Title: Inspiratory loading intensity does not influence lactate clearance during recovery

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Abstract

Purpose: This study examined the effects of different pressure threshold inspiratory loads on lactate clearance and plasma acid-base balance during recovery from maximal exercise. **Methods:** Eight moderately trained males ($\dot{V}O_2$ peak = 4.29 ± 0.46 L·min^{-1}) performed, on different days, four maximal incremental cycling tests (power started at 0 W and increased by 20 W·min^{-1}) of identical duration (exercise time during the first trial was 16.32 ± 1.12 min). During 20 min recovery subjects either rested passively or breathed through a constant pressure threshold inspiratory load of 10 (ITL10), 15 (ITL15), or 20 (ITL20) cmH_2O. Plasma lactate concentration ([La^-]) was measured and acid-base balance was quantified using the physicochemical approach which describes the dependency of [H^+] on the three independent variables: strong ion difference ([SID] = [Na^+] + [K^+] – [Cl^-] + [La^-]), the total concentration of weak acids ([A_{tot}^-]), and the partial pressure of carbon dioxide (PCO_2). **Results:** Peak exercise responses were not significantly different between trials. During recovery the area under the plasma [La^-] curve was not different between trials (pooled mean = 261 ± 60 mEq) and the [La^-] measured at the end of the 20 min recovery was also similar (passive recovery = 9.2 ± 3.1 mEq·L^{-1}; ITL10 = 9.3 ± 3.1 mEq·L^{-1}; ITL15 = 8.7 ± 2.8 mEq·L^{-1}; ITL20 = 8.7 ± 3.2 mEq·L^{-1}). Similarly, changes in other strong ions contributing to [SID], and [A_{tot}^-], PCO_2, and therefore [H^+], were not different between trials. **Conclusion:** These data suggest that, in individuals of moderate endurance training status, inspiratory loading at the intensities used in the present study does not accelerate lactate clearance or modify plasma acid-base balance during recovery from maximal exercise.

Key Words: INSPIRATORY LOADING, RECOVERY, LACTATE CLEARANCE, ACID-BASE BALANCE, CYCLING

Introduction

Paragraph Number 1 Elevated muscle and blood lactate concentrations ([La^-]), and the accompanying H^+ accumulation, have long been associated with impaired muscle function and exercise tolerance (15). Following high-intensity exercise an active recovery using the previously exercised muscles quickens the decline in blood [La^-] (12,29,32,41) and, compared to passive recovery, may result in better maintenance of performance in subsequent exercise bouts (3,31). Conversely, active recovery using the previously exercised muscles may impair subsequent performance due to slower rates of muscle reoxygenation and phosphocreatine resynthesis (6,39). Since the primary fate of lactate during exercise and recovery is oxidative metabolism by skeletal muscle (2,17,18), the high capillary density and oxidative capacity of the inspiratory muscles (33) makes them ideally suited to lactate metabolism. The use of inspiratory loading during recovery may thus provide an alternative strategy by which lactate clearance may be enhanced without compromising the repletion of intramuscular energy stores within the previously exercised locomotor muscles.

Paragraph Number 2 Two recent issues of *Medicine and Science in Sports and Exercise* (5,9) present conflicting evidence regarding whether inspiratory muscle loading can quicken blood lactate clearance following high intensity exercise. Chiappa et al. (9) showed that a pressure threshold inspiratory load (15 cmH_2O) during recovery from high intensity exercise greatly accelerated lactate clearance from the blood. Specifically, when compared with passive recovery the absolute blood [La^-] and area under the curve (AUC) for blood [La^-] were lower with inspiratory loading by around 2.5 $mEq \cdot L^{-1}$ (-24%) and 21 mEq (-16%), respectively. Furthermore, a follow-up study showed that peak cycling power in the second of two successive Wingate tests increased by 25% after this recovery intervention (8). However, despite using similar methodology Brown et al. (5) failed to reproduce the observations of Chiappa et al. (8,9). Specifically, lactate clearance during recovery from high

intensity exercise was accelerated by a 15 cmH_2O pressure threshold inspiratory load only after inspiratory muscle training, and then only by a much smaller degree to that reported by Chiappa et al. (8,9).

Paragraph Number 3 Interestingly, Chiappa et al. (9) reported large reductions in blood [La^-] with inspiratory loading without the concomitant changes in plasma [H^+] and [HCO_3^-] that would be expected to occur. According to the physicochemical approach to acid-base balance (40) [H^+] in intracellular and extracellular fluid is, together with [HCO_3^-], a dependent variable whose value is dependent on the equilibrium state reached by the independent variables: strong ion difference ([SID], fully dissociated cations minus anions, which in plasma is equal to [Na^+] + [K^+] – [Cl^-] + [La^-]), the total concentration of weak acids ([A_{tot}^-]), and PCO_2 (for reviews see refs: 21,23,25). Therefore, for plasma [H^+] to have remained unchanged with inspiratory loading, the large reduction in blood [La^-] observed by Chiappa et al. (9) must have been balanced by corresponding changes in the concentration of other strong ions and/or [A_{tot}^-] (40). This hypothesis was not supported by the work of Brown et al. (5) which showed that concentrations of other strong ions and [A_{tot}^-] were unaffected by inspiratory loading both before and after inspiratory muscle training. Indeed, the lower blood [La^-] with inspiratory loading after inspiratory muscle training was exclusively responsible for an increase in [SID] and, subsequently, a lower [H^+] (5).

Paragraph Number 4 One potential explanation for the disagreement between the findings of Chiappa et al (8,9) and Brown et al. (5) may be that the magnitude of the pressure threshold load is an important determinant of whether inspiratory loading accelerates lactate clearance during recovery. Specifically, after inspiratory muscle training, which increased participants' maximum inspiratory mouth pressure (MIP) by 34%, lactate clearance was accelerated only when inspiratory loading was performed at the same absolute (15 cmH_2O or 10% MIP), but not relative (20 cmH_2O or 13% MIP), intensity (5).

Paragraph Number 5 Therefore, the aim of the present study was to resolve some of the controversies surrounding the effects of inspiratory loading during recovery from high-intensity exercise. Consequently, we investigated the effects of different pressure threshold inspiratory loads (10, 15 and 20 cmH_2O), applied during recovery from high-intensity exercise, on lactate clearance and plasma acid-base balance quantified using the physicochemical approach.

Methods

Participants

Paragraph Number 6 Following approval from Nottingham Trent University's ethics committee, 8 healthy non-smoking males with normal lung function (Table 1) provided written informed consent to participate in the study. Throughout the study participants were instructed to adhere to their habitual training regimen and not to engage in any strenuous exercise the day preceding and the day of a trial. Participants arrived at the laboratory 2 h postprandial having abstained from alcohol and caffeine in the 24 h before testing.

Experimental design

Paragraph Number 7 Participants attended the laboratory on 5 separate occasions, at a similar time of day, separated by at least 48 h but no more than 1 week. During the first laboratory visit, pulmonary function and MIP were measured. During subsequent visits participants performed an incremental cycling exercise test (the first of which was continued to the limit of tolerance) followed by a 20 min recovery period comprising either passive recovery, or breathing against a constant inspiratory pressure threshold load of either 10 (ITL10), 15 (ITL15), or 20 cmH_2O (ITL20). The order of the exercise trials was randomized. The exercise duration achieved during the incremental exercise test of the first trial was replicated in all subsequent trials.

Pulmonary function and maximal inspiratory mouth pressure

Paragraph Number 8 Pulmonary function was assessed according to published guidelines (30) using a pneumotachograph (Pneumotrac, Vitalograph, Buckinghm, UK) calibrated using a 3 L syringe. A hand-held mouth pressure meter (MicroRPM, CareFusion, Hampshire, 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. Maneuvers were performed in an upright standing posture, initiated from residual volume, and sustained for at least 1 s. Repeat efforts separated by 30 s were performed until three serial measures differed by no more than 10% or 10 cmH_2O, whichever was smallest (5). The highest value recorded was used for subsequent analysis. For each participant MIP was compared with predicted values using the equation of Wilson et al. (45), where: $MIP_{predicted} = 142 - (1.03 \times age)$.

Maximal incremental cycling test

Paragraph Number 9 Exercise was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). During the first trial a maximal incremental cycling test was performed, whereby power started at 0 W and increased by 20 $W \cdot min^{-1}$ until participants could not maintain a cadence greater than 60 $revs \cdot min^{-1}$ despite verbal encouragement. In an attempt to standardize the magnitude of elevation of plasma $[La^-]$, the exercise duration achieved during this first test was recorded for each participant and during subsequent tests each participant was required to exercise for an identical duration, at which point the test was terminated by the investigators. Cycling cadence was self-selected during the first test and repeated during subsequent tests. Participants wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) connected to a pneumotachograph and a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri). Respiratory variables were measured breath by breath (ZAN

600USB, Nspire Health, Oberthulba, Germany). The final power attained and the highest oxygen uptake ($\dot{V}O_2$) recorded over any 30 s period defined maximal power ($\dot{W}$ max) and $\dot{V}O_2$ peak, respectively. The prescribed recovery intervention (see below) was imposed immediately upon cessation of exercise.

Passive recovery

Paragraph Number 10 At the cessation of the incremental exercise test a 0.5 m length of wide-bore (35 mm internal diameter) corrugated tubing was attached to the inspiratory port of the two-way valve and participants remained seated at rest on the cycle ergometer for 20 min. The tubing attached to the inspiratory port was used in the inspiratory loading trials to connect the participant to the inspiratory loading device (see below). Blood samples were taken at rest, upon the cessation of exercise and every 4 min during recovery. Upon the cessation of exercise and every 2 min during recovery heart rate was recorded using short-range telemetry (Polar S610, Polar, Kempele, Finland) and arterial oxygen saturation was estimated (SpO_2) using a finger pulse oximeter (Model 8500, Nonin Medical, Minnesota).

Inspiratory pressure threshold loading

Paragraph Number 11 Inspiratory loading trials were identical to the passive recovery trial, except that immediately after exercise the 0.5 m length of wide-bore tube connected to the inspiratory port of the two-way valve was connected distally to a custom-built weighted plunger pressure threshold inspiratory loading device identical to that used previously (5,19,20) and which has been shown to be flow independent over the physiological range (19); for a full description of the device see Johnson et al. (20). The threshold opening pressure during ITL10, ITL15, and ITL20 represented 8 ± 3, 12 ± 4, and 16 ± 5% of MIP, respectively. Absolute pressure threshold loads were used to allow direct comparison with the work of Chiappa et al. (8,10) and Brown et al. (5). We have previously shown that the

performance of our breath by breath gas analyzer is unaffected by the negative pressures generated during ITL (5).

Blood sampling and analysis

Paragraph Number 12 Arterialized venous blood (6 mL) was drawn from a dorsal hand vein via an indwelling 21-G cannula (28). Arterialization was achieved by immersing the hand in water at 40°C for 10 min prior to cannulation and by warming the hand during trials using an infrared lamp. Blood was analyzed immediately for PCO_2 and pH (ABL520, Radiometer, Copenhagen, Denmark), which were subsequently used to calculate plasma bicarbonate concentration ($[HCO_3^-]$) using the Henderson-Hasselbalch equation:

$$pH = pK + \log \frac{[HCO_3^-]}{0.03 \times PCO_2}$$

A portion (5 mL) of each blood sample was immediately centrifuged for 10 min at 3000g and the plasma supernatant was removed. Plasma $[La^-]$ was subsequently determined using an automated analyzer (Biosen C_line Sport, EKF Diagnostics, Barleben, Germany). Plasma $[Na^+]$, $[K^+]$, and $[Cl^-]$ were determined using ion selective electrodes and total protein concentration ($[PPr^-]$) was assayed by immunoturbidimetry (ABX Pentra 400, Horiba, Northampton, UK). $[A_{tot}^-]$ was calculated as 2.45 × $[PPr^-]$ (37). Plasma strong ion difference ([SID]) was calculated as the sum of the strong cations minus the sum of the strong anions (40):

$$[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [La^-])$$

Plasma $[H^+]$ was calculated using the following equation, which describes the dependency of $[H^+]$ on the three independent physicochemical variables ([SID], PCO_2, and $[A_{tot}^-]$), as well as on mass action equilibria, conservation of mass, and electroneutrality (40):

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_{tot}]) - (K_C PCO_2 + K'_W)\}$$
$$\times [H^+]^2 - \{K_A(K_C PCO_2 + K'_W) + (K_3 K_C PCO_2)\}[H^+] - (K_A K_3 K_C PCO_2) = 0$$

Values used for the equilibrium constants were: $K_A = 3.0 \times 10^{-7}$ Eq·L^{-1}, $K_C = 2.45 \times 10^{-11}$ (Eq·L^{-1})^2; $K_3 = 6.0 \times 10^{-11}$ Eq·L^{-1}, and $K_W = 4.4 \times 10^{-14}$ Eq·L^{-1}.

To assess the individual contributions of the three independent variables ([SID], PCO_2, and [A_{tot}^-]) to changes in plasma [H^+], the latter was calculated as one of the three variables was changed to the measured value while the others were held constant at resting values (35,40). Hereafter, the measured and calculated [H^+] are referred to as $[H^+]_{measured}$ and $[H^+]_{calculated}$, respectively.

Data and statistical analysis

Paragraph Number 13 The AUC for $\dot{V}O_2$ and plasma [La^-] during recovery was calculated using the trapezoidal rule. Data were analyzed using repeated measures ANOVA and pairwise comparisons were made according to Bonferroni-adjusted 95% confidence intervals. Statistical significance was set at $P<0.05$. Results are presented as mean ± SD.

Results

Paragraph Number 14 For each participant the exercise duration, and thus $\dot{W}$ max, achieved during the first incremental exercise test (group mean: 16.32 ± 1.12 min and 318 ± 20 W) was replicated in all subsequent tests.

Ventilatory responses, pulmonary gas exchange, heart rate and arterial oxygen saturation

Paragraph Number 15 Breathing pattern, minute ventilation ($\dot{V}_E$) (155 ± 27 L·min^{-1}), $\dot{V}O_2$ peak (4.29 ± 0.46 L·min^{-1}), $\dot{V}CO_2$ peak (5.30 ± 0.75 L·min^{-1}), heart rate (178 ± 9 beats·min^{-1}) and SpO_2 (96 ± 2%) at the end of maximal exercise were not different between trials (values in parentheses are pooled data from all trials). $\dot{V}_E$ throughout recovery was not different between trials (Figure 1). Respiratory frequency (f_R) during passive recovery (23 ±

4 breaths·min^{-1}) was greater than during ITL10 (19 ± 4 breaths·min^{-1}) and ITL15 (18 ± 4 breaths·min^{-1}) (main effect for trial, $P < 0.05$). There was also a trend ($P = 0.09$) for f_R to be higher during passive recovery compared to ITL20 (19 ± 4 breaths·min^{-1}). Tidal volume (V_T) during passive recovery (1.07 ± 0.35 L) was lower (main effect for trial) than during ITL10 (1.43 ± 0.30 L) ($P < 0.01$), ITL15 (1.39 ± 0.27 L) ($P < 0.01$), and ITL20 (1.42 ± 0.31 L) ($P < 0.05$). Duty cycle (T_I/T_{TOT}) during passive recovery (0.44 ± 0.04) was greater than during ITL15 (0.38 ± 0.06) (main effect for trial, $P < 0.05$). There was a trial×time interaction effect for T_I/T_{TOT} ($P < 0.01$) and differences were observed for passive recovery vs. ITL15 and ITL20 in the latter half of recovery (Figure 1). There were no differences between trials for mean $\dot{V}O_2$ during recovery (pooled mean = 0.77 ± 0.28 L·min^{-1}) or the AUC for $\dot{V}O_2$ during recovery (pooled mean = 19.4 ± 2.9 L). Mean $\dot{V}CO_2$ during passive recovery (0.72 ± 0.14 L·min^{-1}) was not different from ITL15 (0.83 ± 0.19 L·min^{-1}), but was less than ITL10 (0.87 ± 0.14 L·min^{-1}) and ITL20 (0.86 ± 0.13 L·min^{-1}) (main effect for trial, $P < 0.05$). Heart rate during recovery was not different between trials (pooled mean = 101 ± 15 beats·min^{-1}). In all trials SpO_2 was 98 ± 1% at rest and 97 ± 1% during recovery.

Plasma ions contributing to [SID]

Paragraph Number 16 Changes in plasma ions contributing to [SID] are shown in Figure 2. Changes in [La^-], [Na^+], [K^+], and [Cl^-] after maximal exercise and during recovery were not different between trials, thus the following data reflect the pooled mean ± SD. Plasma [La^-] increased to 15.4 ± 2.7 mEq·L^{-1} after maximal exercise and peaked at 16.0 ± 2.8 mEq·L^{-1} following 4 min of recovery, after which values progressively declined. The mean AUC for plasma [La^-] during recovery was 261 ± 60 mEq and was not different between trials. Plasma [Na^+] increased 6.2 ± 2.7 mEq·L^{-1} from rest after maximal exercise (main effect for time, $P < 0.01$) and remained elevated after 4 min of recovery ($P < 0.05$). Thereafter values were not

different from rest. Plasma [K^+] increased 1.1 ± 0.3 mEq·L^{-1} from rest after maximal exercise (main effect for time, $P < 0.01$) and returned to rest after 4 min of recovery. Plasma [Cl^-] was unchanged from rest after maximal exercise, but fell by 2.0 ± 1.6 mEq·L^{-1} after 4 min of recovery (main effect for time, $P < 0.05$). Plasma [Cl^-] remained below rest at 8, 12 ($P < 0.01$) and 16 min ($P < 0.05$) of recovery and returned to rest following 20 min of recovery.

Plasma protein and the independent variables [SID], [A_{tot}^-] and PCO_2

Paragraph Number 17 Changes in [PPr^-], [SID], [A_{tot}^-] and PCO_2 after maximal exercise and during recovery were not different between trials (Figure 3), thus the following data reflect the pooled mean ± SD. [PPr^-] increased 0.9 ± 0.3 g·dL^{-1} from rest after maximal exercise (main effect for time, $P < 0.01$) and remained above rest throughout recovery (4-16 min inclusive, $P < 0.01$; 20 min, $P < 0.05$). [SID] fell 7.3 ± 1.8 mEq·L^{-1} below rest after maximal exercise (main effect for time, $P < 0.01$) and remained below rest throughout recovery ($P < 0.01$). [A_{tot}^-] increased 2.2 ± 0.7 mEq·L^{-1} from rest after maximal exercise (main effect for time, $P < 0.01$) and remained above rest throughout recovery (4-16 min inclusive, $P < 0.01$; 20 min, $P < 0.05$). PCO_2 remained unchanged from rest after maximal exercise, but then declined and remained 7.3 ± 4.0 mmHg below rest throughout recovery (main effect for time: 4-16 min inclusive, $P < 0.01$; 20 min, $P < 0.05$).

Changes in the dependent variables [H^+] and [HCO_3^-]

Paragraph Number 18 Changes in $[H^+]_{measured}$ and [HCO_3^-] after maximal exercise and during recovery were not different between trials (Figure 4), thus the following data reflect the pooled mean ± SD. $[H^+]_{measured}$ increased 20.9 ± 8.2 nEq·L^{-1} after maximal exercise (main effect for time, $P < 0.01$) and remained above rest during 12 min of recovery (4 min, $P < 0.01$; 8-12 min, $P < 0.05$). Thereafter values were not different from rest. The mean AUC for $[H^+]_{measured}$ was 1095 ± 137 nEq and was not different between trials. [HCO_3^-] fell 8.2 ± 2.0

$mEq \cdot L^{-1}$ below rest after maximal exercise and remained below rest throughout recovery (main effect for time, $P < 0.01$).

Paragraph Number 19 A change in plasma $[H^+]$ reflects the net change of the three independent variables ([SID], PCO_2, and $[A_{tot}^-]$) (40). The proportion of change in plasma $[H^+]$ caused by a corresponding change in a discrete independent variable was calculated whilst assuming no change from rest in the remaining two independent variables. Similar to previous reports (5,35) excellent agreement was observed between $[H^+]_{measured}$ and $[H^+]_{calculated}$ ($r = 0.848$, $P<0.0001$). Plasma $[H^+]_{calculated}$ and the origins of change in $[H^+]_{calculated}$ due to changes in the 3 independent variables are shown in Figure 5, with positive values representing an acidifying effect and negative values an alkalinizing effect. $[H^+]_{calculated}$ increased by 22.5 $nEq \cdot L^{-1}$ after maximal exercise during the passive recovery trial. Of this increase, 67% (15.1 $nEq \cdot L^{-1}$) was attributable to a decrease in [SID]. The slight increase in PCO_2 after maximal exercise contributed a further 1.6 $nEq \cdot L^{-1}$ (+7%), whereas the increase in $[A_{tot}^-]$ contributed 3.4 $nEq \cdot L^{-1}$ (+15%). $[H^+]_{calculated}$ began to fall after 4 minutes of recovery. This was primarily due to a fall in PCO_2 that reduced $[H^+]_{calculated}$ by 7.2 $nEq \cdot L^{-1}$ and countered the further decrease in [SID], which alone would have increased $[H^+]_{calculated}$ by 20.9 $nEq \cdot L^{-1}$. For the remainder of recovery, the decline in $[H^+]_{calculated}$ towards resting values was primarily due to a gradual increase in [SID] and the maintenance of PCO_2 below rest. As evident in Figure 5, the origins of change in $[H^+]_{calculated}$ during passive recovery were markedly similar to that observed during inspiratory loading trials.

Discussion

Paragraph Number 20 The main finding of the present study was that lactate clearance and changes in plasma acid-base balance during recovery from maximal exercise were not affected by pressure threshold inspiratory loading at 10, 15, or 20 cmH_2O.

Paragraph Number 21 That ITL15 did not influence lactate clearance during recovery after maximal incremental exercise concurs with our previous observations (5). In contrast, using the same inspiratory load and a similar incremental exercise test Chiappa et al. (9) reported an approximate 2.5 $mEq \cdot L^{-1}$ (25%) reduction in blood $[La^-]$ during 15 min recovery. Given the positive relationship between active muscle mass during recovery and rates of lactate clearance (17,32) it is surprising that the relatively small inspiratory muscles can affect lactate clearance to a similar magnitude as the leg muscles (12,29,41). However, the inspiratory muscles do possess a superior capillary density and oxidative capacity compared to limb muscle (33), which makes them ideally suited for lactate metabolism. Chiappa et al. (9) also report large reductions in blood $[La^-]$ with inspiratory loading without corresponding changes in $[H^+]$. In our previous study (5) inspiratory loading lowered blood $[La^-]$ (after inspiratory muscle training) thereby increasing [SID], which lowered $[H^+]$. In the absence of a change in blood PCO_2 the unchanged $[H^+]$ observed by Chiappa et al. (9) was attributed to changes in other (non-measured) strong ions that prevented an increase in [SID]. For this to be the case the net change in the other strong ions must have been positive and equimolar to the reduction in $[La^-]$ (i.e. the net change in cations and $[Cl^-]$ would need to balance the 2.5 $mEq \cdot L^{-1}$ reduction in $[La^-]$). Inspiratory loading-induced reductions in $[K^+]$ and/or $[Na^+]$, and/or increases in $[Cl^-]$, would allow this. After exercise transient increases in $[K^+]$ largely reflect significant efflux from muscle followed by rapid reuptake (22,24). Evidence is lacking to support the notion that inspiratory loading could significantly reduce K^+ efflux from locomotor muscles or accelerate K^+ reuptake. Furthermore, increases in plasma $[Na^+]$ and $[Cl^-]$ after exercise are largely attributed to reduced plasma volume (21,44). We can find no reason why inspiratory loading in recovery would either lessen the post-exercise reduction in plasma volume (thereby causing a relative decrease in $[Na^+]$) or accentuate the decrease in plasma volume (thereby increasing $[Cl^-]$). Besides, any change in plasma volume results in

simultaneous changes in [Na^+] and [Cl^-] causing little net change in [SID] and therefore [H^+]. Our data (Figure 2) and those of others (22,24) also show that post-exercise changes in [Na^+] and [K^+] are transient and therefore unlikely to balance changes in [La^-] over a recovery period of 15 min. Inspiratory loading during recovery is thus likely to influence [SID] only through changing [La^-] (5). Furthermore, inspiratory loading is unlikely to influence plasma [A_{tot}^-], which also increases after maximal exercise secondary to exercise intensity-dependent reductions in plasma volume (21,35). Although Chiappa et al. (9) did not measure strong ion concentrations, our data do not support their assertion that changes in these ions may explain their findings. Thus, given our current understanding of physicochemical principles (40) it is difficult to resolve the reason(s) why their results differ from ours. However, our results demonstrate no effect of inspiratory loading during recovery on lactate kinetics and these data are also supported by our measurement of strong ions.

Paragraph Number 22 The differences between our findings and those of Chiappa et al. (8,9) are intriguing since experimental protocols are very similar. Compared to Chiappa et al. (8,9) we observed a higher $\dot{V}O_2$ peak (present study: 53.6 $mL^{-1}{\cdot}kg^{-1}{\cdot}min$; Brown et al. (5): 52.4 $mL^{-1}{\cdot}kg^{-1}{\cdot}min$; Chiappa et al. (8): 47.9 $mL^{-1}{\cdot}kg^{-1}{\cdot}min$; Chiappa et al. (9): 45.5 $mL^{-1}{\cdot}kg^{-1}{\cdot}min$) and, during recovery, a shorter time to peak [La^-] and a faster decline in [La^-], which implies greater endurance training status in our participants (16,42,43). Since training tends to increase rates of blood and muscle lactate clearance (2,13,16) it seems counterintuitive that individuals with inferior endurance training status would demonstrate greater lactate clearance with inspiratory loading. However, coincident with a lower blood [La^-] with inspiratory loading during recovery, Chiappa et al. (8) also reported increased heart rate and mean arterial blood pressure and, as measured by near-infrared spectroscopy, reduced m. vastus lateralis reoxygenation (suggestive of reduced convective oxygen delivery). These responses were attributed to respiratory muscle metaboreflex activation, whereby increased

afferent discharge from inspiratory muscles and a concurrent increase in sympathetic vasoconstrictor activity results in a redistribution of blood flow from the periphery to the inspiratory muscles (11). A resultant increase in inspiratory muscle perfusion would probably increase lactate uptake by inspiratory muscles (18). Conversely, by virtue of endurance training-induced increases in inspiratory muscle mitochondrial enzyme activity (34), the "threshold" for respiratory muscle metaboreflex activation may have been higher in our participants (1,26,46), thus precluding changes in heart rate, cardiac output distribution and hence systemic lactate distribution. Indeed, recent work by Callegaro et al. (7) demonstrates that compared to sedentary individuals ($\dot{V}O_2$ peak = 36.7 $mL \cdot kg^{-1} \cdot min^{-1}$) those with a history of whole-body endurance training ($\dot{V}O_2$ peak = 52.9 $mL \cdot kg^{-1} \cdot min^{-1}$) are more resistant to activation of the inspiratory muscle metaboreflex. The unchanged heart rate responses during inspiratory loading compared to passive recovery in the present and in our previous study (5), partly supports this notion. Of course, from an ecological validity perspective, if the efficacy of loaded breathing in aiding recovery is restricted to untrained individuals one may question its utility.

Paragraph Number 23 Previously, Brown et al. (5) showed that after inspiratory muscle training, which increased participants' MIP by 34%, lactate clearance was accelerated at the same absolute (15 cmH_2O or 10% MIP), but not relative (20 cmH_2O or 13% MIP), inspiratory load, thus suggesting that the magnitude of the pressure threshold load may be an important determinant of whether inspiratory loading accelerates lactate clearance. The findings of the present study do not support this hypothesis, although the possibility remains that inspiratory loads above 20 cmH_2O may have increased lactate clearance due to activation of a respiratory muscle metaboreflex (see above). However, it is noteworthy that anecdotal reports from our participants suggest that 20 cmH_2O was approaching the maximal tolerable load (most participants reported severe air hunger especially during the first 5 min of

recovery). Notwithstanding this, it seems increasingly likely that a training-induced increase in the oxidative capacity of the inspiratory muscles (4,27,36,46), rather than the intensity of the load relative to MIP, primarily explains our previous observation of increased lactate clearance during recovery with inspiratory loading after inspiratory muscle training (5).

Paragraph Number 24 Consistent with previous observations (5,9) inspiratory loading modified breathing pattern compared to passive recovery. Specifically, the same $\dot{V}_E$ was achieved with a lower f_R and T_I/T_{TOT}, but greater V_T. This breathing strategy was probably generated by the respiratory controller to minimize the energy cost of breathing by reducing the pressure-time integral of the inspiratory muscles (14,47). Furthermore, reducing T_I/T_{TOT} prolongs expiration, thus increasing inspiratory muscle relaxation time and reducing end-expiratory lung volume, which enhances inspiratory muscle force generating capacity (14,38,47). This breathing pattern could also affect gas exchange by increasing the ratio of alveolar to dead space ventilation, which may explain why inspiratory loading in the present study increased $\dot{V}CO_2$ and resulted in a trend toward lower $[H^+]_{measured}$ (see Figure 4). These changes were, however, very small and probably of limited significance.

Paragraph Number 25 In conclusion, pressure threshold inspiratory loading at 10, 15, and 20 cmH_2O had no effect on lactate clearance or plasma acid-base balance when applied during recovery from maximal incremental exercise. These findings support our recent observations (5) but contradict those of Chiappa et al. (8,9). The reason(s) for this disagreement remains unclear, but may be related to inter-individual differences in endurance training status. The findings of the current study and of Brown et al. (5) also suggest that training of the inspiratory muscles may be necessary for this muscle group to engage in measurable lactate uptake with inspiratory loading. The influence of endurance training status on the efficacy of inspiratory muscle loading during recovery thus provides an attractive avenue for future investigation.

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TABLE 1. Descriptive characteristics of the participants. Mean ± SD.

Variable	
Age (yr)	26 ± 6
Body mass (kg)	80 ± 6
Height (cm)	184 ± 4
FVC (L)	5.54 ± 0.51 (101 ± 9)
FEV_1 (L)	4.40 ± 0.52 (96 ± 10)
FEV_1/FVC (%)	80 ± 4 (96 ± 5)
MVV_{10} ($L \cdot min^{-1}$)	190 ± 36 (112 ± 22)
MIP (cmH_2O)	145 ± 39 (126 ± 34)

Values in parentheses represent the percentage of predicted values (26,38). 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.

Figure Legends

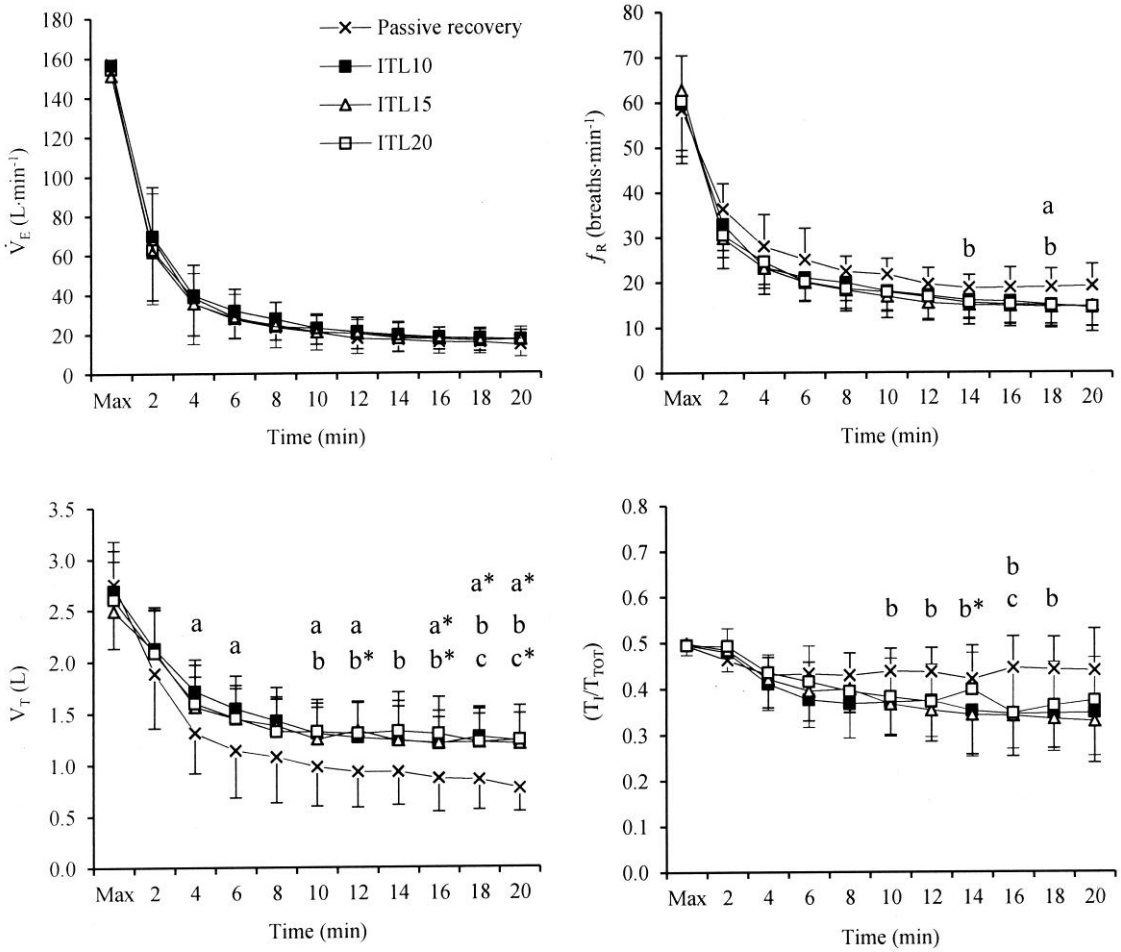

FIGURE 1-Ventilatory responses at the end of maximal exercise and during 20 min recovery. Values are mean ± SD. Difference between trials ($P < 0.05$): a, passive recovery vs. ITL10; b, passive recovery vs. ITL15; c, passive recovery vs. ITL20. *, indicates $P < 0.01$.

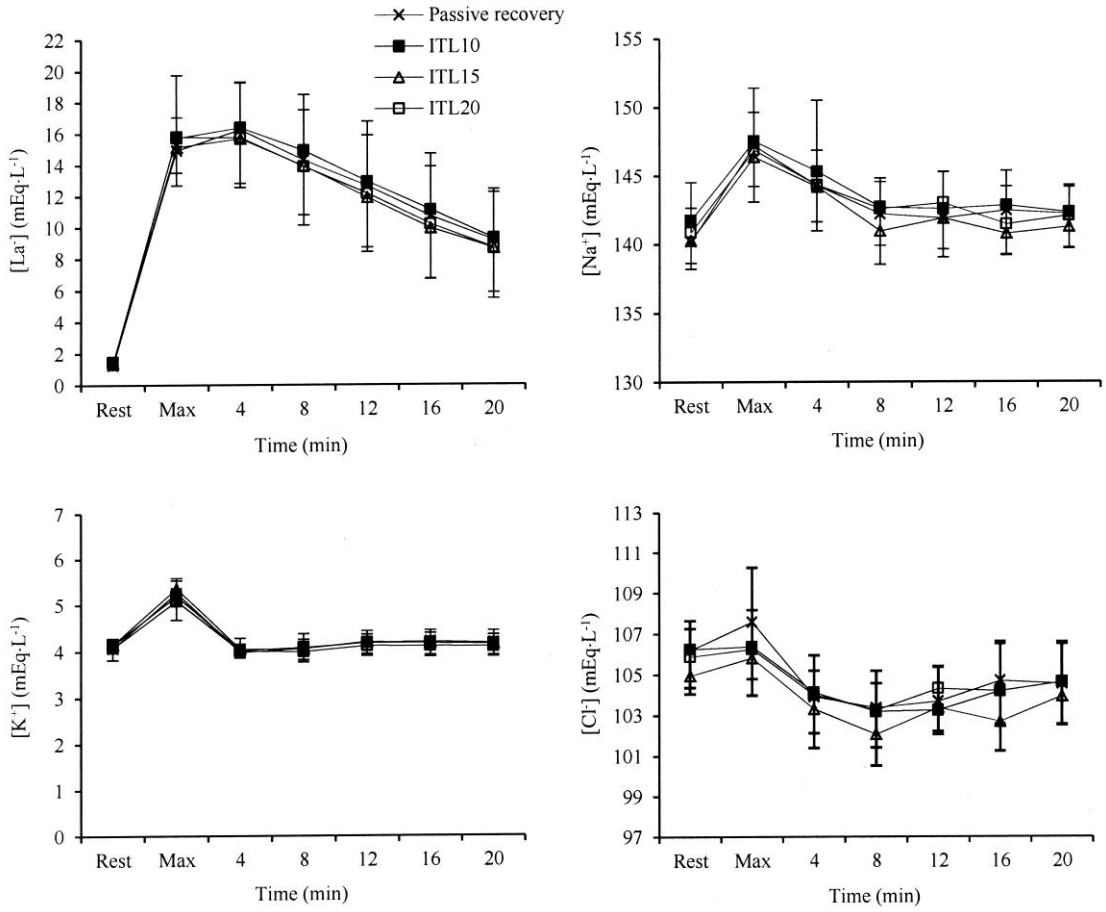

FIGURE 2-Plasma ion concentrations at rest, after maximal exercise, and during 20 min recovery. Values are mean ± SD.

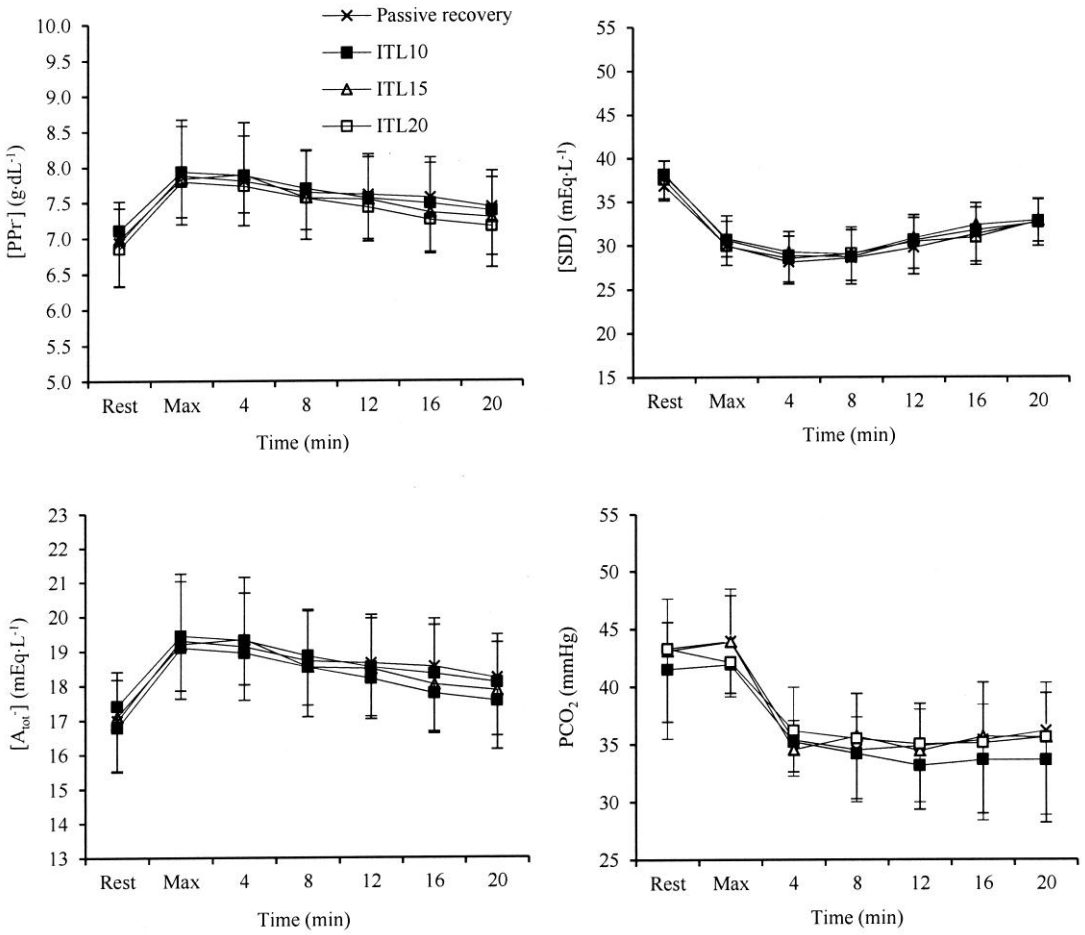

FIGURE 3-Plasma protein concentration ([PPr^-]) and the independent variables [SID], [A_{tot}^-], and PCO_2 at rest, after maximal exercise, and during 20 min recovery. Values are mean ± SD.

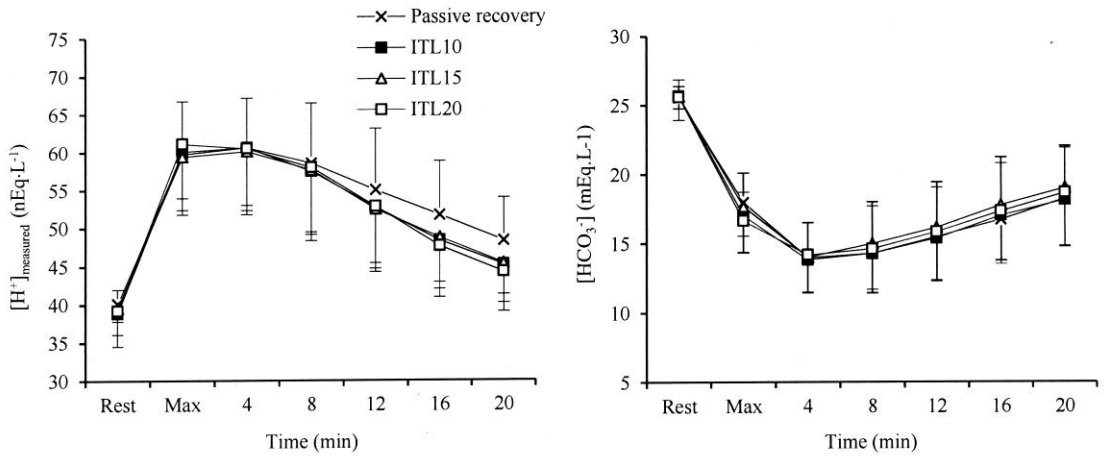

FIGURE 4-Dependent variables $[H^+]_{measured}$ and $[HCO_3^-]$ at rest, after maximal exercise, and during 20 min recovery. Values are mean ± SD.

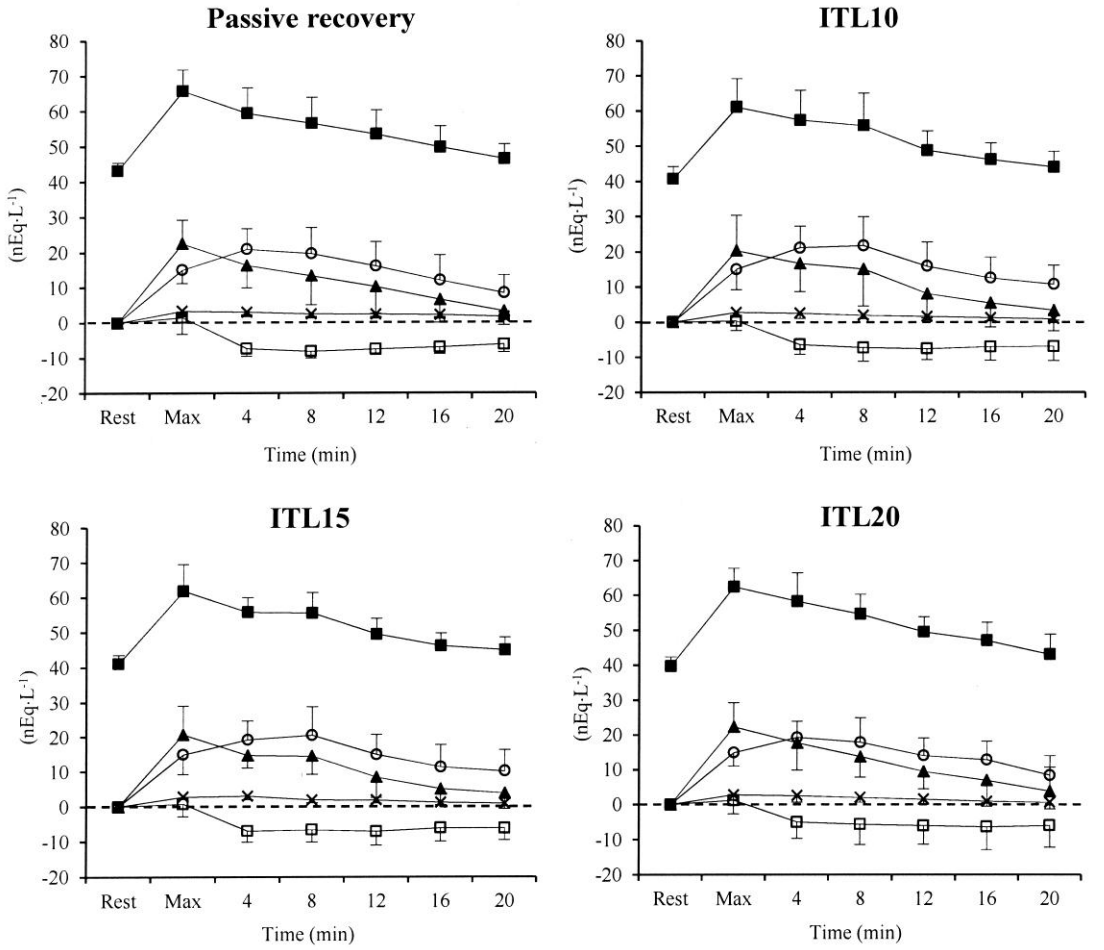

FIGURE 5-Plasma $[H^+]_{calculated}$ (■), change in $[H^+]_{calculated}$ (▲), and origins of change in $[H^+]_{calculated}$ from changes in the 3 independent variables: [SID] (○), PCO_2 (□) and $[A_{tot}^-]$ (×), after maximal exercise and during 20 min recovery. Values are mean ± SD.