be defined by mean oxygen uptake ($\dot{V}O_2$mean) or accumulated training time ≥90% $\dot{V}O_2$max (Midgley et al., 2006; Turnes et al., 2016). This adaptational potential has been attributed to the large metabolic stimulus for myocardial morphological adaptations that increases maximal cardiac stroke volume and also increased peripheral skeletal muscle adaptations (Midgley et al., 2006).

In both prospective and cohort studies, a high weekly running volume has been associated with running-related injuries (Macera et al., 1989; Walter et al., 1989). Although the causes of running injuries are multifactorial, in this context, the runner's interaction with the ground and the resulting reaction force has been considered to be one risk factor (Zadpoor and Nikooyan, 2011; Daoud et al., 2012). Thus, higher loading rates were associated with increased risk of sustaining an injury (Crowell and Davis, 2011; Futrell et al., 2018). More recently, however, in a prospective case control-study in recreational runners, the vertical impact peak and loading rate were not associated with a higher injury rate (Malisoux et al., 2022). Furthermore, in collegiate cross country runners, an higher occurrence rate of bone stress injuries has been linked to a higher step rate, but not higher ground reaction forces (Kliethermes et al., 2021). Nevertheless, besides adequate periodization and polarization models in endurance sports, reducing loading rates is still recommended as an effective means to reduce the risk of developing running injuries (Bowser et al., 2018). In this context, increasing the slope might lead to a significantly lower vertical loading rate during uphill running compared to flat level running (Gottschall and Kram, 2005; Lemire et al., 2022a). Also, increasing the slope from flat level running to 7% was found to reduce flight time and increase floor contact time, in turn resulting in highly significant increases in step frequency (Padulo et al., 2013). Apart from this, previous research revealed an increased energy cost *via* uphill running compared to horizontal running (Lemire et al., 2022b). Additionally, when running at the same velocity, uphill running is more metabolically demanding than horizontal running (Minetti et al., 2002; Vernillo et al., 2017), hence allowing a similar training stimulus at a lower running velocity.

Against this background, this randomized crossover testing examined the peak $\dot{V}O_2$, mean $\dot{V}O_2$ and accumulated time spent ≥90% $\dot{V}O_2$max during moderate slope uphill compared to horizontal HIIT running. We assumed similar $\dot{V}O_2$ data and reduced running speed during uphill HIIT. The findings of the present study might be impactful for designing and integrating HIIT session within polarization models and in terms of training variations to minimize injury risks in runners with high training volumes.

2 Materials and methods

2.1 Participants

G*Power (Version 3.1.9.6) was employed to perform an *a priori* power analysis. Based on increased metabolic costs *via* uphill running (Minetti et al., 1994; 2002; Vernillo et al., 2017) moderate effect sizes (standard mean differences (SMD) = 0.60) between horizontal and uphill HIIT running were assumed. A sample size of n = 13 was determined, using the following statistical indicators (α = 0.05; study power (1-β-error) = 0.95; one tail). Assuming moderate dropouts (15%–20%), n = 17 well-trained runners were enrolled in this acute randomized controlled crossover testing. These participants consisted of 8 female (age: 24.4 ± 3.7 yrs; height: 1.69 ± 0.07 m; body mass: 56.6 ± 5.8 kg; body fat: 14.6 ± 4.8%; $\dot{V}O_2$max: 60.5 ± 2.3 ml/min/kg; running volume: 58.1 ± 18.5 km/week) and 9 male (age: 27.1 ± 8.8 yrs; height: 1.80 ± 0.07 m; body mass: 69.1 ± 5.6 kg; body fat: 9.7 ± 3.1%; $\dot{V}O_2$max: 65.7 ± 4.1 ml/min/kg; running volume: 65.0 ± 20.3 km/week) trained runners. Inclusion criteria were running experience of at least 3 years, running volume of at least 40 km/week, and no medical condition that potentially impedes the completion of testing and training. The study was approved by the local ethical committee (153/2022), fulfilled the international ethical standards, and all participants signed an informed written consent prior to the start of the study.

2.2 Testing procedures

The measurements were conducted within four lab visits over 3 weeks for each participant. Thereby, horizontal and uphill $\dot{V}O_2$max tests (lab visit 1 & 2) as well as horizontal and uphill HIIT protocols (lab visit 3 & 4) were performed. Adapted from previous research (Rønnestad et al., 2019; 2022), the HIIT protocol consisted of four 5-min intervals with 90 s passive rest in between. During HIIT sessions, participants were instructed to run at their maximal sustainable intensity during all four interval bouts (*isoeffort*) (Seiler and Hetlelid, 2005). Therefore, participants could increase or decrease the velocity individually. All measurements were conducted on a motorized treadmill (PPS Med treadmill, Woodway, Waukesha, USA), with the horizontal conditions being performed at 1% incline and the uphill conditions being performed at 8% incline. To avoid sequencing effects, the first two and the last two lab visits were individually performed in a randomized order. At least 96 h rest was ensured between each lab visit. Participants were further instructed to avoid any strenuous exercise 2 days before each testing session. To control for potential circadian effects on performance, all measurements were conducted at similar day times for each participant. A standardized 15-min warm-up (easy running, including knee lift, heel lift, external rotation hip, internal rotation hip, 10 lunges alternating, 10 squats, individual dynamic stretching) was performed prior to each lab session.

Spirometric data during all lab visits were collected using a breath-by-breath system (Zan 600 Oxi USB, Zan Messgeräte, Oberthulba, Germany). This spirometric system was calibrated prior to each test, following the manufacturer's recommendations. To determine uphill and horizontal-running $\dot{V}O_2$max, an incremental ramp testing protocol was performed at horizontal (1% incline) and uphill (8% incline) conditions (lab visit 1 & 2). Adapted from previous research with similar $\dot{V}O_2$max values (Baumgartner et al., 2021), the initial velocity for both ramp tests was set based on prior running experience and estimated 10 km race time for each participant individually at 2, 2.5, or 3 m/s. The ramp protocol then consisted of 0.2% increases every 30 s until the participant reached exhaustion (Midgley et al., 2007). All participants were verbally encouraged and motivated in the same way towards the end of each test. The highest consecutive oxygen uptake values within 30 s during the final part of the ramp tests were considered as $\dot{V}O_2$max. For both conditions, $\dot{V}O_2$max and objective exhaustion were verified for each participant following the corresponding criteria (Midgley et al., 2007). All participants fulfilled these objective exhaustion criteria (i.e., at least 4 out of 6 criteria). Adapted from previous research, the quality of both HIIT sessions were defined by mean $\dot{V}O_2$ and accumulated training time ≥90% $\dot{V}O_2$max (Time90) (Midgley et al., 2006; Thevenet et al., 2007; Turnes et al., 2016). Since both HIIT sessions were time matched with the same work to rest ratio, mean $\dot{V}O_2$ and Time90 were determined based on the entire training session (interval with pauses). Furthermore, to determine Time90, the entire training session (interval with pauses) was normalized to seconds, subsequently seconds with $\dot{V}O_2$ value ≥ $\dot{V}O_2$max were summed up. Thereby, the highest $\dot{V}O_2$max value of the horizontal or incline ramp test was used as reference values. Furthermore, peak oxygen consumption (highest oxygen uptake during the intervals averaged over 30 s; $\dot{V}O_2$peak) during both HIIT protocols was additionally considered. Apart from this, total respiration per minute (minute volume), respiratory frequency (breath frequency), and tidal volume were also used for further data analysis. In addition, capillary blood samples were taken from the earlobe of the participants for lactate analysis (EBIOplus; EKF Diagnostic Sales, Magdeburg, Germany), heart rate (HR) was measured using a heart rate strap (Polar, Kempele, Finland), and perceived exertion levels were assessed based on RPE (CR-10 scale) (Foster et al., 2001) prior to the first interval and immediately after each running interval.

2.3 Statistics

Data are presented as means ± standard deviations. Normal distribution was initially tested using Shapiro-Wilk tests ($p \geq 0.1$). Variance homogeneity was visually confirmed *via* plotting sampled residuals vs. theoretical (ideal) residuals (Kozak and Piepho, 2018). Sphericity was verified *via* Mauchly´s tests. To examine mode differences (horizontal vs. uphill) for the respective outcome measures ($\dot{V}O_2$, $\dot{V}O_2$peak, $\dot{V}O_2$max, Time 90, minute volume, breath frequency, and tidal volume), numerous separate two-way (mode: horizontal vs. uphill) repeated measurement analysis of variances (rANOVA) were conducted. 2 (mode: horizontal vs. uphill) × 4 (time: pre vs. interval 1 vs. interval 2 vs. interval 3 vs. interval 4) rANOVAs were calculated for lactate, HR, and RPE, and running velocity data. rANOVA effect sizes are given as partial eta squared (pes) with ≥0.01, ≥0.06, and ≥0.14 indicating small, moderate, and large effects, respectively (Cohen, 1988). In case of significant mode × time interaction effects, Bonferroni *post hoc* tests were subsequently computed. For pairwise effect size comparison, standard mean differences (SMD) were additionally calculated as the differences between means divided by the pooled standard deviations (trivial: SMD <0.2; small: 0.2 ≤ SMD <0.5; moderate: 0.5 ≤ SMD <0.8; large SMD ≥0.8) (Cohen, 1988). Furthermore, the smallest worthwhile change was calculated as 30% of baseline standard deviation (Hopkins, 2004). Pearson correlation coefficients were calculated in order to define the relationships of the measured variables. A correlation coefficient of | r | ≈ 0.30 is interpreted as low/weak correlation, | r | ≈ 0.50 is interpreted as mean/moderate correlation and | r | ≈ 0.80 is interpreted as large/strong correlation (Cohen, 1988). Statistical analyses were conducted using R (version 4.0.5) and RStudio (version 1.4.1106) software.

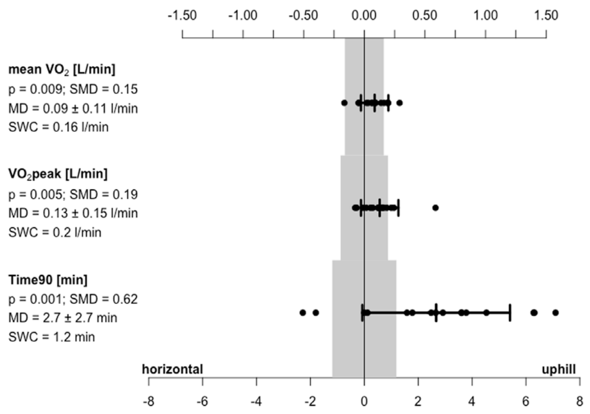

FIGURE 1
Mean difference (MD ± standard deviation) between horizontal and uphill high intensity training protocols for mean oxygen consumption ($\dot{V}O_2$), peak oxygen consumption ($\dot{V}O_2$peak), and accumulated time above 90% of maximal oxygen consumption (Time90). Smallest worthwhile change (SWC) boundaries are marked in grey. Significance levels (*p*) and pairwise effect sizes as standard mean differences (SMD) are presented.

3 Results

3.1 Incremental ramp test

No significant differences (p = 0.100; pes = 0.100; mean difference (MD) = 0.2 ± 0.5 L/min; SMD = 0.28) were found between horizontal (3.9 ± 0.7 L/min) and uphill $\dot{V}O_2$max (4.1 ± 0.7 L/min) during the incremental ramp tests.

3.2 HIIT sessions

rANOVA revealed significant effects ($p \leq 0.012$; pes ≥0.351) regarding $\dot{V}O_2$, $\dot{V}O_2$peak, Time90, minute volume, breath frequency, and tidal volume (Figure 1). Thereby, uphill HIIT showed higher values than horizontal HIIT for $\dot{V}O_2$mean (3.3 ± 0.6 vs. 3.2 ± 0.5 L/min; MD = 0.1 ± 0.1 L/min; SMD = 0.15), $\dot{V}O_2$peak (4.0 ± 0.7 vs. 3.8 ± 0.7 L/min; MD = 0.1 ± 0.2 L/min; SMD = 0.19), Time90 (9.1 ± 4.6 vs. 6.4 ± 4.0 min; MD = 2.7 ± 2.7 L/min; SMD = 0.62), and tidal volume (2144 ± 511 vs. 2061 ± 502 ml; MD = 83 ± 117 ml; SMD = 0.16). In contrast, uphill HIIT revealed lower values than horizontal HIIT for minute volume (94.3 ± 15.1 vs. 101.2 ± 17.3 L/min; MD = 6.9 ± 8.4 L/min; SMD = 0.43) and breath frequency (44.9 ± 6.0 vs. 50.5 ± 9.2 breaths/min, MD = 5.6 ± 5.9 breaths/min; SMD = 0.73). Furthermore, only for Time90, breath

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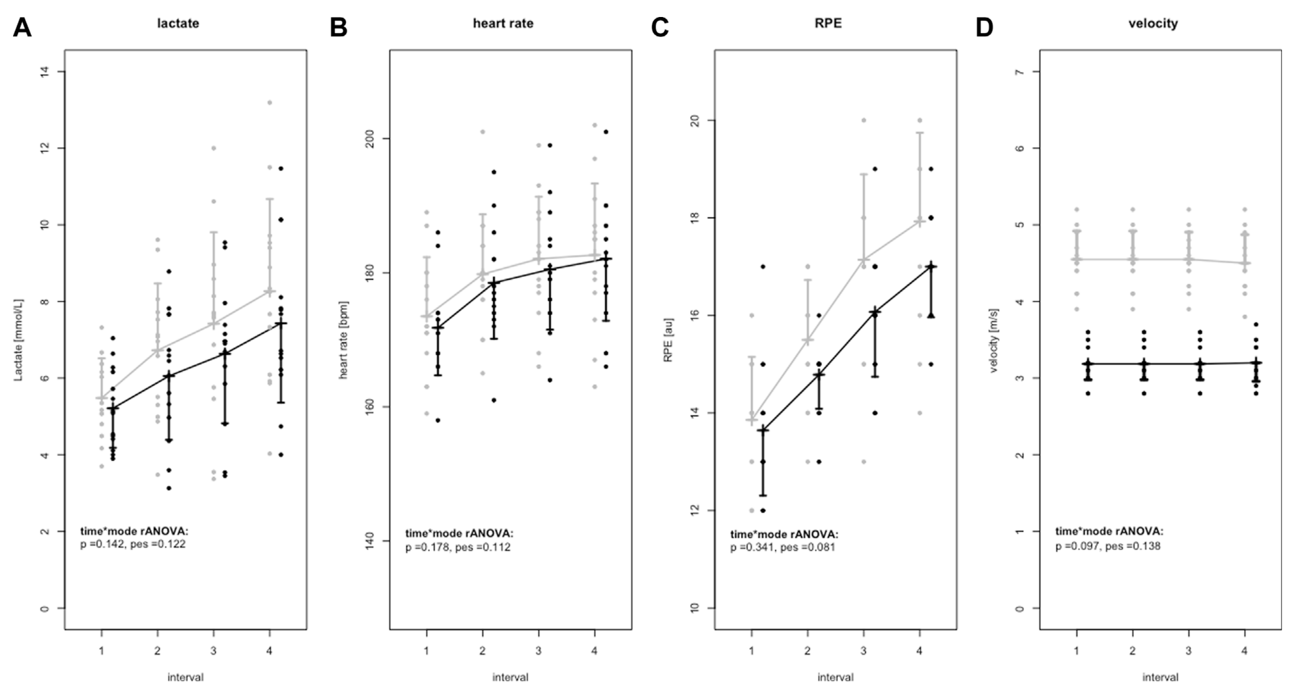

FIGURE 2
Lactate **(A)**, heart rate **(B)**, RPE **(C)**, and running velocity **(D)** data (mean ± standard deviation) of horizontal (grey) and uphill (black) high intensity training protocols. Individual values are marked as points. In addition, *p*-values of time*mode interaction effects (*p*) of the repeated measurement variance analyses (rANOVA) and corresponding effect sizes as partial eta squared (pes) are given.

frequency and minute volume, the differences between conditions exceeded the smallest worthwhile change. Furthermore, Time90 revealed high (r = 0.82) and significant ($p < 0.001$) correlations between horizontal and uphill HIIT.

No significant mode × time rANOVA interaction effects ($p \geq 0.097$; pes ≤0.14) for lactate, HR, RPE and running velocity were found (Figure 2). Nevertheless, running velocity revealed significant time effects ($p \leq 0.001$). Subsequently performed *post hoc* tests ($p \leq 0.001$; SMD ≥3.53) revealed higher running velocity during horizontal HIIT (4.47 ± 0.33 to 4.51 ± 0.35 m/s) compared to uphill HIIT (3.17 ± 0.18 to 3.18 ± 0.21 m/s) during all intervals.

4 Discussion

To the best of our knowledge, this is the first acute randomized controlled crossover study that examined $\dot{V}O_2$, lactate, HR, and RPE response of time- and effort-matched horizontal vs. uphill HIIT running in well-trained runners. Our key findings were increased mean $\dot{V}O_2$, $\dot{V}O_2$peak, and accumulated training time ≥90% $\dot{V}O_2$max *via* uphill HIIT compared to horizontal HIIT. In contrast, lactate, HR, and RPE revealed no significant differences between horizontal and uphill HIIT protocols. Furthermore, horizontal and uphill ramp tests yielded similar $\dot{V}O_2$max values.

A higher acute oxygen consumption during uphill running is commonly explained by the fact that the use of elastic energy may be compromised, so that in turn more mechanical energy (i.e., greater concentric muscle activity) needs to be generated, in order to lift the body's center of gravity upward and subsequently overcome the slope (Snyder and Farley, 2011). Thus, in the present study, uphill running during a HIIT session notably increased the mean time ≥90% $\dot{V}O_2$max by about 42%. Interestingly, this percentage increase is quite similar to previous cycling-related research, which used power-output variation within the work intervals (Bossi et al., 2020). In this previous study, two different interval training sessions, matched for duration and mean power output (6 × 5 min at a mean intensity of 84% of maximal aerobic power (MAP), with 2.5 min of rest between intervals), were performed. By performing several 30s bouts at 100% MAP within these intervals to increase the power-output variation within the work intervals, the mean time ≥90% $\dot{V}O_2$max increased by about 43% (Bossi et al., 2020). It thus seems that variation of the power-output by performing short bouts of sprinting or by employing inclination might be an important factor to increase the time ≥90% $\dot{V}O_2$max during HIIT sessions. In addition, and in line with our findings, lactate, HR, and RPE data reported by Bossi and colleagues (Bossi et al., 2020) were similar for both interval training conditions. However, both studies only focused on short-term effects. Therefore, Bossi and colleagues (Bossi et al., 2020) emphasized the need for longitudinal studies while speculating that performance adaptations will most likely be superior to constant-intensity work intervals. Based on our data, a 6-week period of uphill HIIT (2 sessions per week) would result in about half an hour more accumulated time ≥90% $\dot{V}O_2$max compared to horizontal HIIT. This additional accumulated time ≥90% $\dot{V}O_2$max *via* uphill HIIT is equivalent to 5 horizontal

HIIT sessions. Therefore, superior performance adaptations could be assumed *via* uphill HIIT. This assumption is supported by increased $\dot{V}O_2$max and power output at the lactate threshold adaptations over a 4-week training period, if recreationally-trained cyclists spent about 100s more time above 90% $\dot{V}O_2$max per training session (Turnes et al., 2016). In line with these findings, the accumulated training time ≥90% $\dot{V}O_2$max is frequently considered a highly important marker for efficient HIIT sessions designed to increase $\dot{V}O_2$max (Midgley et al., 2006; Thevenet et al., 2007; Turnes et al., 2016). Our findings of HIIT protocols performed at the maximal sustainable intensity during all four interval bouts (*isoeffort*) (Seiler and Hetlelid, 2005) revealed increased mean $\dot{V}O_2$, $\dot{V}O_2$peak, and accumulated time above 90% $\dot{V}O_2$max at a decreased running velocity during the uphill HIIT condition and similar lactate, HR, and RPE values. However, as at a given speed, uphill running results in higher $\dot{V}O_2$, lactate, HR, and RPE data compared to horizontal running (Minetti et al., 1994; 2002; Vernillo et al., 2017), it might be possible that the maximum oxygen uptake differs between running uphill compared to level running conditions. Nevertheless, we did not find significant differences in $\dot{V}O_2$max in the initial incremental ramp tests performed at horizontal running condition and 8% slope. This is in line with results reported by Lemire and colleagues (Lemire et al., 2020) who reported similar $\dot{V}O_2$max values in well-trained trail runners performing step tests on a treadmill in level and 15% uphill running conditions. However, a different study conducted in well-trained trail runners comparing the physiological responses to step tests with increasing gradient reported significantly higher $\dot{V}O_2$max values at gradients of 40% compared to level running (Cassirame et al., 2022). This has also been described by Margaria and colleagues (Margaria et al., 1963): According to their work, when running on positive gradients up to 15% incline the minimum energy cost of running increases as a function of the incline. At slopes above 20%, however, the energy cost becomes equal to that of concentric muscular work (Minetti et al., 2002). It therefore seems, that at least in special populations (i.e., trail runners) and at very steep inclination (i.e., above 15%) the maximal oxygen uptake might significantly and relevantly differ from level running. Hence, this should be taken into account, when quantifying training load as a percentage value of the maximal oxygen uptake.

Previous research revealed that 19%–79% of runners report musculoskeletal injuries of the lower extremities annually (van Gent et al., 2007). Thereby, loading rate and ground reaction force were repeatedly named as relevant risk factors (Crowell and Davis, 2011; Zadpoor and Nikooyan, 2011; Futrell et al., 2018). These relationships, however, were often established based on retrospective, cross-sectional data. More recently, in prospective case control-studies comprising recreational (Malisoux et al., 2022) and collegiate cross country runners (Kliethermes et al., 2021), the vertical impact peak and loading rate were not associated with a higher injury rate. Nevertheless, reducing loading rates is still recommended as an effective means to reduce the risk of developing running injuries (Bowser et al., 2018). In this context, uphill running revealed decreased ground reaction force data compared to horizontal running (Gottschall and Kram, 2005). Furthermore, we observed decreased running velocities during uphill HIIT compared to horizontal HIIT, which additionally decrease loading rate and ground reaction force (Keller et al., 1996). In detail, previous research revealed a 22%–39% ground reaction force decrease *via* an 6%–9% slope increase (Gottschall and Kram, 2005; Kowalski and Li, 2016). Furthermore, slower running resulted in reduced ground reaction force (Keller et al., 1996). Based on our running velocity differences between horizontal and uphill HIIT, this would result in a ground reaction force reduction of 11%. For the present study a possible reduction of loading rates remains, however, speculative, as these loading rates and ground reaction forces were not measured. Thus, more adequately powered prospective studies are necessary to investigate the association of musculoskeletal injuries of the lower extremities and loading rate as well as the potential prevention effect of uphill running.

Horizontal running has been linked to the stretch-shortening cycle of the muscle-tendon unit of the lower limb (Schöffl et al., 2021), in which part of the mechanical energy of the center of mass (COM) is absorbed during the negative work phase to be restored during the next positive work phase (Nicol et al., 2006). This storage and release of kinetic and potential energy contributes to the acceleration of the body upwards during the propulsive phase and to the reduction of the energy production needed during the concentric phase (Snyder and Farley, 2011; Snyder et al., 2012). In contrast, during uphill running, the center of mass needs to be propelled vertically and does not oscillate around an equilibrium (Dewolf et al., 2016). In detail, the center of mass loses horizontal while simultaneously gaining vertical velocity during the first part of ground contact. Subsequently, during the second part of the contact, a fraction of the energy stored in the elastic elements of the muscle tendon unit is released to increase the kinetic and potential of the center of mass (Dewolf et al., 2016). Accordingly, differences in muscle activation patterns of the lower extremities have been reported between horizontal and uphill running (Yokozawa et al., 2007), with concentric muscle work being dominant during uphill running (Giandolini et al., 2016). Furthermore, to increase the running velocity in flat running conditions, athletes tend to increase their stride length and frequency almost linearly (Ito et al., 1983; Cavanagh and Kram, 1989; Brisswalter and Legros, 1995). Simultaneously, the floor contact time and flight time are reduced (Ito et al., 1983; Cavanagh and Kram, 1989; Brisswalter and Legros, 1995). Even though this pattern is also visible during uphill running compared to flat running, stride length and flight time are significantly reduced, since the foot touches the belt or ground earlier (Padulo et al., 2012; 2013). As the floor contact time does not seem to differ between flat and uphill running, this subsequently leads to a significant reduction in flight time during the uphill running condition (Padulo et al., 2012; 2013). Therefore, it seems possible, that prolonged training sessions running uphill might change the athlete's kinematics, thus resulting in a reduction in running economy at horizontal conditions. Nevertheless, at least for constant running velocities, experienced athletes select an individual combination of stride length and frequency resulting in the least energy cost (Cavanagh and Kram, 1989; Cavagna et al., 1991), while providing the greatest mechanical efficiency (Morgan et al., 1994). Even though only a small fraction of the overall training time is spent on high-intensity running (Stöggl and Sperlich, 2015), a potential longitudinal effect on running economy induced by prolonged uphill running should be addressed in further research.

A limitation that needs to be addressed is the lack of spatiotemporal running parameters including information on stride length and frequency. Thus, further research should try to disentangle the relationship between spatiotemporal running parameters and oxygen uptake during uphill running. In addition, the potential long-term training effects mentioned above should be examined in appropriate longitudinal intervention studies.

In conclusion, this randomized crossover testing revealed increased mean $\dot{V}O_2$, $\dot{V}O_2$peak, and accumulated training time ≥90% $\dot{V}O_2$max *via* uphill HIIT. Thus, uphill running during HIIT sessions appears to be an effective alternative to traditional horizontal HIIT sessions. Whether performance adaptations will be superior to horizontal running work intervals remains to be established by a longitudinal study, but similar lactate, HR, and RPE data suggest that it is unlikely that negative training outcomes occur. Nevertheless, future research should investigate whether training-induced adaptations can be improved *via* uphill HIIT. Furthermore, such further studies should also examine if different muscle activation patterns *via* uphill running (Giandolini et al., 2016) lead to adverse effects in terms of (horizontal) running economy.

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 Ethical committee of the German Sport University Cologne (approval no. 153/2022). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SH, RG, and LD contributed to the conception and design of the study. RG, TW, and JD led the intervention. LR, SH, and TW performed the statistical analysis. SH wrote the first draft of the manuscript. LR, TW, PW, and LD wrote sections of the manuscript. PW copyedited the draft for content, language, and format, and organized the submission and revision/resubmission process. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Restricted nasal-only breathing during self-selected low intensity training does not affect training intensity distribution

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Introduction: Low-intensity endurance training is frequently performed at gradually higher training intensities than intended, resulting in a shift towards threshold training. By restricting oral breathing and only allowing for nasal breathing this shift might be reduced.

Methods: Nineteen physically healthy adults (3 females, age: 26.5 ± 5.1 years; height: 1.77 ± 0.08 m; body mass: 77.3 ± 11.4 kg; VO_2peak: 53.4 ± 6.6 mL·kg^{-1} min^{-1}) performed 60 min of self-selected, similar (144.7 ± 56.3 vs. 147.0 ± 54.2 W, $p = 0.60$) low-intensity cycling with breathing restriction (nasal-only breathing) and without restrictions (oro-nasal breathing). During these sessions heart rate, respiratory gas exchange data and power output data were recorded continuously.

Results: Total ventilation ($p < 0.001$, $\eta_p^2 = 0.45$), carbon dioxide release ($p = 0.02$, $\eta_p^2 = 0.28$), oxygen uptake ($p = 0.03$, $\eta_p^2 = 0.23$), and breathing frequency ($p = 0.01$, $\eta_p^2 = 0.35$) were lower during nasal-only breathing. Furthermore, lower capillary blood lactate concentrations were found towards the end of the training session during nasal-only breathing (time x condition-interaction effect: $p = 0.02$, $\eta_p^2 = 0.17$). Even though discomfort was rated marginally higher during nasal-only breathing ($p = 0.03$, $\eta_p^2 = 0.24$), ratings of perceived effort did not differ between the two conditions ($p \geq 0.06$, $\eta_p^2 = 0.01$). No significant "condition" differences were found for intensity distribution (time spent in training zone quantified by power output and heart rate) ($p \geq 0.24$, $\eta_p^2 \leq 0.07$).

Conclusion: Nasal-only breathing seems to be associated with possible physiological changes that may help to maintain physical health in endurance athletes during low intensity endurance training. However, it did not prevent participants from performing low-intensity training at higher intensities than intended. Longitudinal studies are warranted to evaluate longitudinal responses of changes in breathing patterns.

KEYWORDS

TID, ventilatory LiT, endurance, rating of perceived exertion, heart rate, blood lactate, power

1 Introduction

More than 80% of the target training intensities in endurance sports is spent at low aerobic exercise intensities below the first lactate threshold (Seiler, 2010; Stöggl and Sperlich, 2014). For adequate training regulation in elite athletes, different approaches of quantifying training intensity based on oxygen uptake dynamics (Burnley and Jones, 2007) or blood lactate concentrations [e.g., three -zone model (Seiler, 2010)] have been recommended. Following Seiler (2010), training intensity can be categorized into three different zones: A low lactate zone (Low intensity Training, LiT; intensity corresponding to a blood lactate concentration of ≤ 2 mmol·l^{-1}), a lactate accommodation zone, where blood lactate production and removal rates maintain an equilibrium (Threshold Training, ThT; intensity corresponding to a blood lactate concentration of 2–4 mmol·l^{-1}), and a lactate accumulation zone (High intensity Training, HiT; intensity corresponding to a blood lactate concentration of ≥ 4 mmol·l^{-1}), where blood lactate production exceeds maximum clearance rates. The corresponding external [power output (Poole et al., 2016)] or internal [lactate (Beneke et al., 2011), heart rate (Borges et al., 2020), heart rate variability (Rogers et al., 2021) and perceived efforts (Sanders et al., 2017)] load are variables applied to navigate this training stimulus. These differences in exercise intensity determination and application impede conclusive evidence on optimal intensity dependent dose-responsiveness during endurance training scheduling. For example, whether polarized or pyramidal training is more favorable to induce optimal endurance performance improvements is still not elucidated. Although successful endurance athletes complete a particularly large part of their training volume in the LiT zone (Fiskerstrand and Seiler, 2004; Guellich et al., 2009; Seiler, 2010), LiT is frequently performed at slightly higher real training intensities than intended, resulting in a bottom-up-shift towards ThT (Seiler, 2010; Röhrken et al., 2020). In turn, intended HiT is also shifting top-down towards ThT. Consequently, a clear evidence-based differentiation between both training intensity distribution frameworks to justify optimal training stimuli is hampered. Thus, practical strategies should be explored and examined to comply with the intended target exercise training zone.

With regard to training regulation, monitoring the breathing frequency during exercise has been suggested as a potential parameter, mainly as the breathing frequency is strongly associated with the perceived effort during exercise under normal and special conditions (e.g., heat, hypoxia, glycogen-depleted state) (Nicolò et al., 2017b). Furthermore, an increasing work load is associated with an increasing oxygen demand (Gaesser and Poole, 1996). In professional cyclists, the tidal volume (V_T), breathing frequency (BF) and, subsequently, the total ventilation volume (VE) were found to increase as a function of exercise intensity (Lucía et al., 1999). In this context, it has been reported repeatedly that the nasal contribution to breathing decreases with increasing exercise intensity (Niinimaa et al., 1980; Wheatley et al., 1991; James et al., 1997; Bennet et al., 2003). A turning point from nasal to oronasal breathing has been reported at 38% ± 12% of the predicted maximum physical working capacity for men, and at 55% ± 13% for women in moderately trained young adults (Niinimaa et al., 1980). It is therefore reasonable to assume that athletes breathing exclusively nasally are more likely to comply with lower aerobic exercise intensities (e.g., at an intensity below the first lactate threshold) avoiding the bottom-up trend to ThT.

Against this background, this study examined the effect of nasal-only vs. (non-restricted) oro-nasal breathing during self-selected low intensity cycling on ventilation, power output, oxygen consumption, blood lactate concentration, heart rate response, perceived effort, and perceived discomfort. As restricted nasal-only breathing leads to minimal ventilatory impedance (Tong et al., 2001), this may cause higher perceived efforts. Consequently, athletes may need to adhere to very low exercise intensities to avoid further increases of perceived physical effort. Our findings might help coaches and athletes to guarantee LiT intensities during low intensity training by restricting airway choice.

2 Materials and methods

2.1 Participants and study design

An *a priori* conducted power analysis [$\alpha = 0.05$, study power (1-β-error) = 0.80, r = 0.6, effect size $\eta_p^2 = 0.06$ ($f = 0.25$)] using g*Power (Version 3.1.9.6) revealed a required a sample size of n = 16. Assuming low to moderate (15%–20%) dropouts, n = 19 young, and physically healthy adults [3 females, age: 26.5 ± 5.1 years; height: 1.77 ± 0.08 m; body mass: 77.3 ± 11.4 kg; peak oxygen uptake (VO_2peak): 53.4 ± 6.6 mL·kg^{-1} min^{-1}, power at VO_2peak: 285.7 ± 58.0 W, power (HR) at 2 mmol·l^{-1}: 189.5 ± 64.6 W (146.1 ± 14.4 bpm), power (HR) at 4 mmol·l^{-1}: 234.7 ± 65.8 W (165.6 ± 10.3 bpm)] were enrolled in this acute randomized controlled crossover trial. Inclusion criteria were i) actively pursuing an endurance sport for at least 2 years (training ≥ 3/week) and ii) no medical condition that potentially impede the completion of all experimental sessions. The study was approved by the local ethical committee (033/2022) and all participants signed an informed written consent prior to start of the study.

The study design for this acute randomized crossover study required three lab visits. The first lab visit consisted of anthropometric evaluations and a step test to determine lactate thresholds and VO_2peak. During the second and third lab visit, participants performed 60 min of self-selected low-intensity cycling training in a randomized order with either breathing without restriction (oro-nasal) or exclusively nasal breathing (nasal-only). All three lab visits were conducted at least 48 h apart with examinations completed at the same time of day for each participant to avoid circadian interferences. Furthermore, participants were instructed to avoid any strenuous exercise in the 24 h prior to each lab visit. All lab visits were performed individually with a participant to researcher ratio of 1:1.

2.2 Testing procedures

To determine individual lactate thresholds and assess VO_2peak a step test on a concentric cycle ergometer (Wahoo Kickr V5 Fitness WF133, Wahoo Fitness, Atlanta, United States) until voluntary exhaustion was conducted. Cycling was performed with clipless pedals and participants were instructed to permanently remain seated. The test started at a load of 100 W, which was subsequently increased by 20 W every 3 min until exhaustion. Prior to the start of the test, after each 3 min-step and immediately after exercise cessation, blood lactate samples (20 µL) were obtained from the earlobe (Biosen C-Line; EKF Diagnostic Sales, Magdeburg, Germany). Lactate concentrations were subsequently plotted against the load (in W) and fitted with a third order polynomial function. Based on this function, load and heart rate (HR) corresponding to a blood lactate concentration of 2 $mmol{\cdot}l^{-1}$ and 4 $mmol{\cdot}l^{-1}$ were determined. Furthermore, throughout the whole test, HR (H9; Polar Electro, Kempele, Finnland) and respiratory gas exchange were continuously recorded breath-by-breath comprising a validated metabolic analyzer (Zan Oxi 600, Zan Messgeräte, Germany). Prior to each measurement, this spirometric system was calibrated, following the manufacturer's recommendations. V_T, VE, carbon dioxide output (VCO_2) and oxygen uptake (VO_2) were averaged over 30 s. Furthermore, the respiratory exchange ratio (RER) was calculated by dividing VCO_2 by VO_2. The highest consecutive oxygen uptake values averaged over 30 s were considered as VO_2peak. All athletes were verbally encouraged in a standardized manner until exhaustion.

2.3 Acute intervention protocol

After the first lab visit, participants received a one-page flyer representing the association between blood lactate rise in dependence of exercise intensity: Based on (Seiler, 2010) three training intensity zones representing low intensity training (LiT; ≤2 $mmol{\cdot}l^{-1}$), threshold training (ThT, 2–4 $mmol{\cdot}l^{-1}$), and HiT (> 4 $mmol{\cdot}l^{-1}$) were indicated by vertical lines. Additionally, a short and easy to understand description of this three-zone-model and the respective training intensities for each training zone was provided beneath the schematic depiction (see Supplementary Material S1 for the original flyer in German and Supplementary Material S2 for an English translation). To ensure that the participants had a sufficient understanding of the term LiT, they were instructed to read this flyer before the second lab visit.

Both training sessions at lab visit 2 and 3 consisted of 60 min of cycling. For the oro-nasal condition, participants were only given the instruction to maintain an intensity corresponding to the LiT training zone as described in the aforementioned flyer and to maintain a steady cadence of ~80 rpm. Apart from this, they were allowed to choose their training intensity and gearing. Throughout the whole session, HR and respiratory gas exchange were continuously recorded breath-by-breath. Additionally, every 10 min (T10, T20, T30, T40, T50, T60), blood lactate samples were obtained and participants were asked to rate their perceived effort (RPE; CR-10) (Foster et al., 2001) and discomfort (Steele et al., 2016). Furthermore, power data and cycling cadence were recorded at a rate of 1 Hz, which was subsequently downloaded and transferred to a personal computer. Apart from time left in the session, no feedback (i.e., information on HR, lactate concentration, power, or respiratory gas exchange parameters) was provided during the session. For the nasal-only condition, an identical setup was chosen. However, participants were additionally instructed to only breath through their nose. To provide maximal breathing capacity through the nose, 5 min prior to the session multiple sprays of sea water nasal spray were applied per nostril and nasal dilator strips were taped across the bridge of the nose and sides of the nostrils for holding open the anterior nasal aperture. Furthermore, a strip of tape was applied over the mouth to prohibit breathing through the mouth. This tape was accessible and easy to remove for both the researcher and participant in case of an emergency. No significant differences were found in terms of resting VO_2 [$F(1, 18) = 1.79$, $p = 0.20$, $\eta_p^2 = 0.09$ (nasal-only condition: 0.499 ± 0.144 $L{\cdot}min^{-1}$, oro-nasal condition: 0.469 ± 0.117 $L{\cdot}min^{-1}$)] and blood lactate concentrations [$F(1, 18) = 1.57$, $p = 0.23$, $\eta_p^2 = 0.08$ (restricted: 1.00 ± 0.28 $mmol{\cdot}l^{-1}$, unrestricted: 1.16 ± 0.48 $mmol{\cdot}l^{-1}$)] prior to the two training sessions. To determine gross efficiency (GE), the work accomplished was divided by the energy expended and multiplied by 100 to obtain a percentual value (Gaesser and Brooks, 1975). For all further analyses, HR data, respiratory gas exchange data [V_T, VE, RER, VO_2, VCO_2, BF, end tidal pressure of oxygen ($PETO_2$), end tidal pressure of carbon dioxide ($PETCO_2$)] and ergometer data were averaged over each of the 10 min intervals from T10 to T60. Based on the power output and HR, for each 10-min interval, the percentage of time spent in the respective training zones (LiT, ThT, HiT) was calculated, respectively.

2.4 Statistics

Data are presented as mean ± SD. All data were initially assessed for normal distribution and variance homogeneity via visual inspection. For the respective outcome measures (HR, RPE, V_T, VE, RER, VO_2, VCO_2, BF, $PETO_2$, $PETCO_2$, GE, lactate) separately conducted 2 (condition: oro-nasal vs. nasal-only) × 6 (time: T10, T20, T30, T40, T50, T60) repeated measures of variance (rANOVA) were conducted. To examine "condition" differences (oro-nasal vs. nasal-only) repeated measures of variance (rANOVA) were separately conducted for the respective time spent in training zones (LiT, ThT, HiT). Mauchly's test for sphericity was performed and, if necessary, Greenhouse-Geisser (GG) corrections were applied. Effect sizes for rANOVA are given as partial eta squared (η_p^2) with ≥ 0.01, ≥ 0.06, ≥ 0.14 indicating small, moderate, and large effects, respectively (Cohen, 1988). In case of significant interaction effects, Bonferroni *post hoc* tests were subsequently computed. For pairwise effect size comparison, standard mean differences (SMD) were calculated as differences between means divided by the pooled standard deviations (trivial: $|SMD| < 0.2$, small: $0.2 \leq |SMD| < 0.5$, moderate: $0.5 \leq |SMD| < 0.8$, large: $|SMD| \geq 0.8$) (Cohen, 1988). Statistical analyses were performed using R (version 4.0.5) in its integrated development environment RStudio (version 1.4.1106). A *p*-value below 0.05 was considered as statistically significant.

3 Results

3.1 Performance-related parameters

For the acute oxygen uptake related to the participants respective VO_2peak (%VO_2peak) no significant interaction effect was found [$F(2.5, 44.2) = 1.39$, p (GG) = 0.26, $\eta_p^2 = 0.07$], but

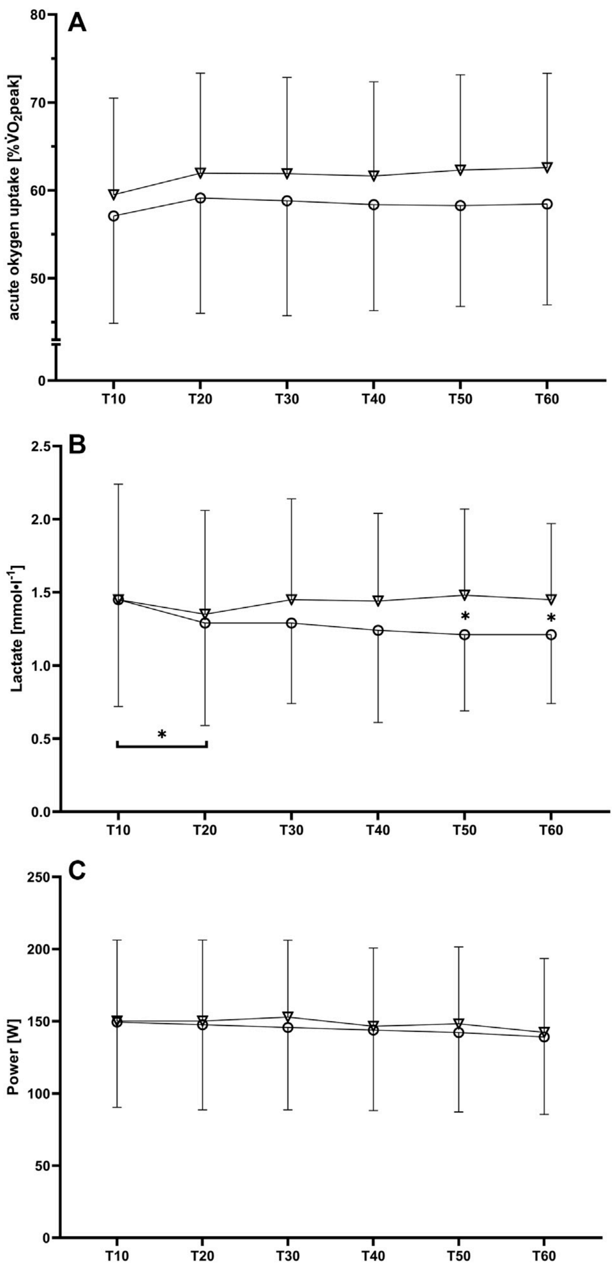

FIGURE 1
Mean values and standard deviations for **(A)** oxygen uptake related to VO_2peak, **(B)** blood lactate concentration and **(C)** power output during the six 10-min intervals (T10 to T60) for the nasal-only (circles) and oro-nasal (triangles) training condition. *$p < 0.05$.

significant and large main effects for both time [F (1.7, 31.1) = 5.73, p (GG) = 0.01, $\eta_p^2 = 0.24$] and condition [F (1, 18) = 5.49, $p = 0.03$, $\eta_p^2 = 0.23$] indicating higher values for the oro-nasal condition (Figure 1A). For blood lactate concentrations, a significant and large interaction effect was found [F (3.2, 57.1) = 3.61, p (GG) = 0.02, $\eta_p^2 = 0.17$]. Subsequently performed *post hoc* testing revealed a significant reduction in blood lactate concentration between T10 and T20 in the nasal-only condition (1.45 ± 0.73 vs. 1.29 ± 0.70 mmol·l^{-1}, $p = 0.03$, SMD = 0.22). Furthermore, significant differences between the nasal-only and oro-nasal conditions were found for T50 condition (1.21 ± 0.52 vs. 1.48 ± 0.59 mmol·l^{-1}, $p = 0.01$, SMD = 0.49) and T60 (1.21 ± 0.47 vs. 1.45 ± 0.52 mmol·l^{-1}, $p = 0.02$, SMD = 0.48) (Figure 1B).

No significant interaction effects were found for power [F (1.6, 24.8) = 0.84, p (GG) = 0.42, $\eta_p^2 = 0.05$], cadence [F (2.9, 46.5) = 2.10, p (GG) = 0.12, $\eta_p^2 = 0.12$], and distance [F (1.8, 28.4) = 0.79, p (GG) = 0.45, $\eta_p^2 = 0.05$] (Table 1).

Furthermore, the rANOVA did not reveal significant interaction effects for VO_2 [F (2.3, 42.2) = 1.44, p (GG) = 0.25, $\eta_p^2 = 0.07$], VCO_2 [F (2.3, 41.5) = 0.67, p (GG) = 0.54, $\eta_p^2 = 0.04$], VE [F (2.3, 41.6) = 1.83, p (GG) = 0.17, $\eta_p^2 = 0.09$], RER [F (2.6, 46.4) = 0.73, p (GG) = 0.52, $\eta_p^2 = 0.04$], BF [F (1.9, 35.0) = 0.31, p (GG) = 0.73, $\eta_p^2 = 0.02$], $PETO_2$ [F (2.4, 42.4) = 0.24, p (GG) = 0.82, $\eta_p^2 = 0.01$], $PETCO_2$ [F (1.9, 34.8) = 0.26, p (GG) = 0.77, $\eta_p^2 = 0.01$], V_T [F (1.9, 34.7) = 0.47, p (GG) = 0.62, $\eta_p^2 = 0.02$], GE [F (1.6, 24.4) = 0.34, p (GG) = 0.67, $\eta_p^2 = 0.02$], HR [F (2.0, 35.2) = 0.41, p (GG) = 0.67, $\eta_p^2 = 0.02$], and discomfort [F (2.6, 47.6) = 2.13, p (GG) = 0.12, $\eta_p^2 = 0.11$], but did reveal significant interaction effects for RPE [F (3.4, 61.8) = 3.38, p (GG) = 0.02, $\eta_p^2 = 0.16$] (Table 2).

3.2 Training zone distribution

The individually conducted 1 x 2 rANOVAs did neither reveal significant "condition" effects for time spent in any of the training zones for power-based calculations of the training zones (Zone 1: (F(1, 18) = 1.45, $p = 0.24$, $\eta_p^2 = 0.07$); Zone 2: (F(1, 18) = 0.98, $p = 0.34$, $\eta_p^2 = 0.05$); Zone 3: (F(1, 18) = 1.03, $p = 0.32$, $\eta_p^2 = 0.05$), Figure 2A) nor heart rate based calculations of the training zones (Zone 1: (F(1, 18) = 0.03, $p = 0.85$, $\eta_p^2 = 0.00$); Zone 2: (F(1, 18) = 0.14, $p = 0.71$, $\eta_p^2 = 0.01$); Zone 3: (F(1, 18) = 0.19, $p = 0.67$, $\eta_p^2 = 0.01$), Figure 2B).

4 Discussion

This randomized-controlled crossover trial aimed at investigating the effect of nasal-only vs. oro-nasal breathing during low intensity cycling on power output, oxygen consumption, blood lactate concentration, heart rate, perceived effort and perceived discomfort. No significant differences were found between the two conditions in terms of training intensity outcomes quantified by power output and heart rate. However, total ventilation, carbon dioxide release, oxygen uptake and breathing frequency were notably lower during nasal-only breathing. Furthermore, lower capillary blood lactate concentrations were found towards the end of the training session during nasal-only breathing. These condition-dependent differences between power output and ventilatory response did not affect training intensity distribution (time spent in the three training zones). Interestingly, even though discomfort was rated marginally higher during cycling with nasal-only breathing, ratings of perceived effort did not differ between both conditions.

Our results of lower breathing frequency, total ventilation volume, carbon dioxide release and oxygen uptake during the training session with nasal breathing restriction are in line with previous research on the influence of nasally restricted breathing on cardiorespiratory parameters during continuous submaximal exercise (Morton et al., 1995; Hostetter et al., 2016; LaComb et al., 2017; Recinto et al., 2017). At

TABLE 1 Performance data (mean value ± standard deviation) for the restricted (nasal-only) and unrestricted condition at each 10min interval (T10-T60). *p*-Values and partial eta squared (η_p^2) of rANOVA are also provided.

Parameter	Condition	T10	T20	T30	T40	T50	T60	rANOVA *p*-value (η_p^2)		
								Time	Condition	Time × condition
Power [W]	Unrestricted	150.1 ± 56.2	150.1 ± 56.1	152.9 ± 53.3	146.6 ± 54.1	148.3 ± 53.2	142.3 ± 51.1	<0.01 (0.37)	0.35 (0.06)	0.42 (0.05)
	Restricted	149.4 ± 59.1	147.6 ± 59.0	145.6 ± 57.0	143.8 ± 55.7	142.2 ± 55.0	139.2 ± 53.7			
Cadence [min^{-1}]	Unrestricted	84.7 ± 3.1	87.6 ± 3.1	87.3 ± 3.1	87.4 ± 2.7	88.1 ± 2.8	87.7 ± 4.3	<0.001 (0.44)	0.38 (0.05)	0.12 (0.12)
	Restricted	83.0 ± 2.6	86.7 ± 3.3	87.4 ± 3.5	87.8 ± 3.1	87.9 ± 3.1	86.6 ± 2.9			
Distance [m]	Unrestricted	3,891 ± 902	4,022 ± 947	3,960 ± 924	4,012 ± 937	4,033 ± 920	4,052 ± 908	0.11 (0.14)	0.51 (0.03)	0.45 (0.05)
	Restricted	4,004 ± 972	4,123 ± 965	4,097 ± 969	4,109 ± 963	4,119 ± 992	4,075 ± 987			

the same load, similar blood lactate concentrations between nasally restricted and unrestricted breathing conditions have been reported (Dallam et al., 2018). This is also fairly in line with our results, as we did not find increased levels of capillary blood lactate concentration during the nasal-only breathing condition, but even slightly lower values towards the end of the session, which, however, might be related to the decreased power output. It therefore seems plausible, that at least during submaximal exercise intensities the oxygen uptake is not limited by the nasal breathing restriction and thus does not hamper the aerobic energy production. A lower breathing frequency at a given total ventilation volume inherently indicates a higher tidal volume (Harbour et al., 2022), which in turn leads to a reduction in the ratio of the volume of the conducting air passages (anatomic dead space) to the total ventilation volume (Harbour et al., 2022). In the present study, however, even though we found a significantly reduced breathing frequency during the nasal-only condition, the tidal volume was only marginally higher, thus resulting in a lower total ventilation. Moreover, we found a significantly higher end tidal partial pressure of carbon dioxide with a simultaneously lower end tidal partial pressure of oxygen during the nasal-only condition. This may indicate that the lower breathing frequency during nasally restricted breathing leads to a longer pulmonary diffusion time (Morton et al., 1995; Hostetter et al., 2016; LaComb et al., 2017). Therefore, it has been hypothesized that this improvement in ventilatory efficiency during nasal-only breathing at submaximal training intensities may in consequence lead to an improved breathing economy (Hostetter et al., 2016; Dallam et al., 2018). In terms of gross efficiency, however, we did not find significant differences between the two conditions in the present study. It thus seems plausible to assume, that the reduction in minute ventilation is probably due to a reduction in both the breathing frequency and carbon dioxide release.

Despite the lower breathing frequency, oxygen uptake and blood lactate concentration, we did not find any significant and meaningful differences in training intensity distribution between the two conditions. A strong correlation has been frequently reported between the breathing frequency and the perceived effort at moderate to high intensities (Robertson and Noble, 1997; Nicolò et al., 2016; 2017a; 2018; Cochrane-Snyman et al., 2019). In this context, it has been speculated, that a lower breathing frequency might decrease the perceived effort at a certain intensity as the participants are misled to feeling exercise to be easier (Harbour et al., 2022). However, even though we found a statistically significantly lower breathing frequency during the nasal-only breathing condition, perceived effort and power output did not differ between both conditions. In well-trained competitive cyclists, Nicolo and colleagues (Nicolò et al., 2018) reported either no or only small changes in the breathing frequency for given workload intensities corresponding to RPE values of 11 or lower on the 6–20 scale, with considerable changes in breathing frequency at intensities corresponding to > 11 on the RPE scale obtained during sinusoidal tests performed across moderate to severe intensities. It was therefore concluded that the breathing frequency may be considered as sensitive for higher, but not low training intensities (Nicolò et al., 2018). The perceived effort at the first lactate threshold is rated by athletes at 10.4 ± 1.7 on the 6–20 scale (Scherr et al., 2013). This corresponds to the intensity at which the breathing frequency shows a substantial response, which in turn is associated with an increase in perceived effort. Therefore, it seems plausible to assume that the intensity, at which the breathing frequency and subsequently the perceived effort show a substantial response, is located slightly above the first lactate threshold. Thus, this threshold might be too high to be used as a measure to remain in the low-intensity training zone.

A limitation of the study that needs to be addressed is that only the acute effects of a single training session without familiarization to the breathing restrictions were assessed. In this context, the slight decrease in power towards the end of sessions may indicate that perhaps too high a load was selected at the beginning of the sessions. However, as no significant interaction effect was found, and blood lactate concentration did not build up throughout the session in either condition, this seems negligible. Nevertheless, possible longitudinal adaptation to the airway restriction and its effect on the air hunger of the participants should be focused on in future research. Moreover, it might be possible that restricting airway choice may lead to deviations in metabolic thresholds determined during the unrestricted ramp test. However, as demonstrated by Dallam and colleagues (2018), at the same load, similar blood lactate concentrations between nasally restricted and unrestricted breathing conditions can be expected (Dallam et al., 2018).

In terms of long-term adaptations, the diaphragmatic function might increase with time, as during nasal-only breathing a smaller airway is utilized (Trevisan et al., 2015). These adaptations may also

TABLE 2 Performance data (mean value ± standard deviation) for the restricted (nasal-only) and unrestricted condition at each 10min interval (T10-T60) for oxygen uptake (VO_2), carbon dioxide release (VCO_2), total ventilation (VE), respiratory exchange value (RER; VCO_2 divided by VO_2), breathing frequency (BF), end tidal pressure of oxygen ($PETO_2$), end tidal pressure of carbon dioxide ($PETCO_2$), tidal volume (VT), gross efficiency (GE; work accomplished divided by energy expenditure and multiplied by 100), heart rate (HR), perceived effort (RPE), and discomfort. *p*-Values and partial eta squared (η_p^2) of rANOVA are also provided.

Parameter	Condition	T10	T20	T30	T40	T50	T60	rANOVA *p*-value (η_p^2)		
								Time	Condition	Time × condition
VO_2 [l·min^{-1}]	Unrestricted	2.49 ± 0.74	2.59 ± 0.76	2.59 ± 0.75	2.57 ± 0.74	2.60 ± 0.75	2.61 ± 0.74	0.01 (0.23)	0.03 (0.23)	0.25 (0.07)
	Restricted	2.39 ± 0.78	2.47 ± 0.83	2.46 ± 0.84	2.44 ± 0.79	2.43 ± 0.76	2.44 ± 0.77			
VCO_2 [l·min^{-1}]	Unrestricted	2.19 ± 0.68	2.34 ± 0.72	2.33 ± 0.72	2.29 ± 0.70	2.30 ± 0.69	2.30 ± 0.67	<0.001 (0.46)	0.02 (0.28)	0.54 (0.04)
	Restricted	2.09 ± 0.66	2.23 ± 0.73	2.22 ± 0.73	2.18 ± 0.70	2.16 ± 0.67	2.15 ± 0.67			
VE [l·min^{-1}]	Unrestricted	53.0 ± 16.1	57.9 ± 17.9	59.0 ± 19.4	59.2 ± 19.3	59.9 ± 18.6	60.0 ± 18.3	<0.001 (0.68)	<0.001 (0.45)	0.17 (0.09)
	Restricted	48.1 ± 14.2	52.2 ± 15.9	53.1 ± 17.0	53.0 ± 16.6	52.8 ± 15.9	53.0 ± 16.1			
RER [au]	Unrestricted	0.88 ± 0.05	0.90 ± 0.04	0.90 ± 0.05	0.89 ± 0.05	0.88 ± 0.05	0.88 ± 0.05	<0.001 (0.54)	0.67 (0.01)	0.52 (0.04)
	Restricted	0.87 ± 0.05	0.90 ± 0.04	0.90 ± 0.05	0.90 ± 0.05	0.89 ± 0.04	0.88 ± 0.04			
BF [min^{-1}]	Unrestricted	26.1 ± 4.4	28.5 ± 4.8	29.7 ± 5.2	30.3 ± 5.2	30.8 ± 5.4	30.7 ± 5.3	<0.001 (0.76)	0.01 (0.35)	0.73 (0.02)
	Restricted	23.3 ± 3.6	25.7 ± 4.0	26.7 ± 4.6	27.2 ± 4.6	27.4 ± 5.0	27.3 ± 4.9			
$PETO_2$ [mmHg]	Unrestricted	97.72 ± 4.48	99.92 ± 3.49	100.58 ± 3.68	100.83 ± 3.74	100.85 ± 3.19	100.85 ± 3.14	<0.001 (0.62)	0.01 (0.37)	0.82 (0.01)
	Restricted	95.27 ± 4.96	97.27 ± 4.37	98.39 ± 3.51	98.44 ± 3.60	98.27 ± 3.60	98.17 ± 3.59			
$PETCO_2$ [mmHg]	Unrestricted	41.50 ± 2.60	41.05 ± 2.59	40.33 ± 2.91	39.70 ± 2.76	39.31 ± 2.49	39.11 ± 2.36	<0.001 (0.68)	<0.001 (0.46)	0.77 (0.01)
	Restricted	43.47 ± 3.12	43.23 ± 3.59	42.40 ± 3.10	41.87 ± 2.91	41.57 ± 2.82	41.38 ± 2.83			
VT [l]	Unrestricted	2.04 ± 0.56	2.04 ± 0.55	1.99 ± 0.52	1.95 ± 0.49	1.95 ± 0.50	1.95 ± 0.49	<0.001 (0.38)	0.50 (0.03)	0.62 (0.02)
	Restricted	2.11 ± 0.67	2.08 ± 0.70	2.02 ± 0.66	1.98 ± 0.62	1.96 ± 0.62	1.97 ± 0.61			
GE [%]	Unrestricted	17.7 ± 2.1	16.5 ± 1.8	16.4 ± 2.0	16.3 ± 2.1	16.0 ± 2.2	15.7 ± 2.2	<0.001 (0.72)	0.08 (0.20)	0.67 (0.02)
	Restricted	18.3 ± 2.5	17.0 ± 2.0	16.9 ± 2.2	16.9 ± 2.2	16.7 ± 2.3	16.3 ± 2.5			
HR [min^{-1}]	Unrestricted	124.2 ± 13.4	130.5 ± 16.2	132.9 ± 17.5	134.3 ± 17.5	136.5 ± 18.0	138.7 ± 18.5	<0.001 (0.77)	0.58 (0.02)	0.67 (0.02)
	Restricted	124.6 ± 16.2	131.4 ± 19.3	133.9 ± 20.0	136.3 ± 21.0	137.6 ± 21.0	139.3 ± 20.7			
RPE [au]	Unrestricted	2.2 ± 0.7[a]	2.6 ± 0.9[c]	2.9 ± 1.0	3.2 ± 1.0	3.4 ± 1.0	3.5 ± 1.0	<0.001 (0.63)	0.75 (0.01)	0.02 (0.16)
	Restricted	2.5 ± 0.8[a]	2.8 ± 0.8[b]	3.0 ± 0.8[d]	3.3 ± 0.8	3.2 ± 0.7	3.2 ± 0.8			
Discomfort [au]	Unrestricted	1.7 ± 0.9	2.1 ± 1.0	2.8 ± 1.1	3.4 ± 1.1	3.8 ± 1.1	4.0 ± 1.2	<0.001 (0.66)	0.03 (0.24)	0.12 (0.11)
	Restricted	2.5 ± 1.6	3.5 ± 1.3	3.7 ± 1.4	3.8 ± 1.7	4.5 ± 1.7	4.9 ± 1.9			

[a]Significantly different from T30, T40, T50, T60 ($p < 0.001$—0.05).
[b]significantly different from T40 ($p < 0.05$).
[c]significantly different from T40, T50, T60 ($p < 0.001$—0.01).
[d]significantly different from T50, T60 ($p < 0.05$—0.01).

help to reduce the higher ratings of perceived discomfort that occurred during nasal-only breathing. Furthermore, the filtration and humidification functions of the nose may help at any exercise intensity to prevent exercise-induced dypnoea and pathogen or particulate inhalation (Aydın et al., 2014). The risk for infections of the upper respiratory tract is significantly reduced when breathing exclusively through the nose during exercise (Walker et al., 2016). By contrast, breathing at submaximal intensities only through the mouth is more likely to cause irritation of the airways, and thus in turn increase the risk of possible exercise-induced laryngeal obstruction (Johansson et al., 2015). Since the head posture and glossopharyngeal mechanics are influenced by different airway choices (Okuro et al., 2011; Sabatucci et al., 2015), breathing predominantly through the nose during submaximal intensities may also prevent exercise-induced laryngeal obstruction (Harbour et al., 2022). Furthermore, by breathing predominantly through the nose, the risk for exercise-induced bronchoconstriction might be reduced (Dallam and Kies, 2020). Therefore, longitudinal studies are necessary to evaluate the long-term effect of nasal-only breathing on perceived effort and physiological parameters in endurance sports.

In conclusion, restricting airway choice did not prevent participants from a tendency to shift from low-intensity training to higher intensities. Nevertheless, temporarily performing low-

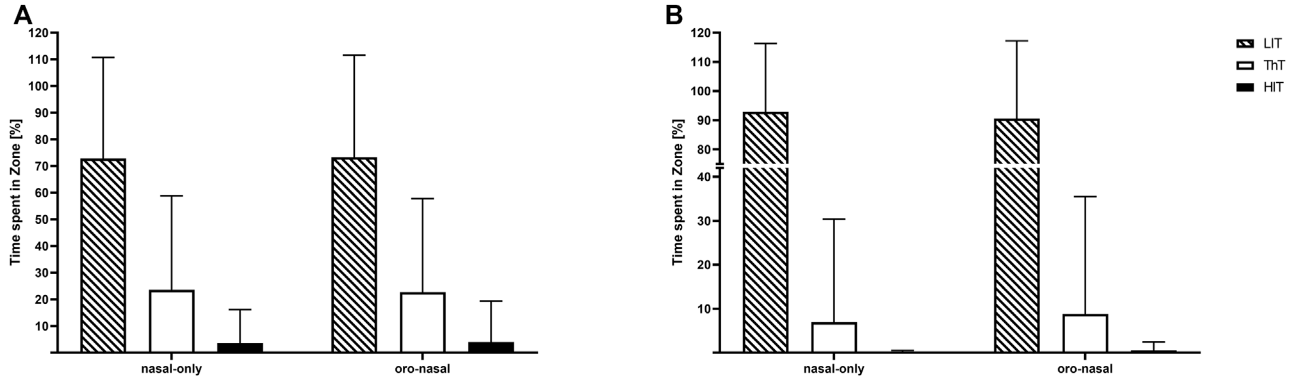

FIGURE 2
Mean values and standard deviations for the relative time spent in training zones [LiT: low-intensity training (dashed); ThT: threshold training (white); HIT: high-intensity training (black)]. Calculated based on power **(A)** and heart rate **(B)** for the nasal-only and oro-nasal training condition.

intensity endurance training under oral breathing restrictions may induce physiological changes that help maintain physical health in endurance athletes.

Data availability statement

The raw data supporting the conclusion 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 Ethics Committee of the German Sport University Cologne. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LR, SH, and LD contributed to the conception and design of the study. JH, TW, and J-PD led the intervention. LR, SH, and TW performed the statistical analysis. LR wrote the first draft of the manuscript. LR, SH, PW, and LD wrote sections of the manuscript. PW copyedited the draft for content, language, and format and organized the submission and revision/resubmission process. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Ventilation and perceived exertion are sensitive to changes in exercise tolerance: arm+leg cycling vs. leg cycling

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Purpose: Growing evidence suggests that respiratory frequency (f_R) is a marker of physical effort and a variable sensitive to changes in exercise tolerance. The comparison between arm+leg cycling (Arm+leg) and leg cycling (Leg) has the potential to further test this notion because a greater exercise tolerance is expected in the Arm+leg modality. We systematically compared Arm+leg vs. Leg using different performance tests.

Methods: Twelve males underwent six performance tests in separate, randomized visits. Three tests were performed in each of the two exercise modalities, i.e. an incremental test and two time-to-exhaustion (TTE) tests performed at 90% or 75% of the peak power output reached in the Leg incremental test (PPO_{Leg}). Exercise tolerance, perceived exertion, and cardiorespiratory variables were recorded during all the tests.

Results: A greater exercise tolerance ($p < 0.001$) was found for Arm+leg in the incremental test (337 ± 32 W vs. 292 ± 28 W), in the TTE test at 90% of PPO_{Leg} (638 ± 154 s vs. 307 ± 67 s), and in the TTE test at 75% of PPO_{Leg} (1,675 ± 525 s vs. 880 ± 363 s). Unlike $\dot{V}O_2$ and heart rate, both f_R and minute ventilation were lower ($p < 0.003$) at isotime in all the Arm+leg tests vs. Leg tests. Furthermore, a lower perceived exertion was observed in the Arm+leg tests, especially during the TTE tests ($p < 0.001$).

Conclusion: Minute ventilation, f_R and perceived exertion are sensitive to the improvements in exercise tolerance observed when comparing Arm+leg vs. Leg, unlike $\dot{V}O_2$ and heart rate.

Abbreviations: Arm+leg, arm+leg cycling; Arm+leg$_{INC}$, arm+leg cycling incremental test; Arm+leg$_{TTE75}$, Arm+leg TTE test at 75% of PPO_{Leg}; Arm+leg$_{TTE90}$, Arm+leg TTE test at 90% of PPO_{Leg}; f_R, respiratory frequency; GET, gas exchange threshold; HR, heart rate; Leg, leg cycling; Leg$_{INC}$, leg cycling incremental test; Leg$_{TTE75}$, Leg TTE test at 75% of PPO_{Leg}; Leg$_{TTE90}$, Leg TTE test at 90% of PPO_{Leg}; P_{ETCO2}, end-tidal partial pressure of carbon dioxide; PPO, Peak power output; PPO_{Leg}, peak power output reached in the Leg$_{INC}$; RCP, respiratory compensation point; RPE, ratings of perceived exertion; TTE, time-to-exhaustion; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_2$peak, peak value of oxygen uptake; V_T, tidal volume.

KEYWORDS

endurance performance, breathing control, respiratory frequency, incremental test, time to exhaustion, fatigue, breathing pattern, oxygen uptake

1 Introduction

The comparison between different exercise modalities has the potential to improve our understanding of the physiology of endurance performance. Classical studies have compared leg cycling (Leg) with arm+leg cycling (Arm+leg) to unravel the mechanisms limiting maximal oxygen uptake (Astrand and Saltin, 1961; Secher et al., 1974; Bergh et al., 1976). Findings from these studies have contributed to outlining the important role of cardiocirculatory factors in setting the upper limit for maximal aerobic power. Indeed, the peak value of oxygen uptake ($\dot{V}O_2$peak) is not always proportional to the differences in the amount of muscle mass involved in various exercise modalities, as the addition of arm work to leg work generally does not increase $\dot{V}O_2$peak more than about 5%–10% (Astrand and Saltin, 1961; Gleser et al., 1974; Secher et al., 1974; Secher and Volianitis, 2006). In fact, the comparison between arm+leg cycling and leg cycling is suitable for gaining insight into other physiological responses that have received less attention so far, including the variables associated with physical effort and changes in exercise tolerance. Exercise tolerance is here defined as the tolerated duration during a time-to-exhaustion (TTE) test performed at a constant work rate or the peak power output (PPO) achieved during an incremental test (Van De Walle and Vukovich, 2018). Some findings have shown that arm+leg cycling results in a greater exercise tolerance compared to leg cycling alone (arms hanging on the participant's side) (Gleser et al., 1974; Secher et al., 1974; Nagle et al., 1984), but it is unclear if this difference is still evident when arm+leg cycling is compared to conventional leg cycling (Secher et al., 1974; Bergh et al., 1976; Nagle et al., 1984). When matched for the same absolute total power output, the greater exercise tolerance that might be expected for arm+leg cycling makes the comparison with (conventional) leg cycling valuable for testing the proposition that improvements in exercise tolerance are accompanied by consistent changes in the responses of respiratory frequency (f_R) and perceived exertion (Nicolò and Sacchetti, 2023).

Growing evidence suggests that f_R is a valid marker of physical effort (Nicolò et al., 2014; Nicolò et al., 2016; Nicolò et al., 2017a; Nicolò et al., 2017b; Nicolò et al., 2019; Nicolò and Sacchetti, 2023) and that its time course reflects changes in exercise tolerance (Nicolò and Sacchetti, 2023). In a variety of conditions where exercise tolerance is reduced (experimentally) or lowered (in a cross-sectional comparison), the rate of increase in f_R is higher, both during incremental and TTE tests. On the other hand, the rate of increase in f_R is lower when assessing exercise strategies, experimental interventions or other conditions leading to an improvement in exercise tolerance (Nicolò and Sacchetti, 2023). The sensitivity of f_R to changes in exercise tolerance and the close association between f_R and perceived exertion are among the factors suggesting that f_R is to a large extent modulated by central command (the activity of motor and premotor brain areas relating to voluntary locomotor muscle contraction) during high-intensity exercise (Nicolò and Sacchetti, 2023). This explains why f_R can be considered a marker of physical effort, which is defined as the degree of motor effort (i.e. the magnitude of central command) (Nicolò et al., 2017b). However, measuring the magnitude of central command during "real" exercise conditions is particularly challenging. Hence, it is important to use different approaches (including the comparison of different exercise modalities) to provide indirect evidence on the contribution of central command to f_R modulation (Nicolò and Sacchetti, 2023).

The comparison between arm+leg cycling and leg cycling may either challenge or reinforce the notion that the increase in f_R during high-intensity exercise reflects changes in exercise tolerance and is influenced by central command. f_R is also modulated by muscle afferent feedback from groups III and IV (hereinafter muscle afferent feedback) (Dempsey et al., 2014; Girardi et al., 2021; Nicolò and Sacchetti, 2023), and this drive to breathe may have a greater relative contribution to f_R modulation when arm muscles assist leg muscles during arm+leg cycling, in view of a potentially larger amount of muscle mass concomitantly involved in exercise (Dempsey et al., 2014; Nicolò and Sacchetti, 2023). Indeed, even the passive movement of the legs leads to a substantial increase in f_R that is at least partially mediated by muscle afferent feedback (Girardi et al., 2021), and this drive to breathe may increase further when adding the movement of the upper limbs. The contribution of muscle afferent feedback to f_R may partially confound the association between f_R and perceived exertion because the latter is supposed to be largely independent of muscle afferent feedback (Marcora, 2009; Bergevin et al., 2023). While arm+leg cycling may also result in a higher $\dot{V}O_2$ compared to leg cycling at the same submaximal power output (Hoffman et al., 1996), f_R largely dissociates from metabolic rate and is not substantially modulated by metabolic inputs, unlike tidal volume (V_T) (Nicolò et al., 2017a; 2018; Nicolò and Sacchetti, 2019; Nicolò et al., 2020a; Nicolò and Sacchetti, 2023). On the other hand, some findings seem to support the association between f_R and perceived exertion during both arm+leg cycling and leg cycling. Robertson et al. (1986) found similar responses of f_R—but not V_T—and the ratings of perceived exertion (RPE) during arm cycling, leg cycling and arm+leg cycling for intensities ranging from 20% to 80% of $\dot{V}O_2$peak. However, the authors neither reported the responses of f_R and RPE when exhaustion was approaching nor described if the two variables were sensitive to between-modality changes in exercise tolerance. Further studies are required to address this issue.

The purpose of the present study was to systematically assess whether exercise tolerance improves with arm+leg cycling vs. (conventional) leg cycling and whether f_R and perceived exertion are sensitive to the expected differences in exercise tolerance. To increase the robustness of our evaluation, we compared arm+leg cycling vs. leg cycling using two exercise paradigms (i.e. incremental test and TTE test) and three comparisons, as the TTE test was performed at two different intensities. We tested the hypotheses that i) arm+leg cycling improves exercise tolerance compared to leg cycling irrespective of the exercise paradigm; and ii) f_R is a good marker of physical effort sensitive to between-modality changes in exercise tolerance, unlike other physiological variables such as $\dot{V}O_2$ and heart rate (HR).

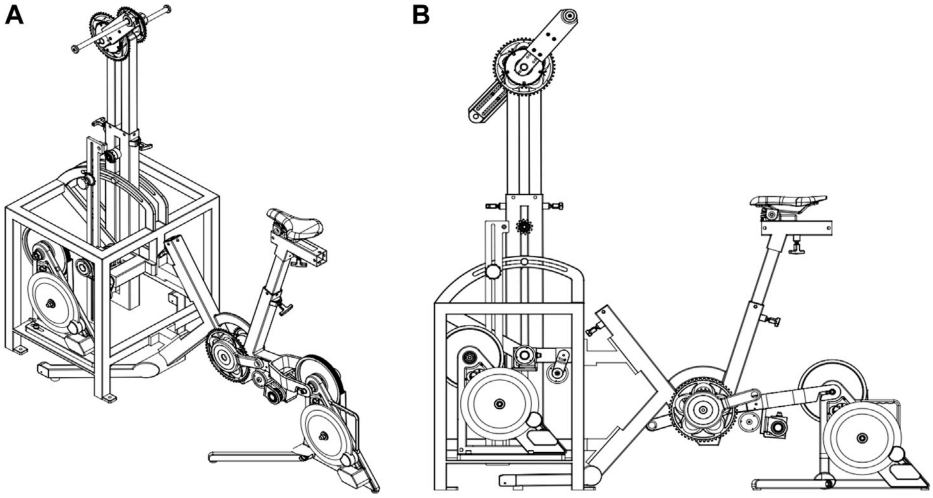

FIGURE 1
Three-dimensional **(A)** and lateral **(B)** views of the multimodal ergometer composed of a grinding ergometer and a cycling ergometer. The multimodal ergometer was custom-made by ORF s.r.l Magnetic Days® (Arezzo, Italy). Note that each of the two ergometers is electromagnetically braked and is equipped with a professional torque transducer.

2 Materials and methods

2.1 Participants

Twelve recreationally trained males (mean ± SD: age 26 ± 4 years; stature 1.79 ± 0.08 m and body mass: 81 ± 10 kg) volunteered to participate in this study. The volunteers recruited participated in one or more sporting activities requiring the use of both arms and legs (e.g. rugby, extreme conditioning program training, and triathlon), as the benefits of arm+leg cycling vs. leg cycling may be more pronounced for individuals exercising with both upper and lower limbs (Secher et al., 1974). The study was approved by the Institutional Review Board of the University of Rome "Foro Italico" in compliance with the *Declaration of Helsinki* (CAR 07/2019). Written informed consent was obtained from all participants. They were asked to refrain from vigorous exercise and the consumption of alcohol and caffeine in the 24 h preceding each laboratory visit.

2.2 Experimental overview

Participants reported to the laboratory on 7 different occasions over a 4-week period, with visits separated by at least 48 h. On the first visit, participants were familiarised with the experimental procedures and tests. On the subsequent visits, participants performed three performance tests to exhaustion in each of the two exercise modalities, i.e. arm+leg cycling and leg cycling. The performance tests consisted of a step incremental test and two TTE tests performed at different intensities. Specifically, the incremental tests ($Arm+leg_{INC}$ and Leg_{INC}) were performed on visits 2 and 3, in random order. The PPO of the Leg_{INC} test (PPO_{Leg}) was used to set the power output of the TTE tests (i.e. 90% and 75% of PPO_{Leg}), which were performed on visits 4–7. The order of Arm+leg and Leg tests was always randomized, as well as the order of the TTE tests at 90% and 75% of PPO_{Leg}. All the tests were performed on a multimodal ergometer custom-made by ORF s.r.l Magnetic Days® (Arezzo, Italy) and specifically developed for performing this study. Exhaustion was defined as the decrease in pedaling cadence below 60 rpm, either with the legs or arms. All testing was completed in a laboratory with a room temperature of 23°C ± 1°C. A cooling fan was used during all the tests, and mechanical, physiological, and perceptual variables were recorded as detailed below.

2.3 Multimodal ergometer

The multimodal ergometer was made up of a grinding ergometer and a cycling ergometer, and their combined use allowed the participants to perform arm+leg cycling (see Figure 1). Both the grinding and cycling ergometers were electromagnetically braked, equipped with professional torque transducers (model RT2A) certified by AEP transducers (Cognento, Modena, Italy), and calibrated according to the manufacturer's instructions. The expanded uncertainty was lower than 0.114% for both torque transducers used. The arm cranks of the grinding ergometer were provided by Harken Italy SPA (Limido Comasco, Como). The chainring of the grinding ergometer was not mechanically connected with that of the cycling ergometer to allow for the separate measurement of the power output provided by the two ergometers. Hence, one of the advantages of this multimodal ergometer is the opportunity to set and register the contribution of arms and legs to the total power output. In all the Arm+leg tests, the relative contribution of the arms was initially set at 20% of the total power output based on the findings reported by Bergh et al. (1976). Thereafter, participants were allowed to request changes in the relative contribution of the arms throughout each test according to preference, and they were familiarised with this procedure on the first visit. The option of individualizing the relative contribution of the arms is supported by previous studies reporting inter-individual variability in preference (Hill et al., 2018) and exercise tolerance (Bergh et al., 1976) for different power output distributions between arms and legs. Participants were free to choose their preferred pedaling cadence in all the tests. In both Arm+leg and Leg modalities, the ergometer settings were set up on the first visit according to participants' anthropometric characteristics and comfort, and were reproduced in the subsequent visits. The Arm+leg tests were performed on the multimodal ergometer, while the Leg tests were performed on the cycling ergometer (the participants were allowed to use the handlebars).

2.4 Step incremental tests

Before the incremental tests, a 5-min warm-up was performed to allow the participants to check the ergometer settings. For both Arm+leg$_{INC}$ and Leg$_{INC}$, the first stage of the incremental test consisted of 3 min at 150 W, and the power output was subsequently increased by 20 W every min. The power output of the first stage was chosen to ensure that at least 20% of the total power output could be sustained by the arms in the Arm+leg modality, and the minimum power output for the arm ergometer was about 25 W. Hence, it was not possible to select a power output lower than 150 W, and this limited the opportunity to rigorously determine the gas exchange threshold (GET) and the respiratory compensation point (RCP). The PPO reached in the incremental test was computed as the work rate of the last completed stage plus the fraction of time spent in the last uncompleted stage multiplied by the work-rate increment (i.e. 20 W). The Borg's 6–20 scale (Borg, 1998) was used to collect ratings of perceived exertion (RPE) data every min. Participants were familiarised with the use of the RPE scale on the first visit and were asked to verbally provide an RPE value, as required by the Arm+leg modality. Breathing artifacts caused by speaking were then removed by data filtering, as described below. Participants did not receive any performance feedback or encouragement during any of the incremental tests performed.

2.5 Time to exhaustion tests

After a 10-min self-paced warm-up, participants performed a TTE test in each of the two exercise modalities (Arm+leg and Leg) and intensities (90% and 75% of PPO$_{Leg}$) in separate visits. Hereinafter, the TTE tests are abbreviated as Arm+leg$_{TTE90}$ (Arm+leg test at 90% of PPO$_{Leg}$), Leg$_{TTE90}$ (Leg test at 90% of PPO$_{Leg}$), Arm+leg$_{TTE75}$ (Arm+leg test at 75% of PPO$_{Leg}$) and Leg$_{TTE75}$ (Leg test at 75% of PPO$_{Leg}$). Perceived exertion data were collected every min, while physiological and mechanical variables were measured continuously. Participants did not receive any performance feedback or encouragement during any of the TTE tests performed in this study.

2.6 Cardiorespiratory measures

f_R, V_T, minute ventilation ($\dot{V}_E$), $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$), end-tidal partial pressure of carbon dioxide (P_{ETCO2}) and HR were measured breath-by-breath during all the tests using a metabolic cart (Quark CPET, Cosmed, Rome, Italy). The metabolic cart was calibrated following the manufacturer's instructions.

2.7 Data analysis

Data were analyzed with MATLAB (R2016a, The Mathworks, Natick, MA, United States). The comparison of the physiological and perceptual responses between arm+leg cycling and leg cycling was performed in all the tests using the "individual isotime" analysis described by Nicolò et al. (2019). This analysis allows for between-condition comparisons while avoiding the data loss that occurs when the variability in TTE is not addressed on an individual basis (Nicolò et al., 2019). Briefly, breath-by-breath data of f_R, V_T, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, P_{ETCO2} and HR were filtered for errant breaths by deleting values greater than 3 standard deviations from the local mean (Lamarra et al., 1987). Subsequently, breath-by-breath data were linearly interpolated and extrapolated every second. Data were then smoothed by a moving average of 60 s. Likewise, RPE data collected every min were linearly interpolated and extrapolated every second. Thereafter, for each individual, the shortest test of each Arm+leg vs. Leg comparison (Arm+leg$_{INC}$ vs. Leg$_{INC}$, Arm+leg$_{TTE90}$ vs. Leg$_{TTE90}$ and Arm+leg$_{TTE75}$ vs. Leg$_{TTE75}$ were compared separately) was segmented into ten timepoints, and the same segmentation was used for the longest test of the same participant. This procedure was performed for all the participants as further detailed by Nicolò et al. (2019).

When reporting the relationship between different variables (i.e. RPE vs. f_R, RPE vs. $\dot{V}_E$, RPE vs. HR, $\dot{V}_E$ vs. V_T, $\dot{V}_E$ vs. $\dot{V}CO_2$, V_T vs. $\dot{V}CO_2$, and f_R vs. $\dot{V}CO_2$), another analysis called "relative isotime" was used as previously suggested (Nicolò et al., 2019). This analysis segments each test into ten timepoints based on the TTE of the test analyzed, and thus results in no data loss for any of the tests.

2.8 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, United States). Data were checked for normality prior to analysis. A paired Student's *t*-test was used to compare the performance values of Arm+leg$_{INC}$ vs. Leg$_{INC}$, Arm+leg$_{TTE90}$ vs. Leg$_{TTE90}$ and Arm+leg$_{TTE75}$ vs. Leg$_{TTE75}$ separately. The Cohen's d effect size for paired *t*-test was then calculated and considered small, moderate or large for values ≥0.2, ≥0.5 and ≥0.8 respectively. A paired Student's *t*-test was also used to compare the end-test values of physiological variables between Arm+leg and Leg tests. A two-way repeated-measures ANOVA (condition × time) was used to compare physiological and perceptual responses (processed with the "individual isotime" method) of Arm+leg vs. Leg for each of the three performance tests separately. When the sphericity assumption was violated, the Greenhouse–Geisser adjustment was performed. Partial eta squared (η_p^2) effect sizes were calculated for the main effect of condition, the main effect of time, and the interaction; η_p^2 values ≥0.01, ≥0.059 and ≥0.138 indicate small, medium and large effects respectively (Cohen, 1988). When a significant interaction was found, pairwise comparisons were performed at each time point

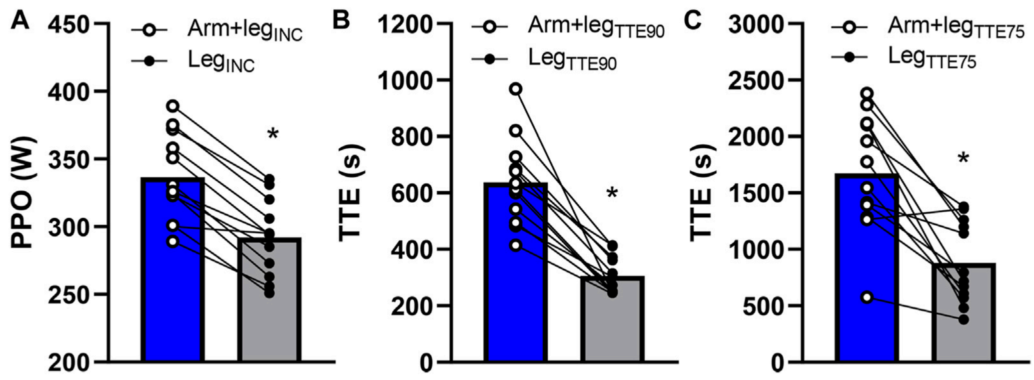

FIGURE 2
Average performance differences between Arm+leg tests (blue bar graphs) and Leg tests (grey bar graphs) for the incremental test **(A)**, the TTE test at 90% of PPO$_{Leg}$ **(B)**, and the TTE test at 75% of PPO$_{Leg}$ **(C)**. The open circles and the filled circles represent individual data during Arm+leg and Leg tests respectively. *p < 0.05 vs. Arm+leg.

using a one-way repeated measures ANOVA to identify differences between Arm+leg and Leg tests. The HR data of the incremental tests and the TTE tests at 75% of PPO$_{Leg}$ were not normally distributed and were analyzed using Friedman's two-way ANOVA. When statistical significance was found, this test was followed up by a Wilcoxon Signed-Rank test to identify where differences between Arm+leg and Leg tests occurred.

After processing data with the "relative isotime" method, the correlations between RPE and f_R, RPE and $\dot{V}_E$, and RPE and HR were analyzed using a previously described method that adjusts for repeated observations within participants (Bland and Altman, 1995). A correlation coefficient (r) and a p-value were obtained by considering all the performance tests together. A p-value < 0.05 was considered statistically significant in all analyses. The results are expressed as mean ± SD in the text and as mean ± SE in the Figures.

3 Results

3.1 Step incremental tests

A significantly greater (p < 0.001; Cohen's d = 2.78) PPO was found in Arm+leg$_{INC}$ (337 ± 32 W) vs. Leg$_{INC}$ (292 ± 28 W) (Figure 2A). The average relative contribution of the arms to the total power output was 22% ± 2% in the Arm+leg$_{INC}$ test. A higher pedaling cadence (p < 0.01) was found in the Leg$_{INC}$ (84 ± 6 rpm) compared to that of the arms (76 ± 6 rpm) and legs (77 ± 7 rpm) of the Arm+leg$_{INC}$ test. The two tests (Arm+leg$_{INC}$ vs. Leg$_{INC}$) showed significant differences (p < 0.043) in the end-test values of $\dot{V}O_2$ (3,913 ± 378 vs. 3,610 ± 310 mL min^{-1}), HR (186 ± 11 vs. 181 ± 10 beats min^{-1}), and V_T (2.92 ± 0.42 vs. 2.84 ± 0.40 L). When comparing the time course of the physiological and perceptual responses between Arm+leg$_{INC}$ and Leg$_{INC}$, a significant (p < 0.001) condition × time interaction was observed for f_R (η_p^2 = 0.75), $\dot{V}_E$ (η_p^2 = 0.74), $\dot{V}CO_2$ (η_p^2 = 0.48), V_T (η_p^2 = 0.22) and P_{ETCO2} (η_p^2 = 0.71). Statistically significant differences (p < 0.001) between Arm+leg$_{INC}$ and Leg$_{INC}$ were also found when evaluating the time course of HR. Figure 3 shows where a simple main effect of condition was found. All the variables reported in Figure 3 showed a main effect of time (p < 0.001; η_p^2 > 0.71). No main effect of condition was found for any of the variables, but some showed p < 0.1, i.e. RPE (p = 0.057), $\dot{V}_E$ (p = 0.055), $\dot{V}O_2$ (p = 0.091), and P_{ETCO2} (p = 0.091). Due to technical problems, HR analysis was performed for 11 participants.

3.2 TTE tests at 90% of PPO$_{Leg}$

A significantly longer (p < 0.001; Cohen's d = 2.27) TTE was found in Arm+leg$_{TTE90}$ (638 ± 154 s) vs. Leg$_{TTE90}$ (307 ± 67 s) (Figure 2B). The average relative contribution of the arms to the total power output was 22% ± 2% in the Arm+leg$_{TTE90}$ test. A higher pedaling cadence (p < 0.033) was found in the Leg$_{INC}$ (83 ± 7 rpm) compared to that of the arms (73 ± 13 rpm) and legs (76 ± 6 rpm) of the Arm+leg$_{INC}$ test. The two tests (Arm+leg$_{TTE90}$ vs. Leg$_{TTE90}$) showed significant differences (p < 0.034) in the end-test values of f_R (59 ± 10 vs. 53 ± 9 breaths min^{-1}), $\dot{V}O_2$ (3,727 ± 361 vs. 3,581 ± 236 mL min^{-1}), $\dot{V}CO_2$ (3,953 ± 319 vs. 4,209 ± 302 mL min^{-1}), HR (183 ± 9 vs. 175 ± 8 beats min^{-1}), V_T (2.68 ± 0.42 vs. 2.91 ± 0.40 L) and P_{ETCO2} (31 ± 3 vs. 33 ± 3 mmHg). When comparing the time course of the physiological and perceptual responses between Arm+leg$_{TTE90}$ and Leg$_{TTE90}$, a significant (p < 0.017) condition × time interaction was observed for RPE (η_p^2 = 0.32), f_R (η_p^2 = 0.52), $\dot{V}_E$ (η_p^2 = 0.67), $\dot{V}CO_2$ (η_p^2 = 0.60), HR (η_p^2 = 0.59) and P_{ETCO2} (η_p^2 = 0.66). $\dot{V}O_2$ showed p = 0.096. Figure 4 shows where a simple main effect of condition was found. All the variables reported in Figure 4 showed a main effect of time (p < 0.001; η_p^2 > 0.76), while a main effect of condition (p < 0.037) was found for RPE (η_p^2 > 0.72), $\dot{V}CO_2$ (η_p^2 > 0.40) and V_T (η_p^2 > 0.34); p = 0.088 was found for HR.

3.3 TTE tests at 75% of PPO$_{Leg}$

A significantly longer (p < 0.001; Cohen's d = 1.53) TTE was found in Arm+leg$_{TTE75}$ (1,675 ± 525 s) vs. Leg$_{TTE75}$ (880 ± 363 s)

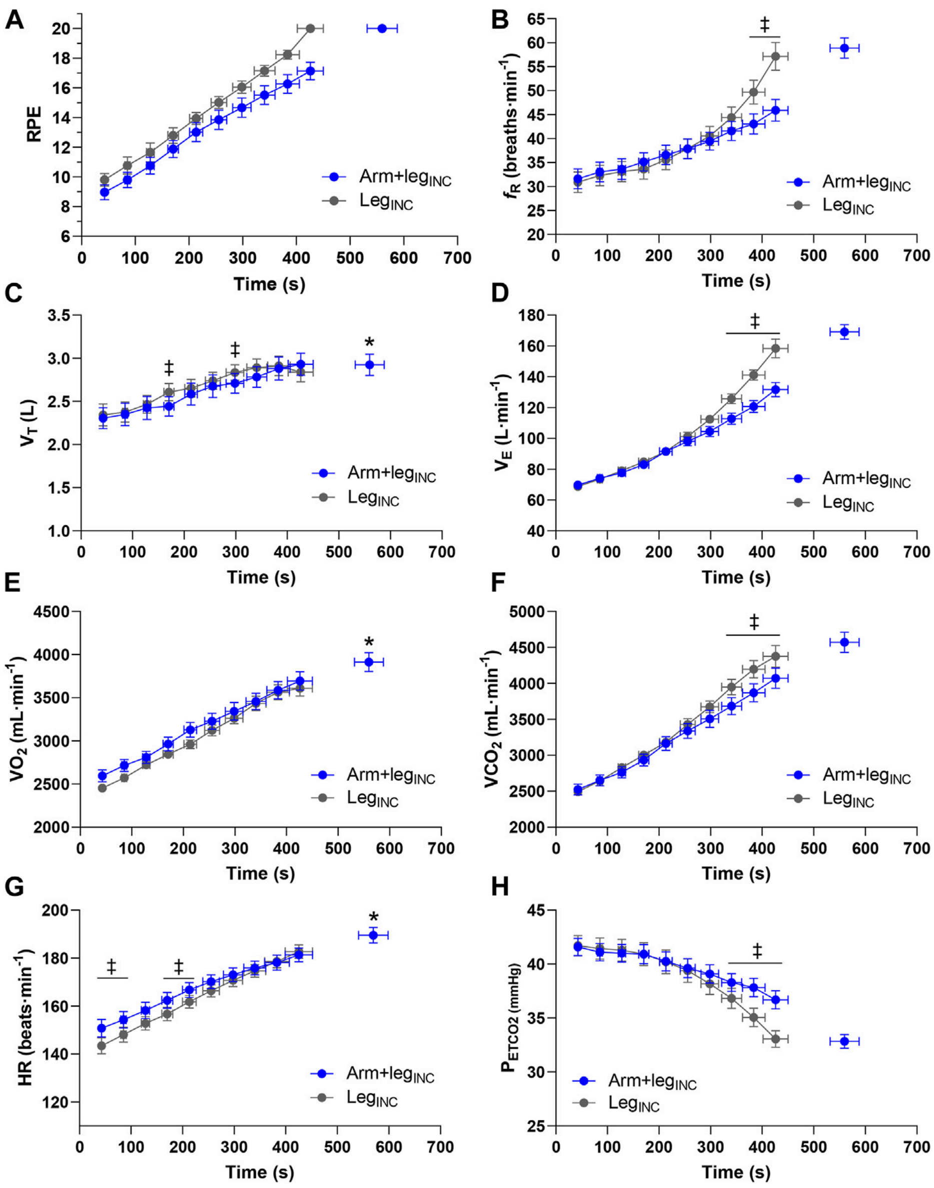

FIGURE 3
Group mean response of ratings of perceived exertion **(A)**, respiratory frequency **(B)**, tidal volume **(C)**, minute ventilation **(D)**, oxygen uptake **(E)**, carbon dioxide output **(F)**, heart rate **(G)** and end-tidal partial pressure of carbon dioxide **(H)** for Arm+leg$_{INC}$ (blue circles) and Leg$_{INC}$ (grey circles). ‡$p <$ 0.05 vs. Arm+leg$_{INC}$, *$p <$ 0.05 vs. Leg$_{INC}$.

(Figure 2C). The average relative contribution of the arms to the total power output was 21% ± 1% in the Arm+leg$_{TTE75}$ test. A higher pedaling cadence ($p < 0.016$) was found in the Leg$_{INC}$ (80 ± 7 rpm) compared to that of the arms (73 ± 8 rpm) and legs (75 ± 6 rpm) of the Arm+leg$_{INC}$ test. The two tests (Arm+leg$_{TTE75}$ vs. Leg$_{TTE75}$) also showed significant differences ($p < 0.042$) in the end-test values of HR (182 ± 10 vs. 175 ± 13 beats min^{-1}) and V_T (2.40 ± 0.30 vs. 2.56 ± 0.40 L), while $p = 0.084$ was found for the end-test values of $\dot{V}_E$ (126 ± 11 vs. 132 ± 14 L min^{-1}) and $\dot{V}CO_2$ (3,321 ± 270 vs. 3,465 ± 384 mL min^{-1}); no significant differences ($p = 0.57$) were found for the end-test values of $\dot{V}O_2$ (3,342 ± 348 and 3,379 ± 294 mL min^{-1}). When comparing the time course of the physiological and perceptual responses between Arm+leg$_{TTE75}$ and Leg$_{TTE75}$, a significant ($p <$ 0.003) condition × time interaction was observed for f_R ($\eta_p^2 = 0.41$), $\dot{V}_E$ ($\eta_p^2 = 0.67$), $\dot{V}O_2$ ($\eta_p^2 = 0.35$), $\dot{V}CO_2$ ($\eta_p^2 = 0.39$), and P_{ETCO2} ($\eta_p^2 = 0.65$). RPE showed $p = 0.068$. Statistically significant differences ($p < 0.001$) between Arm+leg$_{TTE75}$ and Leg$_{TTE75}$ were also found when evaluating the time course of HR. Figure 5 shows where a simple main effect of condition was found. All the variables reported in Figure 5 showed a main effect of time ($p < 0.021$; $\eta_p^2 >$ 0.31), while a main effect of condition ($p < 0.011$) was found for RPE ($\eta_p^2 = 0.77$), $\dot{V}_E$ ($\eta_p^2 = 0.48$), V_T ($\eta_p^2 = 0.53$) and P_{ETCO2} ($\eta_p^2 = 0.47$). Due to technical problems, HR analysis was performed for 11 participants.

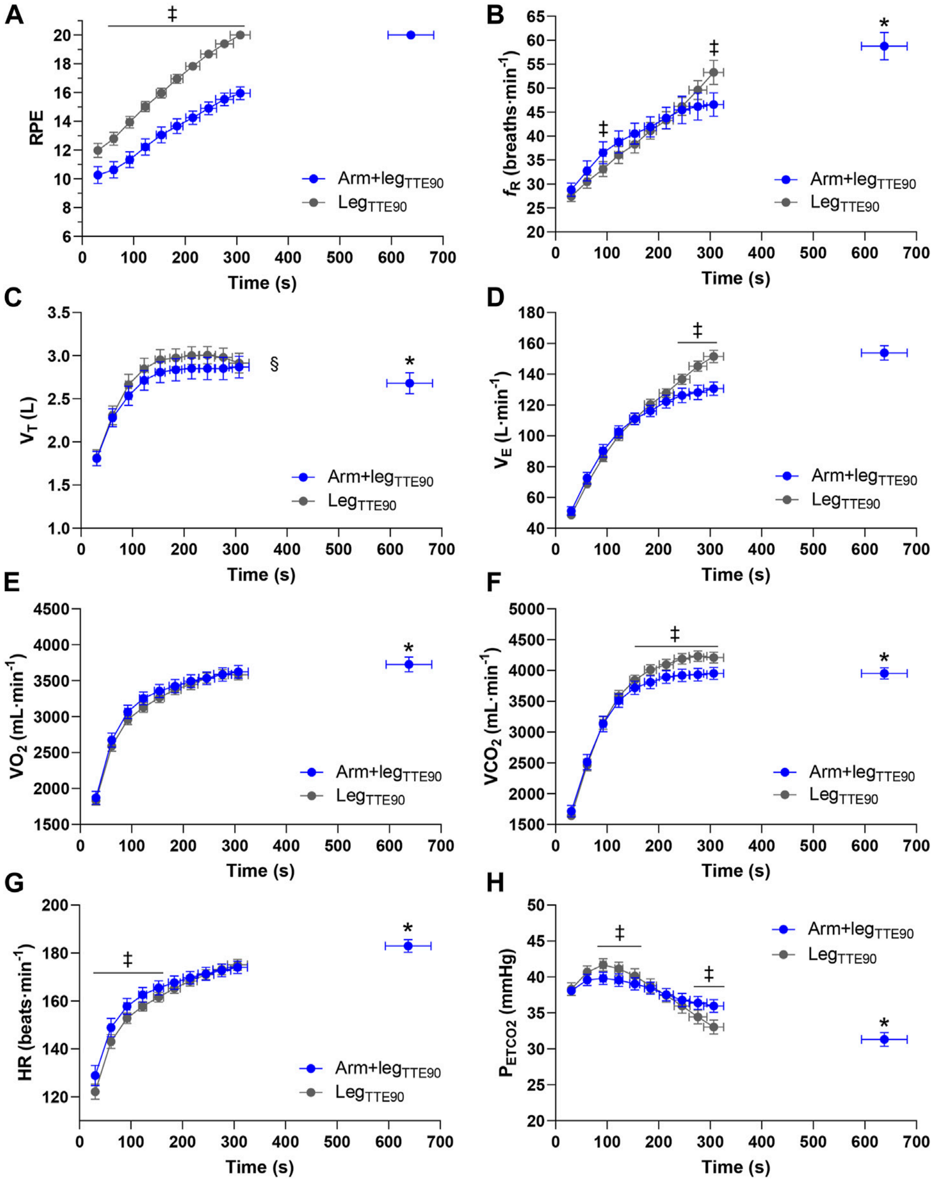

FIGURE 4
Group mean response of ratings of perceived exertion **(A)**, respiratory frequency **(B)**, tidal volume **(C)**, minute ventilation **(D)**, oxygen uptake **(E)**, carbon dioxide output **(F)**, heart rate **(G)** and end-tidal partial pressure of carbon dioxide **(H)** for Arm+leg$_{TTE90}$ (blue circles) and Leg$_{TTE90}$ (grey circles). ‡$p < 0.05$ vs. Arm+leg$_{TTE90}$, *$p < 0.05$ vs. Leg$_{TTE90}$, § main effect of condition.

3.4 The performance tests considered together

When the performance tests were considered together, a significant correlation was found between f_R and RPE ($p < 0.001$; $r = 0.75$), HR and RPE ($p < 0.001$; $r = 0.69$), and $\dot{V}_E$ and RPE ($p < 0.001$; $r = 0.80$). A graphical representation of the correlations between these variables is depicted in Figure 6.

Figure 7 shows the average response of the group when expressing $\dot{V}_E$ as a function of V_T values, and $\dot{V}_E$, V_T and f_R as a function of $\dot{V}CO_2$ values. Note that the inflection point in the $\dot{V}_E$-V_T relationship occurs at different V_T values, especially when comparing Arm+leg$_{TTE75}$ and Leg$_{TTE75}$ with the other four performance tests. A clear dissociation between f_R and $\dot{V}CO_2$ responses is observed for f_R values above 40 breaths min^{-1}.

Figure 8 shows the individual responses of $\dot{V}_E$, V_T and f_R expressed as a function of $\dot{V}CO_2$ values for three participants showing substantially different breathing patterns. The comparison between the responses of the three participants outlines how $\dot{V}_E$ is more closely associated with $\dot{V}CO_2$ than V_T and f_R, and that higher values of f_R for a given $\dot{V}CO_2$ result in higher $\dot{V}_E$ values.

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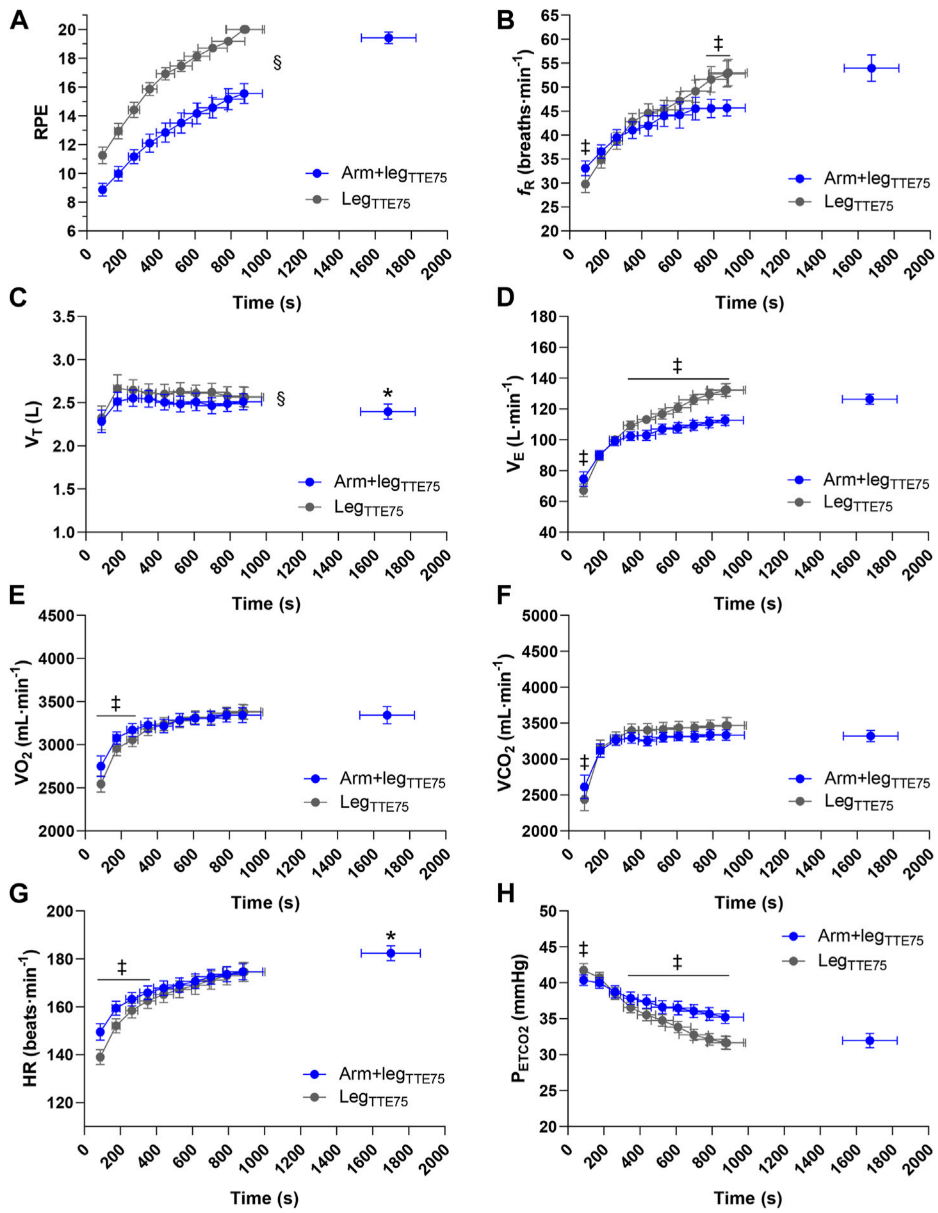

FIGURE 5
Group mean response of ratings of perceived exertion **(A)**, respiratory frequency **(B)**, tidal volume **(C)**, minute ventilation **(D)**, oxygen uptake **(E)**, carbon dioxide output **(F)**, heart rate **(G)** and end-tidal partial pressure of carbon dioxide **(H)** for Arm+leg$_{TTE75}$ (blue circles) and Leg$_{TTE75}$ (grey circles). ‡$p <$ 0.05 vs. Arm+leg$_{TTE75}$, *$p < 0.05$ vs. Leg$_{TTE75}$, § main effect of condition.

4 Discussion

This study aimed to systematically assess whether exercise tolerance improves with arm+leg cycling vs. leg cycling and whether f_R and perceived exertion are sensitive to the expected differences in exercise tolerance. This goal was achieved by comparing the two exercise modalities using three performance tests and two exercise paradigms (i.e. incremental test and TTE test). The main findings of the study are as follows: 1) exercise tolerance was substantially improved in all the Arm+leg tests; 2) perceived exertion, minute ventilation and respiratory frequency were particularly sensitive to the between-modality changes in exercise tolerance observed, unlike $\dot{V}O_2$ and heart rate. These findings support the notion that respiratory frequency is a marker of physical effort during high-intensity exercise and that its time course reflects changes in exercise tolerance. This holds true even during arm+leg cycling, which is an exercise modality where the responses of $\dot{V}O_2$ and heart rate do not reflect the reduction in physical effort and the improvement in exercise tolerance observed when comparing it with leg cycling.

Our findings provide convincing evidence that arm+leg cycling substantially increases exercise tolerance when compared to (conventional) leg cycling, hence expanding on the limited literature dealing with performance differences between the two

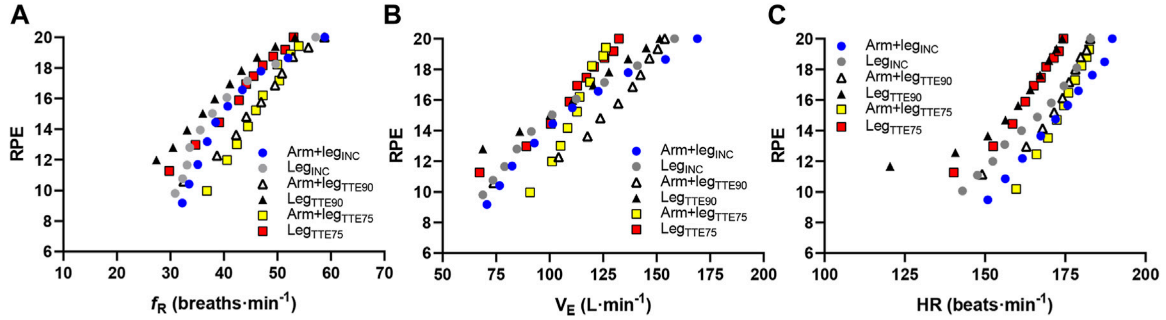

FIGURE 6
Correlations between RPE and respiratory frequency **(A)**, RPE and minute ventilation **(B)**, and RPE and heart rate **(C)** for the Arm+leg$_{INC}$ (blue circles), Leg$_{INC}$ (grey circles), Arm+leg$_{TTE90}$ (open triangles), Leg$_{TTE90}$ (black triangles), Arm+leg$_{TTE75}$ (yellow squares) and Leg$_{TTE75}$ (red squares). Each symbol represents the mean value of all participants at each percentage of the TTE.

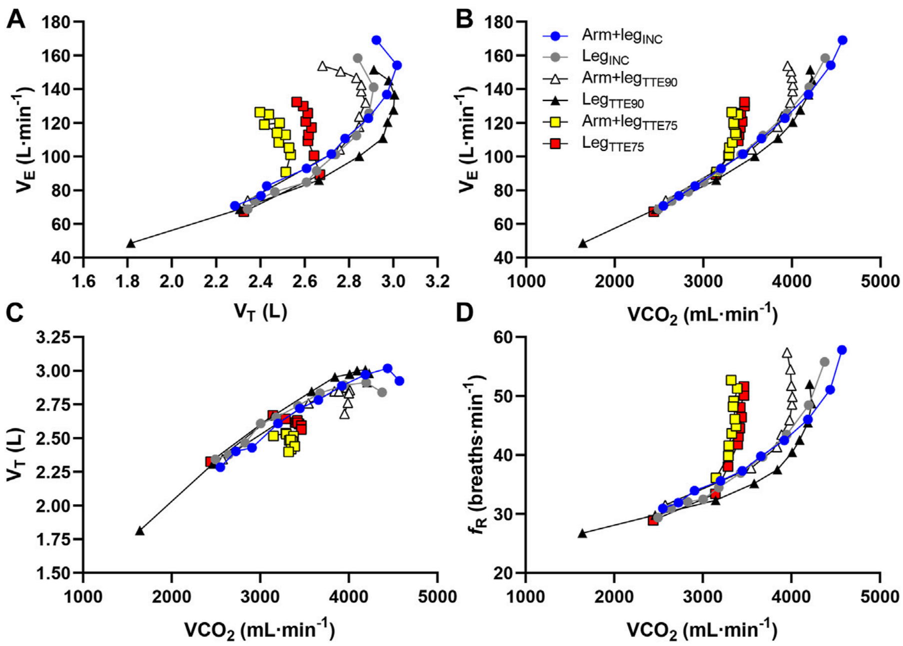

FIGURE 7
Relationship between minute ventilation and tidal volume **(A)**, minute ventilation and carbon dioxide output **(B)**, tidal volume and carbon dioxide output **(C)**, and respiratory frequency and carbon dioxide output **(D)** for the Arm+leg$_{INC}$ (blue circles), Leg$_{INC}$ (grey circles), Arm+leg$_{TTE90}$ (open triangles), Leg$_{TTE90}$ (black triangles), Arm+leg$_{TTE75}$ (yellow squares) and Leg$_{TTE75}$ (red squares). Each symbol represents the mean value of all participants at each percentage of the TTE.

exercise modalities (Secher et al., 1974; Bergh et al., 1976; Nagle et al., 1984). The apparent difference in the percentage improvement in exercise tolerance found between the incremental tests and the TTE tests is, in fact, in line with the different characteristics of the two performance paradigms. Indeed, a 1% improvement in power output in an incremental test, or in a time trial, results in a performance improvement that can exceed 10% in a TTE test (Hopkins et al., 1999). Hence, the average increase in power output of about 15% that we found in the incremental test is compatible with the average increase in TTE found in the TTE tests (i.e. 108% in the Arm+leg$_{TTE90}$ and 90% in the Arm+leg$_{TTE75}$). Such an improvement in exercise tolerance observed in the Arm+leg tests implies that the effort required to sustain a given power output is substantially lower at isotime compared to that of the Leg tests, and this premise is supported by our findings.

The notion that physical effort was lower in the Arm+leg tests than in the Leg tests is substantiated by the lower values of perceived exertion and f_R generally found at isotime. While the decrease in perceived exertion during incremental Arm+leg did not reach statistical significance ($p = 0.057$), it was substantial when

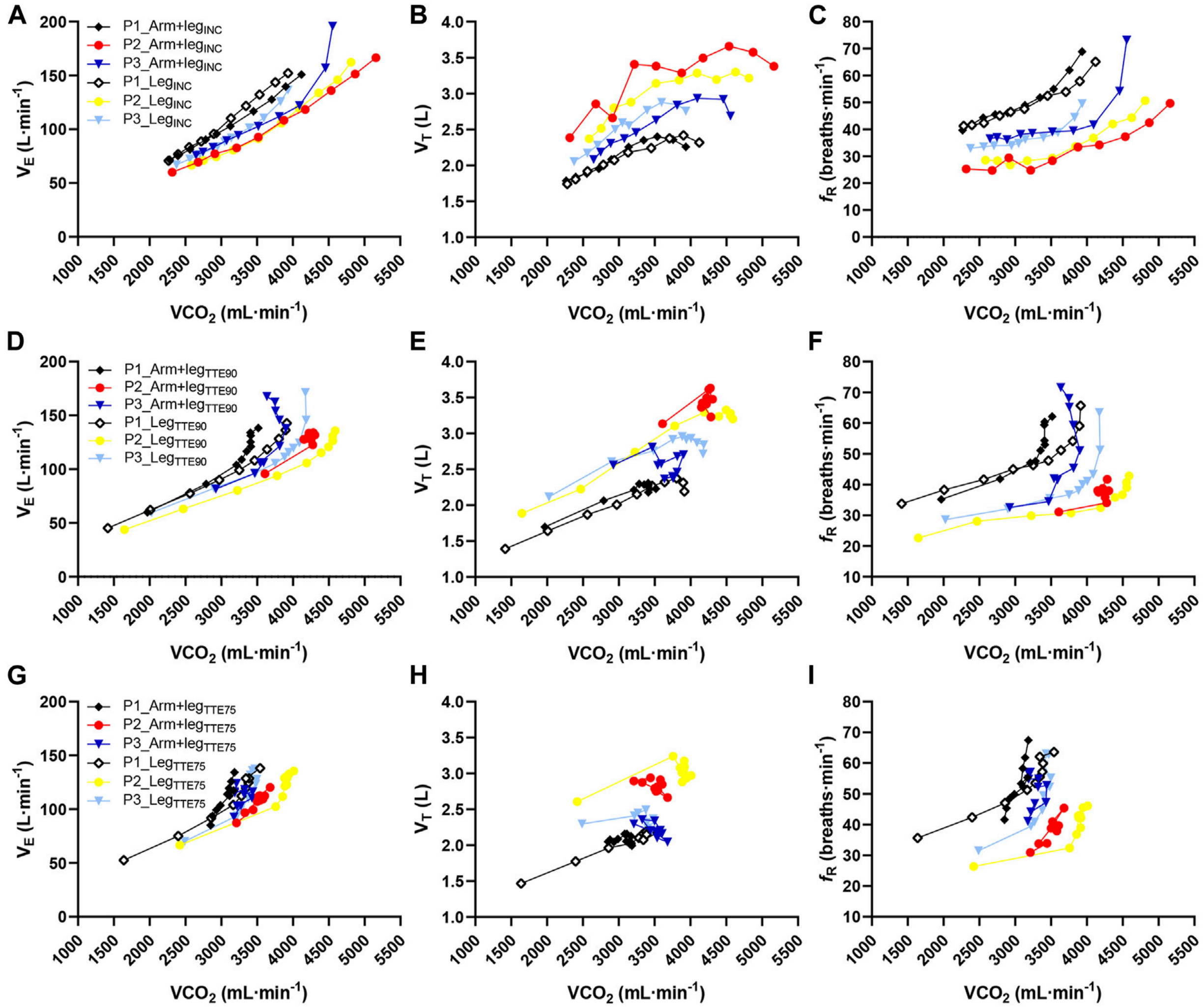

FIGURE 8
Relationship between minute ventilation and carbon dioxide output panels **(A,D,G)**, tidal volume and $\dot{V}CO_2$ **(B,E,H)**, and respiratory frequency and $\dot{V}CO_2$ **(C,F,I)** for three participants (named P1, P2, and P3) showing substantial interindividual differences in the breathing pattern. The Arm+leg tests are represented by black rhombi, red circles and dark blue reversed triangles for P1, P2, and P3 respectively, while the Leg tests are represented by open rhombi, yellow circles and light blue reversed triangles for P1, P2, and P3 respectively. Note that the shape of the relationship between minute ventilation and $\dot{V}CO_2$ shows some similarities with the relationship between f_R and $\dot{V}CO_2$ and that f_R and V_T show opposite responses when making interindividual comparisons.

comparing Arm+leg vs. Leg during the TTE tests. Indeed, perceived exertion was found to be among the most sensitive variables to variations in exercise tolerance during the TTE tests. These findings extend previous findings showing a lower RPE during arm+leg cycling vs. leg cycling at submaximal work rates (Hoffman et al., 1996; Hill et al., 2018). At isotime, f_R was significantly lower ($p < 0.003$; $\eta_P^2 > 0.40$) in the three Arm+leg tests than in the Leg tests, although this difference generally reached statistical significance in the last 20% of the Leg tests. This is an important feature of the f_R response that might have been missed in previous studies that did not compare Arm+leg and Leg until exhaustion (Robertson et al., 1986). Likewise, the reduction in f_R that is observed in the last part of a TTE test when exercise tolerance improves may not appear if the variability in TTE is not addressed on an individual basis when analyzing TTE data (Nicolò et al., 2019). We have overcome this problem by using the previously described "individual isotime" analysis (Nicolò et al., 2019), which reduces extensively the data loss that occurs when using more traditional analyses. Our findings collectively suggest that the time course of f_R reflects changes in exercise tolerance both during incremental exercise and TTE exercise, thus supporting the study hypothesis.

The association found between f_R and RPE and the sensitivity of f_R to changes in exercise tolerance support a substantial modulation of f_R by central command (Nicolò and Sacchetti, 2023). However, a partial dissociation was found between f_R and RPE, which may suggest that also muscle afferent feedback contributed to the f_R response. Although evidence suggests that muscle afferent feedback has a greater relative contribution to f_R during moderate exercise than during high-intensity exercise (Amann et al., 2010; Dempsey et al., 2014; Girardi et al., 2021; Nicolò and Sacchetti, 2023), arm+leg cycling implies the simultaneous use of the muscle mass of both the upper and lower limbs, possibly resulting in a greater magnitude of muscle afferent feedback, and especially of its mechanosensitive component (i.e. mechanoreflex). This may explain the slightly higher f_R shown in the first part of the TTE tests in the Arm+leg vs. the Leg modality. Indeed, it has been suggested that the relative contribution of muscle afferent feedback to ventilation is higher when the muscle mass recruited is larger (Amann et al., 2011; Dempsey et al., 2014; Nicolò and Sacchetti, 2023). Conversely, it is conceivable that the magnitude of the metabosensitive component of muscle afferent feedback (i.e. metaboreflex) was reduced at isotime in the Arm+leg tests because of the lower intramuscular metabolic perturbation. Hence, the metaboreflex cannot be ruled out as an input contributing to the decrease in f_R observed in the Arm+leg tests. Nevertheless, the relative contribution of muscle afferent feedback may reduce over time during a TTE test because the contribution of other inputs increases substantially (e.g. central command) (Dempsey et al., 2014; Nicolò and Sacchetti, 2023). We cannot exclude that afferent feedback from pulmonary mechanoreceptors or alterations in chest wall mechanics might have contributed to the partial dissociation observed between the f_R and RPE responses. Arm movements may increase the contribution to ventilation of afferent feedback from pulmonary mechanoreceptors or alter the mechanics of breathing, although these propositions require further investigation. Furthermore, it cannot be excluded that the lower pedaling cadence observed in the Arm+leg tests might have contributed to the between-modality differences observed in f_R, although variations in pedaling cadence and f_R might not be proportional, especially during high-intensity exercise (Girardi et al., 2021). Conversely, it is less plausible that metabolic acidosis or other metabolic inputs might have provided a substantial direct contribution to the f_R modulation, and the reader is referred to previous studies where evidence supporting this proposition has been reviewed (Nicolò et al., 2020a; Nicolò and Sacchetti, 2023).

The ventilatory responses observed in this study can be interpreted in the light of a recently proposed model of ventilatory control during exercise, which suggests that f_R and V_T are modulated to a large extent by behavioral and metabolic inputs respectively (Nicolò and Sacchetti, 2023). While it was proposed that ventilation is differentially regulated during incremental exercise and TTE exercise (Syabbalo et al., 1994), our findings suggest that the breathing pattern is affected by the magnitude of the inputs modulating ventilation rather than by the type of exercise paradigm. Syabbalo et al. (1994) observed a more rapid and shallow breathing pattern during a TTE test at about 76% of the PPO reached in an incremental exercise than during this latter test. The lower V_T reported by Syabbalo et al. (1994) during the TTE test is in line with the V_T response observed during the TTE tests at 75% of the PPO_{Leg} in this study. However, the breathing pattern we observed in the TTE tests at 90% of the PPO_{Leg} was, conversely, more similar to that found during the incremental exercise than during the TTE tests at 75% of the PPO_{Leg} (see Figure 7). Hence, the rapid and shallow breathing pattern is not a feature of TTE exercise, which is in contrast with what Syabbalo et al. (1994) had suggested. Conversely, the observed findings can be explained by the differential control of f_R and V_T (Nicolò et al., 2017a; Nicolò et al., 2018; Nicolò and Sacchetti, 2019; Nicolò and Sacchetti, 2023). While f_R generally shows similar peak values during incremental exercise and constant work rate exercise (Syabbalo et al., 1994; Nicolò and Sacchetti, 2023), the V_T peak reached during exercise is largely influenced by the magnitude of metabolic inputs and is generally associated with the $\dot{V}CO_2$ peak (Nicolò and Sacchetti, 2023). As such, we found considerably lower V_T and $\dot{V}CO_2$ peak values in the Arm+leg$_{TTE75}$ and Leg$_{TTE75}$ tests than in the incremental tests. Conversely, when the difference in $\dot{V}CO_2$ peak between the incremental tests and the TTE tests was greatly reduced (i.e. when comparing the incremental tests with the Arm+leg$_{TTE90}$ and Leg$_{TTE90}$), the difference in V_T peak values decreased accordingly. Although an association between V_T peak and $\dot{V}CO_2$ peak is commonly found when considering different exercise conditions, populations and levels of exercise capacity (Nicolò and Sacchetti, 2023), the relationship between V_T and $\dot{V}CO_2$ is not always proportional because the V_T response is to some extent influenced by the f_R response (see discussion below). Furthermore, we acknowledge that V_T is influenced by various metabolic inputs that have not been measured in this study, including metabolic acidosis (Nicolò and Sacchetti, 2023).

The association between $\dot{V}CO_2$ and V_T helps explain why $\dot{V}_E$ resulted to be more sensitive than f_R to changes in exercise tolerance in this study. Indeed, both the magnitude of central command and that of metabolic inputs were probably higher in the Leg tests at isotime, thus increasing f_R and V_T respectively. However, the interpretation of the f_R and V_T responses observed requires careful consideration of the interdependence between the two components of $\dot{V}_E$, which has been advocated to explain the close match between alveolar ventilation and metabolic requirements (Haouzi, 2014). Substantial evidence suggests that V_T is fine-tuned based on f_R levels and the magnitude of metabolic inputs (Nicolò and Sacchetti, 2023), and this notion is reinforced by the present findings. While Figure 7 generally shows a consistent increase in V_T with increases in $\dot{V}CO_2$, the responses of the two variables diverge (i.e. V_T stabilizes or even decreases) when f_R starts to increase at a much steeper rate compared to $\dot{V}CO_2$. Notably, the V_T plateau did not occur at specific values of V_T or $\dot{V}CO_2$, and this is especially evident when considering the TTE tests at 75% of PPO_{Leg}, where the steeper increase in f_R occurred at relatively low $\dot{V}CO_2$ levels. Although it has been proposed that the V_T plateau that occurs during high-intensity exercise depends on mechanical constraints (Jensen et al., 1980), evidence suggesting this proposition is scarce (Nicolò et al., 2020a; Nicolò and Sacchetti, 2023). Conversely, evidence suggesting that the stabilization of V_T depends to a large extent on the increase in f_R is substantial (Nicolò et al., 2020a; Nicolò and Sacchetti, 2023), and it is even more convincing during TTE tests performed at relatively low intensities, where pulmonary mechanical limitations in healthy individuals may not occur (Nicolò et al., 2020a; Nicolò and Sacchetti, 2023).

Individual responses further suggest that V_T may not change proportionally to $\dot{V}CO_2$ values because it is affected by f_R values. At given $\dot{V}CO_2$ levels, individuals with lower f_R values show higher V_T values and *vice versa* (see Figure 8). Different combinations of f_R and V_T may guarantee the match between alveolar ventilation and metabolic requirements, and the $\dot{V}_T$ fine-tuning feature is supposed to facilitate such a link (Nicolò and Sacchetti, 2023). Hence, individual responses reveal that $\dot{V}_E$ is more closely associated with $\dot{V}CO_2$ than V_T, as also found in other exercise protocols (Nicolò et al., 2018; Girardi et al., 2021). The ability of the ventilatory control system to adjust V_T according to changes in f_R has nicely been shown both at rest and during exercise in studies replacing spontaneous breathing with different levels of voluntarily imposed f_R (Lamb et al., 1965; Kennard and Martin, 1984; Haouzi and Bell, 2009; Ohashi et al., 2013; Nicolò and Sacchetti, 2023). Conversely, the ventilatory control system appears not to match metabolic requirements effectively when V_T is imposed and f_R is free to vary (Ohashi et al., 2013). In this perspective, V_T may counteract interindividual differences in f_R and guarantee an appropriate match between alveolar ventilation and $\dot{V}CO_2$ for any values of f_R (Nicolò and Sacchetti, 2023). Our findings reveal the potential of comparing different exercise modalities and paradigms to shed light on the f_R and V_T modulation during high-intensity exercise.

The cardiocirculatory adjustments that occur when exercising simultaneously with the upper and lower limbs may provide further mechanistic support to the improvement in exercise tolerance observed in the Arm+leg modality. Arm+leg cycling may result in a greater peak cardiac output than Leg cycling (Secher et al., 1974; Reybrouck et al., 1975), and the higher $\dot{V}O_2$peak found in the Arm+leg$_{INC}$ vs. Leg$_{INC}$ is in line with this notion. Hence, it is conceivable that during Arm+leg$_{TTE90}$ and Arm+leg$_{TTE75}$ participants were exercising at a lower fraction of peak cardiac output compared to Leg$_{TTE90}$ and Leg$_{TTE75}$ tests respectively, especially when similar $\dot{V}O_2$ values were found across conditions at isotime. This may have contributed to accommodating the blood flow requests of both arm and leg muscles, thus improving muscle perfusion. Indeed, the reduction in the leg power output observed in the Arm+leg TTE tests vs. the Leg TTE tests of about 20% may have reduced the leg blood flow demand in the Arm+leg modality. In turn, the lower demand of the legs may have delayed the development of leg muscle fatigue and the increase in the magnitude of central command, thus contributing to the improvement in exercise tolerance observed in the Arm+leg modality. While it has been shown that the addition of (intense) arm work to leg work reduces the leg blood flow observed at a given leg power output (Secher et al., 1977; Secher and Volianitis, 2006), the relatively low intensity sustained by the arms in our study may have not impaired leg blood flow substantially.

The fact that f_R and $\dot{V}_E$ are considerably more sensitive to changes in exercise tolerance than $\dot{V}O_2$ and HR is particularly evident from the present study. Neither $\dot{V}O_2$ nor HR showed lower values in the Arm+leg tests than in the Leg tests at isotime, despite the lower physical effort and the improved exercise tolerance found in the Arm+leg modality. This is not surprising considering that $\dot{V}O_2$ is to a large extent associated with absolute power output during endurance cycling, although it also depends on other factors, including metabolic efficiency, which is lower for arm cycling compared to leg cycling (Cotes et al., 1969; Vokac et al., 1975; Louhevaara et al., 1990; Itoh et al., 2002). However, only a relatively small portion of the total power output is sustained by the arms during arm+leg cycling, and the oxygen uptake of arm+leg cycling has been reported to be minimally higher than that of leg cycling for a given power output (Hoffman et al., 1996). In our study, the addition of arm work to leg work resulted in a slightly higher or similar $\dot{V}O_2$ in the Arm+leg tests compared to the Leg tests. Likewise, similar or slightly higher values were observed for HR in the Arm+leg modality at isotime, and a higher maximal HR was observed in all the Arm+leg tests. In line with our findings, previous studies had raised concerns about HR monitoring during arm+leg cycling because of the different values of maximal HR and HR relative to RPE/%$\dot{V}O_2$peak that are observed when this modality is compared to leg cycling (Kitamura et al., 1981; Hoffman et al., 1996). Hence, the prescription and monitoring of arm+leg cycling should take this HR response into consideration and may benefit from the concomitant measurement of breathing variables (especially f_R), which is technically feasible even in applied settings (Massaroni et al., 2019; Nicolò et al., 2020b).

The between-modality comparison of exercise tolerance, perceived exertion and $\dot{V}O_2$ shows important practical implications of exercising in the Arm+leg modality. Our findings suggest that this exercise modality allows individuals to nearly double the amount of time spent at a given $\dot{V}O_2$ during constant work rate exercise, or, by extension, to exercise at a higher $\dot{V}O_2$ for the same exercise duration and perceived exertion. This consideration is particularly relevant for exercising individuals interested in maximizing energy expenditure, for those willing to maximize the cardiometabolic stimulus of exercise, and for those interested in lowering effort for a given absolute cardiometabolic stimulus (Hill et al., 2018). Indeed, arm+leg cycling has the potential to increase exercise adherence because a high perceived exertion is commonly viewed as one of the main barriers to exercise participation (Cheval and Boisgontier, 2021), and a relatively low perceived exertion may be associated with a sufficient cardiometabolic stimulus in this exercise modality. Furthermore, considering that exercise tolerance is closely associated with morbidity and mortality (Kokkinos et al., 2010; Nesti et al., 2020), arm+leg cycling may have clinical implications. Arm+leg cycling involves the simultaneous use of arm and leg muscles and may result in a time-efficient training strategy for enhancing both health and performance (Zinner et al., 2017). However, further research is needed to test these propositions.

It is worth mentioning that the Arm+leg modality poses some measurement challenges that we had to face in this study. First, we did not attempt to compute the GET and the RCP because of the relatively high initial stage of the incremental tests imposed by the ergometer (i.e. 150 W), which has limited their detection. As such, the TTE tests were not prescribed based on exercise intensity domains. Hence, especially in the Arm+leg$_{TTE75}$ test, it is possible that some participants were exercising in the severe intensity domain while others were in the heavy domain. Second, the measurement of blood lactate is very challenging during the Arm+leg modality, and we decided not to collect it to avoid interfering with the performance tests. Third, perceived exertion can only be rated verbally during the Arm+leg tests, thus generating breathing artifacts and affecting gas exchange measures. However, we used a filtering technique (Lamarra et al., 1987) that addressed this limitation and helped preserve the integrity of the physiological responses. Even the other limitations outlined were partially counteracted by the experimental design and the method of analysis used. Indeed, we performed a detailed between-modality comparison of the responses of some of the main physiological variables commonly used to compute the GET and the RCP. This comparison reveals a greater metabolic perturbation at isotime in the Leg tests, especially when considering the time courses of $\dot{V}_E$, $\dot{V}CO_2$ and P_{ETCO2}. Hence, it is conceivable that participants were exercising at a lower relative exercise intensity during the Arm+leg tests, not only from an effort perspective but also from a metabolic perspective.

5 Conclusion

This study shows that exercise tolerance is substantially higher in the Arm+leg modality than in the leg cycling modality. The average improvement in exercise tolerance was 15% in the incremental test and 108% and 90% in the TTE tests at 90% and 75% of PPO_{Leg} respectively. Perceived exertion, minute ventilation and respiratory frequency were among the most sensitive variables to the improvement in exercise tolerance provided by the Arm+leg modality, hence suggesting that the common mechanism modulating these variables (i.e. central command) plays an important role in endurance performance. These findings reinforce the notion that respiratory frequency is a better marker of physical effort than oxygen uptake and heart rate. Our results have implications for devising exercise strategies to reduce perceived exertion while maintaining a high cardiometabolic demand, and for maximizing energy expenditure for the same level of effort exerted.

Data availability statement

The raw data supporting the conclusion 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 Institutional Review Board of the University of Rome "Foro Italico." The patients/participants provided their written informed consent to participate in this study.

Author contributions

AN, MS, and FF contributed to the conception and design of the study. MG performed data collection. MG and AN analyzed data. AN wrote the first draft of the manuscript. AN, MG, IB, MS, and FF contributed to data interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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