The Effect of Additional Dead Space on Respiratory Exchange Ratio and Carbon Dioxide Production Due to Training

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Abstract

The purpose of the study was to investigate the effects of implementing additional respiratory dead space during cycloergometry-based aerobic training. The primary outcome measures were respiratory exchange ratio (RER) and carbon dioxide production (VCO_2). Two groups of young healthy males: Experimental (Exp, n = 15) and Control (Con, n = 15), participated in this study. The training consisted of 12 sessions, performed twice a week for 6 weeks. A single training session consisted of continuous, constant-rate exercise on a cycle ergometer at 60% of VO_2max which was maintained for 30 minutes. Subjects in Exp group were breathing through additional respiratory dead space (1200ml), while subjects in Con group were breathing without additional dead space. Pre-test and two post-training incremental exercise tests were performed for the detection of gas exchange variables. In all training sessions, pCO_2 was higher and blood pH was lower in the Exp group (p < 0.001) ensuring respiratory acidosis. A 12-session training program resulted in significant increase in performance time in both groups (from 17"29 ± 1"31 to 18"47 ± 1"37 in Exp; p=0.02 and from 17"20 ± 1"18 to 18"45 ± 1"44 in Con; p = 0.02), but has not revealed a significant difference in RER and VCO_2 in both post-training tests, performed at rest and during submaximal workload. We interpret the lack of difference in post-training values of RER and VCO_2 between groups as an absence of inhibition in glycolysis and glycogenolysis during exercise with additional dead space.

Key words: Additional dead space, hypercapnia, respiratory acidosis, aerobic training, exercise physiology.

Introduction

Breathing through additional dead space has been a widely used intervention strategy in varying areas of physiology. For example, it has been used in studies to determine the effects of hypercapnia on long term modulation of pulmonary ventilation (Cathcart et al., 2005; Sumners and Turner, 2003), as well as the effects of respiratory muscle training on muscle endurance (Koppers et al., 2006), effectiveness of interventions for sleep disorders (Khayat et al., 2003) and alterations in blood morphology following training in professional cyclists (Zaton et al., 2010). Rebreathing one's own air, referred to as additional dead space, is a known way to increase blood carbon dioxide pressure (pCO_2) without the need for using complex devices (Khayat et al., 2003; Koppers et al., 2006; McParland et al., 1991; Smejkal et al., 1989; Toklu et al. 2003). The additional dead space volume determines the level of increase in pCO_2 and accompanying respiratory acidosis. Enhanced pCO_2 and the respira-

tory acidosis have been studied to examine their influence on changes in respiratory function (Cathcart et al., 2005, Maruyama et al., 1988; Poon 1989), blood flow distribution (Howden et al., 2004; Ogoh et al., 2009,), muscle contractility (Mador et al., 1997; Vianna et al., 1990) and metabolic pathways of energy production (Graham et al., 1980; 1982; 1986; Graham and Wilson 1983; Kato et al., 2005; McLellan, 1991; Østergard et al., 2012).

Under most conditions enhanced pCO_2 with the accompanying respiratory acidosis will cause a decrease in plasma lactate concentration (LA) during and after a bout of exercise. During an incremental test (Graham et al., 1980; McLellan, 1991) or a constant work test (Ehrsam et al., 1982; Graham et al., 1982; Graham and Wilson 1983, Ishida et al., 1988; Østergard et al., 2012) breathing air with enhanced CO_2 content is accompanied by significant blood pH reduction and decrease in lactate plasma concentration. In the above cited studies, a decrease in plasma lactate concentration occurred despite no significant changes in performance time in an incremental test or workload in a constant work test. The decrease in lactate plasma concentration is mainly associated with enhanced blood hydrogen ion concentration. Likewise, metabolic acidosis (with no changes in carbon dioxide pressure) has been shown to lower lactate concentration (Hollidge-Horvat et al., 1999; Jones et al., 1977; Zoladz et al. 1998). The decrease of lactate concentration might be caused by an impaired lactate transport out of the muscle, increased lactate oxidation (Graham et al., 1986) or decreased lactate production due to the inhibition of glycogenolysis and glycolysis (Graham et al., 1986; Hollidge-Horvat et al., 1999). Glycogenolysis and glycolysis inhibition have been confirmed directly in a study with respiratory acidosis on animals (Graham et al., 1986) and with metabolic acidosis in humans (Hollidge-Horvat et al., 1999). Hollidge-Horvat and colleagues induced metabolic acidosis and reported a significant decrease in glycogen utilization, suppression of phosphofructokinase and pyruvate dehydrogenase activity and decrease in pyruvate production (Hollidge-Horvat et al., 1999).

Decreased respiratory exchange ratio (RER) (Ehrsam et al., 1982; Graham and Wilson, 1983; Graham et al., 1982; Graham et al. 1980; Østergard et al., 2012) or carbon dioxide production (VCO_2) (McLellan, 1991; Østergard et al., 2012) have been reported in studies on respiratory acidosis. These changes in RER were recently confirmed using more sophisticated instrumentation. Østergard and colleagues have shown that RER decreased from 0.98 ± 0.04 to 0.85 ± 0.04, recorded during the last minute of a 6-minute exercise test, performed with the

intensity of 80%VO$_2$max (Østergard et al., 2012). Likewise, metabolic acidosis has been shown to lower RER significantly (Hollidge-Horvat et al., 1999). The decrease of plasma lactate concentration and the decrease in respiratory exchange ratio, provoked by respiratory acidosis, may suggest a substrate shift towards increased lipid utilization (Graham et al., 1980; 1982; Graham and Wilson, 1983; McLellan, 1991; Kato et al., 2005; Østergard et al., 2012). If the respiratory acidosis inhibits glycolysis even in a single session it would be reasonable to expect that a similar or enhanced adaptation would be observed due to multiple training sessions and that the aerobic effects would be accelerated. No studies so far have examined the additive effects of multiple training sessions on respiratory acidosis. Hence, the purpose of this study was to investigate the effects of implementing an additional 1200ml of dead space volume on respiratory exchange ratio and carbon dioxide production following 12, 30-minute sessions of aerobic training using cycle ergometry. We hypothesized that the addition of dead space volume during moderate intensity aerobic training will result in decreased RER and VCO$_2$ measurements in comparison to sessions without adding dead space.

Methods

Subjects
Thirty healthy males were recruited for this study. Subjects led active lifestyles (70 ± 13 minutes per week) but were not competing in any sports. Written consent was obtained from each subject upon explaining the purpose and associated risks of the study protocol. The experiment was approved by Ethics Committee at University School of Physical Education in Wroclaw (Poland).

Experimental protocol
An a-priori randomization was established. Based on that scheme, subjects were assigned to two groups: experimental (Exp, n = 15) and control (Con, n = 15). Participants were ranked from 1 to 30 on the basis of time performance in pre-training test. Men with odd number rankings were assigned to Con and men with even number rankings were assigned to Exp.

Each test and training session was performed in an air conditioned chamber at a temperature of 24ºC, relative humidity of 50% and barometric pressure at 762.75 mmHg. Subjects were instructed to refrain from caffeine, alcohol, and any exercise for 24 hours prior to the testing and training sessions.

Pre-training test
An incremental exercise test until exhaustion constituted the pre-training test. The test was conducted on an electrically braked cycle ergometer (Excalibur, Lode, Netherlands). Exhaustion was defined as the subject refusing to continue the test due to fatigue. The workload, starting from 50 Watt (W), was incrementally increased by 50W every three minutes. The subjects were instructed to keep a constant pedaling rate between 60 and 90rpm. To assure constant workload, pedaling rate was coupled with resistance, i.e., when the rate of pedaling increased the resistance decreased. Gas-exchange (Quark Gas Analyzer, Cosmed, Italy) was recorded continuously beginning three minutes prior to the test, throughout the test and five minutes following completion of the test. Breath-by-breath gas-exchange was averaged every 15 seconds and saved for further analyses.

Training sessions
One week following completion of the Pre-test, all participants began the training period. The training period consisted of twelve cycloergometric (Ergomedic 874E, Monark, Sweden) training sessions. Each training session was carried out at the same time of day, twice a week, three days apart, for six weeks. A single training bout consisted of continuous, constant-rate exercise on a cycle ergometer at 60% of VO$_2$max and maintained for 30 minutes. Subjects in Exp group starting from the third minute before exercise until the fifth minute after exercise were breathing through additional respiratory dead space (1200ml), while subjects in Con group were breathing without additional dead space. Additional dead space was created by a mask, covering nose and mouth, connected with plastic tube of 3 cm diameter. The volume of 1200ml of additional dead space was chosen based upon experience gained in preliminary study. Breathing through additional dead space of 1200ml induced an increase in exercise pCO$_2$ above 45mmHg (hypercapnia) and allowed the subjects to maintain the intensity of 60% of VO$_2$max for 30minutes.

Immediately before and two minutes after, every other training session, each subject undertook fingertip' arterialized blood sampling. One part of each sample was taken for the detection of pCO$_2$ and blood pH, using a heparinized 80μl capillary tube. An additional 20μl of the blood was placed in Eppendorf tubes, containing 0.18 ml of isotonic solution of sodium fluoride and sodium chloride. Blood carbon dioxide pressure and blood pH were determined by using Bayer Rapidlab 248 (Siemens Medical Solutions, Germany).To obtain blood plasma, blood was separated by centrifugation (3000 rpm) then 10μl of supernatant was used to determine LA enzymatically (Sentinel Diagnostics, Italy; EL 240, BIO-TEK™ Instruments, USA).

During every second training session, breath-by-breath respiratory gas analysis (Exp, n = 8; Con, n = 8) was measured. The measurements were continuously recorded beginning at three minutes before the test until fifth minute after exercise. Collected respiratory data were averaged every 15 seconds and saved for further analyses. The implementation of the dead space device required modification in the placement of gas sampling line. During breathing through a tube, the gas sampling line was placed at the end of the tube.

Post-training tests
Two post-training testing sessions were implemented. First, an early follow-up, took place four to six days, after completing the training period, and second, a delayed follow-up took place four weeks after the last training session. The post training incremental tests were the same as described for the pre-training test.

Statistical analysis

All data were processed by means of Statistica software (Statistica 9, StatSoft, USA). Presumption analysis was carried out using the Mauchly's sphericity test followed by, if needed, Greenhouse-Geisser and Huynh-Feldt correction tests. Data obtained during training sessions were analyzed using multivariate analysis of variance (ANOVA) (2 groups x 6 measures). Primary and secondary outcome measures obtained in pre-training and two post-training tests were analyzed multivariate analysis of variance (ANOVA) (2 groups x 3 measures). To compare means in the data obtained during training sessions and in pre-training and two post-training tests, the Duncan post hoc test was used. Significance level was set at $p < 0.05$.

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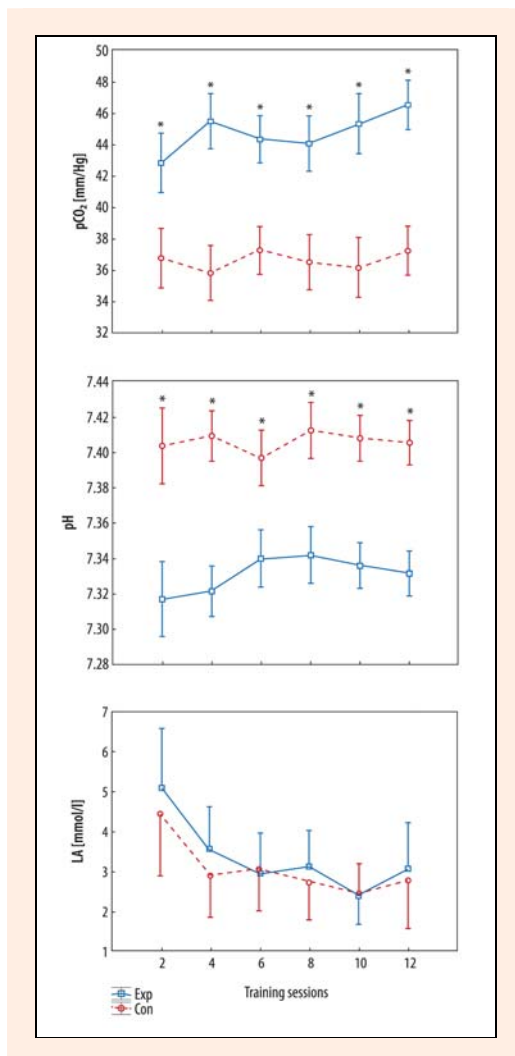

Figure 1. Exercise carbon dioxide pressure (pCO_2), blood pH and plasma lactate concentration (LA) during training sessions 2, 4, 6, 8, 10 and 12. Data were collected two minutes after a particular training session. Squares represent the experimental group (Exp, n=15), and circles the control group (Con, n=15). Each point represents the mean ± one SD. For graphic readability the data points were connected with solid line (Exp) and dashed line (Con). * denotes $p < 0.05$ between groups.

Results

There were no statistically significant differences in age, body height and weight between groups (Table 1).

Evidence for inducing respiratory acidosis

Exercise pCO_2, blood pH and LA in consecutive training sessions are shown in Figure 1. The values of pCO_2 ranged from 46.5 ± 1.7 mmHg to 42.9 ± 2.1 mmHg in Exp group and from 37.3 ± 1.7 mmHg to 35.9 ± 1.8 mmHg in

Con group. pCO_2 was significantly higher in Exp than in Con ($p < 0.001$) during all training sessions. Blood pH ranged from 7.342 ± 0.016 to 7.317 ± 0.023 in Exp and from 7.412 ± 0.016 to 7.397 ± 0.016 in Con. Blood pH was significantly lower in Exp than in Con ($p < 0.001$) during all training sessions. There were no statistically significant differences between Exp and Con in LA.

Table 1. Subject characteristics.

	Experimental (n=15)	Control (n=15)
Age (years)	20.2 (.9)	20.1 (.3)
Height (m)	1.79 (.05)	1.77 (.05)
Weight (kg)	73.6 (7.4)	72.4 (8.6)
VO$_2$ max (ml·min^{-1}·kg^{-1})	49.5 (6.6)	49.5 (4.9)

Data are presented as mean (± SD). No significant differences between the groups.

Exercise RER, in consecutive training sessions, are shown in Figure 2. There were no significant differences in exercise RER between groups except during the 10th training session, when the exercise RER was significantly lower in Exp group (0.89 ± 0.06) than in the Con group (0.97 ± 0.06); $p = 0.04$.
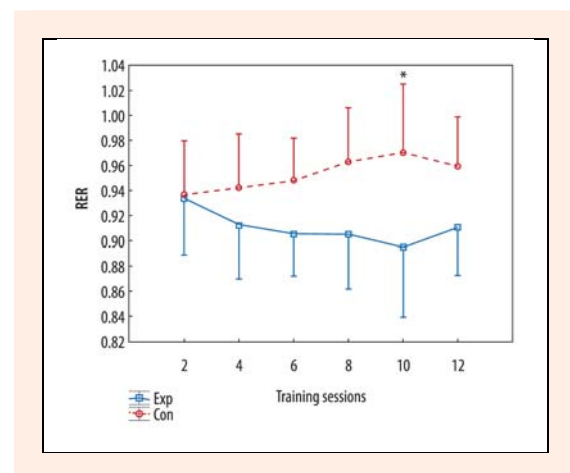

Figure 2. Exercise respiratory exchange ratio (RER) during training sessions 2, 4, 6, 8, 10 and 12. Data were collected during each identified training session and are presented as averaged value between the third and 30th minute of exercise. Squares represent the experimental group (Exp, n=8), and circles the control group (Con, n=8). Each point represents the mean ± one SD. For graphic readability the data points were connected with solid line (Exp) and dashed line (Con). * signifies statistical significance between groups. Significance level was set to $p<0.05$.

Resting RER, VCO_2, oxygen uptake (VO_2), during breathing without tube and during breathing through a tube of 1200ml, are shown in Table 2. Breathing through the additional dead space caused a significant decrease in RER ($p = 0.01$) and VCO_2 ($p = 0.04$). There were no statistically significant differences between breathing through a tube and breathing without the tube in VO_2.

Primary outcome measures: RER, VCO_2

Post training changes of RER and VCO_2 are presented in Table 3 and Table 4. There were no significant difference in RER between Exp and Con, at rest and during sub-

maximal workload, in pre-test and both post-training tests (4-6-days and 4-weeks). RER changed significantly between tests only in the Con group. RER, increased significantly between pre-test and 4-6 days post-training test at rest ($p = 0.03$) and at 50W ($p = 0.04$).

Table 2. Resting respiratory exchange ratio (RER), oxygen uptake (VO$_2$), carbon dioxide production (VCO$_2$) during breathing for three minutes in two conditions: without tube and through a tube (1200ml). During breathing through additional dead space, gas sampling line was placed at the terminal end of the tube. Tests were conducted before training session number 6, on 8 randomly selected subjects. Data are presented as mean (± one SD).

Parameter	Breathing without tube	Breathing with the tube of 1200 ml
RER	.89 (.09)	.68 (.05) **
VO$_2$ (ml·min^{-1})	575 (96)	567 (103)
VCO$_2$ (ml·min^{-1})	571 (143)	382 (75) *

* $p < 0.05$, ** $p < 0.01$.

Table 3. Respiratory exchange ratio (RER) during rest and between 50W and 250W in consecutive incremental exercise tests. Data are presented as mean (± one SD).

Incremental exercise test		Experimental (n=15)	Control (n=15)
REST	Pre	.90 (.07)	.84 (.07)
	4-6 days Post	.93 (.07)	.93 (.13) *
	4 weeks Post	.91 (.12)	.89 (.11)
50W	Pre	.84 (.05)	.81 (.06)
	4-6 days Post	.88 (.06)	.88 (.08) *
	4 weeks Post	.84 (.08)	.84 (.09)
100W	Pre	.89 (.06)	.85 (.04)
	4-6 days Post	.88 (.05)	.89 (.05)
	4 weeks Post	.88 (.07)	.89 (.05)
150W	Pre	.96 (.06)	.93 (.05)
	4-6 days Post	.94 (.05)	.95 (.06)
	4 weeks Post	.96 (.08)	.96 (.04)
200W	Pre	1.01 (.05)	.99 (.04)
	4-6 days Post	1.00 (.04)	1.01 (.06)
	4 weeks Post	1.03 (.09)	1.02 (.06)
250W	Pre	1.08 (.06)	1.07 (.05)
	4-6 days Post	1.07 (.05)	1.08 (.06)
	4 weeks Post	1.10 (.09)	1.10 (.08)

* denotes $p < 0.05$ between pre-test and 4-6 days post-test. There was no statistical difference between groups.

There were no significant differences in VCO$_2$ between Exp and Con, at rest and during submaximal workload, in both post-training tests (4-6 days and 4 weeks). There were two significant differences in VCO$_2$ between Exp and Con at 100W ($p = 0.04$) and at 150W ($p = 0.03$) in pre-test. VCO$_2$ changed significantly between tests only in the Con group. VCO$_2$ decreased significantly between 4-6 days post-training and 4 weeks post-training test at 50W ($p = 0.04$).

Secondary outcome measures: Performance time, maximal oxygen uptake (VO$_2$max) and maximal oxygen uptake in ml·min^{-1}·kg^{-1} (VO$_2$ max/kg)
Post training changes of performance time, VO$_2$ max and VO$_2$ max/kg are presented in Table 5. There were no significant differences in performance time, VO$_2$ max and VO$_2$ max/kg, between Exp and Con groups, in pre-test and both post-training tests (4-6 days and 4 weeks).

Table 4. Carbon dioxide production (VCO$_2$) during rest and between 50W and 250W in consecutive incremental exercise tests. Data are presented as mean (± one SD).

Incremental exercise test		Experimental (n=15)	Control (n=15)
REST	Pre	459 (125)	426 (158)
	4-6 days Post	533 (168)	490 (126)
	4 weeks Post	484 (131)	433 (105)
50W	Pre	981 (137)	931 (185)
	4-6 days Post	998 (186)	956 (137)
	4 weeks Post	909 (165)	853 (166) †
100W	Pre	1452 (120)	1318 (161)*
	4-6 days Post	1387 (228)	1328 (114)
	4 weeks Post	1320 (184)	1276 (149)
150W	Pre	1992 (111)	1844 (158) *
	4-6 days Post	1915 (223)	1845 (123)
	4 weeks Post	1863 (219)	1814 (135)
200W	Pre	2561 (140)	2436 (194)
	4-6 days Post	2521 (221)	2398 (121)
	4 weeks Post	2486 (308)	2402 (173)
250W	Pre	3283 (197)	3133 (228)
	4-6 days Post	3195 (214)	3050 (208)
	4 weeks Post	3203 (385)	3061 (274)

* denotes $p < 0.05$ between the groups. † denotes $p < 0.05$ between 4-6 days post-test and 4 weeks post-test.

Performance time increased significantly between pre-test and 4-6 days post-training test from 17"29 ± 1"31 to 18"47 ± 1"37 in Exp ($p = 0.02$) and from 17"20 ± 1"18 to 18"45 ± 1"44 in Con ($p = 0.02$). Performance time decreased significantly between 4-6 days post-training test and 4 weeks post-training test from 18"47 ± 1"37 to 18 ± 1"47 in Exp ($p = 0.04$) and from 18"45 ± 1"44 to 17"54 ± 1"56 in Con ($p = 0.03$).

Table 5. Performance time and maximal values of: oxygen uptake (VO$_2$, ml·min^{-1}), oxygen uptake in ml·min^{-1}·kg^{-1} (VO$_2$/kg) in consecutive incremental exercise tests.

Incremental exercise test		Experimental (n=15)	Control (n=15)
Performance time [min]	Pre	17"29 (1"31)	17"20 (1"18)
	4-6 days Post	18"47 (1"37)*	18"45 (1"44)*
	4 weeks Post	18"00 (1"47)†	17"54 (1"56)†
VO$_2$ max (ml·min^{-1})	Pre	3633 (508)	3578 (332)
	4-6 days Post	3816 (587)	3569 (503)
	4 weeks Post	3593 (500)	3486 (478)
VO$_2$ max/kg (ml·min^{-1}·kg^{-1})	Pre	49.5 (6.6)	49.5 (4.9)
	4-6 days Post	52.0 (7.3)	49.6 (6.6)
	4 weeks Post	49.0 (6.3)	48.0 (5)

* denotes $p < 0.05$ between pre-test and 4-6 days post-test. † denotes $p < 0.05$ between 4-6 days post-test and 4 weeks post-test. There was no statistical difference between groups.

Discussion

This study examined the longitudinal effect of implementing additional dead space on RER and VCO$_2$. Additional dead space of 1200ml did not induce changes in RER and VCO$_2$ following 12, 30-minute, training sessions.

Our experiment supports the findings of several prior studies by showing that RER and VCO$_2$ were decrease as a result of exercise induced respiratory acidosis (Ehrsam et al., 1982; Graham and Wilson, 1983; Graham et al., 1980; 1982; McLellan 1991; Østergard et al., 2012). RER was consistently lower in the experimental group but reached significance during the 10th training session only.

In previous investigations, the decrease in RER due to respiratory acidosis has been interpreted as a substrate shift towards increased lipid utilization (Graham et al., 1980; 1982, Graham and Wilson, 1983; Kato et al., 2005; McLellan, 1991; Østergard et al. 2012). We question this interpretation on the grounds of the two arguments. First, the tendency to decrease RER in respiratory acidosis may be related to positioning of the gas sampling line of the gas exchange device (Gayda et al., 2010). Gayda and colleagues have shown that lengthening the breathing route, between the month and gas sampling line, lowers VCO_2, which decreases RER (Gayda et al., 2010). Second, as suggested by Østergard and colleagues, the decreases in RER and VCO_2 in respiratory acidosis are also caused by CO_2 retention in lungs (Østergard et al., 2012). Inhaling hypercapnic gas or rebreathing one's own air results in enhanced alveoli CO_2 pressure, which decreases the CO_2 pressure gradient. In turn, the decreased CO_2 pressure gradient results in a decreased CO_2 flow through pulmonary alveoli, thus the value of VCO_2 parameter is decreased. Moreover, our experiment confirmed a decrease in RER and VCO_2 during breathing through a tube even during rest. The decrease in these parameters was observed immediately after the subjects begun to breathe through a tube. We assert that it is unreasonable to expect that the decrease in RER and VCO_2 might have been caused by substrate shift towards increased lipid utilization during the period when the subjects removed the standard face mask and begun to breathe through a tube. Thus, we suggest that either single exercise session or multiple exercises sessions do not cause the substrate shift towards increased lipid utilization.

Breathing through additional dead space induced an increase in pCO_2 and a decrease in blood pH ($p < 0.01$) in all training sessions. The level of respiratory acidosis is similar to the changes in pCO_2 and blood pH obtained in previous studies and corresponds to breathing hypercapnic air with: 5% CO_2 content and light exercise in the study of Ehrsam et al. (1982), 4% CO_2 content and intensity of 65% VO_2max in the studies of Graham et al. (1982) and Graham and Wilson (1983). However, we observed no decrease in LA in all training sessions. The lack of decrease in LA is contrary to previous studies (Graham et al., 1980; 1982; Graham and Wilson, 1983, Ehrsam et al., 1982; McLellan, 1991). However, there are some studies supporting our results. Østergard et al. (2012) have shown only very small and non-significant decrease of LA (from 5.5 ± 1.3 to 5.1 ± 1.7 mmol·l^{-1}). Moreover, Kato et al. (2005) showed no significant changes in LA after incremental tests, consisting of the same performance time. We interpret the lack of decrease in LA on the grounds of three arguments. First, the lack of decrease in LA might have occurred due to the insufficient level of respiratory acidosis. Nonetheless, the lack of decrease in LA was also observed in studies of Kato et al. (2005) and Østergard et al. (2012), despite higher levels of respiratory acidosis than that in our study. In the above cited studies the increase in pCO_2 and a decrease in blood pH are greater than in other similar studies (Ehrsam et al., 1982; Graham et al., 1980; 1982; Graham and Wilson, 1983) and our current study. Thus, we assumed that the

LA does not directly depend on severity of respiratory acidosis. Second, epinephrine and bicarbonate ion concentrations may have an influence on LA (Ehrsam et al., 1982; Hollidge – Horvat et al., 1999). As determined in the study by Ehrsam et al. (1982), subjects with the increase in plasma epinephrine showed no decrease in LA. Furthermore, the suppression of decrease in bicarbonate ion concentration, which occurs during respiratory acidosis, is known to increase the lactate efflux out of the muscle (Ehrsam et al., 1982; Hollidge – Horvat et al., 1999). On the contrary, metabolic acidosis is known to suppress lactate efflux from the intracellular to the extracellular compartment (Hollidge- Horvat et al., 1999; Spriet et al., 1985; Sutton et al., 1981). Third, the lack of decrease in LA occurred due to absence of inhibition in glycolysis and glycogenolysis. We did not include tissue analysis in this study. A tissue analysis might have confirmed the presumptions of the lack of inhibition of glycolysis and glycogenolysis. However, this argument is supported by the absence of longitudinal changes in RER and VCO_2. Moreover, there is one study in the area of respiratory acidosis which directly confirms the inhibition of glycolysis and glycogenolysis in a single exercise. This animal study has shown an inhibition of glycolysis and glycogenolysis in respiratory acidosis dose following electrically stimulating a denervated gastrocnemius-plantaris muscle in anesthetized dogs (Graham et al., 1986). However the RER and VCO_2 may be substantially different during electrical stimulation of a single muscle in an anesthetized mammal, than during exercise involving whole body muscular and cardiorespiratory systems in humans.

There are some limitations which need to be discussed. First, number of training sessions (12) and training duration (30minutes) were lower than those (24 sessions, 45 minutes) in most other studies with moderate intensity training protocols (Malek et al., 2006; Schrauwen et al., 2002; Shono et al., 2002). The number of training sessions is the same as in the study on longitudinal effect of training with respiratory acidosis combined with hypoxia (Woorons et al., 2008). The smaller number of training sessions and shorter training duration might have contributed to the changes in outcome measures. However, the use of our training protocol provided significant improvement in performance time in incremental tests. The increase in performance time demonstrates the increase in physical capacity (Bentley et al., 2007). The training intensity used in our study (60% of VO_2max) corresponds to the workload used in previous studies on respiratory acidosis (Ehrsam et al. 1982; Graham and Wilson, 1983; Graham et al., 1982). On the other hand, using higher training intensity might have contributed to the greater changes in outcome measures. However, the increase in training intensity is limited due to hydrogen ion tolerance. Hydrogen ion tolerance is lowered due to respiratory acidosis. Thus, we assume that the increase in training intensity would likely decrease training duration (Debold et al., 2008; Jonville et al., 2002; Mador et al., 1997; Ueno et al., 2002; Vianna et al., 1990). Second, it is unknown what the changes, in RER, would have been, if a higher level of respiratory acidosis had been induced. Higher level of respiratory acidosis may provide more

influence on the changes in RER and VCO$_2$. However, as we assumed, the range of increases in blood pCO$_2$ are limited by the individuals' tolerance to blood pCO$_2$. In all previous studies related to respiratory acidosis, only single exercise in respiratory acidosis has been used (Graham et al., 1980; 1982; Graham and Wilson, 1983; McLellan, 1991; Kato et al., 2005; Østergard et al. 2012). The tolerance to high pCO$_2$ in a single and short lasting exercise is higher than to repeated and long lasting exercises. During our preliminary studies, we had three subjects who did not complete the experiment due to recurring headaches. Eight subjects included in the current cohort reported headaches but completed the experiment. We suspect the occurrence of headaches may limit the increase of the level of respiratory acidosis. Moreover, increasing the volume of additional dead space may, in turn, cause hyperventilation. Hyperventilation causes a decrease in pCO$_2$, which minimizes the effect of respiratory acidosis. Third, we suggest that future experiments with metabolic acidosis need to be conducted. In metabolic acidosis, the substrate shift towards increased lipid utilization has already been confirmed using tissue analyses (Hollidge-Horvat et al., 1999).

Conclusion

In summary, the present study has examined the effects of implementing additional dead space volume on RER and VCO$_2$ following 12 training sessions. Additional dead space induced respiratory acidosis and caused the increase in pCO$_2$ and decrease in blood pH and did not induce changes in LA. The lack of change in RER and VCO$_2$ as a result of training with additional dead space supports the contention that glycolysis and glycogenolysis were not inhibited.

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Key points

- The purpose of the study was to investigate the effects of implementing additional respiratory dead space during cycloergometry-based aerobic training on respiratory exchange ratio and carbon dioxide production.
- In all training sessions, respiratory acidosis was gained by experimental group only.
- No significant difference in RER and VCO_2 between experimental and control group due to the trainings.
- The lack of difference in post-training values of RER and VCO_2 between groups means absence of inhibition in glycolysis and glycogenolysis during exercise with additional dead space.

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