The difference in respiratory and blood gas values during recovery after exercise with spontaneous versus reduced breathing frequency

Jernej Kapus, Anton Ušaj, Venceslav Kapus and Boro Štrumbelj
University of Ljubljana, Faculty of Sport, Laboratory of Biodynamics, Slovenia

Abstract
Extrapolation from post-exercise measurements has been used to estimate respiratory and blood gas parameters during exercise. This may not be accurate in exercise with reduced breathing frequency (RBF), since spontaneous breathing usually follows exercise. This study was performed to ascertain whether measurement of oxygen saturation and blood gases immediately after exercise accurately reflected their values during exercise with RBF. Eight healthy male subjects performed an incremental cycling test with RBF at 10 breaths per minute. A constant load test with RBF (B10) was then performed to exhaustion at the peak power output obtained during the incremental test. Finally, the subjects repeated the constant load test with spontaneous breathing (SB) using the same protocol as B10. Pulmonary ventilation ($V_{E}$), end-tidal oxygen ($P_{ETO2}$), and carbon dioxide pressures ($P_{ETCO2}$) and oxygen saturation ($SaO_2$) were measured during both constant load tests. The partial pressures of oxygen ($PO_2$) and carbon dioxide ($PCO_2$) in capillary blood were measured during the last minute of exercise, immediately following exercise and during the third minute of recovery. At the end of exercise RBF resulted in lower $P_{ETO2}$, $SaO_2$ and $PO_2$, and higher $P_{ETCO2}$ and $PCO_2$ when compared to spontaneous breathing during exercise. Lower $SaO_2$ and $P_{ETO2}$ were detected only for the first 16s and 20s of recovery after B10 compared to the corresponding period in SB. There were no significant differences in $PO_2$ between SB and B10 measured immediately after the exercise. During recovery from exercise, $P_{ETCO2}$ remained elevated for the first 120s in the B10 trial. There were also significant differences between SB and B10 in $PCO_2$ immediately after exercise. We conclude that RBF during high intensity exercise results in hypoxia; however, due to post-exercise hyperpnoea, measurements of blood gas parameters taken 15s after cessation of exercise did not reflect the changes in $PO_2$ and $SaO_2$ seen during exercise.

Key words: Constant load test, reduced breathing frequency, recovery, respiratory parameters, oxygen saturation, blood gas.

Introduction

In some sports, the environment is inappropriate for direct measurement of respiratory and blood gas parameters during exercise. Moreover, the attachment of cumbersome measurement equipment may influence the motion technique and consequently increase energy cost. To overcome this problem, respiratory and blood gas parameters have been measured at the end of exercise to estimate physiological responses during the exercise. Backward extrapolation of the $O_2$ recovery curve has been used to calculate the peak oxygen uptake during swimming (Rodríguez et al., 2002) and synchronized swimming (Bante et al., 2007). This method requires that measurements are made as soon as possible after the end of exercise. Data collection lasts a few minutes, and the recovery curve is extrapolated back to time zero, i.e., to the end of exercise.

Extrapolation from post-exercise measurements has also been used to estimate changes in partial pressures of blood gases induced by reduced breathing frequency (RBF), as observed in competitive swimming. However, Štrumbelj et al. (2006) found that measurements of respiratory and blood gas parameters taken after the end of a maximal front crawl swimming test did not reflect the conditions which appeared during the swimming test. RBF during swimming increased the alveolar CO$_2$ concentration (Dicker et al., 1980; Peyerebrune et al., 2002; Town and Vanness, 1990; West et al., 2005) and induced higher partial pressure of carbon dioxide in capillary blood after it (Kapus et al., 2002; Kapus et al., 2003). Nonetheless, these studies failed to demonstrate a reduction in oxygen saturation due to RBF either by analysing expired air during swimming (Holmér et al., 1980) or by sampling capillary blood after swimming (Kapus et al., 2002; 2003). On the contrary, RBF during cycle ergometry has been shown to cause a reduction in oxygen saturation and lower partial pressure of oxygen, measured in arterial (Yamamoto et al., 1987) and capillary (Kapus et al., 2007; Sharp et al., 1991) blood. Considering that hypoxia has been detected during cycling but not after swimming, the timing of measurement may be the reason for the apparent difference in response to RBF. The timing of measurement may be especially important when spontaneous breathing follows the exercise with RBF. The present study was designed to elucidate this problem. To date, no data of oxygen saturation and respiratory parameters during recovery after exercise with RBF have been presented. Therefore, the aim of the present study was to ascertain whether measurements of oxygen saturation and blood gases measured immediately after exercise could estimate their values during exercise with RBF.

Methods

Subjects
Eight healthy male subjects (age 25 ± 1 years, height 1.81 ± 0.03 m, weight 80 ± 7 kg, peak oxygen uptake (VO$_2$peak) 44.26 ± 2.93 ml·kg$^{-1}$·min$^{-1}$, forced vital capacity of 5.98 ± 0.58 l and forced expiratory volume of 4.76 ± 0.59 l in 1 s) volunteered to participate in this study. None of the subjects were smokers and were free of respiratory disease at the time of the study. The subjects were fully informed of the purpose and possible risks of the
study before giving their written consent to participate. The study was approved by the University's Research Ethics Committee.

**Procedures**

RBF was defined as 10 breaths per minute and was regulated by a breathing metronome. The breathing metronome was composed of a gas service solenoid valve 24 VDC (Jakiša, Ljubljana, Slovenia) and a semaphore with red and green lights. Both were controlled by a micro automation Logo DC 12/24V (Siemens, Munich, Germany). The subjects were instructed to expire and inhale between a 2 s period of open solenoid valve (the green semaphore light was switched on) and to hold their breath, using almost all lung capacity (breath holding near total lung capacity), for 4 s when the solenoid valve was closed (the red semaphore light was switched on). Prior to the exercise testing, the subjects were familiarized with breathing through the breathing metronome. After familiarization, each subject performed 4 exercise tests on an electromagnetically braked cycle ergometer Ergometrics 900 (Ergoline, Windhagen, Germany) with pedal cadence at ~60 revolutions per minute (rpm). Tests were performed in a prescribed order, each of them on a different day.

**Preliminary tests:** The subjects initially performed an incremental exercise test (IT) to obtain VO_{2peak}. The test began at 30 W and increased by 30 W every 2 min until volitional exhaustion. VO_{2peak} was defined as the highest O_{2} uptake averaged over 60-s interval. The subjects then performed an incremental exercise test with RBF (ITB10) to obtain peak power output. Except breathing, the protocol of this test was identical to the protocol of IT. The peak power output was defined as the highest work stage that each subject completed. From these results, the work rate for the constant load test with RBF was chosen for each subject.

**Experimental protocol:** After preliminary testing, a constant load test with RBF (B10) was performed to exhaustion at the peak power output obtained during ITB10. This test started with a 5 min warm-up at 50 W. After that, the resistance was increased to match the subject’s peak power output and the subject continued to exhaustion. The constant load test was completed by 10 min of active recovery at 20 W with spontaneous breathing. Finally, the subjects repeated the constant load test, however, with spontaneous breathing (SB). The protocol (intensity and duration) of this test was otherwise identical to the protocol of B10.

**Measurements**

During the constant load tests (warm-up, exercise and 10 min recovery; SB and B10), the subjects breathed through a mouthpiece attached to a pneumotachograph. The subject’s expired gas was sampled continuously by a VMAX29 (SensorMedics Corporation, Yorba Linda, USA) metabolic cart for a breath-by-breath determination of respiratory parameters (pulmonary ventilation ($V_E$), end-tidal pressure of oxygen ($P_{ET, O_2}$) and end-tidal pressure of carbon dioxide ($P_{ET, CO_2}$)). The pneumotachograph and the $O_2$ and $CO_2$ analysers were calibrated prior to the test with a standard 3 L syringe and precision reference gases, respectively. For further statistical analysis, breath-by-breath data were averaged for each 10-s interval. During the constant load tests (warm-up, exercise and 10 min recovery), oxygen saturation ($SaO_2$) was measured using a TruStat™ Pulse Oximeter (Datex-Ohmeda, Madison, USA). The pulse oximeter is an indirect oximetry measuring instrument, which displays $SaO_2$ every 4 s. The ear probe was attached to the earlobe after cleaning the area with alcohol. The partial pressure of oxygen ($P_{O_2}$) and carbon dioxide ($P_{CO_2}$) in capillary blood were measured during the last minute of exercise, immediately following exercise (the delay between the subject’s cessation of the exercise and the first measure did not exceed 15 s) and during the third minute of recovery. Capillary blood samples (60 – 80 µl) were taken by micro puncture from an earlobe. Earlobe capillary blood was arterialized by the application of hyperemic cream (Finalgon, Boehringer-Ingelheim, Reims, France) at least 20 min before the first capillary sample. Earlobe samples were collected in heparinized glass capillary tubes and introduced into a blood gas analyser ABL5 (Radiometer, Copenhagen, Denmark) for gas analysis at 37º C.

**Statistical analysis**

The results are presented as means and standard deviations (SD). One-way analysis of variance (ANOVA) for repeated measures was used to test the statistical differences between SB and B10 during entire recovery period. If significance was documented, post hoc, pair wise comparisons of the data of respiratory parameters and $SaO_2$ at the end of exercise and during initial 40 s of recovery were made using paired t test. In addition, the data of respiratory parameters measured every fourth s from initial 40 s period of recovery were also included in these comparisons. Statistical significance was accepted at the p ≤ 0.05 level. All statistical parameters were calculated using the statistics package SPSS (version 15.0, SPSS Inc., Chicago, USA) and the graphical statistics package Sigma Plot (version 9.0, Jandel, Tübingen, Germany).

**Results**

Table 1 depicts VO_{2} responses to incremental cycle ergometry in relation to VO_{2} responses in subjects during the incremental test with RBF.

Data depicted in Table 2, describes the average intensity and time to exhaustion in B10 and yielded 191 W (32 W) and 566 s (332 s), respectively. By design, the intensity and duration of B10 and SB were identical. As expected, $V_E$ was significantly lower (p ≤ 0.01) in B10 than in SB at the end of exercise (time zero of the recovery curve in Figure 1). However, in the B10 trial, $V_E$ dramatically increased post-exercise and it was higher during the initial 200 s of recovery than it was during the corresponding period in the SB trial (p ≤ 0.01). $P_{ET, O_2}$ was significantly lower at the end of exercise and during the initial 20 s of recovery in B10 than in SB (p ≤ 0.01; Figure 2). Similar results were shown for $SaO_2$ (Figure 3), which differed significantly (p ≤ 0.01) between the trials at the end of exercise and for the initial 16 s of recovery. $P_{ET, CO_2}$ was significantly higher for the first 120 s of recovery after B10 than after SB (p ≤ 0.01; Figure 4).
difference in spontaneous versus reduced breathing

Table 1. Individual VO₂ responses to incremental bicycle exercise in two different breathing conditions.

<table>
<thead>
<tr>
<th>subject</th>
<th>power output (W)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
<th>270</th>
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<th>330</th>
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<td>7.72</td>
<td>10.14</td>
<td>13.68</td>
<td>16.44</td>
<td>21.77</td>
<td>24.69</td>
<td>28.37</td>
<td>31.68</td>
<td>35.75</td>
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<td>5.00</td>
<td>9.26</td>
<td>12.11</td>
<td>13.80</td>
<td>17.13</td>
<td>20.75</td>
<td>23.65</td>
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<tr>
<td>IT</td>
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<td>8.44</td>
<td>12.03</td>
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<td>27.37</td>
<td>31.67</td>
<td>36.19</td>
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<td>32.37</td>
<td>35.97</td>
<td>40.19</td>
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<td>13.71</td>
<td>16.48</td>
<td>21.03</td>
<td>24.56</td>
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<td>11.84</td>
<td>15.77</td>
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<td>22.62</td>
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<td>36.68</td>
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<tr>
<td>ITB10</td>
<td>4.01</td>
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<td>12.50</td>
<td>14.77</td>
<td>18.18</td>
<td>18.13</td>
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<tr>
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<td>9.24</td>
<td>12.06</td>
<td>15.60</td>
<td>19.75</td>
<td>23.60</td>
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<tr>
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<td>12.81</td>
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<td>19.06</td>
<td>23.67</td>
<td>26.85</td>
<td>29.45</td>
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</table>

IT and ITB10 denote incremental exercise test with spontaneous breathing and RBF, respectively.

Table 2. Individual values of power output and time to exhaustion during B10.

<table>
<thead>
<tr>
<th>subject</th>
<th>power output (W)</th>
<th>duration (s)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>240</td>
<td>420</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>317</td>
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<tr>
<td>3</td>
<td>210</td>
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</tr>
<tr>
<td>8</td>
<td>210</td>
<td>479</td>
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</tbody>
</table>

Table 3 shows that PO₂ was significantly lower during the last minute of exercise in B10 than during SB (p ≤ 0.01). In addition there were significant differences between SB and B10 in PCO₂, measured during the last minute of exercise and immediately after it (p ≤ 0.01).

Discussion

In accordance with previous studies, RBF during exercise produced a marked reduction in V̇E. V̇E measured at the end of exercise was 49% lower in B10 than SB (Figure 1). With a similar breathing frequency reduction during cycle ergometry, Yamamoto et al. (1987) and Sharp et al. (1991) obtained a smaller reduction of V̇E. However, different testing protocols and intensities of exercise with RBF were used in these studies. Yamamoto et al. (1987) showed a reduction of 30% in V̇E during an interval test with RBF (30 s of exercise at 210 W with RBF alternating with 30 s rest intervals with spontaneous breathing). Sharp et al. (1991) measured a reduction of 25% in V̇E during 8 min of exercise at an intensity above the lactate threshold due to RBF. In the studies of RBF during front crawl swimming, a similar reduction in V̇E, as it was obtained in the present study, was observed with taking a breath every sixth (Town and Vanness, 1990) or eighth (West et al., 2005) stroke cycle as compared to taking a breath every second stroke cycle. However, after cessation of B10, when spontaneous breathing was allowed, V̇E dramatically increased to a peak in the 20th s of recovery. Thereafter, it decreased to the resting values. In contrast, V̇E immediately began to decrease to the resting values after cessation of exercise with SB.

Table 3. Comparison of the PO₂ (kPa) and PCO₂ (kPa) values measured during the last minute of exercise, immediately following exercise, and during the third minute of recovery between the two different breathing conditions.

<table>
<thead>
<tr>
<th>parameter</th>
<th>SB</th>
<th>B10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO₂</td>
<td>11.3 (0.6)</td>
<td>8.7 (1.2) **</td>
</tr>
<tr>
<td>PCO₂</td>
<td>11.8 (1.4)</td>
<td>10.9 (0.9)</td>
</tr>
<tr>
<td>PO₂</td>
<td>12.4 (1.0)</td>
<td>12.5 (1.2)</td>
</tr>
<tr>
<td>PCO₂</td>
<td>5.2 (0.8)</td>
<td>7.3 (0.5) **</td>
</tr>
<tr>
<td>PO₂</td>
<td>5.1 (1.0)</td>
<td>5.9 (1.1) **</td>
</tr>
<tr>
<td>PCO₂</td>
<td>5.0 (0.5)</td>
<td>5.1 (0.5)</td>
</tr>
</tbody>
</table>

** denote p ≤ 0.01 between the exercises in two different breathing conditions.

During the initial 20 s of recovery after B10, the subjects breathed an average 26.4 liters of air compared to 20.5 liters after exercise with SB. This marked hyperventilation influenced changes in other respiratory and blood parameters. As expected, at the end of exercise, RBF resulted in lower PETO₂, SaO₂ and PO₂, when compared to spontaneous breathing during exercise (Figure 2 and 4, Table 3). These data were in accordance with previous studies, which measured these parameters during different cycling exercises with RBF (Kapus et al., 2007; Sharp et al., 1991; Yamamoto et al., 1987). Due to different testing protocols, Yamamoto et al. (1987) and Kapus et al. (2007) reported higher values of SaO₂ than that were measured at
the end of B10. The intensity (subject’s peak power output) and duration (exercise to exhaustion) of B10 were maximal for each subject. Considering that, low values of SaO₂ (83% (8%)) at the end of exercise with RBF were expected in the present study. The results confirmed severe hypoxia during B10. However, hyperventilation after the cessation of B10 induced a rapid recovery of PETO₂, SaO₂, and PO₂. Lower values of O₂ were detected only during the initial 16 s and 20 s after B10 in comparison to SB, measured by SaO₂ (Figure 4) and PETCO₂ (Figure 2) respectively. However, there were no significant differences in PO₂ between SB and B10 measured immediately after the exercise (Table 3). The delay between the subjects’ cessation of the exercise and the first measurement did not exceed 15 s. Nonetheless, this delay was apparently too long to detect hypoxia with measurement of PO₂ after B10. According to our experience, this delay is longer for field testing such as swimming tests in the swimming pool. Considering that, the time of measurement may be the reason why previous studies failed to demonstrate a reduction in PO₂ due to RBF during swimming (Kapus et al., 2002; 2003).

Figure 1. There were significant differences between SB and B10 in VE measured at the end of exercise and during recovery (ANOVA for repeated measures, p ≤ 0.01). The statistically analysed results for comparison at defined time during recovery are marked with standard deviations (paired t test). ** denotes p ≤ 0.01 between the two different breathing conditions.

In accordance with previous studies (Dicker et al., 1980; Kapus et al., 2007; Peyrebrune et al., 2002; Sharp et al., 1991; Town and Vanness, 1990; West et al., 2005, Yamamoto et al., 1987), RBF produced hypercapnia, as evidenced by higher PETCO₂ and PCO₂ in B10 than during SB. Yamamoto et al. (1987) found that arterial partial pressure of carbon dioxide (Paco₂) and hydrogen ion concentration ([H⁺]) continuously increased to the end of an interval test with RBF. Using 8 min of exercise with RBF at an intensity 10 % above lactate threshold workload, Sharp et al. (1991) reported similar results. They concluded that RBF during exercise caused respiratory acidosis at exercise intensities that were not associated with [H⁺] disturbance during unreduced VE. Considering that, it was suggested that the combination of severe hypercapnia, respiratory acidosis and metabolic acidosis was the possible reason for earlier fatigue during exercise at higher intensities, when RBF was used (Kapus et al., 2003). During recovery from exercise, PETCO₂ remained elevated in the B10 trial compared to the SB trial (Figure 4), even after PETO₂ had normalized (Figure 2). Lee et al. (1990) reported a reduction in VCO₂ during exercise with RBF, and a subsequent increase during recovery. They suggested that CO₂ was retained in muscle, plasma and erythrocytes during exercise with RBF and that it was released from these stores during recovery. It seemed that despite hyperventilation during recovery, hypercapnia could be detected by measuring blood gas parameters within 15 s after the exercise with RBF.

Figure 2. There were significant differences between SB and B10 in PETO₂ measured at the end of exercise and during recovery (ANOVA for repeated measures, p ≤ 0.01). The statistically analysed results for comparison at defined time during recovery are marked with standard deviations (paired t test). ** denotes p ≤ 0.01 between the two different breathing conditions.

Figure 3. There were significant differences between SB and B10 in SaO₂ measured at the end of exercise and during recovery (ANOVA for repeated measures, p ≤ 0.01). The statistically analysed results for comparison at defined time during recovery are marked with standard deviations (paired t test). * denote p ≤ 0.05 between the two different breathing conditions.
**Figure 4.** There were significant differences between SB and B10 in PETCO₂ measured at the end of exercise and during recovery (ANOVA for repeated measures, p ≤ 0.01). The statistically analysed results for comparison at defined time during recovery are marked with standard deviations (paired t-test).

* and ** denote p ≤ 0.05 and 0.01 respectively between the two different breathing conditions.

**Possible study limitations:** Ideally blood gases should be obtained in arterial blood. However, indwelling arterial catheters for sampling arterial blood are not always feasible and desirable. Considering that, some indirect methods were used to assess blood gases in the present study. Therefore, the degree to which the actual measurements provide an accurate proxy for arterial measures should be considered. Arterial blood gases (PₐO₂ and PₐCO₂) during exercise could be estimated by using arterialized earlobe blood samples (PₑO₂ and PₑCO₂). Some previous studies found that arterialized earlobe blood samples are in good agreement with arterial blood samples for partial pressure of carbon dioxide, but not for partial pressure of oxygen (Dall’Ava-Santucci, 1996; Fajac et al., 1998; McEvoy and Jones, 1975). During exercise, PₑO₂ was lower than PₐO₂ on average 0.23 kPa (McEvoy and Jones, 1975), 0.63 kPa (Fajac et al., 1998) and 1.2 kPa (Dall’Ava-Santucci, 1996). The main cause of underestimation of PₑO₂ in earlobe samples could be insufficient arterIALIZation of blood due to venous admixture. The earlobe method requires adequate blood flow in the earlobe to enable a sufficient volume of blood to be sampled without additional external pressure during sampling. This was the reason for the delay of up to 15 s between the subject’s cessation of the exercise and the first measure in the present study. In addition, measurement of end-tidal pressure of carbon dioxide (PETCO₂) has been used to estimate PₑCO₂ at rest and during exercise. Most comparative studies have concluded that PETCO₂ provides good index of PₑCO₂ at rest (Jones et al., 1979, Williams and Babb, 1997). However, during exercise, the differences between PₑCO₂ and PₛCO₂ were 0.3 kPa (0.3 kPa) (Williams and Babb, 1997) and 0.4 kPa (0.3 kPa) (Robbins et al., 1990). These differences increased at a higher workload and with increasing tidal volume (Jones et al., 1979). Ear pulse oximeters are often used to provide a non-invasive, continuous estimate of the oxyhemoglobin saturation of arterial blood (SaO₂). In most previous validation studies, ear pulse oximeter estimates during exercise have been shown to be accurate predictors of SaO₂ at least when saturation is above 85% in non-smoking subjects (Mengelkoch et al., 1994, Smyth et al., 1986, Powers et al., 1989, Martin et al., 1992). Considering ear pulse oximeters of Datex-Ohmeda, differences between estimated and measured (via blood sampling) SaO₂ values were 0.87 % (2.6 %), 0.59 % (2.4 %) (Martin et al., 1992) and -0.57 % (1.78 %) (Powers et al., 1989). Thus, the error in the pulse oximeter is not likely greater than 1 %, while significant differences between SB and B10 in SaO₂ were between 5 and 10 % during the initial 16 s of recovery.

**Conclusion**

In conclusion, reduced breathing frequency during high intensity exercise results in hypoxia, however, due to marked post-exercise hyperventilation, measurements of blood gas parameters analyzed 15 s after the cessation of exercise did not accurately reflect the condition during exercise.

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**References**


### Authors Biography

#### Jernej Kapus
**Employment** Assistant, University of Ljubljana, Faculty of Sport
**Degree** PhD
**Research interests** Breathing during swimming, swimming with controlled breathing frequency, training.
**E-mail:** nejc.kapus@fsp.uni-lj.si

#### Anton Ušaj
**Employment** Professor, University of Ljubljana, Faculty of Sport
**Degree** PhD

#### Venčeslav Kapus
**Employment** Associate Professor, University of Ljubljana, Faculty of Sport
**Degree** PhD
**Research interests** Swimming, expert modelling.
**E-mail:** venec.kapus@fsp.uni-lj.si

#### Boro Strumbelj
**Employment** Assistant Professor, University of Ljubljana, Faculty of Sport
**Degree** PhD
**Research interests** Breathing during swimming, acid-base regulation.
**E-mail:** boro.strumbelj@fsp.uni-lj.si

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#### Key points
- In some sports, the environment is inappropriate for direct measurement of respiratory and blood gas parameters during exercise. To overcome this problem, extrapolation from post-exercise measurements has often been used to estimate changes in respiratory and blood gas parameters during exercise.
- The possibility of hypoxia and hypercapnia during exercise with reduced breathing frequency has been tested by measuring capillary blood sampled after the exercise.
- Reduced breathing frequency during high intensity exercise results in hypoxia; however, due to marked post-exercise hyperventilation, measurements of blood gas parameters taken 15 s after the cessation of exercise did not yield any changes in these parameters.
- Despite hyperventilation during recovery, hypercapnia could be detected by measuring blood gas parameters within 15 s after the exercise with reduced breathing frequency.

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Jernej Kapus
University of Ljubljana, Faculty of Sport, Laboratory of Biodynamics, Slovenia
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