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THE EFFECT OF PHONATED BREATHING ON OXYGEN UPTAKE DURING AND AFTER SUBMAXIMAL CYCLING

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ABSTRACT

Purpose: *Positive expiratory pressure (PEP) exhalation during exercise is reported to improve body adaptation to exercise and enhance the exercise tolerance in patients with chronic obstructive pulmonary disease. Wearing mouthguards results in lower oxygen consumption and increased performance by increasing PEP in athletes. Airway resistance during expiration can be manipulated by phonation. Thus, the aim of our study was to examine the effects of phonated breathing on cardiopulmonary adaptation to moderate exercise and subsequent recovery.*

Methods: *26 young healthy participants conducted the same moderate steady cycling protocol using three different breathing patterns: spontaneous breathing (BrP1), phonated breathing pronouncing the sound "h" (BrP2) and phonated breathing pronouncing the sound "sh" (BrP3). Heart rate, oxygen consumption, CO_2 production, respiratory rate, tidal volume, respiratory exchange ratio and ventilatory equivalents were measured (Cosmed, Italy) before, during and 20 minutes after cycling. Data were analyzed using SPSS, with significance level $p<0.05$.*

Results: *The analysis revealed no significant differences related to the breathing economy; respiratory rate was increased, and tidal volume decreased with spontaneous breathing compared to both phonated breathing patterns during exercise; no effect of BrPs on cardiopulmonary parameters was found in recovery.*

Conclusion: *Our results do not confirm the assumption that PEP breathing improves exercise economy probably because of the low exercise intensity applied. Further studies should be conducted at higher exercise loads or in patients with pulmonary dysfunction.*

Keywords: *phonated exhalation, breathing pattern, positive expiratory pressure, metabolic efficiency, moderate exercise*

VPLIV DIHALNEGA VZORCA NA VENTILACIJO IN PRIVZEM KISIKA MED IN PO SUBMAKSIMALNI VADBI NA SOBNEM KOLESU

IZVLEČEK

Cilj: *Izdih s pozitivnim tlakom (PEP) izboljša zmožnost fiziološke prilagoditve na napor ter vadbeno toleranco pri bolnikih s kronično obstruktivno pljučno boleznijo. Nošenje ustnih varoval, ki prav tako povečale upor v dihalnih poteh, zmanjša porabo kisika in poveča zmogljivost športnikov. Upor v dihalnih poteh lahko spreminjamo s fonacijo. Tako je namen naše raziskave ugotoviti, ali fonacija med zmerno telesno vadbo vpliva na spremembe srčnih in dihalnih parametrov med in po taki vadbi*

Metode: *26 mladih prostovoljcev je trikrat izvedlo enako zmerno kolesarjenje, vsakič ob uporabili drugačnega dihalnega vzorca (DV): spontano dihanje (DV1) in izdih ob izgovarjanju glasu H (DV2) oziroma Š (DV3). Pred, med in 20 minut po vadbi smo merili srčno frekvenco, porabo kisika, izločanje ogljikovega dioksida, frekvenco in volumen dihanja, pljučno ventilacijo, respiratorni količnik in ventilacijske ekvivalente z metabometrom Cosmed. Podatke smo obdelali s programom SPSS in postavili mejo signifikantnosti pri $p<0{,}05$.*

Rezultati: *Analiza rezultatov ni pokazala nobenih statistično značilnih sprememb metabolnih parametrov, kakor tudi ne srčne frekvence z ozirom na uporabljen DV. Značilno povečana je bila le frekvenca dihanja in zmanjšan dihalni volumen pri spontanem dihanju glede na oba s fonacijo povezana DV med naporom. V okrevanju po naporu ni bilo nobenih razlik v merjenih parametrih.*

Zaključek: *Rezultati naše študije niso potrdili domneve, da dihanje s PEP, ki ga povzročimo s fonacijo, poveča učinkovitost telesne vadbe, zmanjša porabo kisika in izboljša telesno zmogljivost vendar ocenjujemo, da je bila intenziteta vadbe v naši študiji premajhna, da bi se tovrstne razlike izkazale. Zato bi bilo treba opraviti še nadaljnje raziskave in sicer z večjo obremenitvijo pri zdravih preiskovancih ali pa na bolnikih z respiratorno motnjo.*

Ključne besede: *fonacija, dihalni vzorec, pozitivni tlak v dihalnih poteh med izdihom, metabolna učinkovitost, zmerna vadba*

INTRODUCTION

Breathing patterns (BrP) differ according to breathing frequency, breathing depth, inhalation/exhalation time relationship, and maneuvers applied during expiration (Dallam & Kies, 2020). Expiratory maneuvers are particularly important since changing the resistance of the airways upon expiration can help maintain a positive pressure until the end of exhalation, thus keeping the alveoli and airways open (Francis & Brasher, 1991) longer during the exhalation period. Phonation requires sustained/controlled exhalation. During speech and singing, breath duration and flow rates are controlled to support sound generation by the larynx (Lewis et al., 2021). Regulation of the glottic aperture by laryngeal muscle activity also helps to control ventilation (Lewis et al., 2021). Therefore, the larynx could be considered a key modulator of expiratory flow upon phonation. Pronunciation of different voices can be used to change the resistance in the airways during exhalation (Hoffmann, Torregrosa, & Bardy, 2012). Grunting has been shown to improve force production during exercise (O'Connell et al., 2016).

In elite sports, there is a continuous search for strategies to improve performance (Harbour, Stöggl, Schwameder, & Finkenzeller, 2022). On the other hand, in patients with compromised breathing, strategies to minimize breathing effort during exercise would be appreciated to allow these patients to minimize the exercise associated discomfort (Fagevik Olsén, Lannefors, & Westerdahl, 2015). The way we breathe strongly affects not only the respiratory system itself but also other systems in our body: cardiovascular, nervous, endocrine, lymphatic, immune, digestive (Saoji, Raghavendra, Madle, & Manjunath, 2018).

To manipulate exhalation, pursed lip breathing (Tiep, Burns, Kao, Madison, & Herrera, 1986), specially designed mouthguards (Lässing et al., 2021) or simply phonation of some particular voices could be applied during exercise to increase the airways resistance during exhalation. By breathing out against increased resistance, positive expiratory pressure (PEP) is achieved and it is commonly believed that PEP improves ventilation, at least in patients with pulmonary diseases (Fagevik Olsén & Westerdahl, 2009; Fagevik Olsén , Lannefors, & Westerdahl, 2015), and potentially during exercise (Phimphasak, Ubolsakka-Jones, & Jones, 2018).

There are only a few studies about the effects of BrP on the physiological response to exercise (Green, Benson, & Martin, 2018; Lässing et al., 2021). Our recent study (Klanjšček, 2018) found that BrPs with increased expiratory resistance had a significant favorable effect on the economy of short-lasting trunk stabilization exercise. Expiratory resistance was manipulated by pronouncing different sounds during exercise (Klanjšček, 2018) – sound "sh" (as in push to increase resistance, and "h" (as in host) to decrease it – and compared to spontaneous breathing. Oxygen consumption and carbon dioxide production decreased during short-lasting trunk stabilization exercise in the "sh" breathing pattern compared to the "h" breathing pattern and spontaneous breathing. Additionally, the respiratory quotient, ventilation, and heart rate during exercise decreased while ventilatory equivalents increased in the "sh" pattern compared to the "h" pattern and spontaneous breathing. The participants perceived the “sh” pattern to be significantly easier compared to the other two BrPs (Klanjšček, 2018).

Thus, we aimed to test whether the BrP where exhalation is manipulated by pronouncing different sounds during exercise affects the physiological response to aerobic exercise. To this purpose, the heart rate, oxygen consumption, CO2 production, and ventilation were measured before, during and after moderate cycling at constant load using the three different BrPs: the pronunciation of “sh” or “h” during exhalation, and spontaneous breathing. Crossover design was used to avoid differences between participants.

METHODS

The study was performed in the Exercise Physiology Laboratory of the Institute of Physiology, Medical Faculty, University of Ljubljana. Ethical approval of the study was obtained from the National Ethics Committee (No. 0102-326 / 2018/5).

Subjects

26 healthy participants with comparable levels of physical activity were recruited by public invitation to participate voluntarily in this crossover study. Their physical examination and histories revealed no autonomic dysfunction, chronic diseases, medication usage or smoking. Their ECG and arterial blood pressure values were normal. Written informed consent was obtained before participation. The trial included 18 women and 8 men, 20.85 ± 0.2 years old, with body mass index (BMI) 22.97 ± 0.59 kg/m^2.

Experimental procedure

The study was carried out in a climate controlled laboratory room between 9 and 12 am. The participants refrained from physical exertion for at least 1 day before the first exercise test and were asked not to perform additional physical activities during the experiment period. They were not allowed to consume any alcohol, caffeine or tobacco for at least 2 hours before the beginning of each exercise test and were asked to eat a light meal 1 hour before coming to the laboratory. Each participant visited the laboratory 3 times in February and March with at least one relaxing day between the two consecutive visits. During the three visits, participants performed the same submaximal aerobic cycling with different BrPs during exercise. The BrP applied during a particular visit was chosen randomly and marked BrP1 for spontaneous breathing and BrP2/BrP3 for exhaling upon pronouncing “h”/“sh”, respectively.

Each session started with blood pressure measurement at sitting rest and an explanation of the breathing technique for the selected BrP. A silicone breathing mask was placed upon the mouth and nose (Quark, Cosmed, Italy) to measure oxygen consumption, CO_2 production, and ventilation; ECG electrodes and a finger cuff for continuous blood pressure tracing were attached (Finapres 2300, Ohmeda, USA). The measurement consisted of 5 minutes sitting at rest on a cycloergometer Ergoselect 100 (Ergoline, Germany) (baseline), 5 minutes of cycling at 100 W (women) and 140 W (men), respectively, at a cadence of 60 rpm and followed by 10 minutes of passive recovery. During cycling, a randomly selected BrP was applied.

Data acquisition and statistical analysis

Signals were captured simultaneously breath by breath using Quark CPET hardware and software (Cosmed, Italy); arterial blood pressure and ECG were recorded by DATAQ system (DATAQ instruments Inc., DI-720 series, Ohio, USA). For analysis, three separate intervals were determined: the last three minutes of sitting rest (baseline), the last three minutes of cycling (exercise), and the last three minutes of recovery (recovery). Oxygen consumption per body mass (VO_2/kg), CO_2 production per kg (VCO_2/kg), respiratory exchange ratio (RER), ventilator equivalents (Veq for O_2 and CO_2), respiratory rate (RR), tidal volume (VT), and heart rate (HR) were determined. The data are presented on graphs as mean values ± standard deviation (SD). Additionally, enhanced post-exercise oxygen consumption (O_2 debt) and oxygen deficit (O_2 deficit) at the onset of exercise were determined using Quark CPET analyzing software (Cosmed, Italy) based on the work of Hughson and Morrissey (Hughson and Morrissey 1983).

Statistical analysis was completed using IBM SPSS Statistics, version 27 (IBM, New York, USA). Data were tested for normality and a $p<0.05$ level of confidence was selected. We compared mean differences in measured parameters over time (before and during exercise + recovery) for all three BrPs with a one-way repeated measures ANOVA (rANOVA). The assumption of sphericity was checked using Mauchly's test; Greenhouse-Geisser or Huynh-Feldt corrections were applied when sphericity assumption was violated as published elsewhere (Hopkins, Marshall, Batterham, & Hanin, 2009). When detecting a significant time effect, corresponding contrast tests were used to identify differences between means according to BrP and time interval. For post hoc comparisons a least significant difference test was applied and the Bonferroni correction was used to eliminate type I error in multiple comparisons (Hopkins et al. 2009). In case of significant differences, Cohen’s d was determined to represent the effect size (ES) (Hopkins et al., 2009).

RESULTS

RR increased during exercise in all three BrPs (Figure 1, Table 1), most in BrP1, where the mean value was 22.56 ± 1.1 min^{-1}. RR in BrP1 was significantly higher than in BrP2 ($p < 0.001$) and BrP3 ($p = 0.007$). There were no statistical differences between BrP2 and BrP3 ($p = 0.103$). After exercise, RR dropped as expected, but in the last 3 minutes of recovery it was still higher than the baseline values in all BrPs, but significantly higher only in BrP1 ($p = 0.001$) (Table 1).

Table 1: Effect sizes presented as Cohen's d values for all significant differences.

	RR	VT	VE	RER	VO_2/kg	VCO_2/kg	Veq for O_2	Veq for CO_2
BrP1base/ex	1.59	3.53	6.46	1.34	6.94	6.48	1.50	4.03
BrP1base/rec	0.67	-	1.26	0.80	-	-	0.99	0.60
BrP1ex/rec	1.227	3.42	6.27	0.81	6.71	6.58	2,19	3.23
BrP2base/ex	0.41	3.46	4.00	1.18	6.54	6.59	1.81	2.38
BrP2base/rec	-	-	0.68	-	-	-	0.82	6.82
BrP2ex/rec	0.22	3.54	5.01	1.43	6.69	6.89	2.44	6.89
BrP3base/ex	0.41	3.24	4.42	1.50	7.49	6.76	1.41	2.40
BrP3base/rec	-	0.46	1.10	0.76	-	0.63	1.14	0.63
BrP3ex/rec	0.47	3.23	4.36	0.88	6.55	6.07	1.96	6.37
BrP1ex/BrP2ex	0.72	1.05	-	-	-	-	-	-
BrP1ex/BrP3ex	0.14	0.35	-	-	-	-	-	-
BrP2ex/BrP3ex	-	0.72	-	-	-	-	-	-

BrP1 – breathing pattern 1, BrP2 - breathing pattern 2, BrP3 – breathing pattern 3, base – baseline, ex – exercise, rec – recovery, RR – respiratory rate, VT – tidal volume, VE – ventilation, RER – respiratory exchange ratio, VO_2/kg – oxygen consumption per body mass, VCO_2/kg – carbon dioxide production per body mass, Veq for O_2 – ventilatory equivalent for oxygen, Veq for CO_2 - ventilatory equivalent for carbon dioxide.

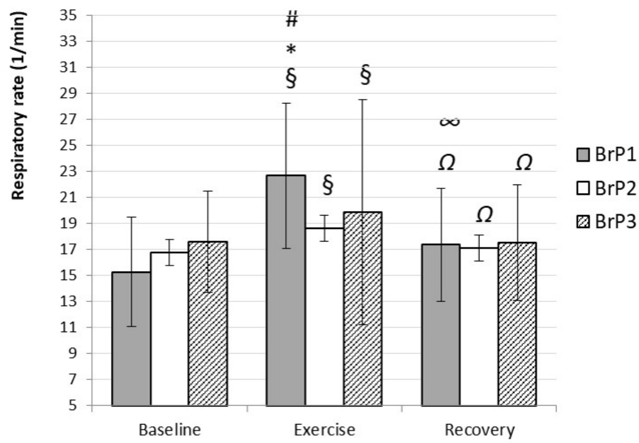

*Figure 1: Changes in RR during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. * - significant difference between BrP1 and BrP2, # - significant difference between BrP1 and BrP3, § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.*

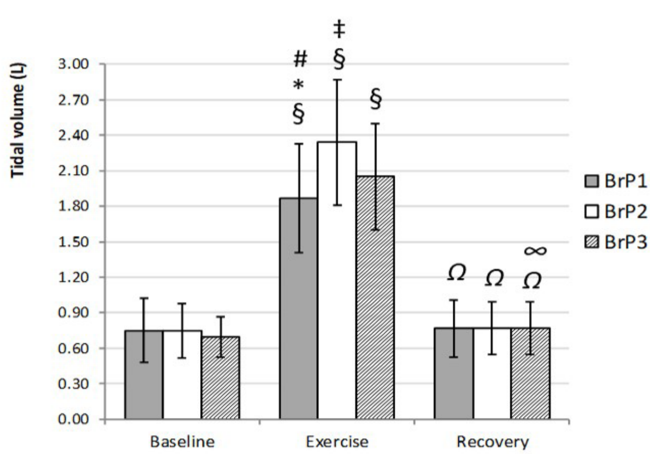

*Figure 2: Changes in tidal volume during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. * - significant difference between BrP1 and BrP2, # - significant difference between BrP1 and BrP3, ‡ significant difference between BrP2 and BrP3, § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.*

Figure 2 shows the changes in VT, related effect sizes are presented in Table 1. The value increased the most in the group with BrP2, which had the lowest RR during exercise. We found significant differences between all three BrP; the statistical difference between BrP1 and BrP2 was $p < 0.001$, between BrP1 and BrP3 $p = 0.016$ and between BrP2 and BrP3 $p = 0.002$. As can be seen from Figure 2, the values returned to baseline values at recovery; only in BrP3 did we find a significant difference between baseline and recovery ($p = 0.008$).

During exercise ventilation increased (Figure 3, Table 1), but no significant differences were found between the groups. During the recovery, the values dropped but not to baseline values. Ventilation in recovery was significantly higher compared to baseline ($p <0.001$ for BrP1 and BrP3, and $p = 0.003$ for BrP2, respectively).

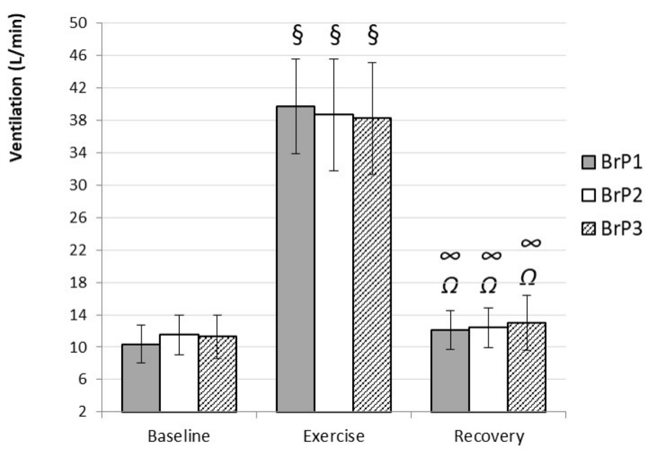

Figure 3: Changes in ventilation during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.

Figure 4 shows the changes in RER. No significant differences were found between BrPs; related effect sizes are reported in Table 1. In recovery, the RER remained elevated above the resting value. It was significantly higher in the groups with BrP1 ($p < 0.001$) and BrP3 ($p = 0.001$); in the group with BrP2 we did not find a difference ($p = 0.333$).

As expected, VO_2/kg increased during exercise and remained increased at the end of the measurement (Fig. 5, Table 1). As can be seen from Figure 5, no significant differences were observed with regard to BrPs, neither at rest nor during exercise or recovery.

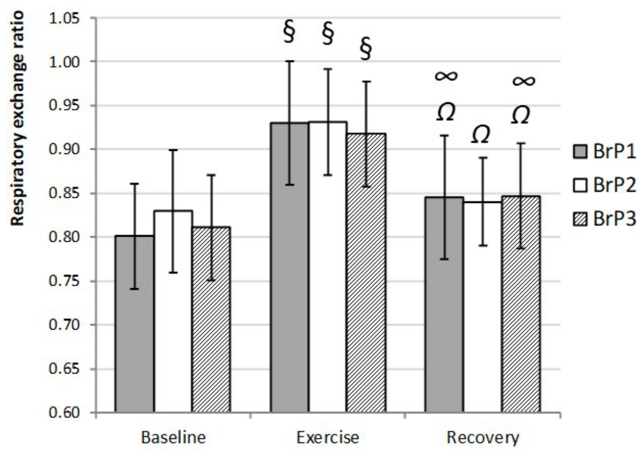

Figure 4: Changes in respiratory exchange ratio during rest, exercise and recovery in different BrPs. Values are presented as the mean ±SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.

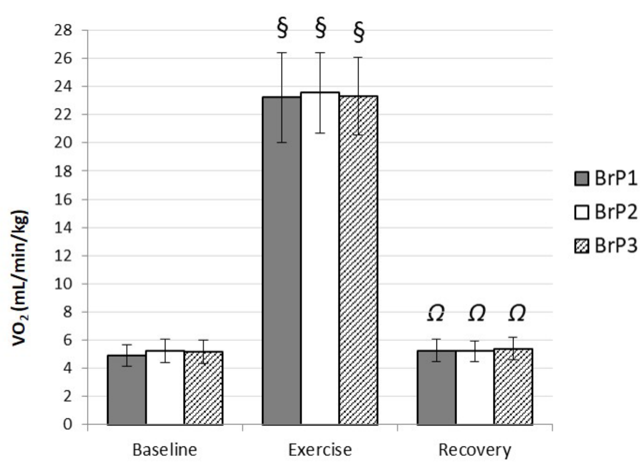

Figure 5: Changes in oxygen consumption during rest, exercise and recovery in different BrPs. Values are presented as the mean ±SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery.

Regarding VCO_2/kg, there were no significant differences between groups during rest and exercise, we did, however, find a significant difference between baseline and recovery in BrP3 ($P < 0.001$) (Fig. 6, Table 1).

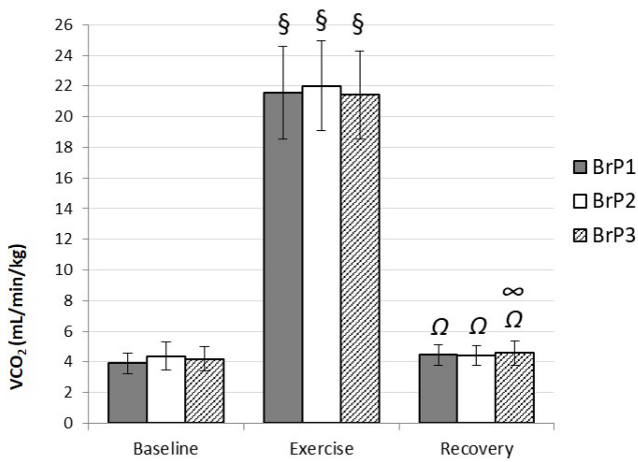

Figure 6: Changes in production of carbon dioxide during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in a particular BrP between rest and recovery.

Figure 7 presents the change in VEq for O_2. No statistical differences were found between the groups in any of the phases, but a higher value was observed in the BrP2 and BrP3 groups at rest and lower during exercise compared to BrP1. We found a statistical difference between baseline and exercise as well as between rest and exercise with respect to recovery, which was $p < 0.001$ in all BrPs (Table 1).

No significant differences between groups were observed during exercise and recovery in Veq for VCO_2 (Figure 8). Statistical differences were observed between baseline and recovery. In the group with BrP1 the difference was $p = 0.009$; in the group with BrP2 $p = 0.007$; and in the group with BrP3 $p = 0.002$. Veq for CO_2 was significantly increased during exercise compared to rest or recovery ($p < 0.001$) (Table 1).

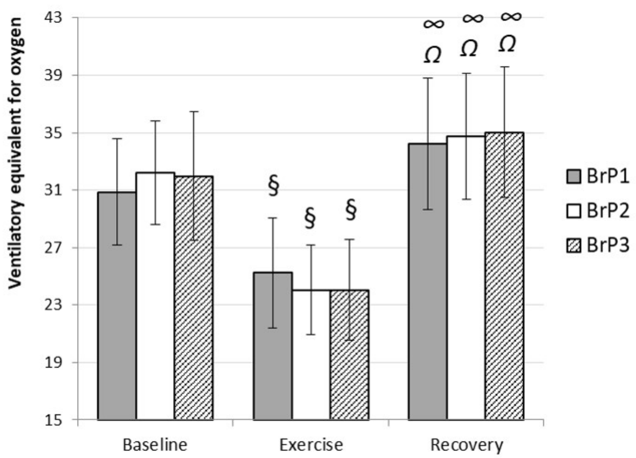

Figure 7: Changes in ventilatory equivalent of oxygen during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.

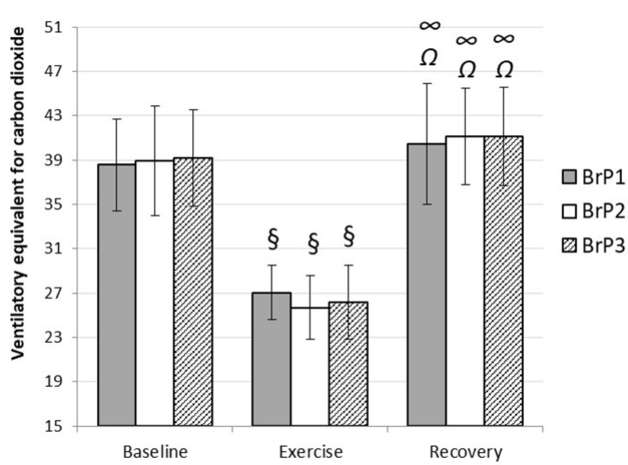

Figure 8: Changes in ventilatory equivalent of carbon dioxide during rest, exercise and recovery in different BrPs. Values are presented as the mean ± SD. § - significant difference in all BrPs between rest and exercise, Ω - significant difference in all BrPs between exercise and recovery, ∞ - significant difference in all BrPs between rest and recovery.

Figure 9 shows O_2 deficit and O_2 debt throughout the three BrPs. O_2 deficit (but not O_2 debt) in spontaneous breathing was significantly higher compared to other BrPs (ES = 0.42 for BrP1 compared to BrP2 and 0.36 for BrP1 compared to BrP3, respectively). O_2 debt was significantly lower compared to O_2 deficit in all BrPs (ES = 0.87 for BrP1, 0.20 for BrP2 and 0.15 for BrP3, respectively).

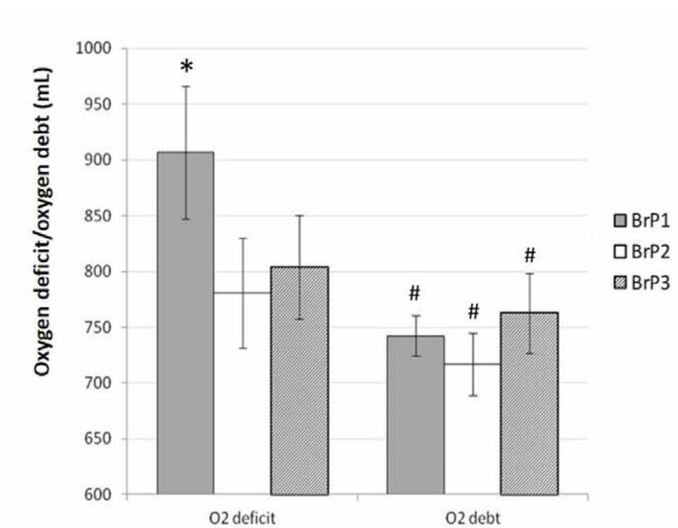

*Figure 9: Changes in oxygen deficit (O2 deficit) and oxygen debt (O2 debt) throughout all breathing patterns: spontaneous breathing (BrP1), pronouncing "h" (BrP2) and pronouncing "sh" (BrP3), respectively. * - significant with respect to BrPs. # - deficit versus debt.*

DISCUSSION

Our first main finding was that phonation during moderate exercise has a minimal effect on the respiratory response to exercise and recovery in young, healthy participants.

Our second main finding was that phonated breathing during moderate exercise provoked an increased rate of perceived exertion compared to spontaneous breathing. And our third main finding was that oxygen deficit at the onset of moderate exercise is significantly higher in spontaneous compared to phonated breathing. On the other hand, EPOC was not affected by BrP, indicating that in a steady state during moderate exercise excessive oxygen deficit was successfully eliminated by aerobic metabolism.

Although not significantly different, evidently lower RER during steady state exercise in BrP3 compared to other BrPs suggested that restricted air flow during expiration may cause CO_2 retention. This assumption was supported by the increased CO_2 exhalation in the recovery phase after exercise (Fig.6) in BrP3. Additionally, during steady state exercise, RR in BrP3 was higher compared to that in BrP2, presumably because of the stimulation of the inspiratory center by increased arterial CO_2 partial pressure. RR during spontaneous breathing was significantly increased compared to those in both BrP2 and BrP3 because of phonation excluding prolonged exhalation. Breathing against increased airway expiratory resistance (pronunciation of "sh") versus open airway expiration (pronunciation of "h") does not appear to alter airway diameter sufficiently to augment the minute ventilation response during moderate exercise. Even upon spontaneous breathing, minute ventilation was not changed compared to phonated breathing patterns.

The similar minute ventilation across all BrPs at steady state moderate exercise corresponding to an oxygen uptake of 23.5 mL/kg/min suggests that all BrPs applied can accommodate a moderate level of exercise intensity in young healthy participants (Plowman and Smith n.d.).

In our study, VO_2 did not differ during moderate physical activity at different BrPs. This finding is not in compliance with our previous study, where the reduced VO_2 in the low-intensity trunk stabilization exercise with hand-oscillation was confirmed upon phonated exhalation while pronouncing "sh" (Klanjšček, 2018). One possible explanation for this could be that the participants breathed in respiratory coupling with locomotion in the previous study. The entrainment could be responsible for better breathing economy (Sporer, Foster, Sheel, & McKenzie, 2007). Further, VO_2 was measured at the onset of trunk stabilization exercise in our previous study and not during the steady state, and thus was accompanied by anaerobic metabolism. When substituting anaerobic metabolism with aerobic in the continuation of trunk stabilization exercise, the differences in VO_2 between different BrPs decreased. This could imply that as the exercise is aerobic, the beneficial effects of PEP exhalation are decreased.

The association between anaerobic metabolism and breathing economy in connection to the use of a mouthguard was established (Schulze, Kwast, & Busse, 2019). The results of the studies examining the effect of different mouthguards on athletes' performance concluded that the economy of breathing is improved by wearing mouthguards at high but not moderate exercise intensities (Lässing et al., 2021; Schulze et al., 2019). The mechanism proposed is altered exhalation, potentially against higher resistance produced by mouthguards (Schulze et al., 2019). The impact of breathing using mouthguards is being researched in connection with sports that due to their nature need this type of protection (such as rugby and hockey) (Phimphasak et al., 2018; Schulze et al., 2019). Studies including young healthy athletes have found that altered exhalation due to wearing this equipment reduces the proportion of anaerobic metabolism at high-intensity exertion, compared to unmodified exhalation when not wearing it (Lässing et al., 2021). Francis and colleagues (Francis & Brasher, 1991) described reduced VO_2 and VEq for O_2 upon mouthguard usage at high-intensity exercise but not at low-intensity exercise. Since we did not confirm the beneficial effect of BrP3 in our study, we may speculate again that the beneficial effect of phonated expiration is limited to predominantly anaerobic or high-intensity load. Additional investigations should be conducted to test this assumption.

Phonated exhalation can be compared to breathing with pursed lips. There are some articles about the effects of pursed lip breathing on the breathing load, but only researched in patients with impaired pulmonary function (Sakhaei, Sadagheyani, Zinalpoor, Markani, & Motaarefi, 2018). Sakhaei and colleagues (Sakhaei et al., 2018) found that in patients with COPD, oxygenation and CO_2 excretion improve – as respiratory work is reduced - upon PEP provoked by pursed lips, while De Araujo (Pereira De Araujo, Karloh, Martins Dos Reis, Palú, & Fleig Mayer, 2015) and colleagues found that this type of breathing reduces dynamic hyperinflation, improving tolerance for exercise and O_2 saturation in the blood during exercise in COPB. Breslin (Breslin, 1992) found that spontaneous rhythmic breathing through pursed lips can affect the coordination of respiratory muscle recruitment and provides patients with a sense of control over ventilation, which results in less anxiety, panic, and consequently reduces dyspnea. Exercise in healthy participants can be seen as a model of impaired pulmonary function, as it increases the load on the respiratory system due to increased need for O_2 and produces more CO_2. Based on our results we can conclude that the intensity of moderate exercise applied in our study was not high enough to reveal the advantages of PEP breathing during exercise in young healthy adults. The physiological effects of phonated breathing in COPB patients should be examined to determine the potential benefits of phonated exhalation (decrease exercise respiratory load and perceived exertion) during exercise in such patients.

We found a significantly increased O_2 deficiency at the onset of moderate exercise upon spontaneous breathing compared to both phonated breathing patterns, indicating that spontaneous respiration was the least economical at the onset of exertion. On the contrary, the EPOC did not differ with regard to BrP. This finding supports our speculation that beneficial effects of breathing against increased airway expiratory resistance, as in the pronunciation of "sh", are manifested only when exercise metabolism is preferentially anaerobic. EPOC is decreased compared to O_2 deficit at spontaneous breathing, presumably because the products of anaerobic metabolism were removed and aerobically metabolized during steady state exercise due to its low intensity. O_2 deficit and EPOC did not differ with respect to the sound pronounced. Further studies should be directed to explain this finding.

Bonsignore and colleagues (Bonsignore, Morici, Abate, Romano, & Bonsignore, 1998) chose the VEq for O_2 and CO_2 as the main measure of respiratory efficiency. In our study, we found that both VEq for O_2 and CO_2 decreased during physical exertion compared to baseline, indicating that moderate exercise made the respiration more efficient (Plowman & Smith, n.d., 2013); and there were no significant differences between individual BrPs. Differences were indicated, yet not significant: both VEq upon BrP2 and BrP3 during exercise were lower than during spontaneous respiration. These observations can be linked to a study by Francis and Brasher (Francis & Brasher, 1991) who found a lower Veq for O_2 in subjects wearing mouthguards, indicating improved alveolar ventilation and oxygenation that allows an individual to maintain a certain level of exercise with less loss to the metabolic system; and to a study by Delaney and Montgomery (Delaney & Montgomery, 2005), who found that mouthguards obstruct airflow and thus affect VO_2 and ventilation during strenuous exercise. Again, both studies have demonstrated greater breathing efficiency in subjects using mouth protective devices only at maximum load.

There are some limitations to this research: in all participants the same load (140 W for males and 100 W for females) was applied as moderate exercise, neglecting individual differences, and the correctness of breathing pattern implementation was not controlled as the participant's mouth was covered by a breathing mask.

In conclusion, we found no physiological benefits of phonation applied upon moderate exercise that could advocate its use for economizing breathing at higher metabolic demand. However, decreased oxygen deficit at the onset of moderate exercise upon phonated compared to spontaneous breathing confirmed its positive effects on the anaerobic phase at the onset of exercise. This finding may potentially provide benefits for patients with compromised respiratory function owing to an enhanced proportion of anaerobic metabolism even at moderate exercise loads, however, further studies need to be conducted to test this assumption. On the other hand, "sh" pronouncing breathing may provoke CO_2 retention at higher exercise intensities. Because phonated breathing during exercise is an atypical breathing pattern, it is subjectively rated as uncomfortable compared to spontaneous breathing, yet the latter does not differ from phonated breathing with regard to breathing efficiency. It is therefore probable that in athletes exercising at higher loads phonated breathing may potentially provide benefits in terms of attenuating oxygen deficit.

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