# INFLUENCE OF INSPIRATORY RESISTIVE LOADING ON EXPIRATORY MUSCLE FATIGUE IN HEALTHY HUMANS

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**NEW FINDINGS** (word count = 86/100)

- What is the central question of this study?

This study is the first to objectively measure both inspiratory and expiratory muscle fatigue after inspiratory resistive loading to determine whether the expiratory muscles are activated to the point of fatigue when specifically loading the inspiratory muscles.

- What is the main finding and its importance?

The absence of abdominal muscle fatigue suggests that future studies attempting to understand the neural and circulatory consequences of diaphragm fatigue can utilize IRL without considering the confounding effects of abdominal muscle fatigue.

**ABSTRACT** (word count = 247/250)

Expiratory resistive loading elicits inspiratory as well as expiratory muscle fatigue, suggesting parallel co-activation of the inspiratory muscles during expiration. It is unknown whether the expiratory muscles are similarly co-activated to the point of fatigue during inspiratory resistive loading (IRL). The purpose of this study was to determine whether IRL elicits expiratory as well as inspiratory muscle fatigue. Healthy male subjects (n=9) underwent isocapnic IRL (60% maximal inspiratory pressure, 15 breaths·min$^{-1}$, 0.7 inspiratory duty cycle) to task failure. Abdominal and diaphragm

contractile function was assessed at baseline and at 3, 15 and 30 min post-IRL by measuring gastric twitch pressure ($P_{ga,tw}$) and transdiaphragmatic twitch pressure ($P_{di,tw}$) in response to potentiated magnetic stimulation of the thoracic and phrenic nerves, respectively. Fatigue was defined as a significant reduction from baseline in $P_{ga,tw}$ or $P_{di,tw}$. Throughout IRL, there was a time-dependent increase in cardiac frequency and mean arterial blood pressure, suggesting activation of the respiratory muscle metaboreflex. $P_{di,tw}$ was significantly lower than baseline (34.3 ± 9.6 $cmH_2O$) at 3 min (23.2 ± 5.7 $cmH_2O$, $P$<0.001), 15 min (24.2 ± 5.1 $cmH_2O$, $P$<0.001) and 30 min post-IRL (26.3 ± 6.0 $cmH_2O$, $P$<0.001). $P_{ga,tw}$ was not significantly different from baseline (37.6 ± 17.1 $cmH_2O$) at 3 min (36.5 ± 14.6 $cmH_2O$), 15 min (33.7 ± 12.4 $cmH_2O$) and 30 min post-IRL (32.9 ± 11.3 $cmH_2O$). IRL elicits objective evidence of diaphragm, but not abdominal, muscle fatigue. Agonist-antagonist interactions for the respiratory muscles appear to be more important during expiratory versus inspiratory loading.

## INTRODUCTION

Fatigue is defined as a reduction in skeletal muscle force and/or velocity generating capacity after exposure to load that is reversible with rest (NHLBI, 1990). It is well known that the respiratory muscles fatigue in response to the substantive ventilatory work associated with heavy exercise (Johnson *et al.*, 1993; Taylor *et al.*, 2006). There are several physiological consequences of fatiguing contractions of inspiratory and expiratory muscles. First, heightened perceptions of dyspnea occur in response to both inspiratory (Gandevia *et al.*, 1981; Supinski *et al.*, 1987) and expiratory muscle fatigue (Chonan *et al.*, 1990; Suzuki *et al.*, 1992). Second, inspiratory (Mador & Acevedo, 1991) and expiratory muscle fatigue (Verges *et al.*, 2007; Taylor & Romer, 2008) reduce subsequent exercise performance. Finally, fatiguing respiratory muscle work has a significant influence on sympathetic vasoconstrictor outflow (St Croix *et al.*, 2000; Sheel *et al.*, 2001, 2002; Derchak *et al.*, 2002). For example, inspiratory muscle fatigue induced by inspiratory resistive loading (IRL) causes a time-

dependent increase in muscle sympathetic nerve activity in an exercising (Katayama *et al.*, 2012) and resting limb (St Croix *et al.*, 2000) as well as decreases in limb blood flow and limb vascular conductance (Sheel *et al.*, 2001). Mean arterial pressure (MAP) and cardiac frequency ($f_c$) also increase in a time-dependent manner during fatiguing diaphragm contractions (Sheel *et al.*, 2001). When the expiratory muscles are fatigued via expiratory resistive loading (ERL), a similar increase in muscle sympathetic nerve activity is seen in the exercising (Katayama *et al.*, 2015) and resting limb and an increase in MAP is also observed (Derchak *et al.*, 2002). Collectively, these data suggest that fatiguing inspiratory and expiratory muscles will evoke reflex effects, which increase sympathetic vasoconstrictor outflow to limb muscle and perhaps explain the observed reductions in limb vascular conductance and blood flow during heavy exercise (Harms *et al.*, 1997, 1998). The physiological consequences of inspiratory and expiratory muscle fatigue are similar; therefore, the ability to determine the impact of each relies on our ability to isolate fatigue to the inspiratory or expiratory muscles.

Magnetic stimulation has been used to objectively assess inspiratory and expiratory muscle fatigue after ERL to task failure (Taylor & Romer, 2009). Subjects completed 4 different ERL trials on 4 different days. Breathing frequency was maintained at 15 breaths/min for all conditions, while expiratory duty cycle was maintained at 0.4 or 0.7 and expiratory gastric pressure ($P_{ga}$) at 40% or 60% of maximum ($ERL_{40\%0.4}$, $ERL_{60\%0.4}$, $ERL_{40\%0.7}$, $ERL_{60\%0.7}$). Transdiaphragmatic twitch pressure ($P_{di,tw}$) and gastric twitch pressure ($P_{ga,tw}$) were reduced below baseline values by 9-15% and 15-22%, respectively, after each ERL trial. The decreases in $P_{di,tw}$ after ERL suggest co-activation of the inspiratory muscles when the load on the expiratory muscles is increased.

The effect of ERL on inspiratory muscle fatigue may also apply to the expiratory muscles during inspiratory loading. Previous work investigating the activity of abdominal muscles during inspiratory loading suggests that the abdominals are recruited when the load on the inspiratory muscles is increased (Martin & De Troyer, 1982; Abbrecht *et al.*, 1991). Recruitment of the

abdominal muscles assists the inspiratory muscles in multiple ways. First, by reducing end-expiratory lung volume (EELV), increases in tidal volume ($V_T$) can occur on the linear portion of the respiratory system compliance curve (Stubbing *et al.*, 1980). Second, as EELV is reduced, the diaphragm is lengthened. Lengthening of the diaphragm enables it to function at a more optimal length for tension development (Smith & Bellemare, 1987). Third, abdominal activity during inspiration increases intra-abdominal pressure, thereby providing a fulcrum for diaphragm contraction (DeTroyer *et al.*, 1982; Reid & Dechman, 1995). Based on the aforementioned observations, we used an IRL protocol known to produce diaphragm fatigue to test the hypothesis that expiratory muscles are co-activated to the point of fatigue during IRL. If fatigue of the expiratory muscles does occur in response to IRL, this will have implications for studies investigating the effect of inspiratory muscle fatigue on dyspnea, exercise tolerance, muscle sympathetic nerve activity, and limb blood flow.

## METHODS

*Ethical Approval.* All study protocols were approved by the Clinical Research Ethics Board at the University of British Columbia (approval number: H14-01208). The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. Subjects provided written informed consent prior to beginning the study.

*Subjects.* Nine healthy young men volunteered to participate. The subjects were young (25±3 yr), had a BMI within the normal range (23±2.5 kg/m$^2$), and were nonsmokers. Pulmonary function was measured using a spirometer (Spirolab II, Medical International Research) and subjects had normal forced vital capacity and forced expiratory volume in 1 s (101 ± 10% and 101 ±9 %, respectively) (Quanjer *et al.*, 1993). Subjects with a $FEV_1$/FVC <80% predicted were excluded from participating.

*Experimental Protocol.* Informed consent, anthropometric and descriptive data were obtained. Subsequently, subjects underwent routine spirometric testing. Skin surface electrodes were placed to

measure electrical activity of the diaphragm, rectus abdominis, and external oblique. Subjects were then instrumented with balloon catheters for the determination of esophageal pressure ($P_{es}$) and gastric pressure ($P_{ga}$). Mouth pressure ($P_m$) was monitored from a side port in the mouthpiece. Tubing connected to the balloon catheters and side port of the mouth piece was connected to pressure transducers calibrated across the physiological range. Five maximal inspiratory pressure maneuvers and five maximal expiratory pressure maneuvers were performed at baseline and the average of the three highest values was defined as the maximum inspiratory and expiratory pressure for that subject. Maneuvers were performed against a device that incorporated a 2 mm orifice to prevent glottic closure during inspiratory maneuvers and to minimise the use of buccal muscles during expiratory maneuvers. Inspiratory maneuvers were initiated from functional residual capacity (FRC) and expiratory maneuvers were initiated from total lung capacity (TLC). Contractile function and membrane excitability of the diaphragm and abdominal muscles were assessed at baseline using a magnetic stimulator (see below). Subjects were then moved into the supine position where resting cardiorespiratory data were collected for 5-10 min. Subjects then breathed against an inspiratory resistive load to the point of task failure (see below). Abdominal and diaphragm contractile function and membrane excitability were assessed at 3 min, 15 min and 30 min after IRL by measuring potentiated $P_{ga,tw}$ and $P_{di,tw}$ in response to magnetic stimulation of the thoracic and phrenic nerves, respectively (see below). Electromyographic (EMG) activity of the diaphragm, rectus abdominis, and external obliques was monitored to ensure consistency of stimulation and to characterize activation patterns throughout IRL.

*Flow, Volume, and Pressure.* Inspired and expired flow was measured using a pair of calibrated pneumotachographs (model 3813, Hans Rudolph, Kansas City, MO) connected to a customized two-way non-rebreathe valve. Volume was determined by numerical integration of the flow signals. Respiratory pressures were assessed using previously described procedures (Milic-Emili *et al.*, 1964;

Dominelli *et al.*, 2015). Briefly, a topical anesthetic (Xylocaine, 2% Lidocaine Hydrochloride) was applied to the subject's nares prior to the insertion of two balloon-tipped catheters (no.47-9005, Ackrad Laboratory, Cranford, NJ). One catheter was inserted into the stomach to measure $P_{ga}$ and the other was inserted into the lower one-third of the esophagus to measure $P_{es}$. The esophageal catheter was placed using the occlusion technique (Baydur *et al.*, 1982). The balloon catheters were connected to calibrated pressure transducers (model MP45, Validyne Engineering). Transdiaphragmatic pressure ($P_{di}$) was obtained online by subtracting $P_{es}$ from $P_{ga}$.

*Diaphragm and Abdominal Muscle Fatigue.* To assess diaphragm fatigue, both phrenic nerves were stimulated using a hand held, 90-mm circular coil attached to a magnetic stimulator (Magstim 200 Mono Pulse, MagStim, Whitland, Wales) (Similowski *et al.*, 1989; Guenette *et al.*, 2010). Subjects sat upright with their necks flexed and the coil was placed between the 5th and 7th cervical vertebrae over the midline. The vertebral level that when stimulated resulted in the highest $P_{di,tw}$ was marked, and the coil positioned at that level for all subsequent stimulations. For the assessment of abdominal fatigue, subjects sat upright with their chest supported by a bench inclined 20° from vertical (Kyroussis *et al.*, 1996; Taylor & Romer, 2009). Once in position, the thoracic nerve roots were stimulated between thoracic vertebrae 8 and 11 using the same circular coil as described above. The vertebral level that when stimulated resulted in the highest $P_{ga,tw}$ was marked and the coil positioned at that level for all subsequent stimulations. The ability of the magnetic stimulator to consistently activate the diaphragm and the abdominal muscles was assessed by constructing recruitment curves. The stimulator was charged to pre-determined percentages of its maximal output (60, 70, 80, 85, 90, 95 and 100%). Three stimulations, each separated by 30 sec to avoid twitch potentiation, were delivered to the phrenic and thoracic nerves at each output setting (Taylor & Romer, 2009). Skin surface electrodes (Kendall H59P Cloth Electrodes) were used to measure electrical activity of the left and right costal diaphragm, rectus abdominis (RA), and external oblique (EO). Electrodes were placed between the 6th and the 8th intercostal space on the anterior axillary line to measure

diaphragm activity (Glerant *et al.*, 2006). Abdominal muscle activity was measured by placing electrodes 3 cm lateral and 7 cm superior to the umbilicus on the RA, and 15 cm lateral to the umbilicus on the EO. EMG signals were amplified (x 200) and band-pass filtered (0.1Hz-3 kHz; Model 78D, Grass Instruments; Oakville, ON, Canada).

The protocol used to assess diaphragm and abdominal fatigue consisted of six 1-Hz potentiated twitches measured at baseline and at 3 min, 15 min, and 30 min after IRL. To potentiate the diaphragm, subjects performed a maximal inspiratory effort, initiated from FRC and maintained against a semi-occluded airway for ~5 sec. The phrenic nerves were then stimulated at the end of the second tidal expiration after the maximal inspiratory effort. Abdominal potentiation consisted of a maximal expiratory effort performed from TLC against a semi-occluded airway for ~5 sec. The thoracic nerves were then stimulated at the end of the second tidal expiration after the potentiation maneuver. All phrenic and thoracic stimulations were delivered at 100% of the stimulator's output and initiated at the same lung volume (FRC) as judged by non-significant changes in end-expiratory $P_{es}$. To ensure consistent degrees of potentiation, the first two twitches at each time point were discarded and $P_{di,tw}$ and $P_{ga,tw}$ were determined based on the average of the latter four stimulations.

*Inspiratory Resistive Loading.* To reduce postural abdominal muscle contribution (Campbell & Green, 1955; Goldman *et al.*, 1987), subjects performed IRL in a supine position by inspiring against a flow resistor until task failure. Target inspiratory pressure was fixed at 60% of maximum inspiratory $P_m$ (determined previously during the maximal inspiratory pressure maneuvers), inspiratory duty cycle ($T_I/T_{TOT}$) at 0.7, and breathing frequency ($f_b$) at 15 breaths·min$^{-1}$. Subjects were instructed to try to maintain $P_m$ at their target throughout the duration of inspiration. Resisted breathing was continued to the point of task failure, defined as the point at which the subject could not achieve or maintain the target $P_m$ for three consecutive breaths despite verbal encouragement. During IRL, subjects viewed a computer monitor with real-time $P_m$ in relation to the target inspiratory pressure and breathed

synchronously to a metronome with distinct inspiratory and expiratory tones. Arterial blood pressure and cardiac frequency ($f_c$) were measured beat-by-beat using finger pulse photoplethysmography (Finometer, FMS, Finapres Medical Systems BV, Arnhem, The Netherlands). An automated sphygmomanometer (BPM-100, VSM MedTech Ltd., Vancouver, Canada) was used to calibrate the Finometer during the first minute of resting data collection. Breath-by-breath measurement of $P_{ET_{CO_2}}$ (model 17630, VacuMed) was performed and $CO_2$ was manually titrated into the inspiratory circuit to maintain eucapnia.

*Data Analysis.* Flow, pressure, $P_{ET_{CO_2}}$, and EMG signals were amplified and A/D converted (model ML 795, PowerLab/16SP, ADInstruments, Colorado Springs, CO) and recorded at a sampling rate of 10 kHz using commercially available software (Chart v5.3, ADInstruments). Inspiration and expiration were defined based on the zero points of mouth pressure. Inspiratory $P_{ga}$ and $P_{di}$ swings were calculated on a breath-by-breath basis by subtracting the end-expiratory pressure from end-inspiratory pressure achieved during each breath and averaging over 1 min intervals. Cumulative force output of the diaphragm was calculated by integrating $P_{di}$ ($\int P_{di}/dt$) down to end-inspiratory pressure over the periods of inspiratory flow for the entire duration of the IRL trial (Taylor & Romer, 2009). To get an indication of abdominal muscle recruitment during loading we integrated $P_{ga}$ ($\int P_{ga}/dt$) down to end-inspiratory pressure over periods of inspiratory flow throughout IRL. Integration of $P_{ga}$ was done during inspiration instead of expiration because gastric pressure was consistently generated only during inspiration. As abdominal muscle and diaphragm contraction will both contribute to inspiratory $P_{ga}$, we named this variable cumulative $P_{ga}$. The average tension-time index for the diaphragm ($TTI_{di}$) was calculated for each min of the IRL trial as the product of $P_{di}/P_{di,max}$ and $T_I/T_{TOT}$ (Bellemare & Grassino, 1982); the highest $P_{di}$ generated throughout loading or during maximal inspiratory pressure maneuvers was defined as $P_{di,max.}$ Respiratory and cardiovascular data were averaged over each min of the IRL trial. The amplitude of the $P_{di,tw}$ and $P_{ga,tw}$ was measured from the initiation of twitch pressure to peak pressure for each percentage of the stimulator's output.

Average $P_{di,tw}$ and $P_{ga,tw}$ amplitude was calculated for all twitches at baseline and at 3 min, 15 min, and 30 min after IRL individually and as a group mean. Average values for diaphragm M-wave amplitude and area in response to phrenic nerve stimulation, and RA and EO M-wave amplitude and area in response to thoracic nerve stimulation, were determined for each subject at increasing percentages of the stimulator's output. Group mean values for M-wave amplitude and area for each percentage of stimulator power output were also calculated. The M-wave amplitude and area were also calculated for all twitches at baseline and at 3 min, 15 min, and 30 min after IRL individually and as a group mean. M-wave amplitude was measured from peak-to-peak (mV). M-wave area was calculated by integrating both the positive and negative portions of the action potential. Neural activation of the abdominal muscles was determined for each minute of loading. A digital band-pass filter with a low and high cut-off frequency of 30-2000 Hz was placed on raw EMG signals prior to transforming them into root mean square with a time constant of 0.1 s. Data were expressed as a percent of maximal EMG activity, defined as the highest level of EMG during maximal static inspiratory or expiratory pressure maneuvers. Peak root mean square data were analyzed during inspiration by manually selecting EMG signals to avoid the influence of cardiac artifact on EMG measurements of the respiratory muscles (Ramsook *et al.*, 2016).

*Statistics.* Repeated measures analysis of variance (ANOVA) was used to compare M-wave amplitude, M-wave area, $P_{ga}$ and $P_{di}$ during the ramp protocol to determine if thoracic and cervical stimulation were supramaximal. Repeated measures ANOVA was also used to compare M-wave amplitude, M-wave area, $P_{ga}$ and $P_{di}$ before and after loading. Following significant main effects, pairwise comparisons were made using Tukey's post-hoc test. Within-occasion coefficient of variation for $P_{di,tw}$ and $P_{ga,tw}$ was determined for each subject at baseline and at 3 min, 15 min, and 30 min after IRL. Repeated measures ANOVA procedures were used to compare cardiorespiratory variables and abdominal EMG activity at equivalent time points during IRL trials. Several physiological variables failed the Shapiro-Wilk normality test and as such were evaluated using

Friedman repeated measures ANOVA on ranks. Pearson's product moment correlations were utilized to determine linear relationships between cumulative force output of the diaphragm and cumulative $P_{ga}$ and the percent reductions from baseline to 3 min post-IRL in $P_{di,tw}$ and $P_{ga,tw}$, respectively. Statistical significance was set at $P<0.05$. Results are expressed as mean ± SD, unless otherwise stated.

**RESULTS**

*Nerve Stimulation.* Figure 1 shows individual subject and group mean $P_{ga,tw}$, $P_{di,tw}$ and M-wave amplitudes at increasing stimulator output. There were proportional increases in $P_{ga,tw}$ and $P_{di,tw}$ as the stimulator output increased. When analyzed as a group mean, $P_{di,tw}$ was not significantly lower at 90% stimulator output than it was at 100% stimulator output. However, when looking at the individual data, values between 90 and 100% stimulator output differed by more than 5% in 4 of the 9 subjects. Despite no difference in $P_{ga,tw}$ between 95% and 100% stimulator output when analyzed as a group mean, there was no clear plateau. Values of $P_{ga,tw}$ at 95 and 100% stimulator output were within 5% in 3 of the 9 subjects. M-wave data were obtained in 3 subjects for the right and left side of the diaphragm, 5 subjects for the RA, and 8 subjects for the EO. For all three muscles, M-wave amplitude was increased as a function of increasing stimulator output.

*Inspiratory Resistive Loading.* Task failure occurred at 15.0 ± 7.5 min (range: 6.8 – 23.0 min). Figure 2 shows raw data from a representative subject during rest, first minute, second minute, and final minute of loading and demonstrates how the main respiratory and cardiovascular variables responded during IRL. Table 1 shows group mean cardiorespiratory and EMG data during rest, the first four minutes, and the final minute of loading. Throughout IRL, subjects maintained a $T_I/T_{TOT}$ of 0.63. End-expiratory $P_{es}$ was not significantly higher than rest at any point during loading. Positive end-

expiratory $P_{es}$ resulted from the supine position. Group mean values of $P_{di}/P_{di,MAX}$ steadily declined throughout loading, and when compared to the first minute, $P_{di}/P_{di,MAX}$ values were significantly lower by the fourth minute. Though significantly higher than resting values during the first, second and fourth minutes, $P_{ga}/P_{ga,MAX}$ did not reach values greater than 12% during inspiration. Expressed as a percent of maximum, $EMG_{RA}$ and $EMG_{EO}$ reached 36% and 25%, respectively. This can be seen in the raw $P_{ga}$, $EMG_{RA}$, and $EMG_{EO}$ trace in Figure 3. Subjects maintained a consistent breathing pattern from the first minute all the way through to the final minute of IRL. From minute to minute, tidal volume ($V_T$) was maintained within 200 mL, minute ventilation ($\dot{V}_E$) within 4 l·min$^{-1}$, and $f_b$ at 16 breaths·min$^{-1}$. When subjects began inspiring against resistance, $P_{ET_{CO_2}}$ fell immediately but was within 5 mmHg of resting values for the remainder of the trial. Values for $f_c$ and MAP were not usable in three subjects due to hand movement hindering measurements from the finger photoplethysmograph. Similar to the representative subject in Figure 2, group mean values for $f_c$ and MAP increased in a time dependent manner.

*Diaphragm and Abdominal Fatigue.* M-wave data were obtained in 3 subjects for both sides of the diaphragm, 5 subjects for the RA, and 8 subjects for the EO. M-wave amplitudes and areas for the left and right diaphragm, RA and EO were not different across time (data not shown). $P_{di,tw}$ was significantly lower than baseline at 3 min, 15 min, and 30 min post-IRL (all $P$<0.001) (Figure 4). $P_{ga,tw}$ was not significantly different from baseline at 3 min, 15 min and 30 min post-IRL (Figure 4). The cumulative force output of the diaphragm, ∫Pdi/dt, was 29307 ± 13784 $cmH_2O/s$ and correlated significantly with the severity of IRL-induced diaphragm fatigue (r =0.81, $P$=0.009). The IRL-induced change in $P_{ga,tw}$ was not significantly associated (r =0.41, $P$=0.3) with the cumulative $P_{ga}$, ∫Pga/dt (9877 ± 6756 $cmH_2O/s$).

## DISCUSSION

*Main Findings.* We tested the hypothesis that expiratory muscles are co-activated to the point of fatigue during IRL. We found that the expiratory muscles are recruited during IRL to assist the diaphragm in generating inspiratory pressure, but are not recruited for active expiration during loading. IRL elicited objective evidence of diaphragm, but not abdominal, muscle fatigue. We interpret our findings to mean that IRL elicits reflexive increases in $f_c$ and MAP owing to fatiguing contractions of the diaphragm with no detectable contribution from abdominal muscle fatigue. This suggests that the diaphragm fatigue associated with IRL elicits neurovascular effects that can be isolated to the inspiratory musculature.

*Interactions of the Inspiratory and Expiratory Muscles.* An agonist-antagonist interaction of the respiratory muscles has been demonstrated by the finding of both inspiratory and expiratory muscle fatigue after *expiratory* resistive loading (Taylor & Romer, 2009). In the present study, we were interested in whether the abdominal muscles are co-activated to the point of fatigue during IRL. The IRL protocol required subjects to target 60% of MIP with a 0.7 $T_I/T_{TOT}$. The tension-time index of the diaphragm ($TTI_{di}$) is defined as the product of mean inspiratory $P_{di}$, as a fraction of $P_{di}MAX$, and inspiratory duty cycle ($T_I/T_{TOT}$) (Bellemare & Grassino, 1982). When $TTI_{di}$ is 0.15 or less, breathing can be sustained indefinitely. Above this threshold, the time that breathing can be sustained decreases as a function of $TTI_{di}$ (Bellemare & Grassino, 1982). In the present study, peak inspiratory $P_{di}$ was increased significantly above resting values throughout IRL, with values ranging from 69% $P_{di}MAX$ over the 1st min to 50% $P_{di}MAX$ over the final min (Table 1). Although subjects maintained a $T_I/T_{TOT}$ lower than the target value of 0.7, the mean $TTI_{di}$ was 0.40 throughout the first four mins of loading and 0.32 over the final min (Table 1). Findings in a canine model of diaphragm fatigue suggest that blood flow to the diaphragm becomes limited during intermittent contractions when $TTI_{di}$ exceeds 0.2 (Bellemare *et al.*, 1983). In humans, a high $TTI_{di}$ during ERL was suggested as a potential

contributor to the 9-15% reduction in $P_{di.tw}$ reported by Taylor and Romer (Taylor & Romer, 2009). They postulated that the high $P_{di}$ generated during inspiration throughout ERL resulted in a $TTI_{di}$ (0.18-0.34) that was great enough to compromise diaphragm blood flow. It is possible that the compromised blood flow to the diaphragm was not improved during periods of expiration during ERL, due to the high expiratory abdominal pressures acting on the vessels perfusing the diaphragm (Buchler *et al.*, 1985). In the current study, the range of $TTI_{di}$ (0.32-0.42) likely caused a reduction in blood flow to the diaphragm (Bellemare *et al.*, 1983), leading to reduced oxygen delivery and fatigue. We reason that blood flow would have been compromised to a greater extent in the subjects who generated the most pressure over time during IRL. This is supported by the significant correlation between the severity of diaphragm fatigue (% reduction $P_{di,tw}$) and the cumulative force output of the diaphragm during IRL ($r = 0.81$, $P$=0.009). Amplitude and area of M-waves for both sides of the diaphragm were not significantly lower than baseline post-IRL (data not shown). However, due to the small sample size for which we have M-wave data, firm conclusions cannot be drawn regarding the mechanisms of diaphragm fatigue.

Contrary to our hypothesis, abdominal fatigue was not detected after IRL, as indicated by no significant pre- to post-IRL reduction of $P_{ga,tw}$ in response to magnetic stimulation of the thoracic nerves. Activation of the abdominal muscles may occur during IRL in order to reduce EELV. At a reduced EELV, the diaphragm can function at an optimal length for force development (Smith & Bellemare, 1987) and elastic energy is stored in the abdominal and thoracic walls to aid subsequent inspiration (Henke *et al.*, 1988). In the present study, no increase in end-expiratory $P_{es}$ during IRL compared to rest was observed (Table 1), suggesting EELV was not reduced during loading by active recruitment of the abdominals during expiration. In contrast, the abdominal muscles were recruited during IRL to aid the diaphragm during inspiratory pressure generation (Figure 3). This is demonstrated by an increase in $P_{ga}/P_{ga}MAX$ to 7-12% during inspiration throughout IRL compared to 2% at rest (Table 1). By comparison, specifically loading the expiratory muscles using ERL resulted

in a peak $P_{di}$ during expiration ≥26% $P_{di,MAX}$ and peak inspiratory $P_{di}$ ≥52% $P_{di,MAX}$ (Taylor & Romer, 2009). When our EMG data was expressed as a percent of maximum activation, RA and EO inspiratory activity during IRL reached peak values of 36% and 25%, respectively. Though EMG values are higher than the $P_{ga}$ values and suggest a higher degree of abdominal muscle activity, it is important to note that the maximum values used for normalization were measured during maximal inspiratory and expiratory pressure maneuvers. Previous research has shown that the maximum activity of the RA and EO occurs during trunk flexion, not respiratory maneuvers (Gandevia *et al.*, 1990). This suggests that the percent of maximum activation calculated in the current study is higher than if we had normalized to EMG activity during trunk flexion. Cumulative $P_{ga}$ during inspiration was 9877 ± 6756 $cmH_2O/s$. When compared to the cumulative force output achieved during ERL protocols that resulted in a reduction in $P_{ga,tw}$ of 15 to 22% (Taylor & Romer, 2009), values during IRL were considerably less than the lowest value achieved during ERL (9877 ± 6756 $cmH_2O/s$ in the present study versus 47224-83455 $cmH_2O/s$). It is likely that the cumulative work history of the abdominal muscles was insufficient to cause fatigue during IRL. The modest increase in $P_{ga}/P_{ga,MAX}$ and relatively low cumulative $P_{ga}$ suggests that although co-activation of the abdominal muscles does occur during IRL, the degree of co-activation is minimal. Limited co-activation of the abdominal muscles during IRL compared to significant activation of the diaphragm during both the inspiratory and expiratory phases of ERL could explain why the diaphragm fatigues during ERL but the abdominals do not fatigue during IRL.

Our finding of limited abdominal recruitment during IRL supports previous findings in an animal model. A supine canine model was used to investigate changes in blood flow to multiple respiratory muscles at three increasing inspiratory resistive loads using a radioactive microsphere technique (Robertson *et al.*, 1977). Only blood flow to the diaphragm was increased significantly above rest at the lowest inspiratory load imposed. At the highest inspiratory resistance, blood flow to

the diaphragm rose 26 times resting values, while flow to the expiratory transverse abdominal and internal intercostals rose only 4 and 5 times resting levels, respectively.

*Physiological Consequences of Inspiratory and Expiratory Muscle Fatigue.* The increased $P_{di}$ associated with IRL likely caused mechanical deformation of the diaphragm and increased activity of mechanically sensitive (type III) afferent fibers within the muscle. Metabolically sensitive (type IV) afferent fibers were likely stimulated by accumulation of lactic acid, inorganic phosphates, and other metabolic by-products, leading to a sympathetically mediated metaboreflex (Dempsey *et al.*, 2002). While muscle sympathetic nerve activity was not measured in the current study, time-dependent increases in $f_c$ and MAP (Table 1) are consistent with previous IRL studies (St Croix *et al.*, 2000; Sheel *et al.*, 2001; Witt *et al.*, 2007). These changes in $f_c$ and MAP are unlikely due to changes in intrathoracic pressures and inspiratory flow. Indeed, when central inspiratory motor output is increased in the absence of diaphragm fatigue, by either increasing inspiratory force output (95% of MIP) or inspiratory flow rate (5 × eupnoea), there is no change in $f_c$, MAP, leg blood flow, or muscle sympathetic nerve activity (St Croix *et al.*, 2000; Sheel *et al.*, 2001). Previous work investigating the metaboreflex originating from the expiratory muscles demonstrates that increases in muscle sympathetic nerve activity only occur during fatiguing contractions of the abdominals and not under conditions of high expiratory motor output or high expiratory flow without task failure (Derchak *et al.*, 2002). That no abdominal muscle fatigue was detected in the present study suggests that the abdominals did not contribute to the sympathetically mediated metaboreflex-induced increases in $f_c$ and MAP.

*Methodological Considerations.* The inclusion of only healthy young men limits the generalizability of our findings. We also acknowledge that the sample size in the present study (n=9) was relatively small. As such, statistical power and the possibility of a type II error must be considered when interpreting our findings. However, it is important to note that diaphragm fatigue occurred in all

subjects (25-42% reductions in $P_{di,tw}$) without evidence of abdominal fatigue, as assessed using non-volitional stimulation techniques. We recognize that stimulation of phrenic and thoracic nerves was submaximal in some subjects (see Figure 1) and we cannot rule out the possibility that this influenced our findings. Although submaximal in some subjects, several steps were taken to ensure that the degree of stimulation remained consistent throughout the study. All stimulations were delivered at 100% of the stimulator's output and the coil position for phrenic and thoracic stimulation was marked before baseline measurements were made to ensure that the coil was positioned the same for all stimulations. End-expiratory $P_{es}$, used as a surrogate for lung volume, was measured before all stimulations and care was taken to avoid stimulating the phrenic and thoracic nerves when the pressure varied by more than a few $cmH_2O$ (Table 2). By maintaining a similar lung volume, we hoped to ensure that the length of the diaphragm remained consistent throughout the stimulation protocol to avoid differences in $P_{di,tw}$ due to the length-tension relationship of this muscle (Smith & Bellemare, 1987). As twitch values are dependent on the voluntary contraction prior to stimulation (Wragg *et al.*, 1994), care was also taken to ensure that the degree of potentiation was consistent at baseline, 3 min, 15 min, and 30 min post-IRL (Table 2). Though subjects were given verbal and visual feedback on their potentiation efforts, MEP values did decline from 3 min post to 30 min post-IRL. This may explain the slight, but insignificant, reduction in $P_{ga,tw}$ post-IRL. Mean values for $P_{di,tw}$ and $P_{ga,tw}$ at baseline were similar to previous studies using cervical magnetic stimulation (Similowski *et al.*, 1989; Taylor & Romer, 2009) and thoracic magnetic stimulation (Taylor *et al.*, 2006). Another important consideration is the potential for axonal hyperpolarization leading to a reduction in axonal excitability (Vagg *et al.*, 1998; Burke *et al.*, 2001). Throughout IRL, repeated contraction of the diaphragm could have caused a reduction in axonal excitability. If excitability of the motor axons that innervate the diaphragm was reduced, this could have contributed to the reduction in post-IRL $P_{di,tw.}$ Previous work has demonstrated that hyperpolarization may take 10-15 min to decay to baseline levels upon cessation of voluntary contractions (Vagg *et al.*, 1998). In the present study, $P_{di,tw}$ remained significantly below baseline values at 15 and 30 min post-IRL (Figure 4). As such, while axonal hyperpolarization may have contributed to the reduction in $P_{di,tw}$ measured 3 min after IRL, we

are confident that the reduced $P_{di,tw}$ at 15 and 30 min post-IRL was due to contractile fatigue rather than changes in axonal excitability. Mean coefficient of variation for $P_{di,tw}$ at baseline and at 3, 15, and 30 min post-IRL was 6.3, 6.6, 8.1, and 6.0% ($P > 0.05$), respectively. The mean coefficient of variation for $P_{ga,tw}$ was 6.0, 9.0, 9.3, and 5.6% at baseline, 3, 15, and 30 min post IRL, respectively ($P > 0.05$). The 9.0% CV at 3 min post-IRL meant that we were unlikely to detect a reduction in $P_{ga,tw}$ <18%. Therefore, it is possible that a small amount of fatigue was present in the abdominal muscles after IRL but was below our threshold of detection.

*Conclusions.* Inspiratory resistive loading to the point of task failure elicits fatigue of the human diaphragm with no evidence of abdominal fatigue. Since others have reported that expiratory resistive loading causes diaphragm fatigue (Taylor & Romer, 2009), we interpret our findings to mean that agonist-antagonist interactions for the respiratory muscles are more important during expiratory loading than inspiratory loading. The time dependent increases in $f_c$ and MAP during inspiratory loading can be attributed to a muscle metaboreflex originating within the diaphragm, with no detectable contribution from the abdominal musculature.

**ADDITIONAL INFORMATION**

*Competing interests:* None declared.

*Author contributions:* CMP, PBD, YMS, LMR, DCM, AWS were responsible of the conception and design of the research. CMP, JFW, PBD and YMS collected data. CMP, JFW, PBD and YMS analysed the data. All authors participated in the interpretation of data. CMP, JFW, PBD, YMS and AWS drafted the manuscript. or revised it critically for important intellectual content. All authors revised, edited and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**Table 1.** Physiological responses during rest, the first 4 min, and the final min of loading.

| | Rest | 1st min | 2nd min | 3rd min | 4th min | Final min |
|---|---|---|---|---|---|---|
| $P_{m,}$ $cmH_2O$ | -0.6±0.3 | -77±14* | -76±13* | -76±17* | -76±15* | -71±13 |
| $T_I/T_{TOT}$ | 0.40±0.02 | 0.61±0.07* | 0.62±0.07* | 0.63±0.07* | 0.63±0.07* | 0.64±0.05* |
| E $P_{es,}$ $cmH_2O$ | 7.8±3.5 | 7.5±5.0 | 8.2±4.6 | 8.9±3.0 | 9.8±5.2 | 8.9±4.3 |
| $P_{di}/P_{diMAX}$, % | 7±3 | 69±13* | 63±11* | 61±11* | 59±11*† | 50±10*† |
| $TTI_{di}$ | 0.03±0.01 | 0.41±0.06* | 0.40±0.04* | 0.38±0.04* | 0.38±0.07* | 0.32±0.07* |
| $P_{ga}/P_{gaMAX}$, % | 2±1 | 12±6* | 10±5* | 8±5 | 10±6* | 7±4 |
| $EMG_{RA}$, %Max | 4±3 | 25±18 | 30±20* | 28±17* | 26±20 | 28±22 |
| $EMG_{EO}$, %Max | 3±2 | 16±11 | 18±10* | 19±13 | 18±13 | 16±12* |
| $V_T$, l | 0.7±0.3 | 2.2±0.8* | 2.1±0.7* | 2.2±0.8* | 2.1±0.7* | 2.0±0.2 |
| $f_b$, breaths·min$^{-1}$ | 15±3 | 16±0.6 | 16±0.3 | 16±0.3 | 16±0.7 | 16±0.3 |
| $\dot{V}_E$, l·min$^{-1}$ | 11±4 | 36±13* | 34±12* | 34±11* | 35±11* | 32±3 |
| $PET_{CO_2}$, mmHg | 41±3 | 32±2* | 37±2 | 39±2 | 39±3 | 40±1 |
| $f_c$, beats·min$^{-1}$ | 52±8 | 86±21* | 94±19* | 95±22* | 100±16* | 106±21*† |
| MAP, mmHg | 86±7 | 83±7 | 92±7 | 92±11 | 97±8*† | 100±10*† |

Values are means ± SD. Note $f_c$ and MAP values represent data for 6 of 9 subjects. $P_m$, mouth pressure; $T_I/T_{TOT}$, inspiratory duty cycle; E $P_{es}$, end-expiratory esophageal pressure; $P_{di}$, peak inspiratory transdiaphragmatic pressure; $P_{diMAX}$, maximum transdiaphragmatic pressure; $TTI_{di}$, tension time index of the diaphragm; $P_{ga}$, peak inspiratory gastric pressure; $P_{gaMAX}$, maximum gastric pressure; $V_T$, tidal volume; $f_b$, breathing frequency; $\dot{V}_E$, minute ventilation; $PET_{CO_2}$, end-tidal carbon dioxide pressure; $f_c$, cardiac frequency; MAP, mean arterial pressure. * significantly different from rest ($P<0.05$); † significantly different from 1st min ($P<0.05$)

**Table 2.** Potentiation efforts and end-expiratory esophageal pressure during nerve stimulation before and after inspiratory resistive loading.

| | Baseline | 3 min post | 15 min post | 30 min post |
|---|---|---|---|---|
| *Diaphragm* | | | | |
| MIP, $cmH_2O$ | -115±22 | -108±23 | -107±21 | -110±22 |
| E $P_{es}$, $cmH_2O$ | -2.5±1.1 | -2.6±2.0 | -1.9±2.1 | -2.3±1.9 |
| *Abdominals* | | | | |
| MEP, $cmH_2O$ | 119±19 | 114±18 | 110±20 | 106±24 |
| E $P_{es}$, $cmH_2O$ | 0.2±4.2 | -0.3±4.6 | 0.6±2.4 | -0.1±3.1 |

Values are means ± SD. MIP, maximum inspiratory mouth pressure; E $P_{es}$, end-expiratory esophageal pressure; MEP, maximum expiratory mouth pressure.

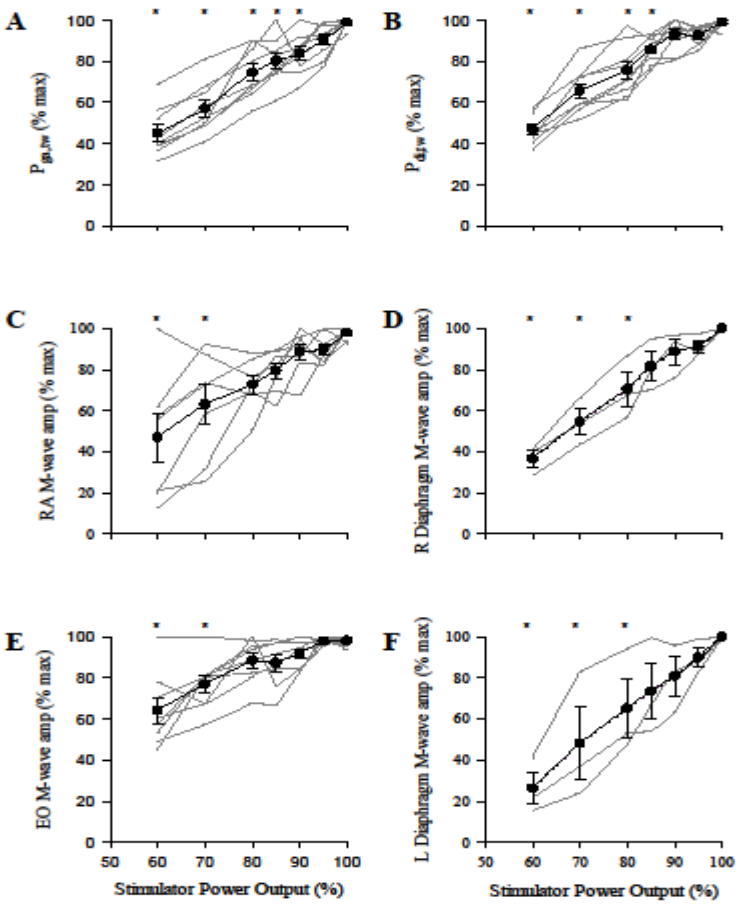


**Figure 1.** Individual subject (thin grey lines) and group mean (thick black lines) gastric twitch pressure ($P_{ga,tw}$) (A), transdiaphragmatic twitch pressure ($P_{di,tw}$) (B), rectus abdominis M-wave amplitude (C), right diaphragm M-wave amplitude (D), external oblique M-wave amplitude (E), and left diaphragm M-wave amplitude (F) in response to 1-Hz magnetic stimulation of increasing stimulation intensity. Values are means ± SE. * group mean values are significantly lower from those at 100% of stimulator output ($P<0.05$).

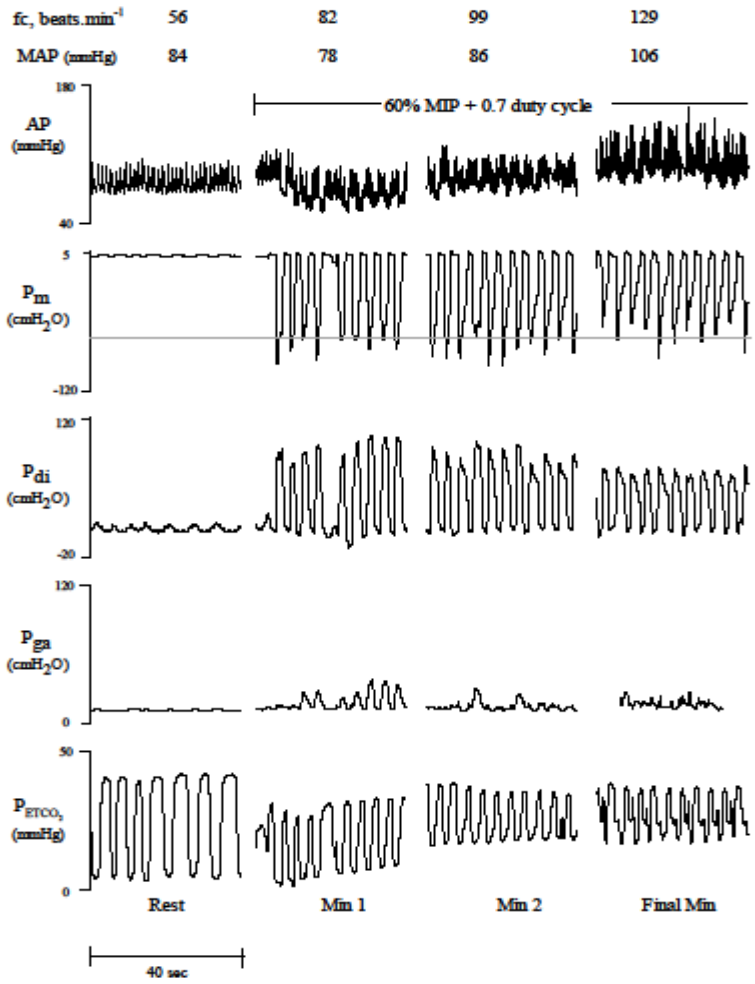


**Figure 2.** Raw data from a representative subject during rest, first min, second min, and final min of inspiratory resistive loading. $f_c$, cardiac frequency; MAP, mean arterial blood pressure; MIP, maximum inspiratory mouth pressure; AP, arterial blood pressure; $P_m$, mouth pressure; $P_{di}$, transdiaphragmatic pressure; $P_{ga}$, gastric pressure; $P_{ET_{CO_2}}$, end-tidal carbon dioxide pressure.

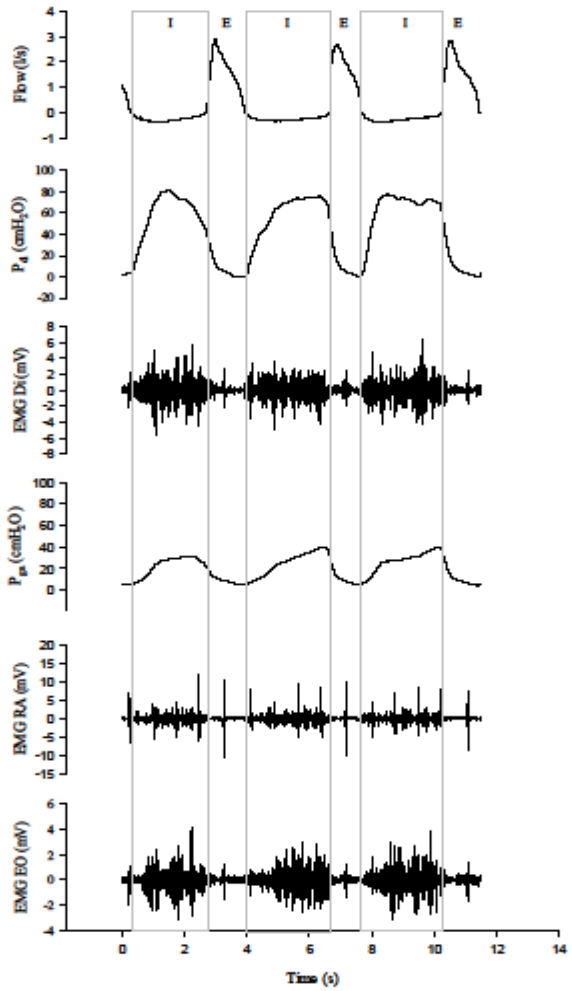


**Figure 3.** Raw data from a representative subject for 3 breaths during the first minute of inspiratory resistive loading. Inspiration (I) is represented by negative airflow. Expiration (E) is represented by positive airflow. $P_{di}$, transdiaphragmatic pressure; Di, diaphragm; $P_{ga}$, gastric pressure; RA, rectus abdominis; EO, external oblique.

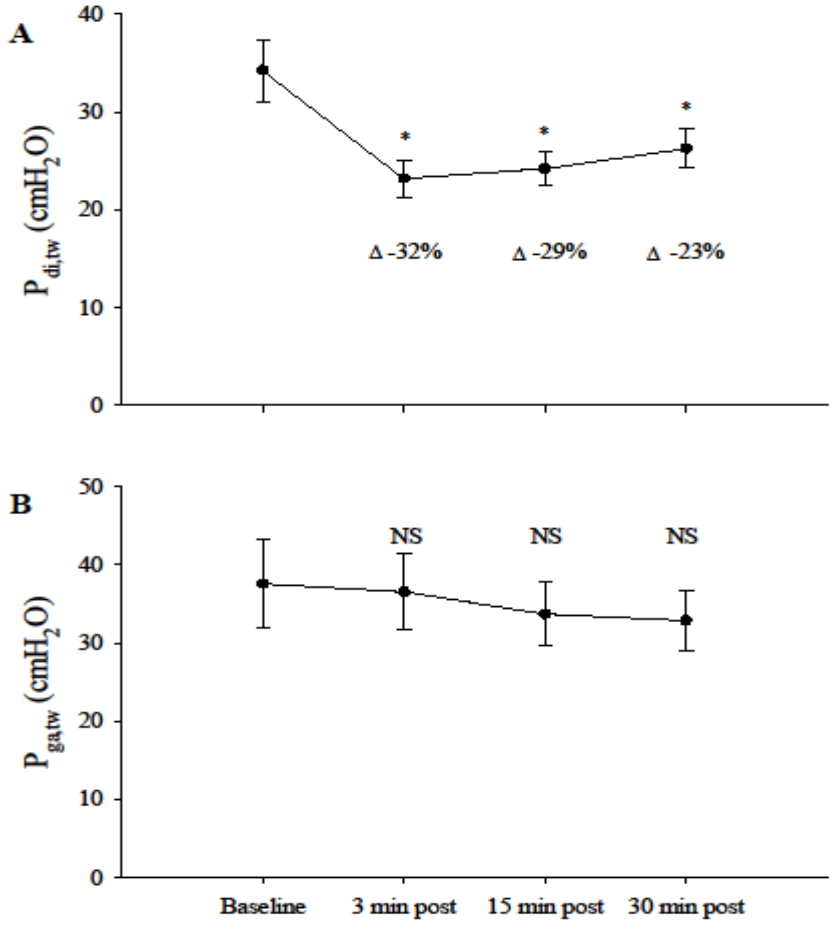


**Figure 4.** Group mean potentiated transdiaphragmatic twitch pressure ($P_{di,tw}$) (A) and gastric twitch pressure ($P_{ga,tw}$) (B) in response to 1-Hz magnetic stimulation at baseline and at 3 min, 15 min, and 30 min after IRL. Values are group means ± SE. * group mean values significantly lower than baseline ($P<0.001$). Group mean values were not significantly reduced below baseline at any time for $P_{ga,tw}$ ($P>0.05$).