Effects of inspiratory muscle training on respiratory muscle electromyography and dyspnea during exercise in healthy men

Authors: Andrew H. Ramsook[1,2], Yannick Molgat-Seon[1,3], Michele R. Schaeffer[1,2], Sabrina S. Wilkie[1,2], Pat G. Camp[1,2], W. Darlene Reid[4], Lee M. Romer[5], Jordan A. Guenette[1,2,3]

[1] Centre for Heart Lung Innovation, University of British Columbia and St. Paul's Hospital, Vancouver, BC
[2] Department of Physical Therapy, University of British Columbia, Vancouver, BC
[3] School of Kinesiology, University of British Columbia, Vancouver, BC
[4] Department of Physical Therapy, University of Toronto, Toronto, ON
[5] Centre for Human Performance, Exercise and Rehabilitation, Brunel University London, Uxbridge, U.K.

Corresponding Author:
Jordan A. Guenette, Ph.D,
UBC Centre for Heart Lung Innovation
166-1081 Burrard Street
Vancouver, BC, Canada, V6Z 1Y6
jordan.guenette@hli.ubc.ca

New & Noteworthy

Exertional dyspnea intensity is thought to reflect an increased awareness of neural respiratory drive, indirectly measured using diaphragmatic electromyography (EMGdi). We examined the effects of inspiratory muscle training (IMT) on dyspnea, EMGdi, and EMG of accessory inspiratory muscles. IMT significantly reduced submaximal dyspnea intensity ratings but did not change EMG of any inspiratory muscles. Improvements in exertional dyspnea following IMT may be the result of non-physiological factors or physiological adaptations unrelated to neural respiratory drive.

ABSTRACT

Inspiratory muscle training (IMT) has consistently been shown to reduce exertional dyspnea in health and disease; however, the physiological mechanisms remain poorly understood. A growing body of literature suggests that dyspnea intensity can largely be explained by an awareness of increased neural respiratory drive, as indirectly measured using diaphragmatic electromyography (EMGdi). Accordingly, we sought to determine if improvements in dyspnea following IMT can be explained by decreases in inspiratory muscle EMG activity.

Twenty-five young, healthy recreationally-active men completed a detailed familiarization visit followed by two maximal incremental cycle exercise tests separated by 5 weeks of randomly assigned pressure threshold IMT or sham control training (SC). The IMT group (n=12) performed 30 inspiratory efforts twice daily against a 30-repetition maximum intensity. The SC group (n=13) performed a daily bout of 60 inspiratory efforts against 10% maximal inspiratory pressure (MIP), with no weekly adjustments. Dyspnea intensity was measured throughout exercise using the modified 0-10 Borg scale. Sternocleidomastoid and scalene EMG were measured using surface electrodes whereas EMGdi was measured using a multi-pair esophageal electrode catheter.

IMT significantly improved MIP (pre:-138±45 vs. post:-160±43cmH_2O, $p<0.01$) whereas the SC intervention did not. Dyspnea was significantly reduced at the highest equivalent work rate (pre:7.6±2.5 vs. post:6.8±2.9Borg units, $p<0.05$), but not in the SC group, with no between-group interaction effects. There were no significant differences in respiratory muscle EMG during exercise in either group. Improvements in dyspnea intensity ratings following IMT in healthy humans cannot be explained by changes in the electrical activity of the inspiratory muscles.

Abstract Word Count: 250

Key Words: Dyspnea, electromyography, inspiratory muscle training, neural respiratory drive.

INTRODUCTION

Inspiratory muscle training (IMT) has been studied extensively in healthy individuals and patients with chronic respiratory diseases but the efficacy of this intervention remains controversial (22, 30). Systematic reviews have concluded that IMT improves whole body exercise performance using a range of performance based exercise tests (13, 16) but does not improve peak aerobic capacity or maximal work rates during incremental exercise tests (13, 16). The purported improvements in exercise performance are thought to be related, at least in part, to reductions in exertional dyspnea ratings (41). While IMT can reduce dyspnea during both performance based and maximal incremental exercise tests in health (13, 34), the physiological mechanisms for this improvement have not been adequately studied.

Previous research in health and disease has demonstrated a strong relationship between diaphragmatic EMG (EMGdi), an indirect measure of neural respiratory drive (NRD), and dyspnea intensity ratings (9, 18, 25, 38). Moreover, the ratio between NRD and the mechanical output of the respiratory system (i.e., neuromechanical coupling) is thought to be an important contributor to both the intensity and qualitative dimensions of exertional dyspnea (28). It follows that improvements in EMGdi and neuromechanical coupling of the respiratory system can reduce dyspnea. Indeed, it has been previously shown that bronchodilator-induced improvements in neuromechanical coupling in COPD are correlated with improvements in dyspnea during exercise (28). IMT may decrease the relative electrical activation of the diaphragm and improve neuromechanical coupling of the respiratory system to perform a given ventilatory task. Recent evidence also suggests that extradiaphragmatic inspiratory muscles, such as the scalene and sternocleidomastoid muscles, are heavily recruited during IMT (31). Thus, reductions in dyspnea following IMT may also be related to changes in the electrical activation of extradiaphragmatic inspiratory muscles. Accordingly, the purpose of this study was to determine if IMT reduces exertional dyspnea intensity ratings in healthy subjects and to determine if improvements in dyspnea are related to improvements in inspiratory muscle EMG and neuromechanical coupling of the

respiratory system. We hypothesized that IMT would reduce dyspnea intensity ratings at submaximal work rates, which would coincide with a reduction in EMG of the diaphragm, scalene and sternocleidomastoid muscles with corresponding improvements in neuromechanical coupling of the respiratory system.

METHODS

Subjects

Twenty-five young and healthy males participated in this study (ClinicalTrials.gov: NCT02243527). All subjects provided written informed consent and all procedures were approved by the Providence Health Care Research Ethics Board at the University of British Columbia and adhered to the *Declaration of Helsinki*. Inclusion criteria were as follows: male; self-reported physical activity levels as 'moderate' or 'high' according to the International Physical Activity Questionnaire (5); spirometry within normal limits; and able to read and understand English. Exclusion criteria were as follows: current or former smokers; history or current symptoms of cardiopulmonary disease; participating in competitive endurance sport at the provincial, national, or international level; ulcer or tumour in the esophagus, a nasal septum deviation, or recent nasopharyngeal surgery; allergies to latex or local anesthetics; and contraindications to exercise testing.

Experimental Overview

Subjects were randomly assigned to either an IMT (n=12) or sham control (SC) training (n=13) program. Subjects completed 3 experimental visits and 5 weeks of training. Visit 1 (V1) served as a detailed familiarization and screening visit where each subject performed pulmonary function testing and a maximal incremental cycle exercise test. Visits 2 (V2) and 3 (V3) served as pre- and post-intervention measurements, respectively, and consisted of the same exercise test performed on V1. Detailed ventilatory, EMG, respiratory mechanical, and sensory responses were measured throughout exercise on V2 and V3.

Training

The IMT group were told they were a part of a 'respiratory muscle strength training' intervention, whereas the SC group were told they were part of a 'respiratory muscle endurance training' intervention. Both IMT and SC groups performed their respective training with a POWERbreathe K3 device (HaB International Ltd., Southam, Warwickshire, UK). The K3 model is a variable flow resistive device that employs an electronically-controlled valve to apply a variable resistance over the course of inspiration. The IMT group trained five days per week for five weeks, at two sessions per day (morning and evening). Each session included 30 sharp inspiratory efforts from residual lung volume. The initial intensity was set at 50% of the participant's MIP, determined on V2. Participants in the IMT group were instructed to increase the training intensity freely, such that they were training at a 30-repetition maximum intensity. The POWERbreathe K3 includes an inspiratory muscle warm-up for the first four breaths, which were completed at an intensity less than the target training intensity. The next 26 repetitions were completed at the target intensity. Any repetition that failed to meet the target intensity did not count towards the total completed repetitions for that session. The SC group also trained for a total of five weeks but at a fixed intensity of 10% of MIP (from V2) once per day for a total of 60 repetitions, five days per week. Each training breath was described as being a slow, protracted, deliberate breath. Previously, a 6-week intervention at 15% of MIP has been shown to be an effective sham protocol that elicits no training effect (34). Both groups performed one supervised session per week in the laboratory in order to monitor MIP and to gauge the appropriateness of their training technique and intensity. The training intensity for the IMT group was increased if mouth pressures were < 50% of current MIP or if subjects were, at the discretion of the research team, performing training lower than a 30 repetition maximum.

Pulmonary Function and Maximal Inspiratory Pressure Measurements

Spirometry, plethysmography, and maximal inspiratory pressure (MIP) from residual volume were collected according to previous recommendations (1, 26, 47) using a commercially available

147 testing system (Vmax Encore 229, V62J Autobox; CareFusion, Yorba Linda, CA) and expressed in
148 absolute terms and relative to predicted values (3, 12, 45, 49).

149 ***Handgrip Strength***

150 Handgrip strength was measured before and after training using a hand held dynamometer
151 (model 76618, Lafayette Instrument Company, Lafayette, IN). This measure assessed each participant's
152 motivation to perform a maximal voluntary contraction. The maximal handgrip strength test involves
153 muscle groups unaffected by IMT. Therefore, an improvement in MIP without an increase in handgrip
154 strength was indicative of a physiological change in MIP, as opposed to a greater voluntary effort in
155 performing MIP manoeuvres.

156 ***Exercise Protocol***

157 Subjects performed incremental exercise tests to exhaustion on an electronically braked cycle
158 ergometer (VIAsprint 200P; Ergoline, Bitz, Germany). Each test began with 6 minutes of steady state
159 rest, followed by a 1 minute warm up of unloaded pedalling. The incremental cycle test began at 25 W
160 and increased by 25 W every 2 minutes until volitional exhaustion. Participants pedalled at a freely
161 chosen cadence (> 60 rpm), and all ergometer measurements (i.e., saddle height, saddle position,
162 handlebar angle/height) were recorded and reproduced for all subsequent exercise tests.

163 ***Inspiratory Muscle EMG***

164 Surface electrodes were used to assess EMG of the sternocleidomastoid (EMGscm) and scalene
165 (EMGsca) muscles as previously described (31). Briefly, bipolar electrodes on the scalenes were placed
166 within the posterior triangle of the neck, at the level of the cricoid cartilage (39). Electrodes were placed
167 along the long axis of the sternocleidomastoid muscle between the mastoid process and the medial
168 clavicle (40). The electrode placement was on the right side of the body and recorded in reference to
169 anatomical landmarks to ensure consistency in electrode placement between visits. A wireless surface
170 EMG system (TeleMyo DDTS; Noraxon USA, Inc., Scottsdale, AZ) was used to evaluate both
171 EMGscm and EMGsca. A combined esophageal electrode–balloon catheter was used to measure

EMGdi, which was connected to a bio-amplifier (bio-amplifier model RA-8; Yinghui Medical Technology Co. Ltd., Guangzhou, China) (38). The catheter was inserted through the nares after application of a topical anaesthetic (Lidodan® Endotracheal Spray; Odan Laboratories Ltd., Montréal, QC, Canada). Position of the catheter was determined when the EMG amplitude was lowest in the center pair and highest at the electrode pairs furthest from the center during spontaneous breathing (21). The catheter was placed at the same depth and in the same nostril on both visits. All EMG data was sampled at 2 kHz and the signals were further digitally processed using LabChart 7.3.7 Pro software (ADInstruments Inc., Colorado Springs, CO) with a band-pass filter between 20 and 500 Hz. All raw EMG data were converted to a root mean square using a time constant of 100 msec and a moving window. In an effort to improve the signal-to-noise ratio in the EMGsca and EMGscm signals, the average root mean square during expiration at baseline was subtracted from all subsequent EMG data. All EMG data were expressed as a percentage of maximal EMG activity achieved during any inspiratory capacity manoeuvre performed at rest or during exercise for a given experimental visit.

Cardiopulmonary Responses

Standard metabolic and ventilatory responses were measured on a breath-by-breath basis using a commercially available metabolic cart (Vmax Encore 229, CareFusion, Yorba Linda, CA, USA). Heart rate and arterial oxygen saturation were measured using a heart rate monitor (Polar T34; Polar Electro, Kempele, Finland) and pulse oximeter (Radical-7 Pulse CO-Oximeter, Masimo Corporation, Irvine, CA, USA), respectively. The inspiratory capacity manoeuvres used for EMG normalization purposes were also used to calculate end-expiratory and end-inspiratory lung volumes (11) and were expressed as a percentage of total lung capacity. Neuromechanical coupling of the respiratory system was determined as the ratio between EMGdi (%max) and V_T (%vital capacity).

Perceived Breathing and Leg Discomfort

Breathing discomfort, defined as “a feeling of laboured or difficult breathing” and leg discomfort, defined as “the feeling of fatigue in [their] leg muscles”, were measured using the modified 0-10

category ratio Borg Scale (2). The end points of the scale were anchored such that 0 represented "no breathing/leg discomfort at all" and 10 represented "the most intense breathing/leg discomfort [they] have every experienced or could ever imagine experiencing". Additionally, at the end of exercise, participants were asked to: first, state their primary reason for stopping exercise (i.e., breathing discomfort, leg discomfort, a combination of the two, or another reason) and to choose applicable qualitative descriptors of breathlessness using a modified version of a previously published questionnaire (42) that we have used previously (4).

Work of Breathing

The esophageal catheter used to measure EMGdi includes an esophageal balloon, which was connected to a calibrated differential pressure transducer (model DP15-34, Validyne Engineering, Northridge, CA, USA) to measure esophageal pressure. The total work of breathing (W_b) was determined as the area within an averaged tidal esophageal pressure-volume loop including a portion of a triangle that fell outside of the loop representing part of the elastic work of breathing (24). The W_b was then multiplied by breathing frequency.

Analysis of Exercise End-Points

All physiological exercise variables were averaged in 30 second epochs. The time between 60-90 seconds of each 2-minute stage was designated as our primary period of data collection. During this time, participants were reminded to look straight forward, minimizing any head or neck movement, keep a loose grip on the handlebars, and to avoid talking or swallowing to minimize contamination of our outcomes of interest. The data obtained during this period was then linked to the breathing and leg discomfort ratings and inspiratory capacity values that were collected during the last 30 seconds of each stage (i.e., from 90-120 seconds). Analyses of EMG and W_b were performed by a blinded assessor. Blinding was achieved through assigning a random identifier to each stored data file during analysis and removing the original file name that identified the subject group and visit. This was done to ensure neutrality when processing data that may involve a bias of selection from the assessor, such as EMGdi

and esophageal pressure-volume loops for assessing the W_b. Following analysis, all files were renamed to their original identifier.

Statistical Analyses

An initial sample size calculation was performed on the basis of previous work (38) showing a decrease in EMGdi by 10 %max correlated with a difference in dyspnea by 1 Borg unit with an alpha of 0.05. This calculation yielded a sample size of 11 subjects per group to detect a significant decrease in EMGdi. Statistical tests were performed using SPSS (Version 21, IBM Corporation, Armonk, NY, USA). Baseline comparisons of pulmonary function and exercise responses between groups were made using unpaired *t*-tests. Pre- vs. post comparisons of subject characteristics, pulmonary function, anthropometry, and exercise measurements were made using paired *t*-tests. Between group differences in the pre-post-changes in dyspnea, leg discomfort, ventilatory responses, and all EMG-derived variables across work rates were tested using repeated measures analysis of variance with a Greenhouse-Geisser correction where appropriate. The between-subject factor tested was SC versus IMT group and the within subject factor was work rate. First, the interaction term was evaluated. As no significant interaction effects were observed in the current data, with the exception of MIP, only the between-subject factors were considered. These *t*-tests were performed on data collected at rest, standardized absolute work rates, the highest equivalent work rate (HEWR) completed by an individual on both visits, and at peak exercise, where peak exercise was defined as the highest work rate maintained for at least 30 seconds. Changes in qualitative descriptors of dyspnea and reasons for stopping exercise were performed with a paired McNemar's test. Significance was set at $p < 0.05$ and all data are presented as mean ± SD.

RESULTS

Subject Characteristics

Subject characteristics and pulmonary function are presented in **Table 1**. Pre-intervention groups

were well-matched for age, mass, pulmonary function, and physical activity levels. There were no changes in self-reported physical activity when comparing pre vs. post intervention in both groups. Peak exercise data can be found in **Table 2.** There were no group differences in any baseline peak exercise responses. There were no statistically significant changes in any peak exercise responses following IMT or SC training.

Training

Adherence to interventions was good in both groups. The IMT group completed 94 ± 9% whereas the SC group completed 88 ± 13% of assigned training sessions. Successful training sessions were monitored digitally by the POWERbreathe device as well as via diary kept by the subject. In the event of a discrepancy between the diary and POWERbreathe recordings of successful sessions, the POWERbreathe data were used. This only occurred in one subject. MIP significantly increased after five-weeks in the IMT group but not in the SC group (see also **Table 1)**.

Inspiratory Muscle EMG

Esophageal catheter-derived measures were obtained in 11 IMT and 11 SC subjects. EMGdi during exercise before and after training are shown in Figure 1 (panels A and D). There were no significant changes in EMGdi following training in both the IMT and SC groups at rest or during exercise. The scalenes were relatively inactive throughout the early stages of exercise with EMGsca activity increasing at the HEWR and peak exercise in both groups (Figure 1, panels B and E). However, there were no statistically significant changes in EMGsca in the IMT or SC groups from baseline. The EMGscm displayed a similar response with low levels of activity up to the HEWR in both groups (Figure 1, panels C and F). A statistically significant decrease in EMGscm was observed at 50 W in the IMT group but there were no significant changes at any other work rate. No changes in EMGscm were observed in the SC group.

Ventilatory Responses

There were no significant changes in minute ventilation, tidal volume, breathing frequency or operating lung volumes (Figure 2) at any work rate in either group following training. Similarly, there was no change in the total W_b or neuromechanical coupling for a given exercise intensity in either group (Figure 3).

Sensory Responses

Figure 4 shows the sensory intensity responses in both IMT and SC groups. Subjects in the IMT group reported significantly lower dyspnea ratings at 125 W (pre: 2.2 ± 1.4 vs. post: 1.6 ± 1.5 Borg units, $p < 0.05$), 150 W (pre: 3.2 ± 1.5 vs. post: 2.3 ± 1.4 Borg units, $p < 0.01$), and the HEWR (pre: 7.6 ± 2.5 vs. post: 6.8 ± 2.9 Borg units, $p < 0.05$) after IMT with no changes in the SC group. There were no changes in leg discomfort ratings in either group. There were no significant changes in the reasons for stopping exercise and the qualitative descriptors of dyspnea upon exercise cessation in both groups following training (data not shown).

DISCUSSION

This study is the first to comprehensively examine the neurophysiological mechanisms associated with reduced dyspnea ratings following IMT in healthy human subjects. The main findings are as follows: 1) Dyspnea intensity ratings were modestly reduced during submaximal exercise intensities in the IMT but not in the SC group. Moreover, IMT had no effect on leg discomfort ratings throughout exercise or on the qualitative dimensions of exertional dyspnea at maximal exercise; 2) IMT had no effect on inspiratory muscle EMG; and 3) IMT had no effect on ventilatory responses, or neuromechanical coupling of the respiratory system during incremental cycle exercise. Collectively, these results suggest that modest improvements in dyspnea intensity ratings following IMT are not explained by improvements in key physiological outcomes known to contribute to dyspnea in health and disease.

Neural Respiratory Drive

Systematic reviews in healthy populations indicate a beneficial effect of IMT on dyspnea ratings (13). Despite this finding, it must be acknowledged that not all studies demonstrate a positive effect of IMT on dyspnea (44, 48) and some studies, like ours, only show modest (i.e., < 1 Borg unit) decreases in dyspnea (27). Nevertheless, several mechanisms have been proposed to explain the apparent improvement in dyspnea following IMT. The most commonly cited, but as of yet, untested mechanism, is that motor outflow (i.e., "neural respiratory drive") decreases for any given level of minute ventilation following IMT (14, 23). This is a reasonable hypothesis given that dyspnea intensity ratings during exercise are largely explained by an increased awareness of NRD (17, 18, 32, 38). Huang *et al.* (15) demonstrated that improvements in inspiratory muscle strength following IMT correlated with a reduction in inspiratory motor command measured using mouth occlusion pressure at 0.1 s. This correlation likely reflects a decrease in the percentage of motor units required for a given ventilatory task. However, the study by Huang *et al.,* (15) did not include a control group and did not evaluate NRD during exercise or examine its association with exertional dyspnea. To our knowledge, the present study is the first to examine the effects of IMT on NRD during exercise in healthy humans. The results of our study suggest that IMT does not affect NRD, as indirectly estimated using invasive measures of crural EMGdi during incremental cycling to exhaustion. A conference abstract based on 10 COPD patients (7 IMT and 3 controls) demonstrated reductions in both EMGdi (by 12%) and dyspnea intensity ratings (by 3.3 Borg scale units) at standardized ventilations during constant load cycling following 8 weeks of IMT (19). One potential explanation for this discrepancy is the fact that these COPD patients had baseline inspiratory muscle weakness and may derive greater benefits from IMT compared to healthy subjects that are not limited during exercise by dyspnea and that have normal baseline inspiratory muscle strength. It is possible that strengthening the inspiratory muscles beyond a certain point confers no additional advantage in reducing NRD during the hyperpnea of exercise.

Neuromechanical Coupling

Neuromechanical uncoupling of the respiratory system is thought to be an important contributor to the intensity and qualitative dimensions of dyspnea (28, 29). In general, the mechanical output of the respiratory system increases proportionally to the level of NRD during exercise in healthy humans. However, when V_T becomes constrained or reaches a plateau/inflection, then the ratio between EMGdi (%max) and V_T (% vital capacity) begins to rise. This often leads to intolerable dyspnea and gives rise to the sensation of "unsatisfied inspiration", particularly in patients with chronic respiratory disease that have severe mechanical constraints on V_T expansion (29). Given the lack of change in EMGdi and V_T in the present study, it is not surprising that there was no change in our measure of neuromechanical coupling following IMT.

Extradiaphragmatic Inspiratory Muscles

There is some evidence to suggest that increases in dyspnea may be associated with extradiaphragmatic inspiratory muscle activity (7). Recent work also suggests that traditional inspiratory muscle strength training protocols, as used in the present study, tend to preferentially recruit extradiaphragmatic inspiratory muscles, particularly those in the neck (31). Thus, improvements in global inspiratory muscle strength following IMT may be due to improvements in extradiaphragmatic muscle strength (e.g., intercostal muscles, scalenes, sternocleidomastoids, etc.). We attempted to address this question by examining changes in EMGscm and EMGsca during exercise. While we observed a statistically significant reduction in EMGscm at one submaximal work rate, this was not sustained throughout exercise and there were no changes in EMGsca at any exercise intensity. Based on these data (Figure 1), we argue that there were no physiologically meaningful changes in EMGscm or EMGsca following IMT. We have previously suggested that diaphragmatic recruitment can be increased significantly if subjects consciously engage the diaphragm during IMT (31). We did not employ this approach in the present study in order to facilitate comparisons with the majority of the IMT

346 literature in healthy subjects. Whether conscious recruitment of the diaphragm during IMT would
347 confer greater benefits on dyspnea and perhaps reduce EMGdi requires further investigation.

348 ***Alternative Mechanisms***

349 Collectively, the results of this study show that IMT has no effect on any physiological
350 measurement related to the hyperpnea of exercise across the full range of ventilations (i.e., rest to
351 maximal exercise). Based on this observation, we speculate that modest decreases in dyspnea intensity
352 ratings following IMT may reflect some form of desensitization to repeated inspiratory muscle loading
353 over several weeks; changes that could not be captured in our physiological measurements during
354 exercise. However, this remains speculative and requires further investigation. Interestingly, we
355 observed no difference in leg discomfort ratings in the current study. This is inconsistent with Romer *et*
356 *al.* (34) during incremental cycling but is consistent with Verges *et al.* (46) during constant load cycling
357 at 85% of peak work rate. If IMT provided a desensitization effect on dyspnea, it stands to reason that
358 our IMT protocol would not impact leg discomfort ratings because IMT would not provide a similar
359 effect to the locomotor muscles. The effect of IMT on perceived leg discomfort remains inconclusive
360 based on the available literature. Discrepancies amongst studies are likely related to varying exercise
361 testing protocols, IMT regimes, and/or differences in subject characteristics (e.g. fitness level).

362 Attenuation of respiratory muscle fatigue is another potential mechanism whereby IMT might
363 improve exertional dyspnea. Respiratory muscle fatigue results in a sympathetically mediated
364 metaboreflex response that reduces limb blood flow and increases perceptions of limb and respiratory
365 discomfort (6, 37). IMT has been shown to improve the fatigue-resistance of the inspiratory muscles
366 (35, 46) and can attenuate the respiratory muscle metaboreflex (50), which may explain, at least in part,
367 reduced dyspnea and leg discomfort ratings following IMT in previous studies. However, we do not
368 believe that diaphragm fatigue played a role in our dyspnea results given that we used an incremental
369 cycling protocol, which does not normally cause diaphragm fatigue (36). We intentionally selected
370 incremental rather than constant load cycling to track the sensory and physiological changes across the

full range of ventilations and to avoid the potential confounding effects of diaphragm fatigue on dyspnea and NRD. Additional studies are needed to determine if respiratory muscle EMG can be reduced during other exercise protocols such as time trials and constant-load exercise tests that are more likely to induce respiratory muscle fatigue.

Limitations

This study has some limitations that must be acknowledged. First, limitations of using multi-pair esophageal electrode catheters for assessing NRD are well established and have been described elsewhere (20, 21, 38). Second, we recognize that there is generally poor between-subject and between-occasion reproducibility of surface EMG measurements. Although reproducible inspiratory muscle EMG during quiet resting breathing and inspiratory threshold loading has been established (8), this has, to our knowledge, not been examined during exercise despite the widespread use of respiratory muscle surface EMG during exercise (7, 33, 39). We attempted to address this problem by carefully standardizing the skin preparation procedures, placing the electrodes in the same position on all visits, and normalizing the data to maximal inspiratory contractions. Third, surface EMG can be influenced by underlying levels of subcutaneous fat. To overcome this limitation, we used lean subjects and we measured skin-fold thickness of the neck and found no changes pre vs. post-training in either group (data not shown). An additional critique is our decision to normalize all EMG data as a percentage of maximum. This was done to standardize the procedures between our catheter derived EMG measurements and our EMG measurements using surface electrodes. Some suggest that it is better to report EMGdi in absolute values when comparing within-subject changes (25, 43). We performed this analysis (data not shown) and our conclusions regarding the lack of change in EMGdi remain the same. Lastly, we acknowledge that there may have been “cross-talk” between our sternocleidomastoid and scalene EMG measurements given the close proximity of these muscles. Thus, we are not able to definitively say that we isolated these specific muscles with our surface electrodes. Nevertheless, our

surface EMG data provide a good global index of extradiaphragmatic inspiratory muscle activation originating from the neck region.

Conclusions

Five weeks of inspiratory muscle strength training resulted in modest reductions in exertional dyspnea intensity but did not change inspiratory muscle EMG, neuromechanical coupling of the respiratory system, or the ventilatory response to exercise. Thus, improvements in dyspnea in healthy individuals following IMT may be driven by non-physiological factors or by some physiological outcomes that were not measured in the present study. Future work is needed to explore the mechanisms of dyspnea relief following both strength and endurance based IMT using various exercise protocols across the spectrum of health and disease.

REFERENCES

1. **American Thoracic Society/European Respiratory Society**. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med* 166: 518-624, 2002.

2. **Borg GA**. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377-381, 1982.

3. **Briscoe WA, Dubois AB**. The relationship between airway resistance, airway conductance and lung volume in subjects of different age and body size. *J Clin Invest* 37: 1279-1285, 1958.

4. **Cory JM, Schaeffer MR, Wilkie SS, Ramsook AH, Puyat JH, Arbour B, Basran R, Lam M, Les C, MacDonald B, Jensen D, Guenette JA**. Sex differences in the intensity and qualitative dimensions of exertional dyspnea in physically active young adults. *J Appl Physiol* 119: 998-1006, 2015.

5. **Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P**. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381-1395, 2003.

6. **Dempsey JA, Romer L, Rodman J, Miller J, Smith C**. Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobiol* 151: 242-250, 2006.

7. **Duiverman ML, de Boer EW, van Eykern LA, de Greef MH, Jansen DF, Wempe JB, Kerstjens HA, Wijkstra PJ**. Respiratory muscle activity and dyspnea during exercise in chronic obstructive pulmonary disease. *Respir Physiol Neurobiol* 167: 195-200, 2009.

8. **Duiverman ML, van Eykern LA, Vennik PW, Koeter GH, Maarsingh EJ, Wijkstra PJ**. Reproducibility and responsiveness of a noninvasive EMG technique of the respiratory muscles in COPD patients and in healthy subjects. *J Appl Physiol* 96: 1723-1729, 2004.

9. **Faisal A, Alghamdi BJ, Ciavaglia CE, Elbehairy AF, Webb KA, Ora J, Neder JA, O'Donnell DE**. Common mechanisms of dyspnea in chronic interstitial and obstructive lung disorders. *Am J Respir Crit Care Med* 193: 299-309, 2016.

10. **Geddes EL, O'Brien K, Reid WD, Brooks D, Crowe J**. Inspiratory muscle training in adults with chronic obstructive pulmonary disease: an update of a systematic review. *Respir Med* 102: 1715-1729, 2008.

11. **Guenette JA, Chin RC, Cory JM, Webb KA, O'Donnell DE**. Inspiratory Capacity during Exercise: Measurement, Analysis, and Interpretation. *Pulm Med* 2013: 956081, 2013.

12. **Gutierrez C, Ghezzo RH, Abboud RT, Cosio MG, Dill JR, Martin RR, McCarthy DS, Morse JL, Zamel N**. Reference values of pulmonary function tests for Canadian Caucasians. *Can Respir J* 11: 414-424, 2004.

13. **HajGhanbari B, Yamabayashi C, Buna TR, Coelho JD, Freedman KD, Morton TA, Palmer SA, Toy MA, Walsh C, Sheel AW, Reid WD**. Effects of respiratory muscle training on performance in athletes: a systematic review with meta-analyses. *J Strength Cond Res* 27: 1643-1663, 2013.

14. **Hill K, Jenkins SC, Hillman DR, Eastwood PR**. Dyspnoea in COPD: Can inspiratory muscle training help? *Aust J Physiother* 50: 169-180, 2004.

15. **Huang CH, Martin AD, Davenport PW**. Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. *J Appl Physiol* 94: 462-468, 2003.

16. **Illi SK, Held U, Frank I, Spengler CM**. Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis. *Sports Med* 42: 707-724, 2012.

17. **Jensen D, Pattinson K, Jolley CJ**. Mechanisms of Breathlessness. In: *European Respiratory Society Monograph* European Respiratory Society Journals, 2016, p. 111-113.

18. **Jolley CJ, Luo YM, Steier J, Rafferty GF, Polkey MI, and Moxham J**. Neural respiratory drive and breathlessness in COPD. *Eur Respir J* 45: 355-364, 2015.

19. **Langer D, Ciavaglia C, Webb K, Preston M, Neder JA, Gosselink R, O'Donnell D**. Inspiratory muscle training reduces respiratory neural drive (RND) during exercise in patients with COPD. *Eur Respir J* 44: 1912, 2014.

20. **Luo YM, Hart N, Mustfa N, Lyall RA, Polkey MI, Moxham J**. Effect of diaphragm fatigue on neural respiratory drive. *J Appl Physiol* 90: 1691-1699, 2001.

21. **Luo YM, Moxham J, Polkey MI**. Diaphragm electromyography using an oesophageal catheter: current concepts. *Clin Sci* 115: 233-244, 2008.

22. **McConnell AK**. CrossTalk opposing view: respiratory muscle training does improve exercise tolerance. *J Physiol* 590: 3397-3398; discussion 3399-3400, 2012.

23. **McConnell AK, Romer LM**. Dyspnoea in health and obstructive pulmonary disease : the role of respiratory muscle function and training. *Sports Med* 34: 117-132, 2004.

24. **McGregor M, Becklake MR**. The relationship of oxygen cost of breathing to respiratory mechanical work and respiratory force. *J Clin Invest* 40: 971-980, 1961.

25. **Mendonca CT, Schaeffer MR, Riley P, Jensen D**. Physiological mechanisms of dyspnea during exercise with external thoracic restriction: role of increased neural respiratory drive. *J Appl Physiol* 116: 570-581, 2014.

26. **Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J**. Standardisation of spirometry. *Eur Respir J* 26: 319-338, 2005.

27. **Nicks CR, Morgan DW, Fuller DK, Caputo JL**. The influence of respiratory muscle training upon intermittent exercise performance. *Int J Sports Med* 30: 16-21, 2009.

28. **O'Donnell DE, Hamilton AL, Webb KA**. Sensory-mechanical relationships during high-intensity, constant-work-rate exercise in COPD. *J Appl Physiol* 101: 1025-1035, 2006.

29. **O'Donnell DE, Ora J, Webb KA, Laveneziana P, Jensen D**. Mechanisms of activity-related dyspnea in pulmonary diseases. *Respir Physiol Neurobiol* 167: 116-132, 2009.

30. **Patel MS, Hart N, Polkey MI**. CrossTalk proposal: training the respiratory muscles does not improve exercise tolerance. *J Physiol* 590: 3393-3395; discussion 3401, 2012.

31. **Ramsook AH, Koo R, Molgat-Seon Y, Dominelli PB, Syed N, Ryerson CJ, Sheel AW, Guenette JA**. Diaphragm recruitment increases during a bout of targeted inspiratory muscle training. *Med Sci Sports Exerc* 48: 1179-1186, 2016.

32. **Reilly CC, Jolley CJ, Ward K, MacBean V, Moxham J, Rafferty GF**. Neural respiratory drive measured during inspiratory threshold loading and acute hypercapnia in healthy individuals. *Exp Physiol* 98: 1190-1198, 2013.

33. **Reilly CC, Ward K, Jolley CJ, Lunt AC, Steier J, Elston C, Polkey MI, Rafferty GF, Moxham J**. Neural respiratory drive, pulmonary mechanics and breathlessness in patients with cystic fibrosis. *Thorax* 66: 240-246, 2011.

34. **Romer LM, McConnell AK, Jones DA**. Effects of inspiratory muscle training on time-trial performance in trained cyclists. *J Sports Sci* 20: 547-562, 2002.

35. **Romer LM, McConnell AK, Jones DA**. Inspiratory muscle fatigue in trained cyclists: effects of inspiratory muscle training. *Med Sci Sports Exerc* 34: 785-792, 2002.

36. **Romer LM, Miller JD, Haverkamp HC, Pegelow DF, Dempsey JA**. Inspiratory muscles do not limit maximal incremental exercise performance in healthy subjects. *Respir Physiol Neurobiol* 156: 353-361, 2007.

37. **Romer LM, Polkey MI**. Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol* 104: 879-888, 2008.

38. **Schaeffer MR, Mendonca CT, Levangie MC, Andersen RE, Taivassalo T, Jensen D**. Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. *Exp Physiol* 99: 427-441, 2014.

39. **Segizbaeva MO, Donina Zh A, Timofeev NN, Korolyov YN, Golubev VN, and Aleksandrova NP**. EMG analysis of human inspiratory muscle resistance to fatigue during exercise. *Adv Exp Med Biol* 788: 197-205, 2013.

40. **Shadgan B, Guenette JA, Sheel AW, Reid WD**. Sternocleidomastoid muscle deoxygenation in response to incremental inspiratory threshold loading measured by near infrared spectroscopy. *Respir Physiol Neurobiol* 178: 202-209, 2011.

41. **Sheel AW**. Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. *Sports Med* 32: 567-581, 2002.

42. **Simon PM, Schwartzstein RM, Weiss JW, Fencl V, Teghtsoonian M, Weinberger SE**. Distinguishable types of dyspnea in patients with shortness of breath. *Am Rev Respir Dis* 142: 1009-1014, 1990.

43. **Singh B, Panizza JA, Finucane KE**. Diaphragm electromyogram root mean square response to hypercapnia and its intersubject and day-to-day variation. *J Appl Physiol* 98: 274-281, 2005.

44. **Suzuki S, Yoshiike Y, Suzuki M, Akahori T, Hasegawa A, Okubo T**. Inspiratory muscle training and respiratory sensation during treadmill exercise. *Chest* 104: 197-202, 1993.

45. **Tan WC, Bourbeau J, Hernandez P, Chapman K, Cowie R, FitzGerald MJ, Aaron S, Marciniuk DD, Maltais F, O'Donnell DE, Goldstein R, Sin D, LHCE investigators**. Canadian prediction equations of spirometric lung function for Caucasian adults 20 to 90 years of age: results from the Canadian Obstructive Lung Disease (COLD) study and the Lung Health Canadian Environment (LHCE) study. *Can Respir J* 18: 321-326, 2011.

46. **Verges S, Lenherr O, Haner AC, Schulz C, Spengler CM**. Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. *Am J Physiol Regul Integr Comp Physiol* 292: R1246-1253, 2007.

47. **Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G**. Standardisation of the measurement of lung volumes. *Eur Respir J* 26: 511-522, 2005.

48. **Wells GD, Plyley M, Thomas S, Goodman L, Duffin J**. Effects of concurrent inspiratory and expiratory muscle training on respiratory and exercise performance in competitive swimmers. *Eur J Appl Physiol* 94: 527-540, 2005.

49. **Wilson SH, Cooke NT, Edwards RH, Spiro SG**. Predicted normal values for maximal respiratory pressures in caucasian adults and children. *Thorax* 39: 535-538, 1984.

50. **Witt JD, Guenette JA, Rupert JL, McKenzie DC, Sheel AW**. Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. *J Physiol* 584: 1019-1028, 2007.

Competing Interests

None declared.

Author Contributions

Conception of work: AHR and JAG. Experimental design: AHR, PGC, WDR, LMR, and JAG. Data collection: AHR, YMS, MRS, and SSW. Analysis and interpretation of data and drafting of the article: AHR, YMS, MRS, SSW, PGC, LMR, and JAG. All authors: approved the final version of the manuscript.

Funding

This research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada and an Infrastructure Grant from the Canada Foundation for Innovation. AHR was supported by the University of British Columbia 4 Year Fellowship (4YF). YMS was supported by a 4YF and a Post Graduate Scholarship from the NSERC. MRS was supported by a 4YF and a fellowship from the British Columbia Lung Association. PGC was supported by a Scholar Award from the Michael Smith Foundation for Health Research (MSFHR). JAG was supported by a Scholar Award from the MSFHR, a Canadian Institutes of Health Research Clinical Rehabilitation New Investigator Award, and a New Investigator Award from the Providence Health Care Research Institute and St. Paul's Hospital Foundation.

Acknowledgments

The authors would like to thank Dr. Joseph Puyat for his assistance with the statistical analysis for this study and to our subjects for their enthusiastic participation.

611 **TABLES**
612

	Pre-IMT	Post-IMT	Pre-SC	Post-SC
Age, years	25 ± 5	-	24 ± 4	-
Height, cm	174 ± 10	-	180 ± 6	-
BMI, $kg \cdot m^{-2}$	24.7 ± 1.9	24.7 ± 1.8	23.0 ± 1.9	23.0 ± 2.0
Self-reported physical activity ($MET \cdot min \cdot week^{-1}$)	3665 ± 1159	3751 ± 1952	3687 ± 1970	3102 ± 1151
Handgrip strength, kg	44 ± 10	44 ± 9	42 ± 7	42 ± 7
(%predicted)	(106 ± 21)	(105 ± 17)	(97 ± 16)	(98 ± 15)
Pulmonary Function				
FEV_1/FVC, %	78. 8 ± 0.78	78.2 ± 0.74	79.8 ± 0.89	80.0 ± 0.91
(%predicted)	(112 ± 21)	(111 ± 20)	(115 ± 25)	(116 ± 26)
FEV_1, L	4.50 ± 1.01	4.49 ± 0.99	4.39 ± 0.89	4.46 ± 0.87
(%predicted)	(100 ± 5)	(100 ± 5)	(92 ± 4)	(94 ± 4)
FVC, L	5.71 ± 1.30	5.73 ± 1.28	5.52 ± 0.89	5.55 ± 0.92
(%predicted)	(106 ± 24)	(107 ± 24)	(96 ± 16)	(97 ± 16)
TLC, L	7.00 ± 1.36	7.17 ± 1.41	6.91 ± 0.88	7.04 ± 0.88
(%predicted)	(99 ± 19)	(101 ± 20)	(92 ± 12)	(93 ± 12)
MIP, cmH_2O	-138 ± 45	-160 ± 43 †*	-134 ± 27	-134 ± 32
(%predicted)	(118 ± 32)	(137 ± 30) †*	(113 ± 33)	(114 ± 38)

613
614 **Table 1.** Participant characteristics. *Abbreviations:* BMI, body mass index; MET, metabolic equivalent;
615 FEV_1, forced expiratory volume in one-second; FVC, forced vital capacity; TLC, total lung capacity;
616 MIP, maximal inspiratory pressure; IMT, inspiratory muscle training; SC sham control. † Significantly
617 different from baseline (pre), $p < 0.01$; * Significantly different from SC (post), $p < 0.05$.
618

	Pre-IMT	Post-IMT	Pre-SC	Post-SC
Work rate, W	285 ± 82	285 ± 83	255 ± 64	254 ± 65
$\dot{V}O_2$, mL·kg^{-1}·min^{-1}	55 ± 10	55 ± 11	50 ± 9	49 ± 11
RER	1.08 ± 0.06	1.09 ± 0.07	1.09 ± 0.05	1.08 ± 0.05
$\dot{V}_E$, L/min	143 ± 35	148 ± 37	124 ± 26	117 ± 25
V_T, L	2.97 ± 0.7	3.02 ± 0.67	2.70 ± 0.57	2.72 ± 0.71
F_b, breaths/min	50 ± 13	50 ± 11	47 ± 10	45 ± 10
$\dot{V}_E$ /$\dot{V}O_2$	35 ± 4	34 ± 6	34 ± 4	32 ± 4
$\dot{V}_E$/$\dot{V}CO_2$	32 ± 4	33 ± 3	31 ± 3	30 ± 3
$PETCO_2$, mmHg	35 ± 4	34 ± 3	36 ± 3	37 ± 3
EELV, %TLC	49 ± 8	50 ± 6	49 ± 5	50 ± 3
EILV, %TLC	92 ± 6	92 ± 4	88 ± 8	88 ± 8
HR, beats/min (%predicted)	180 ± 7 (97 ± 3)	180 ± 11 (97 ± 6)	176 ± 12 (95 ± 7)	178 ± 14 (95 ± 8)
PEFR, L/s	6.4 ± 1.7	6.5 ± 1.6	5.4 ± 0.9	5.3 ± 0.9
T_I, s	0.65 ± 0.24	0.62 ± 0.17	0.65 ± 0.12	0.69 ± 0.14
T_E, s	0.67 ± 0.18	0.66 ± 0.16	0.68 ± 0.13	0.73 ± 0.18
T_I/T_{TOT}	0.48 ± 0.02	0.49 ± 0.02	0.49 ± 0.03	0.49 ± 0.03
Breathing discomfort, (0-10 Borg scale)	8.5 ± 1.9	8.5 ± 2.5	8.2 ± 1.7	7.6 ± 2.4
Leg discomfort, (0-10 Borg scale)	10 ± 0	9.9 ± 0.3	9.3 ± 0.8	8.9 ± 1.5

Table 2. Peak exercise responses. *Abbreviations:* $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; V_T, tidal volume; F_b, breathing frequency; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $PETCO_2$, partial pressure of end-tidal carbon dioxide; EELV, end-expiratory lung volume; TLC, total lung capacity; EILV, end-inspiratory lung volume; HR, heart rate; PEFR, peak expiratory flow rate; T_I, inspiratory time; T_E, expiratory time; T_I/T_{TOT}, inspiratory duty cycle; IMT, inspiratory muscle training; SC sham control. No significant differences were observed within group-pre vs. post or between groups at baseline.

FIGURE LEGEND

Figure 1. Inspiratory muscle electromyography during exercise. First dashed line connects 150 W to HEWR. Second dashed line connects HEWR to peak exercise. *Abbreviations:* HEWR, highest equivalent submaximal work rate; EMGdi, diaphragm electromyography; EMGsca, scalene electromyography; EMGscm, sternocleidomastoid electromyography; IMT, inspiratory muscle training; SC sham control. *, $p < 0.05$.

Figure 2. Ventilatory responses during exercise. First dashed line connects 150 W to HEWR. Second dashed line connects HEWR to peak exercise. Panels D and H represent operating lung volumes during exercise. Grey shaded region represents tidal volume [i.e., the difference between end-expiratory lung volume and end-inspiratory lung volume]. *Abbreviations:* HEWR, highest equivalent submaximal work rate; TLC, total lung capacity; IMT, inspiratory muscle training; SC sham control.

Figure 3. Total work of breathing and neuromechanical coupling of the respiratory system during exercise. First dashed line connects 150 W to HEWR. Second dashed line connects HEWR to peak exercise. *Abbreviations:* HEWR, highest equivalent submaximal work rate; EMGdi, diaphragm electromyography; V_T, tidal volume; VC, vital capacity; IMT, inspiratory muscle training; SC sham control.

Figure 4. Perceived breathing and leg discomfort during exercise. First dashed line connects 150 W to HEWR. Second dashed line connects HEWR to peak exercise. Abbreviations: HEWR, highest equivalent submaximal work rate; IMT, inspiratory muscle training; SC sham control. *, $p < 0.05$.

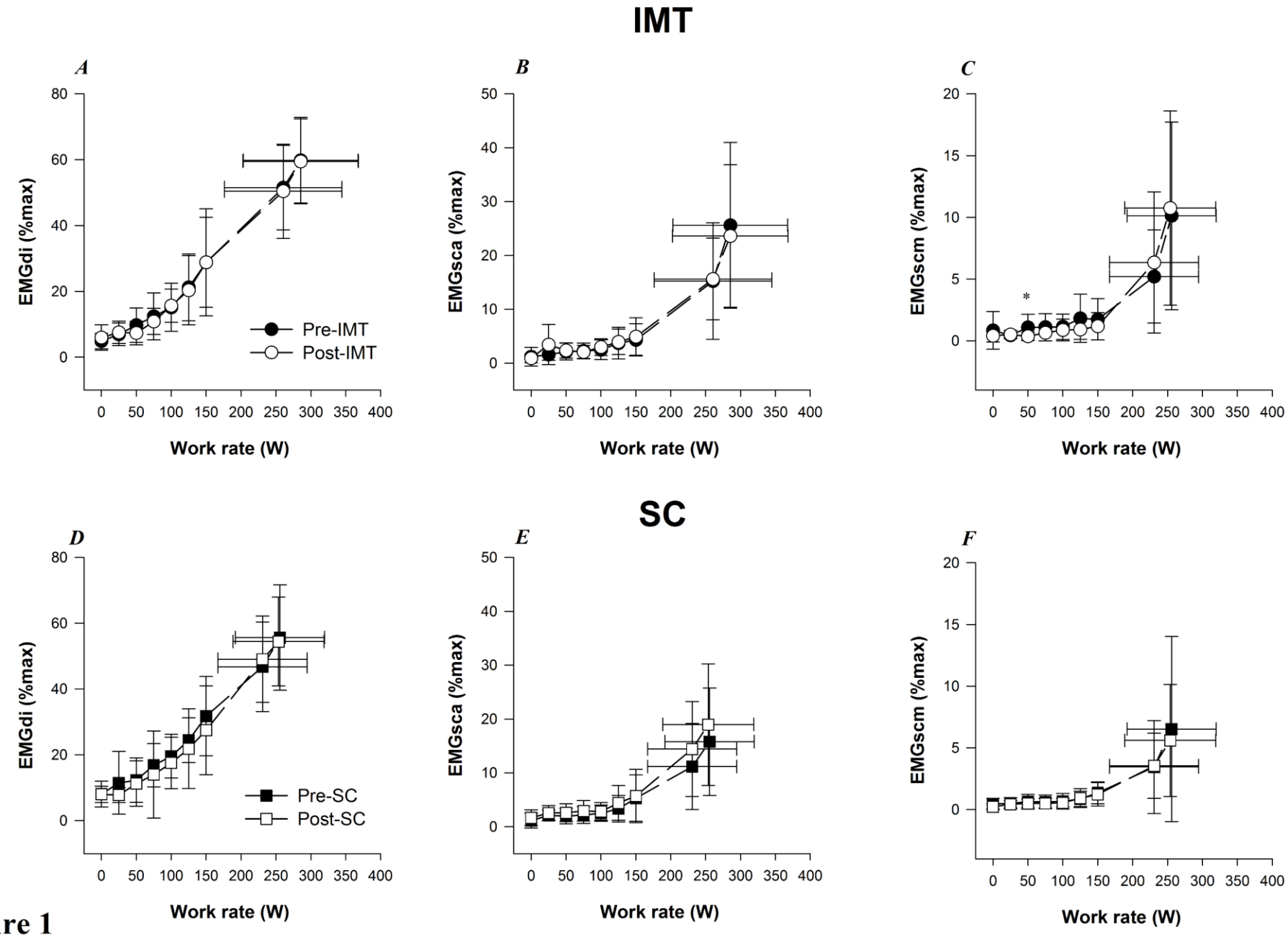
IMT
A
B
C
EMGdi (%max)
EMGsca (%max)
EMGscm (%max)
Work rate (W)
Pre-IMT
Post-IMT
*
SC
D
E
F
Pre-SC
Post-SC

Figure 1

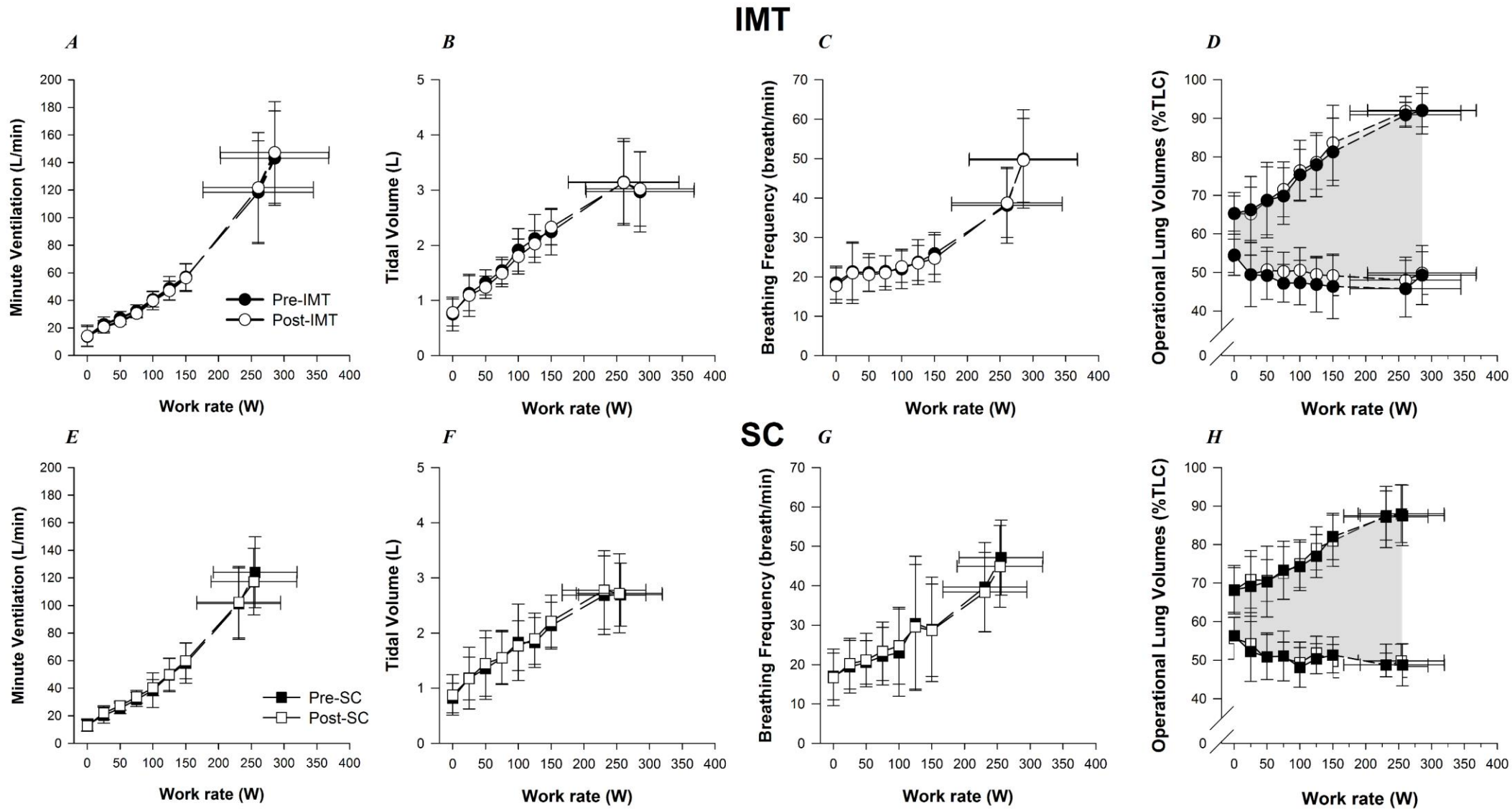
IMT
A
B
C
D
Minute Ventilation (L/min)
Tidal Volume (L)
Breathing Frequency (breath/min)
Operational Lung Volumes (%TLC)
Work rate (W)
Pre-IMT
Post-IMT
SC
E
F
G
H
Pre-SC
Post-SC

Figure 2

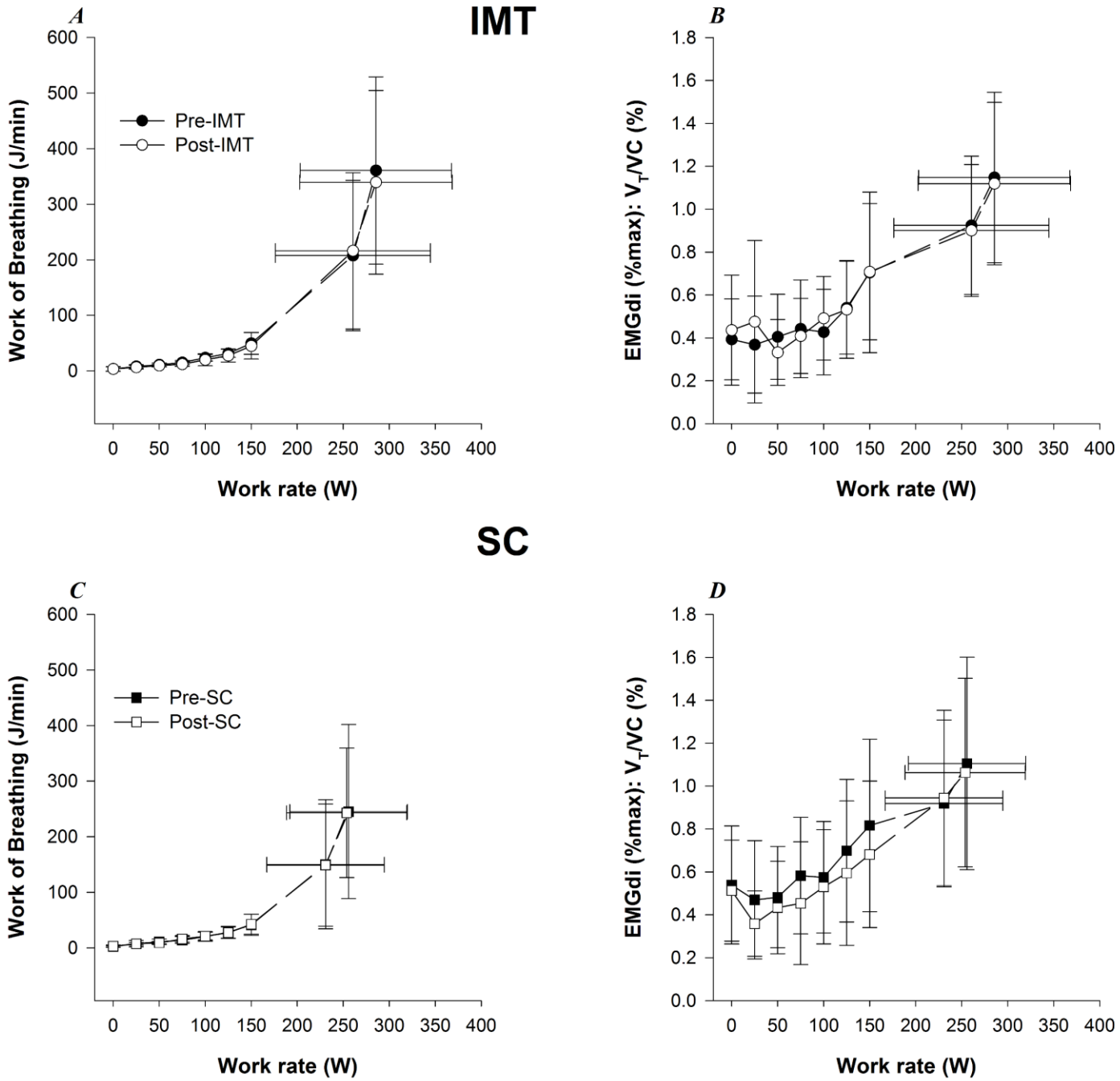
IMT
A
B
Pre-IMT
Post-IMT
Work of Breathing (J/min)
EMGdi (%max): V_T/VC (%)
Work rate (W)
SC
C
D
Pre-SC
Post-SC
Work of Breathing (J/min)
EMGdi (%max): V_T/VC (%)
Work rate (W)

Figure 3

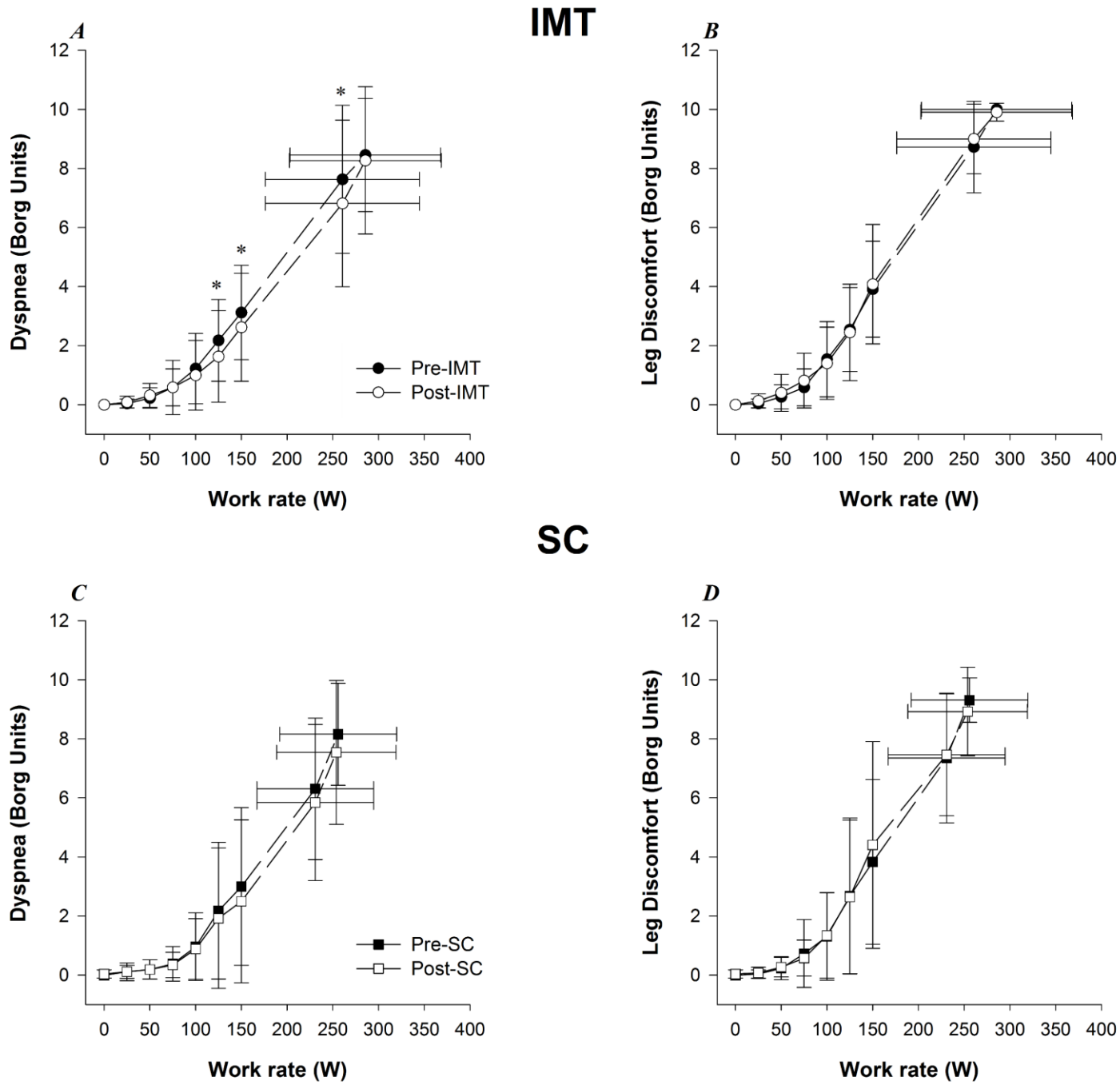
IMT
A
B
Dyspnea (Borg Units)
Leg Discomfort (Borg Units)
Work rate (W)
Pre-IMT
Post-IMT
SC
C
D
Pre-SC
Post-SC

Figure 4