Title:

Loading of trained inspiratory muscles speeds lactate recovery kinetics

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Abstract

Purpose: To investigate the effects of inspiratory muscle loading (ITL) and inspiratory muscle training (IMT) upon blood lactate concentration ($[lac^-]_B$) and acid-base balance following maximal incremental cycling.

Methods: 18 subjects were divided into a control (*n*=9) or IMT group (*n*=9). Prior to and following a 6 wk intervention subjects completed two maximal incremental cycling tests followed by 20 min of recovery with (ITL) or without (passive recovery; PR) a constant inspiratory resistance (15 cmH_2O). The IMT group performed 6 wk pressure threshold IMT at 50% maximal inspiratory mouth pressure (MIP). Throughout recovery, acid-base balance was quantified using the physicochemical approach by measuring the strong ion difference ($[SID]=[Na^+]+[K^+]-[Cl^-]+[lac^-]$), the total concentration of weak acids ($[A_{tot}^-]$) and the partial pressure of carbon dioxide (PCO_2).

Results: Following the intervention MIP increased in the IMT group only (+34%). No differences in lactate clearance were observed between PR and ITL before the intervention in both groups and following the intervention in the control group. Following IMT, relative to PR, $[lac^-]_B$ was reduced throughout ITL (min 2 to 20) by 0.66 ± 1.28 $mmol \cdot L^{-1}$ ($P<0.05$) and both the fast (lactate exchange) and slow (lactate clearance) velocity constants of the lactate recovery kinetics were increased ($P<0.05$). Relative to pre-IMT, ITL reduced plasma $[H^+]$ which was accounted for by an IMT-mediated increase in [SID] due almost exclusively to a 1.7 $mmol \cdot L^{-1}$ reduction in $[lac^-]_B$.

Conclusions: Following maximal exercise ITL affected lactate recovery kinetics only after IMT. Our data support the notion that the inspiratory muscles are capable of lactate clearance which increases [SID] and reduces $[H^+]$. These effects may facilitate subsequent bouts of high-intensity exercise.

Key Words:

Inspiratory muscle training, work of breathing, blood lactate concentration, acid base regulation

Introduction

Paragraph Number 1 Recent evidence suggests that the respiratory muscles become net producers of lactate when the work of breathing exceeds a critical threshold level (2, 16, 40) and that specific training of these muscles reduces their rate of lactate production and / or increases their rate of consumption (2, 40). Reductions in blood lactate concentration ($[lac^-]_B$) have also been reported during exercise following specific respiratory muscle training (RMT; 23, 35, 38) suggesting that at moderate levels of pulmonary ventilation the respiratory muscles may become net lactate consumers (10). These findings are surprising given the small muscle mass (approximately 0.5% total body mass) of the respiratory muscles and collectively suggest an important, previously underestimated role for the respiratory muscles in the regulation of whole body lactate kinetics.

Paragraph Number 2 This theme was recently extended by Chiappa et al. (3) who found that adding an inspiratory resistance (15 cmH_2O) during recovery from maximal incremental cycling exercise significantly reduced $[lac^-]_B$ (~2.5 $mmol \cdot L^{-1}$) compared to a passive recovery. This intriguing finding suggests that inspiratory muscle work accelerates lactate clearance by a similar magnitude to that achieved with an active recovery involving locomotor muscles, but with the benefit of sparing intramuscular energy stores (6). Given that lactate consumption and / or reduced production by the inspiratory muscles is enhanced by training (23, 35, 38) it is attractive to speculate that the finding of Chiappa et al (3) would be magnified after RMT and this was the focus of the present study.

Paragraph Number 3 It is unlikely that increases in [lac^-] *per-se* result in metabolic acidosis and cause skeletal muscle fatigue (34) particularly at physiological temperatures (43). However, according to the integrated physicochemical systems approach, with which it is possible to quantify the mechanisms accounting for disturbances in acid-base balance during and following exercise, the [lac^-] may indirectly affect [H^+] (38). Within a given compartment (e.g. muscle, plasma, erythrocyte) the dependent variables: [H^+] and [HCO_3^-] are determined by the independent variables: strong ion difference ([SID] = [Na^+] + [K^+] - [Cl^-] + [lac^-]), the partial pressure of carbon dioxide (PCO_2) and the total concentration of weak acids ([A_{tot}^-]). Therefore, a reduction in [lac^-] in the systemic circulation may affect the [H^+] by causing a positive shift in [SID] (for reviews: 17, 20). This is especially important given the associations between elevated [H^+] and / or [lac^-] on some intramuscular processes (8) and subsequent exercise performance (30, 40).

Paragraph Number 4 Accordingly, the purpose of the present study was to test the hypothesis that inspiratory pressure threshold loading during recovery from maximal exercise would speed lactate clearance and that this would be further increased following specific inspiratory muscle training (IMT). In order to determine the effect of changes in $[lac^-]_B$ on plasma [H^+], we quantified the contribution of associated physiological variables to the regulation of plasma acid-base homeostasis using the integrated physicochemical approach.

Methods.

Participants

Paragraph Number 5 Following ethical approval and written informed consent, 18 healthy non-smoking participants with normal lung function volunteered for the study (Table 1). Throughout, subjects were instructed to adhere to their normal training regimen and not to

engage in any strenuous exercise the day preceding and the day of a trial. Both habitual training and IMT were recorded throughout the intervention using a training diary. Each subject completed a 24 h diet record prior to their first trial and this was repeated prior to subsequent tests. Subjects arrived at the laboratory 2 h post-prandial having abstained from alcohol and caffeine in the 24 h prior to testing. All exercise trials were performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands), at a similar time of day, separated by 48 h and in similar laboratory conditions (temperature: 21.1 ± 2.7˚C; relative humidity: 46.6 ± 14.4 %).

Experimental design

Paragraph Number 6 Subjects attended the laboratory 3 times prior to a 6 week intervention; each laboratory visit was separated by a minimum of 48 h. During the first laboratory visit subjects completed pulmonary function and maximal inspiratory mouth pressure (MIP) tests and were subsequently familiarized with all testing procedures including maximal incremental exercise. Pulmonary function was assessed using a pneumotachograph (Pneumotrac, Vitalograph, Buckingham, UK) calibrated using a 3 L syringe. Measurement of FVC and FEV_1 was repeated until the difference between the largest and next largest value was less than 100 ml. A minimum of 3 and maximum of eight manoeuvres were performed and the highest value used for subsequent analysis (28). With the exception of peak inspiratory flow lung function is unchanged following IMT (22) and therefore was not measured post-intervention. A hand-held mouth pressure meter (MicroRPM, Micro Medical, Kent, UK) measured MIP as an index of global inspiratory muscle strength. The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure during inspiratory efforts. Manoeuvres were performed in an upright standing posture, were initiated from residual volume, and sustained for at least 1 s. To ensure a true maximal effort, a minimum of 5

manoeuvres were performed. Efforts were repeated every 30 s until 3 serial measures differed by no more than 10% or 10 cmH_2O: whichever was smallest (21). The highest value recorded during repeat measurements was used for subsequent analysis (21). MIP values were compared to predicted values using the equation of Wilson et al. (44) where: $MIP_{PREDICTED}=MIP_{MEASURED}/(142-(1.03\times age)\times 100)$. MIP was re-evaluated throughout the intervention following 2 and 4 wk. Following IMT, MIP was assessed 48 h following the final training session and on a separate day to any exercise testing.

Paragraph Number 7 On two separate occasions subjects completed a maximal incremental exercise test. Immediately following exercise subjects breathed against either a constant pressure threshold inspiratory resistance (15 cmH_2O) for 20 min (ITL) or recovered passively with spontaneous breathing for 20 min (no inspiratory resistance; PR); the order of these trials were randomized and separated by 48 h. Following the pre-intervention trials, subjects were matched for $\dot{W}$ max and divided in to an IMT group (n=9) or a control group (n=9). Following a 6-wk intervention (IMT or no IMT), subjects repeated the pre-intervention trials. Given the increase in MIP expected following IMT, the IMT group completed a third maximal incremental exercise test in which the absolute intensity of ITL during recovery was increased so that the same fraction of MIP was used before and after the intervention. This subsequent trial was defined as ITL%. The order of the post-intervention trials was randomized and separated by 48 h. For each subject, all trials prior to and following the intervention period were performed at a similar time of day (± 1 h)

Passive recovery (PR)

Paragraph Number 8 Subjects performed a maximal incremental cycling test in which the initial power was 0 W and was increased by 20 $W\cdot min^{-1}$ until exercise could no longer be

tolerated ($\dot{W}$max) (3). The highest oxygen uptake ($\dot{V}O_2$) recorded in any 30 s period defined $\dot{V}O_2$max. $[lac^-]_B$ was determined at the cessation of exercise, and every 2 min thereafter; PCO_2 and $[H^+]$ were determined at volitional intolerance and every 5 min thereafter. At the cessation of exercise and following 10 and 20 min, physicochemical variables were determined. Subjects wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) connected to an online expired gas analyser (ZAN 600USB, Nspire Health, Oberthulba, Germany). Breath by breath respiratory variables were averaged over the final 30 s of every 2 min interval. Heart rate (HR) was recorded continuously during exercise using short-range telemetry (Polar S610, Polar, Kempele, Finland).

Inspiratory pressure threshold loading (ITL)

Paragraph Number 9 The ITL trial was identical to PR, however, immediately following exercise, a 1.5 m length of wide bore (35 mm internal diameter) corrugated tubing (Clean-bor tubes, Vacumed, USA) which provided minimal additional resistance (0.16 $cmH_2O \cdot L^{-1} \cdot s^{-1}$) was attached to the inspiratory port of a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) and connected distally to a custom built weighted plunger pressure threshold inspiratory muscle loading device identical to that used previously (13, 14). The 1.5 m length of tubing permitted the subject to remain in the same body position on the cycle ergometer when breathing through the device and provided a small degree of freedom to move the head comfortably and safely. The pressure threshold loading system was previously shown to be flow independent over the physiological range (see ref. 13); a full description of the device is provided elsewhere (14). During ITL, weights were added to the plunger to adjust the threshold opening pressure which was fixed at 15 cmH_2O (3). For the IMT group and the control group this represented 13 ± 3% and 11 ± 3% MIP (pooled data, *n* = 18, 12 ± 3% MIP), respectively. Following 6 wk IMT, and due to the training-induced

increase in MIP, the opening pressure of 15 cmH_2O represented a smaller resistance relative to MIP (10 ± 2% MIP). Thus in the ITL% trial, the absolute resistance was increased to 20 ± 2 cmH_2O which achieved the same relative resistance as the pre-IMT ITL trial (i.e. 13 ± 3%). The measurement accuracy of the online expired gas analyser during ITL was investigated prior to commencement of the study. Comparisons were made with the Douglas bag method at rest and over a range of exercise intensities. The mean bias ± 95% limits of agreement (2 SD) for $\dot{V}_E$ were -1.91 ± 2.19 $L \cdot min^{-1}$; for $\dot{V}O_2$ were -0.08 ± 0.14 $L \cdot min^{-1}$ and for $\dot{V}CO_2$ were -0.07 ± 0.14 $L \cdot min^{-1}$. These data show that the online expired gas analyser performed satisfactorily despite the negative pressures generated during ITL.

Intervention

Paragraph Number 10 IMT was performed using an inspiratory pressure-threshold device (POWERbreathe®, Gaiam, UK). The IMT group performed 30 dynamic inspiratory efforts twice daily for 6 wk against a pressure-threshold load of ~50% MIP. The initial training load was determined by inserting a 0.8 mm hypodermic needle into the mouthpiece of the device which was attached distally to a mouth pressure meter (MicroRPM, Micro Medical, Kent, UK). During repeated maximal efforts, identical to that performed throughout IMT, the opening pressure of the valve was adjusted to 50% MIP. Throughout 6 wk IMT, subjects were instructed to periodically increase the load to a level that would permit them to only just complete 30 breaths. In addition, the resistance of the device was also confirmed using the protocol outlined above after 2 and 4 wk IMT to ensure that the training load did not exceed 50% MIP. Each inspiratory manoeuvre was initiated from residual volume and subjects strove to maximize V_T. This protocol is known to be effective in eliciting an adaptive response (2, 15, 22, 23, 35, 36, 42). Subjects completed a training diary to record IMT adherence and habitual training, which the control group also recorded. The control group

continued with their habitual physical training schedule and were not exposed to an intervention. A placebo treatment was not applied to the control group since the study outcome measures could not be influenced by either motivation or expectation. Subjects were informed that they belonged to a control group prior to commencement of the study and to avoid any possible disadvantage were afforded the opportunity to undertake 6 wk of IMT after completion of the study.

Blood sampling and analysis

Paragraph Number 11 During all exercise trials, arterialized venous blood was drawn from a dorsal hand vein via an indwelling 21-G cannula (9, 25). Arterialization was achieved by immersing the hand in water at ~40˚C for 10 min prior to cannulation and by warming the hand during exercise using an infrared lamp. Blood samples were analyzed immediately for $[lac^-]_B$ (Biosen, EKF Diagnostics, Barleben, Denmark), PCO_2 and pH (ABL520, Radiometer, Copenhagen, Denmark).

Paragraph Number 12 To elucidate the mechanisms accounting for acid-base disturbance the integrated physicochemical systems approach was used (39). At rest, the cessation of maximal exercise and every 10 min thereafter, a 5 ml blood sample was drawn and centrifuged immediately for 10 min at 3000 g. Plasma $[Na^+]$ and $[K^+]$ were measured using inductively coupled plasma optical emission spectrometry (1200DV ICP OES, Perkin Elmer, MA, USA). Plasma $[Cl^-]$ was measured by ion chromatography (DX120, Dionex, CA, USA) and the total concentration of plasma proteins $[PPr^-]$ was assayed in duplicate according to the method of Lowry (19). The total concentration of weak acids ($[A_{tot}^-]$) was subsequently calculated as: $2.45 \times [PPr^-]$ (24). Plasma strong ion difference ([SID]) was calculated as the sum of the strong cations minus the sum of the strong anions (39):

$$[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [lac^-]) \quad [1]$$

Plasma $[H^+]$, along with the contributions of each independent variable (PCO_2, [SID] and $[A_{tot}^-]$) to changes in arterialized venous plasma $[H^+]$ were calculated according to the method of Putman et al. (31).

Lactate recovery kinetics

Paragraph Number 13 The individual $[lac^-]_B$ recovery curves prior to and following the intervention were fitted to the following bi-exponential time function using an iterative non-linear regression technique (11):

$$Lac^-(t) = Lac^-(0) + A_1(1 - e^{\gamma_1 \cdot t}) + A_2(1 - e^{\gamma_2 \cdot t}) \quad [2]$$

Where $Lac^-(t)$ ($mmol \cdot L^{-1}$) denotes the $[lac^-]_B$ for a given time (t; min) of the recovery period and $Lac^-(0)$ ($mmol \cdot L^{-1}$) being the $[lac^-]_B$ at the onset of the recovery period. This equation illustrates that blood lactate kinetics following exercise can be described by two mathematical and physiological processes: one with a fast velocity constant (γ_1; $\cdot min^{-1}$) describing the appearance of lactate in the arterialized blood ($A_1 > 0$; $mmol \cdot L^{-1}$) and an increased $[lac^-]_B$ and a second with a slow velocity constant (γ_2; $\cdot min^{-1}$) describing lactate clearance ($A_2 < 0$; $mmol \cdot L^{-1}$) and a reduction in $[lac^-]_B$. The parameters of the bi-exponential non-linear regression were calculated using SYSTAT (Version 12, SYSTAT software Inc., CA, USA) with the regression method of least mean squares.

Statistical analyses

Paragraph Number 14 Statistical analyses of the dependent variables were performed using SPSS (Version 15, SPSS, Chicago, Illinois, USA). Pre- and post-intervention results and group interactions were assessed using one-way or two-way repeated measures ANOVA across groups (IMT vs. Control), trials (PR vs. ITL) and time (20 min recovery duration or

Pre- vs. Post-intervention). Following a significant *F*-ratio, Tukey's HSD post-hoc analysis was performed. Pearson product-moment correlation coefficients assessed the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

Results

Paragraph Number 15 Training compliance was excellent in the IMT group (92 ± 2%) and inspection of training diaries revealed habitual training remained constant in both groups. MIP was unchanged following the intervention in the control group (pre vs. post: 148.0 ± 35.6 vs. 148.4 ± 37.7 cmH_2O). In contrast, MIP increased from 120.1 ± 27.3 cmH_2O at baseline to 140.0 ± 26.7, 154.8 ± 36.2 and 159.8 ± 34.8 cmH_2O (+34 %) ($P<0.001$) following 2, 4 and 6 wk of IMT, respectively.

Paragraph Number 16 $\dot{W}$ max, $\dot{V}O_2$ max, breathing pattern and HR responses to maximal exercise prior to the intervention are shown in Table 2 for the control and IMT groups, respectively. These responses were similar between trials (PR vs. ITL) and between groups (IMT vs. Control) prior to and following the intervention. Prior to and following the intervention, the coefficient of variation of $\dot{W}$ max in the IMT group was 0.4 ± 1.3% and 0.3 ± 1.0%, respectively, and in the control group was 0.7 ± 1.4% and 1.2 ± 1.9%, respectively. Transient changes in breathing pattern and $\dot{V}O_2$ throughout incremental exercise and recovery from maximal exercise in PR, ITL and ITL% for the IMT group are shown in Figures 1 and 2, respectively. There were no within or between group differences in $\dot{V}_E$, f_R, V_T and $\dot{V}O_2$ during incremental exercise both prior to and following the intervention (Figure 1). During recovery from maximal exercise, $\dot{V}_E$ was similar between trials and between

groups. With ITL, V_T was increased by 0.32 ± 0.16 L and f_R was decreased by 4.5 ± 1.6 breaths·min^{-1}; this increased T_i/T_{tot} in both IMT (absolute increase: 0.020 ± 0.031) and control groups (absolute increase: 0.044 ± 0.047) (Figure 2). These responses were similar following the intervention in both groups and also during the ITL% trial. HR recovery was similar between trials and between groups. Maximal HR was ~180 beats·min^{-1} and decreased to ~100 beats·min^{-1} following 8 min of recovery which was not different from 20 min.

Paragraph Number 17 Pre-intervention, peak and minimum $[lac^-]_B$ were similar in PR and ITL in both groups (Figure 3) and were unchanged in the control group following the intervention. Following IMT the exercise-induced peak, and minimum $[lac^-]_B$ following 20 min recovery were reduced by 1.24 ± 1.32 (P<0.05) and 1.18 ± 1.22 mmol·L^{-1} (P<0.05) in PR, by 1.52 ± 1.26 (P<0.05) and 1.42 ± 1.60 mmol·L^{-1} (P<0.05) in ITL and by 1.50 ± 1.00 (P<0.05) and 1.02 ± 1.01 mmol·L^{-1} (P<0.05) in ITL%, respectively; these reductions were not different between the PR, ITL or ITL% trials. Following IMT only, ITL throughout the 20 min recovery period (mean of 2 to 20 min) reduced $[lac^-]_B$ by 0.66 ± 1.28 mmol·L^{-1} (trial × time interaction effect, P<0.01). When ITL was performed with the same relative inspiratory pressure threshold load as pre-intervention (ITL%), lactate clearance was not different from the post-IMT PR trial (Figure 3).

Paragraph Number 18 The amplitudes and velocity constants for the lactate recovery curves are shown in Table 3. Prior to the intervention, there were no differences between groups or between trials in any parameter, thus ITL throughout recovery failed to affect either lactate exchange or lactate clearance. Following the intervention, all parameters remained unchanged in the control group. Following IMT, relative to the equivalent pre-intervention trial $Lac^-(0)$ and A_2 was reduced in PR (P<0.05). In ITL there was a decrease in A_1 and A_2 and increase in

γ_1 and γ_2 (P<0.05); the reduction in A_2 and increase in γ_2 exceeded those of the control group (group × time × trial interaction effect, P<0.05). In ITL%, relative to the pre-intervention ITL trial there was a reduction in $Lac^-(0)$ and A_2 and increase in γ_1 (P<0.05) although relative to the post-intervention ITL trial γ_2 was slower (P<0.05). In the ITL trial lactate clearance was not correlated with the relative intensity of inspiratory muscle loading (%MIP) prior to the intervention (γ_2; n = 18; see Figure 3: Left panel). Following IMT, there was no correlation between the relative intensity of inspiratory loading and γ_2 when data from both the ITL and ITL% trials were combined (n = 9; see Figure 4: Right panel).

Paragraph Number 19 At rest $[Cl^-]$ was 101.2 ± 3.6 mmol·L^{-1}, $[Na^+]$ was 138.4 ± 5.9 mmol·L^{-1} and $[K^+]$ was 3.9 ± 0.3 mmol·L^{-1} in the IMT group which was not different from the control group. In PR immediately following maximal exercise, $[Cl^-]$ and $[K^+]$ increased by 4.4 ± 0.8 and 1.4 ± 0.5 mmol·L^{-1} in the IMT group and by 4.2 ± 0.6 and 2.5 ± 1.3 mmol·L^{-1}, respectively, in the control group (P<0.05); these increases were similar between groups and between trials (PR vs. ITL). These increases in $[Cl^-]$ and $[K^+]$ were unchanged after the intervention in both groups. $[Na^+]$ remained unchanged after maximal exercise and throughout recovery in both groups and in all trials before and after the intervention. During recovery from maximal exercise $[K^+]$ returned to resting values after 10 min. $[Cl^-]$ remained higher than rest after 10 min of recovery but had returned to resting concentration after 20 min. These patterns were similar in both groups during both PR and ITL trials, and were largely unaffected by the intervention period. The pattern observed in ITL% was not different to that observed after the intervention in the PR trial. After 20 min of the post-IMT ITL trial $[K^+]$ was 0.3 ± 0.3 mmol·L^{-1} greater (P<0.05) than at the same time point of the pre-IMT ITL trial.

Paragraph Number 20 Tables 4 and 5 illustrate the contributions of the independent variables ([SID], $[A_{tot}^-]$ and PCO_2) to changes in plasma $[H^+]$. Before IMT, $[H^+]$ increased significantly from rest (37.3 ± 2.2 nmol·L^{-1}) to maximal exercise (PR: 60.4 ± 7.9 nmol·L^{-1}, ITL: 63.0 ± 7.3 nmol·L^{-1}, P<0.01). Similar changes were observed in the control group. During the final 10 min of the recovery period of the PR trial, 84% of the increase in $[H^+]$ above rest was accounted for by a 9.4 mmol·L^{-1} reduction in [SID] with the remaining 16% due to a 5.2 mmol·L^{-1} increase in $[A_{tot}^-]$. During the recovery period $[H^+]$ was lower by ~3 nmol·L^{-1} in the ITL trial compared to the PR trial although this difference was accounted for by the greater hypocapnia (lower PCO_2) observed during the ITL trial. Similar findings were observed in the control group both prior to and following the intervention. Following IMT, responses in the ITL% trial were similar to those observed in the post-IMT PR trial (Table 4).

Paragraph Number 21 Compared to pre-intervention values, following IMT plasma $[H^+]$ was lower in recovery from maximal exercise in both PR (main effect trial, P<0.05) and ITL (main effect trial, P<0.05). In the same analysis PCO_2 and $[A_{tot}^-]$ after exercise and throughout recovery were not different following IMT. Therefore the reduction in $[H^+]$ was accounted for exclusively by an increased [SID]. The increase in [SID] during PR was accounted for by the reduction in $[lac^-]_B$ and during ITL by the significant 1.7 mmol·L^{-1} (P<0.05) and 0.3 mmol·L^{-1} (P<0.05) decrease and increase in $[lac^-]_B$ and $[K^+]$, respectively.

Discussion

Paragraph Number 22 The primary finding of this study is that the addition of a pressure-threshold inspiratory resistance (15 cmH_2O) during recovery from maximal incremental exercise accelerated blood lactate clearance but only after 6 wk of specific IMT.

Paragraph Number 23 Our finding that pressure-threshold loading of untrained inspiratory muscles immediately following maximal exercise failed to affect systemic lactate clearance (Figure 3) disagrees with the findings of Chiappa et al. (3). An explanation for this disagreement is not readily forthcoming as the experimental protocols were broadly similar (including breathing pattern). However, it must be acknowledged that although the same inspiratory resistance was used the methods of inspiratory muscle loading were somewhat different. The valve opening characteristics of the ITL device may have differed and an additional 1 m length of tubing was used in the present study to connect the device to the subject. Chiappa et al. (3) found that during recovery with an inspiratory resistance [H^+] was unaffected by a large (~2.5 $mmol \cdot L^{-1}$) decrease in $[lac^-]_B$ with no change in PCO_2. The authors suggest that flux in other strong ions (not measured) may explain the unaltered blood acid-base balance despite the large reduction in $[lac^-]_B$. We found no such changes either before or after IMT. Also, lactate clearance is well described by a bi-exponential function following exercise at different intensities (11), with respiratory muscle loading (29; this study) and following both whole body training (26) and IMT (this study). That this pattern was not observed by Chiappa et al. (3) is also difficult to resolve.

Paragraph Number 24 Whilst methodologically disparate our (pre-intervention) findings are similar to those of Perret and Mueller (29) who reported unchanged lactate recovery kinetics following exercise with low intensity isocapnic volitional hyperpnea ($\dot{V}_E$ 61.6 ± 9.3 $L \cdot min^{-1}$, 30 ± 1% of MVV) compared to PR. Therefore the issue of whether increasing the work of breathing offers a method of accelerating lactate clearance remains equivocal. It is possible that the intensity of inspiratory muscle loading is influential: when ITL was performed at the same relative intensity (an absolute pressure threshold of 20 ± 2 cmH_2O; i.e. ITL%) following IMT lactate clearance was not accelerated relative to PR and ITL. This finding is

similar to previous work where relative to high intensity leg exercise (65% $\dot{V}O_2$ max), low intensity leg exercise (35% $\dot{V}O_2$ max) performed immediately after maximal exercise increased lactate clearance (5). The blood flow characteristics of different exercise intensities were proposed as an explanation for their findings (5). Notwithstanding this, the lack of relationship between %MIP of ITL (range: 10% - 19%) and rates of lactate clearance (Figure 4) does not support the notion that the ITL intensity is influential. However, it is interesting to speculate whether a lower inspiratory resistance (<15 cmH_2O) prior to the intervention would have accelerated lactate clearance and further work is warranted to reveal the effects of ITL intensity upon lactate recovery kinetics.

Paragraph Number 25 We also report that IMT reduced peak $[lac^-]_B$ by ~1 $mmol \cdot L^{-1}$ after completion of the maximal incremental exercise test despite no change in the incremental or maximal exercise breathing pattern (Figure 1). This agrees with previous RMT studies showing a lower $[lac^-]_B$ following both maximal incremental (38) and steady-state exercise (23) without a change in the exercise $\dot{V}_E$ and lends further credence to the hypothesis that RMT affects lactate clearance rather than lactate production (2). When comparing $[lac^-]_B$ during the ITL trial pre- and post-IMT the difference was maximal (2.30 $mmol \cdot L^{-1}$) after 8 min. Chiappa et al. (4) recently reported that 10 min ITL accelerated lactate recovery and increased peak power during subsequent 30 s all-out maximal exercise. Whether IMT magnifies such effects also remains an intriguing question.

Paragraph Number 26 We are the first to report that ITL after specific IMT can significantly speed lactate clearance following maximal incremental cycling exercise. Following IMT, A_2 which reflects the amplitude concentration of lactate clearance, was reduced during PR from maximal exercise, however, since the velocity constants were unchanged, this is likely to

reflect the lower absolute $[lac^-]_B$ throughout recovery relative to pre-intervention (Table 3 and Figure 3). Conversely, increasing the work of breathing with ITL immediately following exercise at the same intensity increased the velocity constants and decreased the amplitudes of both exponential terms (Table 3). We observed a significant reduction in PCO_2 throughout the ITL trial and respiratory alkalosis is known to elevate the $[lac^-]_B$ (2). Thus, whether controlling breathing pattern throughout recovery would further lower $[lac^-]_B$ and accelerate lactate recovery kinetics remains to be confirmed. Previous studies have reported similar changes in these parameters following whole-body training (26, 27). After IMT, we observed a 68% increase in γ_1 during ITL indicating an improved capacity for lactate exchange between the previously worked muscle(s) and the systemic circulation (11). Due to the specific nature of IMT this was probably achieved by increasing the concentration gradient between the locomotor muscles and the systemic circulation most likely due to increased lactate clearance by the inspiratory muscles (as confirmed by the 71% increase in γ_2). The increase in γ_2 is similar to that found in whole body exercise training studies in which it was associated with an increase in lactate transport capacity (MCT1, MCT4) and oxidative enzyme activity (26, 27). It has been argued that such adaptations may occur following IMT (cf. 23). In support, oxidative enzyme adaptations occurred in sheep diaphragm following intense resistive RMT (1) and an increased proportion of type I muscle fibres was observed in the external intercostal muscles of COPD patients following 5 wk IMT (33).

Paragraph Number 27 We used the physicochemical approach (39) to quantify the relative contribution of each of the independent variables to changes in acid-base disturbance (31, 39). Similar to a previous study (31), we observed excellent agreement between the measured and calculated $[H^+]$ (r = 0.925, P<0.001). Following IMT the smaller disturbance of $[H^+]$ during ITL compared to PR was due to an increase in [SID]. With the exception of a small

increase in [K^+] no other strong ion was affected. Therefore the increase in [SID] was almost exclusively accounted for by the reductions in [lac^-]$_B$. The defence of plasma acid-base homeostasis is considered of great importance during and following exercise (31), therefore, IMT and ITL may provide a favourable systemic metabolic environment for subsequent bouts of exercise (4, 7).

Paragraph Number 28 $\dot{W}$ max, peak [lac^-]$_B$, acid-base balance and lactate recovery kinetics, were unaltered in the control group after the intervention period. With the exception of one study (37) evidence suggests that placebo effects associated with RMT interventions are minimal (12, 15, 18, 35, 36, 41) thus we feel that the physiological changes observed after IMT in this study are unlikely to be the result of greater subject expectation and/or motivation. Notwithstanding this, a limitation of our study is that a placebo was not used, which may have influenced outcome measures in the control group.

Conclusions

Paragraph Number 29 In stark contrast to the findings of Chiappa et al. (3, 4), we observed no effect of ITL upon lactate recovery kinetics. The novel finding of this investigation is that following IMT, ITL accelerates the capacity for whole body lactate exchange and clearance. Furthermore, IMT also reduced plasma [H^+] which was accounted for by the increase in [SID] due almost exclusively to the IMT-mediated reduction in [lac^-]$_B$. The potential mechanisms affecting lactate recovery kinetics following IMT appear similar to those observed following whole body endurance training. The effects of ITL during recovery from intense exercise on subsequent performance following IMT present novel avenues for future study.

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Table 1. Descriptive characteristics of the subjects.

	Control (*n*=9)	IMT (*n*=9)
Age (years)	27.1 ± 3.7	32.2 ± 6.3 *
Body mass (kg)	81.3 ± 8.0	78.9 ± 16.6
Height (cm)	183.3 ± 6.6	177.0 ± 9.5
FVC (L)	6.03 ± 0.92 (109 ± 14)	5.22 ± 1.03 (107 ± 9)
FEV_1 (L)	4.77 ± 0.63 (103 ± 11)	4.11 ± 0.76 (101 ± 7)
FEV_1/FVC (%)	79.5 ± 5.2 (97 ± 7)	79.3 ± 6.7 (96 ± 7)
MVV_{10} ($L \cdot min^{-1}$)	198.5 ± 23.2 (105 ± 14)	176.6 ± 29.4 (109 ± 9)
MIP (cmH_2O)	148.0 ± 35.7 (114 ± 4)	120.1 ± 27.3 (109 ± 7)
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	4.27 ± 0.49	4.13 ± 0.83
$\dot{W}$max (W)	386 ± 44	378 ± 57

Values are expressed as mean ± SD. Values in parentheses represent the percent of predicted values (28, 37). FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; MVV_{10}, maximal voluntary ventilation in 10 s; MIP, maximal inspiratory mouth pressure; $\dot{V}O_2$max , maximal oxygen consumption; $\dot{W}$max , maximal power output. *, $P<0.05$ control group vs. IMT group.

Table 2. Pre-intervention responses to maximal incremental cycling exercise prior to 20 min passive recovery and 20 min inspiratory threshold loading (ITL).

	Control group		IMT group	
	Passive recovery	ITL	Passive recovery	ITL
$\dot{W}max$ (W)	387 ± 44	387 ± 41	378 ± 57	376 ± 57
$\dot{V}O_2 max$ ($L \cdot min^{-1}$)	4.21 ± 0.66	4.23 ± 0.52	4.10 ± 0.92	4.20 ± 0.75
$\dot{V}_E$ ($L \cdot min^{-1}$)	166.6 ± 22.5	170.1 ± 14.0	163.1 ± 32.3	158.8 ± 34.8
f_R ($breaths \cdot min^{-1}$)	61 ± 13	60 ± 8	60 ± 11	58 ± 11
V_T (L)	2.83 ± 0.62	2.93 ± 0.47	2.90 ± 0.94	2.86 ± 0.93
T_I/T_{TOT}	0.50 ± 0.02	0.50 ± 0.01	0.47 ± 0.04	0.50 ± 0.01
HR ($beats \cdot min^{-1}$)	177 ± 9	178 ± 11	181 ± 10	181 ± 11

Values are expressed as mean ± SD. $\dot{W}max$, maximal power output; $\dot{V}O_2 max$, maximal oxygen uptake; $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume; T_I/T_{TOT}, inspiratory time divided by total breath time (duty cycle); HR, heart rate.

Table 3. Parameters of the bi-exponential non-linear regression model for both the control and IMT groups, respectively. Data from the control group were not different following the intervention and have been omitted.

	Control Group (n=9)		IMT group (n=9)				
	Pre-intervention		Pre-intervention		Post-intervention		
	PR	ITL	PR	ITL	PR	ITL	ITL %
La(0)	10.97 ± 1.22	11.11 ± 1.44	11.25 ± 1.53	11.50 ± 1.52	10.12 ± 1.58[a]	9.91 ± 2.04[a]	9.94 ± 1.32[c]
A_1	4.387 ± 1.443	4.383 ± 1.561	3.933 ± 0.391	3.992 ± 1.900	3.368 ± 0.919	2.554 ± 0.666[ab]	3.209 ± 1.516
γ_1	0.270 ± 0.246	0.313 ± 0.182	0.296 ± 0.084	0.235 ± 0.076	0.308 ± 0.168	0.463 ± 0.266[ab]	0.377 ± 0.199[c]
A_2	-21.765 ± 7.988	-19.166 ± 6.847	-20.624 ± 5.503	-20.172 ± 3.827	-15.161 ± 4.425[a]	-13.132 ± 3.958[ab]	-14.723 ± 3.588[c]
γ_2	0.031 ± 0.014	0.037 ± 0.015	0.031 ± 0.011	0.034 ± 0.009	0.036 ± 0.012	0.056 ± 0.025[ab]	0.038 ± 0.014[d]

Values are mean ± SD. For abbreviations and units see methods. [a], different to the same trial pre-intervention, (P<0.05); [b], different to PR, (P<0.05); [c], different to ITL pre-intervention (P<0.05); [d], different to ITL post-intervention.

Table 4. Independent and dependent acid-base variables immediately following maximal exercise (Max) and after 10 and 20 min recovery in the IMT group only. Data from the control group have been omitted since they were not different from the IMT group pre-intervention and remained unchanged following the intervention.

		IMT group (n=9)									
			Passive recovery			15 cmH_2O ITL			ITL%		
		Rest	Max	10 min	20 min	Max	10 min	20 min	Max	10 min	20 min
Independent variables											
[SID]	Pre	40.4 ± 6.2	28.7 ± 7.3	26.2 ± 7.2†	35.8 ± 7.5	33.2 ± 14.4	32.0 ± 13.3	34.0 ± 6.6	-	-	-
	Post	-	32.3 ± 6.7	28.7 ± 6.7†	36.2 ± 11.0	33.1 ± 9.7	28.7 ± 10.1	40.8 ± 11.8*	31.8 ± 5.4	28.8± 6.5†	34.3 ± 4.5
[A_{tot}^-]	Pre	16.2 ± 8.2	20.5 ± 5.4	23.3 ± 3.6	19.5 ± 4.3	23.6 ± 9.7	22.9 ± 3.4	18.7 ± 6.9	-	-	-
	Post	-	21.1 ± 3.2	23.7 ± 3.1	21.3 ± 7.1	26.7 ± 13.2^{c}	21.5 ± 8.0	22.2 ± 3.3*	22.4 ±4.2	24.6 ± 3.8	21.5 ± 6.1
PCO_2	Pre	39.5 ± 4.1	42.2 ± 8.0	34.4 ± 3.0†	36.1 ± 3.2	44.0 ± 7.7	33.9 ± 4.8	33.2 ± 6.0	-	-	-
	Post	-	42.4 ± 9.0	34.4 ± 2.9	35.7 ± 2.7	42.3 ± 7.4	32.5 ± 4.7†	32.4 ± 5.5	44.2 ± 7.6	34.0 ± 5.0	33.8± 6.6†
Dependent variables											
[H^+]	Pre	37.3 ± 2.2	60.4 ± 7.9†	53.8 ± 5.8†	45.2 ± 4.2$^{\dagger ab}$	63.0 ± 7.3†	51.1 ± 3.8†	41.5 ± 4.0^{ab}	-	-	-
	Post	-	57.0 ± 8.7†	50.6 ± 5.4†	43.2 ± 4.6$^{\dagger ab}$	59.0 ± 10.0†	48.8 ± 7.9^{ab}	40.4 ± 6.3^{ab}	60.2± 8.1†	50.2±5.2$^{\dagger a}$	42.3±6.3$^{\dagger b}$
[HCO_3^-]	Pre	25.3 ± 1.8	16.7 ± 2.0†	15.6 ± 2.1†	19.2 ± 2.4$^{\dagger b}$	16.7 ± 2.1†	15.8 ± 2.0†	19.0 ± 2.4$^{\dagger b}$	-	-	-
	Post	-	17.8 ± 2.5†	16.4 ± 2.1†	19.9 ± 1.9$^{\dagger b}$	17.2 ± 2.1†	16.2 ± 3.0†	19.3 ± 2.7$^{\dagger b}$	17.6± 2.4†	16.2± 1.9†	18.8± 2.3†

Values are expressed as means ± SD. [SID], strong ion difference; [A_{tot}^-], total concentration of weak acids; PCO_2, partial pressure of carbon dioxide; [H^+], hydrogen ion concentration; [HCO_3^-], bicarbonate concentration. Within trials: † significantly different from rest (P<0.05); ^{a} significantly different from max (P<0.05); ^{b} significantly different from 10 min (P<0.05). Between trials: ^{c} time point significantly different from passive recovery (P<0.05); * significantly different from pre-IMT (P<0.05).

Table 5. Contributions of the independent variables PCO_2, [SID] and [A_{tot}^-] to changes in plasma [H^+] following maximal exercise with 20 min passive recovery (PR) and inspiratory threshold loading (ITL) prior to (Pre-IMT) and following-IMT (Post-IMT). Data are the average of min 10 to 20.

	Concentration			Contribution of independent variables [H^+] (nmol·L^{-1})		Δ [H^+] = (exercise) – (rest) (nmol·L^{-1})		Percentage contribution to Δ [H^+] (%)	
Variable	Rest	PR	ITL	PR	ITL	PR	ITL	PR	ITL
Pre-IMT									
[H^+] meas. (nmol·L^{-1})	37.3	49.3	46.4	-	-	+12.0	+9.1	-	-
[H^+] calc. (nmol·L^{-1})	36.1	52.0	44.8	-	-	+15.9	8.7	-	-
PCO_2 (mmHg)	39.5	35.2	33.5	32.2	30.9	-3.9	-5.2	-21	-37
[SID] (mmol·L^{-1})	40.4	31.0	33.0	51.9	47.5	+15.8	+11.4	+84	+81
[A_{tot}^-] (mmol·L^{-1})	16.2	21.4	20.8	39.2	38.8	+3.1	+2.7	+16	+19
Post-IMT									
[H^+] meas. (nmol·L^{-1})	37.3	46.9	44.6	-	-	+9.6	+7.3	-	-
[H^+] calc. (nmol·L^{-1})	36.1	49.7	40.8	-	-	+13.6	+4.7	-	-
PCO_2 (mmHg)	39.5	35.1	32.5	32.2	29.6	-3.9	-6.5	-24	-56
[SID] (mmol·L^{-1})	40.4	32.5	34.8	48.8	44.5	+12.7	+8.4	+77	+72
[A_{tot}^-] (mmol·L^{-1})	16.2	22.5	21.9	39.8	39.4	+3.7	+3.3	+23	+28

For abbreviations see Table 4. meas., measured [H^+]; calc., calculated [H^+] using the method of Putman et al. (27).

List of Figure captions:

Figure 1. Respiratory responses to maximal incremental cycling exercise for the IMT group only prior to and following the 6 wk intervention. ▲, passive recovery trial pre-intervention; □, inspiratory pressure threshold loading trial at 15 cmH_2O pre-intervention; ■, passive recovery trial following the intervention; ∆, ITL trial following the intervention; ●, inspiratory pressure threshold loading trial post-intervention at a higher absolute resistance but the same relative resistance as pre-intervention (ITL%).

Figure 2. Respiratory responses to 20 min of recovery from maximal incremental cycling exercise in the IMT group only prior to and following the 6 wk intervention. 'Max' is defined as the point of exercise intolerance. ▲, passive recovery pre-intervention; □, inspiratory pressure threshold loading at 15 cmH_2O (ITL) pre-intervention; ■, passive recovery following the intervention; ∆, ITL following the intervention; ●, ITL%.

Figure 3. Blood lactate concentration ($[lac^-]_B$) during 20 min of recovery from maximal incremental cycling exercise in the IMT group only prior to and following the 6 wk intervention. 'Max' is defined as the point of exercise intolerance. ▲, passive recovery; □, inspiratory pressure threshold loading at 15 cmH_2O (ITL); ●, ITL%. **, Post-intervention: ITL different to PR (P<0.05).

Figure 4. Inspiratory pressure threshold load relative to the maximal inspiratory pressure (MIP) versus the slow velocity constant (γ_2; $\cdot min^{-1}$) which describes lactate clearance ($A_2 < 0$; $mmol \cdot L^{-1}$). Left panel: pre-intervention pooled data of both control and IMT groups. Right panel: post-IMT data from the ITL and ITL% trials; o, ITL data; □, ITL% data. Note: regression line reflects the pooled data from both the ITL and ITL% trials.