



This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document and is licensed under All Rights Reserved license:

**Turner, Louise A ORCID logoORCID: <https://orcid.org/0000-0002-0153-7075>, Tecklenburg, S. L., Chapman, R., Shei, R. J., Wilhite, D. P. and Mickleborough, T. (2016) The effect of inspiratory muscle training on respiratory and limb locomotor muscle deoxygenation during exercise with resistive inspiratory loading. *International Journal of Sports Medicine*, 37 (8). pp. 598-606. doi:10.1055/s-0042-104198**

Official URL: <https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-0042-104198>

DOI: <http://dx.doi.org/10.1055/s-0042-104198>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/8107>

#### **Disclaimer**

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

**The effect of inspiratory muscle training on respiratory and limb locomotor muscle deoxygenation during exercise with resistive inspiratory loading**

**Running Title:** Inspiratory muscle training and muscle deoxygenation

**Keywords:** Respiratory muscles, muscle oxygenation, near-infrared spectroscopy, respiratory muscle training.

**Word Count (excluding abstract, figure/tables and legends, and references):** 4,485

## Abstract

We investigated how inspiratory muscle training impacted respiratory and locomotor muscle deoxygenation during submaximal exercise with resistive inspiratory loading. Sixteen male cyclists completed 6 weeks of either true (n=8) or sham (n=8) inspiratory muscle training. Pre- and post-training, subjects completed three, 6-minute experimental trials performed at  $\sim 80\% \dot{V}O_{2\text{peak}}$  with interventions of either moderate inspiratory loading, heavy inspiratory loading, or maximal exercise imposed in the final 3 minutes. Locomotor and respiratory muscle oxy-, deoxy-, and total-hemoglobin and myoglobin concentration was continuously monitored using near-infrared spectroscopy. Locomotor muscle deoxygenation changes from  $80\% \dot{V}O_{2\text{peak}}$  to heavy inspiratory loading were significantly reduced pre- to post-training from  $4.3 \pm 5.6 \mu\text{M}$  to  $2.7 \pm 4.7 \mu\text{M}$ . Respiratory muscle deoxygenation was also significantly reduced during the heavy inspiratory loading trial ( $4.6 \pm 3.5 \mu\text{M}$  to  $1.9 \pm 1.5 \mu\text{M}$ ) post-training. There was no significant difference in oxy-, deoxy-, or total-hemoglobin and myoglobin during any of the other loading trials, from pre- to post-training, in either group. After inspiratory muscle training, highly-trained cyclists exhibited decreased locomotor and respiratory muscle deoxygenation during exercise with heavy inspiratory loading. These data suggest that inspiratory muscle training reduces oxygen extraction by the active respiratory and limb muscles, which may reflect changes in respiratory and locomotor muscle oxygen delivery.

## INTRODUCTION

During maximal and near-maximal exercise, the demands of the locomotor musculature, particularly in highly-trained individuals, may place demands on the pulmonary system that exceed the functional capabilities of the ventilatory system [12], thus creating a limiting factor for O<sub>2</sub> transport and utilization [12]. Significant elevations in the work of breathing that occur during maximal exercise are associated with an increased metabolic demand of the respiratory musculature (~10-15% of total oxygen consumption) [3, 21], which requires a significant proportion of cardiac output. As a result, competition may arise between the respiratory musculature and the limb locomotor muscles for blood flow [13].

Oxygen delivery to the active locomotor muscles is likely influenced by high levels of ventilatory work [4, 25], mediated by the accumulation of vasoactive metabolites in the respiratory musculature. These metabolites can activate type IV phrenic afferent fibers [24] and consequently increase sympathetic vasoconstrictor activity [14, 39, 40] and reduce limb perfusion [10, 20, 21, 33, 39]. Therefore, significant increases in the work of breathing during high-intensity, prolonged duration exercise promotes redistribution of cardiac output from the exercising limb locomotor muscles to meet the metabolic demands of the respiratory muscles (increased O<sub>2</sub> requirement). Consequently, exercise tolerance may be impaired [21, 22].

During high levels of ventilatory demand, O<sub>2</sub> delivery to the respiratory musculature is increased while inactive limb locomotor blood flow remains constant [19]. A number of studies have shown that during high-intensity exercise, increased respiratory muscle load increases deoxygenated hemoglobin and myoglobin [deoxy(Hb+Mb)] in respiratory and active limb locomotor muscle [28, 33, 42]. Indeed, a high inspiratory load during submaximal cycling exercise in highly trained cyclists increases the metabolic requirements of the respiratory

musculature, resulting in a concomitant increase in limb locomotor and respiratory muscle [deoxy(Hb+Mb)] [42]. Conversely, unloading the respiratory musculature by reducing the work of breathing, has been shown to increase limb locomotor O<sub>2</sub> delivery and consumption, and increase time to exhaustion during cycling exercise ( $T_{lim} \sim 90\% \dot{V}O_{2peak}$ ) [9, 20-22]. Therefore, taken together, these data suggest that in highly trained individuals ( $\dot{V}O_{2peak}$ , ~60 – 65 ml/kg/min), an increased respiratory load during high-intensity exercise may reduce O<sub>2</sub> delivery to the exercising muscle tissues in order to meet respiratory muscle demands [20, 21, 42].

Recent evidence from our laboratory suggests that following a period of inspiratory muscle training (IMT) the O<sub>2</sub> requirement for a given ventilatory load is reduced [43]. This suggests that an IMT-mediated increase in inspiratory muscle strength may ‘unload’ the respiratory musculature, thereby increasing O<sub>2</sub> availability to the limb locomotor muscles during high-intensity exercise in trained individuals. If so, we would expect to see a reduction in [deoxy(Hb+Mb)] as a marker of oxygen extraction that is independent of the volume of heme-O<sub>2</sub> carriers (i.e., hemoglobin volume) in the muscle tissue. Therefore, the aim of the present study was to evaluate the effect of IMT on respiratory and limb locomotor muscle deoxygenation during cycling exercise with different intensities of resistive inspiratory loading. It was hypothesized that 6-wk of IMT would attenuate the previously reported increase in respiratory and limb locomotor muscle deoxygenation in these highly trained cyclists [42].

## **METHODS**

### ***Subjects***

Sixteen healthy, highly trained, competitive male cyclists with normal pulmonary function (Table 1) were recruited from local cycling teams and volunteered to participate in this study. All subjects provided written informed consent. The Indiana University Institutional

Review Board for Human Subjects approved all tests and procedures. All procedures met the ethical standards set by the *International Journal of Sports Medicine* [23]. Subjects were instructed to adhere to their normal diet and training regime throughout the duration of the study and to arrive at the laboratory for testing sessions fully hydrated, at least 4h postprandial. Subjects were also asked to refrain from alcohol and caffeine for at least 24 h and 6 h prior to each exercise session, respectively and to avoid strenuous exercise 24 h prior to each visit.

### ***Study Design***

On a preliminary visit to the laboratory subjects were thoroughly familiarized with all test procedures, including all respiratory muscle and pulmonary function tests, and completed an incremental cycle ergometer test to the limit of exercise tolerance in order to determine peak oxygen consumption ( $\dot{V}O_{2peak}$ ). On a separate occasion subjects completed an experimental exercise session consisting of a warm-up and 3 consecutive experimental exercise trials: (1) a moderate inspiratory loading trial, (2) a heavy inspiratory loading trial, and (3) a maximal exercise loading trial. The time-course changes in respiratory and limb locomotor muscle deoxygenation during these exercise trials have previously been reported in this group of highly trained cyclists [42]. These data have been included in this study as pre-training data. However, the data presented in this paper are novel in adding the response to IMT. Furthermore, all changes in muscle oxygenation variables were calculated relative to baseline cycling (and not resting conditions as done in our previous study [42]) both pre- and post- training in order to evaluate the influence of IMT using NIRS, and to minimise the effect of biological daily variation in the resting tissue.

Following the completion of the baseline exercise session subjects were randomly assigned in a double-blind manner (participants were advised that they were completing a

strength or endurance training intervention) to either an inspiratory muscle training (IMT;  $n = 8$ ), or a placebo-controlled sham training (CON;  $n = 8$ ) group for 6 weeks. The experimental exercise session was repeated post- training. Subjects completed daily training logs to record cycling training volume and intensity, as well as IMT training, for the duration of the study.

### ***Maximal Incremental Exercise Testing***

An incremental exercise test to the limit of tolerance was completed for determination of  $\dot{V}O_{2peak}$ . All exercise tests were performed on a cycle ergometer (Monark, Model 828E, Varberg, Sweden). Initially, subjects began the test at a workload of 150 W, and the workload increased by 50 W every 3 minutes until volitional exhaustion. The subjects self-selected a cadence prior to the start of the trial, which was held constant throughout all trials. Individual selections ranged from 80-100 rpm (mean  $\pm$  SD  $87 \pm 5$  rpm). The configuration of the saddle and handlebar position was measured and recorded for each subject, and replicated in subsequent exercise tests.  $\dot{V}O_{2peak}$  was determined as the highest 60-s average  $\dot{V}O_2$  value achieved prior to exercise termination. Power output at  $\dot{V}O_{2peak}$  was recorded.

### ***Exercise trials and inspiratory loading***

Prior to the experimental exercise trials, subjects performed pulmonary and respiratory muscle function testing at rest while comfortably seated on a cycle ergometer. Following an initial 6-min cycling warm-up (3-min at 150W followed by 3-min at a power output equivalent to 80% of the power output corresponding to  $\dot{V}O_{2peak}$ ), each subject completed three, 6-min, exercise trials in a randomized order, each separated by 20-min rest as previously described [42]. Briefly, each exercise trial consisted of 3-min of baseline cycling at a power output equivalent to 80%  $\dot{V}O_{2peak}$ , with no loading intervention. Following the initial 3-min of each trial, subjects completed a further 3-min of cycling exercise consisting of an intervention designed to increase

the inspiratory load during exercise. The separate inspiratory loading interventions were either (1) cycling at 80%  $\dot{V}O_{2peak}$  while inspiring against a moderate resistive inspiratory load, (2) cycling at 80%  $\dot{V}O_{2peak}$  while inspiring against a heavy resistive inspiratory load or (3) cycling at 100%  $\dot{V}O_{2peak}$ . A 100%  $\dot{V}O_{2peak}$  trial was included in the protocol to demonstrate the metabolic and ventilatory responses attained during maximal exercise and compare these responses to those obtained during the inspiratory loading trials. During the moderate and heavy inspiratory loading trials, inspiratory resistance was achieved by placing a rubber stopper in the inspiratory line, proximal to the mouthpiece, with an opening of 10mm and 8mm, respectively. Consequently, inspiratory resistance was flow-dependant and the diameter of the openings used in this study were designed to generate an inspiratory resistance of 6cm H<sub>2</sub>O L·s<sup>-1</sup> during the moderate loading trials and 9.5 cmH<sub>2</sub>O L·s<sup>-1</sup> during the heavy loading trial. The moderate and heavy inspiratory loading protocols employed in this study were previously shown to increase whole-body O<sub>2</sub> consumption by 3.8% ± 2.9% and 5.1% ± 3.6% respectively, thereby increasing respiratory and limb locomotor muscle [deoxy(Hb+Mb)] [42]. At rest, subjects are capable of sustaining maximum ventilation for ≥15min without showing signs of fatigue [3]. Thus, even with the imposed increases in inspiratory resistance, 20 min is likely a sufficient amount of time for complete recovery upon commencement of subsequent trials, considering that 1) our subjects are highly-trained and that 2) the imposed ventilatory workload only lasted for 3min. The order of trials completed during the pre- training exercise session was replicated during the post-training session. An outline of the testing protocol is given in Figure 1.

### ***Metabolic and ventilatory measurements***

Metabolic and ventilatory measurements were obtained using open-circuit, indirect calorimetry. Minute ventilation ( $\dot{V}_E$ ) was calculated from the inspired ventilation measured



using a dual thermistor flow probe (Hector Engineering, Ellettsville, IN). Subjects breathed through a low-resistance two-way valve (Hans Rudolph 2700, Kansas City, MO) and expired gases were collected in a 5-L mixing chamber. Fractional concentrations of O<sub>2</sub> and CO<sub>2</sub> were continuously sampled at a rate of 300 mL·min<sup>-1</sup> from the dried expired gases using an Applied Electrochemistry S-3A oxygen analyzer and CD-3A carbon dioxide analyzer (Ametek, Thermox Instruments, Pittsburgh, PA). Mouth pressure was continuously monitored at a port located within the mouthpiece using a pressure transducer (Hector Engineering, Ellettsville, IN). For each trial, in order to estimate the ventilatory workload imposed, analysis of inspiratory mouth pressure (P<sub>m</sub>) was performed on the final 30 sec of baseline cycling at 80%  $\dot{V}O_{2peak}$  and during the final 30 sec of each inspiratory loading intervention. A mean value for the integrated P<sub>m</sub> signal over inspiratory time ( $\int P_m$ ) was multiplied by breathing frequency ( $f_b$ ) to provide an estimate of inspiratory muscle force development ( $\int P_m \times f_b$ ). Heart rate was continuously monitored throughout exercise using short-range telemetry (Polar S610, Polar Electro Oy, Kempele, Finland).

### ***Limb locomotor and respiratory muscle deoxygenation measurements***

Local muscle oxygenation was continuously monitored by near-infrared spectroscopy (NIRS) using a dual-channel tissue spectrometer (ISS oximeter Model 96208, ISS Inc., Champaign, IL) as previously described [42]. A NIRS probe was placed on the vastus lateralis muscle of the subjects' right leg (15 cm above the proximal border of the patella and 5 cm lateral to the midline of the thigh) to monitor limb locomotor (LM) oxygenation status. A second probe was positioned over the right 6th intercostal space at the anterior axillary line, to assess changes in the oxygenation status of the accessory respiratory muscle (RM). The probes were secured to the skin surface to avoid movement of the probe relative to the skin and covered with an

elasticized tensor bandage to minimize the influence of extraneous light, and, while allowing unrestricted movement during exercise. Semi-permanent markings were placed around the optode sites of the limb locomotor and respiratory muscles to ensure precise replication of optode placement for post- training measurements.

The NIRS system utilized in this study emits NIR light to the tissue through fibre optic cables at two different wavelengths (690nm and 830nm). At each wavelength, light is emitted from fibers at four different emitter- to- detector distances (2.0, 2.5, 3.0 and 3.5 cm) to allow a ~1-2cm measurement depth of the tissue. The light reflected from the tissue is detected by a photomultiplier tube and recorded at a sampling rate of 1 Hz, and based on the absorption and scattering coefficients of light at each wavelength (Beers-Lambert Law), concentrations can be estimated for oxygenated ([oxy(Hb+Mb)]), deoxygenated ([deoxy(Hb+Mb)]) and total ([total(Hb+Mb)]) hemoglobin and myoglobin in the muscle tissue. For data analysis, values reported are for the final minute of each exercise trial, and are expressed as changes relative to baseline cycling during each trial (exercise at 80%  $\dot{V}O_{2peak}$ ). The tissue oximeter was calibrated according to the manufacturer's procedures and specifications.

### ***Pulmonary function and maximal inspiratory pressure measurements***

Prior to exercise, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV<sub>1</sub>), and peak expiratory flow rate (PEFR) were assessed in accordance with the American Thoracic Society recommendations [1]. The highest recorded FEV<sub>1</sub> value attained was reported. Maximal inspiratory pressure (PI<sub>max</sub>) was assessed prior to exercise using a portable hand-held mouth pressure meter (Micro Medical Ltd, Kent, UK), both pre- and post- training intervention to provide an index of inspiratory muscle strength [2]. The PI<sub>max</sub> maneuvers were initiated from residual lung volume (RV). A minimum of three PI<sub>max</sub> measurements were performed at 30

second intervals, where the variability of the best values was 5% or within 5 cmH<sub>2</sub>O [44]; the largest value was reported. All tests were conducted in a seated- upright position.

### ***Training Intervention***

Following pre- training intervention testing, subjects were randomly assigned to either an IMT or CON group and completed 6 wk of inspiratory muscle training using a pressure-threshold training device (POWERbreathe®, HaB International Ltd, UK). The IMT group completed 30 dynamic inspiratory manoeuvres, twice daily (am and pm session) for 6 wk at a pressure-threshold load equivalent to 50% of their  $PI_{max}$ ; a training protocol previously shown to improve inspiratory muscle function [8, 36-38]. The CON group completed a 6 wk sham training intervention consisting of 60 breaths, once daily (am or pm session) for the 6 wk training period at 15% of  $PI_{max}$ ; a protocol that shown no changes in inspiratory muscle function [8, 37]. Subjects were instructed to initiate each inspiration from residual lung volume and to continue until total lung capacity was reached. In order to prevent hyperventilation-induced hypocapnea, subjects were encouraged to expire slowly and in a relaxed manner. Compliance to the training intervention was monitored using methods previously described by our group [41, 43].

### **Statistical analysis**

Data were analyzed using SPSS version 17.0 statistical software. The data were assessed for normality using the Kolmogorov-Smirnov test and Levene's test was used to test for homogeneity of variance between tests. A split-plot 2 x 2 (time [pre vs. post] by group [IMT vs. CON]) ANOVA was employed to determine the effect of the training interventions on the physiological variables measured. Significant effects were further explored with a-priori planned comparisons using paired *t*-tests with Bonferroni corrections. Statistical significance was set *a priori* at  $p < 0.05$ . Values are reported as mean  $\pm$  SD.

## RESULTS

### Subjects

There were no significant differences in age, height or weight between the CON and IMT groups. Furthermore, pre-training measures of  $\dot{V}O_{2\text{peak}}$ ,  $\dot{V}_{E\text{max}}$  and power output at  $\dot{V}O_{2\text{peak}}$  was not significantly different between groups. There was no significant difference in pulmonary function between CON and IMT or within groups (pre- to post- training), and all values were within predicted normal values [32]. Pre-training intervention values for respiratory muscle strength ( $PI_{\text{max}}$ ) were not significantly different between the IMT and CON groups;  $114 \pm 12$  cmH<sub>2</sub>O and  $125 \pm 13$  cmH<sub>2</sub>O, respectively. Following 6 wk of training,  $PI_{\text{max}}$  increased by  $26 \pm 19\%$  to  $142 \pm 19$  cmH<sub>2</sub>O in the IMT group ( $p = 0.004$ ). There was no significant change in  $PI_{\text{max}}$  values post- CON ( $126 \pm 14$  cmH<sub>2</sub>O). The self-reported exercise training diaries indicated that subjects did not alter their cycling training volume or intensity for the duration of the study. Adherence to the training intervention was also high in both groups as demonstrated by a compliance rate of 90% for both the IMT and CON groups.

### Metabolic and ventilatory responses to exercise, inspiratory loading and inspiratory muscle training

The addition of a moderate inspiratory load during cycling exercise at  $80\% \dot{V}O_{2\text{peak}}$  increased inspiratory muscle force generation ( $\int P_m \times f_b$ ) from  $-102 \pm 23$  to  $-416 \pm 73$  cmH<sub>2</sub>O/min in the IMT group and from  $-80 \pm 14$  to  $-318 \pm 82$  cmH<sub>2</sub>O/min in the CON group prior to training. There was no significant interaction effect (groupX time) for  $\Delta \int P_m \times f_b$  when going from no inspiratory load to moderate inspiratory loading (Figure 2). Pre- IMT measures of  $\int P_m \times f_b$  increased from  $-92 \pm 19$  during exercise at  $80\% \dot{V}O_{2\text{peak}}$  to  $-691 \pm 164$  cmH<sub>2</sub>O/min with heavy inspiratory loading. Similar values were observed pre- CON ( $-84 \pm 14$  to  $-571 \pm 143$

cmH<sub>2</sub>O/min). There was no significant interaction effect (groupX time) for  $\Delta\dot{P}_{\text{m}} \times f_{\text{b}}$  when going from no inspiratory load to heavy inspiratory loading. In the IMT group, the pre-training change in  $\text{PI}_{\text{max}}$  from pre- to post-moderate inspiratory loading trial, heavy inspiratory loading trial, and maximal exercise trial was  $3 \pm 3$ ,  $-6 \pm 6$ , and  $1 \pm 5$  cmH<sub>2</sub>O, respectively. Similar changes in pre- to post- trial  $\text{PI}_{\text{max}}$  were observed prior to the training intervention in the CON group for moderate inspiratory loading ( $2 \pm 6$  cmH<sub>2</sub>O), heavy inspiratory loading ( $-4 \pm 6$  cmH<sub>2</sub>O) and maximal exercise ( $0 \pm 7$  cmH<sub>2</sub>O). There was no significant interaction effect (group X time; pre- to post- training) for the change  $\text{PI}_{\text{max}}$  for the moderate inspiratory loading trial, heavy inspiratory loading trial, or maximal exercise trial.

There was no significant interaction effect between the IMT or CON groups for  $\Delta\dot{P}_{\text{m}} \times f_{\text{b}}$  during the moderate inspiratory loading, heavy inspiratory loading and maximal exercise loading trials. The metabolic and ventilatory responses to moderate inspiratory loading, heavy inspiratory loading and maximal exercise, pre- and post- training are shown in Table 2. The change in  $\dot{V}\text{O}_2$  from baseline cycling at 80%  $\dot{V}\text{O}_{2\text{peak}}$  to moderate inspiratory loading, heavy inspiratory loading, and maximal exercise in the IMT and CON groups both pre- and post- training are shown in Figure 2 d-f. During the heavy inspiratory loading trial the change in  $\dot{V}\text{O}_2$  from baseline cycling to the addition of the resistive load was significantly smaller from pre- to post- IMT ( $p = 0.02$ ). There was no significant difference in  $\Delta\dot{V}\text{O}_2$  in any other group or exercise trial. Following the 6 wk IMT or sham-training (CON) program there was no significant change in any of the metabolic or ventilatory responses to the loading trials from pre- to post- intervention.

### **Limb locomotor and respiratory muscle oxygenation exercise, inspiratory loading and inspiratory muscle training**

Deoxy, oxy, and total [(Hb+Mb)] during the pre- and post- intervention loading trials are shown in Table 3 (for LM) and Table 4 (for RM). At moderate inspiratory loads and maximal exercise loads, there were no significant differences in any dependent NIRS variables for any muscle group (RM or LM) between pre- and post-intervention in either the IMT or CON groups. However, with heavy inspiratory loading following IMT, the change in LM and RM [deoxy(Hb+Mb)] was significantly reduced by  $1.7 \pm 1.3 \mu\text{M}$  ( $p = 0.02$ ) and  $2.8 \pm 2.6 \mu\text{M}$  ( $p = 0.021$ ), respectively indicating reduced oxygen extraction within those tissues following IMT. There was no significant difference in LM or RM [oxy(Hb+Mb)] and [total(Hb+Mb)] during the heavy inspiratory loading trial from pre- to post- IMT. The [deoxy(Hb+Mb)], [oxy(Hb+Mb)] and [total(Hb+Mb)] response during the heavy inspiratory loading trial was not significantly different from pre- to post- CON. The group mean LM and RM [deoxy(Hb+Mb)] and [total(Hb+Mb)] response across all trials, pre- and post- training for both the IMT and CON groups are shown in Figure 3 and 4, respectively.

## **DISCUSSION**

To our knowledge this is the first study to investigate the effect of IMT on respiratory and limb locomotor muscle deoxygenation status during exercise while undergoing periods of increased ventilatory demand. The main findings of this study are that 6 wk of IMT reduced limb locomotor and accessory respiratory muscle deoxygenation as shown by a significant reduction of the rise in [deoxy(Hb+Mb)] from pre-training levels during constant-load submaximal exercise with high resistive (heavy) inspiratory muscle loading. In contrast to our experimental hypothesis IMT did not alter limb locomotor or respiratory muscle deoxygenation during moderate inspiratory loading or maximal exercise.

Following 6 wk of IMT,  $\dot{V}O_2$  during submaximal exercise with heavy inspiratory muscle loading was significantly reduced, whilst  $\dot{V}_E$  was maintained. This reduction in  $\dot{V}O_2$  suggests that IMT may decrease the relative respiratory load required to generate the same level of ventilation, thereby reducing the metabolic demands of the respiratory musculature [43]. We have previously reported that IMT may act to unload the RM and reduce the RM  $O_2$  demand [43]. Similar to our findings, a decrease in work of breathing during underwater swimming at 70%  $\dot{V}O_{2peak}$  has been observed following 4-wk of combined inspiratory and expiratory muscle training [35].

It has previously been shown that either loading (via mesh screen) or unloading (via proportional-assist ventilator) the RM affects limb vascular resistance and limb blood flow [20, 21]. The load on the RM is therefore an important consideration. We have recently shown that high levels of resistive inspiratory loading during submaximal exercise increases whole-body  $\dot{V}O_2$ , and both limb locomotor and accessory respiratory muscle [deoxy(Hb+Mb)] [42]. It is plausible that increased metabolic demands of the respiratory musculature consequential to resistive inspiratory loading may alter blood flow to the exercising limbs, perhaps compromising  $O_2$  availability. These findings are consistent with previous studies which have demonstrated a sympathetically mediated vasoconstrictor reflex under high ventilatory loads in highly trained individuals [20, 21, 39, 40]. Reducing the relative load of the RM through IMT may delay the activation of this reflex, or reduce its magnitude.

In the present study a significant reduction in the magnitude of change in both respiratory and limb locomotor muscle deoxygenation during submaximal exercise with heavy inspiratory loading was shown following IMT. IMT-induced increases in RM strength and endurance may act to unload the RM by improving RM economy [43], thus reducing RM  $O_2$  demand to sustain a

given ventilation rate. Consequently, RM tissue deoxygenation is reduced, as demonstrated in the present study by a lower RM [deoxy(Hb+Mb)]. Reductions in muscle deoxygenation may be related to increases in oxidative metabolism and a consequent reduction in lactate production (not measured in the present study) [11], thereby reducing O<sub>2</sub> unloading. The reduced  $\Delta[\text{deoxy(Hb+Mb)}]$  from pre- to post- IMT in the RM during heavy inspiratory loading, concomitant with the similar  $\Delta[\text{total(Hb+Mb)}]$  (Figure 3) suggests that 6 wk of IMT may reduce O<sub>2</sub> extraction in this tissue. Both RM and LM [deoxy(Hb+Mb)] were significantly reduced following 6-wk of IMT during submaximal exercise with a high resistive inspiratory load. The observed changes in LM [deoxy(Hb+Mb)] may reflect a reduction in O<sub>2</sub> extraction in the active muscle or an associated increase in limb blood volume [18]. However, [total(Hb+Mb)] was unchanged, suggesting that limb blood volume did not increase. Therefore, our data demonstrate that 1) IMT decreases RM deoxygenation during exercise with heavy inspiratory loading by reducing O<sub>2</sub> extraction of the respiratory musculature; and 2) the associated reduction in LM deoxygenation may be a result of increased O<sub>2</sub> delivery at the LM level or reduced O<sub>2</sub> demand of the RM.

The mechanisms associated with an IMT-mediated reduction in O<sub>2</sub> consumption have previously been discussed [43]. One explanation for the observed reduction in whole-body  $\dot{V}O_2$ , may relate to the increased respiratory muscle strength shown following training in the IMT group. IMT has previously been shown to change respiratory muscle structure. Specifically, IMT increases diaphragm thickness [15, 17] and the proportion of type II muscle fibers in the external intercostals muscles [34]. Increases in muscle fiber cross sectional area are positively correlated to muscle strength [16, 26]. Therefore, the 22% increase in inspiratory muscle strength observed in the present study, which is consistent with previous studies using pressure-



threshold IMT [5, 15, 30, 37, 38, 45], may in part explain the reduction in  $\dot{V}O_2$  during heavy inspiratory loading by lowering the relative intensity at which the RM were working. An alternative explanation for the reduction of the  $\dot{V}O_2$ , may relate to the relationship between force production, fatigue and  $O_2$  consumption. It has been shown that in slow twitch muscle fibers, fatiguing muscle contractions results in a disproportional increase in  $\dot{V}O_2$  relative to force production. Therefore, it plausible that IMT may have reduced fatigue of the respiratory muscles during heavy inspiratory loading through enhanced endurance of the respiratory musculature as previously shown [6, 7]; however, this was not evaluated in the current study. Consequently, during whole-body exercise with an increased ventilatory load, IMT may act to delay the recruitment of accessory respiratory muscles [29] or increase the metabolic efficiency of muscle fibres to lower the  $O_2$  cost while maintaining the required force output.

In contrast to our findings during heavy inspiratory muscle loading, IMT did not alter muscle deoxygenation of the limb or respiratory muscles during moderate inspiratory loading or maximal exercise. Previous studies have demonstrated that moderate inspiratory loading (6.5 cmH<sub>2</sub>O/L/s at a flow of 3 L/s) does not change LM [42] or RM deoxygenation during exercise [27, 33]. Therefore, it is possible that despite a small increase in the metabolic requirements of the RM and LM, imposing a moderate inspiratory load may not be a sufficient stimulus to recruit the accessory respiratory muscles and/or to elicit fatiguing contractions of the respiratory muscles, which is responsible for reducing  $O_2$  availability to the limb locomotor muscles [14, 21, 39, 40].

### ***Limitations of the present study***

Some past studies using NIRS have utilized a physiological calibration using cuff inflation to obtain the physiological minimum (ischemia) and maximum (hyperemia), with

changes in muscle oxygenation variables expressed as a percentage of this range [31]. However, due to the complex nature of the respiratory musculature, it is not possible to apply this method and may in part explain varied magnitude of responses shown in this study. In the present study, the method of measuring NIRS was consistent between groups. Furthermore, changes in [deoxy(Hb+Mb)] [oxy(Hb+Mb)] and [total(Hb+Mb)] for both the LM and RM were expressed relative to baseline steady-state cycling at 80%  $\dot{V}O_{2peak}$  to minimise the effect of biological daily variation in the resting tissue. Nevertheless, this limitation may in part explain the group response presented for  $\Delta$ [deoxy(Hb+Mb)] during the heavy inspiratory loading trial (Figure 2 and 3). Second, it is acknowledged that while the present study assessed inspiratory muscle force development ( $\dot{V}P_m \times f_b$ ), the absence of esophageal pressure measurements to enable the calculation of work of breathing limits the ability to determine whether IMT was able to unload the respiratory muscles during exercise.

Third, while the change in muscle oxygenation using NIRS may be used to gain an insight into the microvascular  $O_2$  delivery and extraction in the field of interrogation, the absence of blood volume or blood flow estimates achieved via more invasive measurement techniques [19, 21] limits the ability to draw definitive conclusions regarding the effect of IMT on the redistribution of cardiac output under increased respiratory demand and warrants further investigation. However, our findings suggest that IMT may act to decrease muscle deoxygenation of the respiratory and locomotor musculature.

## CONCLUSION

The novel findings of this study are that following 6 weeks of pressure-threshold IMT, highly-trained competitive cyclists exhibited a decrease in whole-body  $\dot{V}O_2$ , and LM and RM deoxygenation during exercise with heavy inspiratory loading. These changes suggest that IMT

reduces respiratory muscle demand and decreases oxygen extraction by the active muscles, which may reflect IMT-induced changes in respiratory and limb locomotor muscle oxygen delivery. Importantly, these data provide further insight into the possible mechanisms underpinning improvements in endurance performance previously reported following a period of IMT.

***Conflict of Interest:*** none

## References

1. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med.* 1995; 152: 1107-1136.
2. ATS/ERS Statement on Respiratory Muscle Testing. *Am J Respir Crit Care Med.* 2002; 166: 518-624.
3. Aaron EA, Seow KC, Johnson BD, Dempsey JA. Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol.* 1992; 72: 1818-1825.
4. Babcock MA, Pegelow DF, Johnson BD, Dempsey JA. Aerobic fitness effects on exercise-induced low-frequency diaphragm fatigue. *J Appl Physiol.* 1996; 81: 2156-2164.
5. Bailey SJ, Romer LM, Kelly J, Wilkerson DP, DiMenna FJ, Jones AM. Inspiratory muscle training enhances pulmonary O<sub>2</sub> uptake kinetics and high-intensity exercise tolerance in humans. *J Appl Physiol.* 2010; 109: 457-468.
6. Boutellier U, Büchel R, Kundert A, Spengler C. The respiratory system as an exercise limiting factor in normal trained subjects. *Eur J Appl Physiol.* 1992; 65: 347-353.
7. Boutellier U, Piwko P. The respiratory system as an exercise limiting factor in normal sedentary subjects. *Eur J Appl Physiol.* 1992; 64: 145-152.
8. Caine, McConnell. Development and evaluation of a pressure threshold inspiratory muscle trainer for use in the context of sports performance. *Sports Engineering.* 2000; 3: 149-159.
9. Chiappa GR, Queiroga F, Meda E, Ferreira LF, Diefenthaler F, Nunes M, Vaz MA, Machado MCL, Nery LE, Neder JA. Heliox Improves Oxygen Delivery and Utilization during Dynamic Exercise in Patients with Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med.* 2009; 179: 1004-1010.

10. Chiappa GR, Roseguini BT, Vieira PJC, Alves CN, Tavares A, Winkelmann ER, Ferlin EL, Stein R, Ribeiro JP. Inspiratory Muscle Training Improves Blood Flow to Resting and Exercising Limbs in Patients With Chronic Heart Failure. *Journal of the American College of Cardiology*. 2008; 51: 1663-1671.
11. Costes F, Prieur F, Feasson L, Geyssant A, Barthelemy JC, Denis C. Influence of training on NIRS muscle oxygen saturation during submaximal exercise. *Med Sci Sports Exerc*. 2001; 33: 1484-1489.
12. Dempsey JA. J.B. Wolffe memorial lecture. Is the lung built for exercise? *Med Sci Sports Exerc*. 1986; 18: 143-155.
13. Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobio*. 2006; 151: 242-250.
14. Derchak PA, Sheel AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol*. 2002; 92: 1539-1552.
15. Downey AE, Chenoweth LM, Townsend DK, Ranum JD, Ferguson CS, Harms CA. Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. *Respir Physiol Neurobio*. 2007; 156: 137-146.
16. Edgerton VR, Apor P, Roy RR. Specific tension of human elbow flexor muscles. *Acta physiologica Hungarica*. 1990; 75: 205-216.
17. Enright SJ, Unnithan VB, Heward C, Withnall L, Davies DH. Effect of High-Intensity Inspiratory Muscle Training on Lung Volumes, Diaphragm Thickness, and Exercise Capacity in Subjects Who Are Healthy. *Physical Therapy*. 2006; 86: 345-354.
18. Ferrari M, Mottola L, Quaresima V. Principles, Techniques, and Limitations of Near Infrared Spectroscopy. *Can J Appl Physiol*. 2004; 29: 463-487.

19. Guenette JA, Vogiatzis I, Zakynthinos S, Athanasopoulos D, Koskolou M, Golemati S, Vasilopoulou M, Wagner HE, Roussos C, Wagner PD, Boushel R. Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol.* 2008; 104: 1202-1210.
20. Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Dempsey JA. Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol.* 1997; 82: 1573-1583.
21. Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P, Dempsey JA. Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol.* 1998; 85: 609-618.
22. Harms CA, Wetter TJ, St. Croix CM, Pegelow DF, Dempsey JA. Effects of respiratory muscle work on exercise performance. *J Appl Physiol.* 2000; 89: 131-138.
23. Harriss DJ, Atkinson G. Ethical Standards in Sport and Exercise Science Research: 2014 Update. *Int J Sports Med.* 2013; 34: 1025-1028.
24. Hill JM. Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. *Brain Research.* 2000; 856: 240-244.
25. Johnson BD, Babcock MA, Suman OE, Dempsey JA. Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol.* 1993; 460: 385-405.
26. Kanda K, Hashizume K. Factors causing difference in force output among motor units in the rat medial gastrocnemius muscle. *J Physiol.* 1992; 448: 677-695.
27. Kowalchuk JM, Rossiter HB, Ward SA, Whipp BJ. The Effect of Resistive Breathing on Leg Muscle Oxygenation Using Near-Infrared Spectroscopy During Exercise in Men. *Exp Physiol.* 2002; 87: 601-611.

28. Legrand R, Marles A, Prieur F, Lazzari S, Blondel N, Mucci P. Related trends in locomotor and respiratory muscle oxygenation during exercise. *Med Sci Sports Exerc.* 2007; 39: 91-100.
29. Marcinik EJ, Potts J, Schlabach G, Will S, Dawson P, Hurley BF. Effects of strength training on lactate threshold and endurance performance. *Med Sci Sports Exerc.* 1991; 23: 739-743.
30. McConnell AK, Lomax M. The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. *J Physiol.* 2006; 577: 445-457.
31. McCully KK, Hamaoka T. Near-Infrared Spectroscopy: What Can It Tell Us about Oxygen Saturation in Skeletal Muscle? *Exerc Sport Sci Rev.* 2000; 28: 123-127.
32. Morris JF. Spirometry in the Evaluation of Pulmonary Function. *Western Journal of Medicine.* 1976; 125: 110-118.
33. Nielsen HB, Boesen M, Secher NH. Near-infrared spectroscopy determined brain and muscle oxygenation during exercise with normal and resistive breathing. *Acta Physiol Scand.* 2001; 171: 63-70.
34. Ramírez-Sarmiento A, Orozco-Levi M, Güell R, Barreiro E, Hernandez N, Mota S, Sangenis M, Broquetas JM, Casan P, Gea J. Inspiratory Muscle Training in Patients with Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med.* 2002; 166: 1491-1497.
35. Ray AD, Pendergast DR, Lundgren CEG. Respiratory muscle training reduces the work of breathing at depth. *Eur J Appl Physiol.* 2010; 108: 811-820.
36. Romer LM, McConnell AK. Specificity and Reversibility of Inspiratory Muscle Training. *Med Sci Sports Exerc.* 2003; 35: 237-244.



37. Romer LM, McConnell AK, Jones DA. Effects of inspiratory muscle training on time-trial performance in trained cyclists. *J Sport Sci.* 2002; 20: 547-590.
38. Romer LM, McConnell AK, Jones DA. Inspiratory muscle fatigue in trained cyclists: effects of inspiratory muscle training. *Med Sci Sports Exerc.* 2002; 34: 785-792.
39. Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol.* 2001; 537: 277-289.
40. St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol.* 2000; 529: 493-504.
41. Turner LA, Mickleborough TD, McConnell AK, Stager JM, Tecklenburg-Lund S, Lindley MR. Effect of inspiratory muscle training on exercise tolerance in asthmatic individuals. *Med Sci Sports Exerc.* 2011; 43: 2031-2038.
42. Turner LA, Tecklenburg-Lund S, Chapman RF, Stager JM, Duke JW, Mickleborough TD. Inspiratory loading and limb locomotor and respiratory muscle deoxygenation during cycling exercise. *Respir Physiol Neurobio.* 2013; 185: 506-514.
43. Turner LA, Tecklenburg-Lund SL, Chapman RF, Stager JM, Wilhite DP, Mickleborough TD. Inspiratory muscle training lowers the oxygen cost of voluntary hyperpnea. *J Appl Physiol.* 2012; 112: 127-134.
44. Wen AS, Woo MS, Keens TG. How many maneuvers are required to measure maximal inspiratory pressure accurately? *Chest.* 1997; 111: 802-807.
45. Witt JD, Guenette JA, Rupert JL, McKenzie DC, Sheel AW. Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. *J Physiol.* 2007; 584: 1019-1028.

## Figure Legends

FIGURE 1: Testing protocol for each experimental laboratory testing session..

FIGURE 2: Group mean changes in inspiratory muscle force generation ( $\dot{V}_{Pm} \times f_b$ ) (a-c) and  $\dot{V}O_2$  (d-f) during the moderate inspiratory, heavy inspiratory, and maximal exercise loading trials, pre- (closed bar) and post- (open bar) training. \*Significantly different from pre- training value.

FIGURE 3: Group mean changes in limb locomotor muscle (A) and respiratory muscle (B) deoxygenated hemoglobin and myoglobin concentration [ $deoxy(Hb+Mb)$ ] and total hemoglobin and myoglobin concentration ( $[total(Hb+Mb)]$ ) during the inspiratory loading and maximal exercise trials from pre- to post- IMT intervention. Pre- IMT responses are shown as closed circles and post- IMT responses are shown as open circles. Values are 10 sec averages, expressed as changes relative to baseline cycling exercise at 80%  $\dot{V}O_{2peak}$ . Error bars are not shown for clarity.

FIGURE 4: Group mean changes in limb locomotor muscle (A) and respiratory muscle (B) deoxygenated hemoglobin and myoglobin concentration [ $deoxy(Hb+Mb)$ ] total hemoglobin and myoglobin concentration ( $[total(Hb+Mb)]$ ) during the inspiratory loading and maximal exercise trials from pre- to post- sham training intervention. Pre- CON responses are shown as closed circles and post- CON responses are shown as open circles. Values are 10 sec averages, expressed as changes relative to baseline cycling exercise at 80%  $\dot{V}O_{2peak}$ . Error bars are not shown for clarity.

Figures were generated through Graphpad.