

1 **Title:**

2 Inspiratory muscle training reduces blood lactate concentration during volitional hyperpnoea

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21 **Key Words:**

22 Respiratory muscle training, diaphragm, intercostal muscles, blood lactate concentration,  
23 hyperventilation.

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1 **Abstract**

2 Intense volitional hyperpnoea can increase blood lactate concentration ( $[\text{lac}^-]_{\text{B}}$ ), however,  
3 whether this is reduced following pressure-threshold inspiratory muscle training (IMT) is  
4 unknown. We hypothesised that volitional hyperpnoea at a breathing pattern specific to intense  
5 endurance exercise would increase  $[\text{lac}^-]_{\text{B}}$  and that specific IMT attenuate such a response. 22  
6 physically active males were matched for 85% maximal exercise minute ventilation ( $\dot{V}_{\text{E max}}$ )  
7 and divided equally into an IMT or a control group. Prior to and following a 6 week intervention,  
8 participants performed 10 min of volitional hyperpnoea at the breathing pattern commensurate  
9 with 85%  $\dot{V}_{\text{E max}}$ . The IMT group performed 6 weeks of IMT; the control group performed no  
10 IMT. Maximal inspiratory mouth pressure increased (mean  $\pm$  SD)  $31 \pm 22\%$  following IMT and  
11 was unchanged in the control group. Prior to the intervention in the control group,  $[\text{lac}^-]_{\text{B}}$   
12 increased from  $0.76 \pm 0.24 \text{ mmol}\cdot\text{L}^{-1}$  at rest to  $1.50 \pm 0.60 \text{ mmol}\cdot\text{L}^{-1}$  and in the IMT group from  
13  $0.85 \pm 0.40 \text{ mmol}\cdot\text{L}^{-1}$  at rest to  $2.02 \pm 0.85 \text{ mmol}\cdot\text{L}^{-1}$  following 10 min volitional hyperpnoea  
14 ( $P < 0.05$ ). Following the intervention the  $[\text{lac}^-]_{\text{B}}$  response to volitional hyperpnoea was  
15 unchanged in the control group. Conversely, following IMT,  $[\text{lac}^-]_{\text{B}}$  was reduced by  $17 \pm 37\%$   
16 and  $25 \pm 34\%$  following 8 and 10 min, respectively ( $P < 0.05$ ). In conclusion, increases in  $[\text{lac}^-]_{\text{B}}$   
17 during volitional hyperpnoea at 85%  $\dot{V}_{\text{E max}}$  were attenuated following IMT. These findings  
18 suggest that the inspiratory muscles were the source of at least part of this reduction, and provide  
19 a possible explanation for some of the IMT-mediated reductions in  $[\text{lac}^-]_{\text{B}}$  often observed during  
20 whole-body exercise.

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## 1 **Introduction**

2           Specific respiratory muscle training (RMT) can be performed using either voluntary  
3 isocapnic hyperpnoea (VIH), flow-resistive loading, or pressure-threshold loading; with the  
4 exception of VIH, these are commonly referred to as inspiratory muscle training (IMT).  
5 Ventilatory endurance is enhanced with all three techniques, whereas IMT also increases  
6 diaphragm thickness (Downey et al. 2007; Enright et al. 2006) and the maximal strength,  
7 shortening velocity and power of the inspiratory muscles (for a full review see McConnell and  
8 Romer 2004). Furthermore, well controlled studies have shown improvements in endurance  
9 exercise performance following both IMT (Gething et al. 2004; Griffiths and McConnell 2007;  
10 Johnson et al. 2007; Romer et al. 2002a; Volianitis et al. 2001) and VIH (Leddy et al. 2007).

11           The mechanisms underlying such performance improvements remain speculative but may  
12 include reduced perception of effort (Downey et al. 2007; Gething et al. 2004; Griffiths and  
13 McConnell 2007; Romer et al. 2002a; Verges et al. 2007; Volianitis et al. 2001) and possibly  
14 reductions in both diaphragm fatigue (Verges et al. 2007) and an associated metaboreflex that  
15 attenuates limb blood flow (McConnell and Lomax 2006; Witt et al. 2007). The notion that  
16 genuine physiological adaptation explains, in part, RMT-mediated improvements in endurance  
17 exercise performance is further supported by the frequently observed reduction in blood lactate  
18 concentration ( $[\text{lac}^-]_{\text{B}}$ ) during whole-body exercise following both IMT (Griffiths and  
19 McConnell 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Volianitis et al. 2001) and  
20 VIH (Leddy et al. 2007; Spengler et al. 1999). Furthermore, correlations have been reported  
21 between reductions in  $[\text{lac}^-]_{\text{B}}$  and performance improvements following RMT (Romer et al.  
22 2002b; Spengler et al. 1999), with up to 52% of the variation in performance being attributed to  
23 the reduced  $[\text{lac}^-]_{\text{B}}$  (Romer et al. 2002b).

24           The mechanism(s) by which RMT reduces  $[\text{lac}^-]_{\text{B}}$  remains equivocal. An RMT-mediated  
25 change in minute ventilation ( $\dot{V}_{\text{E}}$ ), which may conceivably alter both the work of breathing and

1 acid base balance, is an unlikely mechanism since reductions in  $[\text{lac}^-]_{\text{B}}$  following RMT have  
2 been observed irrespective of whether  $\dot{V}_{\text{E}}$  is lower (Leddy et al. 2007), unchanged (McConnell  
3 and Sharpe 2005; Spengler et al. 1999; Volianitis et al. 2001), or increased (Kohl et al. 1997). It  
4 thus appears that the specific, targeted nature of RMT elicits respiratory muscle adaptations that  
5 result in the respiratory muscles being the source of at least part of the reductions observed in  
6  $[\text{lac}^-]_{\text{B}}$ .

7 Modest increases in  $[\text{lac}^-]_{\text{B}}$  are observed under resting conditions when  $\dot{V}_{\text{E}}$  is increased  
8 for 5 min at 72 % maximal voluntary ventilation (MVV) (Martin et al. 1984), or sustained to  
9 volitional tolerance at ~70 %MVV (Verges et al. 2007). This increase is reduced during an  
10 exhaustive breathing endurance test following VIH training although the reductions observed  
11 following RMT failed to exceed a control and the authors neglect to explain their findings  
12 (Verges et al. 2007). Notwithstanding these findings, previous studies that have employed a  
13 breathing challenge at a given %MVV have little ecological validity with respect to intense  
14 endurance exercise since the breathing pattern adopted during volitional hyperpnoea can  
15 significantly influence the work of breathing (Coast et al. 1993). Thus for volitional hyperpnoea  
16 to reflect the demands of exercise hyperpnoea,  $\dot{V}_{\text{E}}$ , respiratory frequency ( $f_{\text{R}}$ ), tidal volume ( $V_{\text{T}}$ )  
17 and duty cycle ( $T_{\text{I}}/T_{\text{TOT}}$ ) must be rigorously controlled to that of exercise which has not been  
18 achieved in previous studies. Furthermore, despite VIH reducing  $[\text{lac}^-]_{\text{B}}$  during an intense  
19 respiratory endurance test to volitional tolerance, it is unknown whether strength based  
20 inspiratory muscle training may also reduce systemic  $[\text{lac}^-]_{\text{B}}$  given the discrete differences in  
21 training mode.

22 Therefore, to investigate this issue further the present study examined two hypotheses:  
23 firstly that mimicking at rest the breathing pattern observed during high-intensity endurance  
24 exercise would significantly increase  $[\text{lac}^-]_{\text{B}}$ , and secondly that 6 weeks of IMT would attenuate  
25 such a response.

# 1 **Methods**

## 2 Subjects

3           Following approval from Nottingham Trent University's ethics committee, 22 non-  
4 smoking, recreationally active males provided written informed consent to participate in the  
5 study. Throughout the study subjects were instructed to adhere to their usual training regimen  
6 and not to engage in strenuous exercise the day before test days, during which subjects refrained  
7 from ingesting caffeine and arrived at the laboratory 2 h post-prandial. Descriptive  
8 characteristics of the subjects are presented in Table 1.

9

## 10 Experimental procedure

11           Baseline pulmonary function and maximum inspiratory mouth pressure (MIP) were  
12 measured during the first laboratory visit. On a separate occasion, subjects then performed a  
13 maximal incremental cycling test, and two 10 min isocapnic volitional hyperpnoea tests (the first  
14 being a familiarisation test); all of these tests were separated by a minimum of 48 hours. The  
15 volitional hyperpnoea tests were performed at the  $\dot{V}_E$ , tidal volume ( $V_T$ ), breathing frequency  
16 ( $f_R$ ) and duty cycle ( $TI/T_{TOT}$ ) associated with 85% maximal exercise  $\dot{V}_E$  ( $\dot{V}_E \text{ max}$ ) since pilot  
17 work showed that this was the maximal exercise breathing pattern that could be maintained for  
18 10 min. During the experimental volitional hyperpnoea test expired respiratory and pulmonary  
19 variables were measured breath by breath from min 0 to 10 inclusive and arterialised venous  
20 blood gases, pH and  $[\text{lac}^-]_B$  was measured at rest and every 2 min thereafter. Subjects were  
21 subsequently matched for 85%  $\dot{V}_E \text{ max}$  and divided into an IMT group (n=11) or a control group  
22 (no IMT; n=11). No more than 1 week following a 6 week intervention MIP was measured and  
23 at least 48 hours following this, subjects repeated the volitional hyperpnoea test. Each subject  
24 completed a 24 h diet record prior to the criterion pre-intervention volitional hyperpnoea test and  
25 this was then replicated during the 24 h prior to the post-intervention volitional hyperpnoea test.

1 Pulmonary function, maximal inspiratory pressure, and respiratory measurements

2 Pulmonary function was assessed using a pneumotachograph (ZAN 600USB, Nspire  
3 Health, Oberthulba, Germany) calibrated using a 3 L syringe. Each measurement was repeated 3  
4 times and the highest recorded value was used for subsequent analysis (Quanjer et al. 1993). A  
5 hand-held mouth pressure meter (Ferraris Respiratory Europe, Hertford, UK) measured MIP as  
6 an index of global inspiratory muscle strength. The mouthpiece assembly incorporated a 1 mm  
7 orifice to prevent glottic closure during inspiratory efforts. Manoeuvres were performed in an  
8 upright standing posture, were initiated from residual volume, and sustained for at least 1 s.  
9 Repeat measurements separated by 30 s were taken until 3 values within 5 cmH<sub>2</sub>O of each other  
10 were produced (McConnell 2007). The highest recorded value was used for subsequent analysis.  
11 Throughout hyperpnoea trials and the  $\dot{V}O_2$  max test, respiratory variables were measured breath  
12 by breath (ZAN 600USB, Nspire Health, Oberthulba, Germany). Subjects wore a facemask  
13 (model 7940, Hans Rudolph, Kansas City, Missouri) connected to a pneumotachograph, and  
14 during volitional hyperpnoea tests, a two-way non-rebreathing valve (model 2730, Hans  
15 Rudolph, Kansas City, Missouri) was attached distally to the pneumotachograph allowing  
16 additional CO<sub>2</sub> to be added to the inspire.

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18 Blood sampling and analysis

19 Arterialised venous blood was sampled from a dorsal hand vein via an indwelling  
20 cannula (Forster et al. 1972; McLoughlin et al. 1992). Arterialisation was ensured by immersing  
21 the hand in water at ~40°C for 10 min prior to cannulation and by warming the hand during  
22 volitional hyperpnoea tests using an infrared lamp. Blood samples were drawn into a 2 ml pre-  
23 heparinised syringe (PICO 50, Radiometer, Copenhagen, Denmark) and analysed immediately for  
24 blood gases (ABL520, Radiometer, Copenhagen, Denmark), including the partial pressure of  
25 carbon dioxide ( $PCO_2$ ) and pH, and  $[lac^-]_B$  (Biosen C\_line Sport, EKF Diagnostics, Barleben,

1 Germany). Plasma bicarbonate concentration ( $[\text{HCO}_3^-]$ ) was calculated from  $PCO_2$  and pH  
2 values using the Henderson Hasselbalch equation:

$$3 \quad \text{pH} = \text{pK} + \log \frac{[\text{HCO}_3^-]}{0.03 \times PCO_2}$$

4  
5  $[\text{HCO}_3^-]$  was then subsequently incorporated into the Siggaard-Anderson equation to calculate  
6 base excess of the extracellular fluid ( $BE_{\text{ECF}}$ ) (Siggaard-Anderson and Fogh-Anderson, 1995):

$$8 \quad BE_{\text{ECF}} = 0.93 \times ([\text{HCO}_3^-] - 24.4 + 14.83 \times (\text{pH} - 7.40))$$

9

#### 10 Maximal exercise test

11 Subjects performed a maximal incremental cycling test on an electromagnetically-braked  
12 cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). At the onset of the  
13 exercise test, cycling power was 0 W and subsequently increased by 10 W every 15 s in order to  
14 result in exercise intolerance within approximately 10 min. This rapid incremental protocol was  
15 selected to maximise  $\dot{V}_E$  at the cessation of exercise and reflect intense endurance exercise. The  
16 power at which exercise intolerance ensued defined maximal power output ( $\dot{W}_{\text{max}}$ ), and the  
17 highest oxygen uptake ( $\dot{V}O_2$ ) and  $\dot{V}_E$  recorded in any 30 s period defined  $\dot{V}O_{2 \text{ max}}$  and  
18  $\dot{V}_E \text{ max}$ , respectively.

19

#### 20 Volitional hyperpnoea

21 Volitional hyperpnoea was performed whilst seated on the cycle ergometer in an identical  
22 body position to that adopted during the maximal exercise test. Subjects were instructed to  
23 increase  $\dot{V}_E$  and  $f_R$  in a square wave manner to a level commensurate with 85 %  $\dot{V}_E \text{ max}$ . An

1 audio metronome paced  $f_R$  and real-time visual feedback of  $\dot{V}_E$  was provided throughout the  
2 test. In order to provide a breathing challenge representative of the work of breathing of intense  
3 exercise hyperpnoea, the volitional hyperpnoea tests was performed at the  $\dot{V}_E$ ,  $V_T$ ,  $f_R$  and  
4  $T_I/T_{TOT}$  associated with 85%  $\dot{V}_E$  max since pilot work showed that this was the maximum square  
5 wave response that could be maintained for 10 min. This methodology is deemed superior to an  
6 arbitrary %MVV as it reflects the work of breathing of intense endurance exercise as for a given  
7  $\dot{V}_E$  greater than approximately 60 L·min<sup>-1</sup> the work of breathing of exercise hyperpnoea can  
8 overestimated by as much as 25 % when a spontaneous breathing pattern is adopted during  
9 volitional hyperpnoea (Coast et al. 1993). Isocapnia was maintained during volitional  
10 hyperpnoea by adding CO<sub>2</sub> into the inspiratory circuit in order to maintain resting  $PCO_2$ . Blood  
11 was sampled at rest and at 2 min intervals.

12

### 13 Intervention

14 IMT was performed using an inspiratory pressure-threshold device (POWERbreathe®,  
15 Gaiam, UK). The IMT group performed 30 dynamic inspiratory efforts twice daily for 6 weeks  
16 against a pressure-threshold load of ~50% MIP. Thereafter, subjects periodically increased the  
17 load to a level that would permit them to only just complete 30 manoeuvres. Each inspiratory  
18 manoeuvre was initiated from residual volume and subjects strove to maximise  $V_T$ . This protocol  
19 is known to be effective in eliciting an adaptive response (Johnson et al. 2007; McConnell and  
20 Lomax 2006; McConnell and Sharpe 2005; Romer et al. 2002a,b; Volianitis et al. 2001).  
21 Subjects completed a training diary to record IMT adherence and habitual training, which the  
22 control group also recorded. The control group performed no IMT during the 6 week  
23 intervention since the duration and breathing pattern of the volitional hyperpnoea test was fixed  
24 pre and post-intervention (i.e. no measures of performance) and therefore the responses between  
25 groups were not influenced by either motivation or expectation.



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## Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). Pre- and post-intervention results, differences over time during volitional hyperpnoea and group interactions were compared using one-way or two-way ANOVA for repeated measures and Tukey's HSD post-hoc analysis. Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at  $P \leq 0.05$ . Results are presented as mean  $\pm$  SD.

## Results

### Pulmonary function and maximal inspiratory pressure

Baseline pulmonary function and MIP were all within normal limits (Table 1). The IMT group demonstrated excellent training compliance (91% adherence) and subjects' habitual training remained unchanged in both IMT and control groups. MIP increased from  $147 \pm 27$  to  $189 \pm 27$  cmH<sub>2</sub>O ( $+31 \pm 22\%$ ) following IMT ( $P < 0.01$ ). No change was observed in the control group (pre- vs. post-:  $163 \pm 19$  vs.  $166 \pm 20$  cmH<sub>2</sub>O).

### Responses to volitional hyperpnoea

Ventilatory and acid base responses to volitional hyperpnoea pre- and post-intervention for the control and IMT groups are shown in Table 2. Throughout hyperpnoea pre- and post-intervention (min 0 to min 10) there were no differences in breathing pattern and acid base balance between groups (Table 2).  $\dot{V}_E$  during volitional hyperpnoea represented  $72 \pm 8\%$  and  $81 \pm 19\%$  of  $MVV_{10}$  in control and IMT groups, respectively.  $PCO_2$  was maintained at resting levels throughout hyperpnoea and was not different between groups (Figure 1).

1 Prior to the intervention in the control group,  $[\text{lac}^-]_{\text{B}}$  increased from  $0.76 \pm 0.24 \text{ mmol}\cdot\text{L}^{-1}$   
2 at rest to  $1.50 \pm 0.60 \text{ mmol}\cdot\text{L}^{-1}$  and in the IMT group from  $0.85 \pm 0.40 \text{ mmol}\cdot\text{L}^{-1}$  at rest to  $2.02 \pm$   
3  $0.85 \text{ mmol}\cdot\text{L}^{-1}$  following 10 min volitional hyperpnoea ( $P < 0.05$ ) (Figure 2). The non-significant  
4 difference in the absolute increase in  $[\text{lac}^-]_{\text{B}}$  between groups is likely due to the different relative  
5 loads of the imposed hyperpnoea (control: 72 %MVV; IMT: 81 %MVV). The  $[\text{lac}^-]_{\text{B}}$  response to  
6 volitional hyperpnoea was unchanged in the control group following the intervention.  
7 Conversely,  $[\text{lac}^-]_{\text{B}}$  during volitional hyperpnoea was reduced following IMT, with significant  
8  $\pm 37\%$  and  $25 \pm 34\%$  reductions being observed at 8 and 10 min, respectively. These changes  
9 were different between groups (significant group  $\times$  time  $\times$  trial interaction effect,  $P < 0.05$ ).

#### 10 11 Correlations amongst variables

12 Prior to the intervention, increases in  $[\text{lac}^-]_{\text{B}}$  during volitional hyperpnoea were not  
13 correlated with any measure of pulmonary function, MIP, endurance training status ( $\dot{V}\text{O}_2 \text{ max}$ ,  
14  $\dot{W}_{\text{max}}$ ), or ventilatory responses to volitional hyperpnoea. However, baseline MIP was  
15 negatively correlated with relative IMT-induced increases in MIP ( $r = -0.70$ ,  $P < 0.05$ ).

## 16 17 **Discussion**

### 18 Main findings

19 The main findings of this study were that 10 min of volitional hyperpnoea approximately  
20 doubled resting  $[\text{lac}^-]_{\text{B}}$ , and that 6 weeks of pressure threshold IMT attenuated this increase by  
21 25%. These findings strongly support the notion that the respiratory muscles are capable of  
22 increasing  $[\text{lac}^-]_{\text{B}}$  and are the first to show that this can be attenuated through specific IMT. This  
23 observation may help to explain some of the RMT-mediated reductions in  $[\text{lac}^-]_{\text{B}}$  previously  
24 observed during whole-body exercise.

25

## 1 Volitional hyperpnoea and blood lactate concentration

2 We report an increased  $[\text{lac}^-]_{\text{B}}$  from rest of  $0.96 \pm 0.58 \text{ mmol}\cdot\text{L}^{-1}$  ( $n=22$ ; range: 0.20 –  
3  $2.50 \text{ mmol}\cdot\text{L}^{-1}$ ) during 10 min of intense volitional hyperpnoea at 85%  $\dot{V}_{\text{E max}}$  ( $131 \pm 4.36$   
4  $\text{L}\cdot\text{min}^{-1}$ ;  $n=22$ ). These findings contrast those of Spengler et al. (2000) who reported unchanged  
5  $[\text{lac}^-]_{\text{B}}$  during volitional hyperpnoea at a lower relative  $\dot{V}_{\text{E}}$  ( $\sim 62\% \text{ MVV}$ ;  $122.4 \text{ L}\cdot\text{min}^{-1}$ ),  
6 however, are similar to others with a similar relative breathing challenge (72% MVV, Martin et  
7 al. 1984; 70% MVV, Verges et al. 2007). These data confirm that increases in  $[\text{lac}^-]_{\text{B}}$  during  
8 volitional hyperpnoea are positively related to the ratio of  $\dot{V}_{\text{E}}$  to MVV (Martin et al. 1984;  
9 Johnson et al. 2006) and may, in part, explain the different  $[\text{lac}^-]_{\text{B}}$  responses observed in previous  
10 studies in response to volitional hyperpnoea and between groups in this study. This study  
11 provides novel data that the work of breathing of volitional hyperpnoea when rigorously matched  
12 to high-intensity exercise hyperpnoea is sufficient to result in net lactate release from the  
13 respiratory muscles.

14 The potential for respiratory alkalosis to elevate  $[\text{lac}^-]_{\text{B}}$  is well documented (Davies et al.  
15 1986; LeBlanc et al. 2002). Consequently we were careful to maintain, with considerable  
16 accuracy, resting  $\text{PCO}_2$  throughout the 10 min of volitional hyperpnoea (see Figure 1). Other  
17 measures of acid base status also remained unchanged from rest during volitional hyperpnoea in  
18 both groups pre- and post-intervention. We are thus confident that the increase in  $[\text{lac}^-]_{\text{B}}$  during  
19 volitional hyperpnoea was not a consequence of respiratory alkalosis and we attribute the  
20 increase in  $[\text{lac}^-]_{\text{B}}$  to lactate efflux from the respiratory muscles

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## 22 Inspiratory muscle training and blood lactate concentration

23 The attenuated increase in  $[\text{lac}^-]_{\text{B}}$  during volitional hyperpnoea following IMT is similar  
24 to that observed in healthy subjects performing an exhaustive respiratory endurance test at  $\sim 70$   
25 %MVV following VIH training, although, this reduction did not exceed that of a control (Verges

1 et al. 2007). However, the authors fail to report their attempts to maintain end tidal CO<sub>2</sub> and / or  
2 PCO<sub>2</sub> during the respiratory endurance test, furthermore, subjects were prescribed a pre-  
3 determined arbitrary breathing pattern, of which has been criticised previously for failing to  
4 accurately represent the work of breathing of exercise hyperpnoea (Coast et al. 1993). The IMT-  
5 mediated reduction in [lac<sup>-</sup>]<sub>B</sub> observed in the present study is also similar to the reduction often  
6 observed during submaximal, whole-body exercise following both IMT (Griffiths and  
7 McConnell 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Volianitis et al. 2001) and  
8 VIH (Leddy et al. 2007; Spengler et al. 1999), however, whether these observations during  
9 volitional hyperpnoea and exercise share a common mechanistic explanation is unclear.

10 RMT-mediated reductions in [lac<sup>-</sup>]<sub>B</sub> at submaximal exercise intensities occur (Leddy et  
11 al. 2007; McConnell and Sharpe 2005) when lactate production and release from the respiratory  
12 muscles is probably negligible given the relative ventilatory demand and the reduced activation  
13 of less efficient accessory muscles (Martin et al. 1984; Johnson et al. 2006). Hence, under such  
14 conditions it seems more likely that reductions in [lac<sup>-</sup>]<sub>B</sub> result from increased metabolism of  
15 lactate by the trained respiratory muscles (Spengler et al. 1999) rather than a decrease in net  
16 lactate release. Conversely, during high-intensity exercise where  $\dot{V}_E$  is increased above that of  
17 sub-maximal exercise similar to the  $\dot{V}_E$  of volitional hyperpnoea in this study (Kohl et al. 1997:  
18 130.9 L·min<sup>-1</sup>; Spengler et al. 1999; 147.3 L·min<sup>-1</sup>), it is possible that IMT-mediated inspiratory  
19 muscle adaptation contributed to lowering [lac<sup>-</sup>]<sub>B</sub> through affecting both lactate clearance by and  
20 efflux from the trained inspiratory muscles.

21 The plasticity of the inspiratory muscles has been well documented (McConnell and  
22 Romer 2004; Powers et al. 1997). It is thus attractive to suggest that changes in inspiratory  
23 muscle morphology may explain, in part, the attenuated hyperpnoea-mediated increase in [lac<sup>-</sup>]<sub>B</sub>  
24 following IMT.. An increase in the content of inspiratory muscle monocarboxylate transport  
25 (MCT) proteins (McConnell and Sharpe 2005), which facilitate inter- and intra-cellular lactate

1 shuttling in sarcolemmal and mitochondrial membranes, respectively (Brooks et al. 1999;  
2 Dubouchaud et al. 2000) have been reported following endurance (Baker et al. 1998;  
3 Burgomaster et al. 2007) and strength (Juel et al. 2004) based training regimens. It is possible  
4 that similar adaptations would occur following both IMT (strength-orientated) and VIH  
5 (endurance-orientated) training and may explain, in part, the decrease in  $[\text{lac}^-]_{\text{B}}$  observed during  
6 whole-body exercise and volitional hyperpnoea.

7 Diaphragm hypertrophy has been reported with an approximate 10% increase in  
8 diaphragm thickness (Downey et al. 2007; Enright et al. 2006) and 21 % increase in the size of  
9 type II muscle fibres (Ramírez-Sarmiento et al. 2002) occurring after 6 and 5 weeks of IMT,  
10 respectively. Increasing inspiratory muscle fibre cross-sectional area and subsequently strength  
11 decreases the relative intensity for a given absolute work load, which may reduce/delay fast  
12 twitch fibre recruitment and thus lactate production (Marcinik et al. 1991). A decrease in relative  
13 workload per muscle fibre may also decrease blood flow occlusion, which may influence lactate  
14 production and/or clearance (Marcinik et al. 1991).

15 Finally, the attenuated  $[\text{lac}^-]_{\text{B}}$  response to volitional hyperpnoea following IMT may also  
16 reside in a training-induced increase in the oxidative capacity of the inspiratory muscles. In  
17 support of this notion, Ramírez-Sarmiento et al. (2002) reported 38% increases in the number of  
18 type I muscle fibres in the external intercostals following 5 weeks IMT. Moderate intensity, high  
19 repetitions strength training, similar to the IMT protocol used in the this study can increase  
20 oxidative enzyme activity (Costill et al. 1979; Sale et al. 1990) and reduce  $[\text{lac}^-]_{\text{B}}$  via an increase  
21 in mitochondria derived ATP and lactate oxidation (Holloszy and Coyle 1984). Since it is  
22 probable that similar oxidative adaptations would also occur following VIH (endurance-  
23 orientated) training (Kohl et al. 1997; Leddy et al. 2007; Spengler et al. 1999), this offers an  
24 attractive explanation for the decrease in  $[\text{lac}^-]_{\text{B}}$  observed during whole body exercise (Griffiths  
25 and McConnell 2007; Kohl et al. 1997; Leddy et al. 2007; McConnell and Sharpe 2005; Romer

1 et al. 2002b; Spengler et al. 1999; Volianitis et al. 2001) and volitional hyperpnoea (present  
2 study; Verges et al. 2007) following these dissimilar training stimuli.

### 3 4 Inspiratory muscle strength

5 The 32% increase in MIP following 6 weeks of IMT is consistent with previous studies  
6 (Downey et al. 2007; Edwards and Cooke 2004; Gething et al. 2004; Griffiths and McConnell  
7 2007; McConnell and Sharpe 2005; Romer et al. 2002a,b; Williams et al. 2002). The suggestion  
8 that IMT-mediated increases in MIP are partly dependent upon baseline MIP (Johnson et al.  
9 2007) was substantiated in the present study by the negative correlation ( $r=-0.70$ ) observed  
10 between these variables. These novel data lend credence to the concept that resistance training-  
11 induced increases in strength are partly dependent upon baseline status (Kraemer and Ratamess  
12 2004). However, the significance of our observation is unclear since IMT-mediated increases in  
13 MIP were not related to the reduction in  $[\text{lac}^-]_{\text{B}}$ , suggesting that an increase in inspiratory muscle  
14 strength *per-se* is not an important determinant of the physiological adaptations following-IMT.

### 15 16 **Conclusions**

17 In summary, the present study provides novel evidence that increases in  $[\text{lac}^-]_{\text{B}}$  during  
18 volitional hyperpnoea can be attenuated following IMT. These data thus suggest that the  
19 inspiratory muscles were the source of at least part of this reduction, and provide a possible  
20 explanation for at least some of the IMT-mediated reductions in  $[\text{lac}^-]_{\text{B}}$  previously observed  
21 during whole-body exercise. The precise mechanisms that underpin these changes remain  
22 unknown, but an IMT-mediated increase in the oxidative and/or lactate transport capacity of the  
23 inspiratory muscles is an attractive possibility that merits further investigation.

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2 None

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4 **References**

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1 **Table 1.** Descriptive characteristics of the subjects (mean  $\pm$  SD).

	Control (n=11)	IMT (n=11)
Age (years)	28.5 $\pm$ 4.1	22.4 $\pm$ 4.5 *
Body mass (kg)	75.5 $\pm$ 5.6	78.6 $\pm$ 9.7
Height (cm)	176.9 $\pm$ 7.4	181.6 $\pm$ 7.6
FVC (L)	5.32 $\pm$ 0.55 (104 $\pm$ 8)	5.67 $\pm$ 0.92 (106 $\pm$ 12)
FEV <sub>1</sub> (L)	4.28 $\pm$ 0.62 (99 $\pm$ 11)	4.93 $\pm$ 0.67 (109 $\pm$ 11)
FEV <sub>1</sub> /FVC (%)	80.3 $\pm$ 7.1 (96 $\pm$ 9)	87.7 $\pm$ 8.3 (103 $\pm$ 9) *
MVV <sub>10</sub> (L·min <sup>-1</sup> )	176.3 $\pm$ 15.0 (102.3 $\pm$ 10.9)	173.4 $\pm$ 53.7 (122.4 $\pm$ 30.3))
MIP (cmH <sub>2</sub> O)	163 $\pm$ 19 (113 $\pm$ 4)	147 $\pm$ 27 (119 $\pm$ 5)
$\dot{V}O_2$ max (L·min <sup>-1</sup> )	3.75 $\pm$ 0.55	3.77 $\pm$ 0.75
$\dot{W}$ max (W)	353 $\pm$ 44	362 $\pm$ 38

2 FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; MVV<sub>10</sub>, maximum voluntary  
3 ventilation in 10 s. Values in parenthesis represent the percent of predicted values (Quanjer et al.  
4 1993; Wilson et al. 1984). \*,  $P < 0.05$ .

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1 **Table 2.** Ventilatory and acid-base responses to volitional hyperpnoea prior to and following the  
 2 intervention. Data are mean of min 2 to 10 during volitional hyperpnoea (mean  $\pm$  SD).

	Control (n=11)		IMT (n=11)	
	Pre	Post	Pre	Post
$\dot{V}_E$ (L $\cdot$ min $^{-1}$ )	127.1 $\pm$ 2.3	128.7 $\pm$ 2.4	132.9 $\pm$ 9.6	136.8 $\pm$ 3.2
$V_T$ (L)	2.62 $\pm$ 0.04	2.64 $\pm$ 0.07	2.60 $\pm$ 0.03	2.66 $\pm$ 0.06
$f_R$ (breaths $\cdot$ min $^{-1}$ )	50 $\pm$ 0	50 $\pm$ 0	52 $\pm$ 0	52 $\pm$ 0
$T_I/T_{TOT}$	0.44 $\pm$ 0.00	0.44 $\pm$ 0.00	0.52 $\pm$ 0.00	0.49 $\pm$ 0.00
pH	7.392 $\pm$ 0.031	7.406 $\pm$ 0.024	7.397 $\pm$ 0.023	7.395 $\pm$ 0.014
[H $^+$ ] (nmol $\cdot$ L $^{-1}$ )	40.6 $\pm$ 2.9	39.4 $\pm$ 2.2	40.2 $\pm$ 2.2	40.3 $\pm$ 1.0
[HCO $_3^-$ ] (mmol $\cdot$ L $^{-1}$ )	26.0 $\pm$ 0.9	26.9 $\pm$ 2.5	26.5 $\pm$ 1.4	27.0 $\pm$ 1.3
BE $_{ECF}$ (mEq $\cdot$ L $^{-1}$ )	1.38 $\pm$ 0.91	1.72 $\pm$ 2.04	1.52 $\pm$ 1.11	2.35 $\pm$ 1.23

3  $\dot{V}_E$ , minute ventilation;  $V_T$ , tidal volume;  $f_R$ , respiratory frequency;  $T_I/T_{TOT}$ , duty cycle; [H $^+$ ],  
 4 hydrogen ion concentration; [HCO $_3^-$ ], plasma bicarbonate concentration; BE $_{ECF}$ , base excess of  
 5 the extracellular fluid.  
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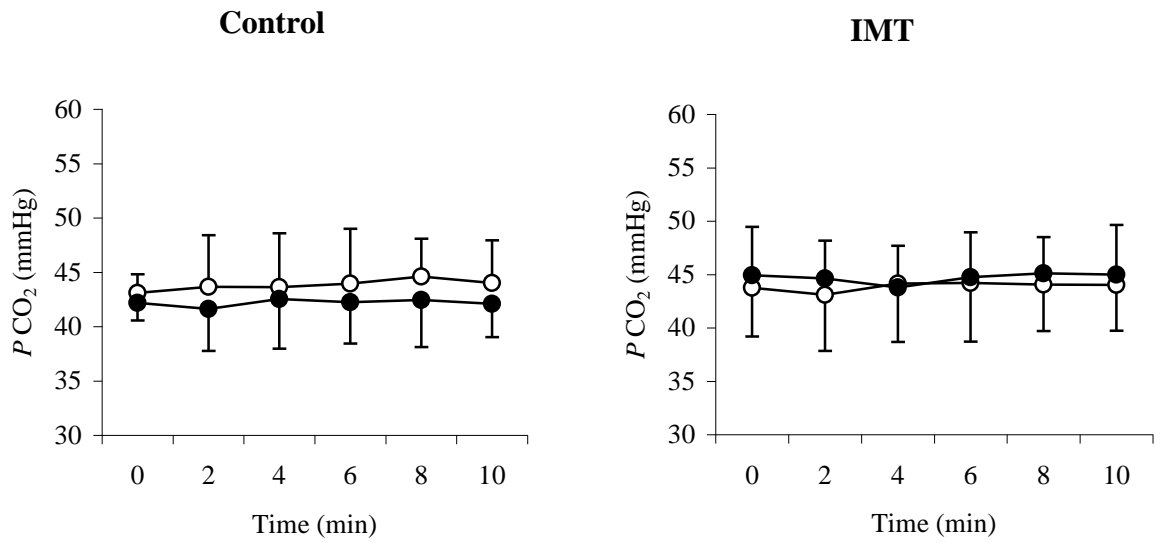
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2 **Fig. 1** Partial pressure of carbon dioxide in arterialised venous blood ( $P_{CO_2}$ ) during volitional  
 3 hyperpnoea pre- (○) and post- (●) intervention in control and IMT groups.

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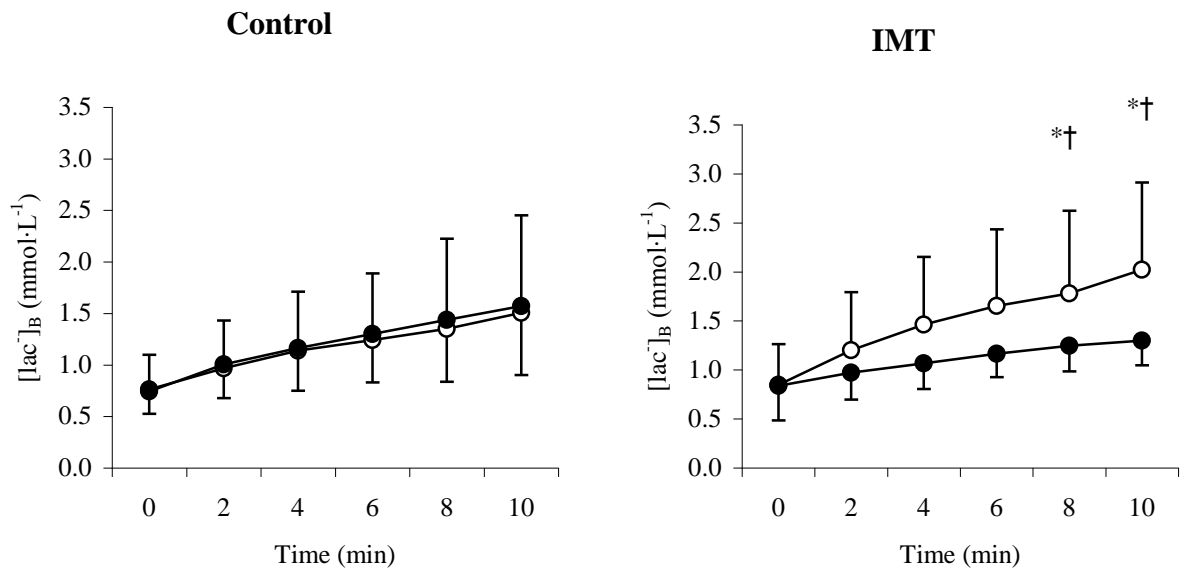
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2 **Fig. 2** Blood lactate concentration ( $[\text{lac}^-]_{\text{B}}$ ) during volitional hyperpnoea pre- ( $\circ$ ) and post- ( $\bullet$ )  
 3 intervention in control and IMT groups. \*Significant difference from pre-IMT ( $P < 0.05$ ).

4  $^\dagger$ Significant group  $\times$  time interaction effect ( $P < 0.05$ ).

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