Warm-up breathing exercises accelerate VO₂ kinetics and reduce subjective strain during incremental cycling exercise in adolescents

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Abstract:
Background: Breathing exercises based on the Wim Hof method, which is a combination of deep breathing and breath holds, increase arterial CO₂ concentration. (i.e., hypercapnia). Induction of hypercapnia prior to exercise is purported to elicit a sympathetic response, leading to an increase in tidal volume and elevated blood flow to skeletal muscle in a manner that may improve exercise performance. We evaluated whether pre-exercise breathing techniques may impact VO₂ kinetics during incremental cycling exercise. Methods: 16 adolescent (16.6±1.4 years) middle- and long-distance runners (8 males and 8 females) participated in our study. Participants performed two incremental cycle ergometer testing sessions consisting of two minute stages at 1, 2, 3, and 4 W·kg⁻¹, which were immediately preceded by either a series of deep breathing exercises and breath holds based on each participant’s CO₂ tolerance, or an equal duration of seated rest, the order of which was randomized. Heart rate, oxygen consumption, and respiratory parameters were continually assessed throughout the cycle ergometer tests and session rating of perceived exertion (Borg scale) was obtained at the end of the test.
Results: Whereas all participants completed the final testing stage (4 W·kg⁻¹) during the session preceded by breathing exercises, five participants were unable to complete this testing stage in the control trial. Oxygen consumption was significantly greater during the testing stages preceded by the breathing techniques (2.4–4.9%; p<0.05) and perceived effort throughout the training session was attenuated (18.5±1.2 vs. 17.4±1.1; p<0.01) following breathing exercises. All participants completed the final testing stage preceded by breathing exercises. Without breathing exercises five participants were unable to complete this stage.
Conclusions: Breathing exercises, incorporating deep breathing and breath holds, performed prior to an acute exercise bout appears to accelerate VO₂ kinetics and reduces subjective strain in young endurance athletes.

Key Words: Breath hold; Load intensity; Human performance; Cardiovascular; Endurance.

Introduction
Breathing is one of the basic automatic coordinated processes in the body that facilitates oxygen delivery and removal of carbon dioxide. Recent work has focused on targeted breathing interventions that manipulate respiratory rhythm (Kenney, Wilmore, & Costill, 2015). Moreover, though retention of breath after inhalation is easier than after exhalation, a breath hold after exhalation may differentially impact host physiology. Whereas retention of breath after inhalation creates tension and pressure in the respiratory muscles, creating temporary hyperinflation that prevents relaxation and proper emptying of the lungs, a hold after exhalation is more relaxing and may lead to an increase in CO₂ tolerance (Chaitow, Bradley, & Gilbert, 2014; Courtney & Cohen, 2008).

Hyperventilation (combination of deep breathing and higher respiratory rate) reduces the CO₂ level in the blood, increases the pH and creates many physiological changes. One of these changes is the constriction of smooth muscle and a reduction in pain threshold (Chaitow et al., 2014). Increasing the level of pH in the blood improves muscle function, which can be seen, for example, in short-term cyclic sprints (Chaitow et al., 2014). During hyperventilation, hemoglobin binds closely to oxygen, reducing the release of oxygen into the tissues, especially the periphery and possibly leading to an increase in muscle lactate. Meanwhile, increased pH also leads to a constriction of smooth muscle, a finding that may explain increased muscle tone in individuals with respiratory pattern disorders (Chaitow et al., 2014; Courtney, 2011; Laffey & Kavanagh, 2002; McArdle, Katch, & Katch, 2016). More recently, hyperventilation has also been demonstrated to yield positive benefits for anaerobic performance (Sakamoto, Naito, & Chow, 2014), a finding that has excited future research pertaining to breathing exercises and athletic performance. Manipulation of breathing with a potential effect on physiological VO₂ and CO₂ levels before exercise through a combination of hyperventilation and breath holding can elicit a sympathetic response that may improve circulating oxygen uptake (Hof et al., 2017; Lindholm & Gennser, 2005; Maestroni, 2006). It could be important in endurance sports (McArdle, Katch, & Katch, 2016).
is known that activation of sympathetic nervous system has impact on cellular metabolism and oxygen consumption (Bravo, 1989).

Many years ago, Ukrainian doctor Konstantina Buteyko proposed a series of breathing exercises as a way to treat asthma and other breathing disorders. Buteyko’s method was based on his belief that carbon dioxide deficiency is a major cause of many chronic diseases. In fact, Buteyko claimed that increasing carbon dioxide in the body can allegedly help in up to 150 diseases (Chaitow et al., 2014). As such, one of the pillars of the Buteyko method was reducing minute ventilation as a way to increase alveolar CO₂. However, in certain patient populations, such as those with anxiety, a compensatory increase in tidal volume reduces the effectiveness of this technique (Conrad et al., 2007). In these patients, other breathing techniques must be chosen to increase CO₂ (Chaitow et al., 2014; Courtney & Cohen, 2008).

If properly implemented, breathing exercises may yield systemic physiological changes. For example, reduced lung hyperinflation, a condition in which residual lung volume is increased due to incomplete exhalation, has been observed (Chaitow et al., 2014). Lung hyperinflation is common in patients with asthma, chronic lung obstruction and other conditions associated with chronic increases in tidal volume, such as anxiety and may lead to dyspnea, diaphragm dysfunction and disruption of neuromechanical breath control (Chaitow et al., 2014; Courtney, van Dixhoorn, Greenwood, & Anthonissen, 2011). Breathing exercises may also lead to relaxation of the nasal passages and relief of acute bronchospasm (Chaitow et al., 2014). In athletes, breathing exercises focused on maximum respiratory retention of carbon dioxide have been suggested to increase endogenous antioxidant production and raise anaerobic threshold (Joulia et al., 2003) and improve of physical kinetics in a manner that may enhance exercise performance.

The Wim Hof breathing method was recently founded by Dutch athlete Wim Hof, and is based on the same principles as the Buteyko method (Allen, 2018; Buijze et al., 2019, Hof, 2015). This method is currently used in patients with Covid-19, where an increase in SpO₂ has been demonstrated (Syiafulloh et al., 2020). Wim Hof breathing techniques are based on three pillars: breathing exercises, concentration and gradual exposure to cold (Agarwal, Chovatiya, & Rana, 2020; Hof, 2020). There are various methods that deal with the individual components of these pillars (Bahenský, Bunc, Marko, & Malátová, 2020; Bahenský, Malátová, & Bunc, 2019; Houtman, 2018; Kox et al., 2012; Kox et al., 2014; Malátová, Bahenský, Mareš, & Rost, 2017; Muzik et al., 2018), which seem to elicit desirable physiological effects (Allen, 2018; van Middendorp, Kox, Pickkers, & Evers, 2016). For example, some studies have demonstrated improvements in anaerobic work capacity following Hof’s breathing techniques (Kairouz et al., 2013; Leithäuser, Böning, Hüttler, & Beneke, 2016), although others have not (Fuji et al., 2015). Meanwhile, less is known regarding the influence of Wim Hof breathing techniques on aerobic performance indicators.

There is a linear dynamic relationship between oxygen consumption (VO₂) and physical work. At exercise intensities below the lactate threshold (LT), VO₂ increases exponentially to steady-state. However, there are factors, such as physical training, age, and some pathological conditions that can alter the kinetic responses of VO₂ at the beginning of exercise (Xu & Rhodes, 1999). Interestingly, ischemic preconditioning is demonstrated to slow VO₂ kinetics during cycling exercise but improve economy (Kilding, Sequeira, & Wood, 2018). Whether pre-exercise breathing techniques to manipulate physiological gas concentrations may similarly impact VO₂ kinetics is unknown. Youth athletes still do not have been finish their muscular development, they also have a different tiredness (Bahenský & Bunc, 2018).

The effect of breathing exercises based on Buteyko and Hof's method on endurance performance has not been thoroughly described. Thus, the aim of this study was to determine whether the application of Wim Hof breathing exercises immediately before training may influence VO₂ kinetics during incremental cycling exercise in young, endurance trained individuals. We hypothesized that these breathing exercises would accelerate VO₂ kinetics in a manner that may enhance exercise performance.

**Material & methods**

**Participants**

The protocol and procedures conforms to the Declaration of Helsinki statements and were approved by The Ethical Committees of Faculty of Education, University of South Bohemia study on October 19, 2018 (002/2018). All subjects provided their written informed consent to participate in the research study. This study was implemented at the Department of Sports Studies, Faculty of Education, University of South Bohemia in the Laboratory of Load Diagnostics.

A total of 16 medium and long distance runners, 8 boys (16.8 ± 1.1 years, weight 69.14 ± 10.6 kg, height 181.5 ± 7.0 cm) and 8 girls (16.4 ± 1.7 years, weight 56.81 ± 4.24 kg, height 166.9 ± 3.6 cm) participated in the study. We specifically chose to study young, endurance-trained individuals to eliminate the possible confounding influence of discrepant physical activity levels throughout the lifespan on exercise responses.

Participants were randomly recruited from a sample of runners who met the training criteria of the Czech Athletic Association (Bahenský & Bunc, 2018). All participants had been consistently running for a least 3 years (≥ 6×/wk) and were between 16–19 years of age. Study participants were randomly divided into two groups (using
the r-and-between function in Excel). The first group performed the cycle ergometer testing sessions first without and then with prior breathing exercises, whereas the order was reversed in the second group. A total of 72 hours were provided between testing visits.

Procedures
Breathing exercises were performed in the lying position on the basis of this position being recommended by Hof due to an improved ability to fully relax while also ensuring maximal safety of the participants (Hof, Jong, & Brown, 2017). Breath holding exercises were based on the CO₂ tolerance of each participant. Participants were told to perform 30–50 full breaths at a metronome rhythm of 20-breaths min⁻¹ and when feeling the first signs of tingling in their fingers to fully exhale and hold their breath. Participants were instructed to hold their breath until the first spontaneous contraction of the diaphragm, or until they felt the strong urge to breathe.

Each breath hold was immediately followed by a full breath and a secondary 15-sec breathhold, designating completion of the first round of breathholds. Three identical rounds of breathholds were completed without a break, lasting between 17–22 min. In the control condition, subjects were asked to rest quietly in the seated position.

Measures
Breath holding exercises or an equal duration seated rest were immediately followed by an incremental testing session performed on a cycle ergometer, which consisted of a warm-up (2 min at 25 W), followed by four two-minute stages at progressive intensities based on each subject’s body mass (1, 2, 3, 4 W·kg⁻¹). Heart rate (HR), and cardiorespiratory parameters including tidal volume (V₄), breathing frequency (BF), respiratory minute volume (V₅), oxygen consumption (VO₂), respiratory exchange ratio (RER) and oxygen pulse (VO₂·HR⁻¹) were continuously monitored using a Metalyzer 3B (Cortex, Leipzig, Germany) and are reported as the mean from the last 60 seconds of each testing stage. If a participant was unable to complete the two-minute testing stage, the last minute they were able to complete was used for analysis and compared to the same time-point during the other test. Borg session rating of perceived exertion (RPE) was surveyed at the completion of the incremental testing protocol.

Statistical analysis
Data were presented as mean and standard deviation. Normality and homogeneity of data were confirmed. A two-way (treatment x time) repeated measures ANOVA was performed to compare the overall effect of breathing exercises on cardiorespiratory parameters and a post-hoc Tukey test was performed to identify mean differences. Between sessions comparison of RPE were performed using a paired student’s t-test. Subsequently, effect size was determined using Cohen’s d. Significance was primary set at the α = 0.05 level and data processing was performed in Excel 2016 (Oregon, WA, USA) and JMP (Cary, NC, USA).

Results
Table 1 shows the measured values of cardiorespiratory parameters during the exercise test. All participants completed the final testing stage (i.e., 2-min at 4 W·kg⁻¹) in the trial preceded by breathing exercises, however, without breathing exercises five participants were unable to complete this stage. Though no interactions were noted, a significant main effect (P<0.05) for workload was observed for all variables at all stages, and post-hoc testing revealed a significant difference among mean values at each load (i.e., 1,2,3,4 W·kg⁻¹; P<0.05). For VO₂, a significant main effect (P<0.05) for treatment (i.e., with vs. without breathing exercises) was also observed, and appeared to be driven by greater VO₂ values at 4 W·kg⁻¹ (medium effect). Though elevated VE values were also observed following breathing exercises (medium effect), these differences were not statistically different (P > 0.05). Subject RPE was significantly lower (P<0.001) following the trial proceeded by breathing exercises.

Table 1. Physiological and psychological responses to the incremental cycling test

<table>
<thead>
<tr>
<th></th>
<th>workload</th>
<th>without breathing exercises</th>
<th>with breathing exercises</th>
<th>% change</th>
<th>Cohen’s d</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ [mL·min⁻¹·kg⁻¹]</td>
<td>1 W·kg⁻¹</td>
<td>24.04 ± 2.32</td>
<td>25.02 ± 2.29</td>
<td>4.33 ± 6.69</td>
<td>0.43*</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>2 W·kg⁻¹</td>
<td>31.24 ± 2.47</td>
<td>32.05 ± 2.29</td>
<td>2.81 ± 6.42</td>
<td>0.34*</td>
<td>P = 0.022</td>
</tr>
<tr>
<td></td>
<td>3 W·kg⁻¹</td>
<td>40.28 ± 2.71</td>
<td>41.13 ± 2.30</td>
<td>2.36 ± 6.54</td>
<td>0.34*</td>
<td>P = 0.182</td>
</tr>
<tr>
<td></td>
<td>4 W·kg⁻¹</td>
<td>47.00 ± 4.24</td>
<td>49.08 ± 3.11</td>
<td>2.89 ± 7.26</td>
<td>0.56**</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>MEANS</td>
<td>35.64 ± 9.24</td>
<td>36.82 ± 9.44</td>
<td>3.60 ± 6.82</td>
<td>0.50*</td>
<td></td>
</tr>
</tbody>
</table>

| VO₂·HR [mL]    | 1 W·kg⁻¹ | 12.15 ± 2.39                 | 12.90 ± 2.91             | 5.87 ± 7.34 | 0.28*    | Treatment |
|                | 2 W·kg⁻¹ | 13.81 ± 2.81                 | 14.59 ± 3.28             | 5.32 ± 7.14 | 0.26*    | P = 0.182|
|                | 3 W·kg⁻¹ | 15.68 ± 3.00                 | 16.37 ± 3.69             | 3.92 ± 7.59 | 0.20*    |         |
|                | 4 W·kg⁻¹ | 16.76 ± 3.38                 | 17.63 ± 3.58             | 5.35 ± 6.17 | 0.25*    | P < 0.001|
|                | MEANS   | 14.60 ± 3.41                 | 15.37 ± 3.82             | 5.12 ± 7.12 | 0.25*    |         |
performed prior to exercise as a way to improve short-duration athletic performance.

Note: Main effects for treatment (i.e., breathing vs. no breathing) and workload (1, 2, 3, 4 W·kg⁻¹) indicated in right hand column. Letters (a, b, c, d) indicate value significantly different from other workloads via post-hoc testing. Cohen’s d: * = small effect size, m = medium effect size, l = large effect size; HR = heart rate, VE = tidal volume, BF = breathing frequency, V̇E = respiratory minute volume, VO₂ = oxygen consumption, RER = respiratory exchange ratio, VO₂·HR⁻¹ = oxygen pulse, RPE = Borg session rating

Discussion
To date, several studies have sought to assess the potential benefits of Wim Hof breathing techniques (Bruton & Holgate, 2005; Kox et al., 2012; Kox et al., 2014; van middendorp et al., 2016). We investigated the effect of this technique on VO₂ kinetics during incremental cycling exercise. The observed differences in cardiorespiratory values between and among stages is in accordance with well-known cardiovascular responses to acute aerobic exercise. Elevated VO₂ and possible VE values in testing stages preceded by Wim Hof breathing exercises provides evidence that the pre-exercise breath training may have accelerated cardiorespiratory responses. Concomitantly, we also observed a reduction in overall perceived exertion in the session preceded by breathing exercises. Accelerated oxygen consumption during physical activity preceded by breathing exercises was similarly observed by sakamoto et al. (2014), who observed a demonstrable increase in VO₂ during repeated anaerobic sprints following 2 minutes of hyperventilation. It has been confirmed that manipulation of breathing with a potential effect on O₂ and CO₂ values before exercise through a combination of hyperventilation and breath holding can elicit a sympathetic response that improves circulating oxygen uptake (Hof et al., 2017; Lindholm & Gennser, 2005; Maestroni, 2006). Activation of sympathetic nervous system has impact on cellular metabolism, including increased oxygen consumption (Bravo, 1989). Reduced subjective strain and faster VO₂ kinetics following Wim Hof breathing exercises may help to improve exercise performance.

Importantly, faster VO₂ consumption following breathing exercises were accompanied by a reduced perception of physical exertion. Moreover, all participants were able to complete the two-minute stage at 4 W·kg⁻¹ following breathing exercises, whereas five individuals had to stop the test prematurely in the control condition. These findings complement previous research demonstrating an improvement in short-term swim performance following hyperventilation (Jacob et al., 2015) and improvements in anaerobic fatigue after Wim Hof breathing exercises (Langewitz, 2020). Additionally, improvements in anaerobic power measured via the Wingate anaerobic test have also been demonstrated following Wim Hof breathing exercises (Kairouz et al., 2013; Leithäuser et al., 2016). Collectively, these findings support the efficacy of Wim Hof breathing exercises performed prior to exercise as a way to improve short-duration athletic performance.

Though previous research has examined the effects of inspiratory muscle warm-up exercises on exercise responses, few have done so using maximal breath holding techniques. In the study of Tong and Fu (2006), two
sets of 30 breaths were used with inspiratory pressure-threshold load equivalent to 15% (IMW_0) for the placebo test and 40% (IMW) maximum inspiratory mouth pressure for the experimental group, meanwhile a control group performed the testing protocol without any prior breathing exercises. VO_2 during an intense intermittent run to exhaustion did not differ among groups (50.6 for control, 49.1 for placebo and 50.9 ml.min^{-1}.kg^{-1} for IMW). In a study by Arend et al. (2015), two rowing tests at 90% of VO_2max were performed. The first one was preceded by a traditional warm-up and second complimented this warm-up with specific inspiratory muscle training at 40% of maximal inspiratory pressure. Once again, VO_2max values did not appear to be affected by prior inspiratory muscle exercises (56.3 ml.min^{-1}.kg^{-1} vs. 55.9 ml.min^{-1}.kg^{-1}). In the study of Voilanitis et al. (2001), a group of well-trained club rowers (n=14) performed a 6-min all-out rowing simulation. After a submaximal rowing warm-up (SWU), a specific rowing warm-up (RWU), and a specific rowing warm-up with the addition of a respiratory warm-up (RWUplus) was used. VO_2 showed no significance difference between tests for each warm-up. This previous research showing lack of an effect of warm-up breathing exercises on VO_2 responses is in contrast to the observed accelerated VO_2 kinetics following Wim Hof breath training. This difference may be explained by the greater stimuli of near maximal effort associated with the Wim Hof technique. Additionally, we examined submaximal VO_2 responses whereas preceding studies primarily measured VO_2 during maximal exercise, where the influence of inspiratory muscle warm-up exercises may be less apparent.

Though the precise physiological mechanisms underlying the observed accelerated VO_2 consumption cannot be identified by the present study, speculatively, it would seem that induction of hypercapnia may have elicited a controlled stress response characterized by activation of the sympathetic nervous system. Thus, at the commencement of exercise preceded by breathing exercises, a greater abundance of vasoactive compounds may facilitate improved oxygen delivery to working tissues (Kox et al., 2012). Additionally, whereas hypocapnic-hyperventilation appears to lower stroke volume (Lewis et al., 2014) we observed no change in O_2 pulse during exercise following breath holding exercises intended to raise arterial CO_2 levels. Speculatively, pre-exercise induction of hypercapnia may have stimulated splenic contractions intended to preserve tissue oxygenation via red blood cell release (Schagatay et al., 2005). If so, increased oxygen carrying capacity following Wim Hof breathing exercises may have underlined the accelerated VO_2 kinetics. More research in a larger number of participants is needed to elucidate the physiological underpinnings of our observations.

Limitations of our study include a relatively small number of participants, their age (needed to confirm in adults), and our very short 2-min warm-up at 25 watts. The minimum warm-up time was chosen so as to minimize the possible confounding influence of a longer warm-up on cardiovascular responses.

Conclusions

Incorporating a 17–22 minute breathing exercise based on deep breathing and breathing restraints according to the Wim Hof method into warming-up before endurance performance altered VO_2 consumption during incremental cycling exercise in a manner that may lead to facilitate endurance performance and reduces subjective strain in young endurance athletes. Interestingly, participants’ average heart rates tended to be lower in stages preceded by warm-up breathing exercises. While the underlying mechanism (i.e., central or peripheral) for this lower heart rate in the presence of increased oxygen consumption cannot be determined by the present study, it is reasonable to hypothesize that this lower heart rate may have contributed to the lower perceived exertion experienced by the participants. Notably, O_2 pulse (i.e., VO_2/HR) values were similar between trials, suggesting a peripheral ergogenic effect. Future research examining whether improved oxygen uptake kinetics following Wim Hof breathing exercises would contribute to meaningful improvements in performance is needed.

Conflicts of Interest: The authors declare no conflict of interest.

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